

Research Article

Multistage A-O Activated Sludge Process for Paraformaldehyde Wastewater Treatment and Microbial Community Structure Analysis

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In recent years, the effect of formaldehyde on microorganisms and body had become a global public health issue. The multistage combination of anaerobic and aerobic process was adopted to treat paraformaldehyde wastewater. Microbial community structure in different reaction stages was analyzed through high-throughput sequencing. Results showed that multistage A-O activated sludge process positively influenced polyformaldehyde wastewater. The removal rates of formaldehyde were basically stable at more than 99% and those of COD were about 89%. Analysis of the microbial diversity index indicated that the microbial diversity of the reactor was high, and the treatment effect was good. Moreover, microbial community had certain similarity in the same system. Microbial communities in different units also showed typical representative characteristics affected by working conditions and influent concentrations. Proteobacteria, Firmicutes, and Bacteroidetes were the dominant fungal genera in the phylum level of community composition. As to family and genus levels, Peptostreptococcaceae was distributed at various stages and the dominant in this system. This bacterium also played an important role in organic matter removal, particularly decomposition of the acidified middle metabolites. In addition, Rhodobacteraceae and Rhodocyclaceae were the formaldehyde-degrading bacteria found in the reactor.

1. Introduction

Formaldehyde is a basic chemical raw material widely used in plastics, chemical, leather, resin, and other production processes. Formaldehyde is soluble in water; the emissions of aqueous solution of this compound can bring serious pollution to water and even kill water creatures. Meanwhile, formaldehyde can produce irritating effect on human body, damage the immune system, and cause cancer. Therefore, China has launched a series of emission regulatory controls for formaldehyde wastewater. For example, the secondary emission standard of formaldehyde must not exceed 2 mg/L in the standard of integrated wastewater discharge, and the formaldehyde content of centralized surface water for domestic and drinking water must not exceed 0.9 mg/L in the standard of surface water environment quality [1, 2]. The large source and quantity of formaldehyde wastewater cause certain difficulties to wastewater treatment. But considering

economic or reality reasons, we cannot ban the application of formaldehyde on the whole. Therefore, we need to treat formaldehyde wastewater from industrial production properly. Physical, chemical, and biological methods are mainly used for wastewater treatment. Physical methods include steam blow-off and adsorption. Blowing can be used as a pretreatment process. The effect of formaldehyde wastewater treatment on adsorption is satisfactory but presents limitations in adsorbent recycling. Chemical methods, including advanced oxidation and condensation/precipitation, are expensive. Biological methods are characterized by low cost, simple operation, and low pollution. Most microbes can use formaldehyde as carbon source and degrade wastewater [3]. Hidalgo [4] used *Rhodococcus erythropolis* UPV-1 in formaldehyde wastewater treatment. Both continuous dosing and intermittent dosing can form stable colony. The formaldehyde and chemical oxygen demand (COD) removal rates are 90% and 56%, respectively. Wang et al. [5] used

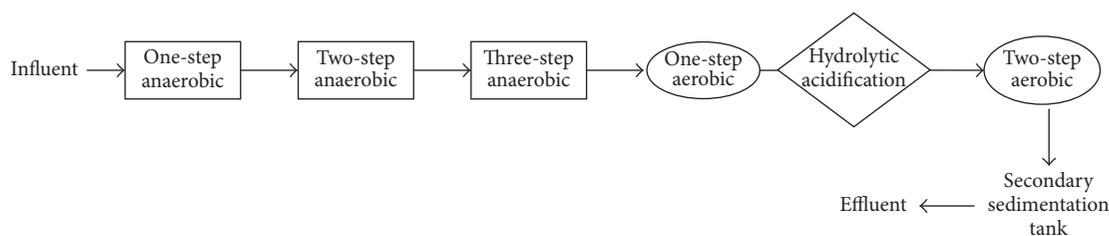


FIGURE 1: Process flow diagram in the field.

TABLE 1: Flooding water quality and sludge properties in the field.

Index	HCHO (mg/L)	COD (mg/L)	pH	MLSS (mg/L)	DO (mg/L)	SV ₃₀ (%)	SVI (mL/g)	SS (mg/L)
Content	650~1200	4000~5800	3.55~3.80	5900~15000	3.40~4.75	90~98	124~683	200~440

activated sludge process in the treatment of formaldehyde wastewater. The results showed that the initial concentration of formaldehyde is 400 mg/L and the sludge concentration is 4 g/L after 10 h; in addition, the removal rates of formaldehyde and COD reach more than 99% and 83%, respectively. *Methylobacillus flagellatus* [6], *Pseudomonas putida* [7], *Ralstonia eutropha* [8], and *Candida maltose* [9] have been reported in formaldehyde degradation. Nevertheless, the degradation effect of these strains shows differences. Most of the strains can only degrade low formaldehyde concentration. Suitable degradation strains should still be determined for high-concentration formaldehyde produced in industrial process. The present study adopted multistage A-O activated sludge process in the treatment of polyformaldehyde wastewater; microbes in the sludge can use formaldehyde as carbon source and degrade wastewater. The process was complex, and the hydraulic retention time was short. This in-depth process can be reproducible in view of high concentration and complex wastewater. We used high-throughput sequencing technology to analyze the change in microbial community, ecological information of colony, and degradation function relationship in different reaction steps in depth. We hoped that our work can provide certain technical and theoretical support for the actual project. Therefore, we can treat formaldehyde wastewater better and reduce its harm to environment and human body.

2. Materials and Methods

2.1. Overview of the Reactor. This study was based on the polyformaldehyde wastewater treatment in the production process in a chemical plant. The experiment process is as follows: raw water → iron and carbon microelectrolysis → one-step anaerobic (A1) → two-step anaerobic (A2) → three-step anaerobic (A3) → one-step aerobic (O1) → hydrolytic acidification → two-step aerobic (O2). The process flow diagram and the flooding water quality and sludge properties in the field are shown in Figure 1 and Table 1.

2.2. Sample Collection. The collection date was June 8, 2015, and the sludge samples were obtained from A1, A2, A3, O1, hydrolytic acidification, and O2. We collected the samples in

TABLE 2: Sludge sample number information in the reactor.

Sample number	Sampling stage
A1D	One-step anaerobic
A2D	Two-step anaerobic
A3D	Three-step anaerobic
O1D	One-step aerobic
BD	Hydrolytic acidification
O2D	Two-step aerobic

TABLE 3: Testing index and method of activated sludge.

Testing index	Testing method
SS	Dry weight
SV ₃₀	Sedimentation method
SVI	Settlement of weighing
MLSS	Weight method

the reactor when it was starting. The sludge sample number information is shown in Table 2.

2.3. Chemical Analysis. Conventional water quality indicators, such as pH, temperature, and COD, were analyzed using the national standard method [10]. The dissolved oxygen (DO) and formaldehyde concentration were analyzed using the DO instrument. Table 3 shows the testing index and method of activated sludge.

2.4. DNA Extraction and High-Throughput Sequencing. DNA was extracted via phenol-chloroform extraction and was purified by purification kit (TIANquick Midi Purification Kit, Tiangen). DNA samples were detected of fragment length through agarose gel electrophoresis detection after purification. The concentration and purity were determined by Nanodrop. PCR (Polymerase Chain Reaction) amplification was carried out, and the amplification products were used for DNA sequencing [11]. The library of sequencing DNA was constructed by TruSeq kit (Illumina, USA) and was determined by Illumina Miseq2500 high-throughput sequencing machine for sequencing.

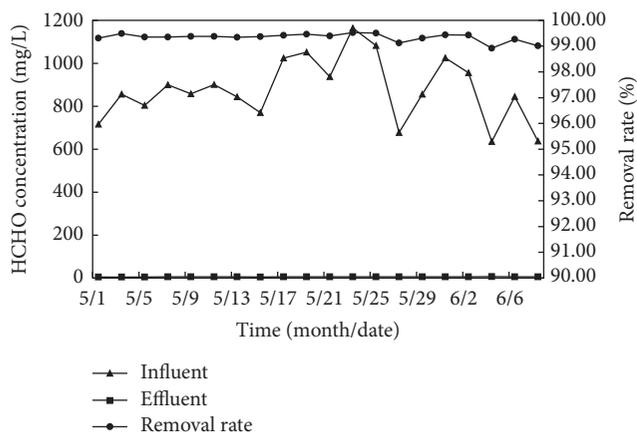


FIGURE 2: Formaldehyde removal from the reactor.

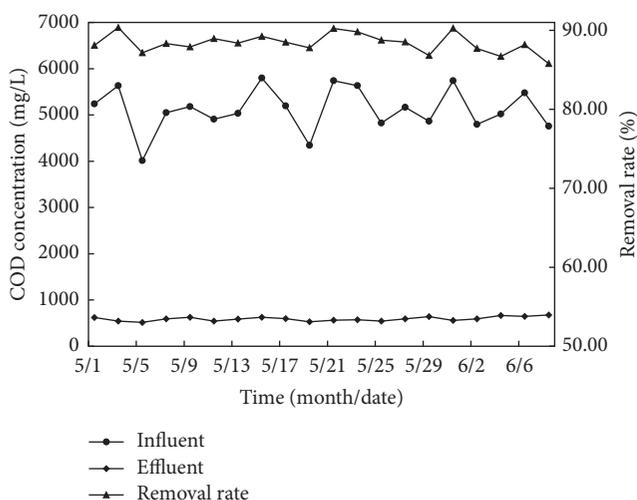


FIGURE 3: COD removal from the reactor.

3. Results and Discussion

3.1. HCHO Removal Performance of the Reactor. Figure 2 shows the formaldehyde removal from the reactor.

Figure 2 shows that influent formaldehyde concentration is 635–1164 mg/L, and the effluent formaldehyde concentration in secondary sedimentation tank is about 5 mg/L. The fluctuation of influent formaldehyde concentration is large because of the complex working conditions in the field. However, the removal rate is basically stable at more than 99%, with the highest at 99.5%. This rate shows that the performance of the technology is ideal, and such technology can respond to the change in external conditions.

3.2. COD Removal Performance of the Reactor. Figure 3 shows the COD removal from the reactor.

Figure 3 shows that influent COD concentration is 4000–5800 mg/L, and the effluent COD concentration in secondary sedimentation tank is 510–670 mg/L. The removal rate of COD is about 89%, and the highest can reach 90.36%. The different removal rates of formaldehyde and COD show that

formaldehyde degradation and its degradation products are not completely synchronous [12, 13]. Longer time is needed before it becomes fully biodegradable.

The system has a total of six units of series reactor, which includes three stages of anaerobic reaction, two stages of aerobic reaction, and a hydrolysis acidification phase. This process is relatively complicated, which can be carried out in in-depth degradation processing in the view of high COD concentration and complex wastewater. Table 4 presents the quality index of polyformaldehyde wastewater.

First, high-concentration wastewater underwent three continuous anaerobic reaction systems with long anaerobic reaction time. Both the flora in graded response and the differentiation of ecological level are abundant. At this stage, most of the carbon sources that can be used are degraded by anaerobic microbes. Thus, most of the reactor formaldehyde and COD are removed during the anaerobic phase, and the removal rate reaches 62.6% and 73.3%, respectively. The biochemical substance content in the effluent water at anaerobic phase is relatively small. Most of these substances are materials that are difficult to use via anaerobic microbes. The organic matter is degraded and mineralized in depth in aerobic phase. Macromolecular organic matter and intermediate metabolites are decomposed sequentially in the hydrolysis acidification phase; these materials are then translated thoroughly into harmless substance and discharged after secondary aerobic phase.

3.3. Sludge Ecology Analysis in the Reactor

3.3.1. Microbial Numbers and Diversity. The results of operational taxonomic unit (OTU), abundance (Chao 1), and diversity (Shannon) index of microbial community were obtained by high-through sequencing. The microbial community diversity in the sludge sample of the reactor is shown in Table 5. The Shannon index of the sediment samples changes in the range of 5.44–6.74. Shannon index is low at the three-step anaerobic, hydrolysis, and acidification stages. Such low value may be attributed to that as the reaction continues; the bacteria, which are not adapted to the environment, gradually lose activity, age, and die because of the continuous change of DO and nutrition matrix. When the hydrolysis acidification phase is reached, the sludge activity is reduced because the life cycle of anaerobic microbes is longer than those of aerobic ones, and the sludge accumulated in the bottom causes inadequate contact with wastewater and poor microbial diversity [14].

Chao 1 and OTU index had a similar change rule with Shannon index. These three indicators describe the microbial diversity and its relationship with the effect of wastewater treatment. The results also reflect that the microbial diversity is high, and the treatment effect is relatively good in the reaction pool [15].

3.3.2. Phylum Level of Community Composition in Sludge Samples. The microbial classification in sludge samples according to phylum is shown in Figure 4. Comparing with sequence in the library construction, we can determine the kinds of microbial communities. Proteobacteria, Firmicutes,

TABLE 4: Quality index of polyformaldehyde wastewater.

	Raw water	Effluent water of iron and carbon microelectrolysis	A1	A2	A3	O1	Hydrolytic acidification	O2
HCHO (mg/L)	638	511	191	9.81	—	10.3	8.6	10.5
COD (mg/L)	4957.2	3772.2	1006.4	920.0	654.0	303.0	283.0	276.4
pH	3.5	5.0	7.0	7.2	7.5	7.5	7.5	8.0
Temperature (°C)	—	—	35.0	36.0	35.0	35.0	36.0	31.0

TABLE 5: Microbial community diversity in the sludge sample of the reactor.

Samples number	Chao 1	Shannon index	OTU number
A1D	1458.35	6.74	1053
A2D	1281.31	6.42	903
A3D	1351.01	5.84	856
O1D	1815.04	6.65	1219
BD	911.64	5.44	663
O2D	1608.42	6.14	991

Bacteroidetes, Actinobacteria, Chloroflexi, Planctomycetes, and Thermotogae are the dominant bacterial communities in the samples.

Proteobacteria (30.1%–67.2%) are dominant in all of the samples [16]. Proteobacteria are one of the largest bacterial categories, which belong to gram-negative bacteria. Their outer membrane is mainly composed of lipopolysaccharide. The metabolic type is different among different members, and most of them is facultative or obligate anaerobic. Proteobacteria are the main groups of bacteria in the wastewater treatment system and plays an important role in the removal of organic matter from wastewater [17, 18]. In wastewater treatment process, the amount of Proteobacteria decreases from 67.2% to 30.1% of hydrolysis acidification phase and subsequently increases to 52.1%. This result might be because Firmicutes and Bacteroidetes begin to multiply and occupy some ecological niches of Proteobacteria [19] through anaerobic fermentation.

Firmicutes is gram-positive bacteria. The peptidoglycan content accounts for 50%–80% of the total quality of cell walls. Firmicutes is absolutely dominant in hydrolysis acidification phase [20, 21]. Its proportion gradually increases from 9.8% to 37.1% of hydrolysis acidification phase in the reactor.

Bacteroidetes is autotrophic bacteria that can translate macromolecular organic matter (protein, starch, and lipid) into small molecules through hydrolysis and fermentation. Bacteroidetes includes some nitrogen-fixing bacteria, which play a part in the denitrification process [22, 23].

3.3.3. Family and Genus Levels of Community Composition in Sludge Samples. The microbial classification in sludge samples according to family and genus is shown in Figure 5. The distribution of family and genus levels can reflect detailed

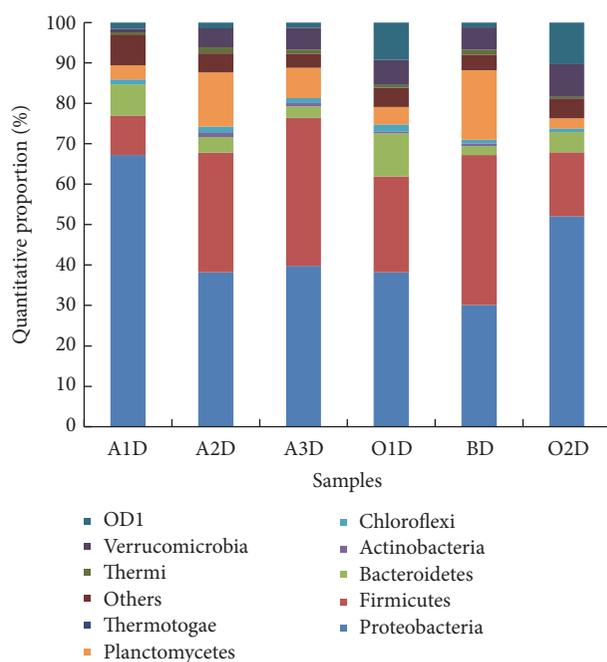


FIGURE 4: Classification of microbes in sludge samples according to phylum.

microbial functional information in the system. Microbial community has certain similarity in the same system. The dominant microflora slightly changes and has certain regularity. Peptostreptococcaceae (3.3%–27.2%), Phycisphaerales (0.9%–14.2%), Rhodobacteraceae (5.0%–15.2%), *Azospirillum* (2.1%–11.9%), Rhodocyclaceae (0.6%–8.8%), *Alphaproteobacteria_BD7-3* (0.2%–6.2%), Piscirickettsiaceae (0.9%–3.5%), and *Candidatus Xiphinematobacter* (0.7%–8.1%) are the dominant bacterial communities in the samples. Most of these communities belong to Proteobacteria.

This process system is further complicated. Different processing units have different functions in the system. The anaerobic phase is relatively different between aerobic phases. Microbial communities in different units show typical representative characteristic affected with working conditions and influent concentration.

Rhodobacteraceae is the dominant microbes in the one-step anaerobic process. These bacteria can accumulate phosphorus in denitrification [24–26]. Rhodobacteraceae and Rhodocyclaceae play an important role in organic matter

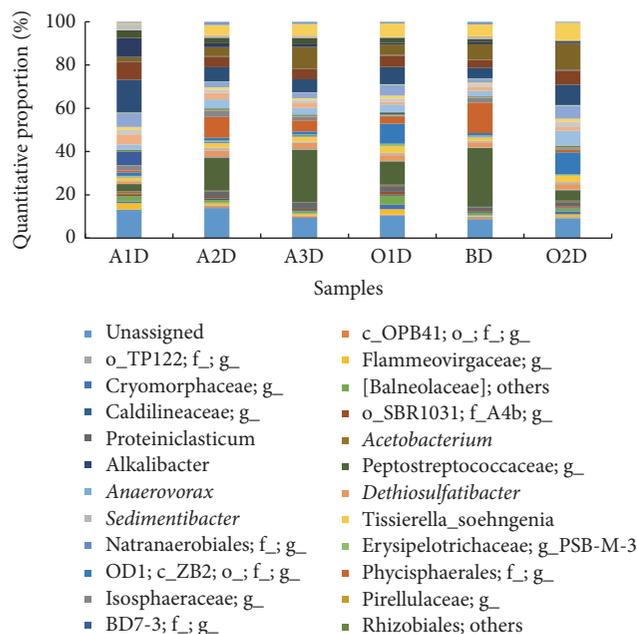


FIGURE 5: Microbial classification in the sludge samples according to family and genus.

degradation [27]. Approximately 60% of the formaldehyde is decomposed during the one-step anaerobic process (Figure 4). Both the numbers of Rhodobacteraceae and Rhodocyclaceae obviously decline with formaldehyde degradation. Therefore, Rhodobacteraceae and Rhodocyclaceae should be the main formaldehyde degradation bacteria in the reactor.

Most of formaldehyde and COD have been degraded in the one-step anaerobic process, and thus two- and three-step anaerobic processes are the main procedures of in-depth anaerobic treatment. The microbial community structures of these two processes are relatively similar, and Peptostreptococcaceae and Phycisphaerales are the dominant bacteria in the system. Phycisphaerales belongs to Planctomycetes and is the anaerobic ammonia oxidation bacterium. These bacteria can create nitrite-oxidizing ammonium and produce nitrogen under anoxic conditions, which contribute to denitrification [28, 29].

After entering the aerobic reaction stage, the diversity of microbial community structure increases in the reactor because the bacterial aerobic metabolism grows quickly, including the increase of nitrifying bacteria, which has high DO demand.

Peptostreptococcaceae is the dominant microbes during hydrolysis acidification. This microbe belongs to Firmicutes and typically uses little or no sugar. It can decompose protein to produce acetic acid [19]. Peptostreptococcaceae reaches about 27.2% in this process, which illustrates that it has already reached the vigorous stage of acid production. Peptostreptococcaceae is also distributed at all stages in the system and has an obvious advantage in the middle of four stages. This microbe is a facultative aerobic bacterium and plays an important role in the removal of organic matter, specifically the acidification decomposition of middle metabolites.

In addition, *Azospirillum* is distributed at all stages in the system; these nitrogen-fixing microbes belong to Proteobacteria and can fix nitrogen with cereals and Gramineae [30]. *Azospirillum* is also part of the denitrifying bacteria groups and can translate nitrate into N_2O or N_2 under enzyme catalysis. This microbe also has a role in the nitrogen cycle [31].

Candidatus Xiphinematobacter grows with the reaction and belongs to Thermotogae. This microbe is a kind of nitrifying bacteria, and little information is available regarding it.

3.3.4. RDA (Redundancy Analysis) of Dominant Bacterium in Sludge Samples. Figure 6 shows the RDA of dominant bacterium and major environmental factor in sludge samples. Rhodobacteraceae and Rhodocyclaceae have a high correlation with formaldehyde removal rate, which is consistent with the conclusions mentioned above.

Piscirickettsiaceae and *Alphaproteobacteria*_BD7-3 also have a high correlation with COD removal rate. Piscirickettsiaceae belongs to γ -Proteobacteria and uses organic matter as the main carbon source. It also plays an important role in COD removal process [28]. *Alphaproteobacteria*_BD7-3 belongs to α -Proteobacteria and has not been named. Most of α -Proteobacteria is saprophytic heterotrophic bacteria, and their main carbon source is the organic matter. Therefore, these bacteria are important in the removal process of COD [30].

4. Conclusions

(1) Multistage A-O-activated sludge process has good treatment effect on polyformaldehyde wastewater. Under the initial formaldehyde concentration of 635–1164 mg/L and COD concentration of 4000–5800 mg/L, the removal rates of formaldehyde are basically stable at more than 99% and those of COD are about 89%. This method can remove pollutant effectively. We hoped that our work can provide certain technical support for the actual project.

(2) The ecology of activated sludge in different reaction stages through high-throughput sequencing was analyzed. The analysis results of microbial diversity indices (Shannon, Chao 1, and OTU) indicated that the microbial diversity of the reactor is high, and the treatment effect is good. Microbial community has certain similarity in the same system. Microbial communities in different units show typical representative characteristic affected with working conditions and influent concentration.

(3) The microbial community structure of the sludge samples was also analyzed. Proteobacteria, Firmicutes, and Bacteroidetes are dominant fungal genera in the phylum level of community composition. Peptostreptococcaceae, Phycisphaerales, and Rhodobacteraceae are dominant fungal genera in the family and genus levels of community composition. Peptostreptococcaceae is distributed at various stages and dominant in this system. This bacterium also plays an important role in organic matter removal, particularly the decomposition in middle metabolite acidification. Rhodobacteraceae and Rhodocyclaceae are formaldehyde-degrading bacteria in the reactor.

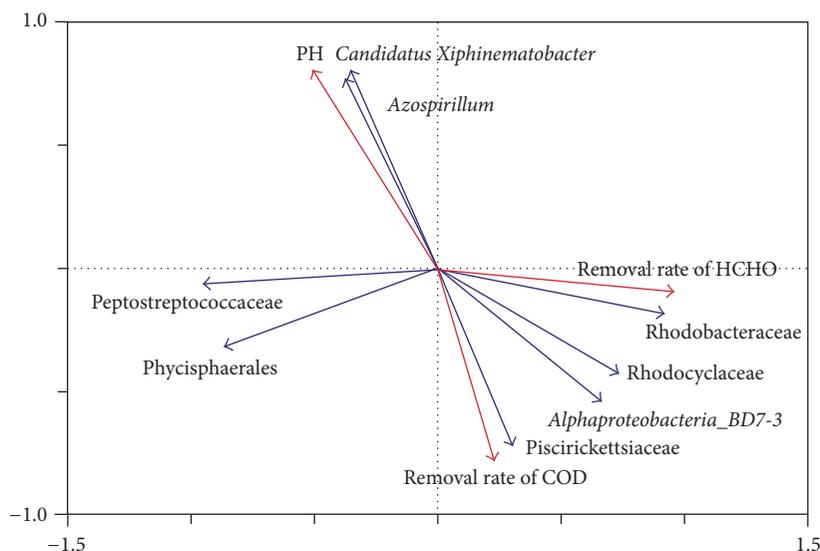


FIGURE 6: RDA of advantage bacterium in sludge samples.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

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