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# Biobleaching of High Lignin Content Kraft Pulps Via Laccase-Mediator Systems

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# BIOBLEACHING OF HIGH LIGNIN CONTENT KRAFT PULPS VIA LACCASE-MEDIATOR SYSTEMS

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## ABSTRACT

The response of a commercial softwood kraft pulp with an initial kappa no. of 97.5 to laccase/N-hydroxybenzotriazole (HBT) treatments was investigated. A series of 84 enzymatic treatments were performed to determine how the bio-delignification of high kappa pulps is influenced by reaction time, dose of laccase, dose of HBT, and pulp extractives. According to the analysis of variance, time, dose of mediator, dose of laccase, and extractives, all had a statistical effect on delignification at a 95% confidence level. Also, the interaction between the variable dose of laccase and extractives, as well as that between the variable dose of mediator and dose of laccase had a statistical effect on delignification. The results from this preliminary study revealed that a laccase-HBT system can effectively delignify high kappa pulps. A drop in kappa no. greater than 16 points was observed in some treatments.

## INTRODUCTION

The production of chemical pulps has been dramatically altered over the past decade in response to new environmental regulations and consumer activism. Although current pulp manufacturing technologies address required environmental performance regulations, new challenges and opportunities are developing. The need for improved manufacturing efficiencies, enhanced wood utilization practices, and continuing environmental concerns has become one of the central research themes of the late 1990's. Recently, significant interest has developed in the production of bleached kraft pulp originating from high lignin content pulps. The primary factor contributing to this research is the well-known loss of pulping selectivity when attempting to remove the last vestiges of lignin in pulps by kraft delignification. Several recent publications have examined the improved yield benefits of utilizing a single or double oxygen stage to delignify high lignin content pulps (1, 2). Bokstrom and Norden (3) have shown that overall yield improvements of 2% can be achieved by stopping a kraft cook at kappa number of 57 instead of the typical value of 27. The high kappa pulp was then further delignified using a two-stage oxygen system followed by a typical D(EOP)$D_N$D sequence.

Unfortunately, chemical consumption and environmental considerations severely limit the types of delignification technologies that can be employed with high-kappa pulps. To date, the two most promising delignification technologies for high lignin content pulps consist of using oxygen delignification or modifying the pulping process (i.e., AQ, polysulfide, AQ/polysulfide). This paper examines a third alternative, the development of a delignification technology for high lignin content pulps.

The use of laccase and a chemical mediator has been shown to be very effective at delignifying typical kraft pulps. By employing a mixture of laccase and N-hydroxybenzotriazole, a 40-60% delignification of hardwood and softwood kraft pulps has been achieved (4, 5). Furthermore, these results are achieved under mild temperatures (40 -55°C) and oxygen pressures (50-140 psig). The mechanism of laccase/mediator delignification is summarized in Figure 1. Studies by Sealey et al. (6), Paice et al. (7), and Poppius-Levlin et al. (8) support this general mechanism.

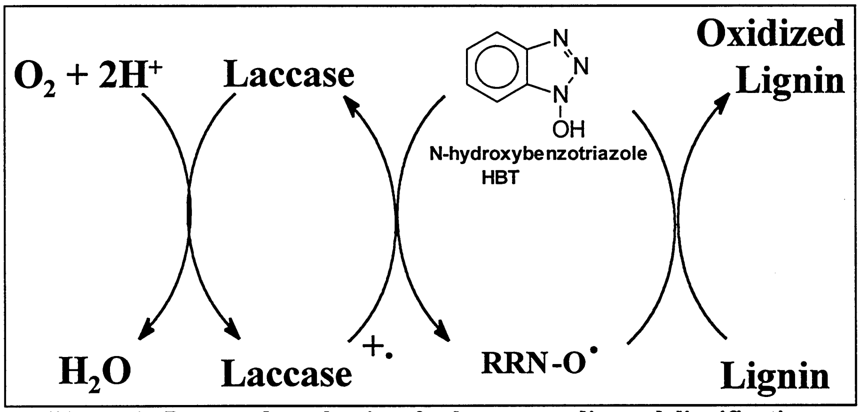


**Figure 1. Proposed mechanism for laccase mediator delignification of kraft pulps.**

In principal, the laccase mediator system is catalytic but recent studies by Bourbonnais et al (9), Sealey and Ragauskas (10), and Potthast (11) have shown that the N-hydroxybenzotriazole is eventually converted to benzotriazole, an inactive agent for the biobleaching stage (see Figure 2).

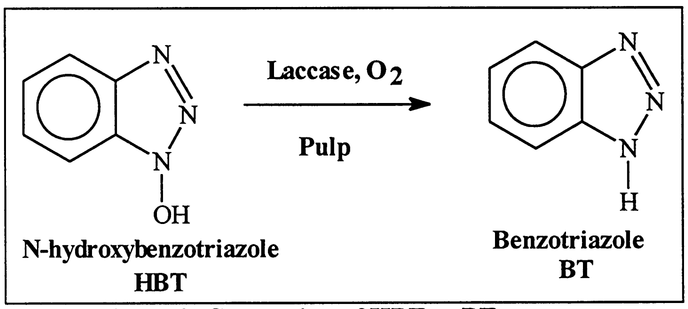


**Figure 2. Conversion of HBT to BT**

Despite these limitations, studies by Call and Muche (12), Sealey et al. (5), and Poppius-Levlin et al. (13) have shown that the laccase/mediator biobleaching system is very selective for lignin removal and appears not to degrade cellulose. Based on these considerations, the use of a laccase/mediator system appeared to be a very attractive technology for the delignification of high kappa kraft pulps.

## EXPERIMENTAL

### Materials

N-hydroxybenzotriazole (HBT), glacial acetic acid, acetone, and 1.0 N NaOH were commercially purchased and used as received. Laccase was isolated from a *Polyporus* fungus and was provided by Novo Nordisk. The enzyme was frozen to -20°C until use. Once thawed, the activity of the enzyme was measured, and the proper dose was added to the pulp.

### Furnish

All laccase-mediator treatments were performed on a commercial softwood Kraft pulp. Prior to the enzymatic treatments, the pulp was washed and screened. A portion of the brownstock (K=97.5) was acetone extracted for 24 hours and thoroughly washed to remove the residual acetone. The acetone extracted pulp (K=95.9) was then used for additional laccase treatments.

**Laccase Activity**

The activity of the laccase was measured by monitoring the rate of oxidation of syringaldazine. One unit of activity was defined as the change in absorbance at 530 nm of 0.001 per minute, per ml of enzyme solution, in a 100 mM phosphate buffer (2.2 ml) and 0.216 mM syringaldazine in methanol (0.3 ml). The test was performed at 23°C. The activity of the enzyme used in this study was 1.87E+06 (U/ml of enzyme solution).

**Laccase-HBT Treatment-General Description**

A 1000 ml capacity Parr Bomb, equipped with a pressure gauge and a stirrer, was charged with 10.0 g of never-dried pulp (solids basis). The pulp consistency was adjusted to 9% by adding distilled water. The slurry was then heated to a temperature of 45°C and was maintained at that temperature for the duration of the enzymatic treatment. The mediator dose was then added to the heated slurry and was allowed to mix for 5 minutes before the pH of the system was adjusted to 4.5 with glacial acetic acid. The proper dose of enzyme was then added and allowed to mix for a minute before the bomb was closed and pressurized with oxygen (145 psig). Subsequent to the laccase treatment, the pulp mixture was filtered, thoroughly washed with distilled water (12L/10g O.D.) and subjected to an alkali extraction. The alkali extraction was performed at 2% NaOH on O.D. pulp for 1 hour at 70°C.

**Pulp Characterization**

The lignin content of the brownstock as well as that of the laccase-treated pulps was expressed as a kappa number and was determined in accordance with Tappi Method T-236 (14). Each reported kappa number in this report represents the average of two individual measurements.

**Experimental Design**

A total of 84 laccase-mediator treatments were performed. The brownstock was subjected to enzymatic treatments at various times, doses of mediator, and enzyme. The experimental protocol for the brownstock is shown in Table 1. The acetone extracted pulp was also subjected to the same experimental schedule as in Table 1. However, the experiments were performed only at 1, 2, and 4 hr intervals (see Table 2).

**Table 1. Experimental Protocol for Brownstock**

| | LACCASE DOSE (U) | | |
|---|---|---|---|
| | **$2.7x10^6$** | **$5.4x10^6$** | **$10.8 x10^6$** |
| **% HBT ON O.D. PULP** | | | |
| **1** | **t= 1,2,4,6 hours** | **t= 1,2,4,6 hours** | **t= 1,2,4,6 hours** |
| **2** | **t= 1,2,4,6 hours** | **t= 1,2,4,6 hours** | **t= 1,2,4,6 hours** |
| **4** | **t= 1,2,4,6 hours** | **t= 1,2,4,6 hours** | **t= 1,2,4,6 hours** |
| **6** | **t= 1,2,4,6 hours** | **t= 1,2,4,6 hours** | **t= 1,2,4,6 hours** |

**Table 2. Experimental Protocol for Acetone Extracted Brownstock**

| LACCASE DOSE (U) | | | |
|---|---|---|---|
| | $2.7x10^6$ | $5.4x10^6$ | $10.8\ x10^6$ |
| % HBT ON O.D. PULP | | | |
| 1 | t= 1,2,4 hours | t= 1,2,4 hours | t= 1,2,4 hours |
| 2 | t= 1,2,4 hours | t= 1,2,4 hours | t= 1,2,4 hours |
| 4 | t= 1,2,4 hours | t= 1,2,4 hours | t= 1,2,4 hours |
| 6 | t= 1,2,4 hours | t= 1,2,4 hours | t= 1,2,4 hours |

## RESULTS AND DISCUSSION

The biobleaching of kraft pulps with laccase/mediator systems continues to receive strong interest, in part due to the discovery of new mediators for laccase. Since Call's discovery of N-hydroxybenzotriazole as a mediator for laccase delignification, several alternative and significantly improved mediators have been found for laccase (15).

The intent of this study was to investigate the response of a high-kappa kraft pulp to a laccase-mediator treatment. The mediator employed for these studies was N-hydroxybenzotriazole (HBT). Although other mediators have been developed for laccase that exhibit improved delignification properties, we selected HBT as the mediator for this study since its fundamental biobleaching chemistry is perhaps the best understood of all the mediators currently available for kraft pulps. This paper describes some of the first successful attempts at delignifying high kappa kraft pulps with a laccase-mediator system.

The laccase/ HBT biobleaching studies were performed on a commercial softwood kraft pulp with an initial kappa of 97.5 to laccase-HBT treatments. A portion of the brownstock was saved and subjected to an acetone extraction prior to enzymatic treatments to free the brownstock from extractives and to determine whether or not extractives had an effect on delignification. The starting kappa no. of the acetone-extracted pulp was 95.9. These two pulps were then used to perform a total of 84 enzymatic treatments at various doses of laccase and HBT as well as at different times (see Tables 1 and 2).

The preliminary data generated from these experiments were subjected to an analysis of variance to determine if the four variables (time, dose of mediator, dose of laccase, and extractives) had a significant effect on delignification. The analysis was also extended to investigate several interaction effects between these variables on delignification. The time interaction with the other variables (i.e., extractives, HBT, laccase) had to be excluded from the analysis due to the unbalanced experimental design. These excluded interactions were added to the residual error. The analysis of variance calculated is shown in Table 3.

**Table 3. ANOVA for delta kappa -Type III Sums of Squares[a]**

| Source | SS | DF | Mean Square | F-ratio | P-value |
|---|---|---|---|---|---|
| **MAIN EFFECTS** | | | | | |
| **A**:Extractives | 19.4 | 1 | 19.4 | 20.0 | **0.0000** |
| **B**:HBT | 519.2 | 3 | 173.1 | 178.2 | **0.0000** |
| **C**:Laccase | 15.5 | 2 | 7.7 | 8.0 | **0.0008** |
| **D**:Time | 16.5 | 3 | 5.5 | 5.6 | **0.0017** |
| | | | | | |
| **INTERACTIONS** | | | | | |
| **AB** | 5.3 | 3 | 1.8 | 1.8 | **0.1555** |
| **AC** | 28.7 | 2 | 14.4 | 14.8 | **0.0000** |
| **BC** | 26.6 | 6 | 4.4 | 4.6 | **0.0007** |
| | | | | | |
| RESIDUAL | 61.2 | 63 | 1.0 | | |
| TOTAL (corrected) | 737.3 | 83 | | | |
| All F-ratios are based on the residual mean square error. | | | | | |

[a] Statgraphics Plus (version 1.4) was used to generate the analysis of variance.

Based on the probability values (P-values) shown in Table 3, the four variables, namely, time, the dose of HBT, the dose of laccase as the well as the extractives, each had a significant effect on delignification at a 95% confidence level. Interestingly, the interaction between the variable extractives and HBT did not have a significant effect on delignification (at 95% confidence level). On the other hand, the interaction between the variable extractives and laccase (AC, see Table 3) as well as the interaction between HBT and laccase (BC, see Table 3) did have a statistically significant effect on delignification. Each of these effects will be discussed next.

**Effect of the Presence of Extractives on Delignification**

The analysis of variance revealed that the presence of extractives had a statistical effect on delignification. Indeed, when considering all the experiments performed in this study, the kappa number fell from 97.5 to 84.5 (Δ kappa=13) for the brownstock, and from 95.9 to 84.0 (Δ=11.9) for the acetone-extracted pulp. Although, there is a statistical significance, change in delignification due to the presence of extractives is minor.

**Effect of HBT Dose on Delignification**

The variable HBT was also shown to have a statistical effect on delignification. Figure 3 depicts the delignification response at the various doses of HBT. As the dose of HBT increases, so does delignification. The drop in kappa number substantially increased as the mediator dose was increased from 1 to 6%. This observation could be attributed to previous studies, which have shown that HBT can be converted to benzotriazole, which is not a delignifying agent (10, 11). Hence, the increase in mediator dose should improve delignification, excluding the enzyme interaction, which is supported by this data.

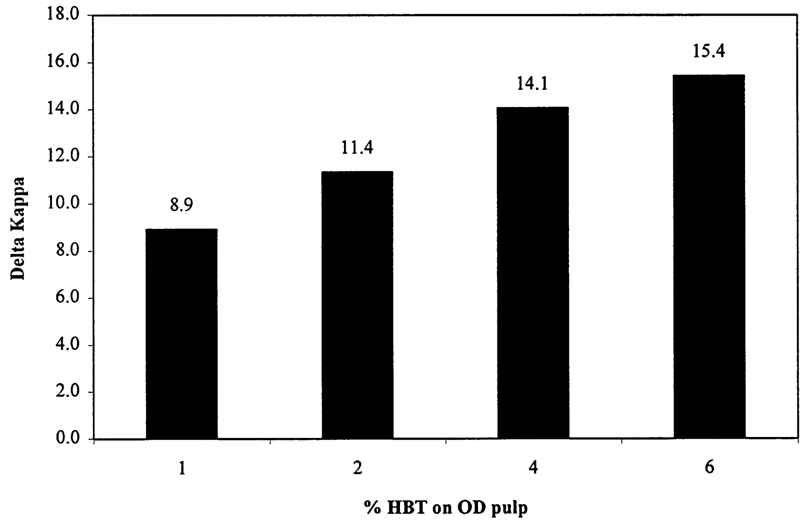


**Figure 3. Delta Kappa vs. % HBT (on OD Pulp) [b]**

[b] The delta kappa values are based on the average of all experiments, which include all doses of laccase, times, and extractives content.

**The Effect of Laccase Dose on Delignification**

The laccase dose was also shown to have a statistical effect on delignification. This observation is graphically depicted in Figure 4. Delignification increased as the enzyme dose was increased from 2.7 x $10^6$ U to 5.4 x $10^6$ U. However, when the laccase dose was further doubled from 5.4 x $10^6$ U to 10.8 x $10^6$ U, delignification seems to level off. This observation suggests that at a laccase dose higher than 5.4 x $10^6$ U, the system is saturated, making any further addition of enzyme ineffective at improving delignification.

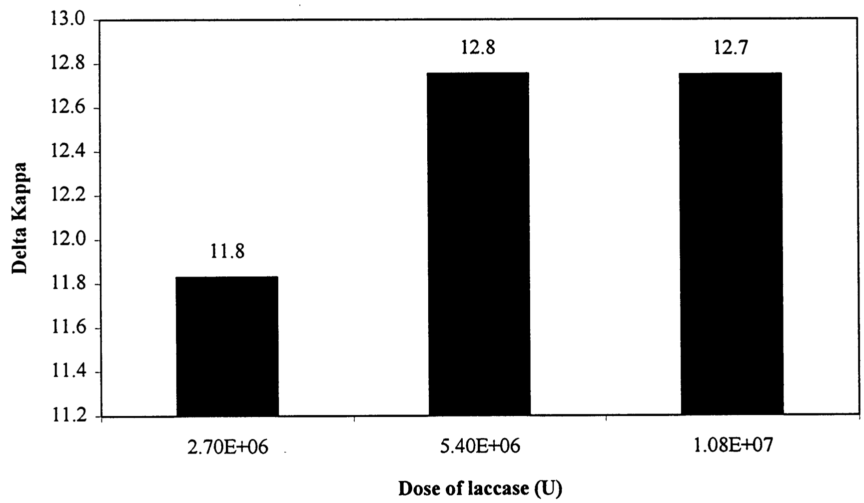


**Figure 4 Delta Kappa vs. Dose of Laccase[c]**

[c] The delta kappa values are based on the average of all experiments, which include all doses of HBT, times, and extractives content.

**The Effect of Reaction Time on Delignification**

Based on the analysis of variance, the variable time also had a statistically significant effect on delignification. The data shown in Figure 5 reveals an increase in delignification as the reaction time is increased from 1 to 6 hr. Although the effect of time on delignification is statistically significant, most of the lignin is removed after a 1 hr treatment, thus highlighting the efficiency of the system. A further increase in reaction time does not yield much higher levels of delignification. In essence, the data seems to suggest that delignification approaches a plateau at reaction times greater than one hour.

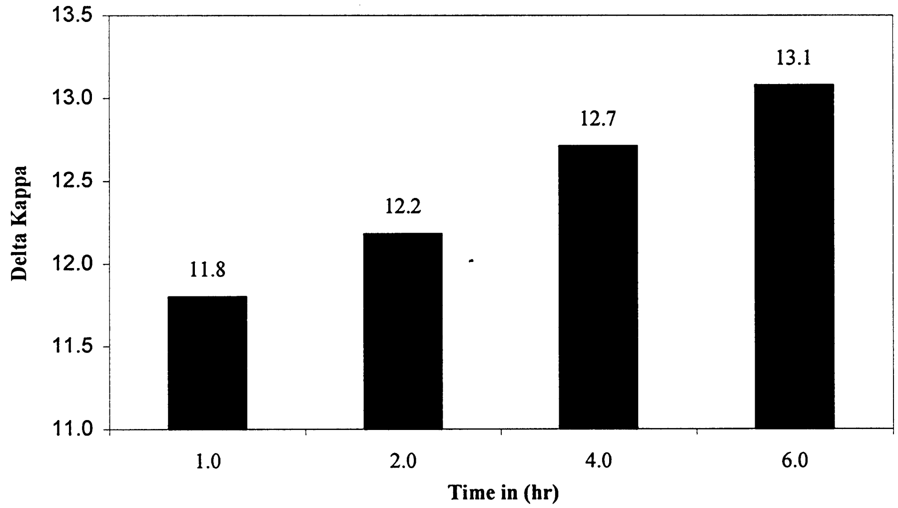


**Figure 5. Delta Kappa vs. Time [d]**

[d] The delta kappa values are based on the average of all experiments, which include all doses of HBT, laccase, and extractives content.

**Interaction Effect of the Extractives Content and Dose of Laccase on Delignification**

The effect of both variables extractives and laccase yielded some very interesting results. An examination of Figure 6 reveals that in the absence of extractives, lower delignification levels were observed when a dose of $5.4 \times 10^6$ U and $10.8 \times 10^6$ U of laccase were employed. At the lower dose of laccase ($2.7 \times 10^6$ U), this effect was not observed. The reasons for this phenomenon remain unclear. Further investigations are underway to address this issue.

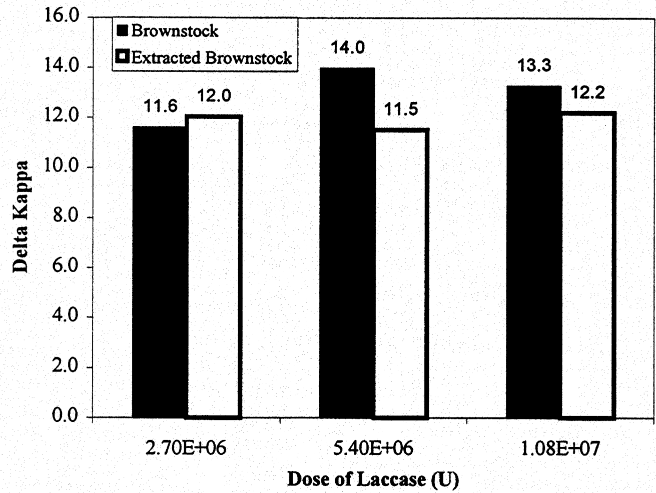


**Figure 6. Effect of Extractives and Dose of Laccase on Delignification** [e]

[e] The delta kappa values are based on the average of all experiments, which include all doses of HBT and all times.

**Interaction Effect of HBT Dose and Laccase Dose on Delignification**

The interaction between the variables HBT and laccase on delignification is shown in Figure 7. Delignification increased as the dose of HBT was increased regardless of the dose of laccase. Although the kappa drop was higher at the 2% dose of HBT than at the 1% dose of HBT, delignification was to a large extent constant at all three levels of laccase. At the 4% and 6% doses of HBT, delignification was more pronounced than at the lower levels of HBT. Interestingly, at these doses of HBT, more laccase was required to achieve a better delignification response. This observation supports some of the earlier findings on the capability of HBT to inhibit the enzyme (15). Hence, as more HBT is added to the system, more laccase is needed to achieve substantial delignification.

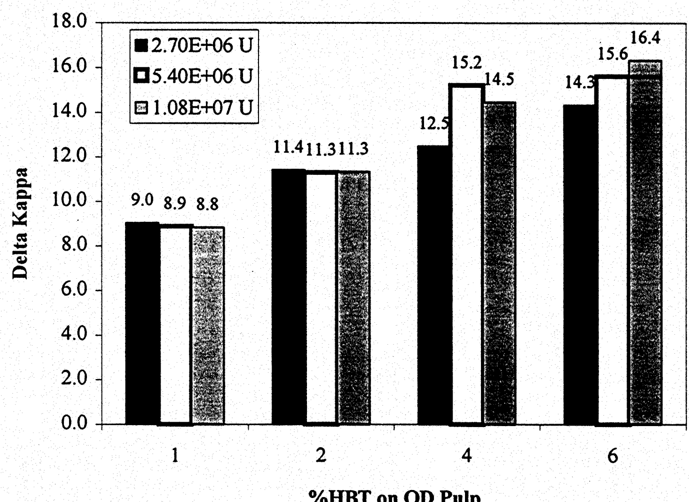


**Figure 7. Effect of Laccase and HBT on Delignification** [f]

[f] The delta kappa values are based on the average of all experiments, which include all reaction times and extractives.

## CONCLUSIONS

Although preliminary in nature, this study is the first of its kind to examine the effects of a laccase-mediator system on high kappa pulps. The data presented in this report demonstrated that a laccase/N-hydroxybenzotriazole treatment could efficiently remove lignin from high kappa pulps. The main variables, time, dose of laccase, dose of HBT, and the extractives content, all had a statistical effect on delignification. An increase in the dose of HBT from 1% to 6% (on O.D. pulp) yielded a substantial increase in delignification ($\Delta$ kappa =8.9 vs. 15.4). When considering reaction times, most of the lignin was removed within the first hour. However, small increases in delignification were seen for each increment in reaction time. A statistical effect on delignification was observed as the dose of laccase was increased from $2.7 \times 10^6$ U to $10.8 \times 10^6$ U. However, the delignification response seemed to level off at a laccase dose of $5.4 \times 10^6$ U. The key interactions between the major variables were between the dose of laccase and extractives as well as between the dose of mediator and the dose of laccase. Further investigations will be necessary to understand the trends seen between laccase and extractives. Furthermore, additional mechanistic studies will be performed to investigate the reactions between laccase/mediator and extractives on high lignin content pulps. Future studies will address these issues and also examine alternative mediators that will exhibit improved stability and delignification properties for high lignin content pulps.

## ACKNOWLEDGMENTS

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