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Transportation and Removal of *E.coli* K-12 Through Selenium Doped Zeolite Packed bed Reactor

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Abstract

At present, water pollution has become a serious threat throughout the world. WHO recommends *Escherichia coli* as a faecal indicator to analyze the quality of water. This investigation aimed to study the fate and its removal from drinking water and secondary treated wastewater in the zeolite (Z) and selenium doped zeolite composite (Se-Z) packed column system. A series of experiments were carried out to establish the breakthrough curve and to assess the column performance. Breakthrough curve analysis was conducted by varying bed height (2 to 4 cm) and flow rate (1 to 2.5 mL/min) with an initial *E. coli* concentration of 4 $\times 10^{10}$ mg/L. The removal efficiency of Z and Se-Z were compared. In comparison, Se-Z exhibited a higher percentage of *E. coli* removal of 66.0% for drinking water and 70.18% for secondary treated wastewater. *E. coli* was efficiently removed by the fixed bed reactor packed with Se-Z with an adsorption efficiency of 14.4×10^{10} mg/g at bed height of 4 cm with a volumetric flow rate of 1.5 mL/min. The maximum adsorption capacity was obtained with an increased height of the bed and decreased volumetric flow rate. Selenium doped zeolite packed column exhibited improved elimination of *E. coli*, which can be utilized in the future to treat water-containing pathogenic microorganisms.

Keywords: *Escherichia coli*, zeolite, selenium, composite, breakthrough curves, pathogenic microbes.

Introduction

Water is an essential constituent of life. Universally almost 100 crores of people lack proper access to drinking water, and 2.5 billion people have insufficient sanitation facilities. Worldwide around 2 billion people are affected by water-related diseases [1], such as diarrhoea, dysentery, typhoid, cholera, and polio. Water-related diseases (diarrhoea) were estimated to cause 5,02,000 deaths each year [2,3]. The water-related disorder's key reason is mixing fecal matter containing pathogens to the water system [4, 5]. Hence the WHO focuses on the treatment and prevention of pathogens entering the water system. Water quality is determined by analyzing its chemical, microbial and radiological characteristics. *Escherichia coli* belongs to normal microflora found by both humans and other mammals. It is an opportunistic pathogen that enables them to cause a variety of enteric diseases [6]. The WHO recommends *Escherichia coli* as a faecal indicator to detect the presence of faecal contamination in water. *Escherichia coli* was not seen in water unless the water had been polluted with faecal matter [7].

The most critical parameter in the study of water quality is the presence or absence

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of contaminants. Water treatment technologies are driven by three fundamental factors: finding pollutants, the announcement of water quality standards, and cost [8]. Water treatment technology uses chlorination and antimicrobial nanomaterials for microbial contaminants removal [9]. The chlorination process results in the creation of disinfectant by-products that are carcinogenic and toxic. [10]. There is also a need to establish an alternative technique for the removal of pathogens present in the water [11]. Nanotechnological processes are superior and cost-effective for water and wastewater treatment. It can be utilised to sanitize and use alternative water sources in a cost-effective way [12, 13].

Zeolite is used in wastewater treatment due to its ion exchange and adsorption property. In the treatment of wastewater, it is used for the heavy metal removals (in-organic anions) such as phosphates, nitrates, chromates, fluorides, and arsenates and also used in bio-filter for microorganism capturing due to its large surface area [14, 15]. Scolecite is a small pore zeolite with an eight-membrane ring structure. The most crucial feature of zeolite is its selective adsorption towards specific microorganisms [16]. Selenium has interaction with pathogenic bacteria due to its increased surface tension. Selenium remains less- toxic to the human system. According to the WHO International standards for drinking water, the permissible limit of selenium in drinking water is 0.01 mg/ Litre [17]. It has antibacterial, antiviral, and antioxidant activities. Selenium nanoparticles have an inhibition effect on both Gram-positive and Gram-negative bacterial growth [18].

Water pollution leads to microorganisms' movement through soil resulting in numerous water-borne diseases worldwide [19]. Hydrological features of the drainage basin and adsorption, desorption, biological and mechanical factors will influence the transport of faecal indicators and pathogens [20]. Hence it is essential to study the fate and transport of faecal indicator. This work aims to produce a selenium-doped zeolite composite for the removal of pathogenic organisms present in the water system. Also, the fate and removal of *E. coli* were analysed through the porous bed in different water samples using zeolite and selenium doped zeolite composite.

Materials and Method

Sodium selenite, Ascorbic acid, Luria Bertani broth, Ethanol were purchased from Himedia Chemicals Limited. The zeolite (400 mm diameter) were purchased from Ajanta minerals, Aurangabad and *Escherichia coli* K-12 (MTCC- 1302) purchased from MTTC, Chandigarh. Wastewater was collected from the sewage treatment plant, VIT, Vellore. Drinking water has been obtained from VIT, Vellore.

Synthesis of zeolite nanocomposite: For the synthesis of Zeolite nanocomposite, 20 ml of 100 mM sodium selenite solution and 4 grams of zeolite were taken and kept on the magnetic stirrer. To that, add 80 ml of 50 mM Ascorbic acid solution slowly. The process was continued for 30 mins and then kept stirring for around 2 hr for selenium's adsorption onto zeolite. After 2hr of stirring, excess selenium was removed by washing with distilled water. Then the zeolite was dried in a hot air oven at 80 ℃ overnight.

Characterization: The XRD pattern of zeolite and selenium doped zeolite was made to predict the crystallographic size and structure. The FTIR pattern was analysed to classify the functional groups, which further confirm the selenium doped zeolite composite formation. The surface morphology and the efficiency of elemental selenium adsorbed onto the zeolite were observed using SEM. Energy dispersive x-ray microanalysis was performed to confirm the elemental or chemical configuration of the composite.

Column experiment: The fate and removal of *E. coli* through packed bed reactor was studied in a glass column (1.5 cm diameter * 20 cm height) with the matrix. Glass wool was packed up to 1 cm height to prevent the loss of adsorbent and to provide mechanical support for the

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adsorbent bed. Zeolite or selenium doped zeolite composite are used as packaging material for the study. The bed height was changed from 2 cm to 4 cm according to the requirement. The desired flow rate was maintained using a peristaltic pump (1.0 to 2.5 mL/min). Figure 3 demonstrates the pictorial representation of the packed bed column.

Preparation of water sample for column experiment: *Escherichia coli K-12* (MTCC Id 1302) were used for the microbial transport studies. *E. coli* cells were grown in Luria Bertani broth for 24 hr at 37 ˚C in an orbital shaker. The suspension was centrifuged for 10 min at 9000 rpm, and the supernatant was removed and replaced with distilled water, followed by centrifugation. These steps were repeated twice to completely remove any trace of growth medium present along with the cell suspension. The pellet was then dissolved and added to 50 ml of secondary treated autoclaved wastewater and drinking water by setting the O.D to 0.5 at 600 nm. The resulting bacterial suspension was then used in the column experiment.

Study of column performance

Parameters affecting the column performance: The fate and removal of *E. coli* in different water sources have been studied by various factors such as volumetric flow rate and bed height. The influence of volumetric flow rate on the removal of *E.coli* from wastewater and drinking water was tested by varying the volumetric flow rates from 1.0 mL/min, 1.5 mL/min, 2.0 mL/min, and 2.5 mL/min with the bed height of 4 cm and with constant influent concentration ($4*10^{10}$ mg/L). The effect of height of the bed on column performance and *E.coli* removal was analysed with varying bed heights (2 cm, 3 cm, and 4 cm) with unchanged influent ($4*10^{10}$ mg/L) and volumetric flow rate of (1 ml/min, 1.5 ml/min, 2 ml/min, 2.5 ml/min).

Breakthrough analysis calculation: The performance of the column was studied based on the shape of the breakthrough curve. The breakthrough curve was plotted between the normalized outlet concentration (C_t/C_0) and time (t), where C_t and C_0 are the concentration of *E. coli* in inlet and outlet (mg/L), respectively. The length of the mass transfer zone of *E. coli* was computed from the following equation (Eq.1); [20]

$$Zm = Z(1 - \frac{t_b}{te}) \qquad \text{(Eq. - 1)}$$

Where Zm is the mass transfer zone length (cm), Z is the bed height (cm), t_b is the breakthrough time (h) and t_e is the exhaustion time (min). The treated effluent volume, V_e was given as (Eq.2);

$$Ve = Qt_e \text{ (Eq-2)}$$

Where Q is the volumetric flow volume rate (mL/min) and t_e is the exhaustion time (min)

The *E. coli* interacted and got adsorbed onto the matrix was given by the equation (Eq.3) [21, 22]

$$q_b = \int_0^{V_b} \frac{C_0 - C_t}{M} dV \text{ (Eq-3)}$$

Where q_b is the breakthrough capacity (mg/g), C_0 and C_t are the influent and effluent concentration (mg/L), M is adsorbent mass (g), and V_b is the total solution passed until the breakthrough point (mL). The equation gave the capacity of adsorbent at the exhaustion point of both zeolite and the selenium doped zeolite composite (Eq.4); [23]

$$q_e = \int_0^{Ve} \frac{C_0 - C_t}{M} dV \text{ (Eq-4)}$$

Where q_e is the exhaustion point capacity (mg/g), and V_e is the volume of sample passed until exhaustion happens (mL).

The entire amount of *E. coli* (m total (g)) enter into the column was calculated by the following equation (Eq.5); [20]

$$\mathbf{m\ (total)} = \frac{C_\circ Qte}{1000} \text{(Eq-5)}$$

The total percentage of the *E. coli* removal concerning flow volume was calculated by Eq.6

$$\%\ \mathbf{removal} = \frac{m(ad)}{m(total)} \times 100 \qquad \text{(Eq-6)}$$

Where m (ad) is the complete *E. coli* held in col-

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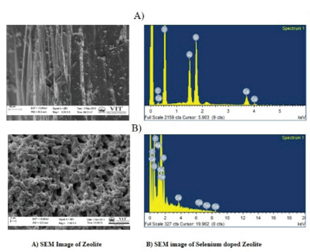
umn (g), which is the region contained above the breakthrough curve and is attained by numerical integration.

Results and Discussion

Characterization of the composite

The self-assembly of selenium nanoparticles formed the selenium doped zeolite composite onto the surface of the zeolite. Scanning electron microscopy image (Figure 1) revealed a spherical selenium nanoparticle formed on the zeolite. The Se element group's inclusion in the EDAX analysis confirmed selenium nanoparticles' assembling over the zeolite. Figure 2A shows the XRD pattern of selenium nanoparticle, zeolite, and selenium doped zeolite. The peaks appeared at 2 θ of 28.67°, 30.21°, 40.07°, and 48.24° are due to the assembling of selenium on to zeolite [23]. The peaks at 13.55°, 19.05°, 20.59°, 31.06°, 37.41°, 77.04°, and 80.83° are the characteristics of zeolite [24, 25]. The XRD pattern of selenium nanoparticles and the zeolite was compared with the standard (For selenium JCPDS No – 03-0226 and for zeolite 39-1383, 25-1349 and 47-1870 ICDD 1993) [26].



C)

Element	Weight%	Atomic%
C K	1.55	2.54
O K	56.79	69.81
Al K	12.14	8.85
Si K	20.62	14.44
Ca K	8.89	4.36
Totals	100.00	

D)

Element	Weight%	Atomic%
C K	13.19	33.12
O K	19.54	36.84
F K	2.31	3.67
Na K	1.75	2.30
Si K	2.87	3.08
Ca K	0.89	0.67
Se L	47.68	18.21
Tm M	11.77	2.10
Totals	100.00	

Figure 1. SEM and EDAX analysis of Zeolite and Selenium doped Zeolite

Figure 2B shows the FTIR pattern of selenium nanoparticles (Black Line), zeolite (Red line), and selenium doped zeolite (Blue line). The peaks at a wavenumber of 1018.41, 985.62, and 929.69 cm^{-1} are due to the internal tetrahedral asymmetric stretch of zeolite. A symmetric stretch of Si-O-Si and bending mode of water at 1099.43 and 715.59 cm^{-1}. Peaks at 1654.92 cm^{-1} are due to H_2O bend, which showed the better-advanced property of the zeolite. The peak at 435.91 and 588.29 cm^{-1} belongs to metal groups, which further confirms selenium's binding onto the zeolite [27].

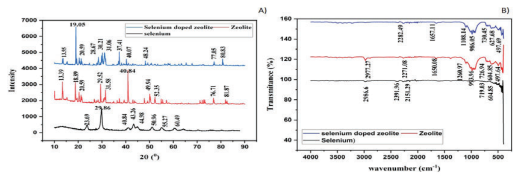


Figure 2: XRD (A) and FTIR (B) pattern of selenium nanoparticle, zeolite, and Selenium doped zeolite

Column experiment

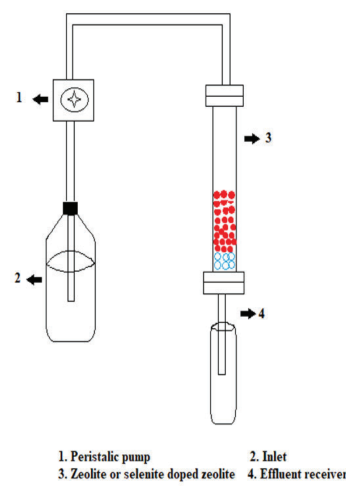


Figure 3: Schematic diagram of the overall column Setup. 1. Peristaltic pump, 2. Inlet, 3. Zoolite or selenite doped zoolite, 4. Effluent receiver.

The vertical column setup was prepared to analyse the fate and removal of *E.coli,* which is shown in figure 3. The rate of *E.coli* removal was studied by varying the volumetric flow rate and height of the bed. The performance of the

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column was analyzed using a breakthrough curve. The percentage of *E.coli* removal was measured in terms of optical density at 600 nm.

Effect of volumetric flow rate:

To analysis, the influence of different volumetric flow rates (Table 1A and B) (1.0 mL/min, 1.5 mL/min, 2.0 mL/min, and 2.5 mL/min), the bed height of 4 cm (zeolite and composite as matrix) and influent concentration ($4*10^{10}$ mg/L) were kept unchanged throughout the experiment. It was observed that as the volumetric flow rate increased, the level of effluent treated also increased, and the percentage removal of *E. coli* decreased.

It was seen that as the volumetric flow rate increased from 1 ml/min 2.5 ml/min, the total *E. coli* percentage removal through zeolite decreased from 65.32% to 46.48% for secondary treated wastewater. The total *E. coli* removal percentage through zeolite was decreased from 63.2% to 47.12% in drinking water. For selenium doped zeolite column at the volumetric flow rate of 1.5 mL/min, the uppermost *E. coli* removal of 70.18% and maximum adsorption capacity at breakthrough and exhaustion was obtained at $10.65*10^{10}$ mg/g and $14.581*10^{10}$ mg/g, respectively for secondary treated wastewater (Figure 4B). In drinking water, 66.0% of *E. coli* removal and maximum adsorption capacity at breakthrough and exhaustion points were obtained at $10.74*10^{10}$ mg/g and $14.325*10^{10}$ mg/g (Figure 4A), respectively. This confirms that at a high flow rate, there will be a weaker dispersal of *E.coli* in the zeolite bed resulting in inadequate time for the dispersion of *E.coli* inside the adsorbent bed, where the liquid leaves the bed in advance the steady-state was obtained, resulted in lesser uptake capacity and removal efficacy [20]. The flow of the *E. coli* from the liquid to the composite's surface was made likely by improving a concentration incline at the borderline. Greater flow disrupt the film adjoining the composite particles, thereby dropping the linkage of *E. coli* onto the surface of the composite [27]. The Zm is observed to rise from 0.80 cm to 1.78 cm with varying volumetric flow (1.0 mL/min, 1.5 mL/min, 2.0 mL/min, and 2.5 mL/min). A larger mass transfer zone causes the breakthrough and exhaustion points to occur within a short time.

Effect of bed height

According to tables 2A and B, different results were obtained using zeolite and selenium doped zeolite composite as the adsorbent with varying bed height. Here the initial concentration of *E. coli* was kept at $4*10^{10}$ mg/l and the flow rate at 1.5 ml/min. It was seen that with increased bed height, the level effluent treated and the total *E. coli* removal percentage was also improvised [25].

As the bed height increased from 2 to 4 cm, the total removal of *E. coli* has also increased from 51.32% to 64.56% for zeolite packed columns with secondary treated wastewater. For drinking water with zeolite bed, the total percentage of *E.coli* removal also increased from 50.28% to 62.51%. The higher the bed height for the selenium doped zeolite column showed an increased total *E.coli* removal percentage from 58.72% to 76.21% in the secondary treated wastewater [25]. The total rate of E. coli removal also increased from 56.80% to 75.3% for drinking water. Increased bed height designates a higher quantity of adsorbent, which resulted in increased binding sites for *E. coli* cells [26].

The total *E. coli* adsorbed at a breakthrough time, $q_{b,}$ and at exhaustion time, q_e considerably better with increasing bed height [27]. On the lesser height of bed (2cm), axial dispersal was directed by the mass transfer mechanism, which indirectly showed that there is no proper interval for the *E.coli* to pass throughout the composite bed, resulting in a shorter breakthrough time (Figure 4A and B) [28].

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Table 1A: Effect of flow rate on column performance using zeolite

Effect of flow rate for *E. coli* removal in wastewater					
Different flow rate (ml/min)	Veff (ml)	q_b (10^{10}mg/g)	q_e (10^{10} mg/g)	Percentage of Total removal	Zm (cm)
1	20	11.19	13.20	65.32%	1.00
1.5	25	9.98	11.98	60.82%	0.8
2	30	8.68	10.92	56.55%	0.66
2.5	35	6.23	8.69	46.48%	0.5714
Effect of flow rate for *E. coli* removal in drinking water					
1	20	10.52	13.32	63.2%	1.00
1.5	25	9.58	11.54	60.04%	0.8
2	30	8.12	10.81	57.32%	0.66
2.5	35	5.43	8.45	47.12%	0.5714

Table 1b: effect of flow rate on column performance using selenium doped zeolite composite

Effect of flow rate for *E. coli* removal in wastewater					
Different flow rate (ml/min)	Veff (ml)	q_b (10^{10}mg/g)	q_e (10^{10} mg/g)	Percentage of Total removal	Zm (cm)
1	25	9.80	11.56	63.00%	0.8
1.5	30	10.65	14.581	70.18%	1.00
2	32.5	7.80	11.25	59.83%	1.33
2.5	35	4.34	7.82	40.26%	1.78
Effect of flow rate for *E. coli* removal in drinking water					
1	25	9.5	11.87	60.0%	0.8
1.5	30	10.74	14.325	66.0%	1.00
2	32.5	7.5	11.00	57.76%	1.33
2.5	35	3.18	6.375	33.21%	1.78

Table 2a: effect of bed height on column performance using zeolite

Effect of bed height for *E. coli* removal in wastewater					
Different bed height (cm)	Veff (ml)	q_b (10^{10} mg/g)	q_e (10^{10} mg/g)	Percentage of Total removal	Zm (cm)
2	25	12.54	15.38	51.32%	0.400
3	30	13.63	17.79	58.21%	0.500
4	35	15.78	19.31	64.56%	0.571
Effect of bed height for *E. coli* removal in drinking water					
2	25	12.32	15.28	50.28%	0.400
3	30	13.76	17.86	57.82%	0.500
4	35	15.8	19.25	62.51%	0.571

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Table 2B: Effect of bed height on column performance using selenium doped zeolite composite

Effect of bed height for *E. coli* removal in wastewater					
Different bed height (cm)	Veff (ml)	q_b (10^{10} mg/g)	q_e (10^{10} mg/g)	Percentage of Total removal	Zm (cm)
2	25	11.61	16.89	58.72%	0.400
3	30	14.38	19.21	69.23%	0.500
4	35	16.59	21.74	76.21%	0.571
Effect of bed height for *E. coli* removal in drinking water					
2	22.5	11.52	17.28	56.80%	0.66
3	30	14.4	19.20	66.50%	0.75
4	37.5	16.20	20.25	75.3%	0.80

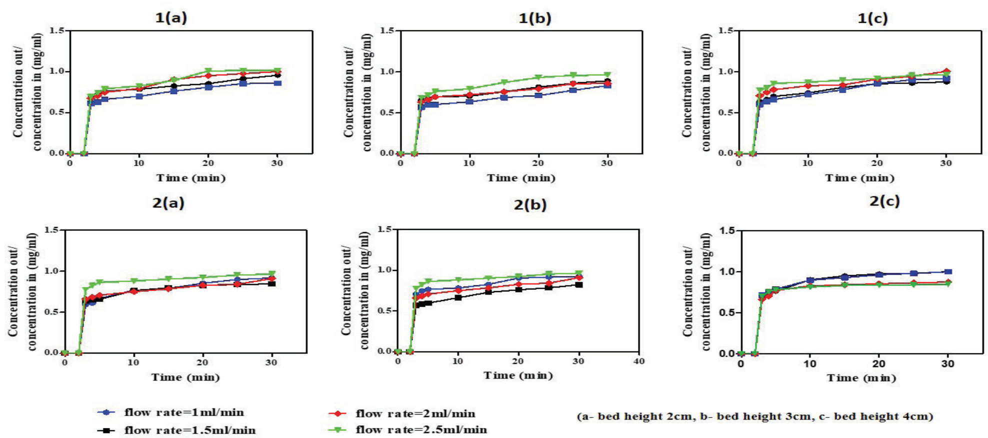

Figure 4a: breakthrough curve for the effect of flow rate and bed height for e.Coli removal in drinking water.

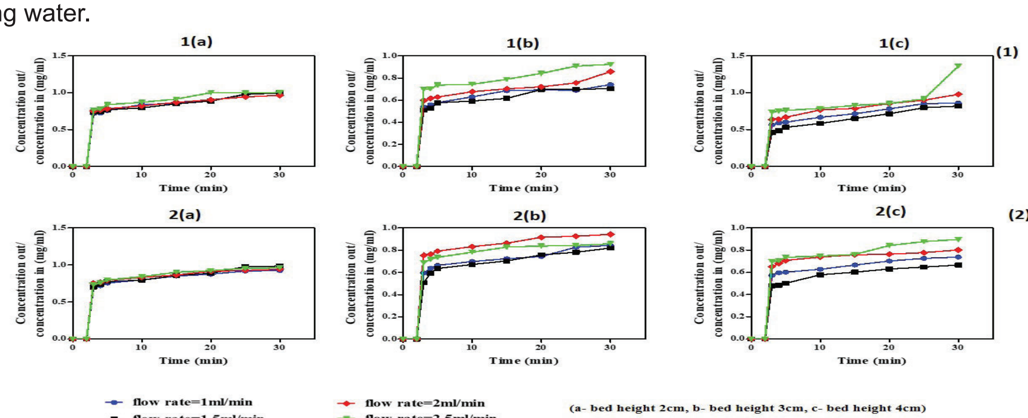

Figure 4b: breakthrough curve for the effect of flow rate and bed height for *e.Coli* removal in wastewater.

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1. Zeolite, 2 – Selenium doped zeolite composite

Conclusion

The removal of *E. coli* in a packed bed system using zeolite and selenium doped zeolite composite as an adsorbent material is an operative and achievable method. The breakthrough curve profile and adsorbent bed capacity for *E. coli* uptake were strongly dependent on the bed height and flow rate. A more extended breakthrough and exhaustion time have arisen at a greater bed height (4 cm), and a lesser flow rate (1 ml/min). Higher *E. coli* removal was attained for the increase in bed height due to higher adsorbent loading. In contrast, there was lesser *E. coli* removal for the increased in flow rate, due to lesser adsorbent-adsorbate interaction. Further, by comparing the results between the zeolite and selenium doped zeolite composite material as adsorbent, it was observed that Se-Z showed better *E. coli* removal. This could be because of the different binding sites and possibly the antibacterial activity of selenium doped zeolite composite. In this study, the composite material has a higher adsorption capacity in comparison to zeolite. Thus, the data is necessary for water treatment plants' theoretical designs to remove *E. coli* from different water sources.

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