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# Different Types of Inoculated Sludge for Chemical Wastewater Treatment: Acclimation and Microbial Community

**Dong Yingbo*(**)† and Lin Hai *(**)**
*School of Civil and Environment Engineering, University of Science and Technology Beijing, Beijing 100083, China
**Beijing Key Laboratory of Resource-oriented Treatment of Industrial Pollutants, Beijing 100083, China
†Corresponding author: Dong Yingbo

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## ABSTRACT

A laboratory-scale reactor for hydrolytic acidification-biological contact oxidation (A/O) was constructed and inoculated with different types of sludge to treat synthetic chemical wastewater. Biodegradation of degradation-resistant organic matters, such as epoxy propane, glycerol and aniline, in saline water were evaluated, and acclimation of chemical and sewage seeding sludge was contrastively analysed. An average COD reduction rate of 93% by A/O reactor was achieved after the 22 d and 31 d of acclimation of chemical and sewage seeding sludge, respectively. Salt-tolerance acclimation of chemical seeding sludge required 35 d and 14 g/L NaCl for effective pollution degradation, whereas, chemical sludge required 32 d and had salt-tolerance of 16 g/L NaCl. The microbial community structure differed between chemical and sewage seeding sludge. Sewage sludge presented higher average Shannon diversity index (3.34) and more uniform microbial distribution than chemical sludge. The results can provide the basis for selection, cultivation and acclimation of inoculation sludge for field debugging of treating chemical wastewater through biological membrane method.

## INTRODUCTION

Wastewater treatment in chemical industries has been a research hotspot and challenge because the industrial process involves complex compounds, and feature high organic matters and salt (Lin et al. 2011). Pollutants in chemical wastewater are usually toxic and refractory, resulting in high treatment cost and difficult processing (Lefebvre & Moletta 2006). Currently, wastewater from chemical industries is usually treated through neutralized sedimentation, electrolysis, redox reaction, membrane separation, ion exchange, adsorption, and biological treatment (Campos et al. 2002, Yang et al. 2003, L'Amoura et al. 2008, Yan et al. 2010). Biological treatment is a simple, inexpensive, effective and universal method (Loukidou & Zouboulis 2001, Dosta et al. 2011). In recent years, research on biological treatment has focused on improving various physical and chemical properties of sludge; these properties include settle ability, filter and particle size distribution, and contents of extracellular polymeric substance, bacterial protein polysaccharide and humus (Ramos et al. 2007, Aloui et al. 2009, Zhang et al. 2012, Li et al. 2013). Different types of inoculation sludge exhibit varied effects on bacterial culture, domestication, and start-up process during treatment of saline wastewater from chemical industries (Palmeiro-Sánchez et al. 2013). Thus far, few studies have conducted contrast analysis on different types of inoculation sludge.

In this work, hydrolytic acidification-biological contact oxidation (A/O) was utilized to treat synthetic solutions prepared using epoxy propane, glycerol, aniline and sodium chloride. Biodegradation of pollutant in synthetic solutions was assessed using the A/O bioreactor with different types of seeding sludge (chemical and sewage). The biological domestication, microbial community structure, microbial diversity, and COD removal of different types of seeding sludge was contrastively investigated. The results will provide a basis for selection, cultivation and domestication of inoculation sludge for practical engineering applications to treat high-salt chemical wastewater through biological membrane method.

## MATERIALS AND METHODS

**Inoculation of sludge samples:** Sewage sludge was obtained from aerobic and anaerobic tank of Qinghe Municipal Wastewater Treatment Plant in Beijing. The mixed liquor suspended solids (MLSS) and SV30 were 2000-3500 mg/L and 35%, respectively, for aerobic sludge and 5000 mg/L and 60%, respectively, for anaerobic sludge.

Chemical sludge was obtained from aeration and a secondary sedimentation tank of Yanshan Petrochemical Wastewater Treatment Plant in Beijing. The MLSS and SV30 of aerobic sludge were 4000 mg/L and 30%, respectively.

**Synthetic solutions:** According to the condition of mixed

Table 1: Feed solution characteristics.

| Item | COD | NaCl | Epoxy propane | Glycerol | Aniline |
|---|---|---|---|---|---|
| Content(mg/L) | 1500 | 14000-16000 | 505±0.5 | 386±0.5 | 59±0.5 |

chemical wastewater produced from a plant in China, synthetic solutions prepared using epoxy propane, glycerol, aniline and NaCl were fed in the reactor to assess their biodegradation. Trace elements as $MgSO_4$, $FeCl_2$, $CoCl_2$, $ZnSO_4$, $CuSO_4$, $NiSO_4$ and $MnCl_2$ were added in minute quantities. In order to balance nutrients, each solution was mixed with glucose, urea and $KH_2PO_4$ to acquire a C: N: P mass ratio of 100: 5: 1 (w/w/w). The pH of the solution was 7.8-8.0, and the other characteristics are presented in Table 1.

**Laboratory reactor:** A laboratory reactor was constructed to remove chemical pollutants from the synthetic solution. The schematic diagram of the experimental biological membrane reactor is shown in Fig. 1. The reactor was made of Plexiglas and contained two main functional sections, namely, hydrolytic acidification and biological contact oxidation. The upper end of the hydrolytic acidification tank (A tank) with a volume of 7.5 L was cylindrical (350 mm in height and 150 mm in diameter), and the lower end was a conical (150 mm in height and 150 mm in diameter). The biological contact oxidation tank (O tank) with a volume of 15 L was cuboidal (300 mm in length, 250 mm in width and 200 mm in height). The O tank was provided with a water inlet pipe, drainage pipe and mud discharging pipe. An aeration device was installed at the bottom of the O tank. One baffle was fixed at the water inlet pipe to ensure uniformity and prevent short flow, and another baffle was fixed at the end of the O tank to form a precipitation chamber. The combined filter material containing polyethylene and polyester was fixed in the O tank with a 60% filling rate. The specific surface area and porosity of the filter materials were 120 $m^2/m^3$ and 96%, respectively.

**Culturing of biological membrane:** According to the technical characteristics, a piecewise continuous hanging membrane was used for hanging membrane phase of filter material. The natural hanging membrane was used for the A tank, and the residual sludge inoculation method was used for the O tank. Two sections were joined after both biological membranes were developed. Considering the poor biodegradability of epoxy propane, glycerol and aniline, the prepared solution was mixed with glucose solution at ratios of 50:50, 75:25 and 100:0, respectively, for biomass acclimation. The COD removal rate was used as an indicator to evaluate the performance of the bioreactor during the start-up period. A COD removal of 75% for a 300 mg/L prepared solution was achieved after 18 and 16 d of inoculation and acclimation, respectively, of sewage sludge and chemical sludge. The reactor was used for normal operation.

**Operation of the reactor:** The water flow in the A tank was up-flow, that is, from the intake at the bottom to the outlet at the top by a water pump. The water flowed into the O tank under the action of gravity. The aeration port was set at the bottom of the O tank to supply air for biochemical reaction. Air flow rates were adjusted to maintain the dissolved oxygen concentration at 2-4 mg/L. The influent pH values were regulated at 7.8-8.0. The hydraulic retention time of the A and O tanks were 12 h and 24 h, respectively. During the experiment, the system was operated under ambient temperature ranging from 20 to 30°C.

After the reactor was operated under stable condition, microorganisms in the biological contact oxidation tanks with different types of inoculation sludge were collected for DNA extraction. The microbial community structures of different biological membranes with inoculation sewage or chemical sludge were contrastively analysed through PCR-DGGE technology to study species diversity and similarity.

**Analytical methods:** Influent and effluent chemical oxygen demand (COD) was determined according to standard method. Temperature and pH were routinely monitored during the experimental period. Each experiment was carried out in triplicate.

## RESULTS AND DISCUSSION

**Sludge acclimation effect of different inoculants:** As a large number of refractory organic compounds and a high concentration of salt may inhibit the growth of microorgan-

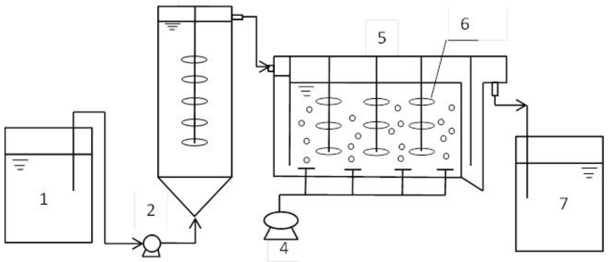
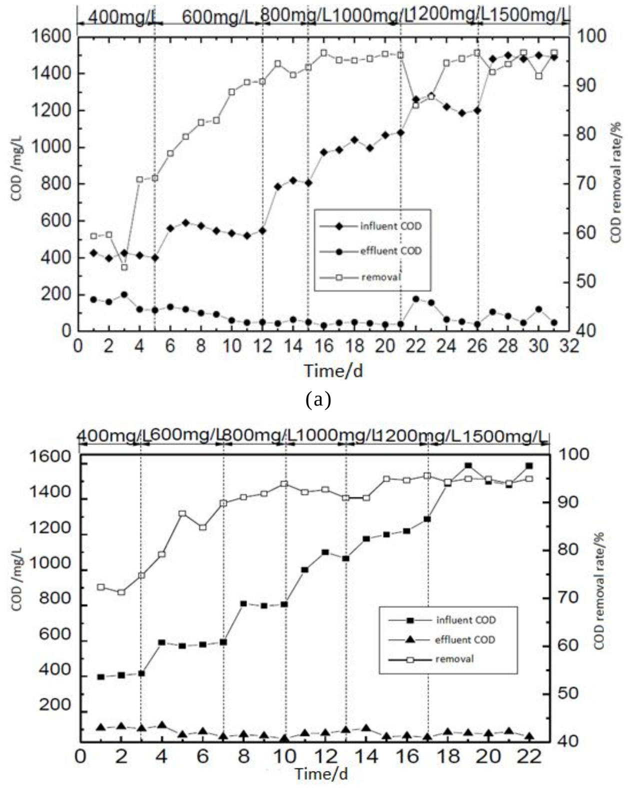


Fig.1: Schematic diagram of the experimental set-up.
(1-Influent tank, 2-Metering pump, 3- Hydrolytic acidification tank, 4-Aeration pump, 5-Biological contact oxidation tank and 6-Filter materials)



(a)

(b)

Fig.2: COD removal by the reactor with different types of inoculation sludge during the first acclimation phase (a-sewage sludge and b-chemical sludge).

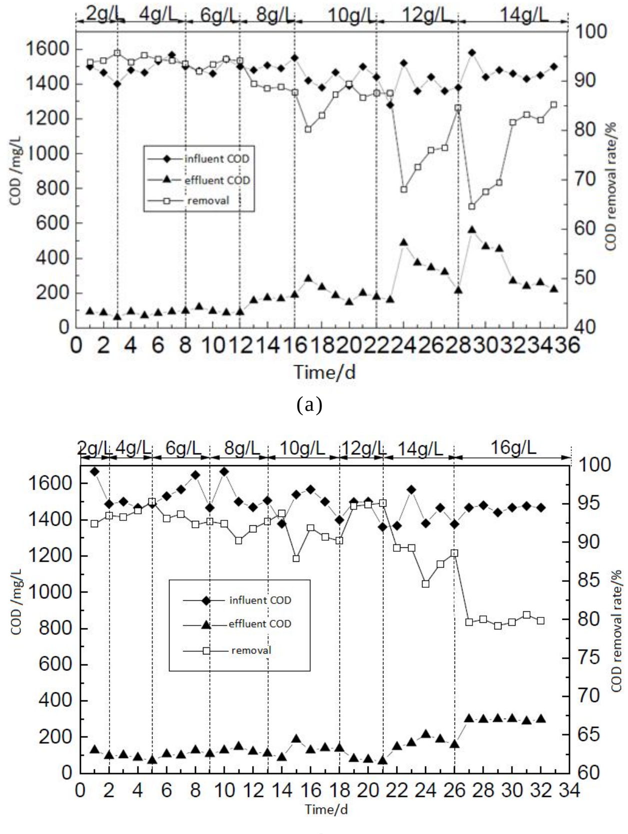
isms, biological membrane acclimation should be conducted in two stages to allow adaptation to the high concentrations of pollutants and salt. The influence of different types of inoculation sludge on acclimation time and treatment results was studied. During the first stage of acclimation, the salt concentration of prepared solution was maintained at 2g/L, and COD was gradually increased to 400, 600, 800, 1000, 1200 and 1500 mg/L. Effluent COD was determined daily. Effluent COD and COD removal rate of different types of inoculation sludge are shown in Fig. 2.

In Fig. 2, biological membranes from different types of inoculation sludge began to adapt to increasing of COD; and microorganisms with ability to degrade the target pollutants enriched. Thus, COD removal rate followed an increasing trend. When the influent COD was 400 mg/L, the COD removal rates of sewage and chemical sludge were 59.3% (Fig. 2(a)) and 72.4% (Fig. 2(b)), respectively. These results showed that microorganisms in chemical sludge can rapidly adapt to wastewater and exhibited a higher pollutant degradation rate of epoxy propane, glycerol and aniline (Sridang et al. 2004, Ferro et al. 2013). As seen in Fig. 2(a), the COD removal rate of sewage sludge decreased from 96.3% to 86.03% as the influent COD increased from 1000 mg/L to 1200 mg/L. After that the removal rate reverted to the original level and then continuously increased. However, the COD removal rate of chemical sludge exhibited a steady increase without large fluctuations. When the influent COD increased to the highest value of 1500 mg/L, the effluent COD of sewage sludge and chemical sludge reached 47-120 mg/L and 60-85 mg/L, respectively. Furthermore, the COD removal reached 92.8%-96.8% and 94.0%-96.2% for sewage sludge and chemical sludge, respectively. These results indicated that microorganisms of sewage sludge and chemical sludge adapted to wastewater and exhibited a high degradation rate of pollutants during the first stage of acclimation. However, acclimation time differed between sewage sludge and chemical sludge. Chemical sludge completed the first stage of acclimation after 22 d, and sewage sludge required 31 d.

During the second stage of acclimation, the COD of prepared solution was maintained at 1500 mg/L, and the salt concentration was gradually increased to 2, 4, 6, 8, 10, 12, 14 and 16 g/L. Effluent COD was determined daily. Effluent COD and COD removal rate of different types of inoculation sludge are shown in Fig. 3.

As shown in Fig. 3, the reactor sustained a stable operation, and the COD removal rate was maintained at more than 90% when the NaCl concentrations in sewage sludge and chemical sludge systems were 2-6 g/L and 2-8 g/L, respectively. When NaCl concentration was higher than the above range, microorganisms in the biological membranes adapted to the environment condition to maintain osmotic balance and protect internal proteins from damage (Uygur & Kargi 2004). This characteristic enabled microorganisms to maintain normal metabolism. When the NaCl concentration was 10 g/L, the COD removal rates of sewage and chemical sludge presented a minimal decrease, but were quickly restored to the initial level. After the NaCl concentration was increased to 12 g/L, the COD removal rate of sewage sludge suddenly decreased to 68% and that of chemical sludge was maintained at approximately 94%. However, when the increase in NaCl concentration continued, the COD removal rate of chemical sludge began to significantly decrease. The exorbitant salinity increased the osmotic pressure of microbial cells, resulting in cell wall separation and reduced dehydrogenase activity (Ferro et al. 2013). In addition, the increase in salinity strengthened the lysis of cells and the release of cellular components. This process continued and resulted in a relatively low degradation effect.

The COD removal rate of different types of inoculation sludge under high NaCl concentration gradually increased with prolonged domestication time. This result showed that



(a)

(b)

Fig. 3: COD removal rate by the reactor with different types of inoculation sludge during the second acclimation phase (a-sewage sludge and b- chemical sludge).

microorganisms are salt-tolerant. In the reactor inoculated with sewage sludge, the effluent COD was 220-269 mg/L, the COD removal rate was 81.6%-85.3% and the reactor was stable under the conditions of 1500 mg/L influent COD and 14 g/L NaCl. When NaCl content continued to increase, the COD removal rate significantly decreased and a considerable amount of biological membrane in the O tank was desquamated. The reactor was still in abnormal situation after days of continuous feeding. By contrast, in the reactor inoculated with chemical sludge, the effluent COD was 287-300 mg/L, the COD removal rate was approximately 80% and the reactor was stable under the conditions of 1500 mg/L influent COD and 16 g/L NaCl.

Overall, the biological membrane of chemical sludge was adjusted better to salt shock and its salinity gradient acclimation required shorter time than that of sewage sludge. The second stage of acclimation of sewage sludge required 35 d, the NaCl concentration limit was 14 g/L. By contrast, chemical sludge acclimation required 32 d and the NaCl concentration limit was 16 g/L.

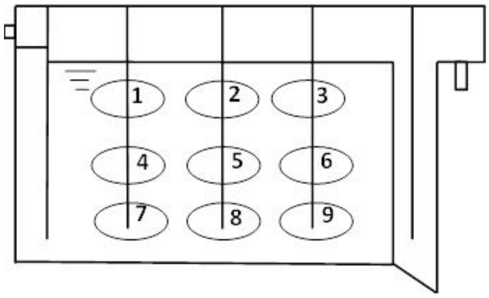


Fig. 4: Schematic diagram of sampling points in the contact oxidation tank.

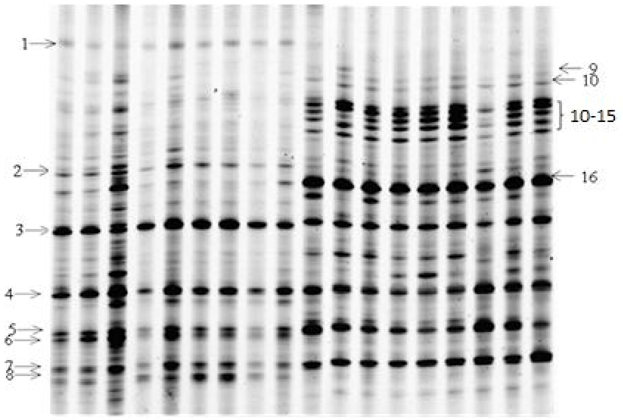


Fig. 5: DNA profiles analysis of DGGE electrophoresis (From left to right: C1-C9, D1-D9).

**Microbial community structure from different inoculants:** Biological samples for DGGE electrophoresis analysis were collected from different locations in the contact oxidation tank, and the sampling points were set as shown in Fig. 4. The samples from the chemical and sewage sludge biological membranes were marked as C1- C9 and D1-D9, respectively. The electrophoresis was conducted at 100 V for 600 min. After electrophoresis, the samples were dyed using SYBR Green I and the results of DNA fingerprint through DGGE electrophoresis are shown in Fig. 5.

As shown in Fig. 5, the microbial community structure differed between the two inoculation sludge. The microbial community structures varied in different sampling points. For example, in the lanes of samples D1-D9, bands 9 to 16 were evident. By contrast, the bands 1, 2, 6 and 8 were pronounced in the lanes of samples C1-C9. However, bands 3, 4, 5 and 7 were all pronounced in the lanes of samples D1-D9 and C1-C9. These results indicated that microorganisms represented by bands 3, 4, 5 and 7 were dominant in the biological membrane of both sewage and chemical sludge (Baloch et al. 2008, Persson et al. 2014).

Table 2: Diversity characteristics of different samples.

| **Samples** | **C1** | **C2** | **C3** | **C4** | **C5** | **C6** | **C7** | **C8** | **C9** | **Average value** |
|---|---|---|---|---|---|---|---|---|---|---|
| S | 37 | 39 | 47 | 41 | 42 | 39 | 32 | 38 | 40 | 39 |
| H | 3.25 | 3.21 | 3.66 | 3.09 | 3.47 | 3.16 | 2.94 | 3.11 | 3.42 | 3.26 |
| E | 0.90 | 0.88 | 0.95 | 0.83 | 0.92 | 0.86 | 0.85 | 0.88 | 0.87 | 0.88 |
| **Samples** | **D1** | **D2** | **D3** | **D4** | **D5** | **D6** | **D7** | **D8** | **D9** | **Average value** |
| S | 41 | 38 | 42 | 42 | 38 | 42 | 37 | 42 | 39 | 40 |
| H | 3.32 | 3.32 | 3.51 | 3.29 | 3.31 | 3.36 | 3.25 | 3.39 | 3.30 | 3.34 |
| E | 0.89 | 0.91 | 0.91 | 0.88 | 0.91 | 0.90 | 0.89 | 0.92 | 0.91 | 0.90 |

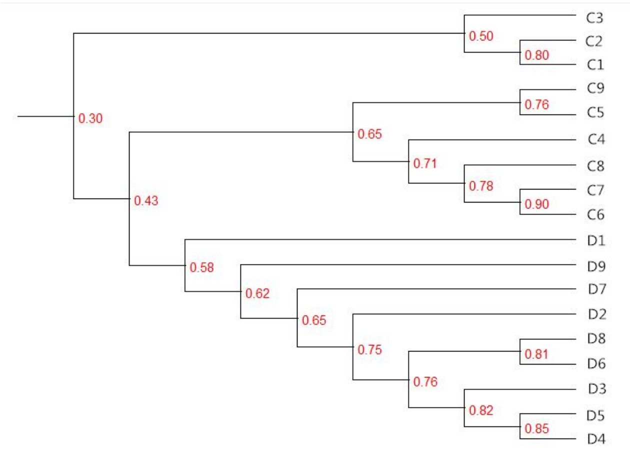


Fig. 6: UPGMA cluster analysis of DGGE profile.

**Microbial diversity analysis:** The microbial diversity characteristics of the biological membrane samples from different types of inoculation sludge were determined using the Shannon diversity index (H), Pielou evenness index (E) and Patrick index (S). Patrick index refers to the total number of all species in the sample. Shannon diversity index can be calculated based on the position and band intensity of the DGGE band. Band intensity is expressed by the peak area of the band. Shannon diversity index can be calculated as:

$$H = -\sum_{i=1}^{S} P_i \ln P_i \qquad ...(1)$$

$$P_i = n_i / N \qquad ...(2)$$

Where, $P_i$ is the percentage of colony number of the *i* species in all colonies, $n_i$ is the peak area of the *i* species and N is the total area of all peaks. E indicates the degree of uniformity of strain distribution; the formula is as follows:

$$E = -\sum P_i \ln P_i / \ln S \qquad ...(3)$$

Where, lnS is the maximum potential diversity of the sample.

The diversity characteristics of different samples are given in Table 2. Regardless of the type of inoculation sludge, the S and H of sample No. 7 were the lowest, which were 32 (C7), 37(D7), 2.94 (C7) and 3.25(D7), respectively. By contrast, the S and H of sample No. 3 were the highest, which were 47(C3), 42(D3), 3.66(C3) and 3.51(D3), respectively. In the contact oxidation tank, microorganisms in sample No. 7 became in contact with wastewater earlier than those in sample No. 3. Thus, wastewater contained a high concentration of pollutants with complex organic structure, which are toxic to microorganisms. Some microbial species that cannot adapt to pollutants were eliminated, resulting in low abundance and diversity.

Overall, the microorganism population in sewage sludge was slightly higher than that of chemical sludge. For example, the average H of sewage sludge was 3.34, which was higher than that of chemical sludge (3.26). Moreover, the microbial distribution of sewage sludge was more uniform and E was relatively stable within the range of 0.88-0.92. The E of chemical sludge was between 0.83 and 0.95.

Cluster analysis of different samples was conducted using unweighted pair-group method with arithmetic means (UPGMA). As shown in Fig. 6, 18 samples can be divided into three categories. The first category included C1-C3, the second category included C4-C9 and the third category included D1-D9. The degree of similarity among microorganisms from sewage sludge was high, and nine samples were divided into one class. The samples from chemical sludge were divided into two classes. These results showed that the community structure of chemical sludge can be easily affected by the environment.

## CONCLUSION

1. Two stages of acclimation of chemical and sewage seeding sludge were contrastively analysed. The acclimation of chemical and sewage seeding sludge required 22 d and 31 d, respectively, to degraded gradation-resistant organic matter with a COD of 1500 mg/L. Moreover, the

COD removal rate was maintained at 94.0%-96.2% and 92.8%-96.8% for chemical and sewage seeding sludge, respectively. After 35 d salt-tolerance domestication, the system seeded with sewage sludge can effectively degrade pollutants from wastewater containing 14 g/L NaCl. By contrast, chemical sludge only required 32 d and tolerated 16 g/L NaCl.

2. The microbial community structure differed between chemical and sewage seeding sludge. Sewage sludge presented higher average H (3.34) than chemical sludge (3.26). Moreover, the microbial distribution of sewage sludge is more uniform. Cluster analysis showed that 18 samples can be divided into three categories. The first category included C1-C3, the second category included C4-C9 and the third category included D1-D9.

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