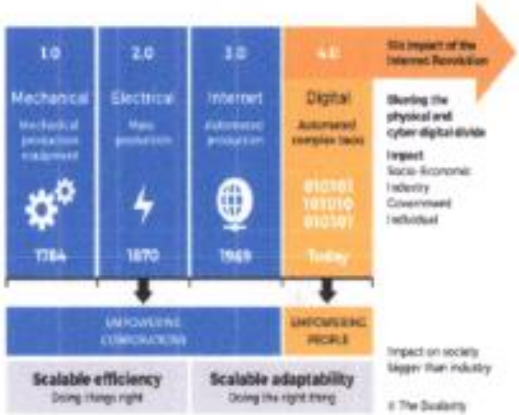
Dept. Biology ITS Surabaya

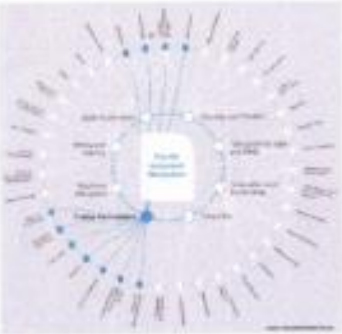
- Biomaterial and Enzyme Technology Research Group
  - > 50 peer reviewed publications
  - 2 book chapters
- Antioxidant measuring system
- Polymer processing
  - Functionalisation of synthetic and natural polymers
  - Enzymes: Hydrolases,Oxidoreductases
- Enzymology
  - New enzymes, screening procedures
  - Biochemical characterisation
  - Biotransformation pathways
- **Contact: endry@bio.its.ac.id**

Welcome to the Industrial Revolution 4.0



INDUSTRY 4.0
FUSING DIGITAL, PHYSICAL & BIOLOGICAL

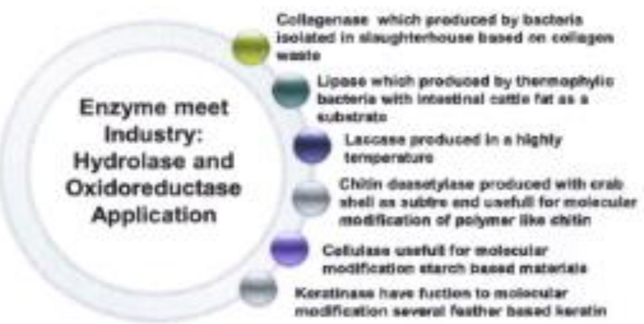
"The fourth industrial revolution, is not only about the smart and connected machines and systems. Its scope is much wider. Occurring simultaneouslyare waves of further breakthroughs in areas ranging from gene sequencing to nanotechnology, from renewables to quantum computing. It is the fusion of these technologies and their interaction across the physical, digital and biological domains that make the fourth industrial revolution fundamentally different from previous revolutions." Klaus Schwab, founder and Executive Chairman of the World Economic Forum

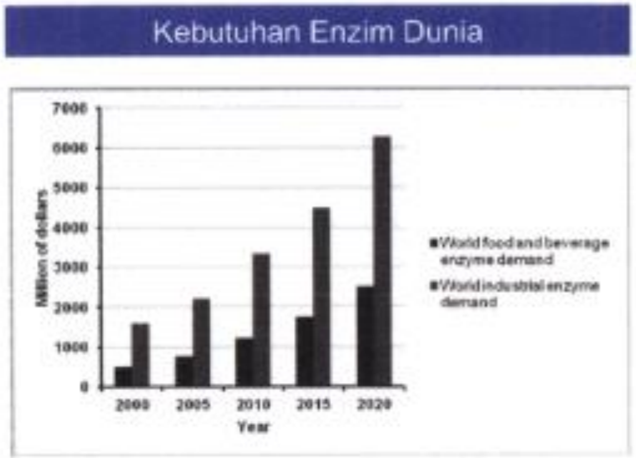


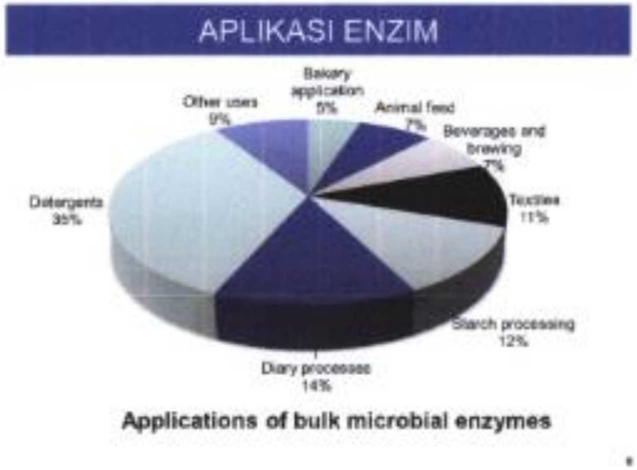
"I think the biggest innovations of the 21st century will be at the intersection of biology and technology. A new era is beginning."
-Steve Jobs (2011)

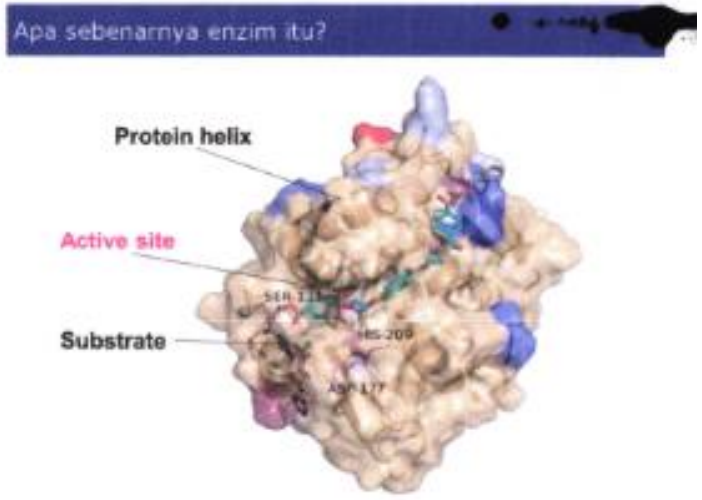
ENZYME. NOWDAYS

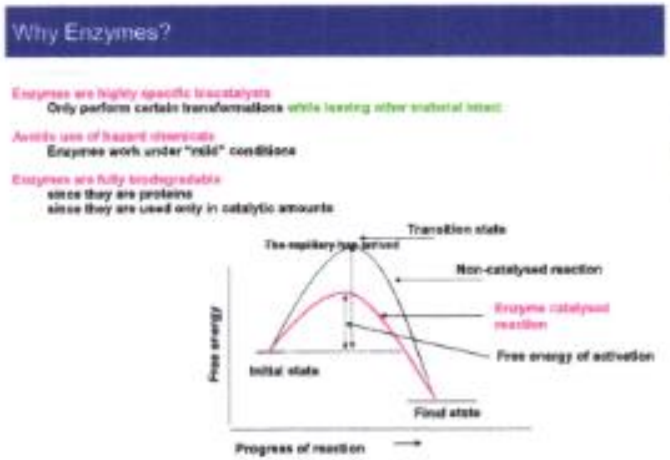
Biomaterial and enzyme technology research group well managed to develop several enzymes

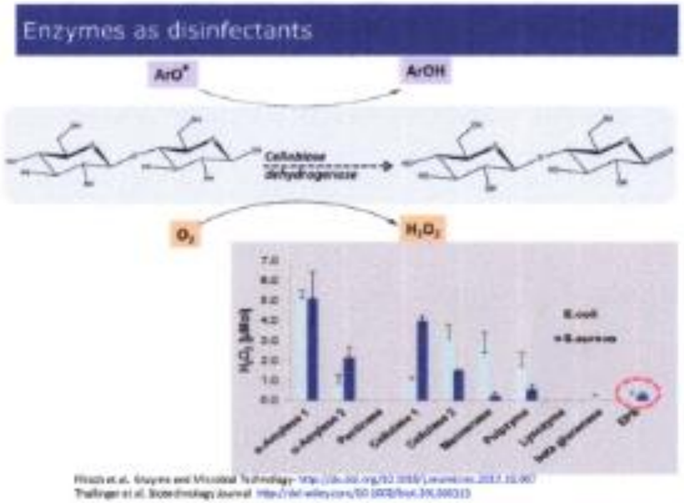



Kebutuhan Enzim Dunia
7000
6000
5000
4000
3000
2000
1000
0
Million of dollars
2000
2005
2010
2015
2020
Year
World food and beverage enzyme demand
World industrial enzyme demand

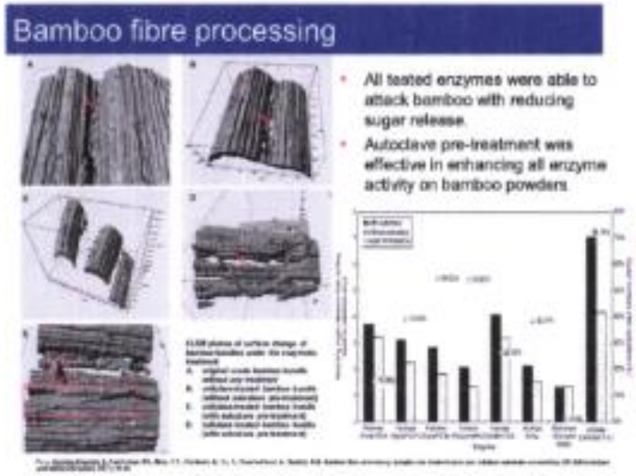

APLIKASI ENZIM
Bakery application 5%
Animal feed 7%
Beverages and brewing 7%
Textiles 11%
Starch processing 12%
Diary processes 14%
Detergents 35%
Other uses 9%
Applications of bulk microbial enzymes

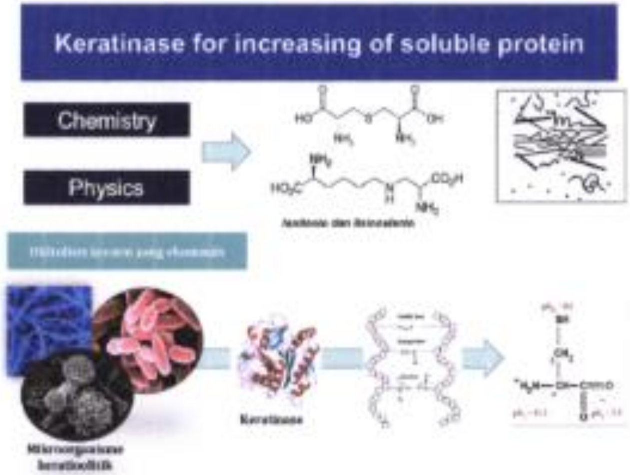

Apa sebenarnya enzim itu?
Protein helix
Active site
Substrate


Why Enzymes?
Enzymes are highly specific biocatalysts
Only perform certain transformations while leaving other material intact
Avoids use of hazard chemicals
Enzymes work under "mild" conditions
Enzymes are fully biodegradable
since they are proteins
since they are used only in catalytic amounts
Transition state
Non-catalysed reaction
Enzyme catalysed reaction
Free energy of activation
Free energy
Initial state
Final state
Progress of reaction

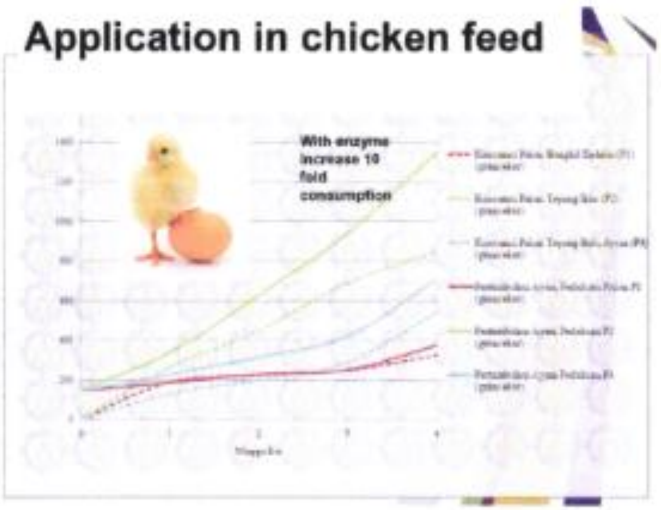

Enzymes as disinfectants
ArO*
ArOH
Cellobiose dehydrogenase
O_2
H_2O_2
E.coli
S.aureus

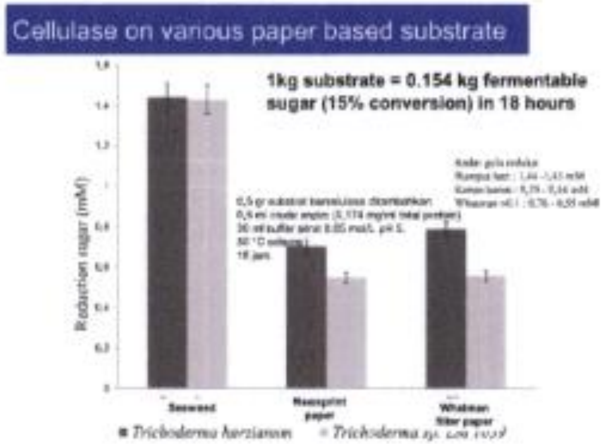

Environmental friendly processes
Desizing
Amylases
Scouring
Bleaching
Glucose oxidase, Lignin peroxidase, Manganese peroxidase, Laccase
Stone washing
1 Kg of stones for 1 Kg of jeans
Cellulases and laccase-mediator systems
Bleach Clean-up
Catalase
Waste effluents, dyes
oxidoreductases
CDH

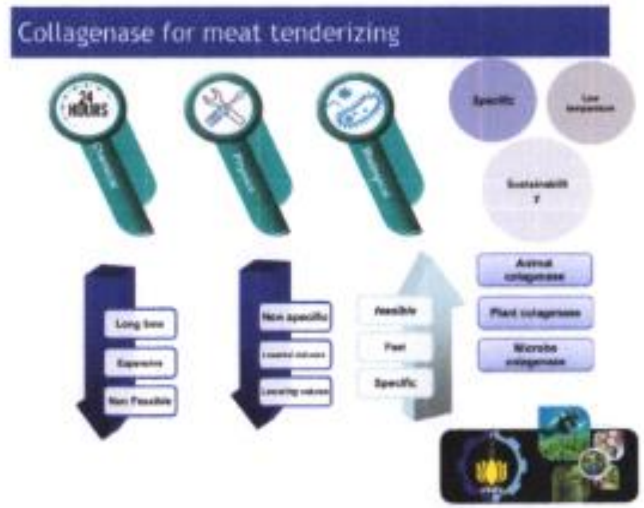

Bamboo fibre processing
All tested enzymes were able to attack bamboo with reducing sugar release.
Autoclave pre-treatment was effective in enhancing all enzyme activity on bamboo powders.


Keratinase for increasing of soluble protein
Chemistry
Physics
Keratinase

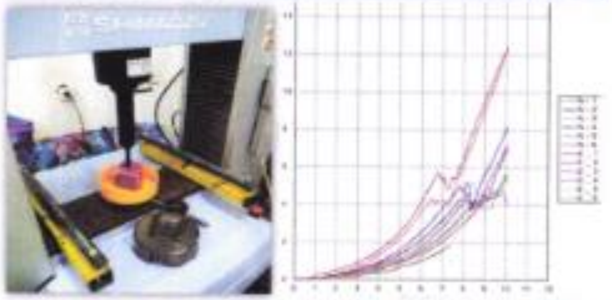

Biocatalysis keratin based waste
Rambut Sapi
Rambut Manusia
Rambut Anjing
Rambut Domba


Application in chicken feed
With enzyme increase 10 fold consumption


Cellulase on various paper based substrate
1kg substrate = 0.154 kg fermentable sugar (15% conversion) in 18 hours
Reduction sugar (mM)
Seaweed
Newsprint paper
Whatman filter paper
Trichoderma harzianum

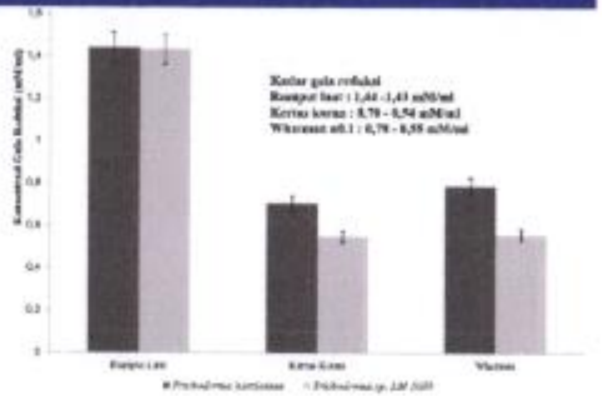

Collagenase for meat tenderizing
Specific
Long time
Expensive
Non Feasible
Non specific
Feasible
Fast
Specific
Animal collagenase
Plant collagenase
Microbe collagenase

Meat softness test

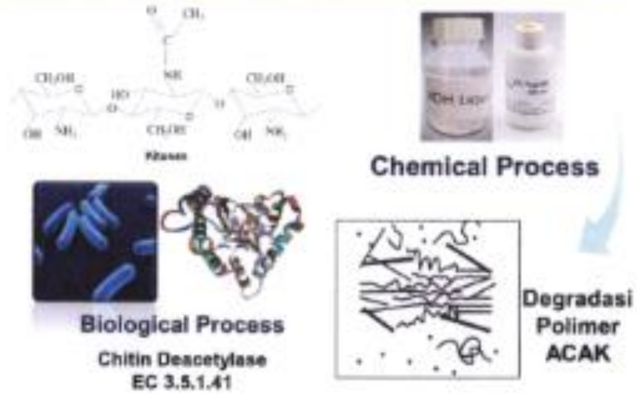


21% tenderized meat in 15 min by collagenase treatment at room temperature

Aplikasi Enzim Pada Substrat



Enzymatic production of chitosan



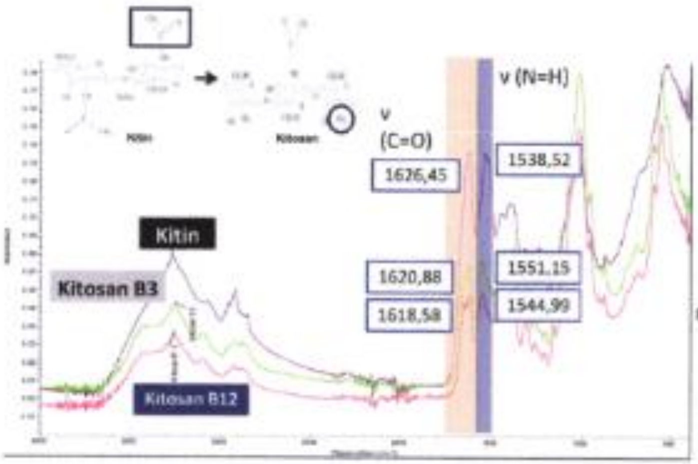
Degree of deacetylation by different source of chitin deacetylase

Reaction at 50 °C for 48 hr

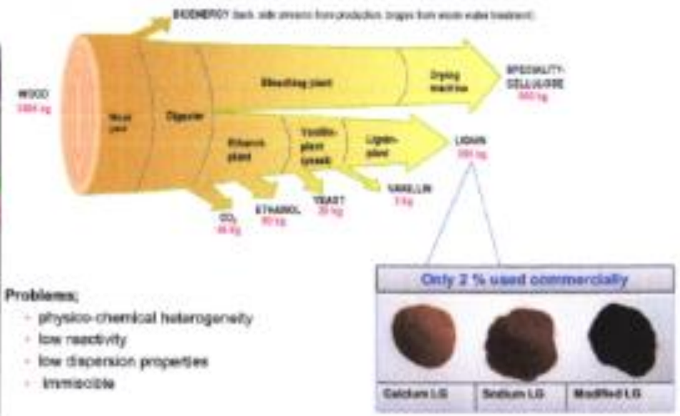
Analysis	[illegible]	Degree of deacetylation after enzymatic treatment			
		[illegible]	[illegible]	[illegible]	[illegible]
Glucosamine standard (μM)	219	585	454	483	533
Degree of deacetylation (%)	< 50	63	52	55	59

Synowiecki et al., (2003), degree of deacetylation of chitin more than 50% has transformed become chitosan

FTIR spectra of enzymatic chitosan

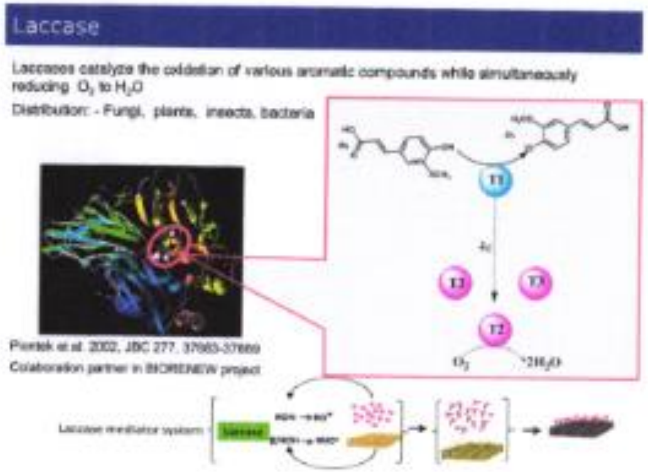


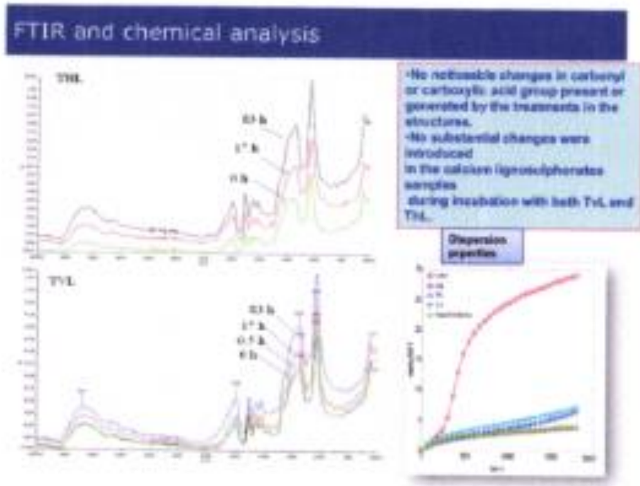
Lignin

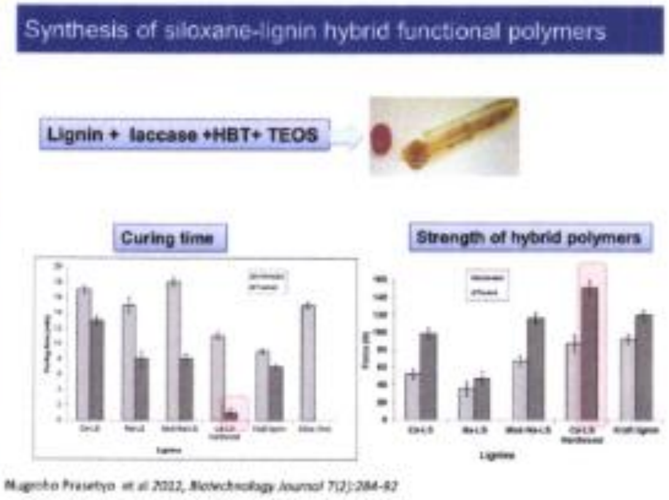


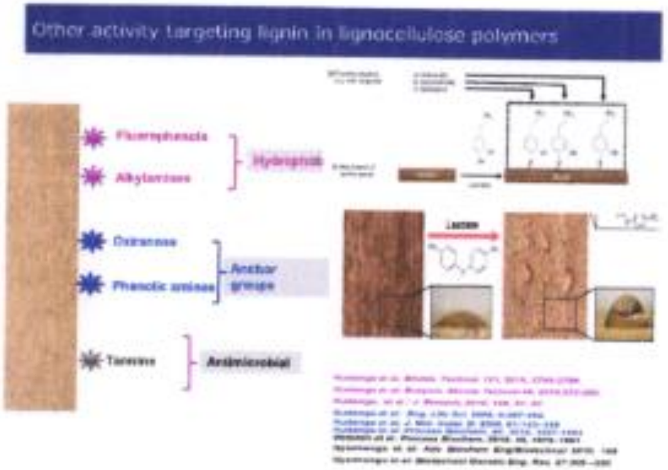
Problems:

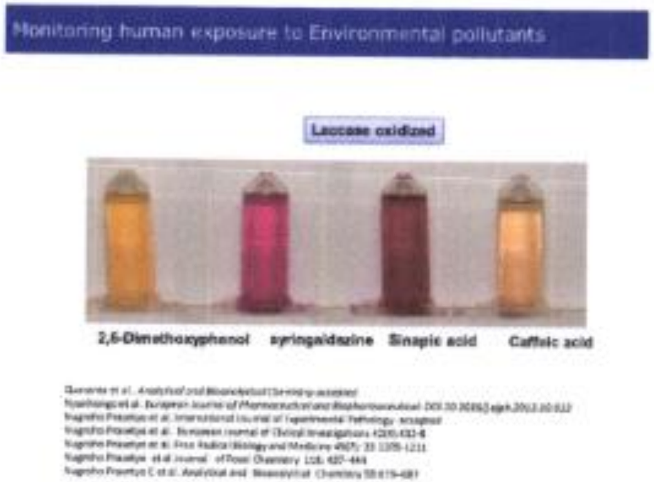
- physico-chemical heterogeneity
- low reactivity
- low dispersion properties
- immiscible

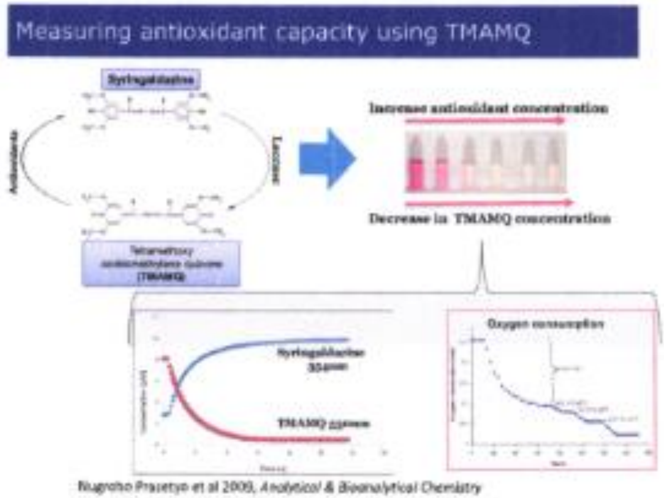

Laccase
Laccases catalyze the oxidation of various aromatic compounds while simultaneously reducing O_2 to H_2O
Distribution: - Fungi, plants, insects, bacteria
T1
T2
T3
Piontek et al. 2002, JBC 277, 37663-37669
Collaboration partner in BIORENEW project
Laccase mediator system
Laccase


FTIR and chemical analysis
ThL
TvL
-No noticeable changes in carbonyl or carboxylic acid group present or generated by the treatments in the structures.
-No substantial changes were introduced in the calcium lignosulphonates samples during incubation with both TvL and ThL.
Dispersion properties

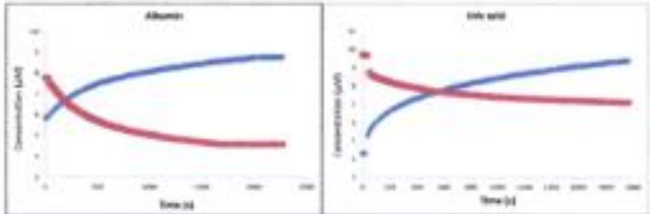

Synthesis of siloxane-lignin hybrid functional polymers
Lignin + laccase +HBT+ TEOS
Curing time
Strength of hybrid polymers
Nugroho Prasetyo et al 2012, Biotechnology Journal 7(2):284-92


Other activity targeting lignin in lignocellulose polymers
Fluorophenols
Alkylamines
Hydrophob
Coirenose
Phenolic amines
Anchor groups
Tannins
Antimicrobial
Laccase


Monitoring human exposure to Environmental pollutants
Laccase oxidized
2,6-Dimethoxyphenol
syringaldazine
Sinapic acid
Caffeic acid

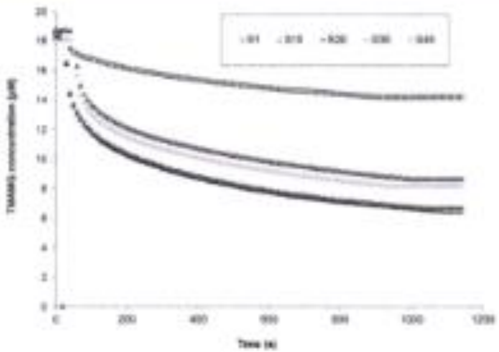

Measuring antioxidant capacity using TMAMQ
Syringaldazine
Antioxidants
Laccase
Increase antioxidant concentration
Decrease in TMAMQ concentration
Oxygen consumption
TMAMQ
Nugroho Prasetyo et al 2009, Analytical & Bioanalytical Chemistry

Monitoring reduction of TMAMQ and formation syringaldazine

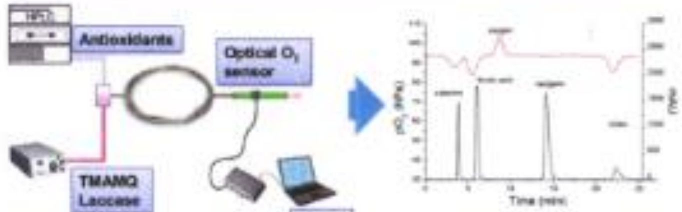


Antioxidant	Reduced TMAMQ (µM)	Formed syringaldazine (µM)
Alpha Tocopherol	5.09 ± 0.07	4.98 ± 0.9
Gamma Tocopherol	1.18 ± 0.04	1.16 ± 0.05
Trolox	4.75 ± 0.04	4.64 ± 0.05
Uric acid	2.60 ± 0.02	2.82 ± 0.03
Ascorbic acid	4.44 ± 0.03	4.50 ± 0.05

Monitoring antioxidant capacity of human serum samples

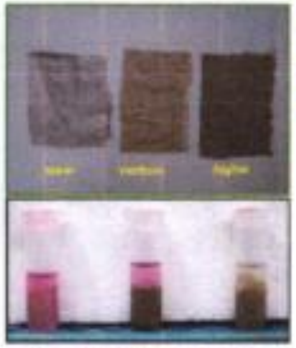


Online HPLC and rapid test antioxidant activity



Quaranta et al 2013 *Analytical and Bioanalytical chemistry*

Fabric antioxidant



Blind fabrics samples to which flavonoids had been enzymatically grafted were able to quench TMAMQ by concentration rank of grafted flavanoid

Sample code	TMAMQ consumed (µmol/cm² of fabric)
1	[illegible]
2	[illegible]
3	[illegible]
4	[illegible]
5	[illegible]
6	[illegible]
7	[illegible]
8	[illegible]
9	[illegible]
10	[illegible]
11	[illegible]
12	[illegible]
13	[illegible]
14	[illegible]
15	[illegible]

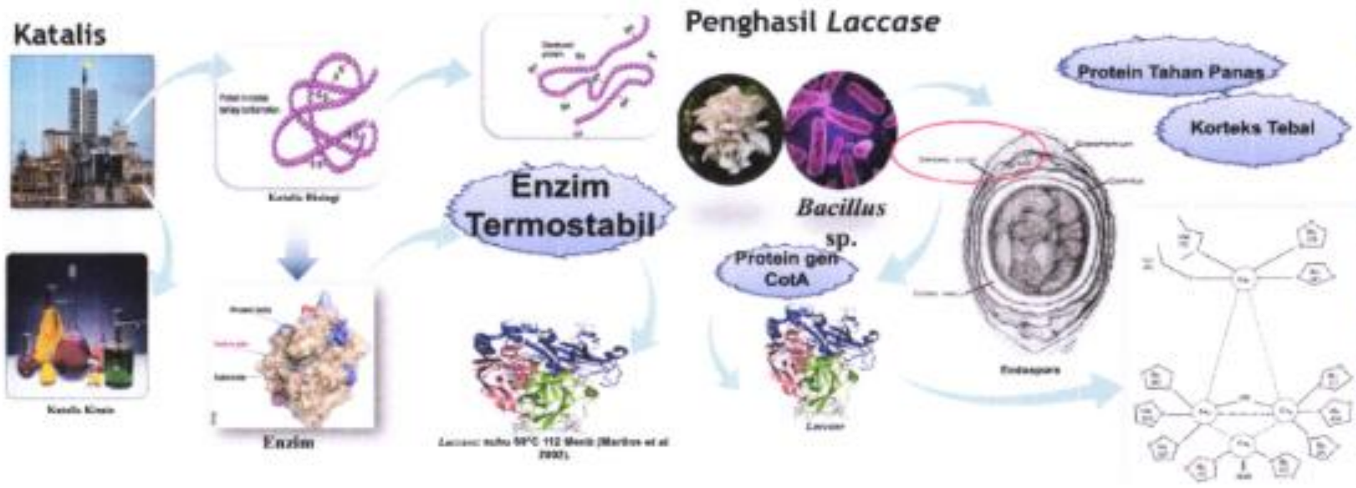
Phenols removal from Olive Oil Mill Waste (OMW) by CDH-Laccase combination treatment for biogas plant

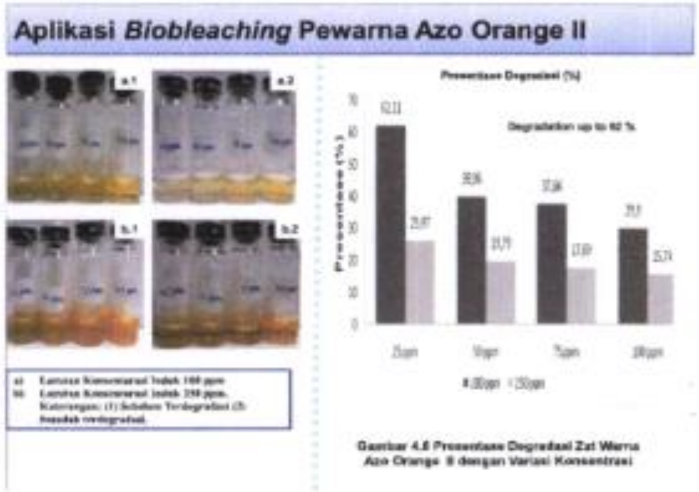
Decrease in phenols concentration

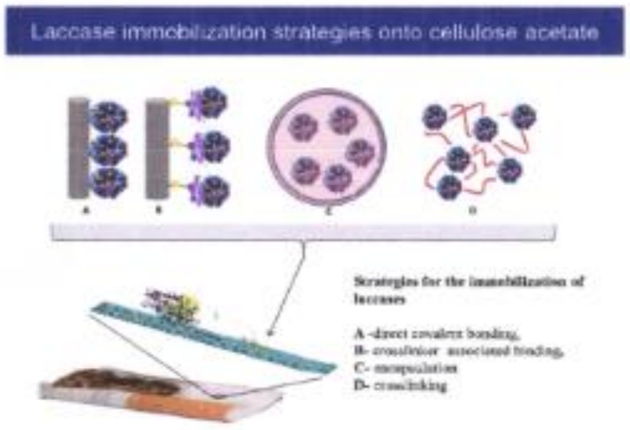
Labscale biogas plant

Fabrics bleaching by CDH

- **Figure 2: G1:** amylase, bleaching with GOX + glucose
- **C1:** amylase, bleaching with CDH + lactose,
- **C4:** same procedure as C1, but half volume of buffer,
- **K:** standard scouring and bleaching with 35 % H_2O_2
- **U:** untreated cotton fabrics,
- **K2:** bleaching with 35 % H_2O_2







Cigarettes smoke toxicant removal

Different strategies used to remove different smoke toxicants	
Substance	**Elimination strategy**
Acrylonitrile	Copolymerization with hydroquinone
2-Aminonaphthalene (naphthylamine)	Direct oxidation
Catechol	Direct oxidation
Hydroquinone	Direct oxidation
Crotonaldehyde	copolymerization
Nitric oxide	Copolymerization with hydroquinone, catechol and methylquinone
NNK	Copolymerization with hydroquinone, catechol and methylquinone
NNN	Copolymerization with hydroquinone
Acetaldehyde	Copolymerization with hydroquinone, catechol and methylquinone
Acrolein	Copolymerization with hydroquinone, catechol and methylquinone
Benzene	No oxidation and not possible to copolymerize
Benzo[a]pyrene	Copolymerization with hydroquinone, catechol and methylquinone
Formaldehyde	Copolymerization with hydroquinone, catechol and methylquinone

Decreased formaldehyde in the presence of laccase phenolic substrates						
Sample/Medium	**Formaldehyde concentration with Laccase [µM]**	**Std. dev.**	**Decrease in %**	**Formaldehyde concentration without Laccase [µM]**	**Std. Dev.**	**Decrease in %**
without Mediator/coupling agent	[illegible]	[illegible]		[illegible]	[illegible]	[illegible]
Hydroquinone	[illegible]	[illegible]	2,72	[illegible]	[illegible]	[illegible]
Methylhydroquinone	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Trimethylhydroquinone	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Catechol	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Methylcatechol	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2-Naphthylamine	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Future perspectives

- Cold resistant collagenase
- Novel natural mediator for laccase
- Biobleaching on natural cotton
- Development of enyzme based analytical method for antiaging agent
- Enzymatic based fertilizer
- Enzyme based gelling agent
- Enzyme base grouting materials
- Enzyme based phenolic sensor
- Enzimatic modification of drug carrier

Partners

HCML Husky-CNOOC Madura Limited

TU Graz

biorenew

FFG

SSAAMP

acib

JALA TANI PERTIWI FOUNDATION

MADUAGROMARINE

ITS

DAAD

oead

COTTON BLEACH

3 postdoc
2 PhD
3 Master
25 Bachelor

Thank you