BIOCATALYTIC PRODUCTION OF LACTOBIONIC ACID FROM LACTOSE USING FUNGAL OXIDOREDUCTASES

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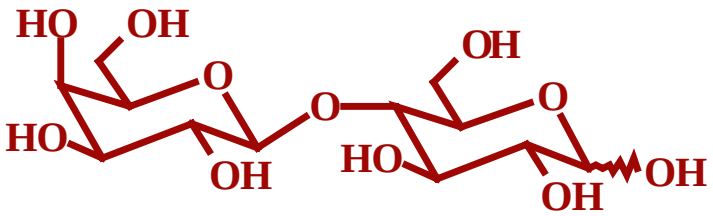
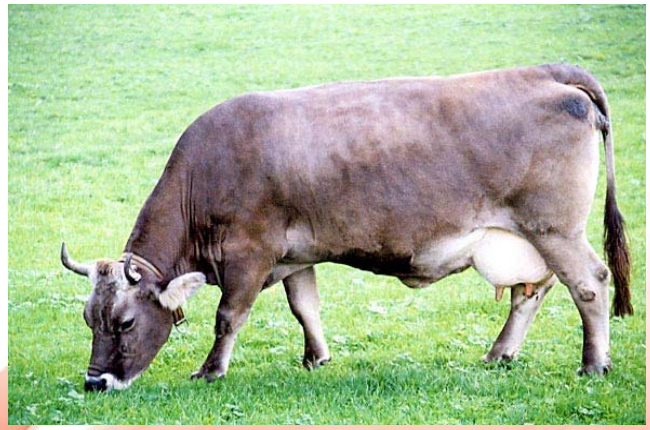
Lactose

- Main source: whey; 70–75% of dry weight is lactose
- approx. 3.2 million t/a worldwide, mainly in Europe
- Used in food, confectionary, beverages, pharmaceuticals



β-D-galactopyranosyl-(1∏4)-D-glucopyranose



Lactobionic acid: C1 oxidation product of lactose

applications: skin care products, pharmaceuticals,sport drink ingredient, lactobiono-alkyl-amides as detergents

Production of Lactobionic Acid

A. Chemical synthesis:

oxidation of lactose by bromine water (Fischer & Meyer 1889)

B. Electrochemical:

electrooxidation on modified platinum electrodes in alkaline medium

(Druliolle et al. 1995)

C. Fermentative:

Pseudomonas mucidolens submerged cultures (El-Refai et al. 1994)

Production of Lactobionic Acid

D. Enzymatic:

Oligosaccharide oxidase from *Acremonium strictum* (Lin et al. 1993)

Hexose oxidase from *Chondrus crispus* (Savary et al. 2001)

Cellobiose Dehydrogenase from *Sclerotium rolfsii* (Baminger et al. 2001

Flavoproteins in Biocatalysis

- Oxidation of alcohols

glucose oxidase, alcohol oxidase

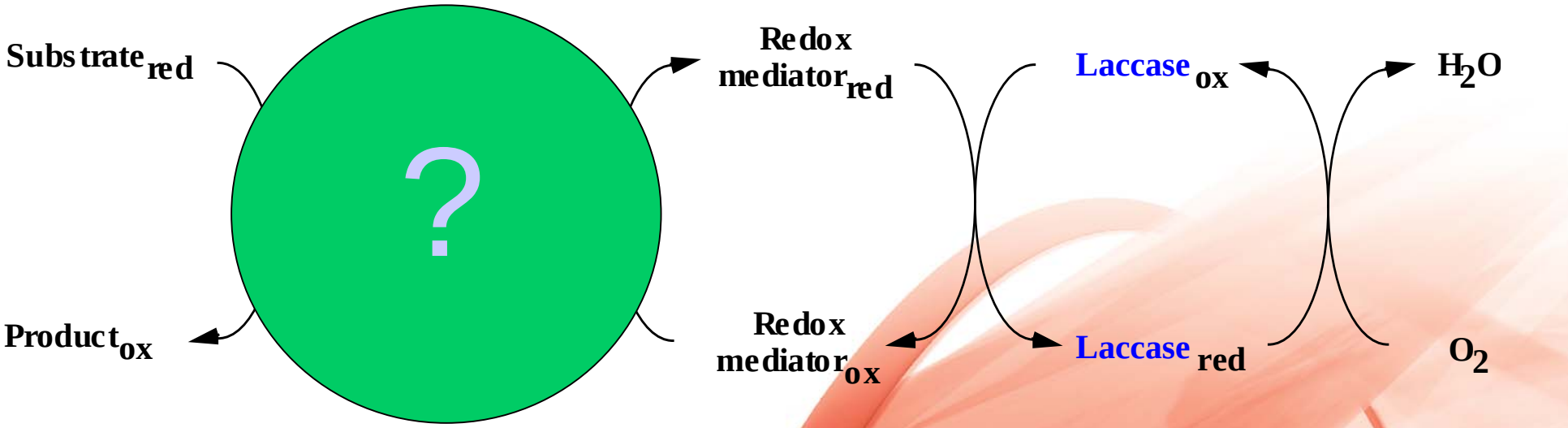
- Oxidation of phenols

vanillyl-alcohol oxidase

- Oxidation of the C-N bond

amino acid oxidases, monoamine oxidase

Regeneration of Electron Acceptors used by Flavoenzymes



Sources of Cellobiose Dehydrogenase

Widespread among fungal species

white rot: *Phanerochaete spp.*

Trametes spp.

brown rot: *Coniophora puteana*

soft rot: *Humicola insolens*

Corynascus thermophilum

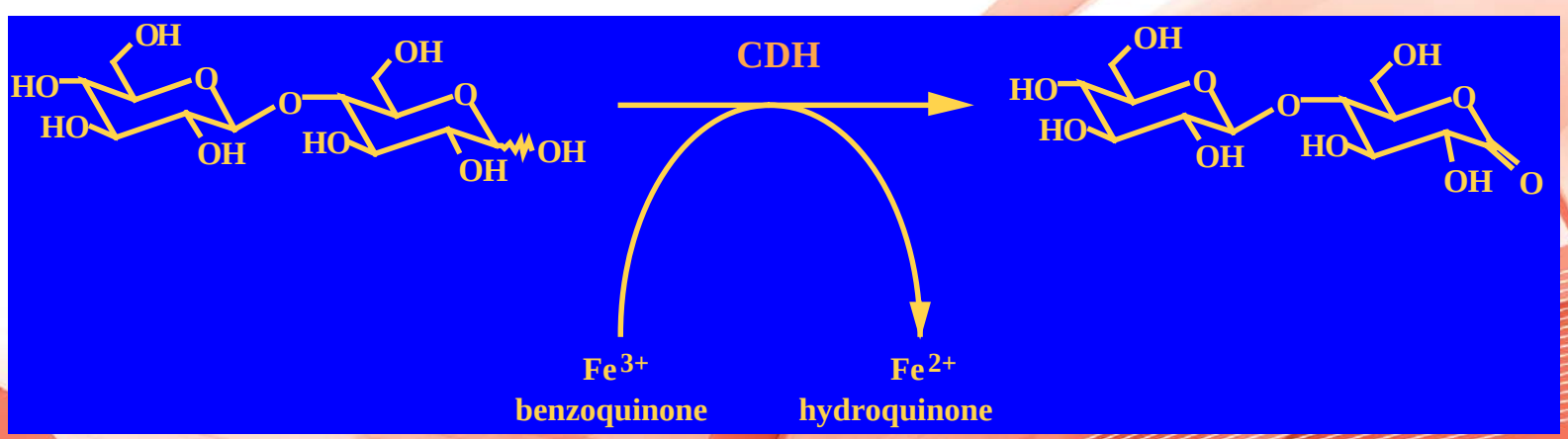
Myriococcum thermophilum

plant pathogen: *Sclerotium rolfsii*

Catalytic Properties of Cellobiose Dehydrogenase (CDH)

- **substrates / electron donors:**
- cellobiose and cellooligomers
- **lactose**
- xylobiose, maltose, mannobiose
- glucose and mannose

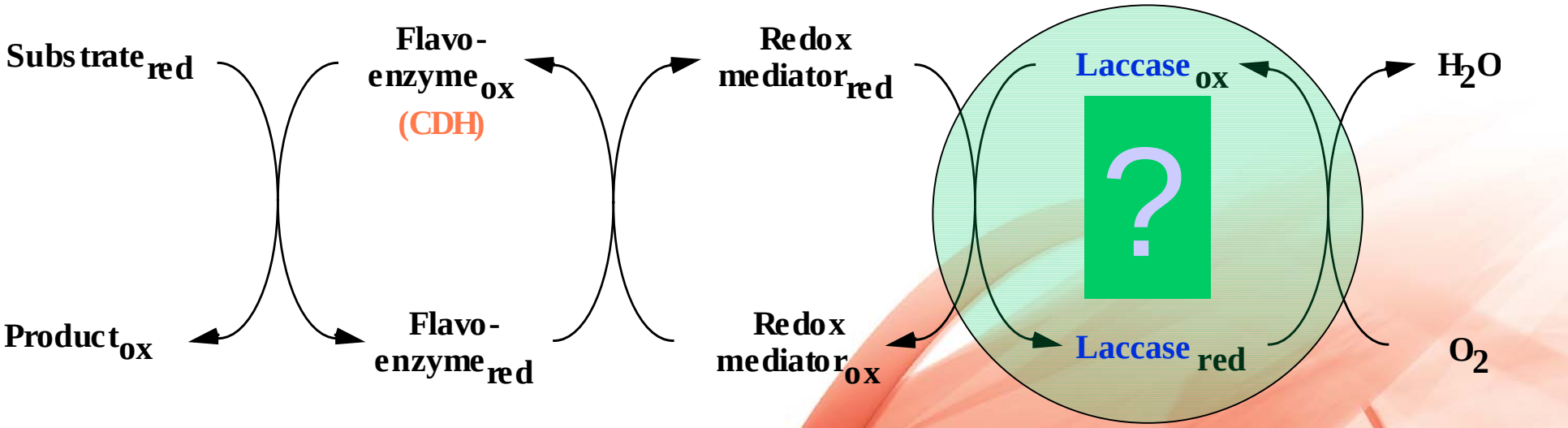
- **cosubstrates / electron acceptors:**
- two electron acceptors:
- O_2, DCIP, quinones
- one electron acceptors:
- Fe^{3+}, Cu^{2+}, Mn^{3+}, $K_3Fe(CN)_6$
- *cyt c*, ABTS radical



Catalytic Constants of Cellobiose Dehydrogenase (CDH)

substrate	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m ($mM^{-1} \cdot s^{-1}$)
cellobiose	120	27	225
lactose	2,400	26	11
1,4-benzoquinone	25	30	1,200
3,5-di-*tert*-butyl-1,2-bq	53	23	434
DCIP	15	30	2,000
methylene green	4.3	18	4,190
ferricenium ion	0.83	39	47,500
$K_3Fe(CN)_6$	20	37	1,850
ABTS cation radical	0.4	27	67,500

Regeneration of Electron Acceptors used by Flavoenzymes



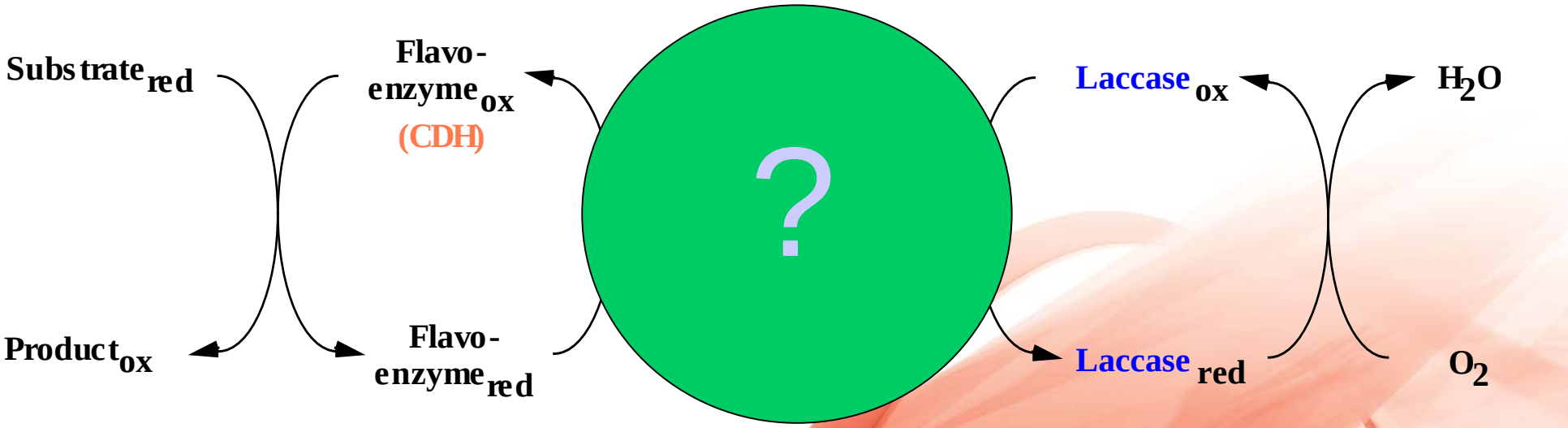
Fungal Laccases

- Common enzymes in wood degrading fungi
- Blue multicopper oxidases
- Catalyse the oxidation of a very wide range of organic and inorganic substrates (polyphenols)
- Very efficient enzymes (kcat 130 – 2900 s-1, kcat/Km up to 48 ´ 106 M-1 ·s-1)

Laccases from *Trametes pubescens*

Substrate	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m (M^{-1} s^{-1})
ABTS	14	690	48×10^6
Ferrocyanide	43	850	2×10^6
Hydroquinone	390	320	0.82×10^6
Oxygen	410	2,900	7.0×10^6

Regeneration of Electron Acceptors used by Flavoenzymes



Selection of Suitable Redox Mediators

Screening experiments

1 U laccase

0.5 U CDH

30 µmol lactose (15 mM)

0.2 µmol redox mediator

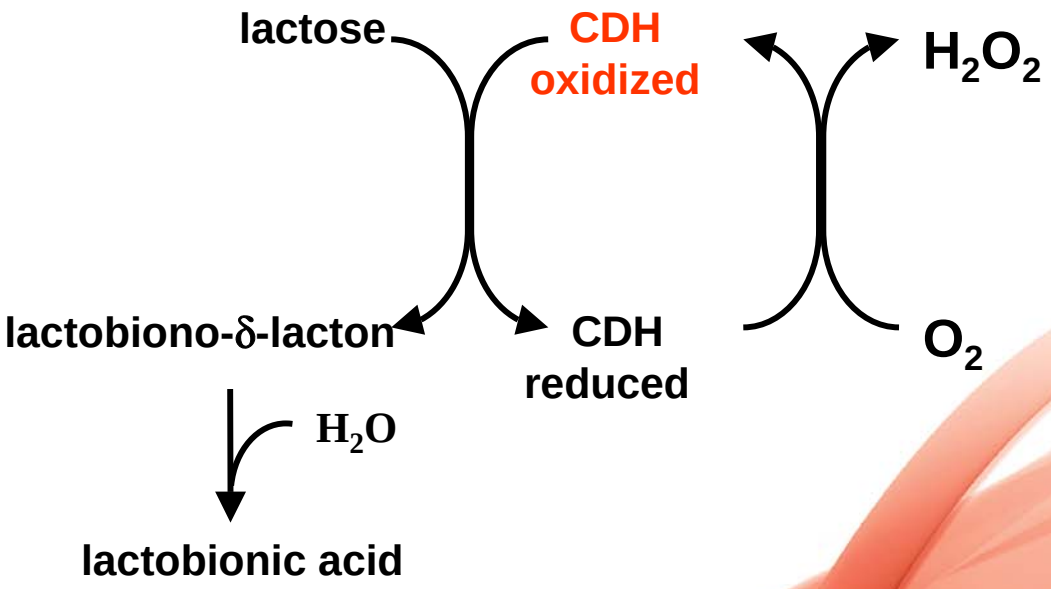
2 ml Na acetate buffer pH 4.5

30°C, 2 h

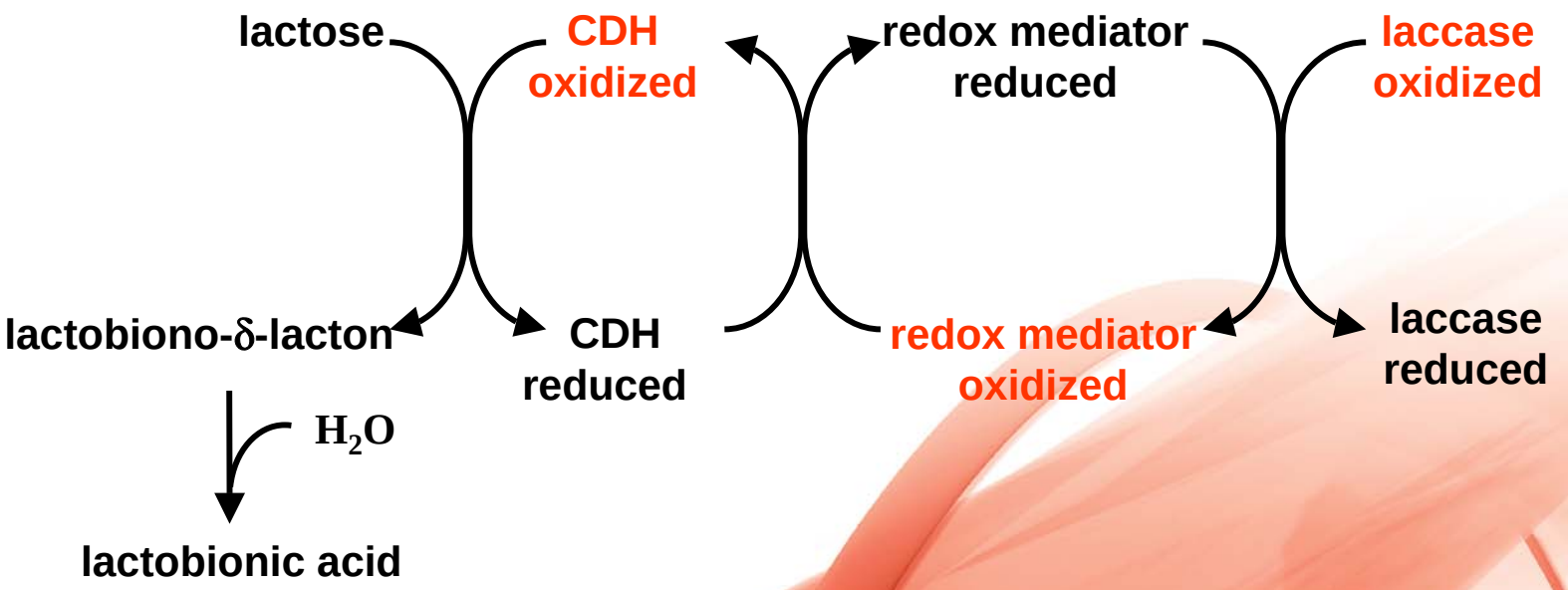
Selection of Suitable Redox Mediators

redox mediator	turnover (%)	redox mediator	turnover (%)
1,4-benzoquinone	~ 100	indophenol	82.6
tetrachloro-1,4-bq	~ 100	2,6-dichloro-indophenol	~ 100
tetrabromo-1,4-bq	~ 100	ABTS	~ 100
tetrafluoro-1,4-bq	75.8	methylene green	84.0
2-chloro-1,4-bq	~ 100	methylene blue	41.4
methyl-1,4-bq	92.5	meldola‘s blue	74.4
3,5-di-*tert*-butyl-1,2-bq	31.2	phenazine methosulfate	90.6
dimethyl-1,4-bq	30.0		
1,2,4-trihydroxybenzene	57.3	ferricenium	47.4
		ferricyanide	32.1
oxygen + catalase	*5.0*	Cu^{2+}	8.6
		Fe^{3+}	8.2

Lactose Oxidation Scheme

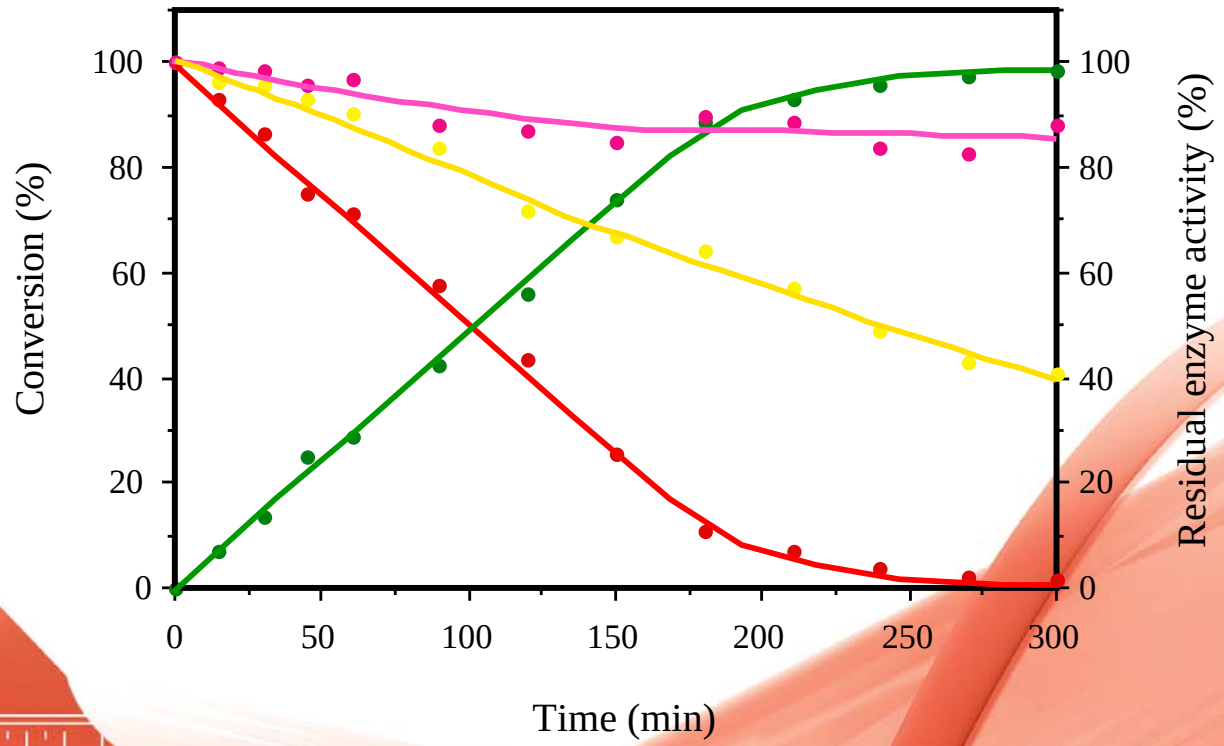


Regeneration System



Conversion of Lactose 1

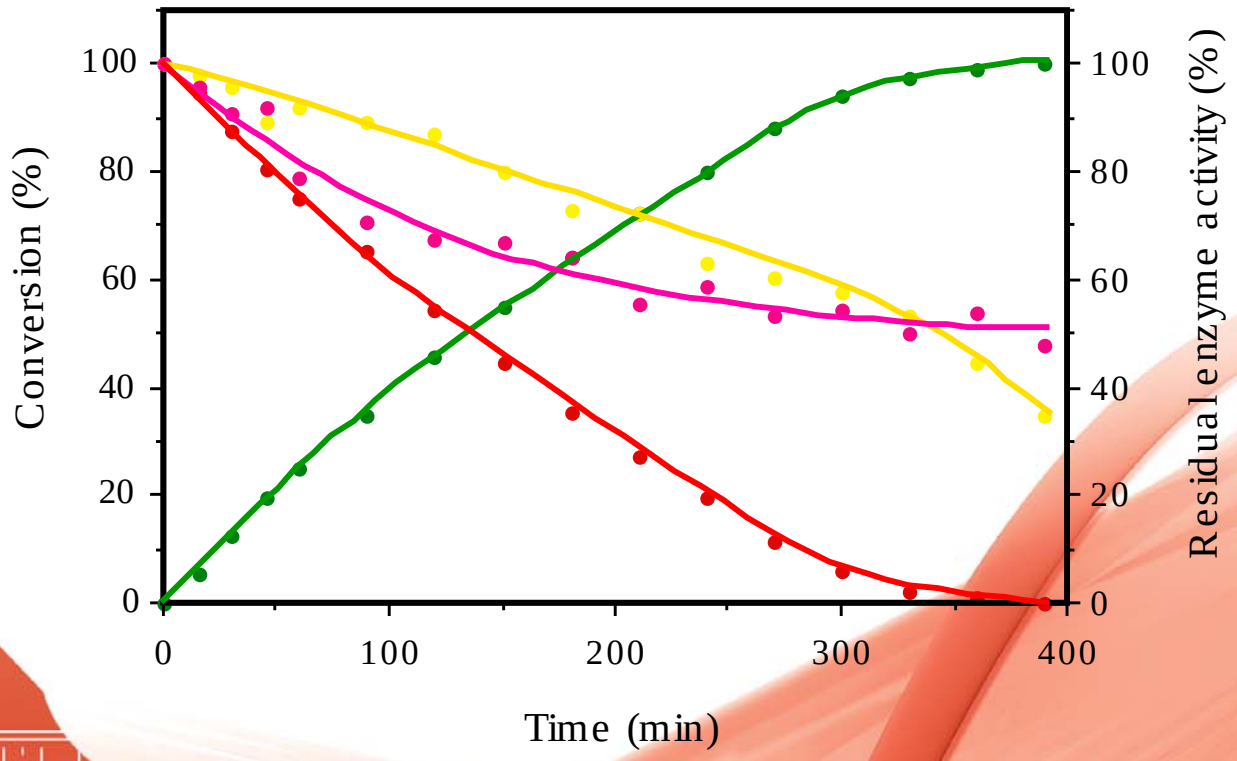
1 U/mL CDH, 2 U/mL laccase
200 mM lactose, 1.75 mM benzoquinone; 30 °C, pH 4.0



- Lactose
- Lactobionic acid
- CDH activity
- Laccase act.

Conversion of Lactose 2

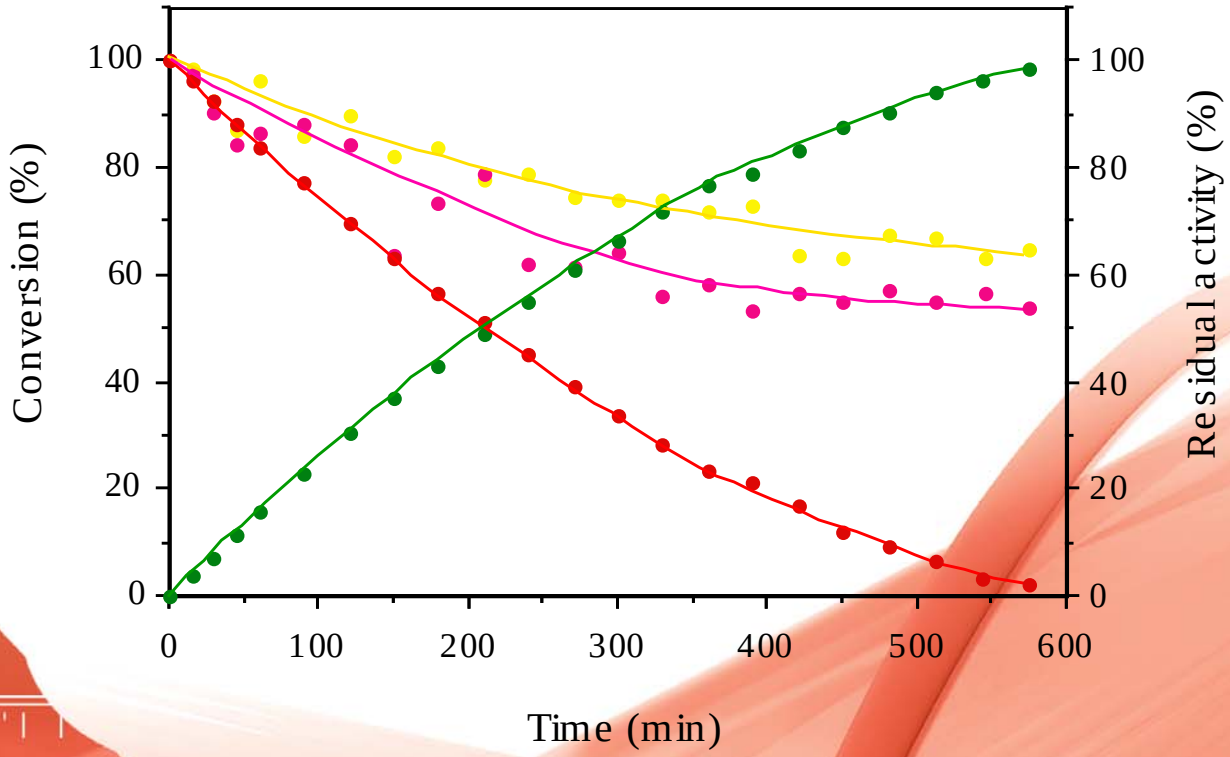
1 U/mL CDH, 2 U/mL laccase
200 mM lactose, 0.2 mM ABTS; 30 °C, pH 4.0



- Lactose
- Lactobionic acid
- CDH activity
- Laccase act.

Conversion of Lactose 3

1 U/mL CDH, 2 U/mL laccase
200 mM lactose, 0.2 mM dimethylaminomethyl-ferrocene; 30 °C, pH 4.0



- Lactose
- Lactobionic acid
- CDH activity
- Laccase act.

Characterization of the Biotransformation

- Complete conversion of lactose, no by-products detectable
- 40% CDH, 85 % laccase activity remaining
- Productivity of 21 g/L·h or 21 g/kU·h
- Turnover number TTN_{CDH} 1.25106
  $TTN_{laccase}$ 6.05106
  TTN_{bq} 115

Characterization of the Biotransformation

Redox mediator	Productivity $g \cdot (kU \cdot h)^{-1}$	CDH inactivation (U/mmol)
ABTS	19.3	2.1
DCIP	6.8	1.6
benzoquinone	21.4	2.7
methylene green	9.1	1.4
ferricyanide	3.2	2.0
ferrocene	9.1	1.9
Fe^{3+}	0.37	11.8
Cu^{2+}	2.0	26.1

Enzyme Inactivation during Turnover

- By the hydroxyl radical when Cu^{2+}, Fe^{3+}, or Mn^{3+} are used as redox mediator (formed by the Fenton reaction)

- At the air-liquid interface in the presence of high velocity gradients

Effect of Aeration

Reaction condition	Productivity $g \cdot (h \cdot kU)^{-1}$	CDH inactiva. $U \cdot mmol^{-1}$
10% DOT, O_2	11.2	0.02
20% DOT, O_2	16.0	0.81
20% DOT, air	16.6	2.34
40% DOT, O_2	17.8	1.46
60% DOT, O_2	20.4	1.48

The People:

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Thank You for Your Attention!