Energy Efficient Catalytic Activation of Hydrogen peroxide for Green Chemical Processes

Final Report

Grant

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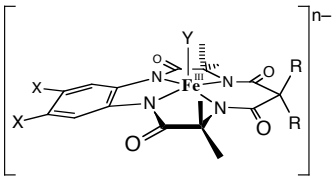
Prepared by Terrence J. Collins (11/10/2004)

Decontamination of chemical and biological warfare agents with TAML/peroxide oxidant system

During the grant period, major developments were achieved in the development of a catalytic oxidation technology based upon our patented TAML catalysts for destroying spores of *B. atrophaeus*. Spores of *B. atrophaeus* are simulants both of the biological warfare and terrorism agent, anthrax, and the difficult-to-kill water borne protozoans that threaten US drinking water supplies, *Cryptosporidium parvum* and *Giardia*. Our stated goal was to achieve the kill rate to 7-log or 99.99999% with TAML/peroxide process in a short time. Achievement of this goal would make the TAML/process potentially useful for decontamination in cases of anthrax attacks and for disinfection of the drinking water supply on a global basis as a more economical and practical alternative to ozone treatment.

Here we report that we achieved this goal in the grant period, a 7-log kill of *Bacillus atrophaeus* spores, which we have achieved unambiguously and in extremely short times. Several innovative approaches lead to this significantly enhanced kill rate. We employed *tert*-butyl hydroperoxide (TBHP) for its hydrophobic character with an especially active TAML activator. The use of TBHP has proven to be very effective in enhancing penetration of the hydrophobic coat of the spores. Additionally, we incorporated a very low concentration (0.03% w/v) of the cationic surfactant, CTAB. It is known that cationic surfactants are effective at killing Gram-positive bacteria by softening and disrupting the spore cortex. This, we assumed, would result in breaches through the spore coat and cortex through which the oxidizing agent could gain access to the spore core. Whatever the underlying mechanism is, our ideas have resulted in achieving a 7-log kill in 1 hr at 0.3M concentration of TBHP and 5μM TAML activator [**1e**]$^-$. At a TBHP concentration of 0.5M, a 7-log kill is achieved in ca. 45 minutes (Fig. 1a). The data pattern suggests that inactivation goes beyond the 7-log value in 1 hr (Fig 1b). However, the estimation of sporicidal activity beyond 7-log is technically difficult with only 10E8 colony forming units of bacterial spores available in one milliliter aqueous suspension. An exchange of the cationic component of the TAML activator, [**1e**]$^-$, from Li+ to CTA+ has resulted in enhanced reactivity; treatment with 5 μM [CTA][**1e**] and TBHP achieved in 7-log kill in around 30 minutes.

Finally, ***a 7-log kill can be achieved in 15 minutes*** using 50 μM [CTA][**1e**] with 0.5 M TBHP, pH 10 (0.1 m Na_2CO_3/0.1 M $NaHCO_3$), 0.03% w/v CTAB.

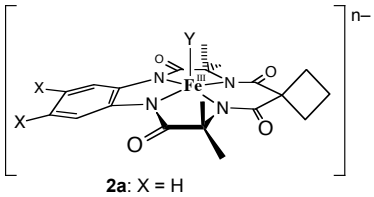


1a: X = H; R = CH_3
1b: X = Cl; R = CH_3
1c: X = OCH_3; R = CH_3

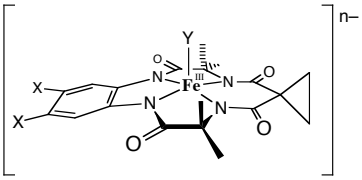
1d: X = H; R = F
1e: X = Cl; R = F
1f: X = F; R = F

1g: X = NO_2, H; R = CH_3
1h: X = CH_3, H; R = CH_3
1i: X = NH_2, H; R = CH_3

1j: X = H; R = Et
1k: X = Cl; R = Et
1l: X = OCH_3; R = Et
1m: X = CH_3;R = Et



2a: X = H
2b: X = Cl
2c: X = OCH_3



3a: X = H
3b: X = Cl
3c: X = OCH_3

In the parent case, Y=H_2O and is omitted in the abbreviation, "**1a**". But with other axila ligands, e.g., Y=Cl^-, the abbreviation would be "**1a**-Cl".

Currently available TAML activators (19) that differ in lifetimes and reactivities based on the X and R substituents.

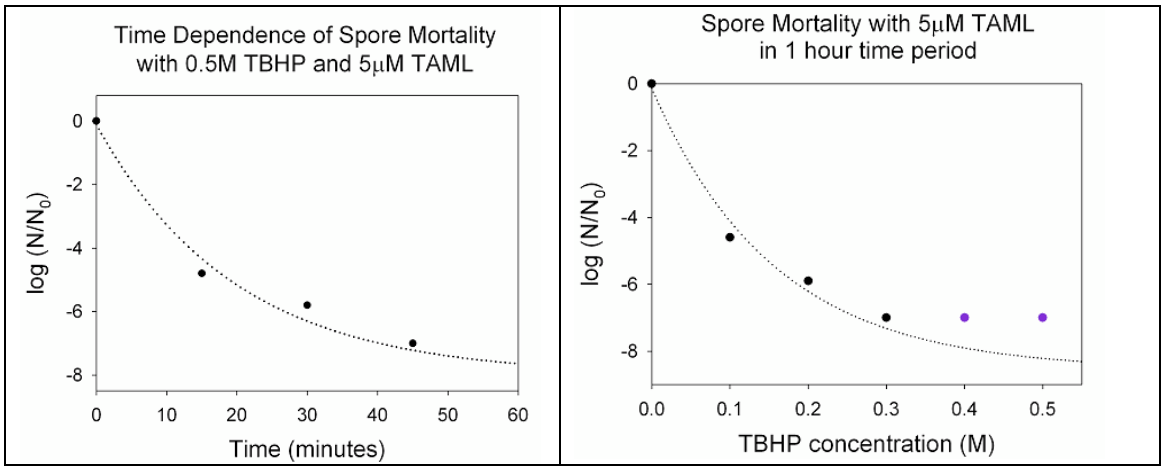
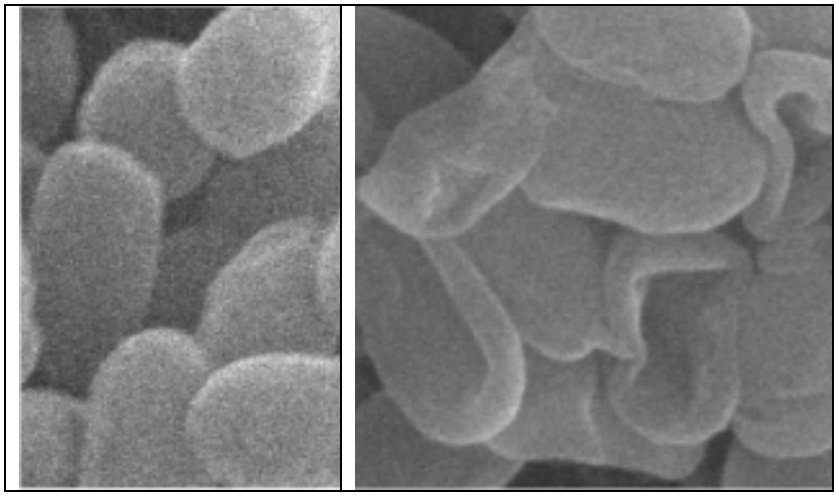


Figure 1. Optimization for TAML/TBHP *B. atrophaeus* spore inactivation, a) Time; b) [TBHP]

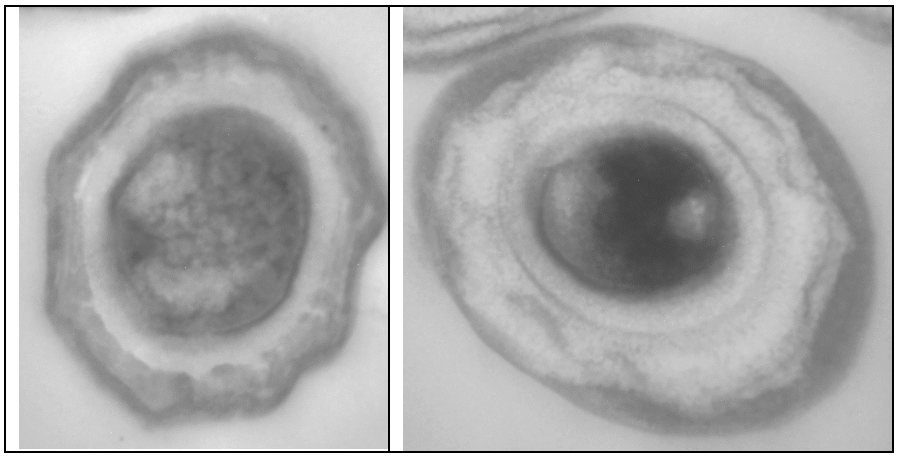
The scanning electron micrographs (SEM) of the bacterial spores before (Fig. 2A) and after treatment (Fig 2B) show significant structural changes. The cylindrical spores of *B. atrophaeus* on treatment with TAML/peroxide are seen here as emptied out shells. It is apparent that cortex destruction results in structural collapse of the spore. A drained out shell like structure is probably the result of the spores being subjected to dehydration process for scanning electron micrographs.



A B

Figures 2. SEM of *B. atrophaeus* spores, a) Control; (b) After treatment with TAML/TBHP .

The TEM micrographs of *B. atrophaeus* spores, the control (Fig 3A) and treated (Fig. 3B) show a pronounced change in the outer layers as well as in the core. After the treatment, the layer that consists of spore cortex appears enlarged, less dense and relatively clear, while the core appears to be compressed and denser than in the untreated spore. Peroxygen compounds are known to oxidize cellular components and disrupt membranes and proteins. It is likely that after the destruction of the cortex, which provides strength and shape to the spore, the cell membrane made up of a bilayer of phospholipids ruptures easily under the osmotic pressure created by the cytoplasm.



A B

Figure 3. TEM of *B. atrophaeus* spore a) Control; b) After treatment with TAML/TBHP

Our achievement of a 7-log kill in 15 minutes means we have reached the gold standard being sought by the Department of Defense for a superior decontamination system for biological warfare agents.

Decontamination of chemical warfare Agents

In the ensuing period, we have studied the decomposition of thiophosphate pesticide with [**1d**]$^-$ /peroxide at ambient conditions and found these to undergo facile total degradation. The reactions proceed with a facile oxidative hydrolysis followed by oxidative degradation of the hydrolysates to small aliphatic acids and partial mineralization. A minor formation of phosphonates due to oxidative desulfuration can also take place under some conditions. These are often more toxic than the original compounds, but it is important to emphasize that we have found conditions where phosphonate formation is not detectable. The presence of peroxyanions formed under high pH conditions of the reaction, facilitate nucleophilic hydrolysis of the thiophosphates and the phosphonates (for conditions where these form) followed by oxidative degradation of the hydrolysates (Fig. 4).

a

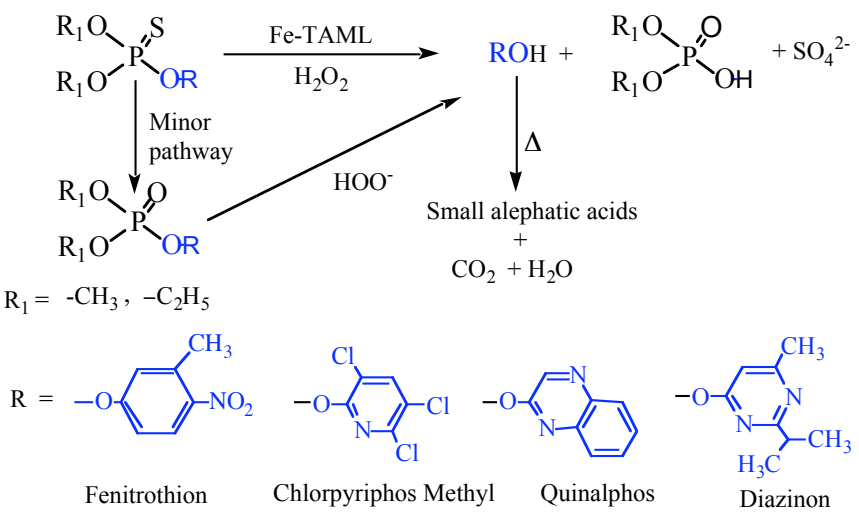


Figure 4. A generalized scheme for the degradation of thiophosphate pesticides on treatment with [**1d**]$^-$/peroxide.

The mechanism of the reaction is believed to closely mimic oxygenase and peroxidase enzymatic pathways (Fig. 5). Some spectroscopic evidence for the intermediates has been observed.

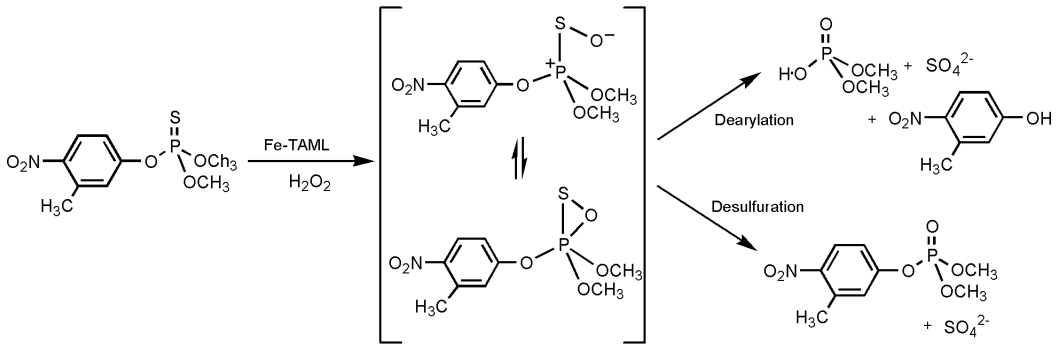


Figure 5. A mechanism for FE-TAML/peroxide treatment of fenitrothion rationalizes oxidative hydrolysis and oxidative desulfuration products obtained initially.

These studies on the total degradation of thiophosphates and phosphonates on treatment with TAML/peroxide have opened up potential applications for the decontamination system for phosphonate structured warfare agents, for inexpensive, easy to perform degradation of stored and aged pesticide stocks (especially in Africa and Asia), for remediation of polluted sites and water bodies, and for the destruction of chemical warfare agent stockpiles.

Normally, the electron-withdrawing nature of nitro groups in aromatic compounds makes them resistant to chemical or biological oxidation and hydrolysis. However, nitro and chloro substituted phenolates, products of hydrolysis of thiophosphate esters, on treatment with TAML/peroxide have been found to undergo degradation readily. 3-methyl-4-nitrophenol and trichloro-2-pyridinol are, respectively, components of the hydrolysates of the thiophosphate pesticides, fenitrothion and chlorpyriphos. These are rapidly degraded by TAML/peroxide to small aliphatic acids and partial mineralization (CO_2, HCl, NO_3^-). The degradation of these hydroxyaromatic compounds bearing electron-withdrawing nitro- and chloro-substituents have opened an attractive possibility that TAML/H_2O_2 treatment of other electron-poor aromatic compounds could lead to their facile degradation. Electron-withdrawing substituents often make organic compounds persistent in the environment. This TAML/peroxide technology has unprecedented efficacy for destroying such compounds, as we have publ;ished recently in *Science* for the USEPA priority pollutants, pentachlorophenol and 2,4,6-trichlorophenol.

Similarly, we have achieved outstanding degradation results with HD surrogates, such as di-n-butyl sulfide, which is selectively oxidized to the sulfoxide derivative (Fig. 6) in just about 5 minutes.

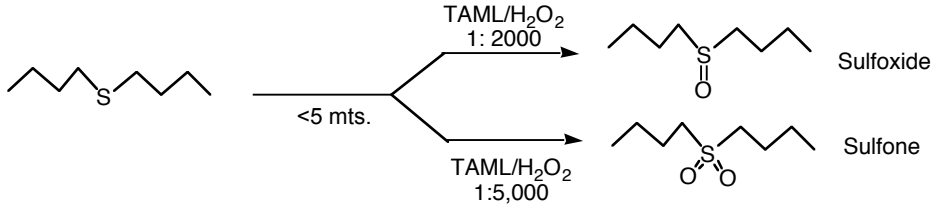


Figure 6. TAML-activated H_2O_2 treatment of di-n-butylsulfide results in the selective formation of di-n-butyl sulfoxide by controlling the [**1d**]$^-$:peroxide ratio at 1:2000.

We have established a research relationship with the British Government's Defense Laboratories at Porton Down to work with the "live agents". They have confirmed the most rapid kinetics for any catalytic system and the exclusive formation of the desired sulfoxide derivative on treatment of TAML/peroxide system with HD agent, bis(2-chloroethyl)sulfide or "sulfur mustard".

Field trial of color removal by TAML catalyst/H_2O_2 from the process stream of a bleached Kraft pulp mill.

Much of the effort in the grant period was devoted to producing bulk quantities oft the prototype TAML® activator, Na[**1a**], for use in field trials in the pulp and paper filed of use. Twenty-five kilograms of Na[**1a**] was produced by a US-based Company and by an efficient and cost-effective methodology developed in collaboration with our Carnegie Mellon Laboratories. A field trial was then conducted at a mill in Pennsylvania. The mill uses a three stage bleaching process: D, E_p, and D.

It had previously been shown from a two-week field trial at another pulp mill (in New Zealand) using a similar bleaching sequence that the TAML activator could effectively remove color from the effluent stream of the caustic extraction stage. The caustic extraction stage accounts for the majority of the colored waste-stream produced during the bleaching process and for the entire mill. However, after extensive consultation with the PA mill, it was decided that a field trial of an in-process treatment would fit their needs best. The goal for the trial was color reduction from the E_p drain. It is expected that by reducing the color from the extraction stage, whole mill effluent color will be reduced. Currently, the mill uses a polymer immediately prior to discharge into the receiving creek and they wish to cease this operation for cost.

Over a five day period, Na[**1a**] was added directly into the caustic extraction stage (E_p) of the PA bleached Kraft pulp mill. The flow rate of brownstock, sourced from pine-wood into the bleaching tower was ca. 5,500 L/m. The rate of flow of the pulp through the bleaching tower was approx. 300 tonnes/day. All mill operations were carried out under their standard conditions with respect to time, temperature, and caustic (1% on pulp) and hydrogen peroxide (0.65% on pulp) concentrations.

The Na[**1a**] catalyst solution was metered into the existing line feeding the caustic soda sprays of the 1st. stage pulp washer from a 55 gallon drum using a standard diaphragm pump, (Figures 7, 8). Hydrogen peroxide was added to the pulp downstream. Efficient mixing of pulp, peroxide, FeB*, and caustic was provided by the re-pulper and pug-mill that precede the bleaching tower.

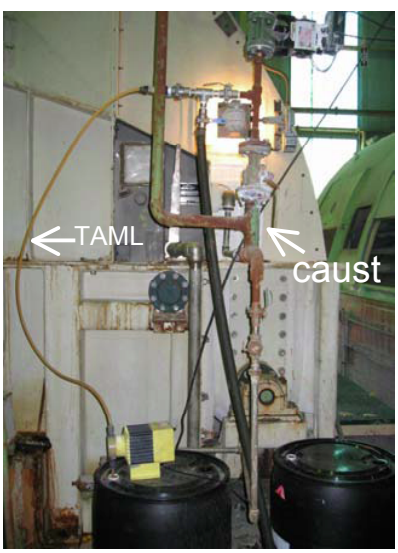
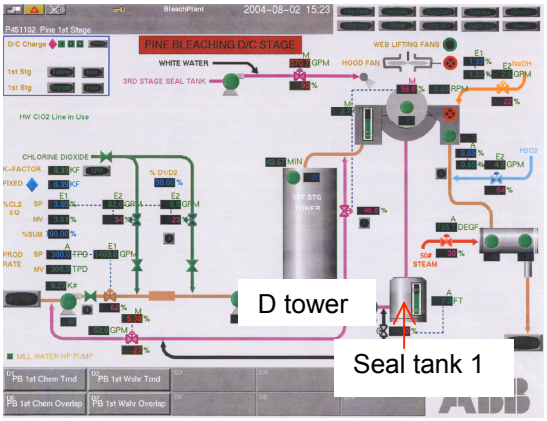


Figure 7 (left) shows addition point of TAML catalyst to the caustic line feeding the sprayers for the first stage pulp washer.
Figure 8 (right) shows the first stage pulp washer.

The wash-waters from the bleaching stages immediately preceding (Fig. 9 – Seal tank 1) and following (Fig. 10 – Seal tank 2) treatment with the Na[**1a**] catalyst were sampled hourly for absolute absorbance and color analysis was determined every two hours using NCASI 253 by the

plant operators. The diagrams in Figs. 9 and 10 reveal the counter-current washing systems commonly used by pulp mills. The shower flows onto each of the pulp washers were monitored hourly along with other parameters relating to the brownstock in order to obtain a mass balance for the process. Our mill partner is further analyzing this data. During the week of the field trial, the Na[**1a**] TAML catalyst was added at a rate consistent with its known reactivity at low micromolar concentrations – 0.25, 0.5, and 1.0 μM.



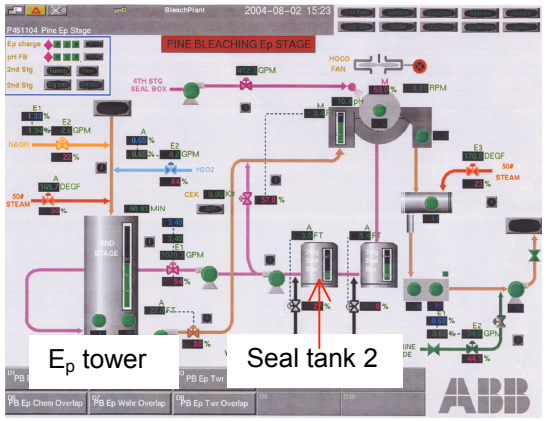


Figure 9 (left): Schematic showing D (ClO_2) bleaching of brownstock pulp, first stage washer, and seal tank 1 for D color samples.
Figure 10 (right): Schematic showing E_p bleaching of pulp, second stage pulp washer, second stage seal tank for E_p color samples.

The washwaters from the bleaching stages immediately preceding (Fig. 9 – Seal tank 1) and following (Fig. 10 – Seal tank 2) treatment with the [**1a**]$^-$ catalyst were sampled hourly for absolute absorbance and color analysis was determined every two hours using NCASI 253 by the plant operators. The diagrams in Figs. 9 and 10 reveal the counter-current washing systems commonly used by pulp mills. The shower flows onto each of the pulp washers were monitored hourly along with other parameters relating to the brownstock in order to obtain a mass balance for the process. During the week of the field trial, the Na[**1a**] TAML catalyst was added at a rate consistent with its known reactivity at low micromolar concentrations – 0.25, 0.5, and 1.0 μM on pulp concentrations were investigated.

On the first day of the field trial, a reduction in absorbance of the wash water from the 2nd stage washer (seal tank 2) of greater than 50% (~1,200 pcu to ~490 pcu) was observed about twelve hours after the initial addition of the TAML catalyst. A picture of seal tank 2 samples taken before and after TAML addition shows color reduction clearly visible to the naked eye, Fig. 11. The color reduction is depicted graphically in Fig. 12, which shows color as a function of time during the trial (each data point is two hours – Na[**1a**] TAML was added at the first data point). An unexpected loss of electrical power at the mill (first * on Fig. 12) caused the trial to be halted temporarily for approximately 12 h. A second shutdown for nearly 2 hours was encountered during the trial in order to repair an acid line to one of the D bleaching towers.

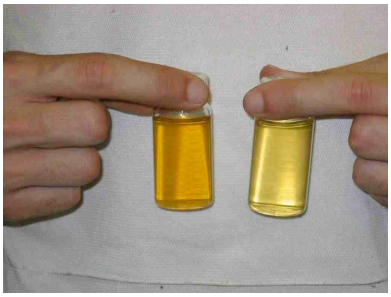


Figure 11: Seal tank 2 effluents before (left) and after (right) treatment with the TAML catalystt (0.25 μM TAML).

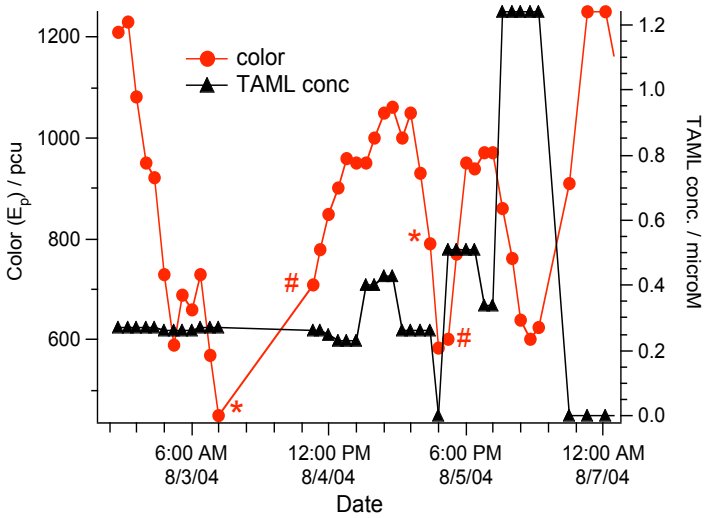


Figure 12: Graphical representation of the color data (pcu) as a function of time and TAML catalyst concentration. *indicates unexpected mill shutdowns and cessation of catalyst addition and # denotes restart of the TAML addition.

The complexity of the mill environment and the compromises to data collection caused by unexpected mill shutdowns and the subsequent start-ups, makes the data difficult to interpret precisely. This is because water flows, brownstock pulp, ClO_2 levels adjust considerably during this period. However, the data do indicate that whenever the TAML activator was present, color levels were always lower than at the start of the field trial. The plant operators reported that the brightness characteristics of the pulp were unaltered by the addition of TAML to the pulp bleaching process. Thus the catalyst had no negative impacts on the mill operations and there is every reason to believe that the extremely positive results encountered before the mill's technical problems occurred will be found under stable operating conditions. A longer field trial is planned for the near future.

Desulfurization of Transportation Fuels

Catalytic oxidation of dibenzothiophene (DBT) and 4,6-dimethy-dibenzothiophene (4,6-DMDBT) in a bi-phasic system was performed using the Fe-TAML activators Li[**1e**] and CTA[**1e**] (CTA = cetyltrimethylammonium), at pH 7 and 8 (0.01 M KH_2PO_4 buffer) at 50° C with H_2O_2. A water/t-BuOH mixture (7:3 v/v) was used as the solvent system as no reaction was found without the t-BuOH. The t-BuOH may help to partially solubilize the DBT or 4,6-DMDBT in the aqueous phase where the oxidation takes place although neither could be detected in the aqueous phase by GC-MS.

In a typical oxidation reaction, DBT or 4,6-DMDBT was dissolved in a hydrocarbon (decane or octane). A two-phase system consisting of 3.0 mL of 0.01 M pH 8 phosphate buffer:t-BuOH (7:3) and 1 mL of DBT (2.7×10^{-3} M) in hydrocarbon was then prepared. The mixture was stirred vigorously at 50° C and then CTA[**1e**] and H_2O_2 were added in six equal portions over a 3 h period such that the final quantities of CTA[**1e**] and H_2O_2 were 2.7×10^{-8} moles and 2.7×10^{-4} moles, respectively. This corresponds to a catalyst:substrate:H_2O_2 molar ratio of 1:100:10,000. The addition of catalyst and H_2O_2 in portions minimizes H_2O_2 disproportionation that might occur because of the low concentration of substrate in the aqueous phase relative to catalyst and H_2O_2. When the reaction was completed, the hydrocarbon layer was separated after centrifugation and subjected to GC-MS analysis and sulfur specific GC-flame photometric detector (GC-FPD) analysis. We found no trace of DBT in the hydrocarbon phase with either technique. This corresponds to the removal of sulfur from >500 ppm to <0.1 ppm from the hydrocarbon with no additional processing. The t-BuOH was removed in vacuo from the water-t-BuOH mixture and then the water layer extracted with diethyl ether. The diethyl ether layer was separated and

analyzed by both GC-MS and GC-FPD. The only observable products were the DBT sulfoxide and DBT sulfone. The results demonstrate that the oxidation of DBT and its derivatives takes place in a step-wise process. Interestingly, when this reaction is performed under homogeneous conditions, i.e. rigorous exclusion of hydrocarbons, the only oxidized species observed is the DBT sulfone. However, the most significant point of the TAML catalyzed oxidation process is the simultaneous oxidation and extraction process, which no other oxidation technology yet claims. The same experimental protocol was used for the oxidation and removal of 4,6-DMDBT from a hydrocarbon phase and the quantitative oxidation/extraction again was observed.

Residual H_2O_2 measurements using UV-vis spectroscopy reveal that 5.5% of available H_2O_2 was consumed whereas 2% is stoichiometric for sulfone and 1% for sulfoxide formation. This indicates that any side reactions, such as oxidation of compounds other than the dibenzothiophenes and more importantly H_2O_2 disproportionation are negligible. This constitutes a green process as high atom efficiency with regard to H_2O_2 is achieved, and the only byproduct is water. This atom efficiency makes the process promising for future applications.

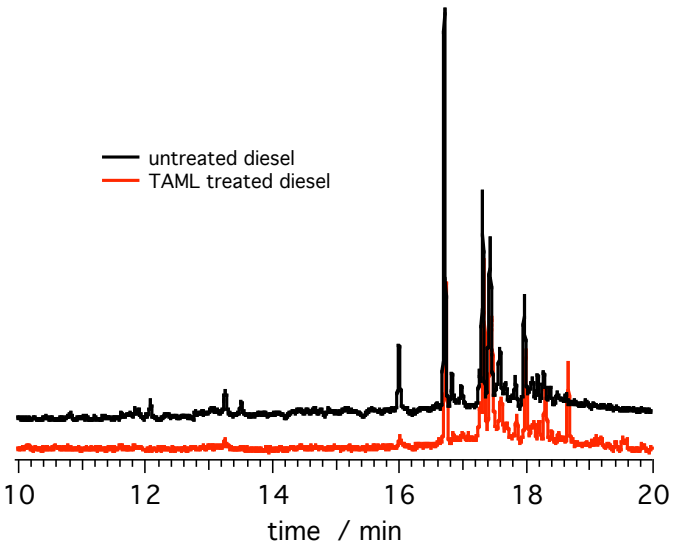


Figure 13: Chromatogram (FPD) of commercial diesel before and after treatment with TAML/H_2O_2. Total sulfur removal is >70% based on integration of all signals and baseline for a single treatment.

A commercially available diesel was purchased in order to determine if sulfur compounds could be removed from this complex mixture. A two-phase system consisting of 3.0 mL of 0.01 M pH 8 phosphate buffer:t-BuOH (7:3) and 1 mL of diesel was prepared. For purposes of deciding on how much catalyst/peroxide to add, it was assumed that the total sulfur content of the diesel was 500 ppm. The mixture was stirred at 50° C and then CTA[**1e**] and H_2O_2 were added such that the final quantities of catalyst and H_2O_2 were 2.6 x 10^{-8} moles and 2.6 x 10^{-4} moles, respectively. The catalyst and H_2O_2 were added in six equal portions over a 3 h period. Analysis of the diesel layer by GC-FPD (specific detection for sulfur compounds) following centrifugation revealed ~70% removal of total sulfur present in the diesel, Fig. 13. All sulfur components decreased. Some, including DBT, have been converted completely to oxidized forms as their GC-FPD peaks completely disappear. However, at this point in time, some DBT-sulfone remains in the diesel, in contrast with the octane or decane experiments. Further experiments are being conducted to optimize DBT sulfone transfer from the diesel to the water/t-butanol phase. Total sulfur analysis remains to be conducted to give a second method for checking on the accuracy of the GC-FPD method for sulfur removal. A commercial analytical lab has been identified and specific solutions will soon be tested.

Scale-up Synthesis of TAML catalysts

In order to clearly demonstrate the industrial viability of the TAML catalysts, it was necessary to produce them by a commercial source. Six mid-size companies made proposals to Carnegie Mellon to produce the prototype TAML catalyst. Two were chosen for small-scale batches of 100 g. Then, one was chosen for production of the catalyst for the full-scale field trial at the pulp mill.

This US based company ultimately produced >25 kg of the catalyst. Carnegie Mellon purchased from unrestricted funds, 20 kg of this material and a potential licensee of the TAML technology, purchased 5 kg from the same supplier.