Effect of continuously dosing Cu(II) on pollutant removal and soluble microbial products in a sequencing batch reactor

YangWei Yan, YuWen Wang, Yan Liu, Xiang Liu, ChenChao Yao and LuMing Ma

ABSTRACT

The effects of synthetic wastewater that contained 20 mg/L Cu(II) on the removal of organic pollutants in a sequencing batch reactor were investigated. Results of continuous 20 mg/L Cu(II) exposure for 120 days demonstrated that the chemical oxygen demand (COD) removal efficiency decreased to 42% initially, followed by a subsequent gradual recovery, which peaked at 78% by day 97. Effluent volatile fatty acid (VFA) concentration contributed 67 to 89% of the influent COD in the experimental reactor, which indicated that the degradation of the organic substances ceased at the VFA production step. Meanwhile, the varieties of soluble microbial products (SMP) content and main components (protein, polysaccharide, and DNA) were discussed to reveal the response of activated sludge to the toxicity of 20 mg/L Cu(II). The determination of Cu(II) concentrations in extracellular polymeric substances (EPS) and SMP throughout the experiment indicated an inverse relationship between extracellular Cu(II) concentration and COD removal efficiency.

INTRODUCTION

The content of heavy metals in industrial wastewaters (such as those from the electroplating, metal finishing, automobile, paint, and petroleum industries) has increased significantly over the years. The combination of industrial and municipal wastewater treatment threatens the performance of biotreatment systems in sewage plants.

Cu(II), one of the most common heavy metals, has severe toxic effects on microorganisms. The effect of Cu(II) on the pollutant removal ability of activated sludge has been investigated by using chemical oxygen demand (COD) and ammonia as indicators (Nicolau et al. 2005; Wang et al. 2010). Previous studies have considered only short-term inhibitory responses, indicating that shock loads of high-level Cu(II) (40 and 80 mg/L) led to complete failure of biological processes on batch results (Wong 1999). Other papers have focused on Cu(II) inhibition on continuous reactors (less than 50 days) after a single dose (Hu et al. 2004). However, the effects of continuous exposure to Cu(II) on activated sludge remain unclear. A few studies have provided insights into the changes of the degradation reaction of organic substances under the inhibition of Cu(II), but they have not explicitly demonstrated which step in the biochemical reaction is responsible for the incomplete degradation of organic substances. The inhibition of Cu(II) on ammonia removal ability and nitrification has rarely been considered in relation to N conversion.

Soluble microbial products (SMP) are generally defined as products that are released into solution from substrate metabolism and biomass decay by hydrolysis and solubilization of extracellular polymeric substances (EPS) or as a response to a stress condition (Barker & Stuckey 1999). EPS are products that result from active secretions, cellular lysis, and adsorption of organics from the environment; these products are also related to the physicochemical characteristics of activated sludge (Wingender et al. 1999). Protein, polysaccharide, humic substances, and DNA are the four main biochemical constituents of SMP and EPS (Pérez Silva et al. 2009). Previous studies have shown the adsorption ability of SMP and EPS for heavy metals (Comte et al. 2006; Yin et al. 2013). Reported study results related to SMP have also focused on the formation of SMP.
under different conditions, such as temperature changes, osmotic shocks, and nutrient deficiency (Aquino & Stuckey 2004). However, the effect of heavy metals on SMP production and the relationship between SMP and COD have rarely been considered. Furthermore, reports on the distribution of heavy metals in EPS and SMP are limited.

This study investigated the effects of continuous exposure to Cu(II) on pollutant removal ability and SMP production in a sequencing batch reactor (SBR). The objectives were to determine the vital step in the degradation process of organic substances by monitoring the production of intermediate products (e.g., volatile fatty acids (VFA)) and SMP, as well as to analyze N conversion based on measurements of nitrite and nitrate. Finally, the relationship between Cu(II) distribution and inhibition effects on an activated sludge system was examined by determining the Cu(II) concentrations in EPS and SMP.

MATERIALS AND METHODS

Experimental set-up

Two parallel SBRs with an overall volume of 5.6 L and a working volume of 4 L under the same operating conditions were used in this study. One SBR was set as an experimental reactor (ER) dosed with 20 mg/L Cu(II) by adding CuSO₄·5H₂O, and the other SBR was set as a control reactor (CR) without Cu(II) exposure. The SBRs were operated in a 12-h cycle. Each cycle consisted of five stages, namely, fill (0.5 hour), aeration (6 hours), settle (2 hours), decant (0.5 hours), and idle (3 hours). At the decant stage, 2 L of fresh synthetic wastewater was fed to replace the supernatant of the same volume.

Activated sludge was collected from a backflow tank in a wastewater treatment plant in Shanghai. The two reactors were operated for more than 40 days on synthetic wastewater devoid of Cu(II) to obtain a dense culture of acclimatized biomass. During the 160 days of the experiment, the SBRs were run at 20 ºC to 30 ºC with 2.0–2.5 mg/L dissolved oxygen concentration at the aeration stage. The pH ranged from 6.5 to 8.0, sludge retention time was 20 days, and mixed liquor suspended solids (MLSS) were maintained at 2,000–8,000 mg/L. After the steady state, 20 mg/L Cu(II) was dosed continuously into the influent of the ER from day 42.

Water composition

Synthetic wastewater, which contained C₆H₅COONa as C source, NH₄Cl as N source, and KH₂PO₄ as P source with a COD/N/P ratio of 100:20:1, was used as the influent. Mineral nutrients (Ca, Mg, Fe, Co, Mn, Zn, and others) were supplied. pH ranged from 6.5 to 8.0 and was adjusted by NaHCO₃ solution. The COD and ammonia concentrations in the influent were maintained at approximately 800 and 40 mg/L, respectively, throughout the experiment (Beardsley & Coffey 1985; Li et al. 2011a).

Analysis methods

During the experiment, COD and ammonia were analyzed to indicate the organic pollutant and ammonia removal ability of the activated sludge. The concentrations of nitrite and nitrate were monitored to determine the nitrification inhibition and the conversion of N. All analyses and the measurement of MLSS were conducted according to Standard Methods (APHA 1995).

The mixed liquor samples of activated sludge (10 mL) were collected and filtered through a 0.22 μm filter membrane to obtain SMP, and EPS was extracted according to the procedure used by Li et al. (2011a). To extract EPS, the mixed liquor samples (5 mL) were dewatered at 1,063 × g for 10 minutes and the supernatant filtered through a 0.22 μm filter membrane was considered to be the low speed centrifugation product (LSCP). After being re-suspended in 0.85% sodium chloride solution, filtered samples were re-centrifuged at 9,564 × g for 20 minutes and the supernatant was filtered through a 0.22 μm membrane to ensure the soluble EPS (EPSₗ) was separated from the biomass. The bound EPS (EPSₘ) was extracted by heating, where the sludge pellets after EPSₗ extraction were heated at 80 °C for 30 minutes and then centrifuged at 9,564 × g for 20 minutes. The supernatant filtered through a 0.22 μm membrane was considered as EPSₘ. Loosely bound EPS (LB-EPS) was defined as the sum of LSCP and EPSₗ, while tightly bound EPS (TB-EPS) was EPSₘ. Total EPS (EPSₗ) includes LB-EPS and TB-EPS. The chemical components of SMP (carbohydrates, protein, humic contents and DNA) were analyzed by the methods of Li et al. (2011a).

Cu(II) concentrations in the extracted SMP and EPS were measured by flame atomic absorption spectrometry (Hitachi Z-5000, Japan) at 324.8 nm wavelength.

Supernatant samples were collected at the start and end of the aeration cycle to analyze VFA using a gas chromatograph (Agilent 6890N, USA) equipped with a 2 m × 2 mm (inner diameter) glass column, which was packed with Porapak GDX-105 (80–100 mesh) (Ren et al. 2005). All of the measured values of VFA as mg/L were converted into
COD values as mg/L according to suitable oxidation equations (Aquino & Stuckey 2004).

\[
VFA \text{ (as COD)} = 0.35 \text{ (formate)} + 1.07 \text{ (acetate)} + 1.51 \text{ (propionate)} + 1.82 \text{ (butyrate + isobutyrate)} + 2.04 \text{ (valerate + isovalerate)}.
\]

RESULTS AND DISCUSSION

Effect of Cu(II) on organic pollutant removal ability

Figure 1 shows that before ER was exposed to 20 mg/L Cu(II), the COD values in the influent for CR and ER were 760 mg/L on average, with approximately 95% COD removal efficiency. On day 42, 20 mg/L Cu(II) was continuously dosed into the influent of ER. The changes of the COD removal ability in ER underwent three stages. Stage I: a sharp decrease from 96 to 18% and a fluctuation with a range of 2–16% (days 43–80). Stage II: recovery to the maximum of 78% (days 81–97). Stage III: decline to nearly 0% (days 98–160). After Cu(II) addition, the effluent COD in ER increased by up to 457 mg/L. During the subsequent 37 days, the effluent COD in ER maintained a relatively high level from 524 to 768 mg/L. The effluent COD removal efficiency decreased to the minimum (2%) on day 56. The effluent COD removal efficiency increased from 15% (on day 80) to 57% on day 89. In the following 17 days, the COD removal efficiency of ER tended to recover with a continuous increase up to 78%. However, after 97 days of the experiment, the removal efficiency decreased to nearly 0% by day 160. The recovery in Stage II might indicate a short-term adaptation of the microorganisms to Cu(II), but long-term, continuous dosing absolutely inhibited sludge activity and resulted in a decline of COD removal efficiency in stage III (Li et al. 2011a). Cu(II) accumulated in EPSt (see Table 1) eventually and the Cu(II) toxicity led to loss of cell activity. The organic pollutant removal ability of ER was completely inhibited. One probable reason for Cu(II) inhibition was that when Cu(II) was transported into the cells, its ionic form transformed into a metal complex by reacting with intracellular materials, such as protein and nucleic acid. Heavy metals blocked the enzyme systems or interfered with some essential cellular metabolites of bacteria and protozoa (Madoni et al. 1996); thus, microbial cells were inactive and unable to survive. Li et al. (2011a) found that the organic pollutant removal ability of activated

![Figure 1](https://iwaponline.com/wst/article-pdf/72/9/1653/466079/wst072091653.pdf)

**Table 1** Copper concentration in different parts of EPS and SMP in ER (mg/L)

<table>
<thead>
<tr>
<th>Time/d</th>
<th>7</th>
<th>55</th>
<th>61</th>
<th>77</th>
<th>104</th>
<th>129</th>
<th>134</th>
<th>143</th>
<th>161</th>
</tr>
</thead>
<tbody>
<tr>
<td>LB-EPS</td>
<td>0.00</td>
<td>1.12</td>
<td>0.80</td>
<td>4.08</td>
<td>0.16</td>
<td>1.84</td>
<td>6.12</td>
<td>7.63</td>
<td>10.4</td>
</tr>
<tr>
<td>TB-EPS</td>
<td>0.08</td>
<td>1.12</td>
<td>1.05</td>
<td>0.60</td>
<td>0.09</td>
<td>0.89</td>
<td>1.50</td>
<td>0.30</td>
<td>0.46</td>
</tr>
<tr>
<td>EPSt</td>
<td>0.08</td>
<td>2.24</td>
<td>1.85</td>
<td>4.68</td>
<td>0.25</td>
<td>2.73</td>
<td>7.62</td>
<td>7.93</td>
<td>10.9</td>
</tr>
<tr>
<td>SMP</td>
<td>0.00</td>
<td>1.39</td>
<td>0.98</td>
<td>0.61</td>
<td>0.18</td>
<td>0.96</td>
<td>7.66</td>
<td>4.18</td>
<td>7.57</td>
</tr>
</tbody>
</table>
sludge in SBR recovered under continuous exposure to 120 mg/L Ni(II) because of the existence of Ni-resistant species. Han et al. (2013) reported similar results, i.e., activated sludge could endure 400 mg/L Zn(II) based on a continuous dosing experiment. However, they showed that although temporary recovery was likely to occur to a certain extent without any special measures, the organic pollutant removal ability of ER was inhibited heavily by 20 mg/L Cu(II) and unable to recover eventually. Therefore, Cu(II) was more toxic than Ni(II) and Zn(II) on the pollutant removal ability of activated sludge.

**Effect of Cu(II) on ammonia removal and nitrification in SBR**

Figure 2 illustrates a constant ammonia removal with less than 8 mg-N/L effluent ammonia and over 80% ammonia removal efficiency in CR and ER before Cu(II) addition. During the entire experiment, the ammonia removal ability of CR remained steady with the effluent ammonia below 10 mg-N/L. In the 4 days after the addition of 20 mg/L Cu(II), the effluent ammonia continued increasing to 39.9 mg-N/L, whereas ammonia removal efficiency kept declining. The effluent ammonia concentration in ER fluctuated in the range of 21.7–49.5 mg-N/L with 45 to 80% removal efficiencies until day 129. The effluent ammonia remained at 32–40 mg-N/L, and the removal efficiency remained low at 4.5 to 8% thereafter. The ammonia removal ability of ER was unable to recover during the 160-day experimental period. Thus, continuous exposure to 20 mg/L Cu(II) had a remarkable inhibitory effect on the ammonia removal ability of activated sludge. The results were totally different from that of the COD removal ability, in which recovery occurred, thereby indicating that 20 mg/L Cu(II) had a stronger inhibitory effect on the ammonia removal ability than the organic pollutant removal ability of activated sludge. Hu et al. (2004) reported that heavy metals always block active sites of the enzymes responsible for the conversion of ammonia to nitrite or nitrate.

From day 45 to 119, the detected concentration of ammonia in the effluent was greater than that in the influent. This phenomenon probably resulted from the high amount of intracellular material excreted by broken cells that resulted from the mass death of microorganisms caused by Cu(II) toxicity (Madoni et al. 1996).

Figure 3 shows that before Cu(II) exposure, the nitrite concentration in the effluent increased by 1–3 mg-N/L in one cycle, with nitrate concentration increasing by 2–5 mg-N/L. The contribution of nitrification of the influent ammonia was 53.2%. It indicated that ammonia was effectively converted to nitrite and nitrate; thus, nitrification was effective in the activated sludge system.

After the addition of Cu(II), nitrification in ER was seriously inhibited with a drastic reduction of nitrite and nitrate concentrations. After day 45, no nitrate formation was observed in the effluent. After day 59, the nitrite was also below the detectable limit. Nitrification is generally considered the vital step in biological N removal because of the slow growth rate and environmental sensitivity of nitrifying bacteria (Grunditz & Dalhammar 2001). The biological removal of N in an activated sludge system involved the conversion of ammonia to nitrite and nitrate by nitrifying autotrophic bacteria and the conversion of nitrite/nitrate to gaseous N compounds by denitrifying microorganisms. The conversion of ammonia to nitrite could have been retarded because both nitrite bacteria and nitrobacteria activities were inhibited. Nitrite
accumulation can be observed despite the absence of nitrate accumulation in the first several days of Cu(II) addition, which reflected that nitrobacteria were more susceptible to Cu(II) toxicity than nitrite bacteria (Semerci & Çeçen 2013).

**Effect of Cu(II) on organic matters in effluent**

The effluent organic matter in biological reactors comprised not only the influent non-degraded compounds and intermediate products but also SMP that were produced and excreted by microorganisms (Aquino & Stuckey 2007).

Figure 4 illustrates the variation of total SMP in ER. Prior to Cu(II) exposure, the SMP content in ER was 44 mg/L. After Cu(II) was dosed in ER, the SMP concentration varied along with the COD level (Figure 4). An increasing trend in SMP concentration was observed, and the peak SMP production was up to 105 mg/L on day 49. The sudden addition of 20 mg/L Cu(II) to ER may also accelerate the death of some bacteria. SMP may be produced as a result of this process (Barker & Stuckey 1999). SMP monitoring showed that the production from days 77 to 105 decreased to 29, 40, and 27 mg/L, less than the 44 mg/L production before Cu(II) addition. This result may be due to the utilization of SMP as a secondary metabolite by microorganisms (Feng et al. 2008). An increase in SMP accumulation can be observed following the recovery
period (stage III) and reached 83 mg/L on day 134. Such SMP were biomass-associated products released into the medium as a result of stressful conditions and cell death (Mesquita et al. 2010).

Figure 5 illustrates the change of three major constituents in SMP and their correlation with the effluent COD in ER. Before exposure to 20 mg/L Cu(II), the protein, polysaccharide, humic substances, and DNA content in ER were nil, 24, 7, and 15 mg/L, respectively. When Cu(II) had been added continuously for 3 days, the protein and humic substances increased significantly from nil to 9 mg/L and from 7 mg/L to 25 mg/L, respectively. The polysaccharide content increased thereafter and reached its maximum of 58 mg/L on day 49. Protein, humic substances, and polysaccharides have played an important function in the adaptation of microorganisms to the presence of heavy metals by absorption and enzymatic detoxification. The subsequent excretion of these three components may be the result of enzymatic reactions. A remarkable increase in the DNA content to 69 mg/L was observed on day 67, and then dropped to nil by the end of stage I. Because DNA mainly distributes in cellular nucleus, the release of intracellular DNA into the effluent suggested that numerous microorganisms were unable to survive under 20 mg/L Cu(II), and their cells ruptured. According to Li et al. (2011), a general increase in DNA was observed during days 45 to 61 in EPS, which indicated that DNA was initially absorbed by EPS and then released into SMP (day 67). During the recovery period (stage II), the DNA content was below the detectable limit. Therefore, almost no DNA substance was released into the effluent. This phenomenon was probably caused by the existence of Cu-resistant microorganisms that may adapt to the high-Cu(II)-concentration environment (Li et al. 2011a, b).

In stage III, the protein, polysaccharide, humic substance, and DNA contents had the same change patterns as those in stage I, with a substantial increase on days 105, 129, 129, and 134, respectively. This result could be attributed to the death of Cu-resistant microorganisms in response to the long-term 20 mg/L Cu(II) exposure.

### Cu(II) distribution in SBR

The determined Cu(II) concentration in EPS, SMP, and effluent throughout the experiment that reflected the distribution of Cu(II) in microbial products and aqueous phase is summarized in Table 1.

The data for day 7 before Cu(II) addition showed that almost no Cu(II) accumulation was observed in EPS and SMP. As shown in Table 1, the Cu(II) concentration in total EPS increased after dosing with Cu(II) and reached 4.68 mg/L on day 77 when the COD removal efficiency was maintained at a low level (stage I) (see Figure 1). The minimum Cu(II) concentration (0.25 mg/L) in EPS was measured on day 104 after the recovery of the COD removal efficiency occurred (stage II). However, in the following days, poor COD removal was observed. As the Cu(II) concentration in EPS increased, COD removal efficiency decreased accordingly. Cu(II) accumulated again in EPS and eventually achieved the maximum of 10.9 mg/L (stage III).

The probable reason for the decrease of Cu(II) in EPS during the COD removal recovery was that the extracellular Cu(II) was transported through the membrane and turned into intracellular forms or accumulated in the cells of Cu-resistant microorganisms (Li et al. 2011a, b). Thereafter, the significant Cu(II) increase in EPS was possibly caused by
the limited absorptive ability of EPS for Cu(II), which was continuously dosed into the reactor and released during cell lysis (Sheng et al. 2010). Significant Cu(II) accumulation (4.18–7.66 mg/L) was also observed in SMP in the late experimental period as a result of EPS release into the bulk solution and cell lysis in response to the extreme environment with the presence of Cu(II) (Fukushi et al. 2014). Cu(II) also accumulated more in LB-EPS than in TB-EPS because LB-EPS with higher concentration of polysaccharides may have a greater adsorptive ability for Cu(II) than TB-EPS (Comte et al. 2006).

Analysis of organic pollutant degradation

The degradation pathway of C₆H₅COONa was considered to involve the degradation to central metabolites (such as pyruvate), the catabolism to VFA, and the conversion of VFA to inorganic components, including CO₂ (Díaz et al. 2016). In the late experimental period (stage III) (see Figure 1), the concentrations of VFA were determined in CR and ER for further discussion. Table 2 summarizes the initial and final values of VFA from each cycle on days 129, 134, and 143.

As shown in Table 2, the VFA detected in the effluent of the two reactors were composed of propionate, butyrate, isobutyrate, valerate, and isovalerate. The initial and final VFA values of CR were maintained at 10–15 mg-COD/L. No VFA accumulation was observed in CR in the aeration cycle, which indicated that the degradation of organic substances in CR was completed. However, the total VFA values of ER were more than 300 mg-COD/L, except that on day 129, with an initial VFA value of 360 mg-COD/L. The VFA increased by 93–249 mg-COD/L in the operation cycle in ER because of the incomplete degradation of organic substrates. Comparison of the initial VFA values of ER in these 3 days revealed that the VFA accumulated during the long-term experiment. Table 3 presents the percentage contribution of VFA to COD and the conversion of organic substrates in SBR. The final COD components mainly consisted of SMP, VFA, undegraded benzoate, and other intermediate products. Most of the benzoate was degraded in CR with its relatively low final VFA concentration of no more than 15 mg-COD/L, which accounted for less than 41% of the effluent COD. By contrast, the final VFA concentration of ER was obviously higher than that in CR with a considerable proportion of the effluent COD (more than 70%). This result indicated that the degradation of most C₆H₅COONa in ER was interrupted at the biodegradation of VFA after one operation cycle.

Figures 4 and 5 show that the variation of total SMP, polysaccharides, and humic substances correlated well with that of VFA. Meanwhile, Cu(II) accumulated in the SMP and peaked at 7.66 mg/L on day 134 (Table 1). Most of the benzoate could be degraded into intermediate products of VFA in the presence of Cu(II), but the decomposition of VFA was inhibited. The accumulation of Cu(II) blocked the conversion of VFA to inorganic substances in biological organic degradation. Kuo & Genthner (1996) reported that benzoate biodegradation was sensitive to additional Cu(II). Thus, differences in metal sensitivity between benzoate-degrading species and VFA-degrading species may lead to the interruption of organic degradation.

CONCLUSIONS

1. The continuous exposure to 20 mg/L Cu(II) resulted in an inhibitory effect on the COD and ammonia removal.

<table>
<thead>
<tr>
<th>Time</th>
<th>Propionate</th>
<th>Butyrate</th>
<th>Isobutyrate</th>
<th>Valerate</th>
<th>Isovalerate</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 129</td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>CR</td>
<td>1.56</td>
<td>0.00</td>
<td>36.6</td>
<td>30.4</td>
<td>3.27</td>
<td>0.00</td>
</tr>
<tr>
<td>ER</td>
<td>2.43</td>
<td>3.19</td>
<td>34.7</td>
<td>0.00</td>
<td>5.40</td>
<td>3.38</td>
</tr>
<tr>
<td>Day 134</td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>CR</td>
<td>3.44</td>
<td>5.57</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>ER</td>
<td>2.70</td>
<td>3.12</td>
<td>67.1</td>
<td>377</td>
<td>2.84</td>
<td>5.22</td>
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<tr>
<td>Day 143</td>
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<td>Final</td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>CR</td>
<td>0.00</td>
<td>0.00</td>
<td>46.6</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>ER</td>
<td>0.00</td>
<td>0.00</td>
<td>46.6</td>
<td>0.00</td>
<td>3.27</td>
<td>5.48</td>
</tr>
</tbody>
</table>

The initial value was the VFA concentration in the reactor at the start of the aeration cycle; the final value represented the VFA concentration in the effluent at the end of the aeration cycle.
3. Cu(II) accumulated in EPS and SMP eventually. As the Cu(II) concentration in EPS increased, COD removal recovery was undetectable during recovery. The DNA content was undetectable during the COD removal recovery. The DNA content was undetectable during the COD removal recovery. The DNA content was undetectable during the COD removal recovery. The DNA content was undetectable during the COD removal recovery. The DNA content was undetectable during the COD removal recovery. The DNA content was undetectable during the COD removal recovery.

4. The degradation of organic substances ceased at the conversion of VFA to inorganic substances.

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REFERENCES


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