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Short Communication

Enhancement of anaerobic digestion of ciprofloxacin wastewater by nano zero-valent iron immobilized onto biochar

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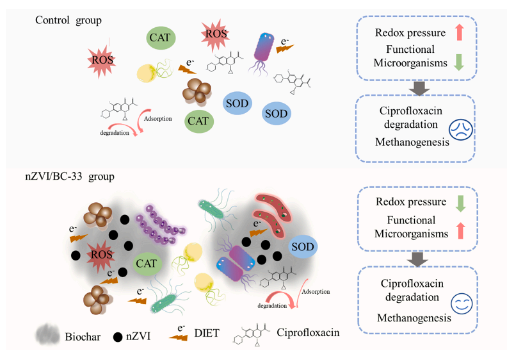
HIGHLIGHTS

- Adding iron-carbon produced methane of 143 mL/g COD under ciprofloxacin pressure.
- Iron-carbon promoted the degradation of ciprofloxacin with an efficiency of 87%.
- Iron-carbon alleviated microorganism subjected to reactive oxygen species.
- Iron-carbon enriched microbes related to ciprofloxacin removal and methanogenesis.

GRAPHICAL ABSTRACT



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ABSTRACT

The commonly used antibiotic ciprofloxacin (CIP) can significantly inhibit and interfere with the anaerobic digestion (AD) performance. This work was developed to explore the effectiveness and feasibility of nano iron-carbon composites to simultaneously enhance methane production and CIP removal during AD under CIP stress. The results demonstrated that when the nano-zero-valent iron (nZVI) content immobilized on biochar (BC) was 33% (nZVI/BC-33), the CIP degradation efficiency reached 87% and the methanogenesis reached 143 mL/g COD, both higher than Control. Reactive oxygen species analysis demonstrated that nZVI/BC-33 could effectively mitigate microorganisms subjected to the dual redox pressure from CIP and nZVI, and reduce a series of oxidative stress reactions. The microbial community depicted that nZVI/BC-33 enriched functional microorganisms related to CIP degradation and methane production and facilitated direct electron transfer processes. Nano iron-carbon composites can effectively alleviate the stress of CIP on AD and enhance methanogenesis.

1. Introduction

Antibiotics are widely employed to treat bacterial diseases in animals and humans because they inhibit or kill bacteria. Antibiotics have been detected in municipal, hospital, livestock, and pharmaceutical wastewater at concentrations ranging from ng/L to mg/L. Pharmaceutical wastewater has become an important reservoir of antibiotics at concentrations several orders of magnitude higher than others.

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Ciprofloxacin (CIP), a third-generation fluoroquinolone, has been detected in several pharmaceutical wastewaters at higher concentrations than many other antibiotics, approximately 1–20 mg/L (Wang et al., 2021). CIP can affect anaerobic methanogenic performance by inhibiting the activity of enzymes in microorganisms by binding to deoxyribonucleic acid pro-cyclase (Shen et al., 1989). Anaerobic digestion (AD) is a sustainable technology with biogas output, low sludge production, and energy consumption (Mai et al., 2018). The presence of CIP in wastewater can destabilize the anaerobic system and affect the recovery of biogas. Do et al. (2022) found that CIP concentrations above 5 mg/L resulted in a 40%-50% reduction in methanogenesis. Tang et al. (2022) demonstrated that a CIP concentration of 2 mg/L affected microbial activity and functional enzyme secretion, and methane production decreased from 331 to 218 mL/g VS. Therefore, weakening or eliminating CIP negative effects on AD, especially for pharmaceutical wastewater containing high CIP concentrations, is crucial for controlling antibiotics and promoting methanogenesis in wastewater treatment.

Nano zero-valent iron (nZVI) can be utilized for CIP removal due to its strong reducing property. In addition, adding nZVI to AD was reported to improve AD reactor performance. However, nZVI particles tend to agglomerate due to their own van der Waals forces and passivate due to strong reactivity, requiring nZVI modification. Moreover, nZVI, at the nanoscale, can enter the cell and disrupt the cell membrane and internal structure (Kong et al., 2021). Applying nZVI to AD of CIP wastewater reasonably and effectively is worthy of further exploration. Biochar (BC) is a carbon-sequestering organic material rich in functional groups, easy to obtain, economical, and environmentally friendly. BC can be used as a carbon skeleton to support and disperse nZVI particles, alleviating nZVI agglomeration and passivation. More importantly, BC has been recognized as a conductive material that can improve the AD performance by facilitating direct interspecies electron transfer (DIET) (Bu et al., 2022).

Currently, adding BC to AD is generally considered to improve methanogenic performance, but there is still controversy about whether nZVI can facilitate AD due to the cell penetration and excitation of excessive reactive oxygen species (ROS). More importantly, more attention has been focused on the role of nZVI and BC in AD without antibiotic stress; whether they can improve methanogenesis in pharmaceuticals wastewater with high concentrations of antibiotics requires further exploration. Distinguishing from previous studies, this work aims to investigate the feasibility and effectiveness of nano iron-carbon composites in simultaneously removing CIP and improving methanogenesis under the stress of antibiotic CIP. In addition, this work seeks to reveal the response of anaerobic system to nano iron-carbon composites through oxidative stress and functional microbial communities.

2. Materials and methods

2.1. Reagents and materials

BC was formed by pyrolytic carbonizing waste tea leaves at 600 °C for 3 h. nZVI/BC were prepared as mentioned in previous study (Yao et al., 2019). nZVI/BC composites with nZVI percentages of 16%, 33%, and 66%, were referred to nZVI/BC-16, nZVI/BC-33, and nZVI/BC-66, respectively.

Total solids (TS) and volatile total solids (VS) of anaerobic sludge were 16.50 (±0.20) and 8.30 (±0.30) g/L, respectively. To simulate pharmaceutical wastewater containing a high concentration of CIP, 9.37 g/L glucose, 0.96 g/L NH_4Cl, 0.22 g/L KH_2PO_4, and 10 mg/L CIP was added in aqueous solution as substrate (Lv et al., 2017; Wang et al., 2021). The pH value of synthetic CIP wastewater was 8.30 ± 0.10, adjusted using sodium bicarbonate.

2.2. Experimental procedure

Six groups were set up for the experiment, with three parallel samples established for each group: Blank, Control, BC, nZVI/BC-16, nZVI/BC-33, and nZVI/BC-66 group, with the parameters set as displayed in Supplementary Material. The experiments were conducted in 250 mL brown serum bottles containing 50 mL of anaerobic sludge and 150 mL of CIP wastewater. The anaerobic systems were purged under high-purity nitrogen for 5 min and placed in a constant temperature incubator to incubate at 37 °C and 150 rpm. Biogas was collected and monitored daily, and the supernatant was filtered to determine anaerobic physicochemical indicators.

2.3. Analytical methods

The contents of H_2 and CH_4 were determined using gas chromatography (GC2088, China) equipped with a thermal conductivity detector. VFAs were obtained using gas chromatography (GC9720Plus, Fuli Instruments, China) equipped with a hydrogen flame ion detector (FID) and an RB-FFAP column. The CIP adsorbed on the solid phase was extracted and enriched to determine the concentration, as described in previous study (Tang et al., 2022). CIP concentrations in aqueous solution were determined using a high-performance liquid chromatograph (Waters e2695, USA) with a C18 column (3.5 µm, 4.6 × 50 mm, Waters) under a mobile phase with a 3:7 (v/v) ratio of acetonitrile to 0.1% formic acid in water. ROS, catalase (CAT), and superoxide dismutase (SOD) enzymes were determined using the kit (Shanghai Ruifan Biological Technology Co., Ltd.). Microbial community diversity were determined by Shanghai Meiji Biomedical Technology Co (Tang et al., 2022).

2.4. Statistical analysis

According to mass conservation, M_t (mg) $= M_s + M_l + M_d$, where M_t, M_s, M_l and M_d represented the amount CIP of total, solid phase adsorption, liquid phase residual, and degradation, respectively. Degradation efficiency (%) $= M_d/M_t * 100$.

All analyses are expressed as the mean ± standard deviation of triplicate samples. Analysis of variance (ANOVA) was performed using SPSS 27.0. $P > 0.05$ was considered not statistically significant.

3. Results and discussion

3.1. Effect of nano iron-carbon composites on anaerobic digestion performance

AD was not disrupted by CIP in Blank, and its methanogenic process was completed within 12 days (Fig. 1a). Compared to Control, which was severely affected by CIP, the methanogenesis improved after adding materials, and its cumulative methane production slowly increased, indicating that the anaerobic system gradually adapted to the environment. Compared with BC, nZVI/BC-16, and nZVI/BC-66, nZVI/BC-33 displayed superior cumulative methane production of 143 mL/g COD (see supplementary material).

H_2 production of nZVI/BC-33 and nZVI/BC-66 with higher nZVI content was significantly higher ($p < 0.05$) than the other groups on day 2 (see supplementary material). Excluding the rapid H_2 release from nZVI, the iron-carbon microelectrolysis reaction also increases H_2. H_2 can pull the hydrogen nutrient methanogenesis and the homotypic acetic acid production process.

Short-chain VFAs are excellent precursors for methanogenesis during AD. Compared to Blank, total VFAs increased in the experimental groups with added materials (see supplementary material), consistent with the

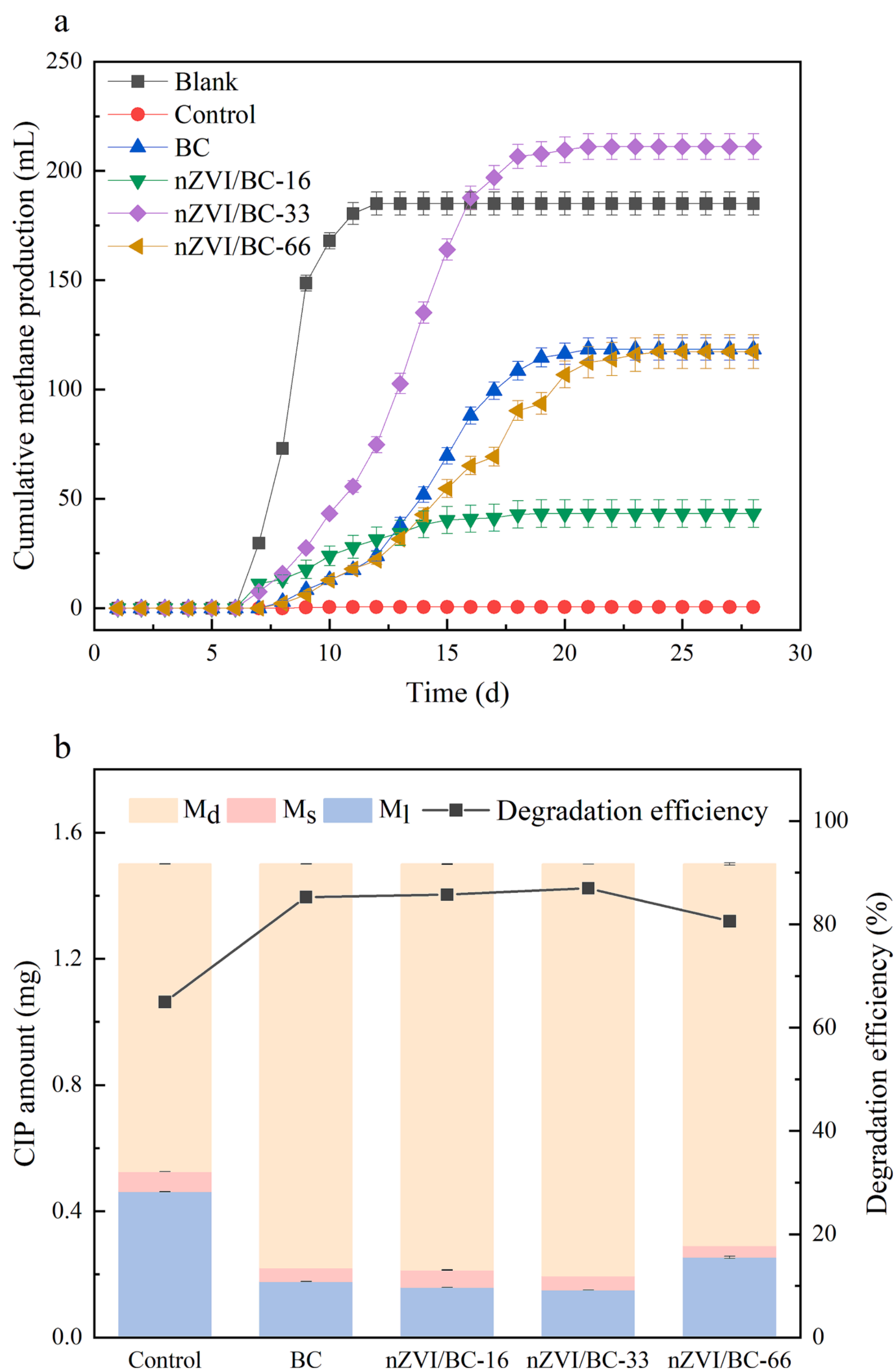

Fig. 1. The cumulative methane production during AD (a) and the degradation efficiency of CIP after AD (b).

studies by Qin et al. (2020) and Bu et al. (2021) that nZVI and carbon-based materials could promote hydrolysis acid production. Although the production of VFAs in nZVI/BC-66 was significantly higher ($p < 0.05$) than in the other groups, its methane production was not. This might be due to the inhibition of methanogens activity by excess iron nanoparticles (Dong et al., 2022). nZVI/BC-33 did not depict the highest increase, probably due to the appropriate amount of nZVI fixed on the BC that avoided acid accumulation and promoted rapid and effective methanation of VFAs.

3.2. *Ciprofloxacin removal in anaerobic digestion*

In addition to improving the methanogenic performance of CIP wastewater, adding materials improved the degradation efficiency of CIP. The CIP degradation efficiencies of BC, nZVI/BC-16, nZVI/BC-33 and nZVI/BC-66 group were 85%, 86%, 87% and 80% (Fig. 1b), respectively, higher than 65% in Control. The adsorption in the solid (M_s) and residual in the liquid phase (M_l) of CIP were higher in Control than in the other groups, resulting in a much lower cumulative methane yield (Fig. 1a). CIP degradation in BC, nZVI/BC-16, and nZVI/BC-33

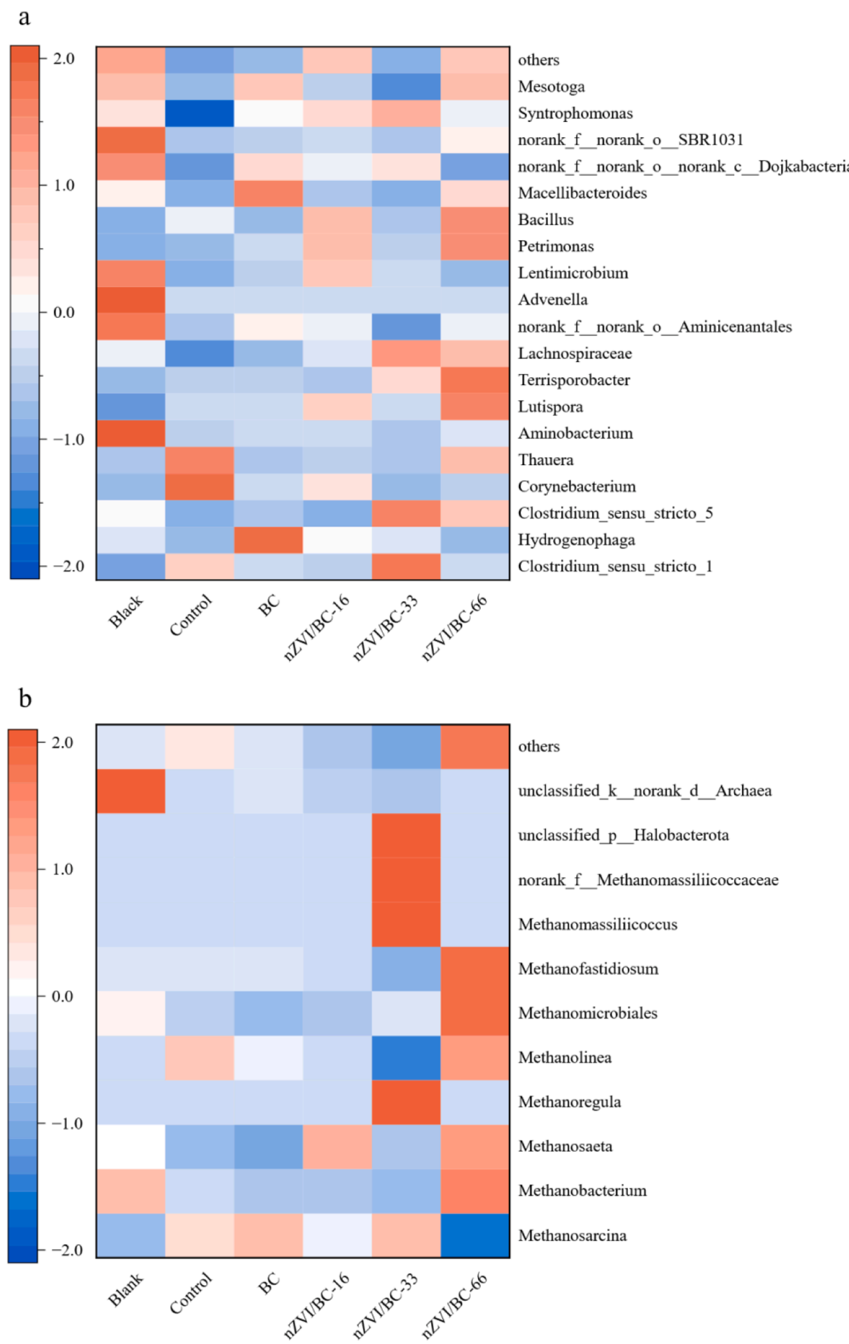

Fig. 2. Bacterial (a) and archaeal (b) communities at the genus level.

groups might be due to BC and nZVI itself or its mediated microbial metabolic activity. The reduced CIP degradation efficiency in nZVI/BC-66 system was attributed to the excessive nZVI coating on the BC surface. It not only made futile efforts to mitigate the agglomeration of nZVI, but also the high iron content affected the biodegradation of CIP (Dong et al., 2017; Wei et al., 2018).

3.3. Response of anaerobic systems to nano iron-carbon composites

3.3.1. Redox pressure

ROS are natural byproducts of oxygen metabolism in organisms and generally include oxygen-containing free radicals and peroxides. ROS accumulation in Control at day 23 compared to Blank was due to CIP (see supplementary material), and excessive ROS could degrade the lipid membrane of microbial cells, damaging the cell membrane and causing cell injury or death, which could further explain the reduced methanogenic performance in Control (Van der Paal et al., 2016). nZVI/BC-16, nZVI/BC-33, and nZVI/BC-66 demonstrated a decrease in ROS on day 14, probably due to active cell metabolism along with high methane production (Fig. 1a). On day 23, four groups with added materials had significantly lower ROS than the Control ($p < 0.05$), owing to the ability of BC and nZVI to enhance CIP degradation in AD (Fig. 2a). nZVI/BC-66 released excess nZVI in AD, mediating a high level of ROS and then hindering methane production (Xie et al., 2021). The nZVI/BC-33 system exhibited the best methanogenic performance because the dual pressure of microorganisms exposed to CIP and nano iron particles was relieved, resulting in the lowest ROS on day 14 and day 23.

SOD and CAT are essential enzymes for cells to resist oxidative stress. Except for nZVI/BC-66, which regulated intracellular redox stress mainly by catalyzing hydrogen peroxide, all other groups were co-regulated by catalyzing both superoxide radicals and hydrogen peroxide (see supplementary material) (Castro-Alférez et al., 2017). The Control group had the highest ROS and SOD on day 7, day 14, and day 23 compared to the group with added material, implying that it was subjected to the strongest oxidative stress and the strongest intracellular stress response. Compared with BC and nZVI/BC-16, nZVI/BC-33 had lower SOD and CAT on Days 7 and 23, indicating that intracellular ROS accumulation and detoxification were better balanced and could carry out the normal methanogenic processes.

3.3.2. Microbial communities

Bacterial community structure was significantly altered at the genus level (see supplementary material). Distinguished from Blank, the dominant species in Control were mainly *Clostridium_sensu_stricto_1*, *Corynebacterium* and *Thauera* (Fig. 2a). *Thauera* and *Clostridium_sensu_stricto_1* can degrade complex organic pollutants, which explains the decrease in CIP concentration (Fig. 1b) (Li et al., 2022; Zhou et al., 2018). The dominant species *Hydrogenophaga* in the BC group achieved 32.7% and could degrade a wide range of pollutants, in addition to breaking down glucose (Feng et al., 2022). Different mass ratios of iron to carbon induced different dominant species. nZVI/BC-33 revealed a higher percentage of *Clostridium_sensu_stricto_1* (55.3%) than the other groups. This implies that CIP can be chemically degraded by highly active nZVI dispersed on BC and biodegraded by microorganisms of the dominant species, as shown in Fig. 1b. Notably, *Syntrophomonas* in nZVI/BC-33 group was higher than in the other groups. *Syntrophomonas* not only converted carbohydrates into acetate and hydrogen but was also a typical DIET-mediating bacterium that carbon materials could enrich (Wu et al., 2022). In addition, the *Clostridium_sensu_stricto_1* and *Clostridium_sensu_stricto_5* belonged to the order *Clostridium*, were electro generators and could provide electrons for subsequent methanogenesis via DIET (Qin et al., 2020).

The six groups included *Methanosarcina*, *Methanobacterium*, and *Methanosaeta* (Fig. 2b). *Methanosarcina* was dominant in six groups, accounting for 55%, 74%, 79%, 66%, 81%, and 42%. *Methanosarcina* could produce methane using various metabolic pathways such as acetic acid, hydrogen and methyl, and could participate in DIET as an electron acceptor. In addition, *Methanosarcina* had strong viability to survive against external stresses and promote methanogenesis despite unfavorable living conditions, and it is more abundant in tannery wastewater, slaughter wastewater, and solid waste digestion in the presence of antibiotics compared to other archaea (Alex Kibangou et al., 2022). *Methanomassiliicoccus* and *Methanoregula* increased in nZVI/BC-33 and produced energy from acetate and hydrogen. *Methanoregula* is also tolerant to fluorinated organisms and possesses the potential to degrade pollutants (Wu et al., 2021). Functional microorganisms related to CIP removal and methanogenesis accounted for the highest cumulative methane production in nZVI/BC-33 group.

4. Conclusion

Adding nano iron-carbon composites (nZVI/BC-33) to simultaneously enhance methane production and remove CIP is feasible in AD under CIP stress. The highest methanogenesis and CIP degradation efficiency were 143 mL/g COD and 87%, weakening the inhibitory effect of CIP on AD performance. nZVI/BC-33 mitigated microorganisms subjected to dual redox pressure from CIP and nZVI and reduced a series of oxidative stress reactions. The microbial community results demonstrated an elevated abundance of functional microorganisms associated with CIP degradation and methanogenesis, including those involved in DIET. nZVI/BC-33 has the potential to facilitate pollutant removal and methanogenesis in AD of CIP wastewater.

CRediT authorship contribution statement

Bing Yao: Conceptualization, Investigation, Methodology, Data curation, Writing – original draft. **Min Liu:** Supervision, Writing – review & editing, Resources. **Taotao Tang:** Supervision, Writing – review & editing. **Xuan Hu:** Investigation, Writing – review & editing. **Chengyu Yang:** Investigation, Writing – review & editing. **Ying Chen:** Supervision, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors are unable or have chosen not to specify which data has been used.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2023.129462.

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