Verdartis | Desenvolvimento Biotecnológico

04 - 06
OUTUBRO 2010
TRANSAMERICA EXPO CENTER
SÃO PAULO - BRASIL

PersoZyme - Personalized Enzymes for Biobleaching of Cellulose Pulps.

ABTCP-TAPPI 2010
43º CONGRESSO E EXPOSIÇÃO INTERNACIONAL DE CELULOSE E PAPEL

43º Congresso e Exposição Internacional de Celulose e Papel

43rd Pulp and Paper International Congress & Exhibition

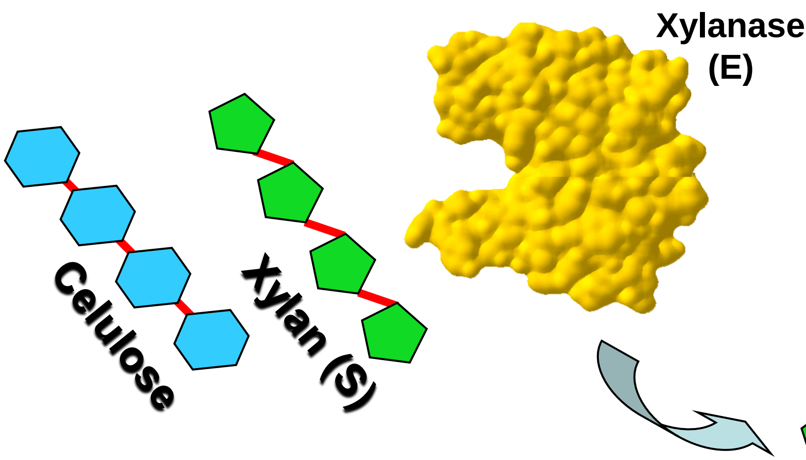
ABTCP

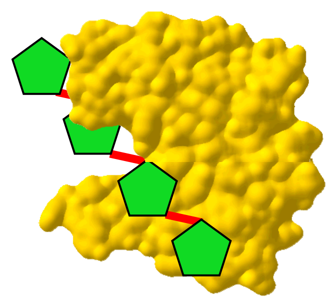
TAPPI

Summary

- Enzymes and their production in microorganisms
- Use of enzymes in pulp bleaching
- Obtaining low cost enzymes for efficient pulp bleaching
- Protein engineering of thermostable xylanases.
- Pulp bleaching tests with engineered xylanases.
- Conclusions.

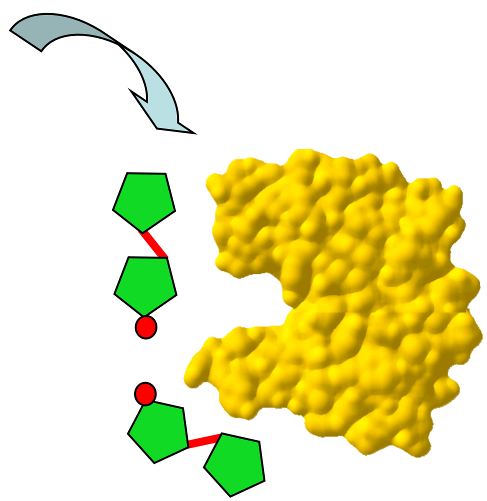
Enzymes are Good Catalysts





(1) Specific interaction between the enzyme and its substrate.
Enzymes are highly selective

(2) An exact chemical reaction is catalyzed by the enzyme.
Enzymes are highly specific



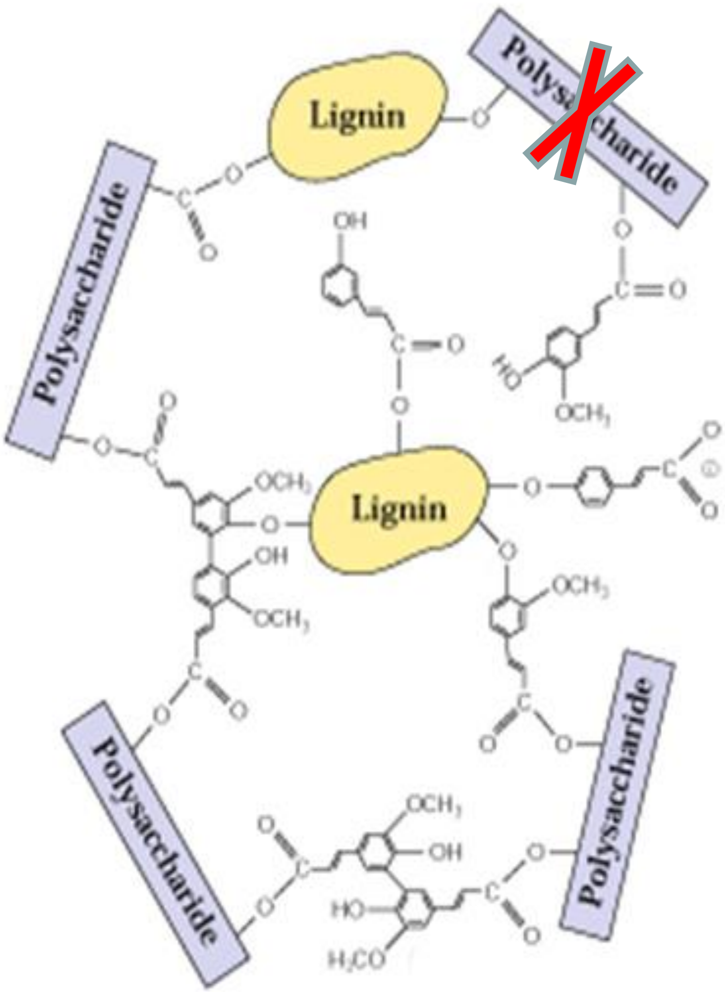
Enzymes are not consumed during the reaction

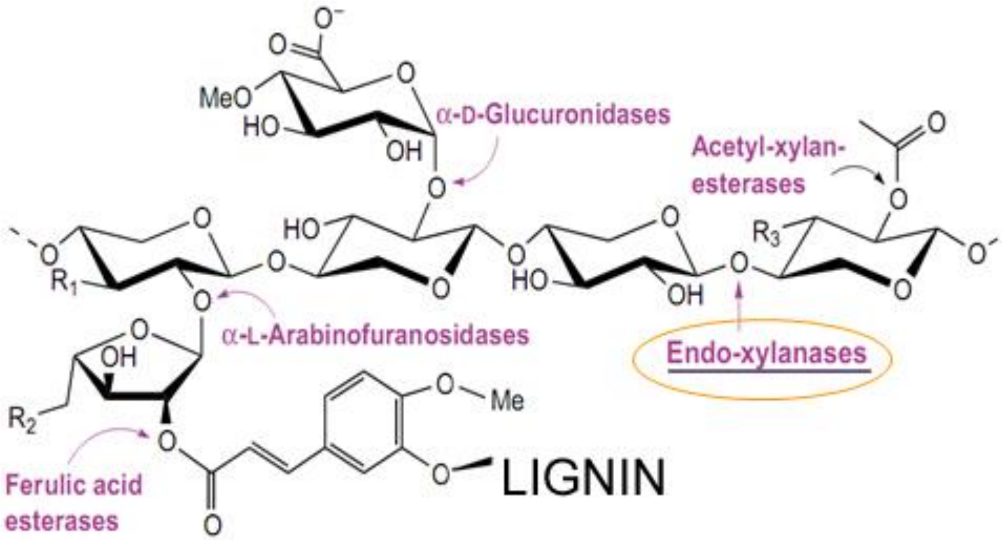
$$E + S \rightleftarrows ES \longrightarrow E + P$$

Sources of Enzymes

1) Production by microorganisms found in natural environments.	Usually low productivity	Mixture of enzymes (ex; contamination by celulases).	High cost
2) Production by genetically improved microorganismos	Improved productivity	Contamination may still be found.	Reduced cost
3) Production by genetically modified microorganismos	High productivity	No contamination	Low cost

Xylanase for Biobleaching





Xylanases do not act directly on lignin, rather they weaken the hemicelulose/lignin matrix and facilitate lignin extraction by conventional processes.

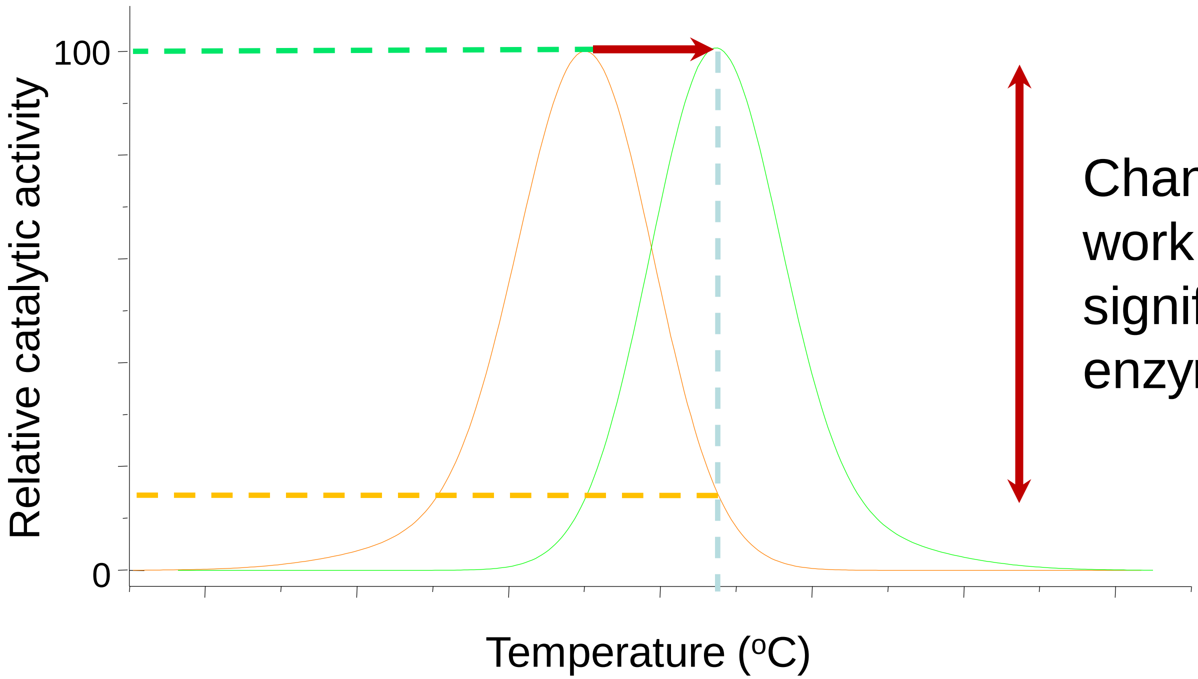
Enzyme application between alkaline treatment and pulp washing/pressing ensures increased lignin extraction and a decreased chlorine dioxide demand in downstream chemical bleaching steps.

Desired Enzyme Properties

- Working temperature ~ 80 - 85^{o}C
- Retention of activity for ~ 1 hour
- pH ~ 7
- Low material (mass) losses to effluents

Strategy for Improving Enzyme Performance

Enzymes have Optimum Working Temperatures



Changes in the optimum working temperature have significant effects on enzyme activity.

How can we obtain an enzyme that works at a desired temperature?

Enzyme engineering

How Enzymes have Evolved in Nature

Random Mutation + Natural Selection = *Evolution*

↓

Biodiversity

↓

Enzymes found in nature

TIME SPAN?

Millions of years!

Bioprospection programs aim to isolate spectific enzymes with certain pH and temperature optima.

TIME SPAN?

Maybe soon, maybe never!

How to Mimick This Process in the Laboratory

Directed Mutation + Artificial Selection
= *Directed evolution*

1 - 2 years

Directed Evolution + Bioinformatics analysis
<u>= *Accelerated evolution*</u>

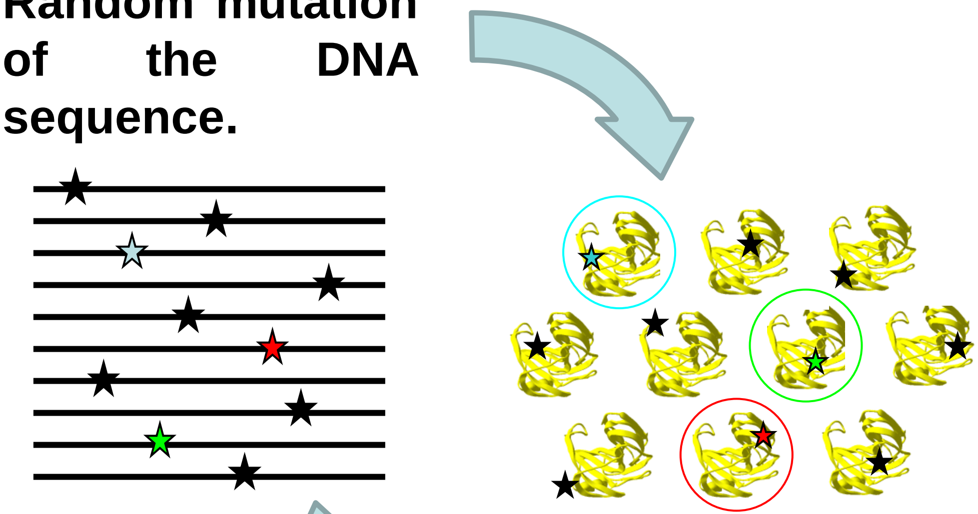
6 months

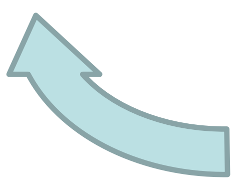
Enzymes created in the laboratory with chosen, personalyzed, catalytic properties

Personalyzed Enzymes: ***PersoZyme***

Improving Xylanase Thermostability by Directed Evolution - Method

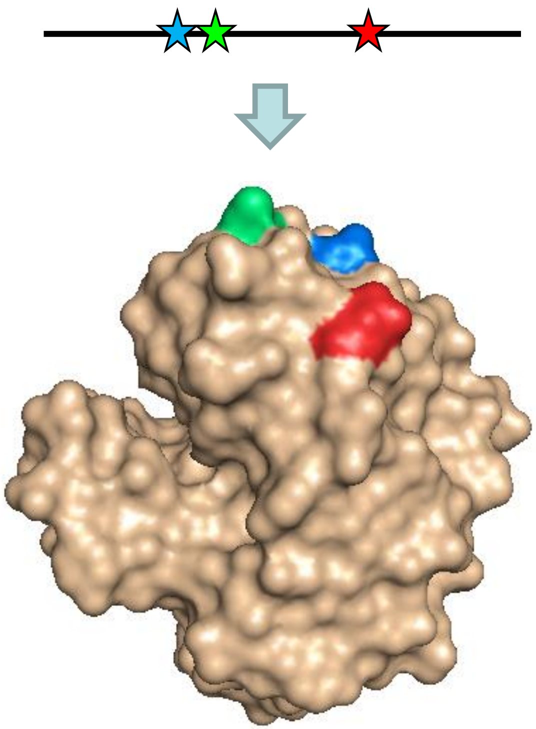
Random mutation of the DNA sequence.





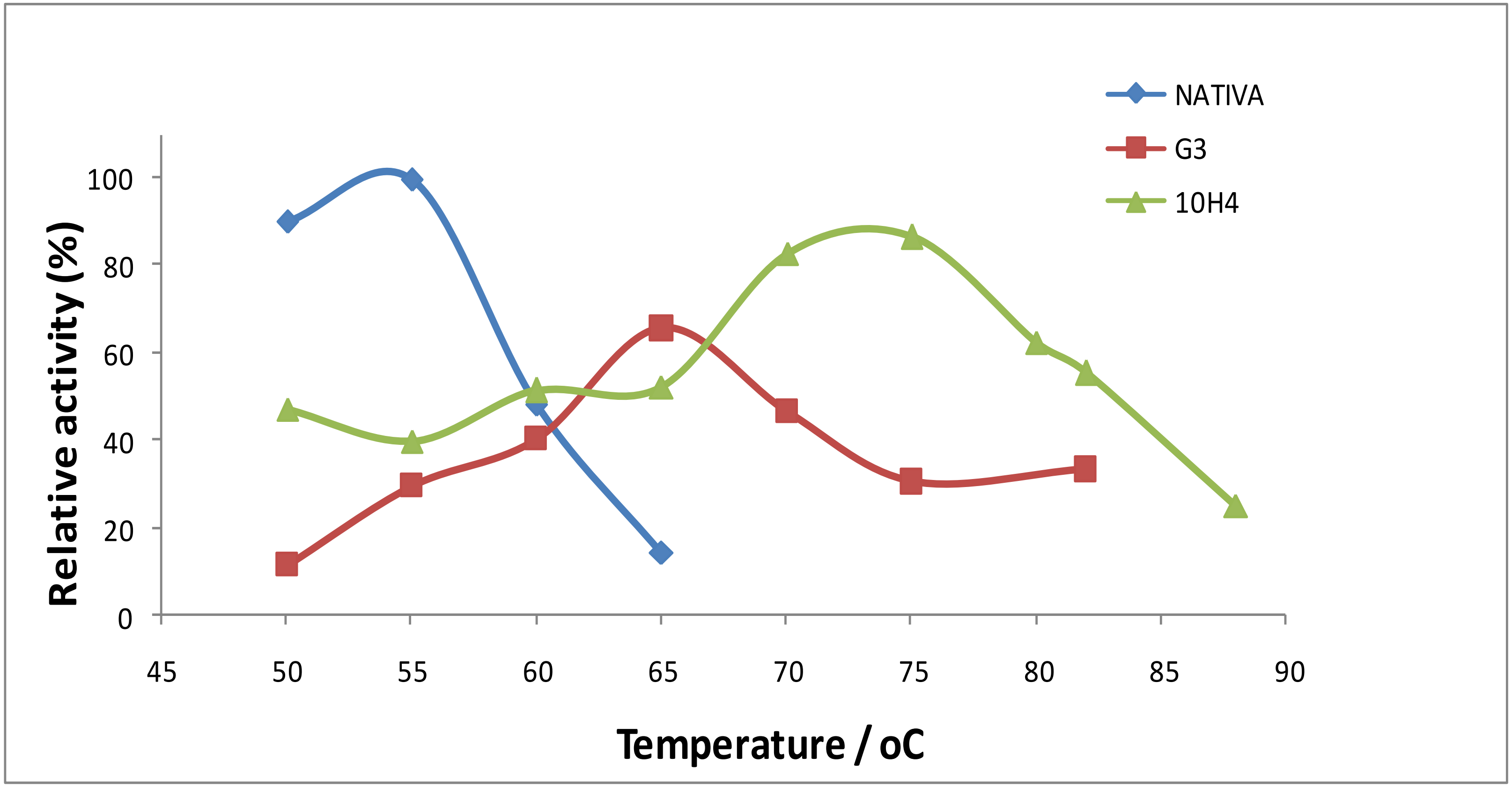
Seleçt the variants showing thermostability.

Cycles of Directed Evolution accumulate favorable mutants



Improving Xylanase Thermostability by Directed Evolution - Result

Successive cycles produce xylanases showing increasing thermostability



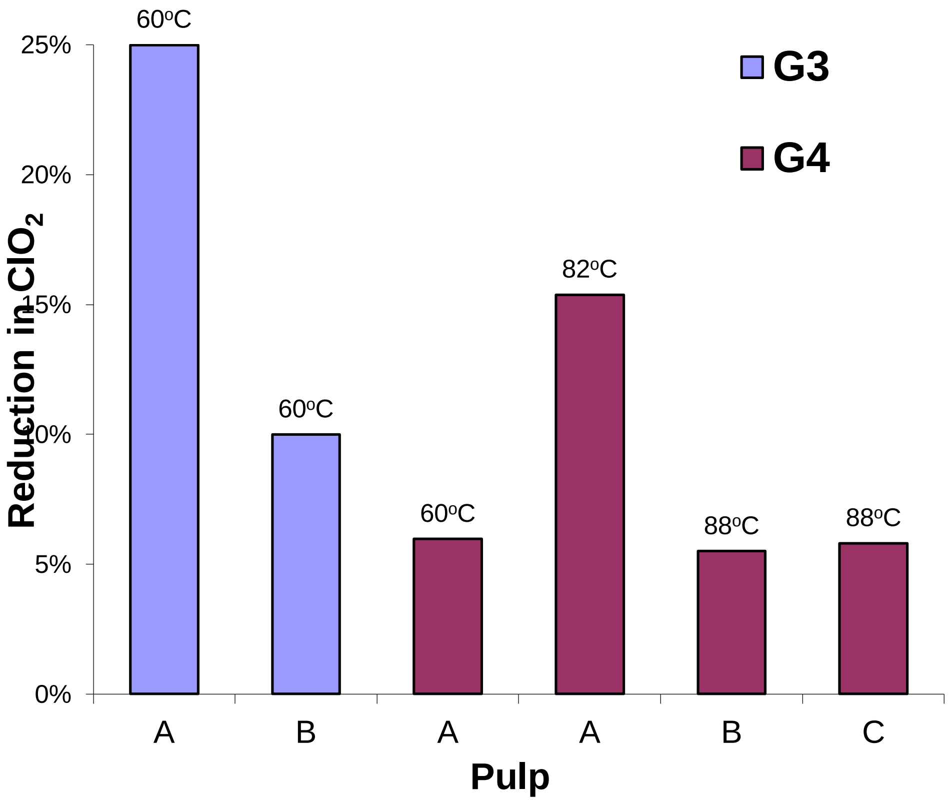
Pulp Treatment

- Industrial kraft pulps pre-delignified with oxygen from SUZANO PAPEL e CELULOSE S/A production units A, B and C.
- A= ENZYME - D*-(EP)-D-P
- B= ENZYME - A/D-(EPO)-D-P
- C= ENZYME - D-(PO)-D-P
- Optimize dioxide treatment step D1
- pH = 6
- Final whiteness = 90 %ISO

Pulp Analysis Methods

Kappa	TAPPI um 245
Viscosity	TAPPI T230 om 82
Whiteness	TAPPI T525 om 86
Whiteness reversion	4 h, 105 C, 0% UR, after conditioning sheets for 4 hr in an acclimatized room
Hexurinic acid	HUT Method: Vuorinen, T., Teleman, A., Fagerstrom, P., Buchert, J., and Tenkanen, M., Selective hydrolysis of hexenuronic acid groups and its application in ECF and TCF bleaching of kraft pulps. Proc. 1996 Intl. Pulp Bleaching Conf., Tappi Press 1:43-51 (1996).
Titration of solutions and bleaching residues	Kraft, P., In: Pulp & Paper Manufacture, Vol. 1, McDonald, R.G. (editor), 2nd ed., McGraw-Hill Book Company, New York, 1967, p. 628-725
Effluent COD	CPPA H.3
Yield with Enzyme Treatment	Yield loss(%)=0,3974*TOC(kgC/t)+0,1124
Bleaching yield	Yield loss(%)=0,0993*TOC(kgC/t)+1,706
Effluent AOX	SCAN-W9:89

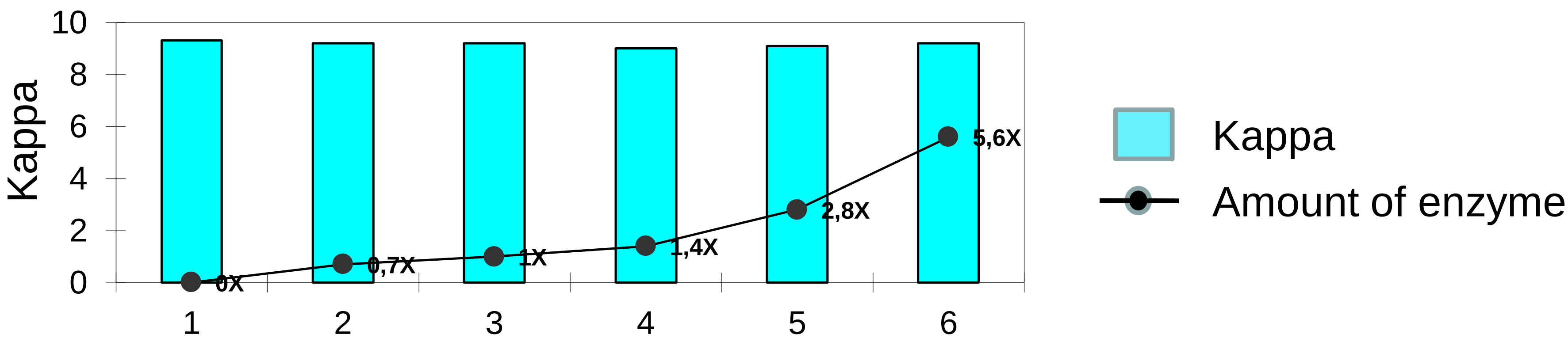
Pulp Treatment with Enzymes G3 and G4.

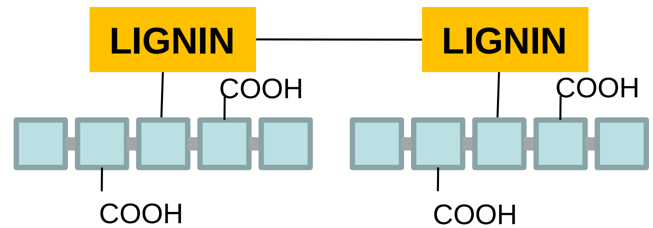


1) Treatment of pulps A, B and C with enzimas G3 e G4 reduces chlorine dioxide demand.

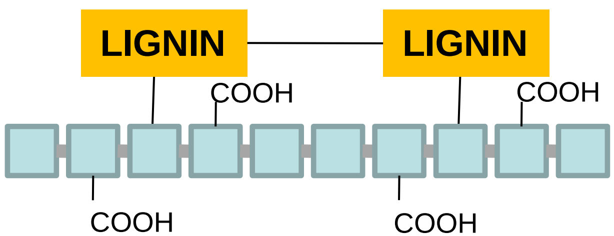
2) The effect of the enzyme varies from one pulp to another – a single enzyme may not be suitable for all processes.

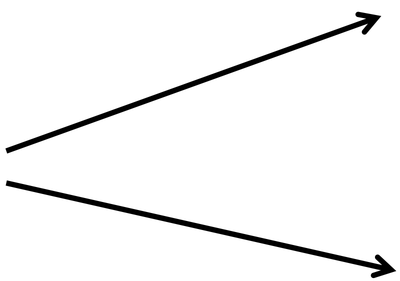
Kappa of Pulp A is Independent of the Amount of Enzyme.

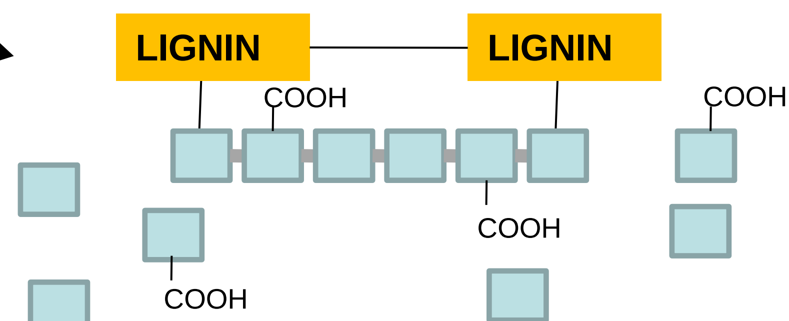




G4 limits mass loss and HexA extraction

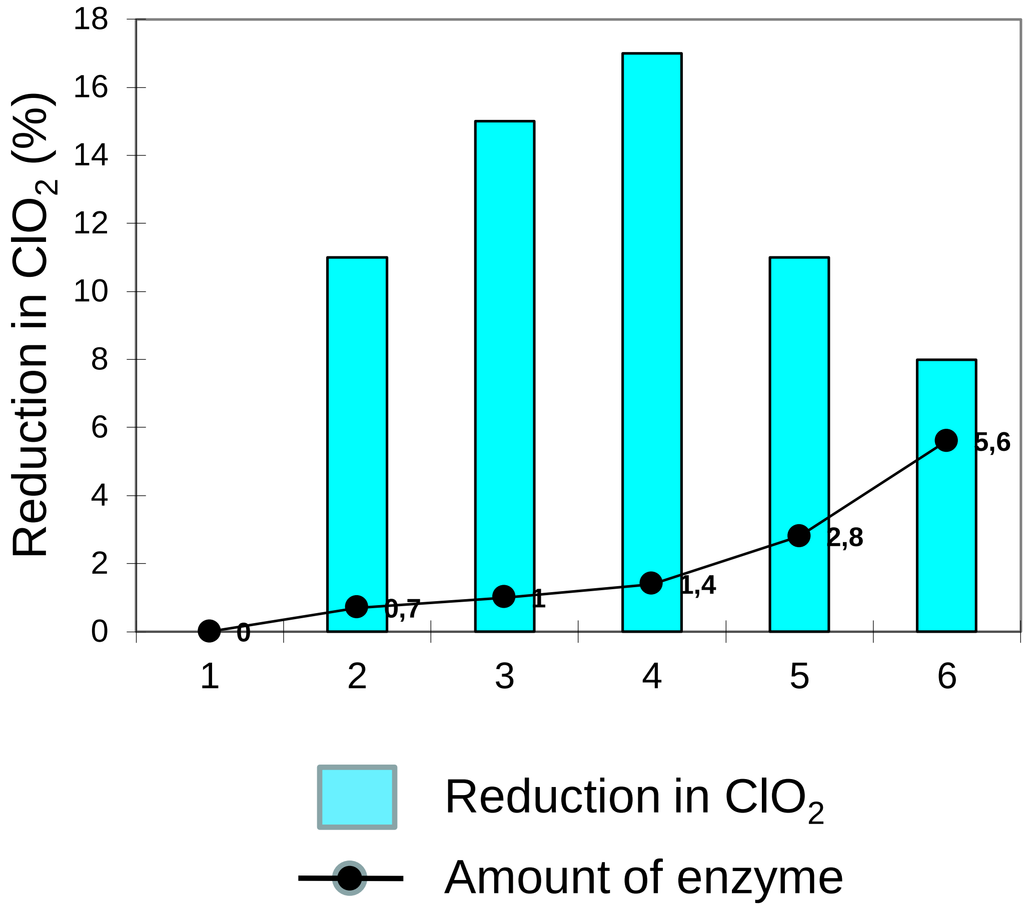






Extensive modification by commercial enzymes results in mass loss and HexA extraction

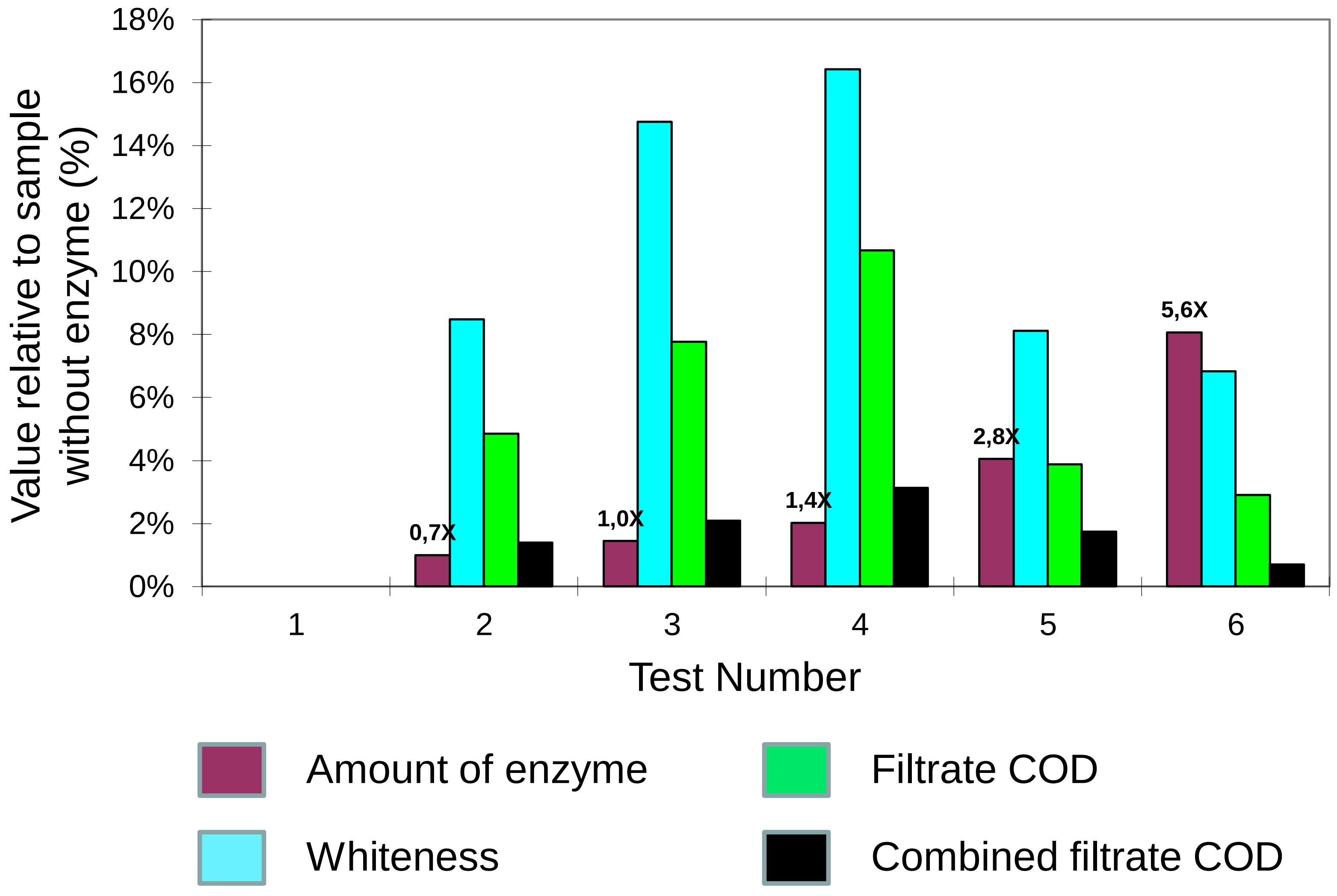
Working Enzyme Concentration Should be Optimized.



Maximum chlorine dioxide economy is found at an enzyme dose of 1.4x.

Values above this amount are less effective – Enzymes are inhibited by their reaction products.

Enzyme Activity Influences Whiteness and COD.



Technical Summary of the Effect of Enzyme on Bleaching of Pulp A.

	Pulp A, 82ºC					
Test Number	**1**	**2**	**3**	**4**	**5**	**6**
Enzyme Quantity (x standard)	Reference (0)	0,7	1,0	1,4	2,8	5,6
ENZYME, X/300 g pulp A	0,00	4,14	5,93	8,30	16,55	33,11
Economy of ClO_2, kg/t for 90% ISO	-	**1,00**	**1,40**	**1,57**	**1,00**	**0,72**
Final whiteness, % ISO	90,0	90,0	90,2	90,4	90,0	89,8
Reverted whiteness,% ISO	88,6	88,7	88,7	89,0	88,5	88,3
Whiteness reversion, % ISO	1,4	1,3	1,5	1,4	1,5	1,5
Yield, %	98,1	98,0	98,0	98,1	98,1	98,0
Viscosity, mPa.s	14,4	14,4	14,3	14,5	14,9	15,6
Kappa (immediately following enzyme treatment)	9,3	9,2	9,2	9,0	9,1	9,2
Whiteness (immediately following enzyme treatment), % ISO	54,2	58,8	62,2	63,1	58,6	57,9
Filtrate COD, kgO_2/t	10,3	10,8	11,1	11,4	10,7	10,6
Combined filtrate COD, kgO_2/t	28,7	29,1	29,3	29,6	29,2	28,9

Comparison of G4 with Comercial Enzymes.

	Comercial Enzymes (Eiras et al, ABTCP 2009)	Enzima G4
• Kappa:	Reduction	Maintained
• Whiteness %ISO:	Increase ~5,4% (44)	Increase 8,9% (54)
• Xylan & Hexe. Ac.	Reduction	Maintained
• Yield.	Loss (1,6%)	Unchanged
• COD:	84 a 314%	10,68%
• Economy of ClO_2:	42 to 52%	18%

- **Reduction in Chemical Cost (calculated to a whiteness of 90.00% ISO) = US$ 3,00 US$ por tonne.**

Conclusions

- The technical viability for enzyme based biobleaching depends on lignin extraction by water.
- The action of the G4 enzyme facilitates the extraction of lignin in an aqueous environment.
- Enzymes are inactivated by their reaction products – the amount of enzyme must therefore be optimized for a given application.

Acknowledgements and Perspectives

Enzyme technology under development for:

Acknowledgements.

FAPESP

FINEP

CNPq

Biobleaching

Cellulose refining

Second Generation (2G) Ethanol

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