A new General Purpose Decontamination System for Chemical and Biological Warfare and Terrorism agents

Sushil Khetan, Deboshri Banerjee, Arani Chanda, and Terry Collins

Institute for Green Oxidation Chemistry
Carnegie Mellon University, Pittsburgh, PA 15213

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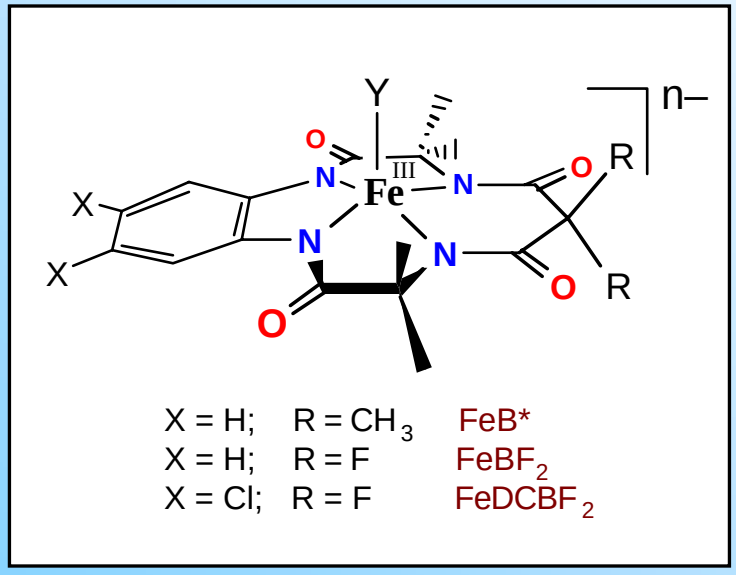
Fe-TAML® Activator of Peroxide



X = H; R = CH_3 FeB*
X = H; R = F $FeBF_2$
X = Cl; R = F $FeDCBF_2$

TAML® Activators developed at Carnegie Mellon University

New Applications

Rapid Inactivation of Bacterial Spores and degradation of organophosphorus triesters as surrogates of Biological and chemical Warfare Agents

'Green' Oxidizing System[1]

- Biomimetic System
- Non-toxic and non-corrosive
- Efficient user of peroxide
- High turnover in oxidative environment

Tested Applications[1,2]

- Effluent Treatment
- Bleaching in Pulp and Paper
- Desulfurization of Diesel
- Dye Transfer Inhibition Agent

1. Collins, T. J. *Accounts of Chemical Research* **2002**, *35*, 782-790
2. http:// www.cmu.edu/Greenchemistry

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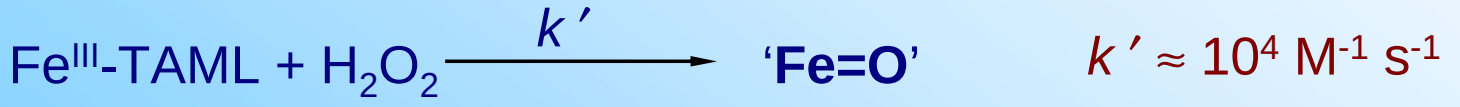
Activators of Hydrogen peroxide

Relative Rates of Reactive Intermediates Formation

- Bicarbonate Activated Peroxide* System

  (Aqueous Foam decon by Sandia National Laboratory)

- Fe-TAML® activators of hydrogen peroxide



– Deep Oxidation capability
– Non-toxic and non-corrosive

$k1/k = 10^7$

* Richardson, D. E. *et al. J. Am. Chem. Soc.* **2000**, *122*, 1729

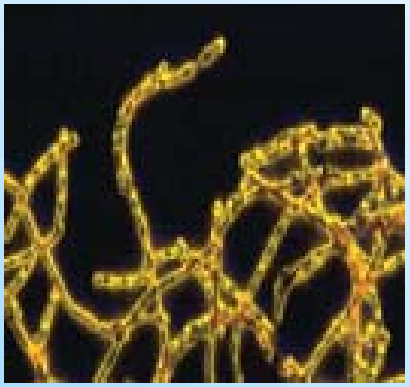
Biological Warfare Agents

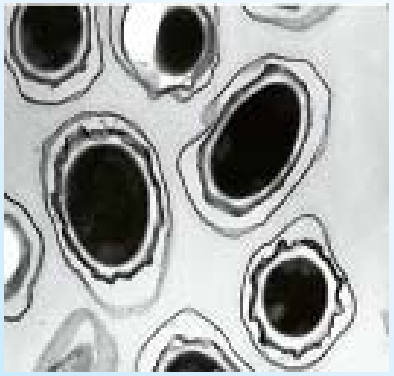
A microorganism or its by-product (toxin), which causes disease in man, plants or deterioration in material; used as weapons of warfare and/or terrorism

Major Threats

- Bacterial Diseases
  - Anthrax
  - Tularemia
  - Plague
- Viral Diseases
  - Smallpox
  - Viral hemorrhagic fevers
- Toxins
  - Botulinum Toxins
  - Ricin

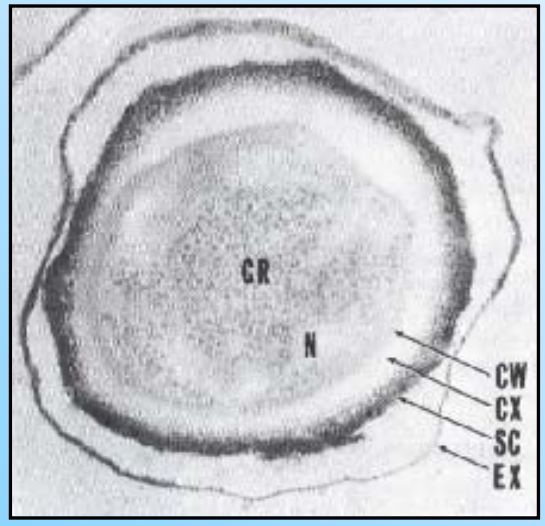
Anthrax Spores



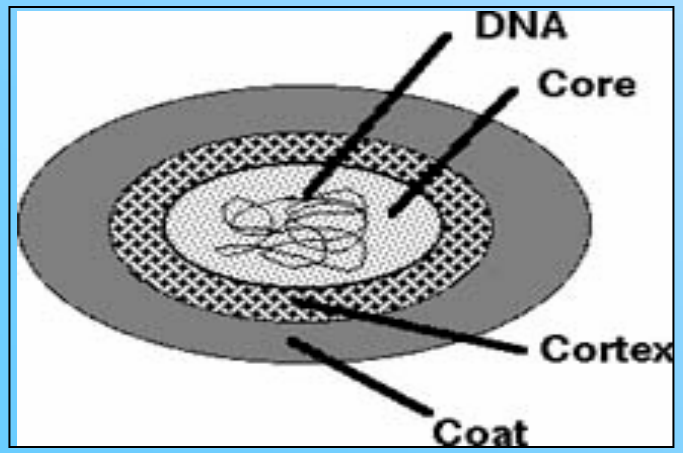


- Dormant survival form of the vegetative bacterium
- Resistant to stress conditions, e.g. heat, UV radiations and chemical treatments
- Germinates on encountering favorable conditions

Bacterial Endospore



Source: L. M. Prescott, Microbiology, McGraw-Hill, NY, 5th Ed., 2002



Spore resistance is due to two protective shells that encase the organism

Spore Coat

Multi-layered highly cross-linked polypeptide structure with numerous disulfide linkages

Spore Cortex

Thick layer of loosely cross-linked peptidoglycan structure with an overall negative charge

Spore Core

- Normal cell structures with ribosomes and a nucleoid
- Metabolically inactive and largely dehydrated

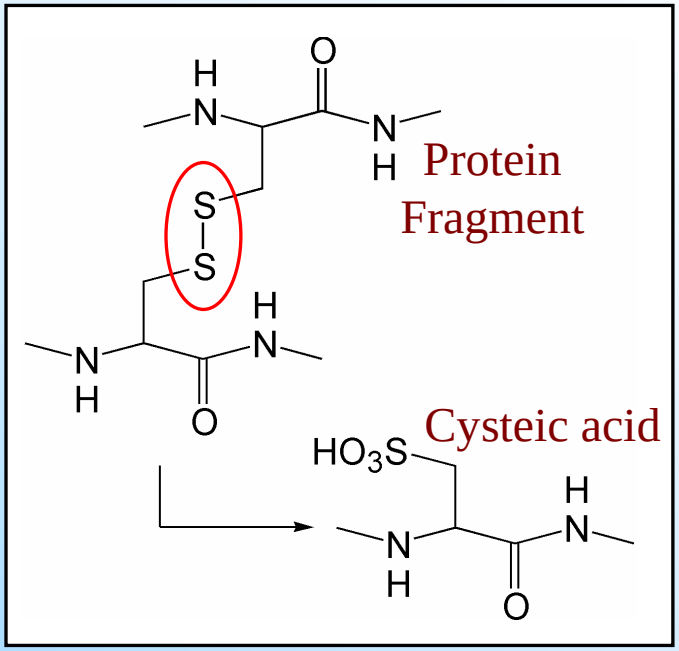
Bacterial Spore Deactivation

Strategies and Mechanism

Strategies

- Penetration of the spore coat with subsequent degradation of bacterial DNA
- Dissolution of spore peptidoglycan structure, exposing the vegetative cell elements
- Initiation of germination with weakening of spore wall followed by deactivation
- Inactivation of spore germination apparatus by destruction of germination-specific lytic enzymes

Weakening of spore coat through oxidation of the disulfide bonds



Model compound:
Dialkyldisulfide (e.g. Cystine)

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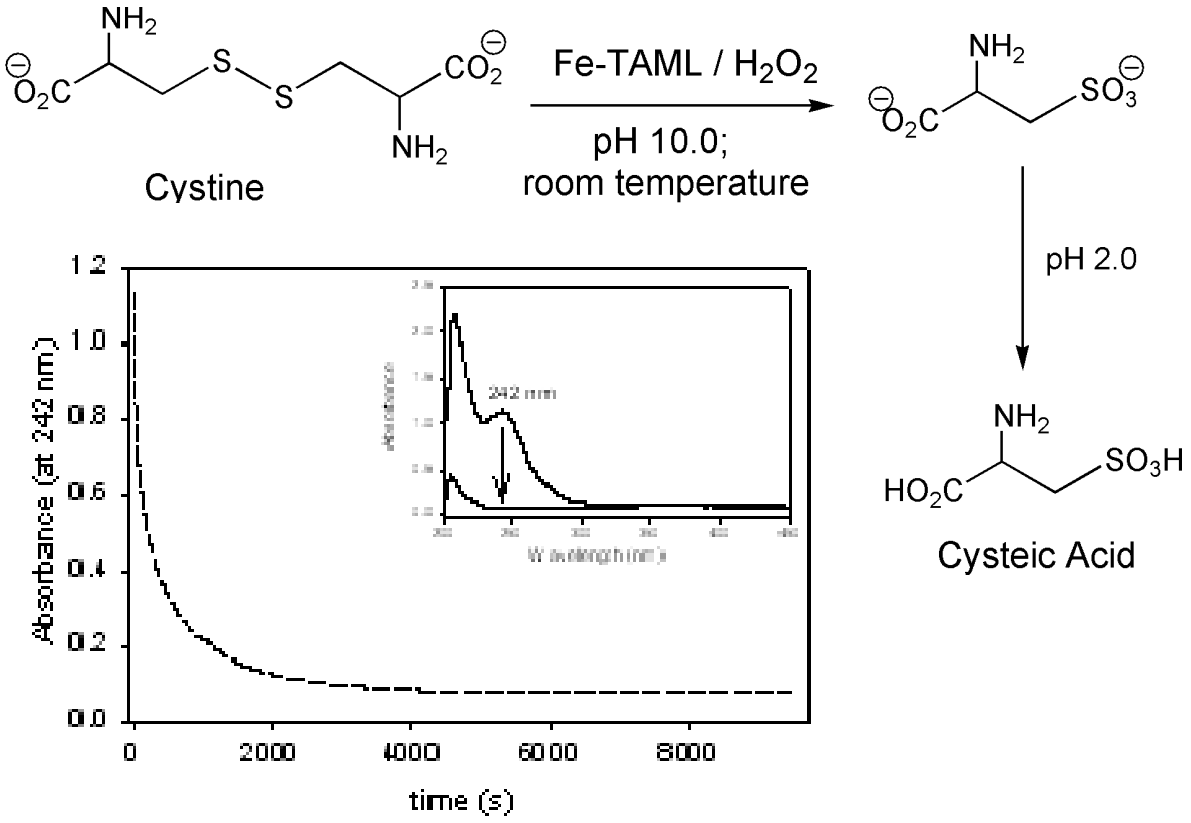
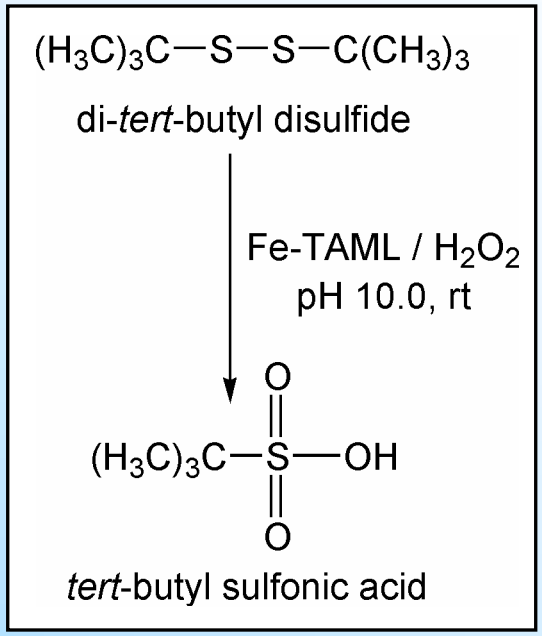
Modeling Studies

Oxidation of Di-alkyl Disulfides



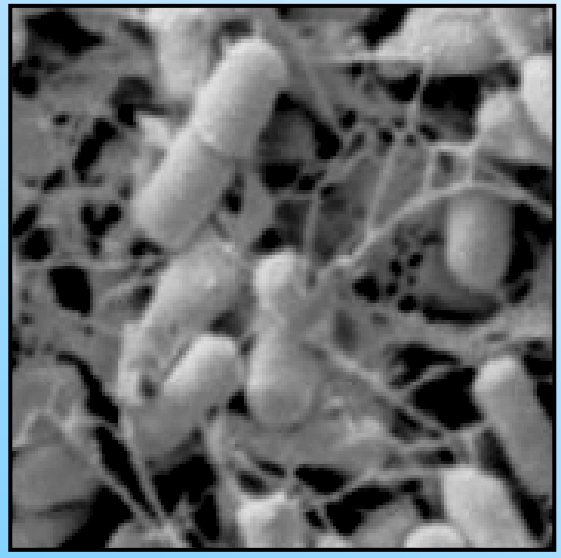
Cysteic acid formation confirmed by HPLC and UV-Visible Spectroscopy

Dissociation of disulfide bonds also observed in di-*tert*-butyl disulfide



Result obtained from ESI-MS studies

Deactivation Studies with *Bacillus* spores



Bacillus atrophaeus
(formerly *B. globigii*)

Spore-forming harmless soil bacterium *B. atrophaeus* (ATCC 9372) was tested as surrogate for *Bacillus anthracis* in spore deactivation studies

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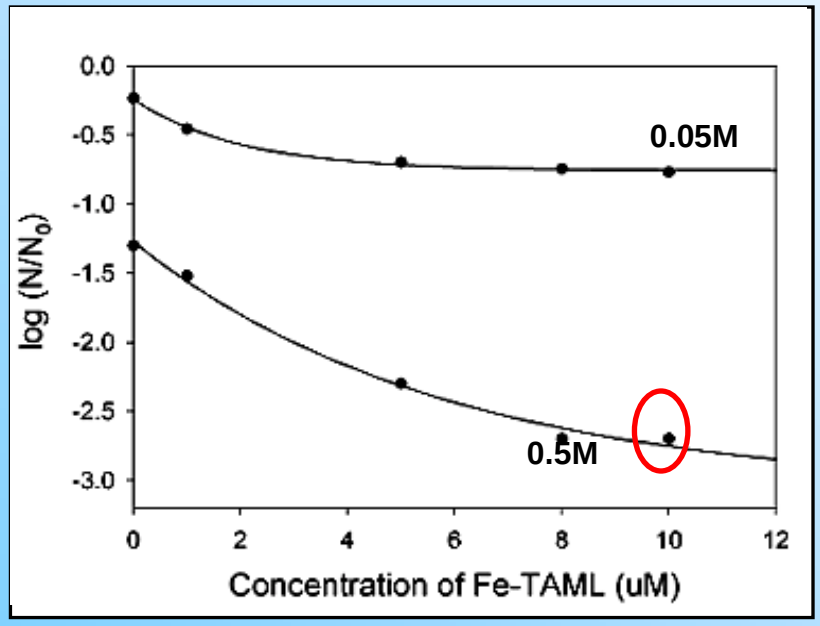
Optimization of Reaction Conditions

Variation of Fe-TAML® concentrations



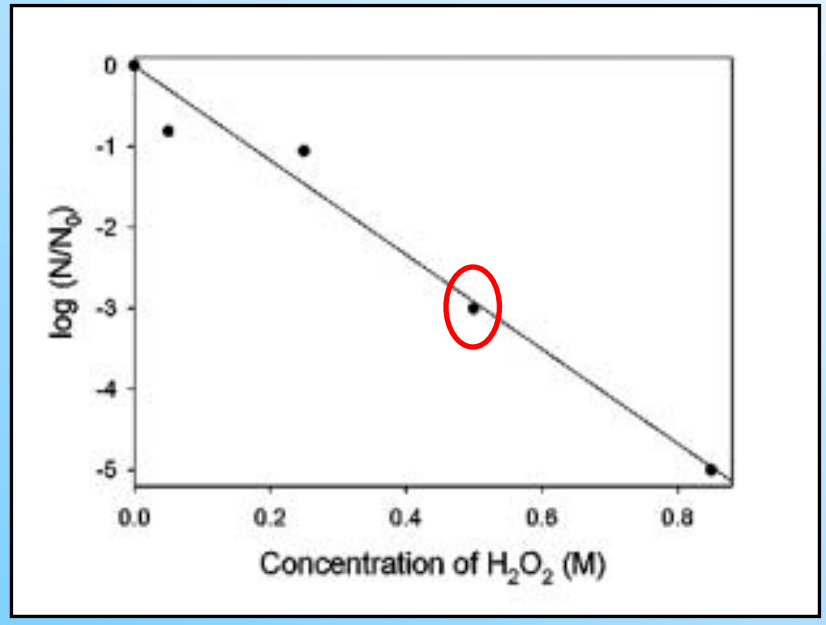
N_0 = Initial number of spores
N = Number of surviving spores

- Studies conducted at two H_2O_2 concentrations
- Exponential relationship between spore deactivation and Fe-TAML® concentration
- Optimized Fe-TAML® concentration: 10 μM

- Reactions carried out for 1 hour at 30°C
- Spore Population of 5×10^7 CFU/ml
- Na-carbonate/bicarbonate (0.1 M) buffer, pH 10.0

Optimization of Reaction Conditions

Variation of H_2O_2 concentrations



- Linear relationship between spore deactivation and concentration of H_2O_2
- Optimized H_2O_2 concentration: 0.5M

N_0 = Initial number of spores
N = Number of surviving spores

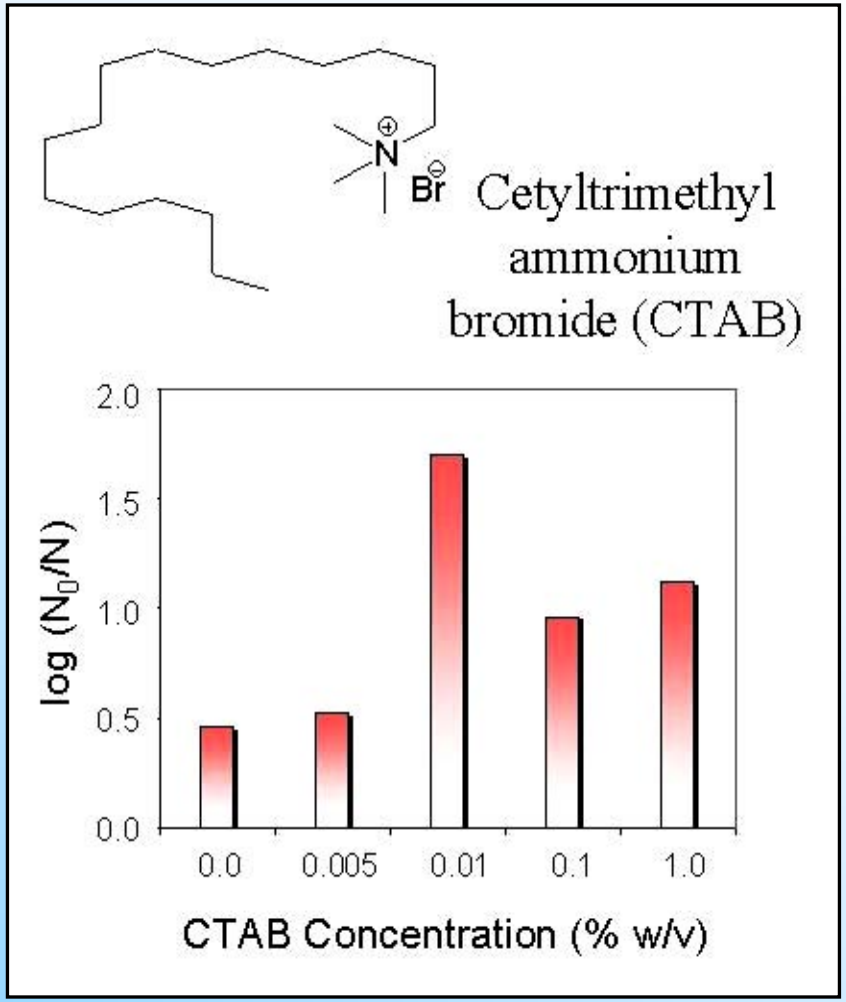
- Reactions carried out for 1 hour at 30°C
- Spore Population of 5×10^7 CFU/ml
- Na-carbonate/bicarbonate (0.1 M) buffer, pH 10.0

Use of Cationic Surfactant

Cationic Surfactants

- Enhance penetrability of Fe-TAML® activators across the spore coat
- Increase dispersion of hydrophobic spores in aqueous phase
- Can cause collapse of spore peptidoglycan structure through ionic interactions

Optimized concentration: 0.03%
(close to cmc value)



N_0 = Initial number of spores
N = Number of surviving spores

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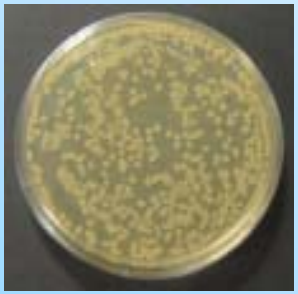
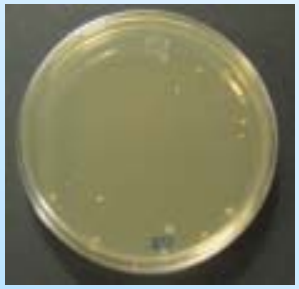
Time Dependence of Spore Kill



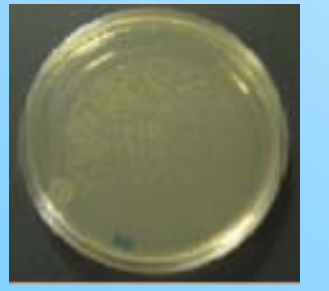
10,000×

Control



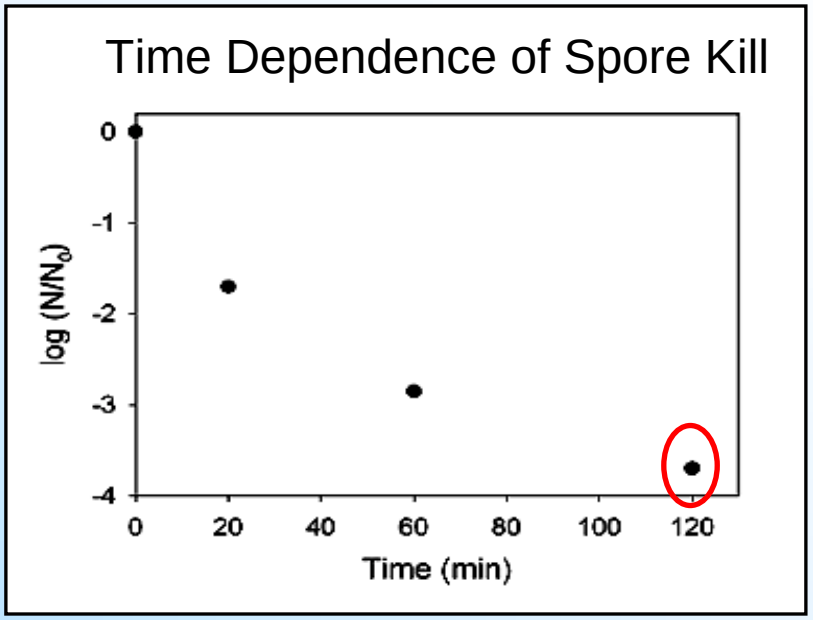
10,000×

95% mortality
with hydrogen peroxide



10,000×

99.98% mortality
with Fe-TAML® activator,
and hydrogen peroxide



N_0 = Initial number of spores
N = Number of surviving spores

- 99.98% (4-log) kill of spores
- Treatment time: 2 hours
- Fe-TAML®: 10 μM; H_2O_2: 0.5 M
- Spore population: 1×10^8 cfu/ml

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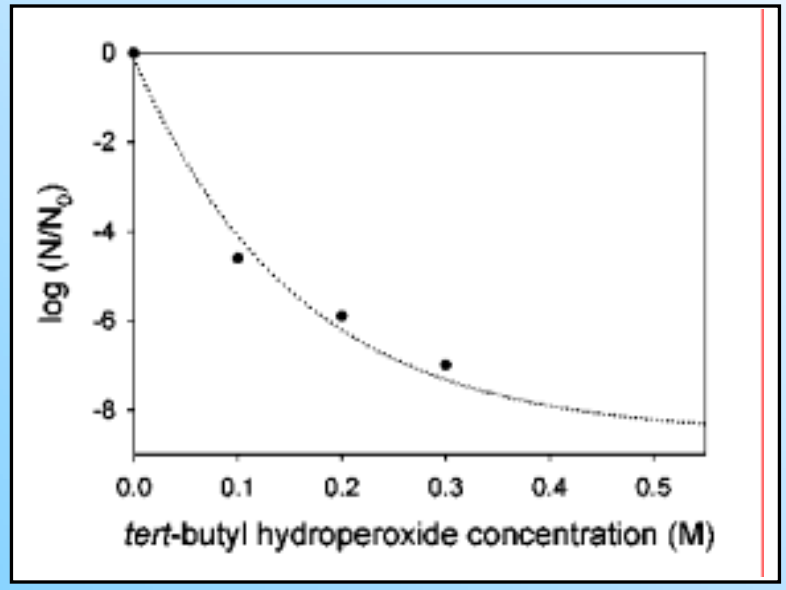
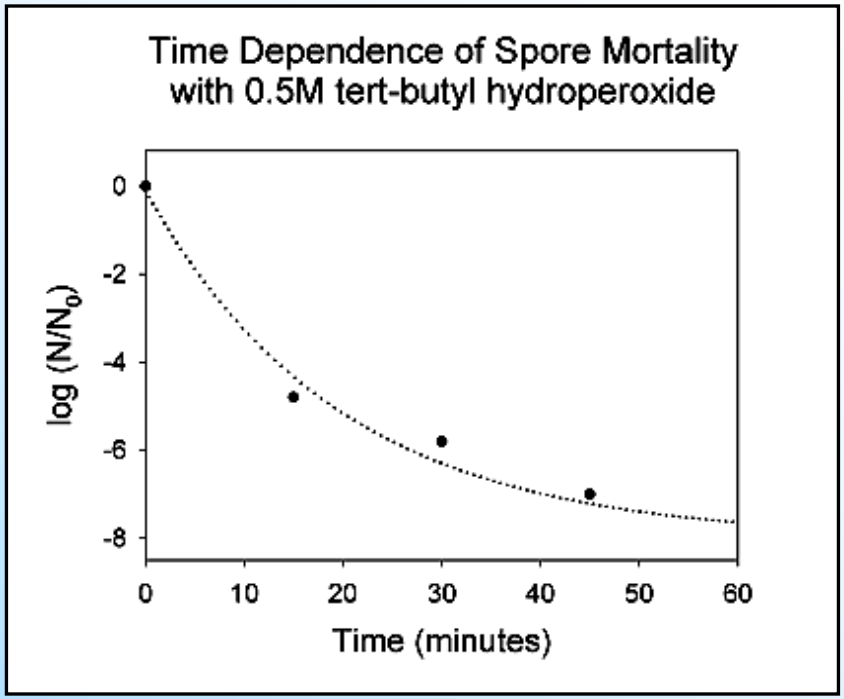
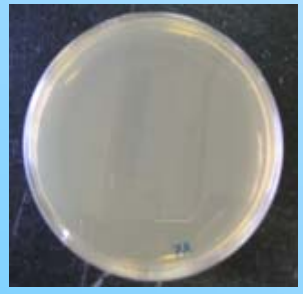
Enhanced Spore Mortality



N_0 = Initial number of spores
N = Number of surviving spores



75% with ^{t}BuOOH

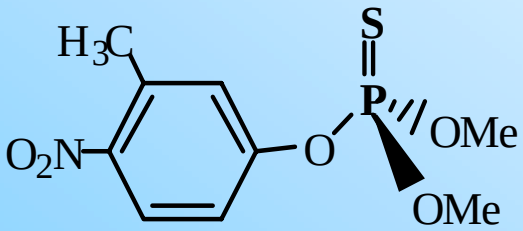


99.99999% with Fe-TAML® + ^{t}BuOOH

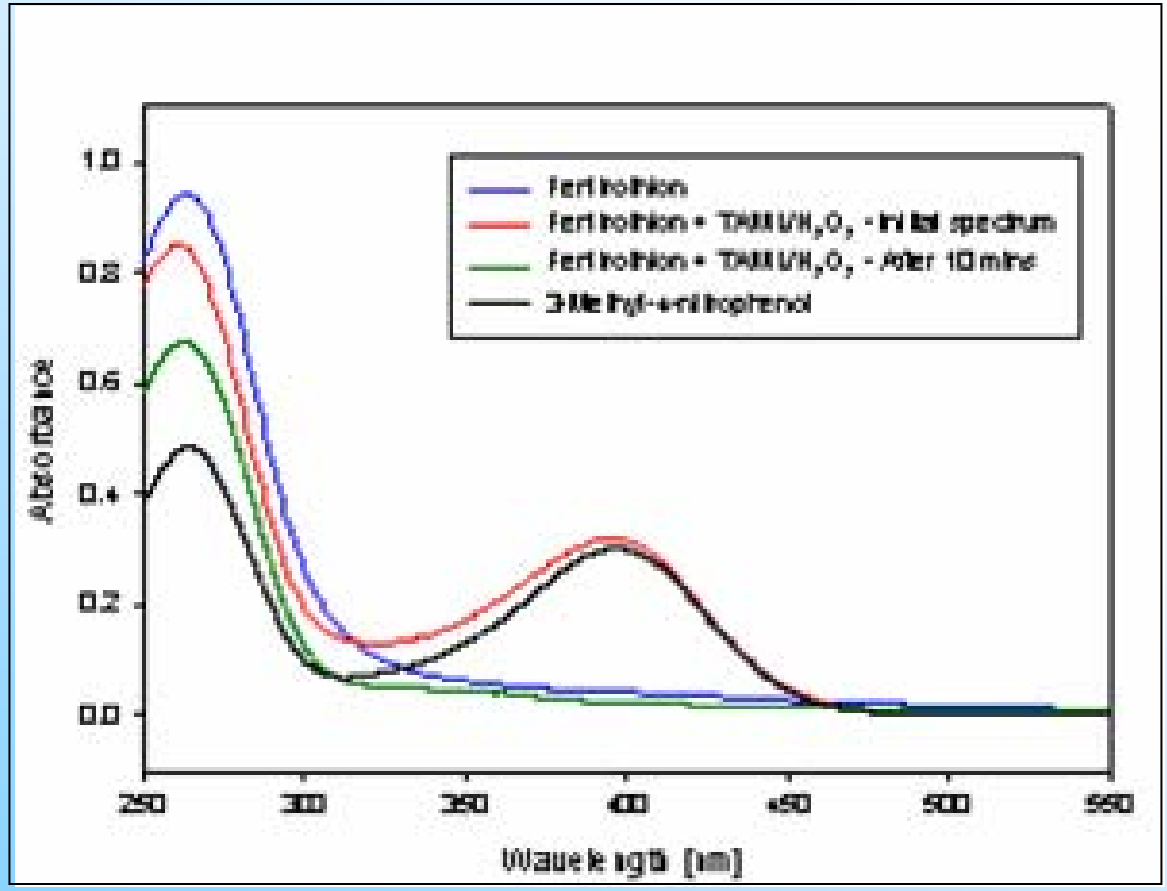
- 99.99999% (7-log) kill of spores
- Treatment time: 1 hour
- Fe-TAML®: 5 μM; ^{t}BuOOH: 0.3 M
- Spore population: 1×10^{8} cfu/ml

Oxidative Detoxification of Organophosphorus Triesters and Dialkyl sulfides

TAML®-activated H_2O_2 Treatment of Fenitrothion

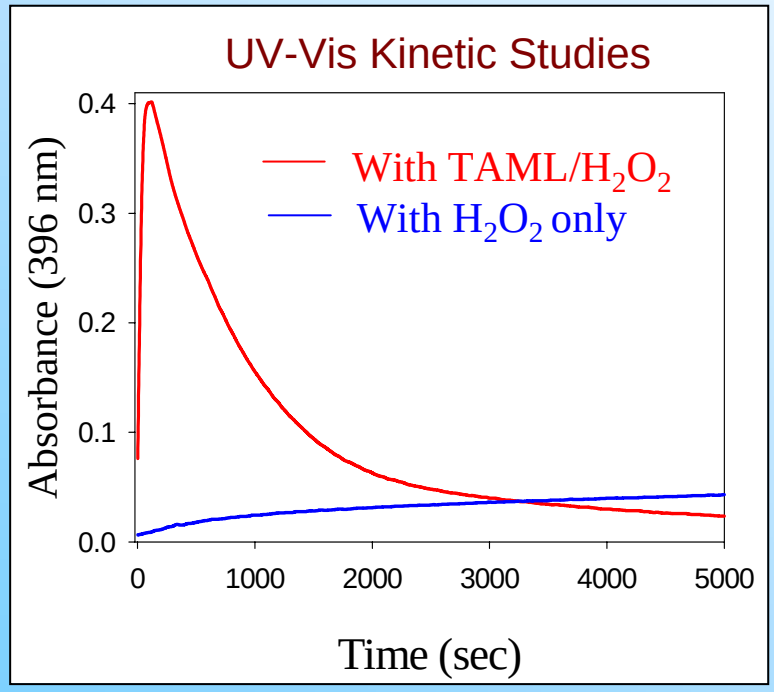


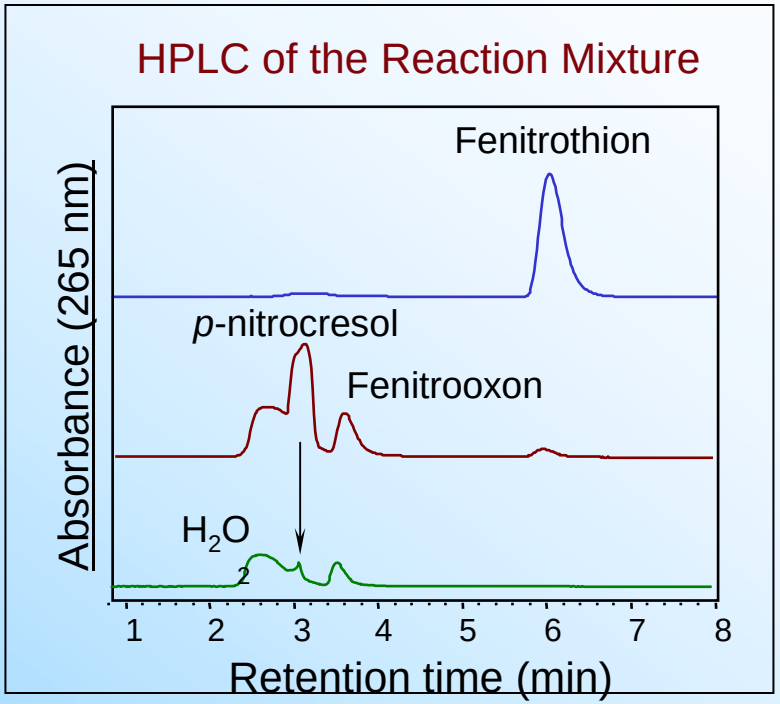
Fenitrothion



UV-Visible spectroscopic study

TAML®-activated H_2O_2 Treatment of Fenitrothion

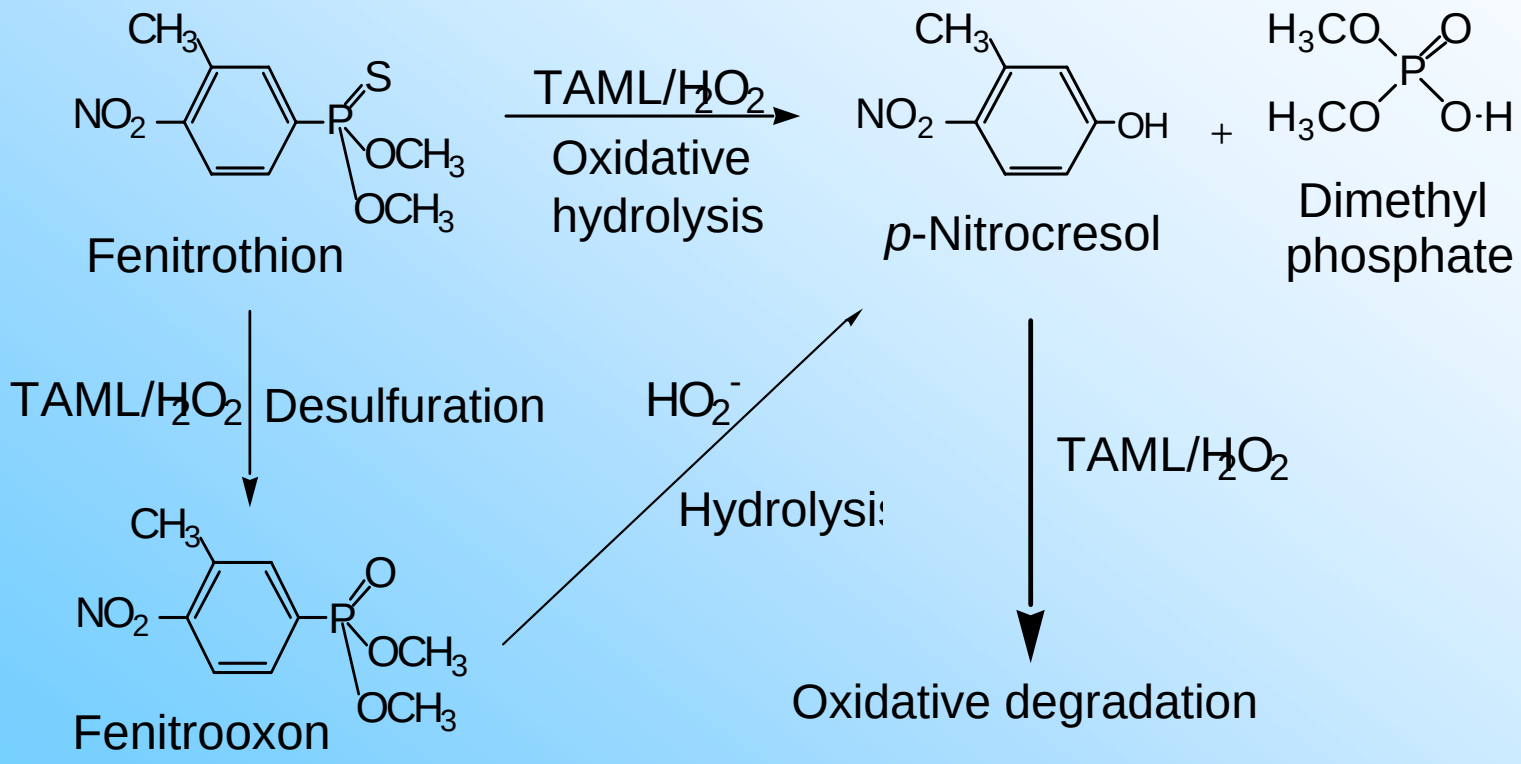




Kinetics of decomposition of fenitrothion is followed through absorption at 396 nm In UV-Vis. Rapid hydrolysis is seen followed by degradation of *p*-nitrocresol.

Time-lapsed analysis of the reaction mixture by HPLC shows initial formation of *p*-nitrocresol and fenitrooxon. In subsequent stage, most of *p*-nitrocresol is degraded.

TAML-activated peroxide decomposition of Fenitrothion



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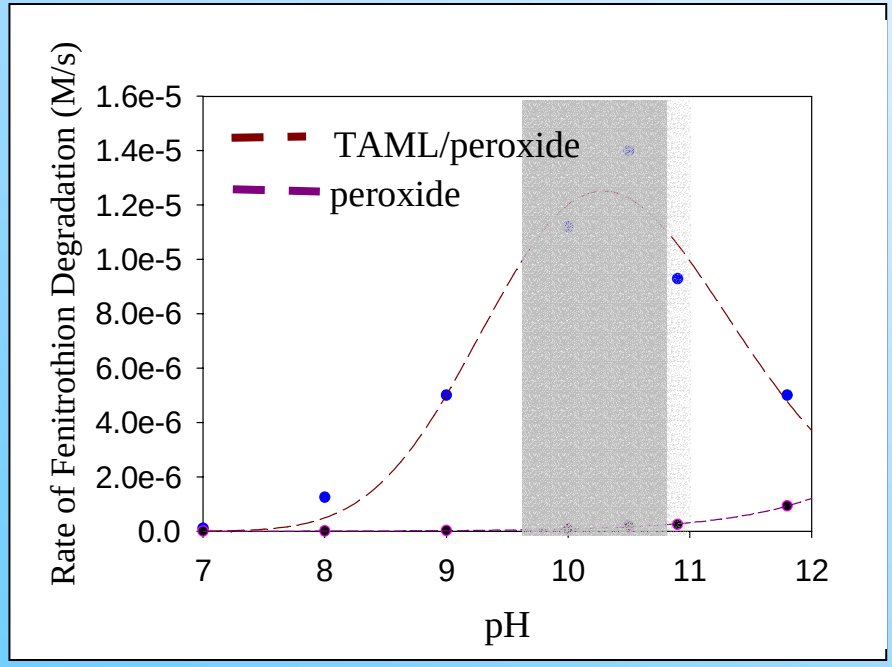
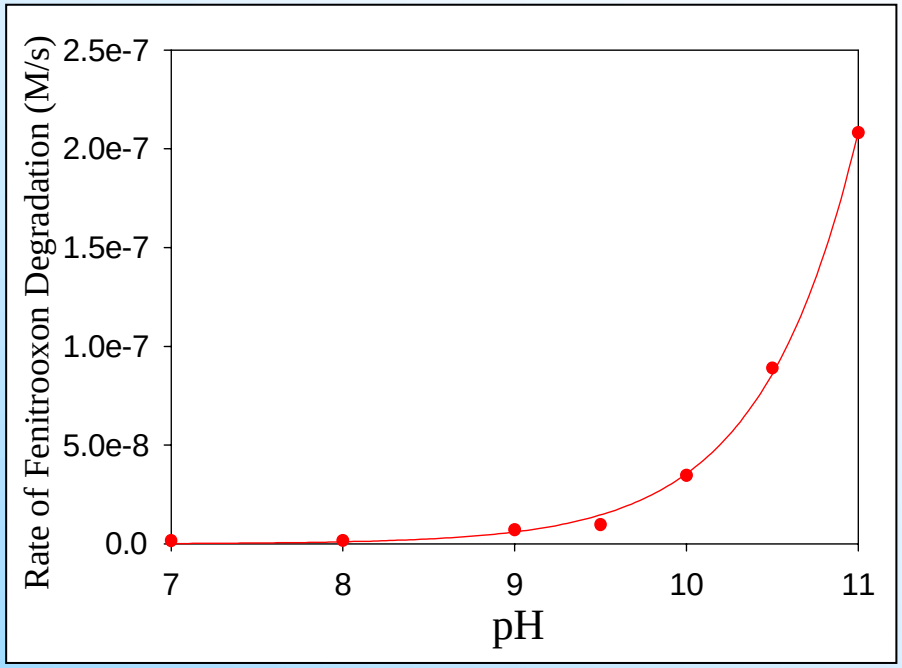
Fenitrothion and Fenitrooxon Degradation - pH Dependence

Initial rate measurements following *p*-nitrocresol formation (395 nm)

TAML/H_2O_2 mediated Fenitrothion degradation



Peroxide assisted Fenitrooxon degradation



TAML®: Fenitrothion: peroxide (1: 25: 50,000) in phosphate buffer (0.1M)

Fenitrooxon: peroxide (1:2000) in phosphate buffer (0.1M)

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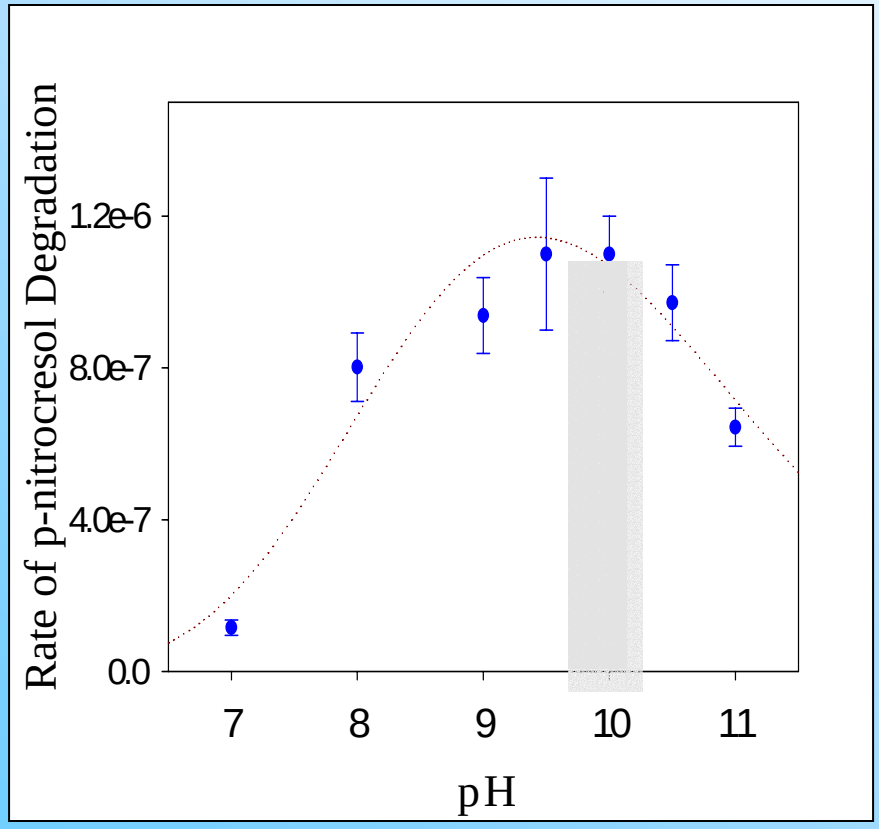
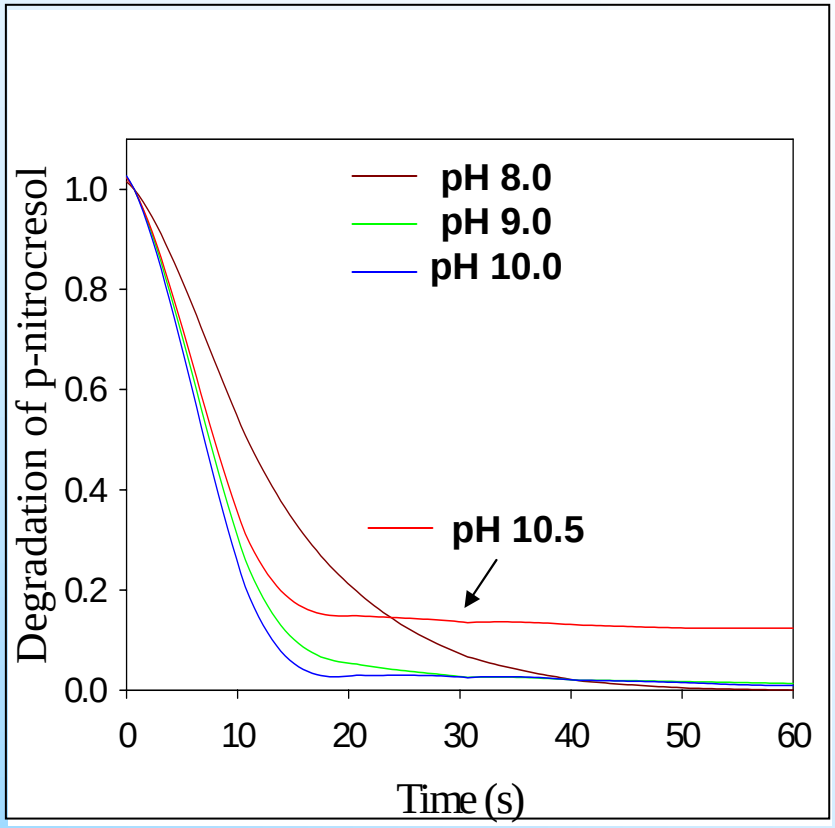
p-Nitrocresol Degradation – pH dependence

Optimization of Reaction Conditions

Initial rate measurements



Kinetics of oxidative degradation



At higher pH, the reaction rate increases, but catalyst gets inactivated faster

Optimum pH range 9.5-10.0

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Summary of Fenitrothion degradation Study

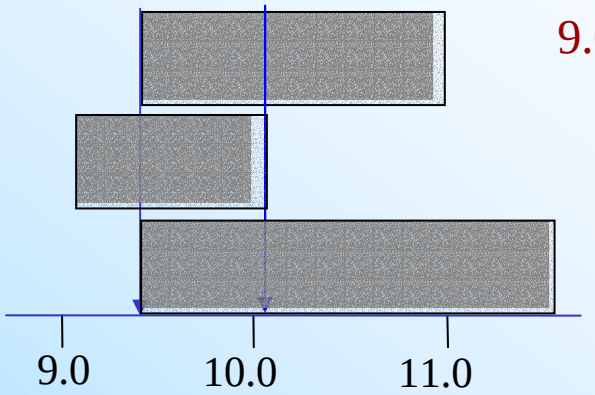
Optimal pH

Limiting condition

Fenitrothion – Oxidative hydrolysis

p-Nitrocresol – Oxidative degradation

Fenitrooxon – Peroxy anion assisted hydrolysis

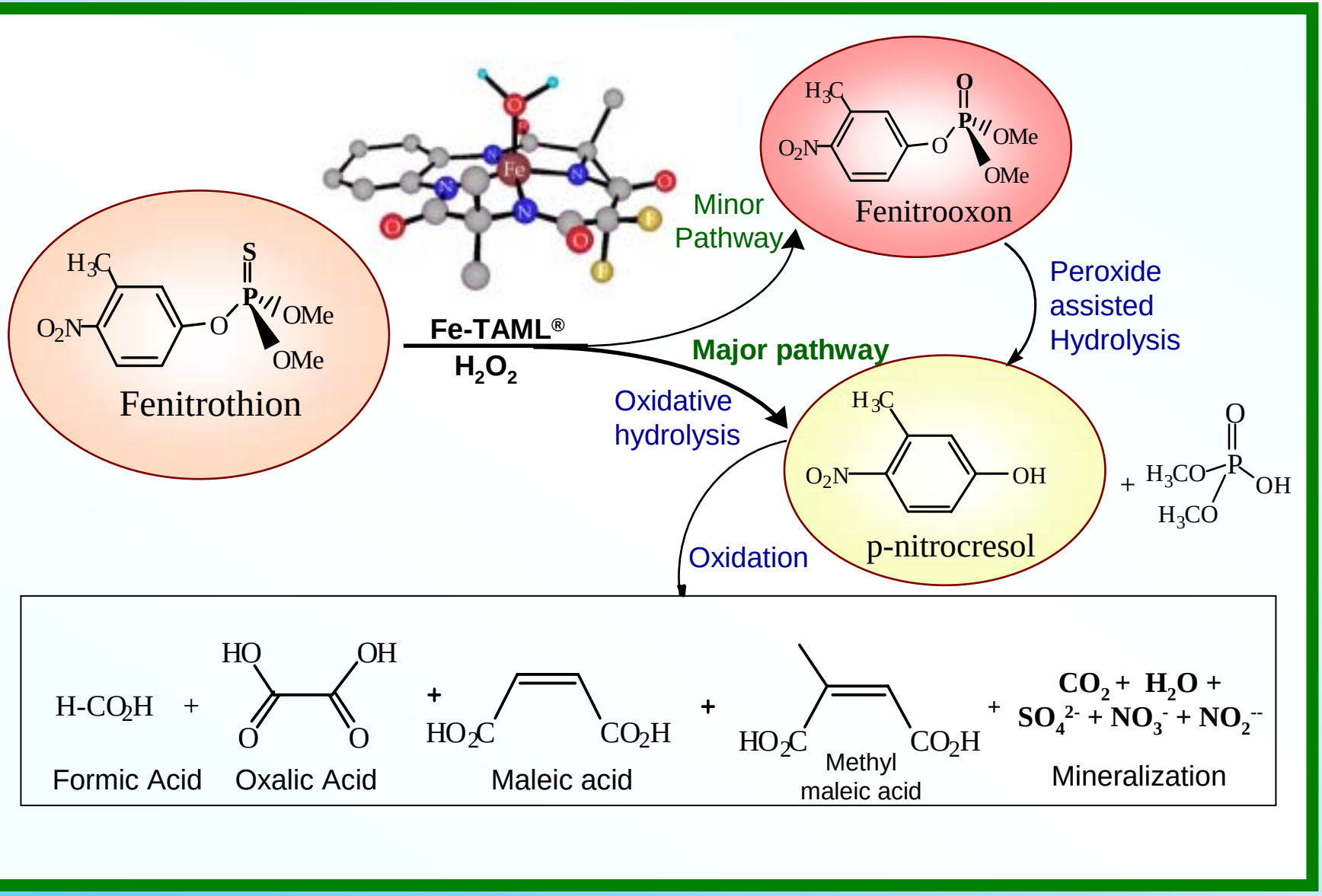


Optimal reaction conditions for Total degradation of fenitrothion

pH 9.5-10.0, phosphate buffer (0.1 M), 25°C

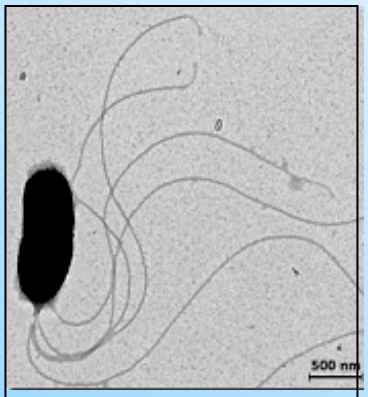
TAML®: Fenitrothion: Peroxide 1 : 25 : 50,000

Total Degradation of Fenitrothion

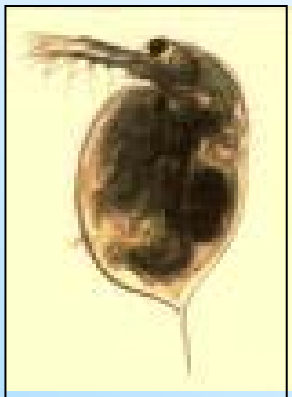


Fenitrothion Degradation

Aquatic Toxicity



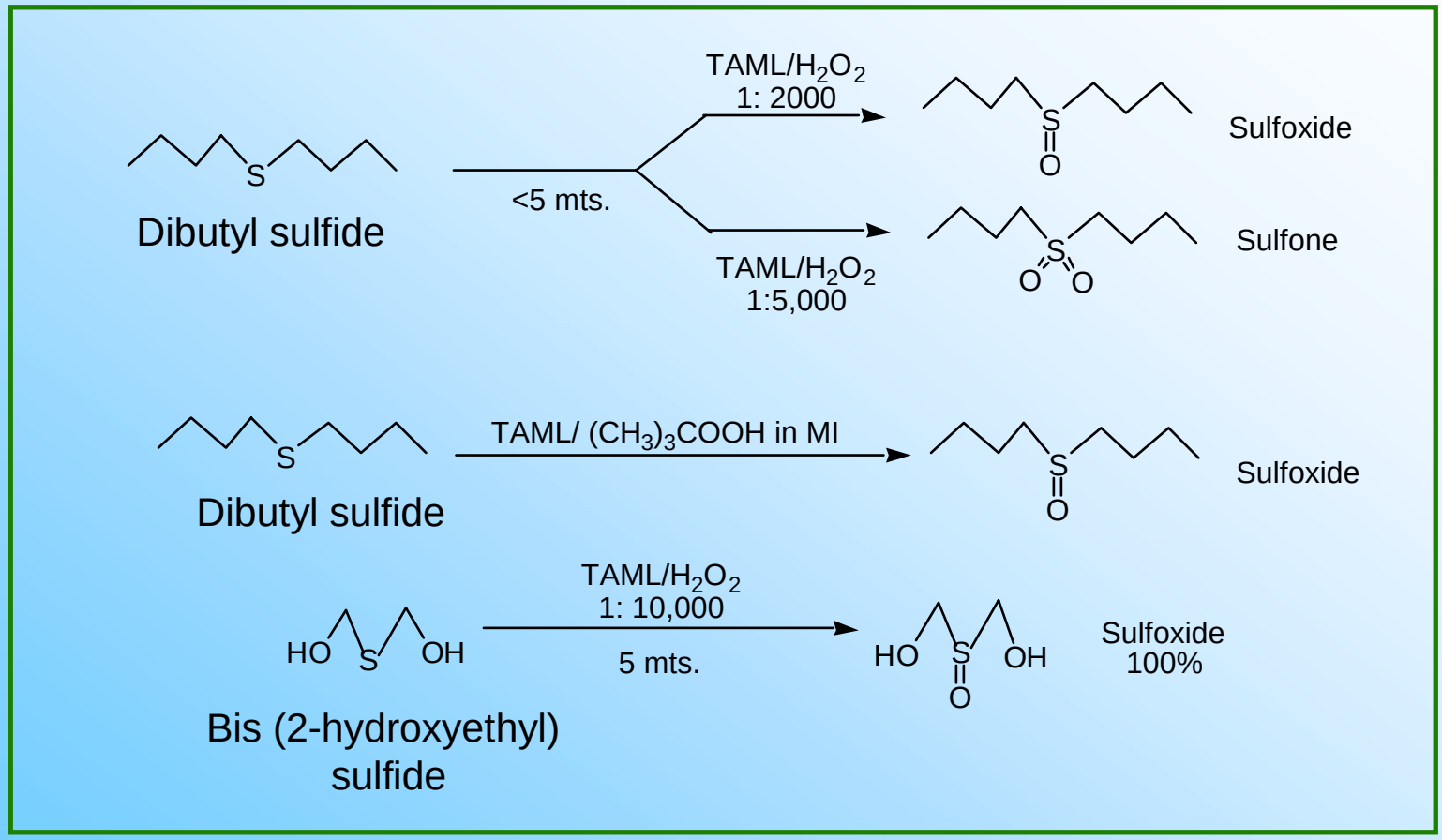
Vibrio fischeri



Dafnia Magna

	MicroTox EC_{50} (15 min.) Mg/L	*D. Magna* EC_{50} Mg/L
Fenitrothion (99%)	2.33	14.1
TAML catalyst ($FeBF_2$)	58.00	NA
Reaction mixture (pH 10, quenched with *catalase*)	57.25	>530
Reduction in toxicity	25-fold	>38-fold

Reaction of Dialkyl sulfides with TAML/peroxide



TAML:substrate = 1:1,000; pH 8; Phosphate buffer, 25°C

Conclusions

TAML®-peroxide technology:

- Effectively deactivate bacterial spores, the toughest of all microorganisms, in aqueous solution achieving 99.99999% (7-log) of spore destruction
- Rapidly detoxify organo-phosphorus triesters, followed by the deep oxidation of hydrolysates
- Selectively oxidize dialkyl sulfides to less toxic sufoxide
- Promises an environmentally friendlier superior technology for destruction of all chemical-biological warfare agents

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New Decon System Features

- **Catalytic –** Requires very low catalyst and low peroxide concentration
- **Designed to be Non-toxic –** No toxic elements or functionality
- **Aqueous based –** Compatible with wide variety of surfaces and technologies; can be used on sensitive equipment
- **Broad-spectrum activity –** Detoxify and degrade large-range of chemicals and inactivate bacterial spores
- **Performance previously unavailable –** Truly biomimetic with deep oxidation capability (leaves no toxic biproducts)
- **Robust system –** Stable and functional over wide range of pH
- **Rapid acting and safe** - for people and environment
- **Easy to use –** Used at ambient conditions, offers a practical approach

Acknowledgements

Anindya Ghosh

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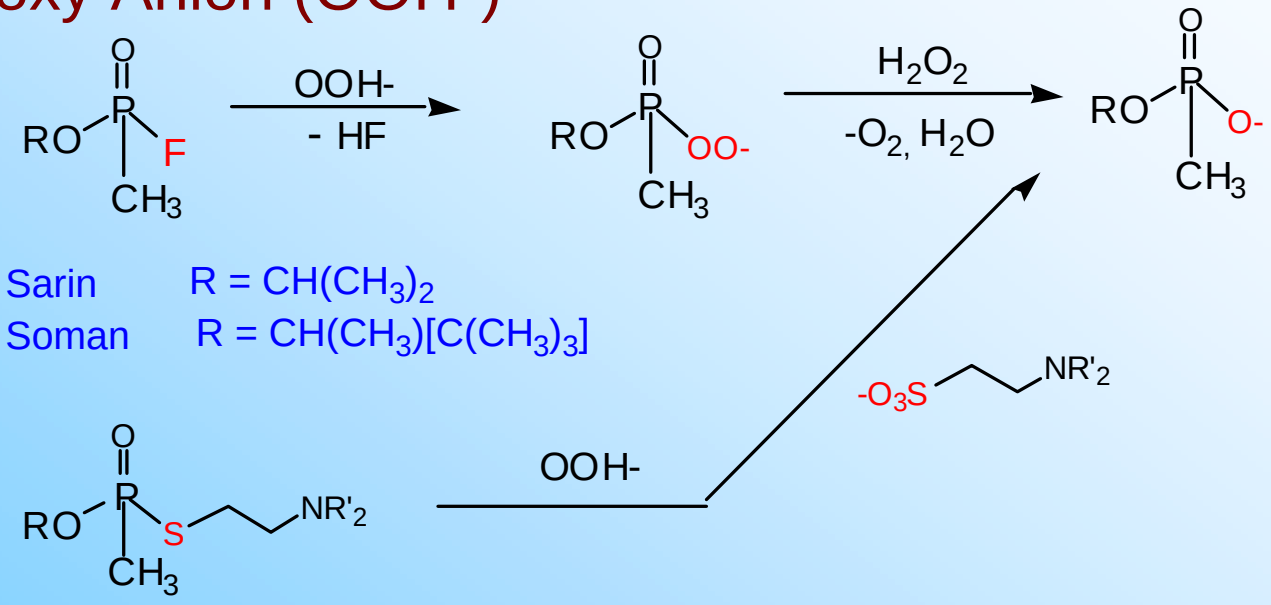
NSF

DURIP

Nucleophile assisted Hydrolytic Detoxification of Chemical Warfare Agents

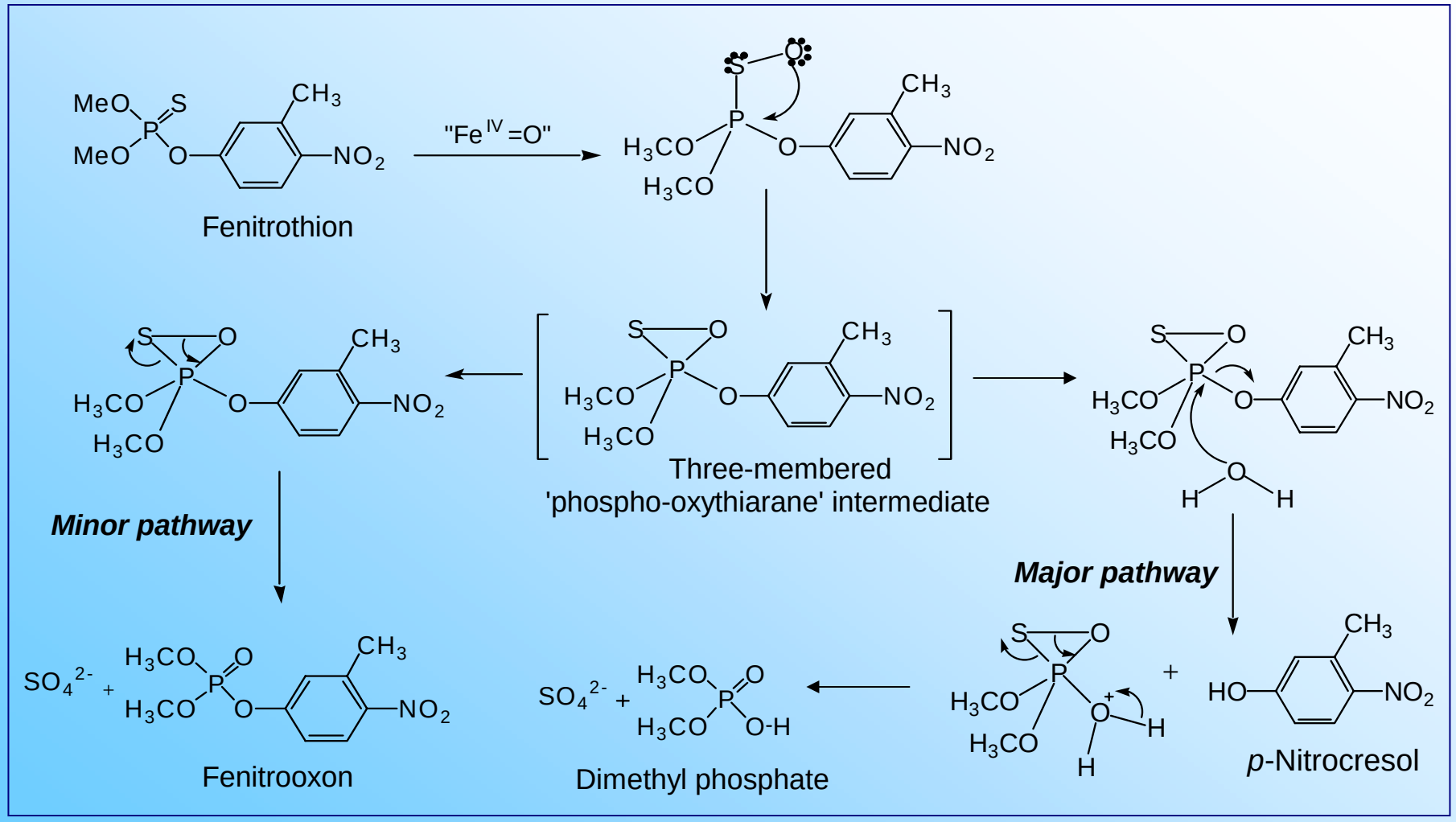
Peroxy Anion (OOH-)



The rate of nucleophile aided hydrolysis of esters is increased by cationic micelles (e.g. $^{-}$OOH/CTABr).

Wagner and Yang, 2002.
Ind. Eng. Chem. Res., 41(8), 1925-1928

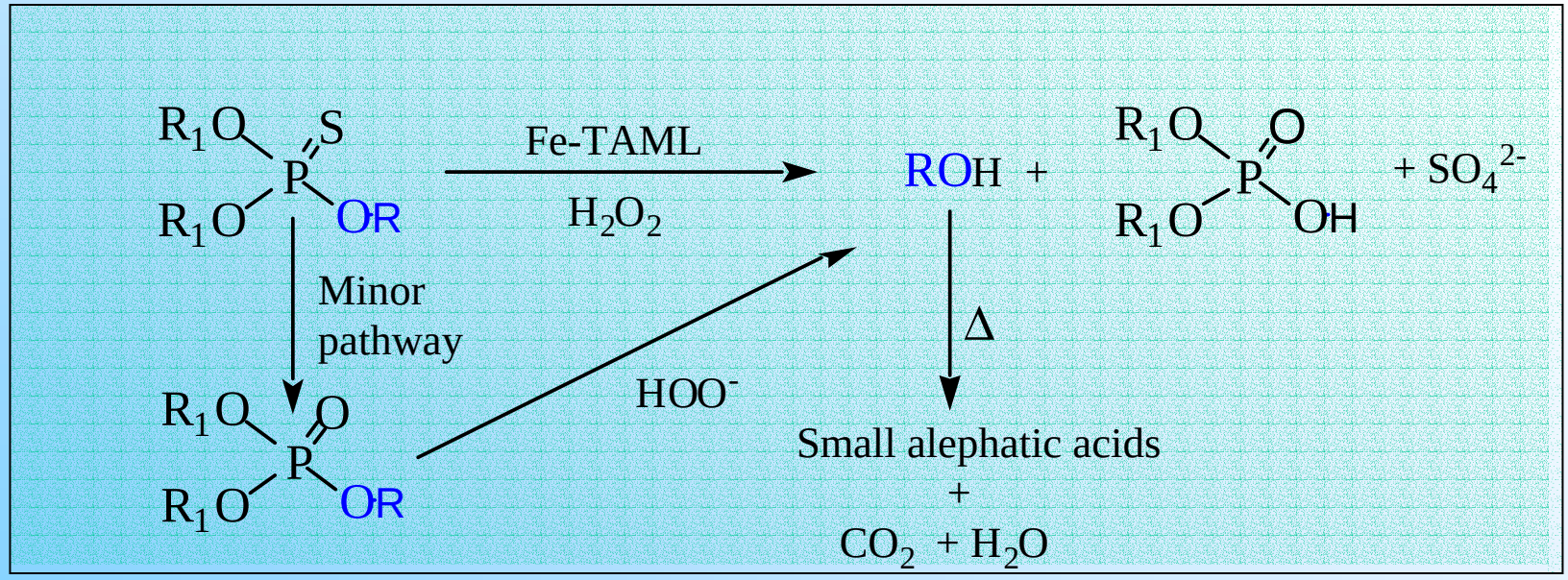
Fe-TAML peroxide oxidant system mimics Cytochrome 450



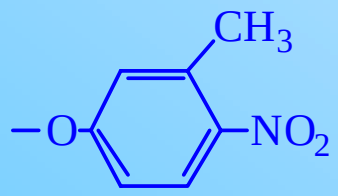
TAML-activated peroxide treatment of fenitrothion possibly results in a common 3-membered ring intermediate formation leading to fenitrooxon and p-nitrocresol

TAML/H_2O_2 Degradation of Organophosphorus Triesters

A Versatile and Robust Process

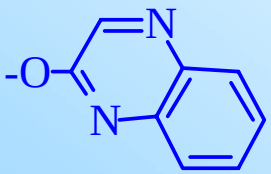


R =



Fenitrothion

Chlorpyriphos



Quinalphos

Diazinon

R_1 = $-CH_3$, $-C_2H_5$

Catalysis of Phosphate Triester Hydrolysis by Cationic Micelles

- Nucleophile (such as peroxide anion) aided hydrolysis is the most preferred reaction to detoxify phosphorus esters.
- The rate of nucleophile aided hydrolysis of esters is increased by cationic micelles (e.g. $^{-}$OOH/CTABr).[1,2]
- CTABr has significantly enhanced hydrolytic rate of phosphorus esters, (depending on substrate, 20-300 fold enhancement) with hypochlorite.[1]
- Aqueous cationic micelles accelerate spontaneous hydrolysis of dinitrophenyl phosphate and acyl phosphate dianions, with an extensive P-O bond cleavage in the transition state.[3]

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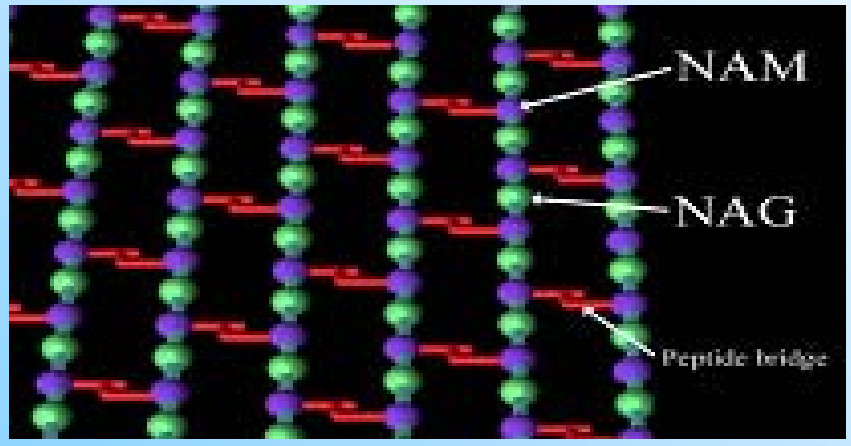
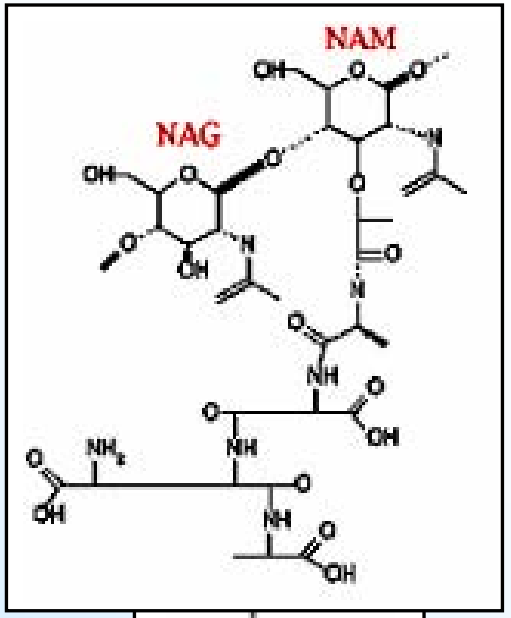
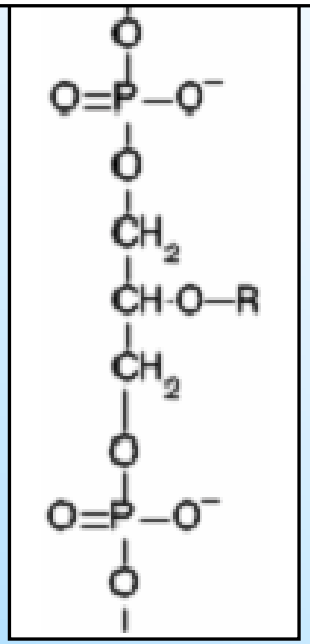
1. Dubey, Gupta et al., *Langmuir*, **2002**, 18, 10489-10492
2. Couderc and Toullec, *Lanmuir*, **2001**, 17, 3819-3828.
3. Brinchi, profio et al., *Langmuir*, **2000**, 16, 10101-10105

Bacterial Endospore
Spore Cortex







Teichoic Acid

- Loosely cross-linked peptidoglycan composed of *N*-acetyl glucosamine and *N*-acetylmuramic acid with short peptide side-chains
- Maintains spore dormancy and heat resistance; hydrolyzes during germination
- An overall negative charge — from the phosphate backbone of teichoic acid (20-40% of dry weight of cortex)