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POTENTIAL USE OF BAGASSE AS A CHEAP SOURCE OF CELLULOSE FOR THE IMMOBILIZATION OF UREASE ENZYME BY ADSORPTION

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Abstract

Bagasse is a valuable biomaterial produced as a waste of sugar processing industry. In Pakistan it is mainly used as source of fuel and in paper industry. Bagasse can be used as an economical source for the production of cellulose and cellulose derivatives, which are widely used in industries and pharmaceuticals. In the present work cellulose was extracted from bagasse using acid and basic hydrolysis followed by bleaching with sodium chlorate. The extracted cellulose was activated thermally at 250°C. The extracted cellulose (native) and thermally activated cellulose was used as carrier for the immobilization of Urease enzyme. The immobilization efficiency of the different carriers was studied as a function of contact time, temperature, pH, carrier dosage and enzyme concentration. The maximum immobilization efficiency for the different types of carriers was found at contact time: 1.0 H at adsorbent dose: 0.5 g., pH: 7, temp: 25C◦, Enzyme conc: 1mg/mL. The results of this study show that bagasse and other agriculture residues which are rich in lignocelluloses' materials can be efficiently employed in cost effective immobilization technologies.

Key words: Bagasse, cellulose, urease, immobilization, adsorption

Introduction

The immobilization of biocatalysts is a new area of interest for scientist throughout the world for biotechnological applications in various fields. The immobilized enzymes are believed to be thermally more stable as compared to their free form (Singh *et al*. 2013). Immobilized enzymes are widely used in food processing, textile, detergents, biotechnology, and pharmaceutical industries a (Harrer *et al*. 2007). Number of methods can be applied to increase the thermal stability of enzymes (Singh *et al*. 2013). These include chemical modification (Datta *et al*. 2013), Protein engineering (Homaei *et al*. 2013), and enzyme immobilization (Hassan *et al*. 2019), these modifications are found to increase the thermal stability of enzymes and resistance toward denaturation (Mohammad *et al*. 2015). The simplest method of immobilizing enzyme is the adsorption of an enzyme on a carrier or support material (Jesionowski *et al*. 2014). Adsorption involves weak non-covalent linkages such as ionic or hydrophobic interactions, hydrogen bonding and van der Waals forces (Krishnamoorthi *et al*. 2015; Weng *et al*. 2004) between the enzyme and the support material (Nakamura *et al*. 2002). Most of the support materials are either inorganic or Organic (Hudson *et al.,* 2008). The inorganic carriers are usually alumina, ceramics, activated carbon and kaolinite, while organic are both synthetic carriers (nylon, polystyrene), and natural organic polymers (chatoyant, dextran, gelatin, cellulose, starch).

Sugarcane is an important cash crop of Pakistan. It is grown on an area of about 1.07 million hectares (ha) in Pakistan. The province wise break up is as follow, Punjab (62.4%), Sind (26%), and Frontier (11.2%). The total cane production according to Government of Pakistan is 53.4 million tone/year. The average yields being around 50

tones/ha. There are about 81 sugar mills in Pakistan, which depends on this sugarcane for the sugar production. Beside these sugar mills also produce bagasse to the tune of 16.6 million tons annually as against 54 million tones world production annually. One tone of sugarcane can produce153kg of sugar, 273kg of bagasse (50% moisture content) and165kg of straw (15% moisture content) (Kamel *et al*. 2012).

Bagasse is the main bio-waste, which can be utilized as a cheap source of cellulose for various uses in industries. Since bagasse are principally composed of cellulose, hemicelluloses, and lignin. So further chemical treatment of bagasse can produce other useful by products, which may be the source of income on one hand and may also help in the reduction of agriculture related pollution on the other hand (Kamel *et al*. 2012). In the present study we tried bagasse and bagasse cellulose to study its ability to acts as a carrier for enzyme urease.

Materials and methods

Materials

Bagasse, Sodium Hydroxide, Sulphuric acid, Phosphate Buffers (pH 5- 8), Jack bean urease were commercial grade chemicals obtained from local market in Peshawar Pakistan.

Methods

Air Drying: Sufficient amount of Bagasse was taken and was kept in a dry place for 2-4 days to make it air dried. While drying, it was kept away from sun-light so as to keep its components unchanged.

Oven Drying: A labeled china dish was washed and kept in an oven for 4-5 hours to make it dry and its weight in dry condition was determined. China dish containing 10 g of the air dried sample was weighed and kept in oven at 100ºC for 24 hours to remove water. The moisture content of the sample was determined by the following formula:

$$\text{Moisture Content (\%)} = \frac{\text{Weight of dry bagasse}}{\text{Weight of wet bagasse}} \times 100$$

The sample was used on dry weight basis for subsequent studies.

Preparation of adsorbents

Pre-Hydrolysis of bagasse with 10% H_2SO_4 (AT: Acid treated bagasse): A weighed quantity of the raw material (oven dried bagasse) was taken in 0.5dm^3 flask and 10% H_2SO_4 was added to the flask (liquor ratio 1:15). The contents of the flask were heated under reflux for 3 hours. The contents were cooled under tap water and finally washed with distilled water till neutral (tested with indicator) the wet sample was stored in a fridge at 4°C (Kamel *et al*. 2012).

Alkaline Hydrolysis (BT: Base treated bagasse): A weighed quantity of the wet sample was taken and enough water was added to make the liquor ration 1:15.10% NaOH solution was added to the flask and was heated under reflux for 3 hours. The contents were washed first with tap water and then with distilled water till become neutral (tested with indicator) the wet sample was placed in fridge at 4°C (Kamel *et al*. 2012).

Charred Bagasse (C: Charred bagasse): A weighed quantity of bagasse was heated in muffle furnace for 6 hrs at 250°C to get charred bagasse.

Method of immobilization of urease on chemically and thermally treated bagasse

A known weight of carrier was taken in a test tube. A known volume of enzyme solution prepared in phosphate buffers of known pH was added, the contents were agitated on a shaker at 20 rpm for 10 minutes. The contents were placed at

various temperatures for different time. The contents were filtered on filter paper (what man No.40). The concentration of the residual protein in the filtrate was determined spectrophotometerically at 520 nm. The effect of contact time, pH, temperature, and carrier dose and enzyme concentration were studied and the immobilization efficiency was calculated as follow (Jesionowski *et al*. 2014).

$$Residual\ protein\ in\ the\ filtrate\ (X) = (Absorbance\ of\ the\ filtrate) / (absorbance\ of\ the\ test\ solution)\ x\ 100$$

% Immobilization yield = 100- X

Results and Discussion

Chemical composition of bagasse:

The chemical composition of the three types of carrier was determined. The results are shown in table 01.

Table 01: Chemical composition of bagasse and modified bagasse

Content %	Materials		
	Bagasse	Unbleached Bagasse	Bleached Bagasse
Wax and resin	05.0	00.0	00.0
Lignin	16.0	09.2	05.2
α- Cellulose	46.6	60.3	68.7
Silica	05.0	04.3	03.9
Hemicelluloses	13.7	12.1	10.6
Ash	10.0	07.5	05.3

Immobilization

The immobilization of urease on chemically and thermally treated bagasse was carried by the adsorption method (Jesionowski *et al*. 2014). The immobilization conditions like time, adsorbent dose, effect of pH, effect of temperature and enzyme concentrations were optimized for the immobilization procedure. The results are discussed below:

Effect of contact time

The effect of time on the immobilization of urease on chemically and thermally treated bagasse was studied from 1-5 hours. The results are shown in fig 1. The results show that maximum immobilization yield is obtained for urease in one hour on acid treated bagasse (84%), basic treated bagasse (72%) and charred bagasse (68%). On further increasing the time of contact, there is an irregular trend in immobilization yield for both chemically and thermally treated bagasse. This may be due to the weak Vander Waal's forces between the carrier and the enzyme urease which leak the enzyme back into the solution and hence immobilization yield is not regularly increasing (Nakamura *et al*. 2002).

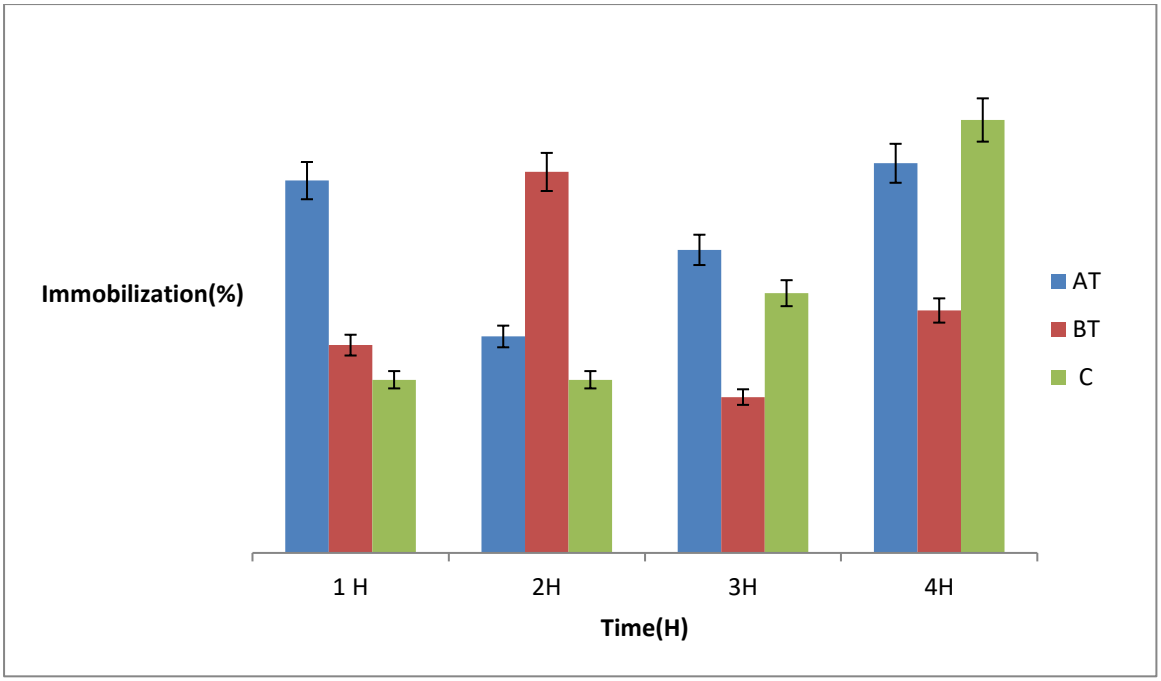

Fig.1: Effect of time on the immobilization of urease: at adsorbent dose: 0.5g, pH: 7, temp: 25C◦, Enzyme conc: 1mg/mL

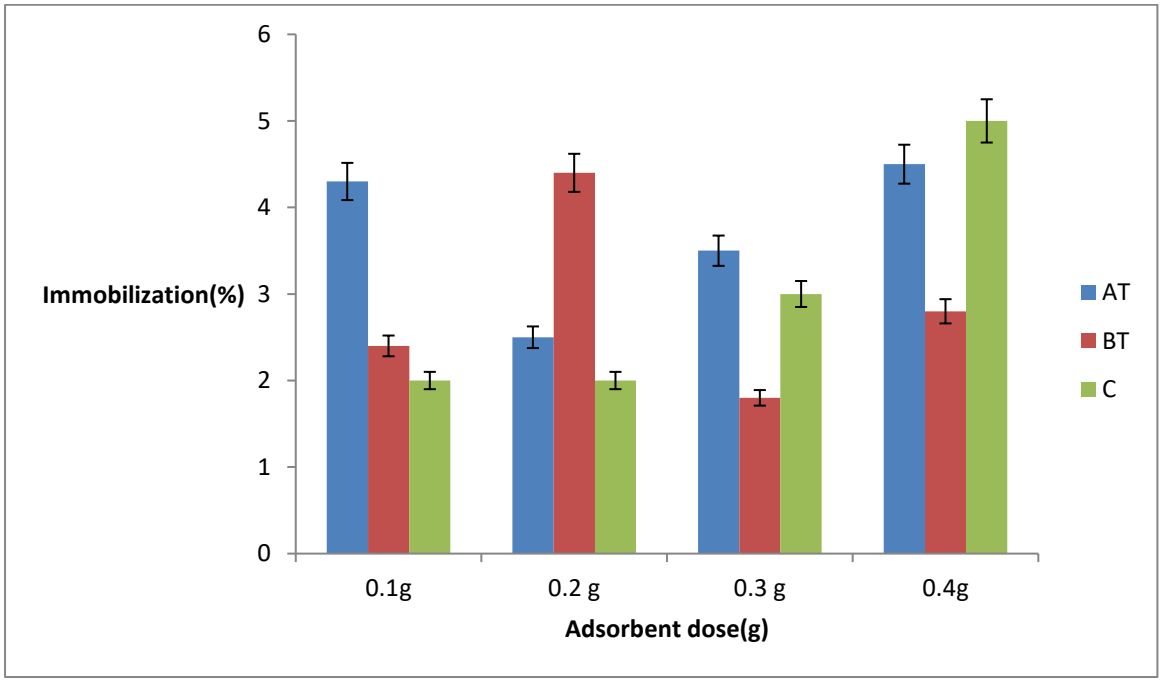

Fig 2: Effect of carrier dose on the immobilization of urease: At adsorbent dose: 0.5 g, pH: 7, Temp: 25˚C, Enzyme conc.: 1mg/mL

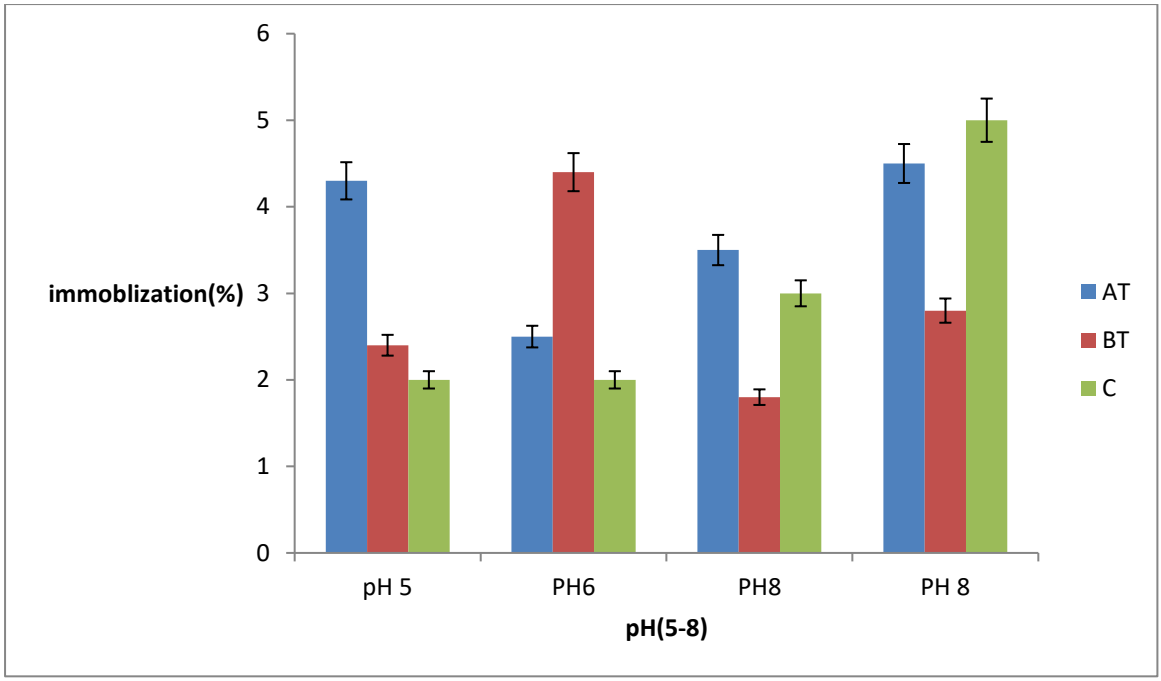

Fig.3: Effect of pH on the immobilization of urease: At adsorbent dose: 0.5 g, pH: 7, temp: 25C◦, Enzyme conc.: 1mg/mL

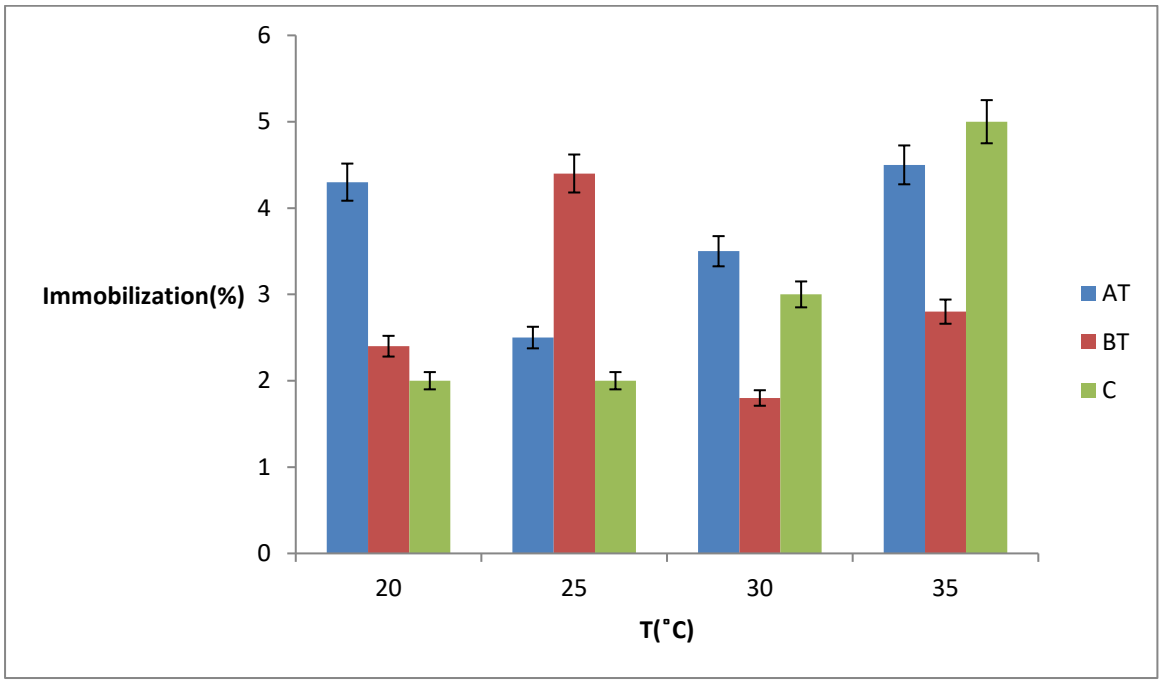

Fig.4: Effect of temperature on the immobilization of urease: At adsorbent dose: 0.5 g, pH: 7, temp: 25℃, Enzyme conc: 1mg/mL

Effect of carrier Dose

The effect of carrier dose on the immobilization yield of urease on chemically and thermally treated bagasse was studied in the range of 0.1g to 0.5g. The results are shown in the fig.2. It is clear from the fig that for acid treated bagasse, 0.1g of adsorbent was enough for maximum immobilization. By further increasing the adsorbent beyond 0.1g, there is decrease in immobilization efficiency. For basic treated bagasse, the optimum dose is 0.4g and after that there is decrease in immobilization efficiency. For charred bagasse, the optimum dose is 0.3g and after that there is decrease in immobilization efficiency.

Effect of PH

The effect of pH on the immobilization of urease on chemically treated and thermally treated bagasse was studied in the range of 5-8 keeping all other conditions constant. The results are depicted in figure 3. The result show that for acid treated and basic treated bagasse, the maximum immobilization efficiency is obtained at pH=6 but for charred bagasse, it is pH=7. The change in pH is due to the difference in the surface charges of the two carrier substances (Weng *et al*. 2003).

Effect of Temperature

The effect of temperature on the immobilization of urease on thermally and chemically treated bagasse was studied in the range of 20-35 °C. The results are depicted in fig.4. The optimum temperature for the maximum immobilization was found to be 25°C for basic treated bagasse and charred bagasse while for acid treated bagasse, it was 35°C. The difference in optimum temperature may be attributed due to the difference in the chemical nature as well as reactive groups.

Similar results of model enzyme, carboxyl esterase (CE) immobilization on Bio mineralized Calcium Carbonate Microspheres was observed. The free and the cross-linked CE were compared for their stability at various (pH8-10) at 25°C. Highest CE activity was observed at pH 8. Moreover immobilized CE was found to be more stable than the free CE. This could be due the multipoint attachment of CE with inorganic $CaCO_3$ microspheres and electrostatic interactions with the negative charged hydrophobic $CaCO_3$ surfaces (Vescovi *et al*. 2016).

The effect of temperature on the free and immobilized CE was also studied in the range of 15–55°C at optimized pH 8.0. During temperature profiling, the immobilized CE was found to be more stable than free CE. It was found that immobilized CE showed 94% activity while free CE lost its activity by 62%. This result showed that temperature profile of immobilized CE was broader than free CE activity at all temperatures and the enzyme activity was retained after immobilization process.

Effect of Enzyme Concentration

The concentration of urease was studied in the range of 0.2-1 mg/mL. The maximum immobilization efficiency was achieved at 0.2 mg/mL of enzyme concentration for both acid treated and charred bagasse and 0.4 mg/mL for basic treated bagasse.

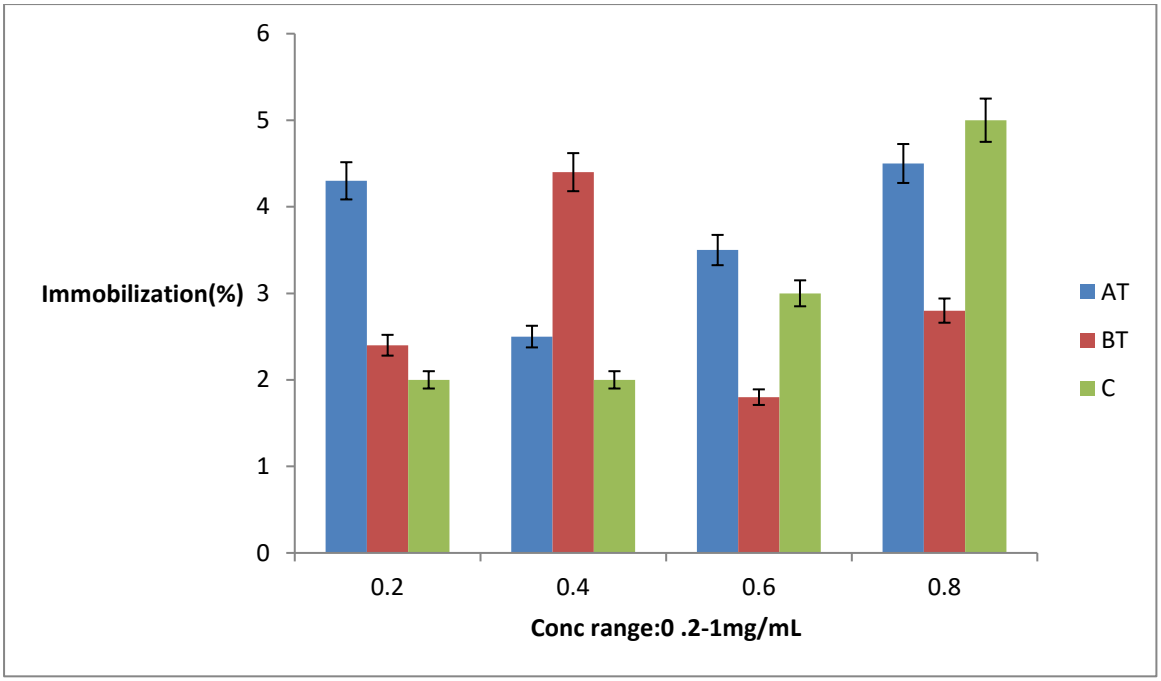

Fig.5: Effect of unease conc. on the immobilization of unease: at adsorbent dose: 0.5 g, pH: 7, temp: 25℃, Enzyme conc.: 1mg/mL

Conclusion

Biopolymers including cellulose possess a unique set of properties, like natural origin, biodegradability, affinity to protein due to the presence of reactive hydroxyl group; biocompatibility and non-toxic nature make them suitable supports for enzymes. Moreover, these biomaterials are renewable and easily to available which make them inexpensive and reduce the costs associated with the immobilization process. The results of this study shows that bagasse and other agriculture residues which are rich in lignocelluloses' materials can be efficiently employed in cost effective immobilization technologies.

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Conflict of Interest

None

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Author's Contribution:

All the authors contributed equally

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