

CORROSION OF COPPER IN A MEDIUM OF BACTERIA SULFATE REDUCTION PROCEEDING

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Received 08 January 2018
Accepted 05 December 2018

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ABSTRACT

The paper deals with copper (99.9%) biocorrosion in neutral water-salt medium with sulfate-reducing bacteria of *Desulfovibrio*, *Desulfomicrobium* strains and the enrichment culture, gathered from the metal equipment surfaces of sewage treatment constructions. Using inversion voltammetry, gravimetric, analytical and SEM methods, it is found that the studied bacteria cultures differ in their corrosive and metabolic activity and are able to form a biofilm on the copper surface. Copper appeared to be more resistant to microbial corrosion initiated by *Desulfomicrobium* bacteria compared to that by *Desulfovibrio* bacteria. The corrosive aggressiveness of sulfate-reducing bacteria in the enrichment culture gathered from the biofilm on the sewage treatment constructions is sufficiently high. The corrosion rate observed equals that caused by *Desulfovibrio* genus.

Keywords: copper, biocorrosion, sulfate-reducing bacteria.

INTRODUCTION

Copper is resistant to corrosion in neutral water media and is widely used as a structural material in heat exchangers and pipelines [1 - 3]. Such systems create anaerobic conditions which are suitable for sulfate-reducing bacteria (SRB) growth. Their enzymatic systems can engage in the electrochemical corrosion process. In particular, bacteria hydrogenases accept electrons from the metal surface and provide sulfur reduction from the sulfate-ions present [4]. Simultaneous formation of biogenic hydrogen sulfide proceeds. According to V. Madina et al. [5], metallic copper oxidizes to Cu^+ in the medium, where bacterial sulfate reduction takes place. When interacting with biogenic hydrogen sulfide, chalcocite, Cu_2S , is produced. A biofilm (a body of microorganisms and their metabolic products) is formed over time on the metal surface [6]. Its

heterogeneity creates local differences and expansion of the active areas, where corrosive processes occur [7]. The mechanism of copper microbe corrosion is considered to be sufficiently complex due to thermodynamic complications [5].

The copper corrosion in the medium containing sulfate-reducing bacteria is studied by Fu Wenjie et al. [5], Madina et al. [8], K. Elmouaden [9] and others. These investigations are carried out with certain cultures of sulfate-reducing bacteria (SRB). As the corrosive aggressiveness of SRB from different natural groups differs, it is important to conduct a comparative study of copper microbial corrosion in presence of SRB of different genera as a part of the natural microbe community.

The objective of this paper is to study copper microbial corrosion in neutral water-salt medium in presence of sulfate-reducing bacteria of *Desulfovibrio*, *Desulfomicrobium* genera and the enrichment culture

gathered from the metal equipment surfaces of sewage treatment constructions.

EXPERIMENTAL

Materials and Apparatus

Copper (copper content 99.9 %) coupons were used. Their surface area was $3.6 \times 10^{-4} \text{ m}^2$. They were polished to the 4 - 5 class of accuracy. Then they were immersed in 120 ml sealed glass tanks. The coupons exposure time was 5 days, 10 days, 15 days and 20 days. The temperature was maintained under 27°C as this was the optimal temperature for the development of sulfate-reducing bacteria.

The model Postgate "B" medium [10, 11] containing sulfate-reducing bacteria was used as a testing corrosive medium. Its composition per liter referred to 0.5 g of KH_2PO_4 , 1.0 g of NH_4Cl , 1.0 g of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, 2.0 g of MgSO_4 , 3.5 g of calcium lactate, 10 ml of 5 % yeast extract, 10 ml $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (5 % solution in 1 % HCl), 2 ml of 5 % ascorbic acid, and NaHCO_3 (5 %) in a quantity for pH adjustment to 7.5 (measured with pH-meter/ionomer pH/ION 340i). The initial number of the sulfate-reducing bacteria in the corrosive medium was $10^9 \text{ cell ml}^{-1}$.

Sulfate-reducing bacteria of *Desulfovibrio sp.* M.4.1 strains were used as test microorganisms. They were previously gathered by us from the corroded iron coating of the subterranean gas pipeline [12]. *Desulfomicrobium sp.* TC4 and *Desulfovibrio sp.* 10 bought from the collection of the Department of General and Soil microbiology, D. K. Zabolotny Institute of Microbiology and Virology, the National Academy of Sciences of Ukraine were also investigated. The strain *Desulfomicrobium sp.* TC4 was gathered from the corroded fouling of the central heating brass pipelines, while that of *Desulfovibrio sp.* 10 originated from the corroded steel reinforcement of the Dniproges (The Dnieper Hydroelectric Station, Zaporizhia City). The enrichment culture used was gathered from the metal equipment surfaces of the sewage treatment constructions (Chernihiv) [13]. It contained sulfate-reducing bacteria ($2.5 \times 10^9 \text{ cell ml}^{-1}$), iron-reducing bacteria ($1.3 \times 10^3 \text{ cell ml}^{-1}$), denitrifying bacteria ($6.0 \times 10^1 \text{ cell ml}^{-1}$) and ammonifying bacteria ($7.0 \times 10^6 \text{ cell ml}^{-1}$). The number of the microorganisms in the enrichment culture was evaluated using the Most Probable Number method inoculated to the correspondent selective

medium: the Postgate «B» medium for the sulfate-reducing bacteria, the Kalinenko medium for the iron-reducing bacteria (IRB), to the Giltay medium for the denitrifying bacteria (DNB) and the meat peptone broth [14] for the ammonifying bacteria (AMB).

Inversion voltammetry

The Postgate "B" medium was tested following the copper coupons immersion. The voltammetry method was applied to identify the copper solubility. The programmable two-chamber furnace PDP - Lab (Research and Development Enterprise "Tomanalit" RF) was used to transfer into the solution copper ions in an electrochemically active form. The samples were prepared in the same way as for the sewage. The corrosive medium of 10 ml was dried in a porcelain cup at a temperature in the range of 150°C - 180°C . Then 2.0 ml of concentrated nitric acid and 1.0 ml of 30 % solution of hydrogen peroxide were added. It was subjected to evaporation within 60 min - 70 min at a temperature in the range of 150°C - 350°C . The sample was ashed within 30 min at 450°C . The steps of nitric acid and hydrogen peroxide introduction, evaporation and ashing were repeated two-three times until getting a homogeneous ash of a white, a yellow or a grey color. The ash was dissolved in 0.2 ml of concentrated formic acid. Double distilled water was added to reach a volume of 10 ml. In addition 10 ml of distilled water, 0.2 ml of concentrated formic acid and a sample aliquot of a volume of 20 μl were introduced using a quartz electrochemical chamber.

The content of Cu^{2+} ions was determined on the voltammetry analyzer TA-Lab (Research and Development Enterprise "Tomanalit" RF) using a three-electrode electrochemical cell. An amalgam electrode was used as an indicator electrode, while an electrode filled with 1M solution of potassium chloride served as an auxiliary electrode. A silver chloride reference electrode was used. The background electrolyte containing 200 μl of concentrated chemically pure formic acid was analyzed. The procedure applied consisted of electrochemical purification of the indicator electrode at a potential value of +0.050 V, metals accumulation on the surface of the indicator electrode within 10 s at a potential value of 1.500 V, solution calming for 5 s at a potential value of 1.300 V and anodic oxidation at a sweep rate of 80 mV s^{-1} .

The analysis of a probe of each sample was carried out three times. The copper ions determination was performed by the method of additives using a standard solution. The latter contained 1 mg l⁻¹ of Cu⁺². It was prepared on the ground of standard samples and double distilled water. The device TA - Lab (specialized computer program, version 3.6.10) was used.

Gravimetric method

The corrosion tests were performed by the gravimetric method [15]. Prior to the introduction to the corrosive medium, the copper coupons were cleaned with acetone, and weighed with analytical scales with an accuracy of 5·10⁻⁵ g. They were immersed in the corrosive medium for 10 days and then weighed again. The corrosion rate was calculated on the ground of:

$$k_m = \frac{\Delta m}{S \cdot \tau} \quad (1)$$

where Δm was the weight loss in g; S was the area in m², while τ was the exposure time in h.

The statistical analysis of the experimental data (the corrosion rate) at a reliability level of 95 % was conducted with the help of Microsoft Excel. The experiments were conducted three times.

SEM investigations

The biofilms grown on the steel surface were fixed through immersion for 1 h in a 2 % glutaraldehyde solution and successive dehydration for 15 min using 25 vol. %,

50 vol. %, 75 vol. % and 100 vol. % ethanol solutions. Then they were air dried overnight [16]. After fixation, the coupons were examined using field emission scanning electron microscopy (FEIE-SEM XL 30). The maximum residual pressure in the microscope pillar was not greater than 6.7·10⁻⁴ Pa under a gun current of 76 mA.

Analytical methods

The concentration of the biogenic hydrogen sulfide was measured by iodometric titration.

The redox potential (E, mV) of the corrosive medium was defined by using the oxidation-reduction potential sensor and recording device LabQuest 2 (Vernier Software & Technology). Measurement error was ± 0.5 mV.

RESULTS AND DISCUSSION

The Postgate “B” medium containing SRB culture is a multicomponent system with a constantly changing composition. It is so because SRB produce different metabolic products, the main being hydrogen sulfide. The accumulation of the metabolic products affects the medium redox potential, which correspondingly determines the further course of the processes.

The test results referring to the redox potential dynamics of Postgate “B” medium with SRB cultures in absence and presence of a copper coupon are presented in Fig. 1. It should be noted that the presence of *Desulfomicrobium*, *Desulfovibrio* genera bacteria

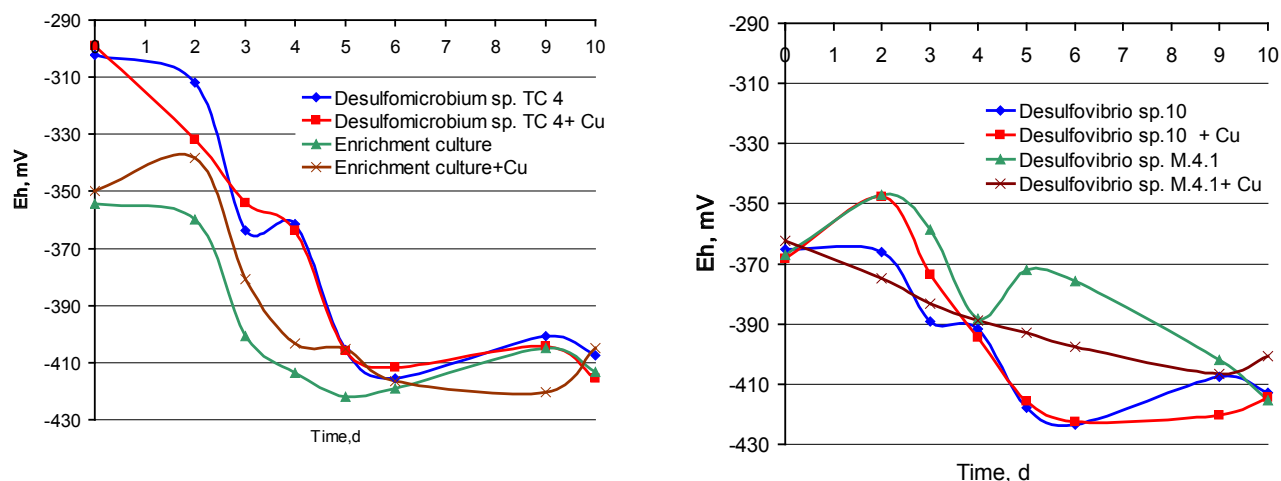


Fig. 1. Dependence of redox potential of Postgate “B” medium on the time under the growth of sulfate-reducing bacteria and under copper microbial corrosion: a) enrichment culture and *Desulfomicrobium* sp. б) *Desulfovibrio* sp. M.4.1 and *Desulfovibrio* sp. 10.

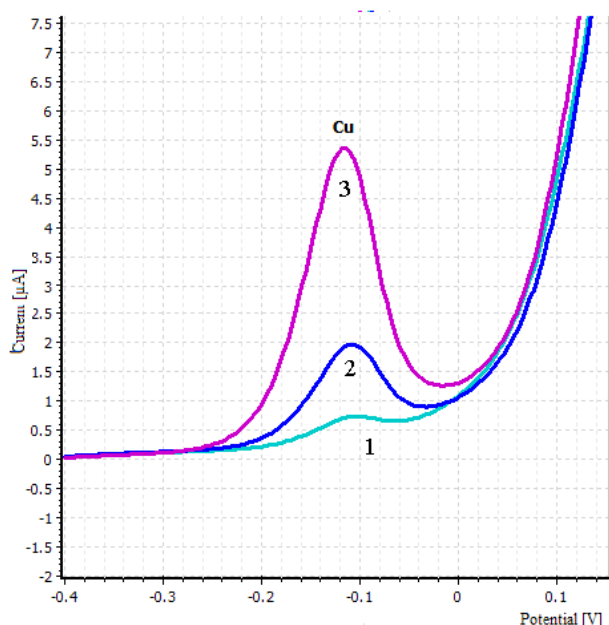


Fig. 2. Voltammetry curves: 1 - background solution; 2 - Postgate "B" medium with *Desulfomicrobium sp.* TC4 culture after 10-day copper sample exposure; 3 - standard solution.

and the enrichment culture cause different initial values of redox potential and, hence, provide a different oxidizing capacity [17]. The initial E value in Postgate "B" medium with bacteria *Desulfomicrobium sp.* TC4 (Fig. 1(a)) is by 70 mV more positive when compared to that in the medium containing bacteria of *Desulfovibrio genus* (Fig. 1(b)). The presence of a copper coupon in Postgate "B" medium affects the redox potential. This can be connected with changes in the sulfate-reducing activity of the bacteria, their binding to the metal surface forming a biofilm, and copper sulfides formation.

The dynamics of the redox potential for the *Desulfomicrobium sp.* TC4 bacteria under the conditions of bacteria growth in absence and presence of copper differs during the first 4 days (Fig. 1(a)). During the time of the experiment the redox potential decreases by 70 mV and reaches its minimal value (-415 mV) on the 6th day. This is caused by the active production of hydrogen sulfide. The latter accumulates, which results in SRB development depression and deceleration of their growth. B The nature of the redox potential change with time is the same for the enrichment culture of SRB, although the E value at most checkpoints differs by 10 mV - 20 mV.

A certain decrease of the redox potential is initially observed in presence of bacteria of *Desulfovibrio genus* (Fig. 1 (b)). It is followed by fluctuation. In

general, E varies from -345 mV до -420 mV. The redox potential grows slowly with time within 9 days in case of copper corrosion in presence of *Desulfovibrio sp.* M.4.1. For all cultures studied the potential change with time indicates a complex nature determined by the oxidation-reduction processes in the medium. The copper corrosion is accompanied by metal ions transition to the solution. No oxidation peaks are observed (Fig. 2, curve 1) in the voltammetry curve of the background solution within the potential interval from -400 mV to +100 mV. This indicates the purity of the background electrolyte, namely the absence of Cu^{2+} ions in it. There is a maximum at -50 mV in the voltammetry curves of experimental samples (Fig. 2, curve 2). It corresponds to the processes of copper anodic oxidation. The peak current increases with the addition of a standard solution, containing the studied metal. The increase is proportional to the metal concentration increase (Fig. 2, curve 3).

The change of Cu^{2+} ions concentration in Postgate "B" medium in case of copper microbial corrosion in presence of the enrichment culture of SRB is demonstrated in Fig. 3. On the 5th day, the concentration of Cu ions reaches a value of $0.16 \text{ mg}\cdot\text{l}^{-1}$, which is indicative of copper corrosion. The decrease of Cu ions concentration on the 10th day can be explained by their binding on the coupon surface with the sulfides produced. According to V. Madina et al. [5] the most probable product is chalcocite (Cu_2S). The increase of the micro-galvanic pairs on the copper surface facilitates the corrosion and the further accumulation of ions in the

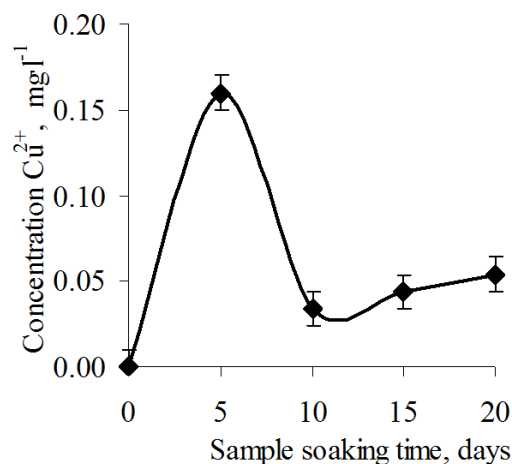


Fig. 3. Correlation between Cu^{2+} ions concentration in Postgate "B" medium, inoculated with the SRB enrichment culture, and copper coupons exposure time.

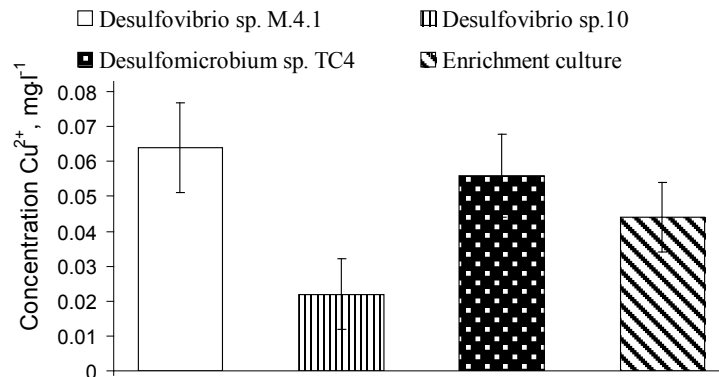


Fig. 4. Cu²⁺ ions concentration in Postgate “B” medium, inoculated with SRB cultures after copper coupons exposure (10 days).

solution. In case of copper corrosion in Postgate “B” medium in bacteria absence, the Cu ions concentration on the 10th day reaches a value of 0.26 mg×l⁻¹. It is of an order higher to that observed in case of microbial corrosion. This corresponds with the research results [18, 19] showing the ability of copper dissolution in sulfate-containing media. It also indicates that Cu ions are incorporated into sulfides when interacting with

biogenic hydrogen sulfide.

The comparison of Cu ions concentration in the corrosive medium on the 10th day of copper coupons exposure shows the highest concentration in presence of *Desulfovibrio sp. M.4.1*, while the lowest – in that of *Desulfovibrio sp.10* (Fig. 4). This corresponds to the results of the redox potential study and indicates a different corrosive activity of the studied SRB cultures.

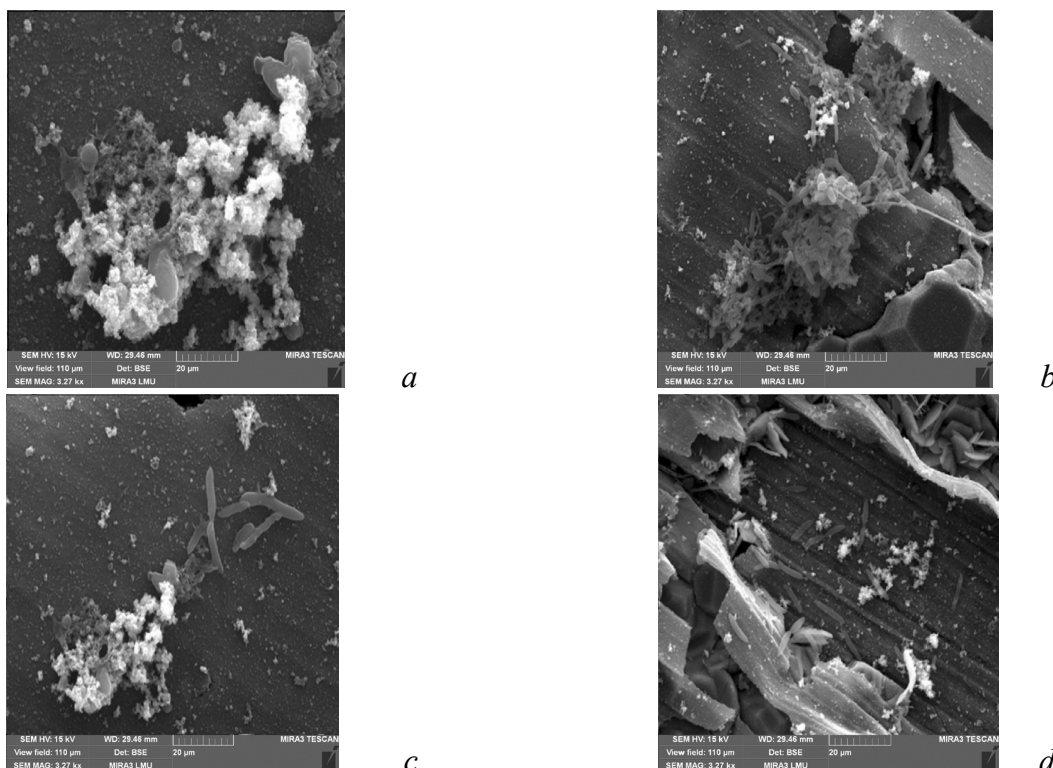


Fig 5. FE-SEM images (×10 000) of the biofilm formed on copper surface after exposure to Postgate «B» medium with sulfate-reducing bacteria culture: a) *Desulfovibrio sp. M.4.1*; b) *Desulfovibrio sp.10*; c) *Desulfomicrobium sp. TC4*; d) SRB enrichment culture.

Table 1. Concentration of biogenic hydrogen sulfide in the Postgate «B» medium.

Strain	<i>Desulfovibrio</i> <i>sp. M.4.1</i>		<i>Desulfovibrio</i> <i>sp.10</i>		<i>Desulfomicrobium</i> <i>sp. TC 4</i>		Enrichment culture	
	without Cu	with Cu	without C	with Cu	without Cu	with Cu	without Cu	with Cu
Concentration H ₂ S, mg·l ⁻¹	445	510	535,5	539	561	488	512	489

Table 2. Speed rate of microbial corrosion of copper, induced with the enrichment culture and certain SRB strains.

Strain	<i>Desulfovibrio</i> <i>sp. M.4.1</i>	<i>Desulfovibrio</i> <i>sp.10</i>	<i>Desulfomicrobium</i> <i>sp. TC 4</i>	Enrichment culture
k, g·m ⁻² ·h ⁻¹ · 10 ³	6,7±0,5	3.9±0,4	5.0±0,5	6.7±0,5

The concentration of the biogenic hydrogen sulfide accumulated in Postgate “B” medium after 10 days of copper coupons exposure is demonstrated in Table 1.

The concentrations of hydrogen sulfide in presence of *Desulfomicrobium* sp. TC4 and the enrichment culture are lower than those in absence of copper. The biggest difference (73 mg·l⁻¹) is defined for *Desulfomicrobium* sp. TC4. This can be explained by the active participation of biogenic hydrogen sulfide in forming Cu sulfides on the metal coupons surface. For *Desulfovibrio* sp. M.4.1 the concentration of H₂S in the test with copper is higher by 65 mg l⁻¹. This indicates the high sulfate-reducing activity of the bacteria in case of copper microbial corrosion. For the culture *Desulfovibrio* sp.10 the concentration of biogenic hydrogen sulfide in absence and presence of a copper coupon differs insignificantly.

The morphology of the copper coupons surface after 10 days of exposure to the corrosive medium with bacterial sulfate-reduction is presented in Fig. 5. The photos show that the studied SRB cultures are able to colonize the copper surface and form a biofilm of a different composition. For the strains *Desulfovibrio* sp. M.4.1; *Desulfovibrio* sp.10 and *Desulfomicrobium* sp. TC4 (Fig. 5a-c) the colonies are regular in outline but differ in size and appearance. A chaotic accumulation of bacteria is observed in the polymer matrix (Fig. 5d) for the enrichment culture.

According to the gravimetric data the highest copper microbial corrosion rate is observed in presence of *Desulfovibrio* sp. M.4.1 and the enrichment culture (Table 2).

CONCLUSIONS

In the media of bacterial sulfate-reduction proceeding the copper is subject to microbial corrosion. The bacteria of *Desulfomicrobium*, *Desulfovibrio* genera and the enrichment culture of SRB, gathered from the biofilm from sewage treatment constructions, are able to colonize the copper surface. The sulfate-reducing bacteria *Desulfovibrio* sp. M.4.1. demonstrate the highest corrosive aggressiveness towards copper. The research results should be considered during copper constructions operation under anaerobic conditions.

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