# Bioleaching of rare earth elements (REEs) from Indonesian red mud by the bacterium *Bacillus nitratireducens* strain SKC/L-2

*Aisyah Minzikrina Masbar* Rus[1], *Ronny* Winarko[2], *Siti Khodijah* Chaerun[1,3*], *Fika Rofiek* Mufakhir[4], *Widi* Astuti[4], *Wahyudin Prawira* Minwal[1]

[1]Department of Metallurgical Engineering, Faculty of Mining and Petroleum Engineering, Institut Teknologi Bandung, Ganesha 10, Bandung 40132, West Java, Indonesia
[2]Department of Materials Engineering, University of British Columbia, 309-6350 Stores Road, Vancouver, BC, Canada
[3]Geomicrobiology-Biomining & Biocorrosion Laboratory, Microbial Culture Collection Laboratory, Biosciences and Biotechnology Research Center (BBRC), Institut Teknologi Bandung, Ganesha 10, Bandung 40132, West Java, Indonesia
[4]Research Unit for Mineral Technology, National Research, and Innovation Agency (BRIN), Jl. Ir. Sutami KM. 15, Tanjung Bintang, Lampung Selatan, 35361, Indonesia

**Abstract**. Red mud, a residue of the bauxite industry, represents a secondary source of rare earth elements (REEs) with substantial commercial value and untapped potential. Bioleaching, an innovative, cost-effective, and environmentally friendly method, offers a means of extracting valuable metals from mining wastes. This study explored the bioleaching of Indonesian red mud using *Bacillus nitratireducens* strain SKC/L-2 to recover REEs. The experiments were carried out over three days at 25 °C with different concentrations of red mud (1.5, 3, and 6 g/L) and a 10% v/v bacterial inoculum in a specific bioleaching medium. The findings indicated a slight reduction in REEs extraction by the bacterium as the red mud concentration increased from 1.5 to 6 g/L in the direct bioleaching process. In the experiment using 1.5 g/L red mud, 16 REEs were successfully extracted, with high extraction levels for specific elements such as Lu (92.0%), Tb (80.61%), and Gd (67.42%). However, when the red mud concentration was increased to 6 g/L, the survival potential of *Bacillus nitratireducens* strain SKC/L-2 decreased, leading to reduced recovery of elements such as Lu (76.80%), Tb (70.30%), and Gd (55.83%). The study reveals the behaviour of *Bacillus nitratireducens* in interacting with red mud and enduring high alkalinity, resulting in REEs extraction. These findings enhance the understanding of microbial interactions with red mud and provide insights into potential resource recovery applications.

**Keywords:** *Bacillus nitratireducens*; Bauxite residue; Bioleaching; Rare earth elements (REEs); Red mud

## 1 Introduction

Red mud is an alkaline residue generated during the Bayer process, which is used to convert bauxite into alumina. It represents one of the most complex and abundant secondary resources and possesses significant economic potential [1]. In the production of one ton of alumina, approximately four tons of raw bauxite are consumed, resulting in the generation of over two tons of red mud. Typically, red mud is stored in land-based bauxite residue disposal facilities. Its storage not only occupies space but also poses serious environmental risks and threatens ecosystems due to its high alkalinity, caustic nature, and the presence of radioactive elements [2].

In recent years, the utilization of rare earth elements (REEs) has expanded in high-tech, aerospace, and defense sectors, leading to an increased demand. This surge in demand has prompted the European Commission to categorize REEs as critical raw materials (CRMs) due to the associated supply risk. Many researchers have explored the potential of using red mud as a secondary source for REEs. Red mud, a alkaline residue of the conversion from bauxite ore to alumina, has been found to contain between 0.05-0.17 wt.% of REEs [3]. The extraction of REEs from red mud has garnered attention, and various methods have been

*Corresponding author: skchaerun@gmail.com; skchaerun@itb.ac.id

explored. Inorganic acids have been utilized as leaching agents to recover REEs, but this approach is associated with significant drawbacks, including high energy requirements, elevated costs, potential environmental harm, and risks related to the handling of potentially hazardous chemicals during the process [4]. Another method for REEs extraction is pyrometallurgy, conducted at extreme temperatures (1500–1700°C). This method is often considered inefficient in terms of energy consumption and is associated with the risk of releasing hazardous gases, such as sulfur monoxide (SO). Consequently, both of these strategies are typically constrained by considerations related to energy efficiency, environmental impact, and economic feasibility [5].

The biohydrometallurgical approach, commonly referred to as bioleaching, offers an efficient method for extracting metals without the use of concentrated acids or bases, and it is both energy-efficient and free from toxic gas emissions. This process leverages naturally occurring bacteria, which also facilitate the continuous renewal of beneficial oxidants such as ferric ions ($Fe^{3+}$). The combination of cost-effectiveness and environmental sustainability renders bioleaching an attractive option, garnering interest from researchers [5]. In bioleaching, specific bacteria are employed to recover metals from insoluble ores and secondary waste materials, solubilizing the metals contained within the sulfides and oxides as metal cations in the leaching medium. Red mud, which contains REEs, can be subjected to bioleaching through various methods, including one-step and two-step contact bioleaching processes, as well as non-contact or spent-medium bioleaching.

One-step bioleaching involves the simultaneous introduction of microbial culture and REE-containing material into the same incubation medium [4]. This method offers several advantages, including the potential for increased leaching efficiency through the following mechanisms: (a) direct formation of bio lixiviants on the surface of the REE-containing material, thereby reducing mass-transfer limitations via surface adsorption, (b) alteration of the culture's metal equilibrium due to metal uptake, adsorption, or complexation by excreted compounds, and (c) microbial consumption of constituents released from the solid REE sources (e.g., phosphate in monazite), which minimizes the precipitation of solubilized REEs. These attributes contribute to the growing favorability of bioleaching as a method for extracting REEs [3].

Chemolithoautotrophic, chemoorganotrophic, and mixotrophic bacteria are three types commonly used in bioleaching. Red mud increases the pH of the bioleaching medium significantly, and lacks the energy sources (sulphur or reduced iron) necessary for the growth of chemolithoautotrophic bacteria, such as acidophilic *Acidithiobacillus* species. These factors render chemolithoautotrophic microbes unsuitable for the bioleaching of red mud. Conversely, mixotrophic bacteria present multiple advantages for extracting metals from red mud. Firstly, they can tolerate environments with high alkalinity and elevated concentrations of metals such as iron and aluminium. Secondly, these bacteria have the ability to secrete metabolites, including organic acids, amino acids, and proteins. These substances form complexes with the toxic metal ions found in red mud, consequently mitigating the detrimental effects on the microorganisms' metabolic functions [4].

The genus *Bacillus*, comprising aerobic, spore-forming, and Gram-positive bacteria, is characterized by its ability to secrete organic acids and polysaccharides, which may facilitate mineral dissolution [6]. Various species within the genus *Bacillus* have been examined for their bioleaching potential, showing efficacy in mobilizing metals and altering the chemical, mineralogical, and morphological properties of diverse materials [7]. However, *Bacillus nitratireducens* has not been reported for use in bioleaching processes. Instead, it has been employed as a bioflocculant in a study designed to flocculate heavy metals such as Pb, Ni, Cd, Zn, and Cu from water [8]. In that study, *Bacillus nitratireducens* exhibited emulsifying activity, thereby producing biosurfactants [8]. These biosurfactants can maintain ferric ions in soluble form even under alkaline conditions by complexing with them, resulting in soluble ferric ions at elevated pH levels. Consequently, *the Bacillus nitratireducens* strain SKC/L-2 was chosen for bioleaching tests in this study. This strain, a mixotrophic bacterium, can obtain energy and carbon sources from both organic and inorganic compounds, making it suitable for survival in red mud environments with high pH and metal content. Therefore, the objective of this study is to evaluate the bioleaching of Indonesian red mud using *Bacillus nitratireducens* strain SKC/L-2 and investigate its potential to recover REEs. This research offers an alternative, ecologically sound and cost-effective method for recovering valuable metals in red mud. Additionally, the findings may contribute valuable insights for future research in bioleaching red mud, highlighting the behaviour of specific bacterial species in interacting with red mud and thriving in high alkalinity environments, ultimately leading to elevated extraction levels of REEs.

## 2 Materials and methods

### 2.1 Red mud sample

The red mud sample, kindly provided by PT Indonesia Chemical Alumina (PT ANTAM) in Tayan, West Kalimantan, Indonesia, exhibited a pH of approximately 11.2 and a moisture content of around 26%. The characteristics of the red mud were analysed using X-ray fluorescence (XRF), X-ray diffraction (XRD), and scanning electron microscopy (SEM) techniques. The XRF analysis was conducted to ascertain the elemental composition of the material. Prior to analysis, the red mud samples were pre-treated by being dried in an oven for 24 hours to eliminate any water content. A freshly prepared red mud sample was then utilized for the bioleaching experiment.

### 2.2 Bacterium and cultivation medium

*Bacillus nitratireducens* strain SKC/L-2 (coded as Lusi 2.1), a mixotrophic bacterium capable of oxidizing iron and sulfur and producing significant amounts of extracellular polymeric substances (EPS), was isolated from an Indonesian mine site and employed in the experiment. The bacterium was cultivated in a medium containing the following components (g/L): 10 glucose, 5 yeast extract, 0.5 $Na_2S_2O_3.5H_2O$, 0.25 $FeSO_4.7H_2O$, 0.1 KCl, 0.5 $K_2HPO_4$, 1 $(NH_4)_2SO_4$, and 0.5 $MgSO_4.7H_2O$. The 250 mL Erlenmeyer flasks, each containing 100 mL of the medium, were autoclaved at 121 °C and 1.5 atm for 30 minutes to achieve sterilization. Each flask was then inoculated with the pure bacterial strain at a concentration of 10% v/v, followed by activation for 48 hours at room temperature (25°C) in a shaking incubator.

### 2.3 Bioleaching experiments

The bioleaching experiment employed a one-step (direct) bioleaching process in which the bacterium was introduced directly into the bioleaching medium containing red mud. This direct bioleaching was conducted over a 72-hour period at room temperature (25 °C), with flasks shaking and rotating at 180 rpm. The 250 mL Erlenmeyer flasks were each filled with 150 mL of leaching solution containing sterilized red mud at varied pulp densities of 1.5 g/L, 3 g/L, and 6 g/L, referred to as adaptation levels 1, 2, and 3, respectively. The medium was autoclaved at 121°C for 30 minutes to ensure sterilization, and its composition included (g/L): 4 glucose, 6.5 $FeSO_4.7H_2O$, 5 $Na_2S_2O_3.5H_2O$, 0.1 KCl, 0.5 $K_2HPO_4$, 3 $(NH_4)_2SO_4$, and 0.5 $MgSO_4.7H_2O$. During the experiment, 10 mL samples were taken at regular intervals from each treatment to measure pH values, bacterial growth (using Optical Density, $OD_{600\ nm}$), redox potential (Eh), and REEs concentration, which were analyzed using inductively coupled plasma-mass spectrometry (ICP-MS).

## 3 Results and discussion

### 3.1 Characteristics of red mud sample

The elemental composition of red mud was analyzed using XRF, as presented in Table 1. The primary metallic constituents of the red mud were Fe (39.4%), Al (17.90%), Ca (14.21%), Ti (2.85%), while the most dominant non-metallic element was Si (15.2%). Figure 1 illustrates XRD results for the original red mud sample. The XRD peaks revealed that the main mineral components of the red mud were $Fe_2O_3$ (hematite), $Al_2O_3$ (gibbsite), CaO (calcium oxide), and $SiO_2$ (silica). The rare-earth mineral phases were present in minor quantities, with shallow peaks that made distinguishing them in the bauxite residue challenging. These findings are consistent with other study conducted by Borra et al. [9]. The morphology of the red mud was characterized using SEM. As depicted in Figure 2, the red mud comprised a coarse, divaricate structure, and irregular and angular particles, with significant fracturing evident on its surface. Additionally, the red mud exhibited low porosity and a highly crystalline structure. The material was observed to be very fine, forming aggregates.

**Table 1**. Elemental composition of red mud analyzed by XRF

| Element | Wt (%) | Element | (ppm) | Element | (ppm) |
|---|---|---|---|---|---|
| Al | 17.90 | Cr | 694.60 | Te | 178.13 |
| Si | 15.20 | Ni | 265.80 | Hg | 96.10 |
| Ca | 14.21 | Cu | 268.60 | Sn | 437.60 |
| Ti | 2.85 | Ga | 88.37 | | |
| P | 0.375 | Rb | 337.12 | | |
| S | 0.342 | Sr | 103.47 | | |
| K | 0.256 | Zr | 806.87 | | |
| V | 0.270 | Re | 466.25 | | |
| Mn | 0.117 | Pb | 501.57 | | |
| Fe | 39.40 | Bi | 307.52 | | |

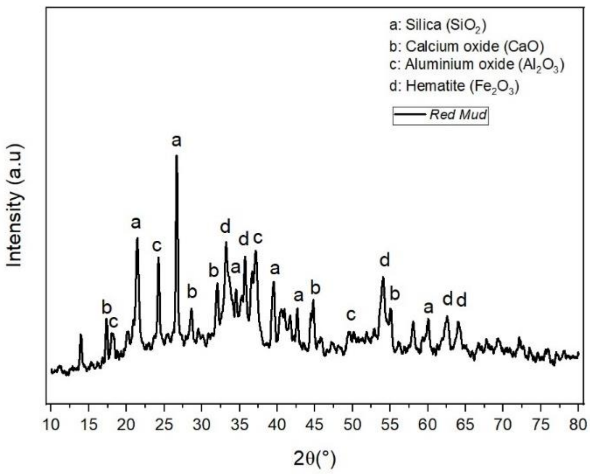


**Fig. 1.** X-ray powder diffraction analysis of the original red mud sample

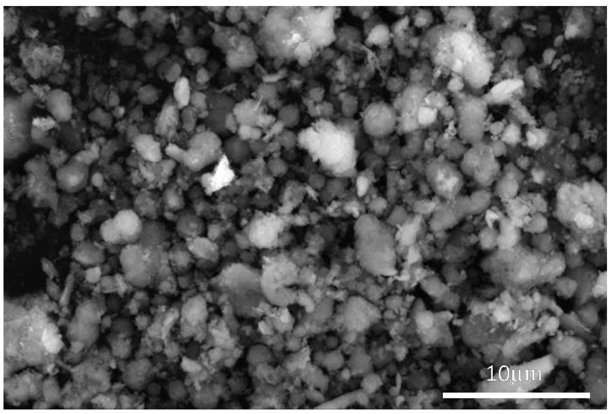


**Fig. 2.** SEM image of the original red mud sample

## 3.2 Bioleaching of red mud

Figure 3 delineates the alterations in pH across different concentrations of red mud during a one-step bioleaching process utilizing *Bacillus nitratireducens* strain SKC/L-2 (coded Lusi 2.1). Typically, the initial pH values in the bioleaching medium for adaptations 1, 2, and 3 exhibited a continuous decline over the course of 72 hours, substantiating the bacterial influence in reducing pH levels throughout the bioleaching experiments. Notably, the decrement in pH was less significant with increasing concentrations of red mud. Consequently, the pH values observed during the bioleaching followed the sequence: adaptation 1 < adaptation 2 < adaptation 3. These pH values are essential in evaluating the efficiency of bioleaching, which involves the production of both inorganic (e.g., $H_2SO_4$) and organic acids [10]. Enhanced acidity levels promote more facile dissolution of metals [11]. However, the synthesis of these acids may be inhibited at higher concentrations of red mud. Prior research has indicated that augmenting the pulp densities of red mud generally reduces leaching efficacy under each bioleaching condition. This phenomenon is likely due to two primary reasons: (i) an elevated concentration of red mud results in an increased pH value within the leaching solution, and (ii) growth of the microorganism is hampered by the toxic properties of red mud in both one- and two-step bioleaching processes, leading to a reduction in the production of metabolites such as organic acids and biosurfactants by the bacterial strains [12]. Recent research supports this observation, suggesting that bacteria are likely to flourish on mineral surfaces where they can form a microenvironment to alleviate environmental stress and enhance metabolic activity through cytomembrane stress responses [13].

Another vital factor influencing leaching behaviour is the Eh value. Figure 4 displays how the Eh values of the bioleaching suspension augmented as the pH dropped. The Eh values for adaptations 1, 2, and 3 mirrored the pH trends during the 72-hour bioleaching period, typically increasing during the first 0-8 hours. In adaptations 1 and 3, the Eh values sharply rose from 0 to 8 hours, whereas in adaptation 2, this change was observed from 0 to 12 hours. Furthermore, the initial Eh value for the bacterium in adaptation 1 began at a low Eh of 250 mV and gradually climbed to a high Eh of 558 mV. In adaptations 2 and 3, the starting Eh values were roughly 300-378 mV, rising within the 0 to 12-hour range and subsequently fluctuating slightly for the remaining bioleaching time. After 72 hours, the Eh values followed the order: adaptation 1 < adaptation 2 < adaptation 3. The continued increase in Eh values during the bioleaching period is interpreted as biooxidation of the ferrous ion to the ferric ion, thereby enhancing mineral dissolution. This trend further indicates that the bacterium creates a particular electrochemical environment that considerably impacts red mud leaching, since red mud leaching is primarily governed by the electrochemical potential of leaching solution.

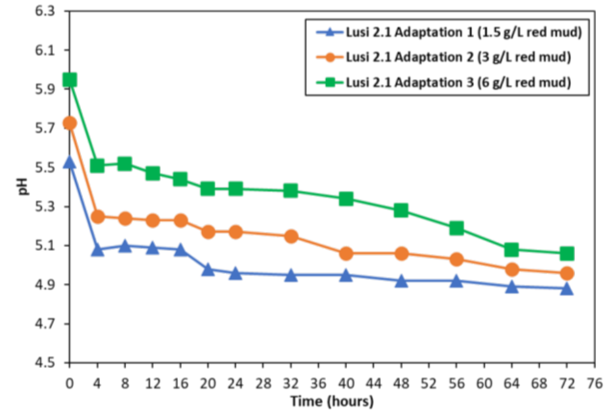


**Fig. 3.** Suspension pH during bioleaching under different pulp densities (1.5, 3, 6 g/L red mud) using *Bacillus nitratireducens* strain SKC/L-2 (coded Lusi 2.1)

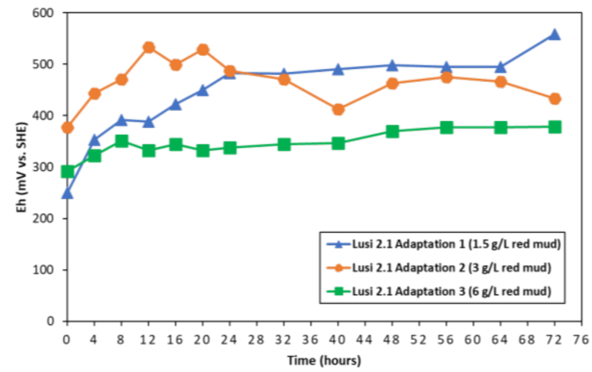


**Fig. 4.** Suspension Eh (mV vs. SHE) during bioleaching under different pulp densities (1.5, 3, 6 g/L red mud) using *Bacillus nitratireducens* strain SKC/L-2 (coded Lusi 2.1)

Figure 5 illustrates the optical density (OD) over a 72-hour growth period, reflecting the bacterial growth rate in bioleaching suspension. The change in optical density, representing medium turbidity, signifies bacterial proliferation. In adaptations 2 and 3, the bacteria reached 80% of the exponential phase at 8 hours of bioleaching time, whereas adaptation 1 reached this phase at 12 hours. The stationary phase, commencing after 16 hours, was characterised by a relatively constant flat curve. This pattern of growth underlines the bacterium's ability to adapt to and resist extreme or toxic conditions within the growth environment. Furthermore, the growth characteristics of the bacteria vary according to the specific conditions of their environment [14].

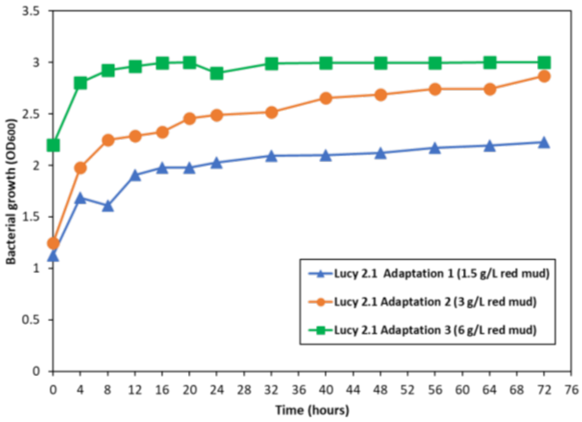


**Fig. 5.** Bacterial growth of *Bacillus nitratireducens* strain SKC/L-2 (coded Lusi 2.1) during bioleaching under different pulp densities (1.5, 3, 6 g/L red mud)

In the experiment employing the bacterium *Bacillus nitratireducens*, successful extraction of 16 REEs elements (Lu, Tm, Yb, Gd, Ho, Er, Tb, Dy, Sc, Y, Nd, La, Eu, Ce, Sm, and Pr) was achieved. Figure 6 depicts the extraction levels of REEs under direct bioleaching processes across various red mud concentrations (adaptations 1, 2, and 3). A notable trend was observed: as the concentration of red mud increased, the recovery efficiency of REEs decreased. Among the bioleaching experiments, five major REE elements exhibited significant recovery levels: Lu, Gd, Tb, Nd, and Pr. Adaptation 1 resulted in the highest bioleaching recovery for these elements, with extraction levels of 92%, 67.42%, 80.61%, 26.70%, and 10.41%, respectively. In contrast, adaptation 2 yielded extraction levels of 76.80%, 55.83%, 70.30%, 31.17%, and 5.92% for the same elements. The recovery of the element Nd was slightly higher in adaptation 2 than in adaptation 1. However, adaptation 3 showed the lowest extraction levels for four significant elements (Lu: 43.60%, Gd: 33.04%, Tb: 40.91%, Nd: 17.16%), while the element Pr (27.69%) had the highest extraction level compared to adaptations 1 and 2.

A previous study indicated that in bioleaching, organic acids play two essential roles: first, they facilitate the dissolution of metal ions from leaching materials by chelating metals released into the solution and weakening the bonds between surface metals and bulk-leaching materials [12]; second, they mitigate the detrimental effects that metal ions might have on microorganisms [4]. Apart from the five REE elements with notable recovery levels, the elements Tm, Yb, Ho, Er, Dy, Sc, Y, La, Eu, Ce, and Sm demonstrated recovery levels of less than 10% across adaptations 1, 2, and 3. Specifically, the extraction of elements Tm and Yb ranged from 4% to 8%, while the remaining elements were below 4%. The efficiency of bioleaching is significantly affected by the pH value, which is indicative of both inorganic ($H_2SO_4$) and organic acid formation [15]. In this particular bioleaching experiment, the bacterium did not reduce the pH value below 4. Consequently, the organic acids and biosurfactants produced by the bacterium are instrumental in maintaining the solubility of ferric ions through complexation actions, even under high pH or alkaline conditions.

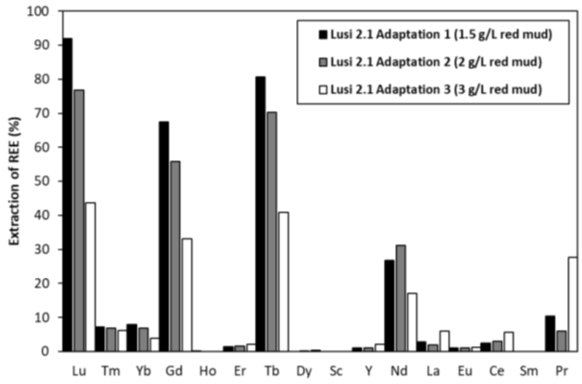


**Fig. 6.** Extraction levels of REEs in bioleaching using *Bacillus nitratireducens* strain SKC/L-2 (coded Lusi 2.1) after 3 days of bioleaching under various pulp densities (1.5, 3, 6 g/L red mud).

## 4 Conclusion

The bacterium *Bacillus nitratireducens* strain SKC/L-2 demonstrated the ability to extract 16 REEs, with the extraction efficiency being inversely proportional to red mud concentrations (1.5, 3, 6 g/L). Five specific elements, namely Lu, Gd, Tb, Nd, and Pr, were extracted with substantial efficiency, while the remaining elements, including Sc, Y, La, Ce, Sm, Eu, Dy, Ho, Er, Tm, and Yb, were recovered at lower levels (less than 10% recovery). This investigation provides critical understanding of the extraction behaviour of *Bacillus nitratireducens* strain SKC/L-2 in the context of red mud bioleaching. The trends and data observed not only highlight the potential of the strain in extracting REEs but also provide essential knowledge that could inform future research and application in bioleaching, particularly in environments characterized by high alkalinity.

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