Developing an enzyme-based antifouling coating, part I:

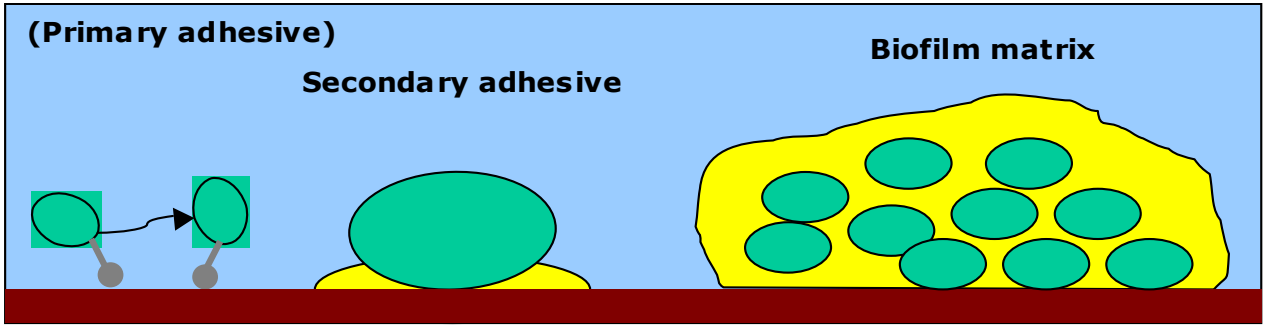
THE ENZYME SYSTEM

Genencor – A Danisco Division, Brabrand, Denmark
iNANO – Interdisciplinary Nanoscience Center, University of Aarhus, Denmark

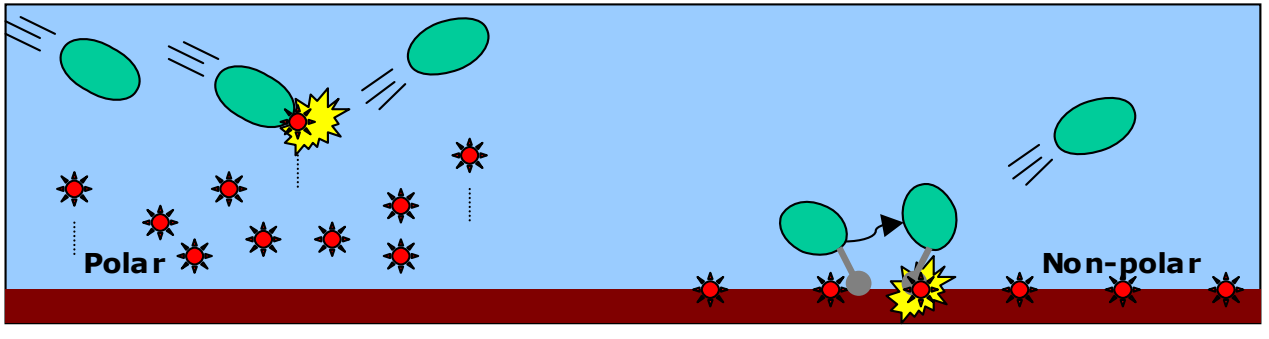
Jakob Broberg Kristensen
g8jgk@danisco.com

14th ICMCF
Kobe, Japan
July 30, 2008

Enzyme-based antifouling



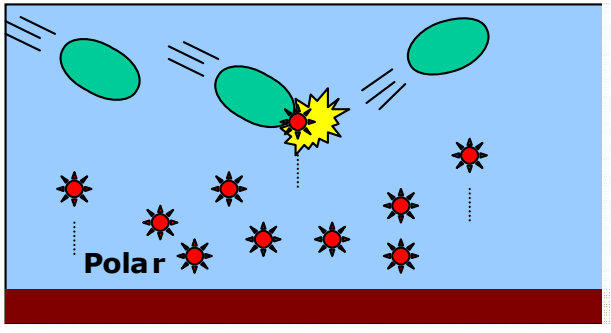
Silicone, super-hydrophobic, super-hydrophilic, nano/micro textured, sol-gel
Hydrolases



Traditional biocide (TBT, CuO), Co-biocides
In-situ generated deterrent

Kristensen *et al.*,
Antifouling enzymes and the biochemistry of marine settlement,
Biotechnology Advances, 2008, 26:471-481

Enzyme-based antifouling



- Hydrogen peroxide produced *in-situ* from starch by an enzyme catalysed reaction
- Continuous release
- Strong case for paint compatibility and antifouling effect
- Prototype coatings in field trials

Kristensen *et al.*,
Antifouling enzymes and the biochemistry of marine settlement,
Biotechnology Advances, 2008, 26:471-481

Key points in developing antifouling coatings

GENENCOR®
A Danisco Division

- Effective release of **ANTIFOULING AGENT**
- Well defined (limited) **ENVIRONMENTAL FATE** and impact
- **REGULATORY** registration
- **PAINT COMPATIBILITY**
- **COLLABORATION** between agent developers and paint producers

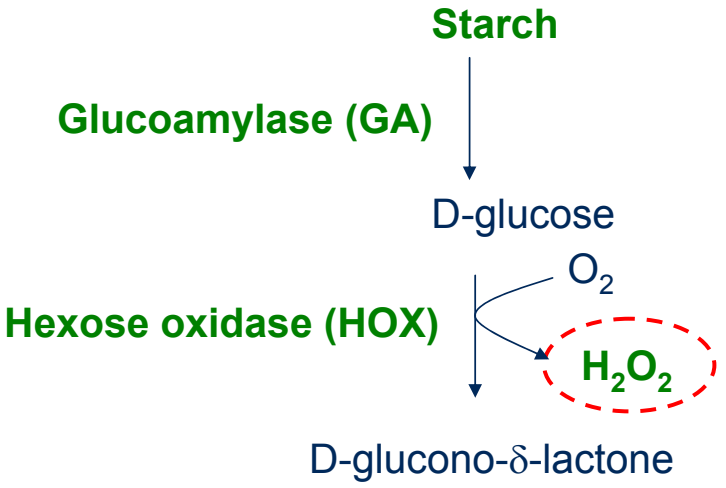
Adapted from Dan Rittschof *et al*. (2003) *Biofouling* 19:207-212

iNANO

ANTIFOULING AGENT

Enzymatic formation of hydrogen peroxide

GENENCOR®
A Danisco Division



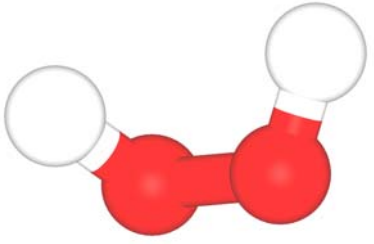
Chondrus cripus

iNANO

ANTIFOULING AGENT
Hydrogen peroxide effects

GENENCOR®
A Danisco Division

- Remove ectoparasites from Atlantic salmon[1].
- Moderate toxicity to aquatic organisms[2]
  - Algae
  - Daphnia
  - Fish
- Reduce macrofouling in marine cooling water systems[3]
  - + ferrous ions → active oxygen radical



1 Thomassen J. In: Boxshall G, DeFaye D, editors. Pathogens of wild and farmed fish: sea lice. Ellis Horwood Limited, 1993. p. 290-5

2 ECETOC. Joint Assessments of Commodity Chemicals No. 22, Hydrogen Peroxide. 1993

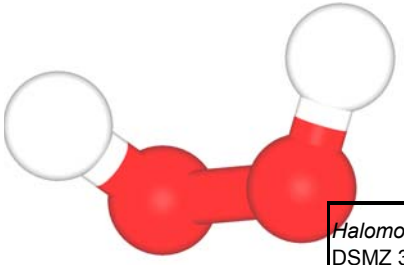
3 Nishimura K et al. Marine Biology 1988;99:145-50

iNANO

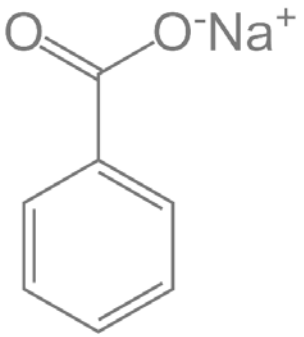
ANTIFOULING AGENT

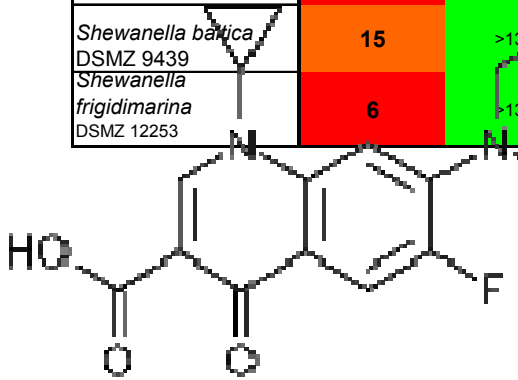
Bacterial growth inhibition (Minimum Inhibitory Concentration)

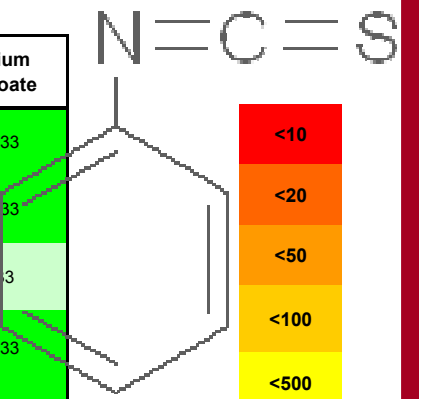
GENENCOR® A Danisco Division

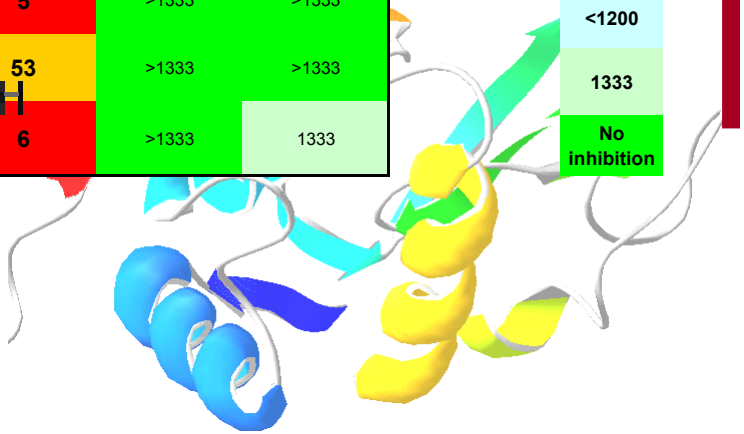


	Ciprofloxacin	Phenyl isothio-cyanate	Hydrogen peroxide	Lysozyme	Sodium benzoate
Halomonas variabilis DSMZ 3051	4	>1333	119	>1333	>1333
Cobetia marina DSMZ 4741	4	>1333	99	>1333	>1333
Halomonas pacifica DSMZ 4742	4	>1333	5	>1333	1333
Shewanella putrefaciens DSMZ 6067	15	>1333	79	>1333	>1333
Pseudoalteromonas atlantica DSMZ 6839	24	>1333	1200	>1333	>1333
Pseudoalteromonas citrea DSMZ 8771	9	>1333	5	>1333	>1333
Shewanella baltica DSMZ 9439	15	>1333	53	>1333	>1333
Shewanella frigidimarina DSMZ 12253	6	>1333	6	>1333	1333









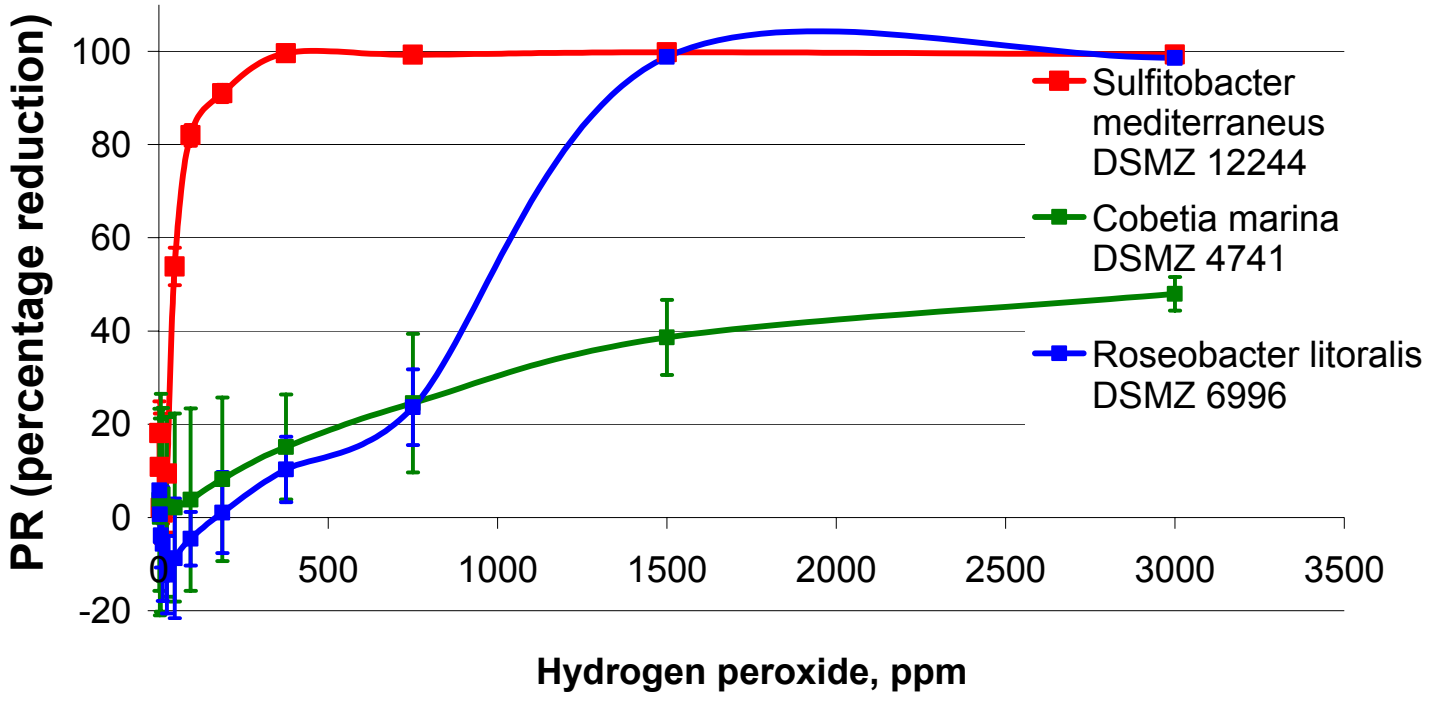
<10
<20
<50
<100
<500
<1000
<1200
1333
No inhibition

iNANO

ANTIFOULING AGENT
Biofilm

GENENCOR
A Danisco Division

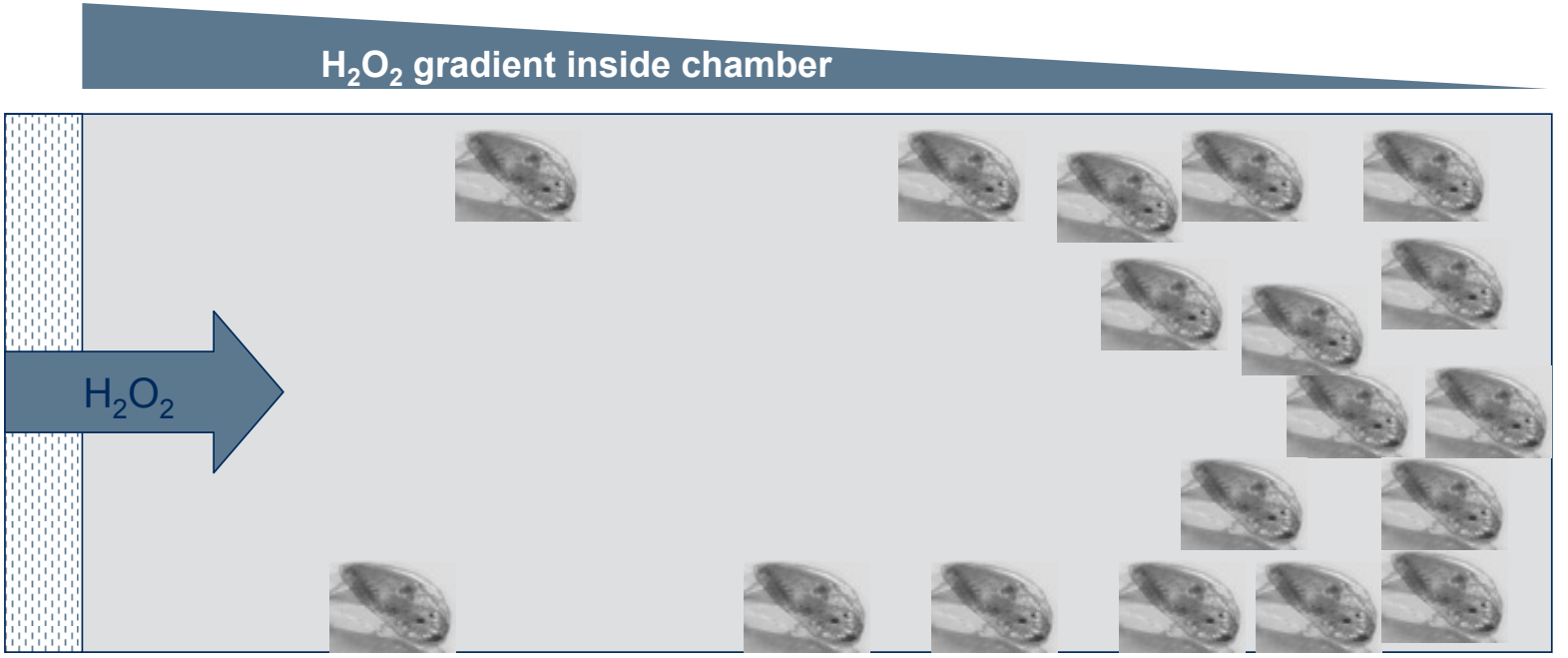
Biofilm inhibition in microtiter plates



iNANO

ANTIFOULING AGENT

Flux chamber experiment



Based on observations from Olsen, SM (Hempel, 2007)

ANTIFOULING AGENT
The REAL test

- Raft trials - Hempel
  - Barnacles
  - Seaweed
- Microscope slide tests – TNO
  - Barnacle settlement
  - Ulva zoospore adhesion test
- Raft trials - TNO

Photos courtesy of Hempel A/S

ENVIRONMENTAL FATE and impact

- $2\ H_2O_{2(aq)} \rightarrow 2\ H_2O_{(l)} + O_{2(aq)}$
  - ΔH= -98.20 kJ/mol[1]
- Bioaccumulation unlikely[2]
- Potentially an efficient non-ecotoxic biocide
- Considerable environmental improvement over current antifouling biocide technologies.
- Enzymes degraded upon release

1. Eul W et al. Hydrogen peroxide. In: Kirk-Othmer Encyclopedia of Chemical Technology. 8. ed., Wiley-VCH, 2002.
2. Jacobi S. Hydrogen peroxide. In: Ullmann's Encyclopedia of Industrial Chemistry. 7. ed., Wiley-VCH, 2002.

REGULATORY
From food to paint

GENENCOR®
A Danisco Division

- All enzymes and substrates are used in food technology
- Extensive documentation of toxicology
- **G**enerally **R**ecognized **A**s **S**afe

- Hydrogen peroxide is well described
- Extensively used
- GRAS

- Large portfolio ready for registration
- ➲ Shorter time-to-market

iNANO

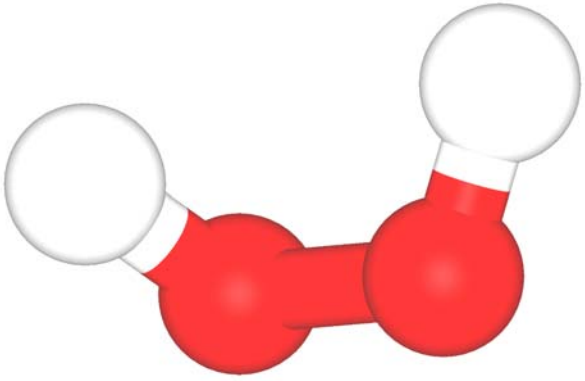
PAINT COMPATIBILITY

Why we need enzymes in the coating!

GENENCOR®
A Danisco Division

Hydrogen peroxide – not exactly paint-friendly!

- Highly oxidative
  - High concentrations may damage coating
- Unstable
  - Will not persist through coating lifetime



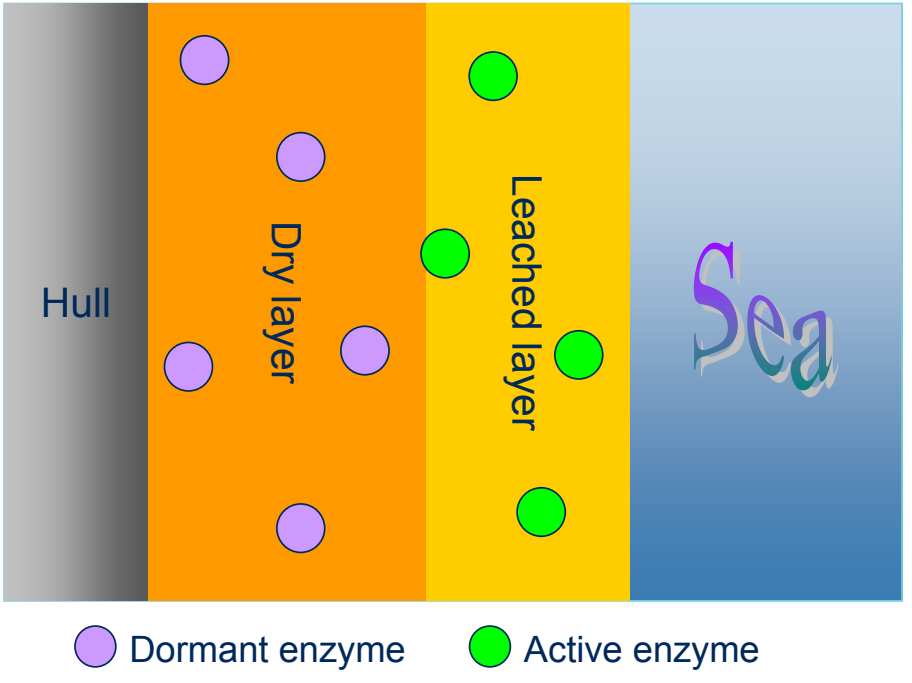
- Need for production *in-situ* and when needed for transport out of coating

iNANO

PAINT COMPATIBILITY

”On-demand” enzyme activity

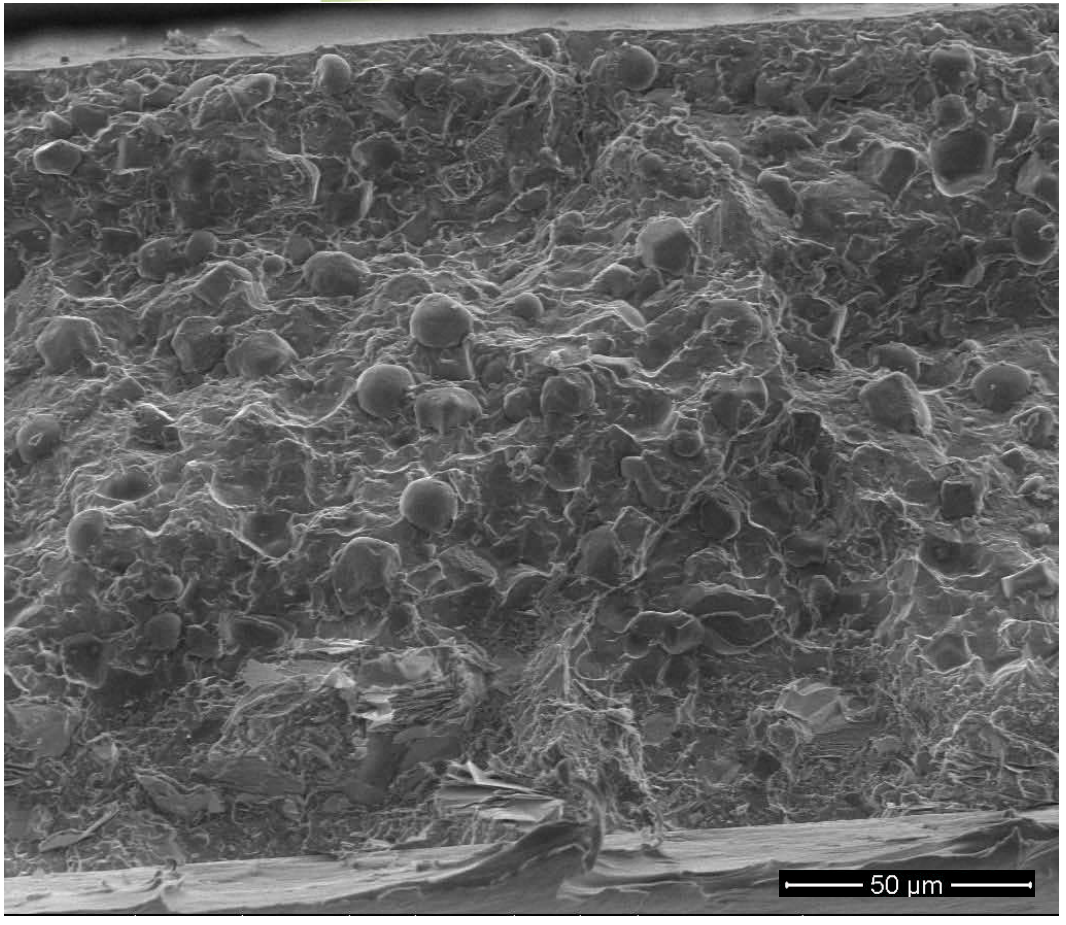
GENENCOR®
A Danisco Division



iNANO

PAINT COMPATIBILITY

SEM of starch granules in a paint

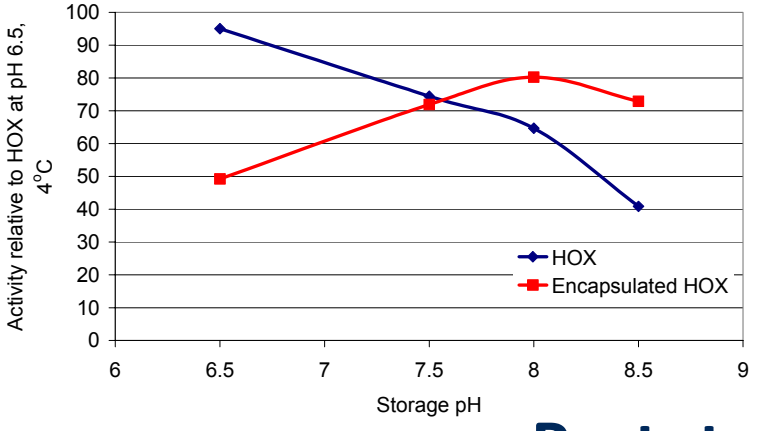


PAINT COMPATIBILITY

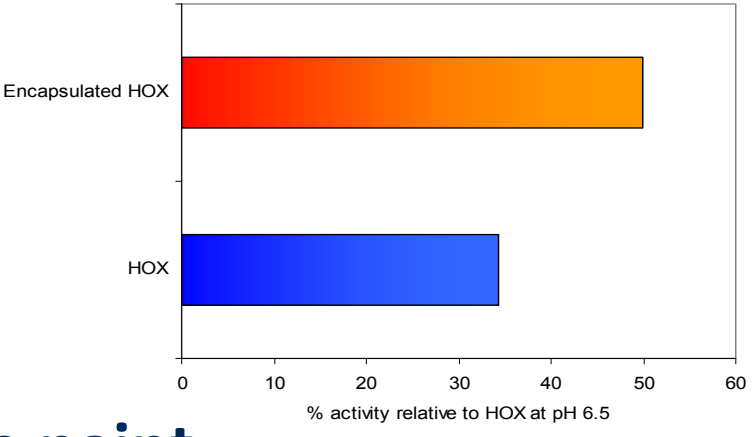
Enhanced marine enzyme stability by encapsulation

GENENCOR® A Danisco Division

Activity after 24h storage at RT



Activity at pH 8



Prototype paint:

More than 150nmol H_2O_2 released per cm^2 per day after 2 months in seawater without polishing

iNANO

COLLABORATION
Acknowledgments

Brian Søgaard LAURSEN, Scientist, supervisor

Charlotte Horsmans POULSEN, Development director, senior supervisor

Charlotte Adamczky BAK, Laboratory technician

Rikke Louise MEYER, Associate professor, supervisor

Flemming BESENBACHER, Professor, senior supervisor

ACADEMIA

INDUSTRY

Stefan Møller OLSEN, Industrial PhD student

Lars Thorslund PEDERSEN, Scientist

Søren KIIL, Associate professor

Merete Høgh HERMANN, Scientist

Summary

- The Starch-Glucoamylase-Hexose oxidase system producing hydrogen peroxide is effective against marine bacteria and biofilm.
- Macrofouling studies and field trials are under way
- Prototype coatings work as intended
- Biodegradable, non-persistent and non-bioaccumulating active components
- Safe, easily implementable production process
- The ”holistic” approach: design enzymes and coatings interdependently