CIMEE

INTERNATIONAL SYMPOSIUM ON MATERIALS, ELECTROCHEMISTRY & ENVIRONMENT

LUT University

Blue laccases for green textile wastewater treatment

International Symposium on Materials, Electrochemistry and Environment, CIMEE’25

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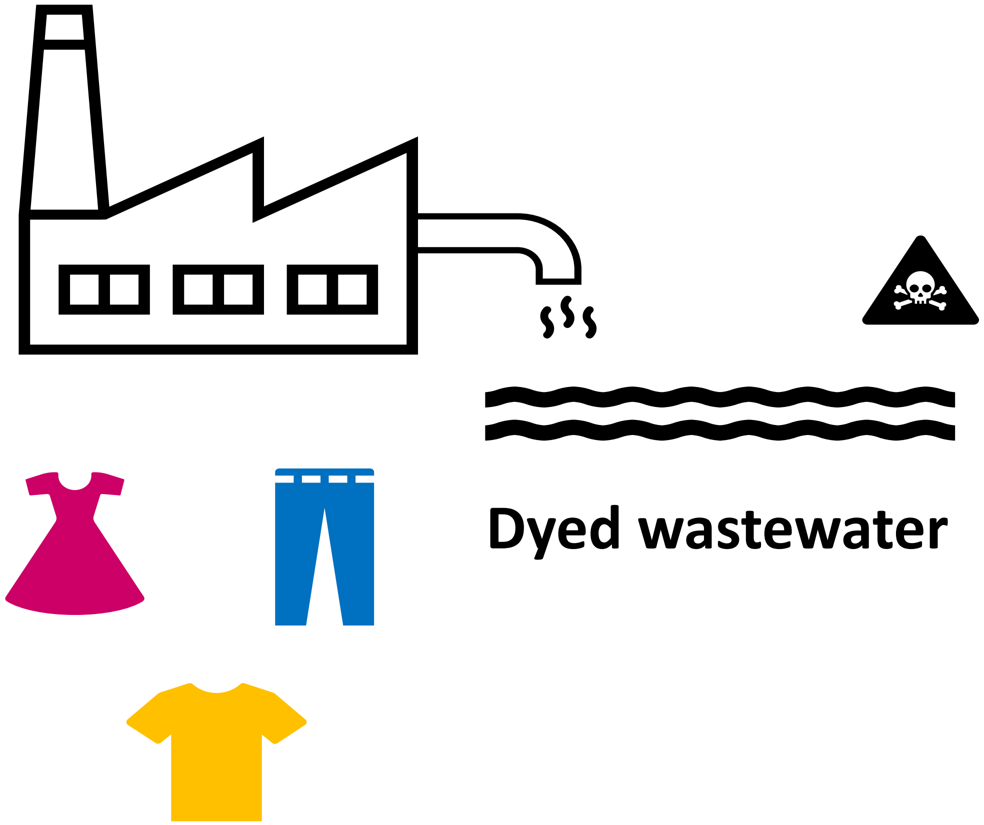
TEXTILE INDUSTRY



responsible for 20% of global water pollution

TEXTILE WASTEWATER CHARACTERISTICS

- Different synthetic dyes ⇒ Toxic, Mutagenic, Teratogenic, Carcinogenic
- Suspended solids
- Salts
- Additives
- Surfactants
- High COD
- High BOD
- High temperature
- Variable pH

⇒ Challenging to treat

DYES USED IN THE TEXTILE INDUSTRY

Type of dye	Example	Colour Index	Fabric
Disperse	Disperse Red 60	60756	Polyamide, nylon
Azo	Aniline Yellow	11000	Cotton, nylon
Acid	Acid Blue 78	62105	Wool
Reactive	Reactive Red 1	18158	Wool, silk, nylon
Sulphur	Thiazine	52000-52999	Silk, cotton
Mordant	Mordant Red 11	58000	Wool
Direct	Direct Yellow II	23640/40000	Leather, cotton

IMPACT OF TEXTILE DYES ON THE ENVIRONMENT

- Produce algal bloom.
- Cause mutagenic effects on aquatic flora and fauna.
- Degrade soil quality.
- Groundwater pollution.
- Human health issues through the food chain.

TREATMENT OF TEXTILE WASTEWATER

- ✓ The traditional physicochemical and activated sludge processes are inefficient.
- ✓ Emerging technologies are expensive, energy intensive, generate sludge and toxic byproducts.

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Need for new low-cost, efficient and environmentally friendly technologies

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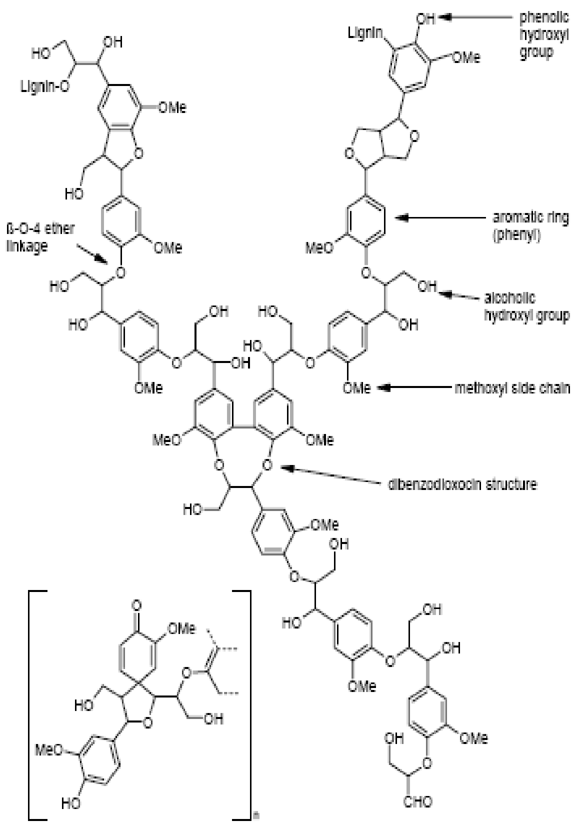
Ligninolytic enzymes

LIGNINOLYTIC ENZYMES



Lignin peroxidase (LiP, EC 1.11.1.14)

Manganese-dependent peroxidase (MnP, EC 1.11.1.13)

Other peroxidases and oxidases

Laccase (EC 1.10.3.2)

WHY LACCASES?

- They have broad substrate specificity.
- They are green enzymes that function with molecular oxygen (easily available from air) producing water as the only by-product.
- They operate under mild reaction conditions.
- They are produced ecologically from living microorganisms.
- They are naturally biodegradable.

WHAT ARE LACCASES?

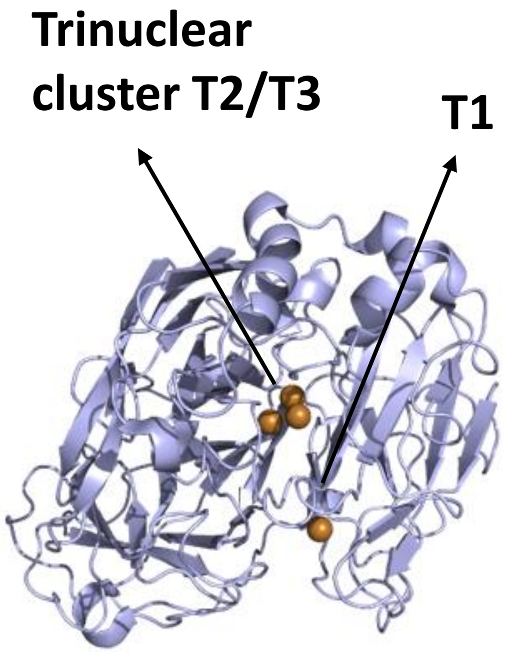
- They are multicopper-containing oxidase enzymes (EC 1.10.3.2, p-diphenol: oxygen oxidoreductase).
- They are found in higher plants (*Rhus vernicifera*), bacteria (*Azospirillum lipoferum*), **fungi** (*Trametes versicolor*) and insects (Bombyx).

→ **White-rot basidiomycetes**

WHY FUNGAL LACCASES?

- They are mainly extracellular enzymes.
- They have a high redox potential (E^0 > 720 mV).
- They have broad substrate specificity.
- Their glycosylation (10-25%) gives them stability and protects them from proteolysis.
- They degrade dyes into phenolic compounds instead of generating toxic amines as other oxidases do.

FUNGAL LACCASE STRUCTURE



Structure of a *Trametes versicolor* laccase

It comprises about 520-550 amino acids.

Its molecular weight ranges from 60 to 80 kDa.

It is typically a monomeric enzyme.

It contains 4 copper atoms:

-1 copper type 1 (T1)

-1 copper type 2 (T2) } trinuclear cluster

-2 coppers type 3 (T3) } trinuclear cluster

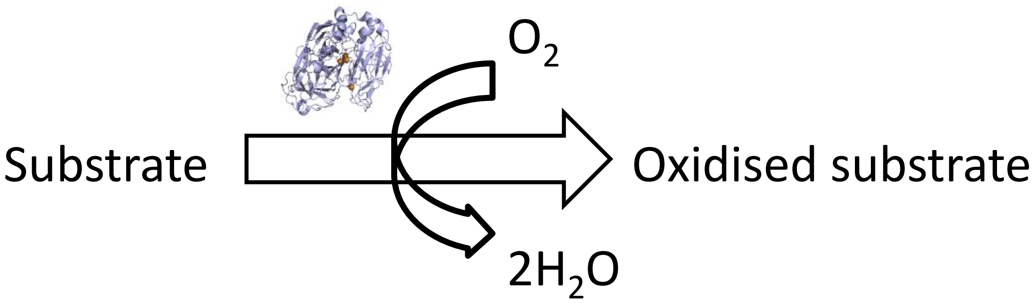
The reduction potential (E^0) of the T1 site determines the oxidation efficiency on substrates.

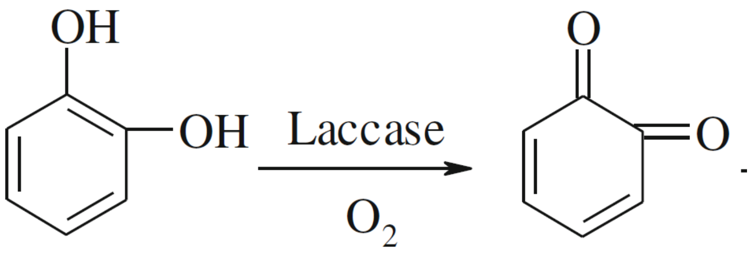
STATUS OF COPPER IN FUNGAL LACCASES

	Cu Type	Cu atoms/ protein	EPR signal	Features	Functions
	1	1	+	“**Blue Cu^{2+}**”, absorbance at 610 nm, redox potential +785 mV	**Substrate oxidation**
Trinuclear cluster	2	1	+	“Non-blue Cu^{2+}”, no absorption in the visible spectrum	**O_2 reduction to H_2O**
Trinuclear cluster	3	2	-	Absorbance at 330 nm	**O_2 reduction to H_2O**

LACCASE CATALYSIS

Direct oxidation of aromatic compounds (e.g., phenols, anilines) with the concomitant reduction of molecular oxygen to water.

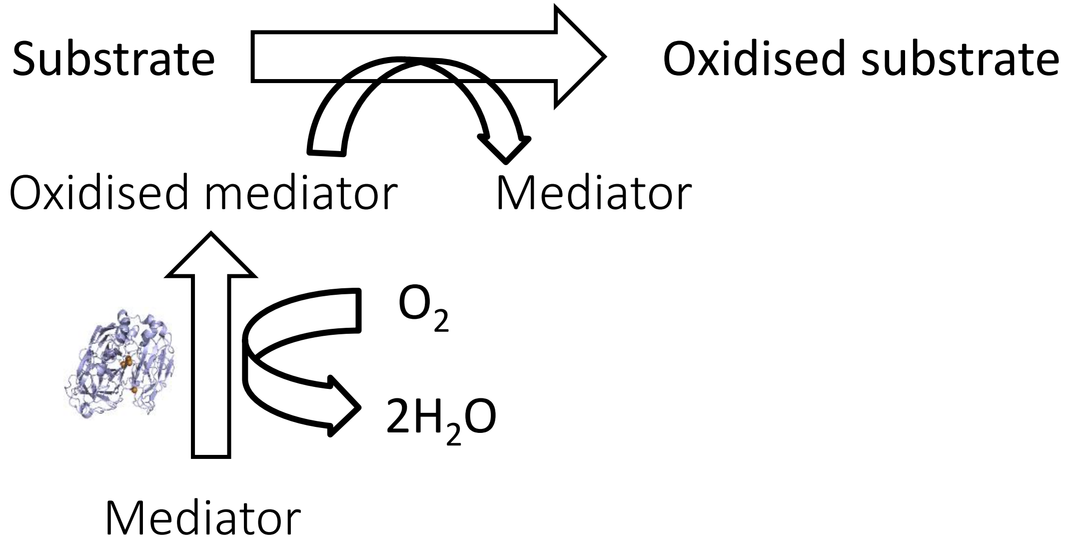




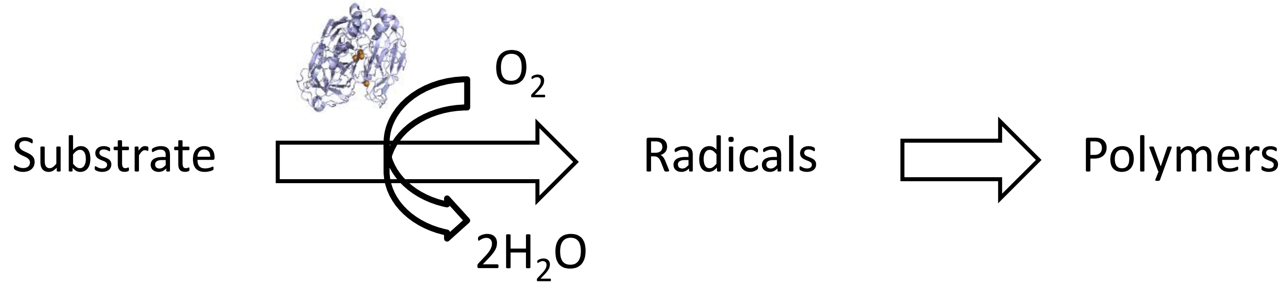
Catechol

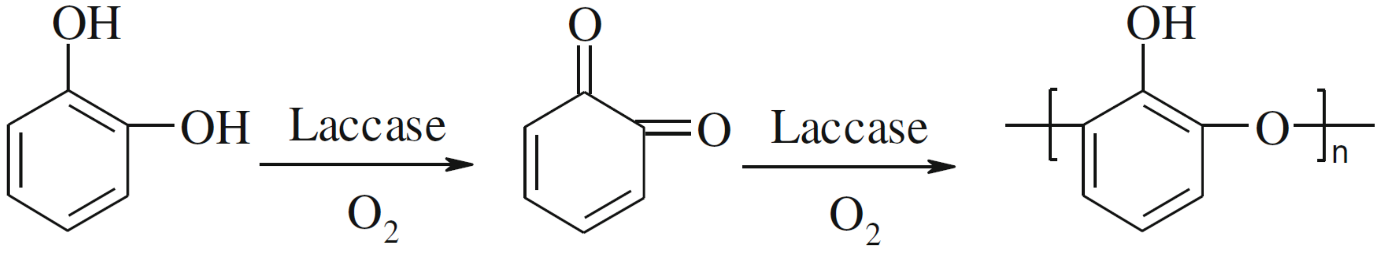
Quinone

Indirect oxidation of non-laccase substrates (e.g., large molecules and high redox compounds) in the presence of a redox mediator: laccase-mediator system (LMS).



Polymerisation: laccase creates radicals from phenolic monomers that then undergo homo- and hetero-coupling reactions to form polymers.





Catechol

Polycatechol

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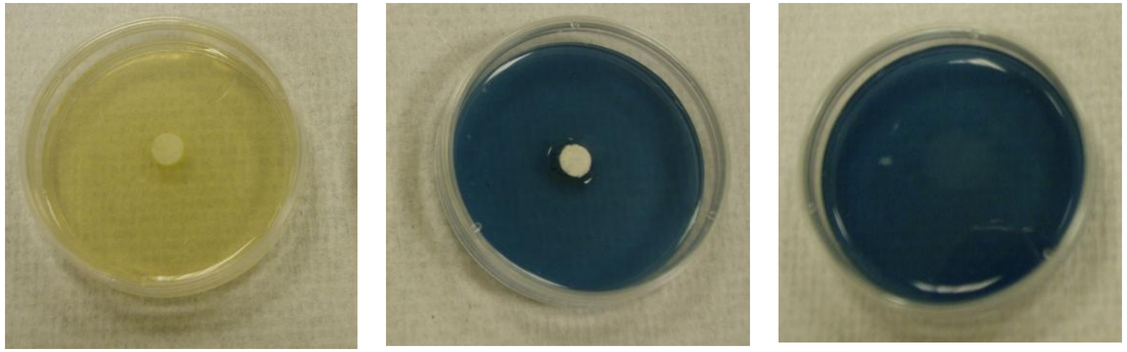
DYE DECOLOURATION BY *TRAMETES PUBESCENS* ON PETRI PLATES

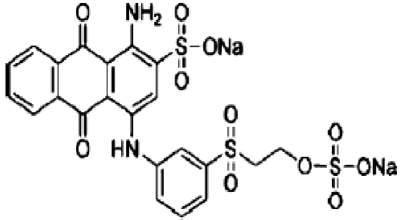
Biotic control | **Test** | **Abiotic control**



Time 0

Time 6 days

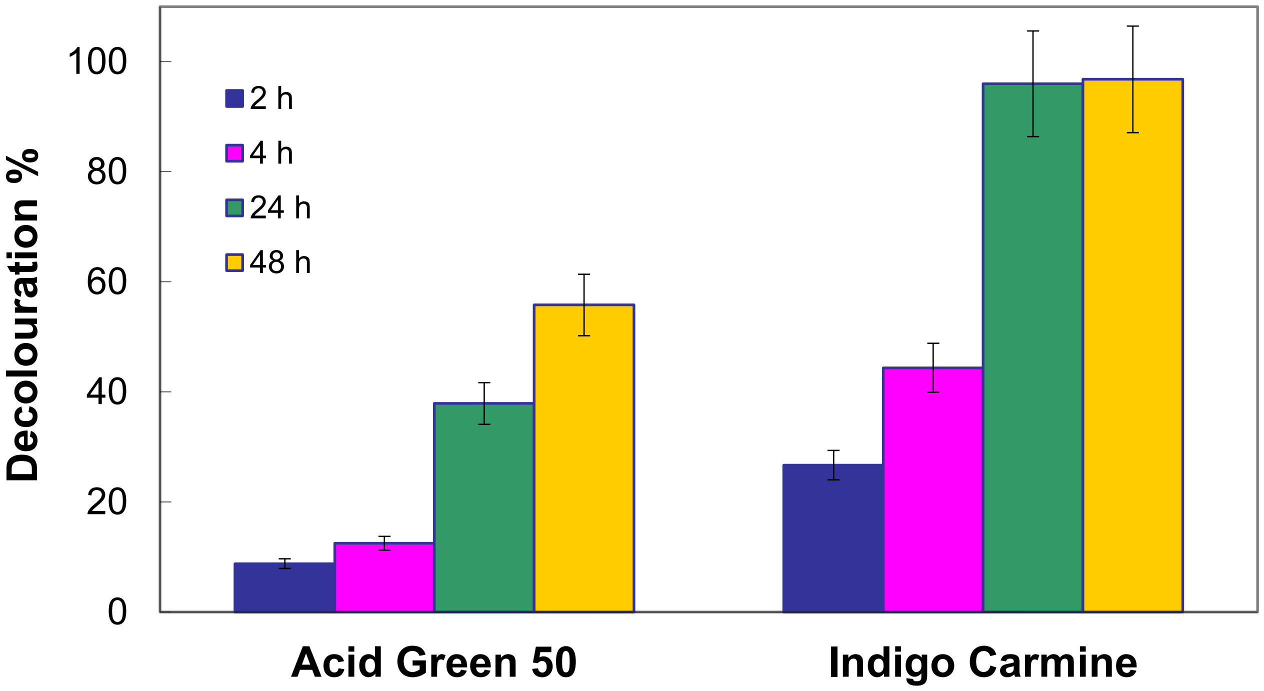
Medium composition: malt agar extract
0.4 g/L RBBR (test)
0.01% antibiotic
pH 5.0
30°C



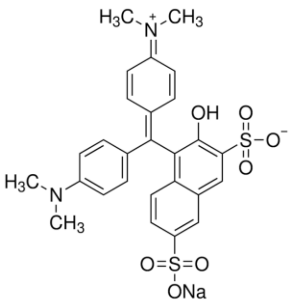
Remazol Brilliant Blue R

INTERNATIONAL SYMPOSIUM ON MATERIALS, ELECTROCHEMISTRY & ENVIRONMENT

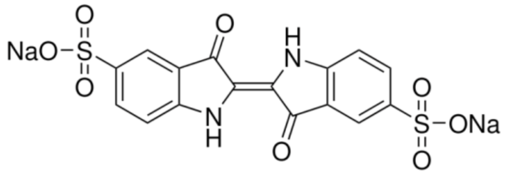
DYE DECOLOURATION BY CRUDE LACCASE FROM *TRAMETES HIRSUTA*



Reaction mixture: 500 U/L crude laccase
100 mg/L AG 50 ; 300 mg/L IC
pH 5.0
room temperature



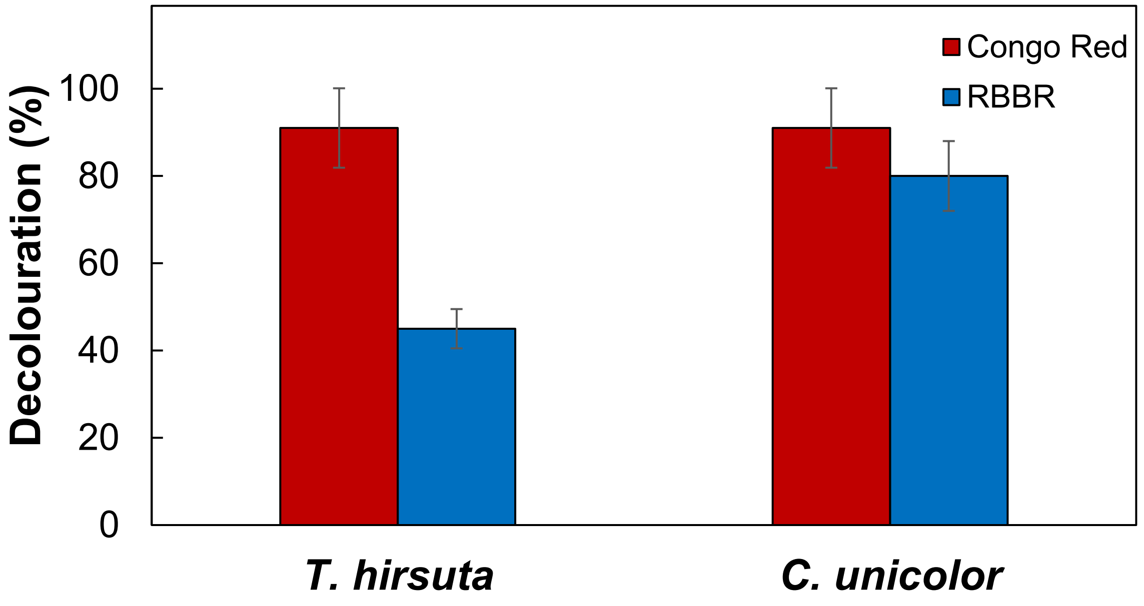
Acid Green 50



Indigo Carmine

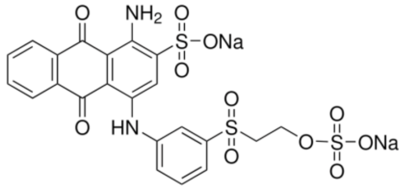
J.F. Osma, M. Delcea, J.L. Toca Herrera and S. Rodríguez Couto. Laccase production by Trametes hirsuta grown on paper cuttings, 6th ANQUE International Congress of Chemistry. Puerto de la Cruz (Tenerife), 5-7 December 2006

DECOLOURATION OF A SIMULATED TEXTILE EFFLUENT BY CRUDE LACCASE FROM *TRAMETES HIRSUTA* AND *CERRENA UNICOLOR*

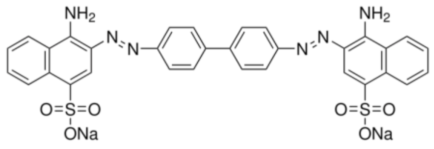


Simulated textile effluent: hydrolysed dye (100 mg/L RBBR; 12.5 mg/L CR)
2.9 g/L hydrolysed starch
0.15 g/L NaCl
0.53 g/L acetic acid
2 g/L $NaHCO_3$
500 U/L crude laccase

19.5 h



Remazol Brilliant Blue R



Congo Red

U. Moilanen, J.F. Osma, E. Winquist, M. Leisola and S. Rodríguez-Couto (2010). Decolorization of simulated textile dye baths by crude laccases from Trametes hirsuta and Cerrena unicolor. Engineering in Life Sciences, 10: 242-247, DOI: 10.1002/elsc.200900095.

CHALLENGES THAT PREVENT THE USE OF LACCASES TO TREAT TEXTILE WASTEWATER

- Low operational stability and shelf-life.
- Commercially available products have limited applications due to their low redox potential.
- Laccases can be deactivated under the harsh conditions existing in wastewater.
- Cumbersome recovery and re-use.

⇩

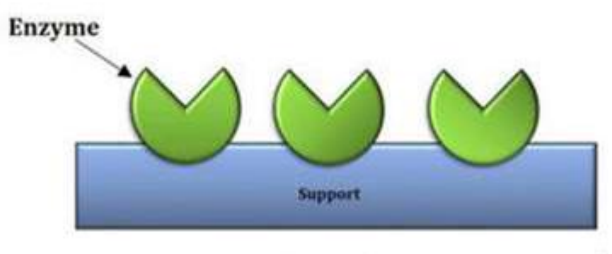
Solution: immobilisation

ADVANTAGES OF LACCASE IMMOBILISATION

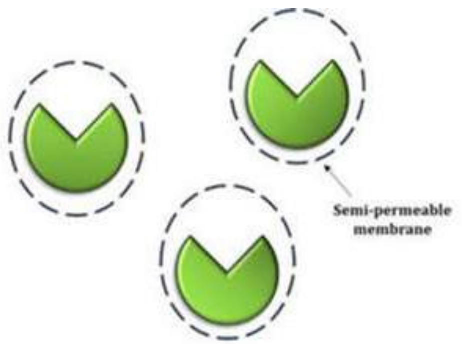
- Enhance stability to pH and temperature.
- Decrease inhibition.
- Enhance life-shelf storage.
- Allows enzyme recovery and reuse.
- Easier downstream processing
- Different bioreactor configurations possible.
- Allows continuous operation.

ENZYME IMMOBILISATION TECHNIQUES

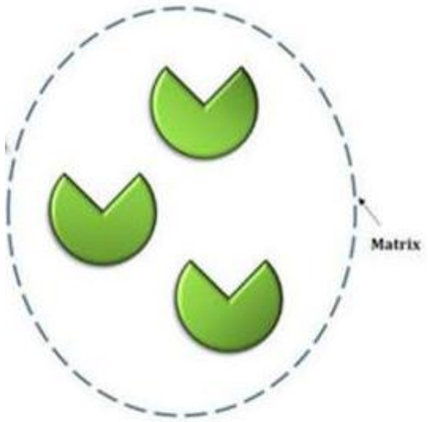
Physical



Adsorption



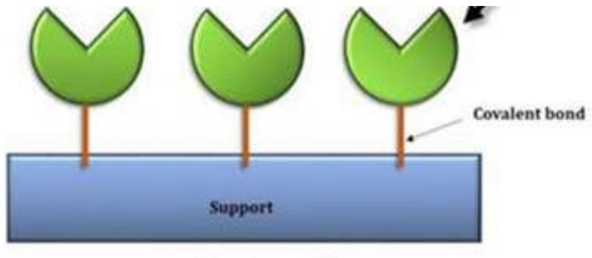
Encapsulation



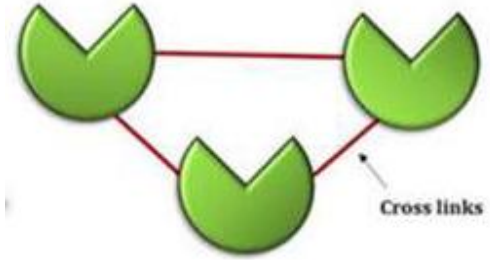
Entrapment

ENZYME IMMOBILISATION TECHNIQUES

Chemical



Covalent bonding



Crosslinking

SUPPORT MATERIALS FOR ENZYME IMMOBILISATION

- **Organic materials**
  - Natural polymers: alginate, chitosan, cellulose
  - Synthetic polymers: polyvinyl chloride, polystyrene, polyamide
  - Agricultural waste
  - Carbon

SUPPORT MATERIALS FOR ENZYME IMMOBILISATION

- **Inorganic materials**
  - Silica
  - Clay
  - Glass
  - Alumina

SUPPORT MATERIALS FOR ENZYME IMMOBILISATION

- **Hybrid materials**
  - Polyvinyl alcohol/alginate
  - Chitosan/clay
  - Chitosan/silica
  - Alginate/chitosan
  - Silica/magnetite
  - Silica/alginate

SUPPORT MATERIALS FOR ENZYME IMMOBILISATION

- **Nanomaterials**
  - Magnetic nanoparticles
  - Graphene
  - Graphene oxide
  - Carbon nanotubes
  - Metal organic framework (MOF) nanoparticles

LACCASE IMMOBILISATION

Covalent bonding on alumina pellets

IY: 68%

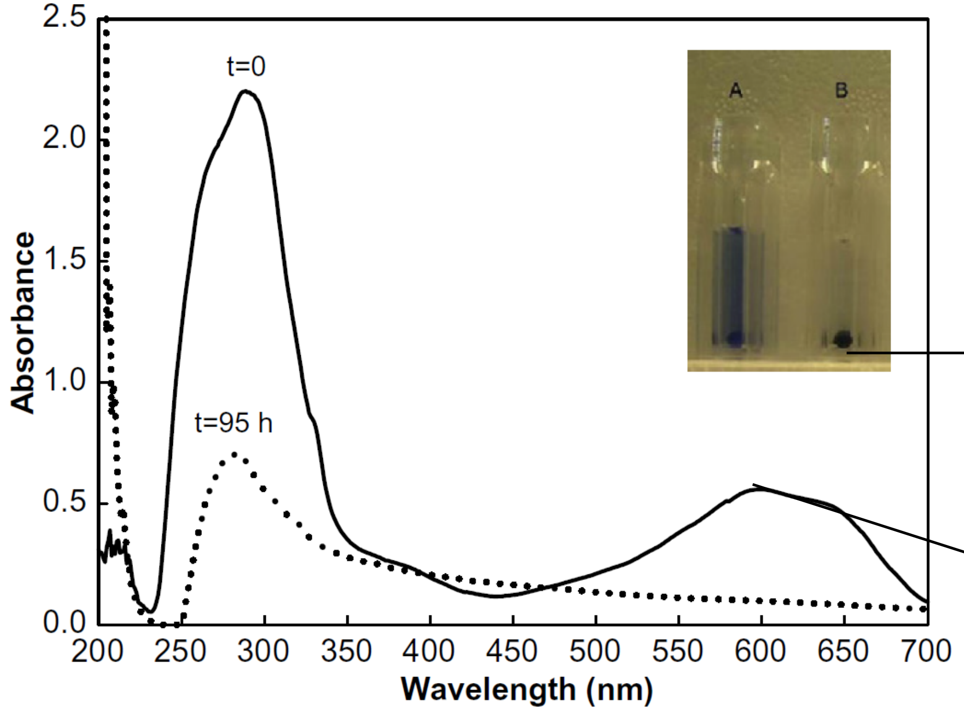
Entrapment in Ca-alginate beads

IY: 62%

S. Rodríguez-Couto, J.F. Osma,, G.M. Gübitz and J.L. Toca-Herrera (2007). Coating of immobilised laccase for stability enhancement: a novel approach. Applied Catalysis A: General 329: 156-160.

R. Genc and S. Rodríguez-Couto (2009). Using biotechnology in the laboratory: using an immobilized-laccase reactor-system to learn about wastewater treatment. Biochemistry and Molecular Biology Education 37: 182-185.

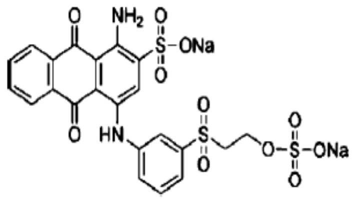
DYE DECOLOURATION BY IMMOBILISED LACCASE FROM *TRAMETES PUBESCENS*



Reaction mixture: 500 U/L laccase immobilised on alumina pellets
133 mg/L RBB
room temperature

RBBR adsorption < 5%

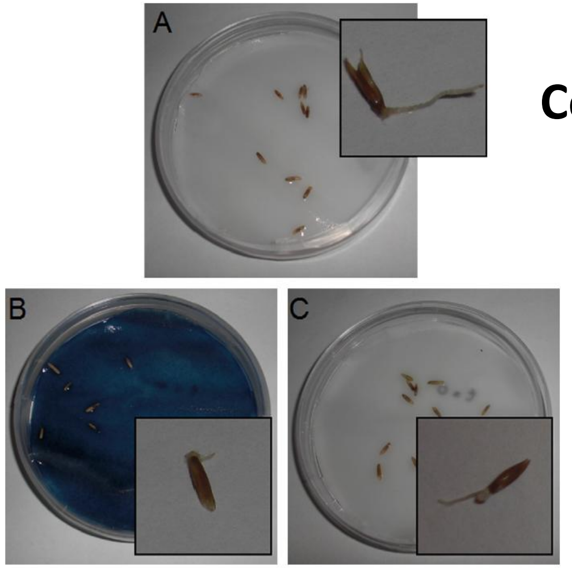
Chromophore peak



Remazol Brilliant Blue R

DETOXIFICATION STUDIES

Phytotoxicity studies (ryegrass seeds)



Control (water)

RBBR

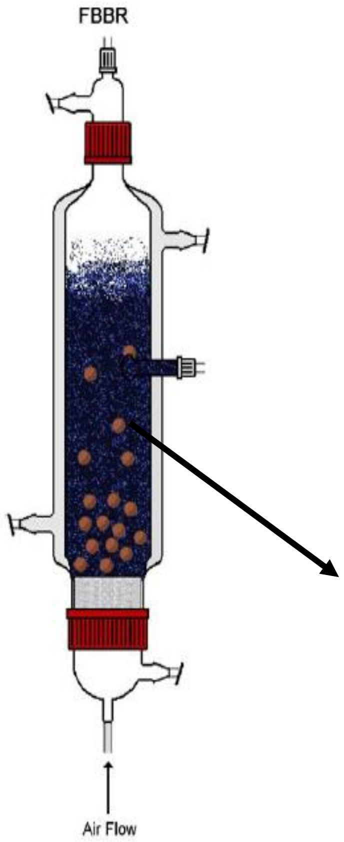
Degradation products

	GI (%)
Control	100
RBBR (133 mg/L)	26
RBBR degradation products	69

The degradation products were less toxic than the parent dye

J.F. Osma, J.L. Toca-Herrera and S. Rodríguez-Couto (2010). Transformation pathway of Remazol Brilliant Blue R by immobilised laccase. Bioresource Technology, 101: 8509-8514.

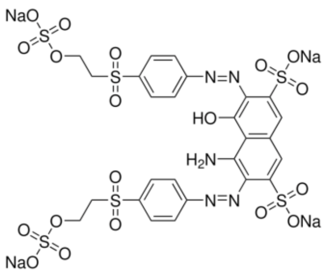
TREATMENT OF A SIMULATED TEXTILE EFFLUENT BY IMMOBILISED LACCASE FROM *TRAMETES PUBESCENS*



Immobilised laccase

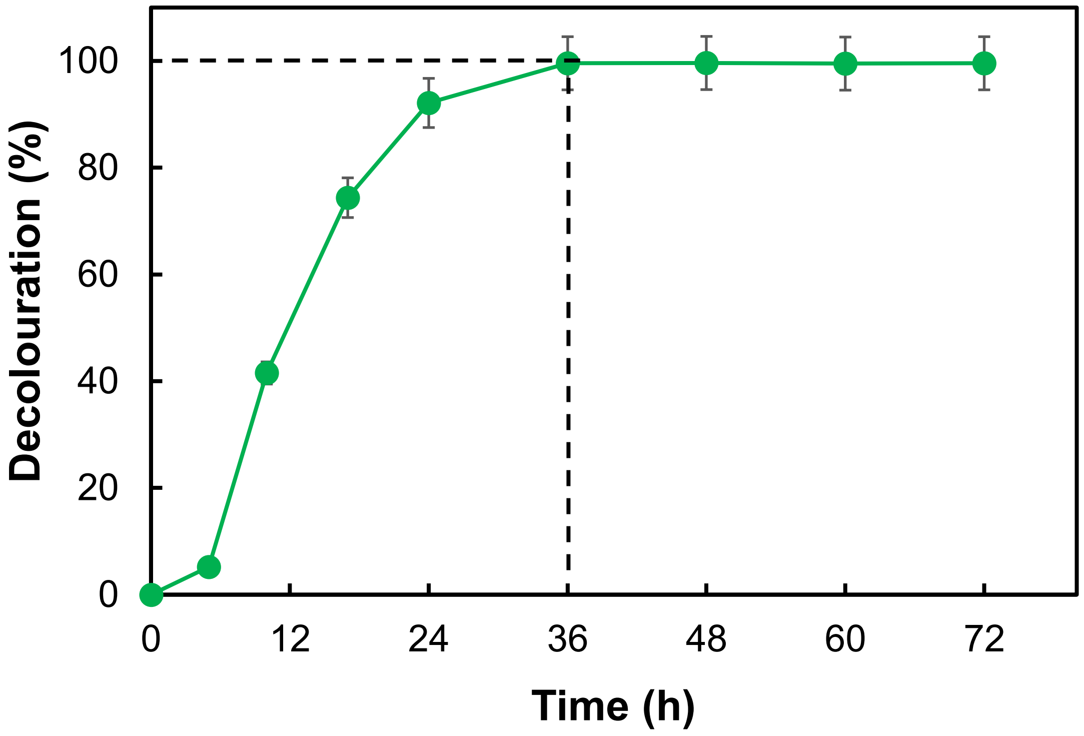
Operation conditions: fluidised-bed reactor (working volume 200 mL); aeration: 0.5 vvm; room temperature

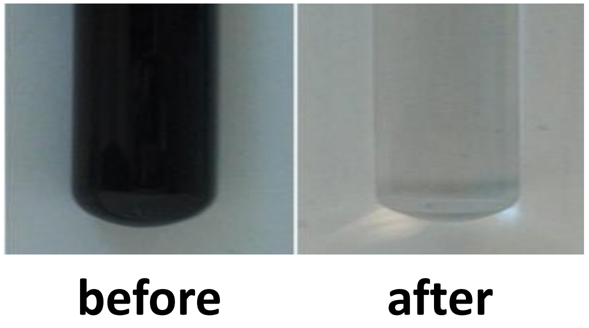
Simulated textile effluent (Bezema, Switzerland): 0.5 g/L **Reactive Black 5** (diazo)
30 g/L NaCl,
5 g/L Na_2CO_3
1.5 mL/L de 32.5% NaOH
pH 4.5



Reactive Black 5

BATCH OPERATION

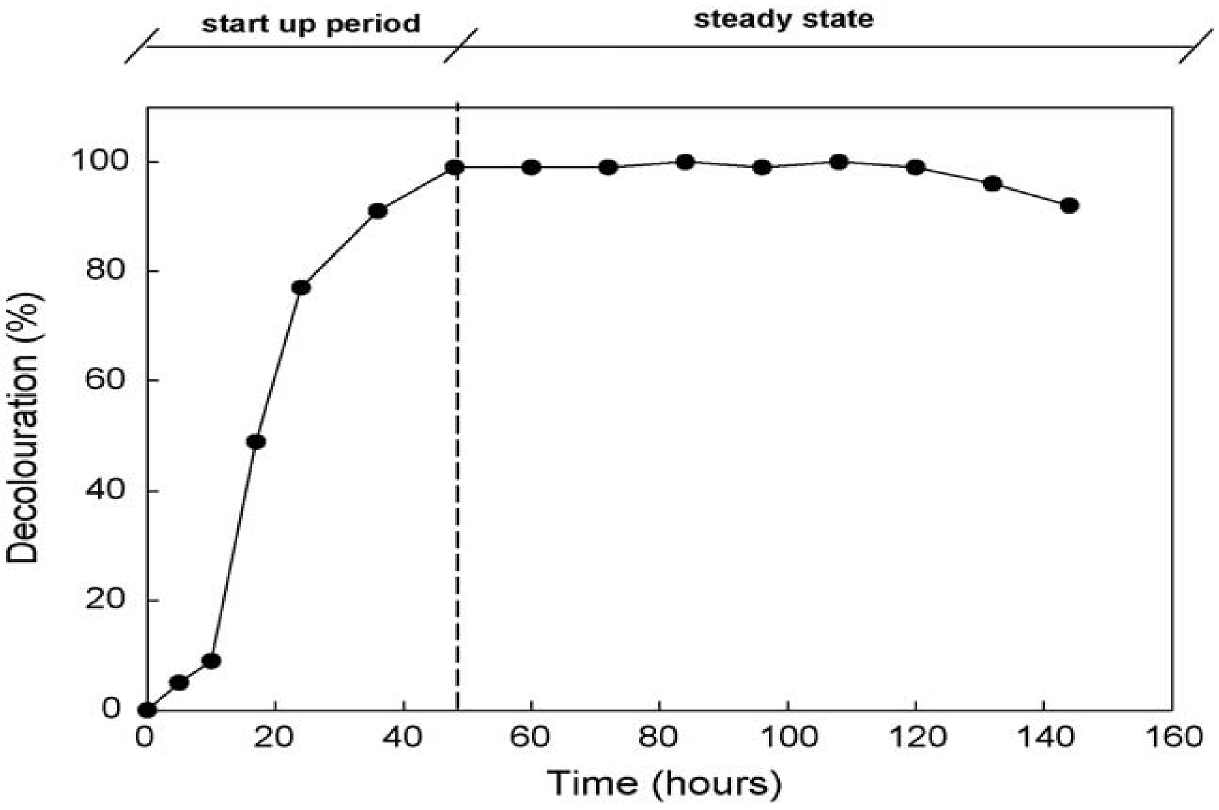




100% in 36 h

(dye adsorption on the carrier<5%)

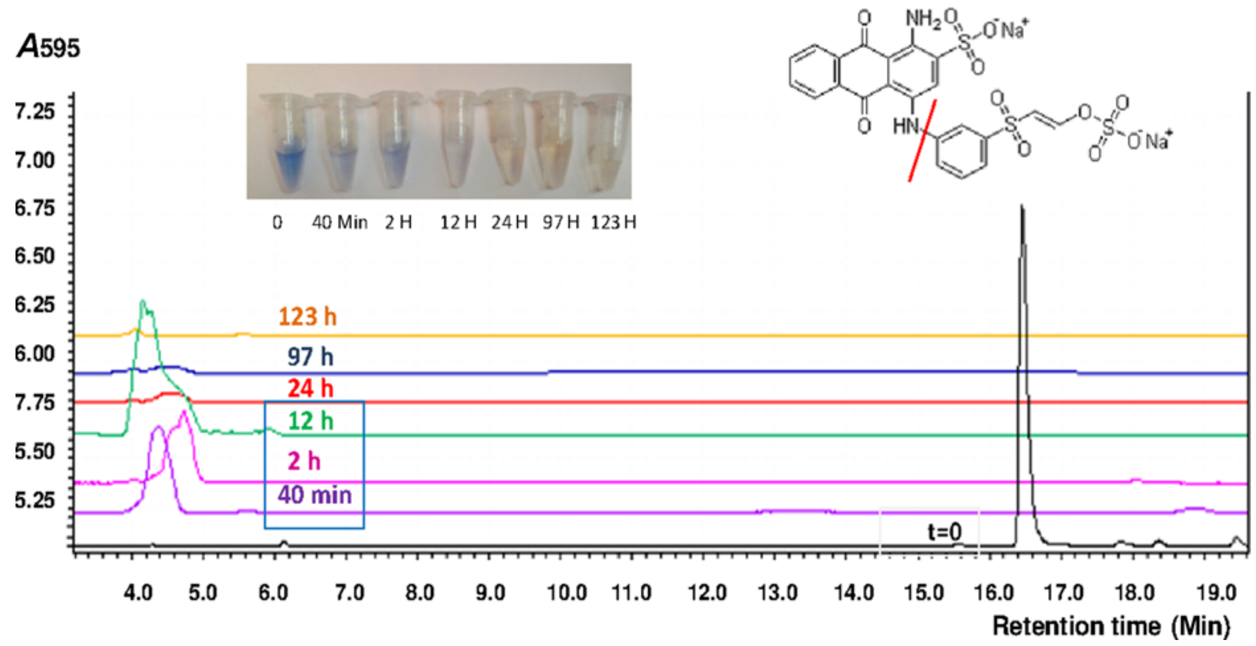
CONTINUOUS OPERATION



HRT 33 h

J.F. Osma, J.L. Toca-Herrera and S. Rodríguez-Couto (2010). Biodegradation of a simulated textile effluent by immobilised-coated laccase in laboratory-scale reactors. *Applied Catalysis A: General*, 373: 147-153, DOI: 10.1016/j.apcata.2009.11.009.

DECOLOURATION OF REMAZOL BRILLIANT BLUE R BY IMMOBILISED LACCASE FROM *TRAMETES VILLOSA*



Reaction mixture: 667 U/L laccase immobilised on agarose
130 mg/L RBBR
pH 4.8
22 °C

The RBBR peak disappeared in 40 min and a new peak appeared at 595 nm which disappeared after 24 h.

L. Gioia, S. Rodríguez-Couto, P. Menéndez, C. Manta and K. Ovsejevi (2015). Reversible covalent immobilization of Trametes villosa laccase onto thiolsulfinate-agarose: an insoluble biocatalyst with potential for decolouring recalcitrant dyes. Biotechnology and Applied Biochemistry, 62: 502-513, DOI: 10.1002/bab.1287.

CONCLUSIONS

- **Fungal laccases** hold great potential to treat textile wastewater efficiently and environmentally friendly.
- **Immobilisation** is imperative for the development of continuous laccase-based wastewater treatment processes.
- **More research under real conditions** is needed to assess the true potential of laccase enzymes for the treatment of textile wastewater.

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Thank you very much for your attention.

https://www.lut.fi/en/studies/technology/water-technology