Bioethanol production from date palm fibers: Effect of alkaline hydrogen peroxide pretreatment and fermentation conditions

I. Ben Atitallah[1,2], I. Ntaikou[3], G. Antonopoulou[3], C. Bredai[4], G. Lyberatos[3,5], T. Mechichi[2]

[1]Laboratory of Enzyme Engineering and Microbiology, National School of Engineers of Sfax, University of Sfax, BP 1173, 3038 Sfax, Tunisia.
[2]Laboratory of Biochemistry and Enzyme Engineering of Lipases, National School of Engineers of Sfax, University of Sfax, BP 1173, 3038 Sfax, Tunisia.
[3]Institute of Chemical Engineering Sciences, 11 Stadiou st., Platani, Patras, GR 26504, Greece
[4]Electromechanical Systems Laboratory LASEM, National Engineering School of Sfax, University of Sfax, Soukra km 4, 3038 Sfax, Tunisia
[5]School of Chemical Engineering, National Technical University of Athens, GR 15780 Athens, Greece.
ntaikou@iceht.forth.gr

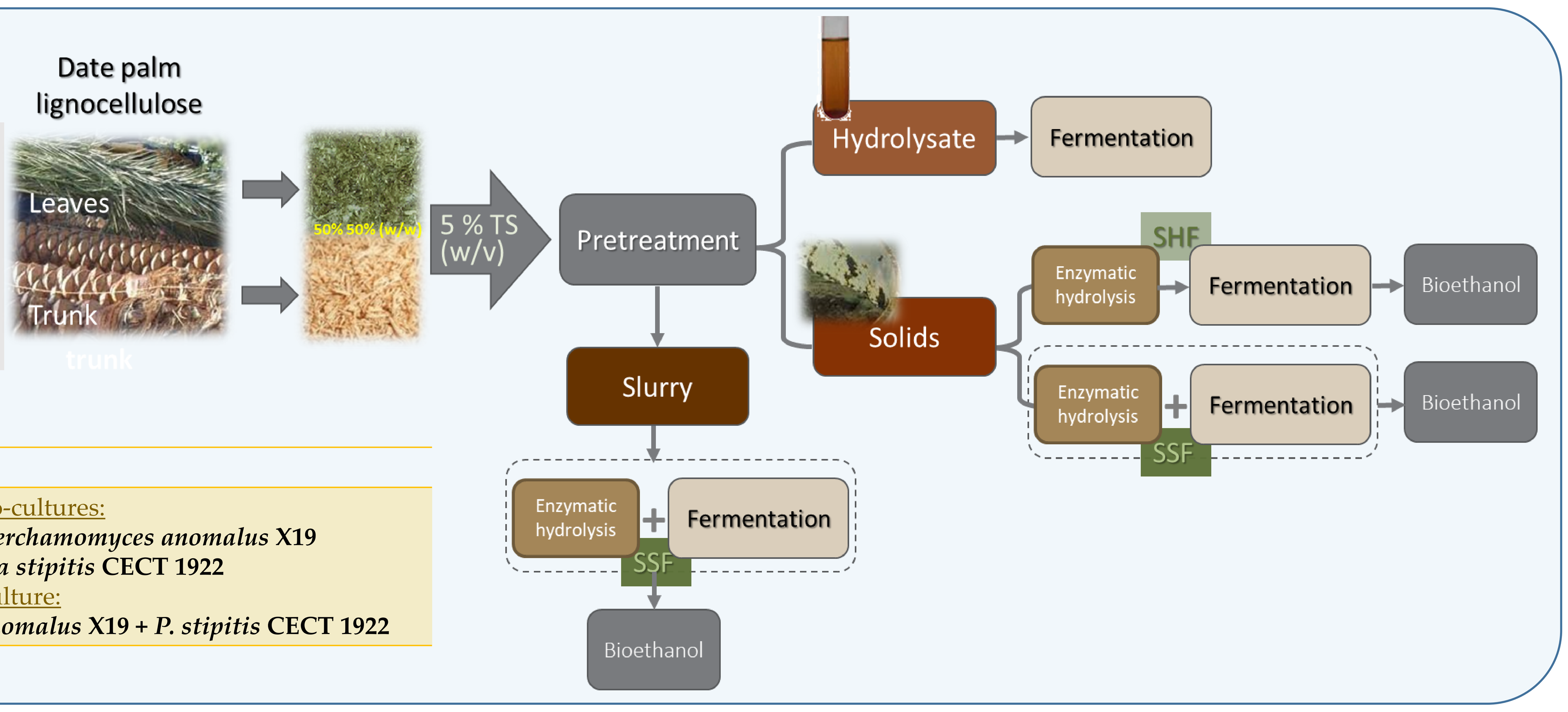
INTRODUCTION

Bioethanol is considered to be one of the most promising renewable fuel candidates but in order to be sustainable, its production has to be based on renewable carbon sources. Lignocellulosic biomass represents viable substrates for bioethanol production due to its great availability and low cost. Pretreatment of the lignocellulosic biomass is the most crucial step, since it influences all subsequent process steps. Several pretreatment methods have been developed in the effort of removing the structural and compositional barriers and for improving the yield of the enzymatic hydrolysis of lignocelluloses. Among them, alkaline hydrogen peroxide pretreatment has been shown to be a promising approach as it leads to high glucose yields and can be carried out in conditions for moderate temperature and pressure without acids which leads to inhibitors formation. Furthermore, pretreatment with peroxides improves the enzymatic efficiency through oxidative delignification and decrease the crystallinity of the cellulose. The date palm (*Phoenix dactylifera*) is one of the most cultivated palms in the arid and semi-arid regions of the world. The removal of dry leaves and trunk fibers after harvesting of date fruits generates an important quantity of date palm lignocellulosic residues. A rational way of valorizing this abundant renewable resource could be its use as substrate for biofuels production such as lignocellulosic ethanol.

Approach & Methodology

Aim: Exploitation of date palm fibbers towards second bioethanol production via yeasts, by exploring the:

- ✓ *fractionation and saccharification of lignocellulose via novel thermochemical pretreatment*
- ✓ *maximisation of ethanol yields via yeast mono- and co-cultures*

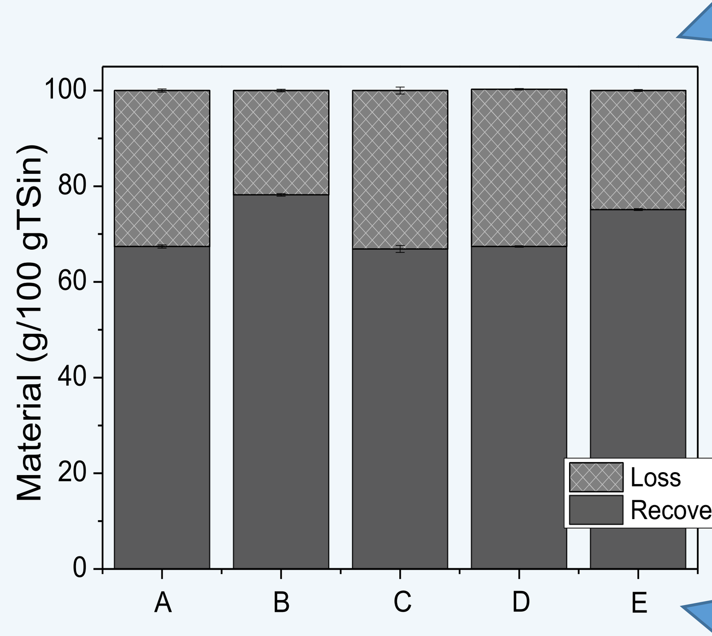


A. NaOH, 0.5% (w/v)
B. H_2O_2 0.5% (v/v)
C. H_2O_2 1% (v/v) + NaOH, 1% (w/v)
D. NaOH, 0.5% (w/v) → H_2O_2 30% (v/v)
E. H_2O_2 30% (v/v) → NaOH, 0.5% (w/v)

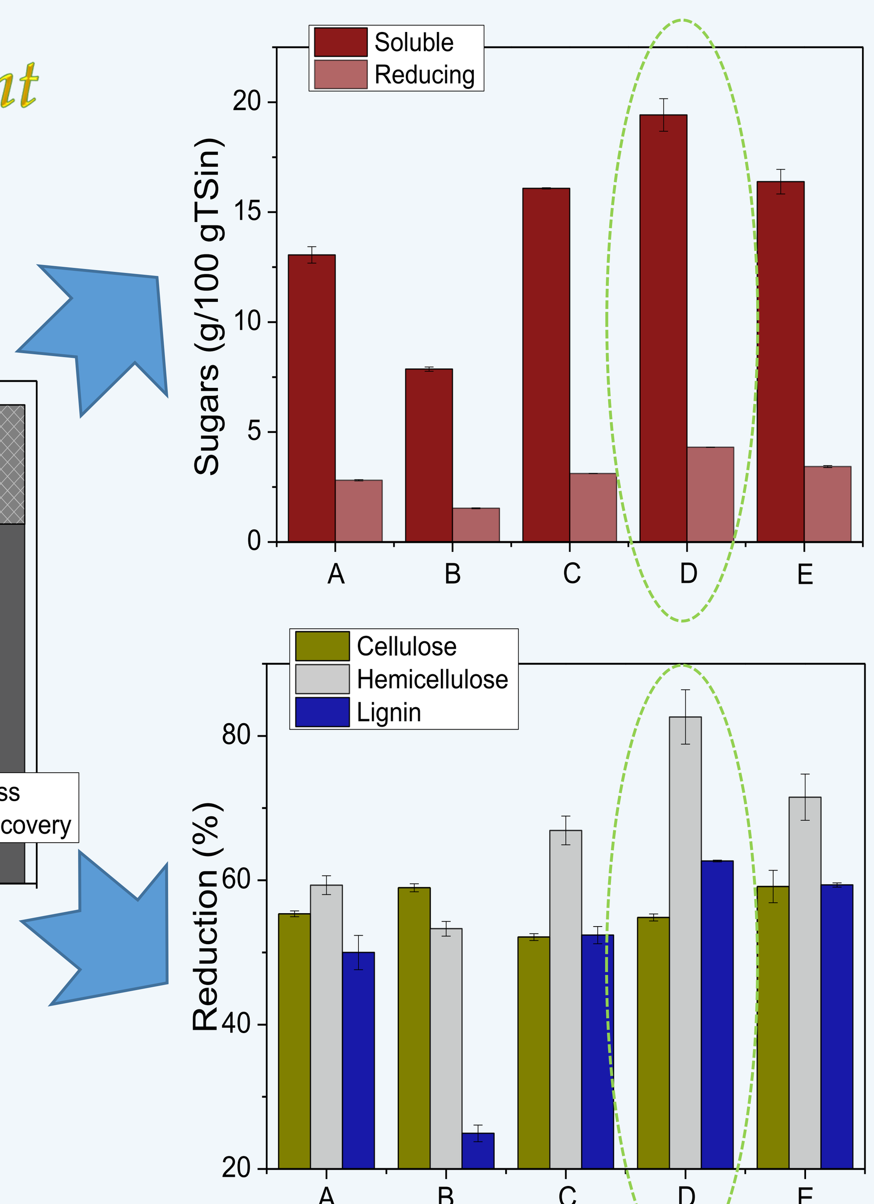
Enzyme
Cellulase blend, pH=4.8, 50°C, 30 FPU Cellic/gTS

Yeasts
- ✓ Mono-cultures:
- - *Wickerchamomyces anomalus* X19
- - *Pichia stipitis* CECT 1922
- ✓ Co-culture:
- - *W. anomalus* X19 + *P. stipitis* CECT 1922

Results : *pretreatment*



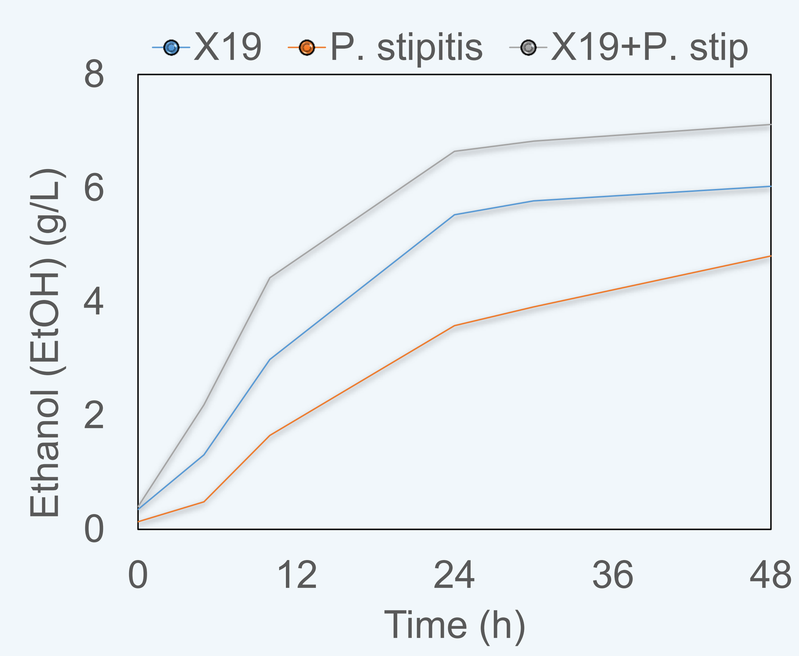
- Cellulose : 42.30 ± 0.25
- Hemicellulose : 23.82 ± 1.11
- Lignin : 27.17 ± 0.79



- ✓ The material recovery (MR) was high in all cases (67-78%)
- ✓ Single step H_2O_2 treatment led to the highest MR and lowest yield of free sugars
- ✓ Reducing to free sugars ratio was approximately 20% in all cases

- ✓ 2 step treatments were more effective on
  - *Hemicellulose solubilisation*
  - *Lignin removal*
- ✓ Cellulose removal ranged from 52%-59%, with H2O2 treatments either in single step or two steps process leading to higher solubilisation

Results : *fermentation*



- ✓ Co-cultures resulted to higher ethanol production in all cases
- ✓ Fermentation of pretreated DPF using co-cultures of *W. anomalus* and *P. stipitis* leaded to the highest ethanol titter of 7.12 g/L (6.70 g/L at SHF).
- ✓ Ethanol production efficiency from the pretreated slurry and solids was not affected by the fermentation mode, neither for the monocultures, not the co-culture,

Strain	Slurry			
	SSF		SHF	
W. anomalus	0.38 ± 0.01	128.01 ± 3.12	0.38 ± 0.01	142.40 ± 4.44
P. stipitis	0.37 ± 0.01	101.82 ± 1.99	0.37 ± 0.01	118.05 ± 2.65
Co-culture	0.41 ± 0.01	151.07 ± 2.47	0.40 ± 0.01	158.26 ± 3.52

Strain	Hydrolysate		Solid			
			SSF		SHF	
W. anomalus	-	-	0.36 ± 0.01	52.45 ± 3.01	0.30 ± 0.00	50.24 ± 3.01
P. stipitis	0.16 ± 0.01	30.83 ± 1.82	0.37 ± 0.00	48.29 ± 2.18	0.31 ± 0.01	45.77 ± 3.01
Co-culture	-	-	0.38 ± 0.02	66.12 ± 3.31	0.36 ± 0.01	61.44 ± 3.01

CONCLUSIONS

- ✓ Hydrogen peroxide pretreatment can be quite effective for the delignification of lignocellulosic biomass when combined with alkaline pretreatment in a two steps process
- ✓ Pretreated date palm fibers could be a promising substrate to produce lignocellulosic ethanol via the coculture of the non-conventional yeasts *W. anomalus* and *P. stipitis*.

We acknowledge financial support of this work by the project "Research infrastructure for Waste Valorization and Sustainable Management of Resources, INVALOR" (MIS 5002495) which is implemented under the "Action for the Strategic Development on the Research and Technological Sector", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund).