Different Strategies for Lignocellulose Sugars Conversion into Ethanol from Phosphoric Acid Steam Exploded Olive Tree Pruning

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CIEMAT Spanish Research Center in Energy, Environment and Technology.

Mission: To promote and execute research, innovation and technical development in the energy sector to incorporate new energy technologies into society with minimum environmental impact and in an economic way.

Areas of activity:

- Nuclear: Fission and Fusion
- Residues valorization: Combustion and Gasification
- Renewable Energies: Solar , Wind, Energy efficiency and saving, Biomass and Biofuels
- Environment
- Technology
- Knowledge Transfer and Capacity Building

Personnel : 1.500 staff

Budget : 92 M€ / year

LIQUID BIOFUELS UNIT

OBJECTIVE

To develop processes and technologies for converting lignocellulosic materials into ethanol in an efficient and cost-effective manner to facilitate the adoption of these processes by industry

JOINT RESEARCH UNIT CIEMAT-IMDEA ENERGy

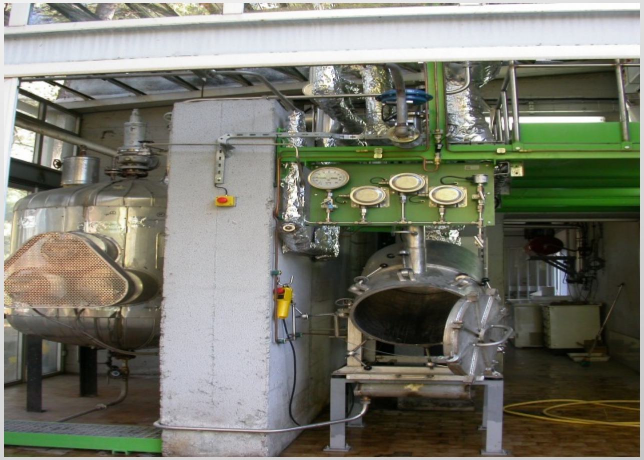
Annual average budget: 1M€
Personnel: 10 permanent senior researchers, 7 technicians and PhD students

OBJETIVE: To advance in the study of processes to produce biofuels

- Increasing the robustness of fermentative microorganisms
- Microalgae for biofuels and bioproducts

FACILITIES

- **Pilot plants:**
  - **Pretreatment:** Steam explosion (2 and 10 L), Parr reactors (0.5 -5 L), Continuous Twin Screw Extruder
  - Enzymatic hydrolysis (30 L Stirred Bioreactors , drum reactor)
  - Microalgae: 1 m^3 close photobioreactors and 1 m^3 open Ponds
- **Molecular biology laboratory** equipped with state-of-the-art instrumentation for recombinant DNA technology
- **Microbiology and biotechnology lab**, equipped with laminar flow cabins, orbital shakers, pH meters, autoclaves, lab bioreactors and photobioreactors, drums reactors
- **Analytical lab**, equipped with UV-VIS spectrophotometers, GC, HPLC equipped with a variety of columns and detectors (IR, MS, UV-VIS, HPAEC-PAD).



Different Strategies for Lignocellulose Sugars Conversion into Ethanol from Phosphoric Acid Steam Exploded Olive Tree Pruning

Why Olive Tree Pruning Biomass?

- Olive trees are cultivated in more than 40 countries (10.4 million ha).
- Andalusia 1.5 million ha (300 million olive trees).
- Pruning: 3 ton of residual biomass per hectare

Additional cost: 50 €/ha

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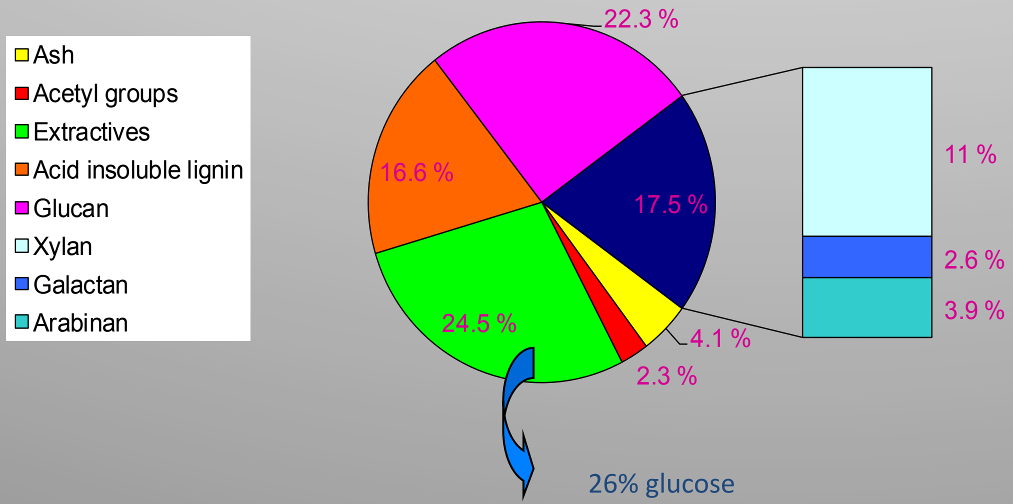
- OTP constitutes an important source for energy and chemicals production. Till date it is not being used.
- Biotechnological valorization of OTP presents significant challenges due to its heterogeneity (50% of thin branches, 25% of wood and 25% of leaves) with high amount of soluble compounds (extractives).

OTPB composition



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Antioxidant capacity
ABTS (5057 mg TE/100 g)

OLIVE TREE PRUNING (OTP)

↓

Water extraction
10% (w/v) at 120 °C for 60 min

↓

EXTRACTED OTP (EOTP)

Component	EOTP(%)
Cellulose	26.9
Hemicellulose	20.1
Lignin	26.0
Ash	4.8
Extractives	9.0

EOTP was impregnated phosphoric acid solution 1% (w/w) and pretreated at 195 ºC, 10 min.

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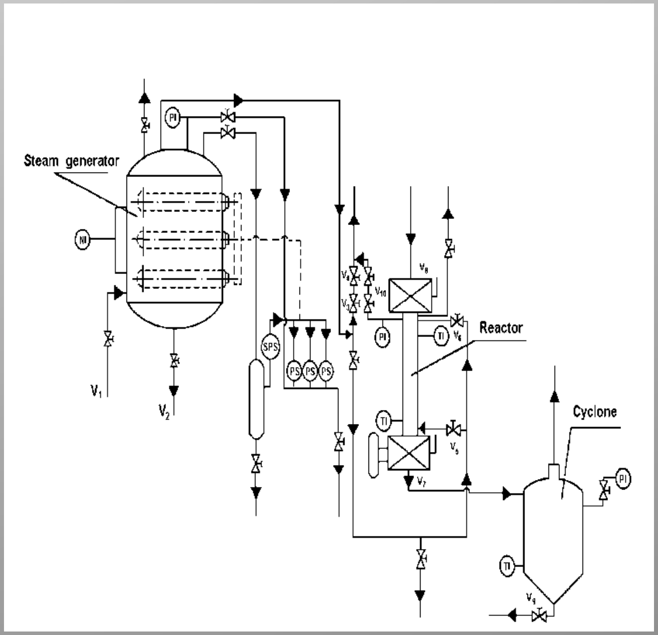
Why Phosphoric Acid Steam Explosion?

- Steam pretreatment with dilute mineral acids serves as an efficient approach to depolymerize hemicellulose and enhance cellulose digestion with enzymes.
- Side products (furfural, 5-hydroxymethyl furfural, acetate, phenolics and others) formed during pretreatment inhibit microbial growth and retard fermentation.
- Weaker acids such as phosphoric acid produce lower levels of toxic side products and lower corrosiveness (no need for exotic metal alloys).
- Phosphate is an obligatory nutrient in fermentation media.
- The higher cost of phosphoric acid as compared to sulfuric acid could be offset in part by recovery and reuse as a dilute fertilizer when crops are nearby.

STEAM EXPLOSION PRETREATMENT

- ✓ Uses saturated steam at temperatures 170-220ºC → sudden depressurization → break up of lignocellulose fiber
- ✓ Combines chemical effect and mechanical forces
- ✓ It is developed at commercial scale



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OLIVE TREE PRUNING (OTP)

↓

Water extraction

↓

EXTRACTED OTP (EOTP)

↓

Pretreatment
1% (w/w) H3PO4, 195 ºC, 10 min.

↓

PRETREATED EOTP (PEOTP)

↓

Filtration

LIQUID FRACTION (LF-PEOTP)

	LF-PEOTP(g/L)		
Sugars		**Inhibitors**	
Glucose	6.6	Acetic acid	3.9
Xylose	15.9	Furfural	2.3
Arabinose	4.2	5-HMF	0.5
Galactose	4.1	Formic acid	0.3
Mannose	0.9	Vanillin	0.02
		Syringaldehyde	0.04

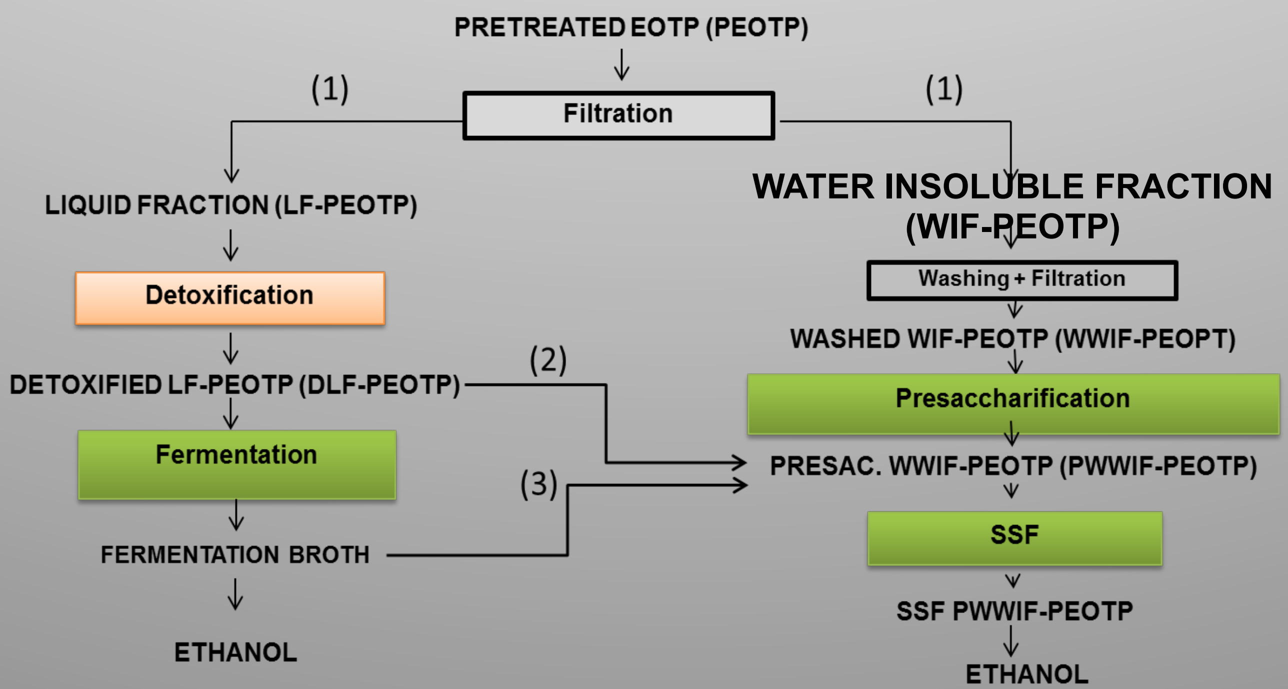
WATER INSOLUBLE FRACTION (WIF-PEOTP)

	WWIF-PEOTP(%)
Component	
Cellulose	42.8
Hemicellulose	1.3
Lignin	47.9
Ash	---
Extractives	---

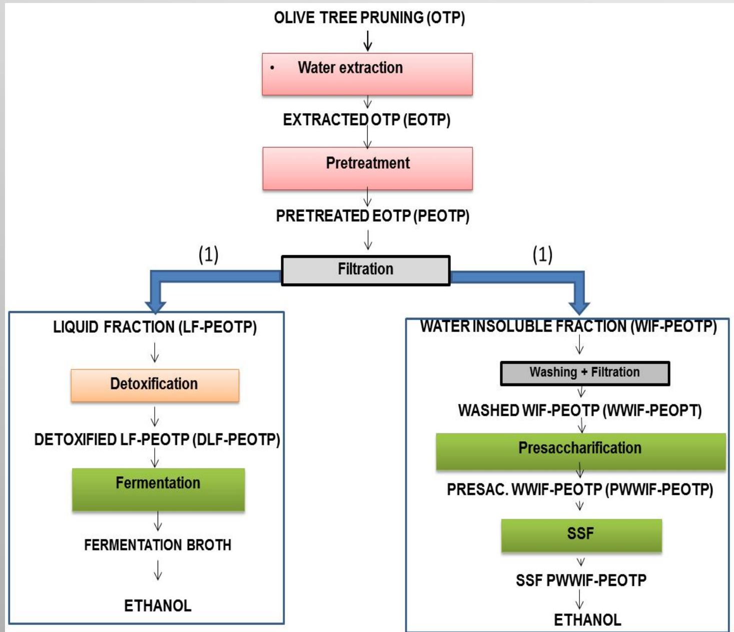
Different strategies for ethanol production from all sugars contained in the pretreated OTP

- Saccharomyces cerevisiae F12 (Recombinant strain with XYL1 and XYL2 from *Scheffermomyces stipitis*)
- Presacc. 18% substrate loading, 0.12 mL/g Cellic CTec2

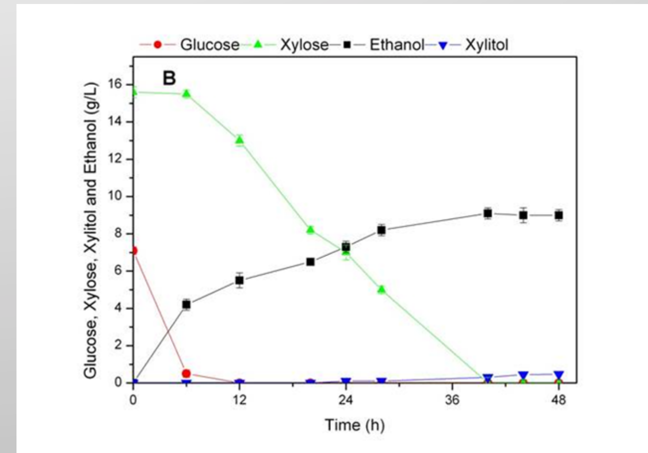
1) Separate fermentation of prehydrolyzate (C5 sugars) and hydrolyzate (C6 sugars).
2) Combined fermentation of prehydrolyzate (C5 sugars) and hydrolyzate (C6 sugars).
3) Sequential fermentation of prehydrolyzate (C5 sugars) and hydrolyzate (C6 sugars).



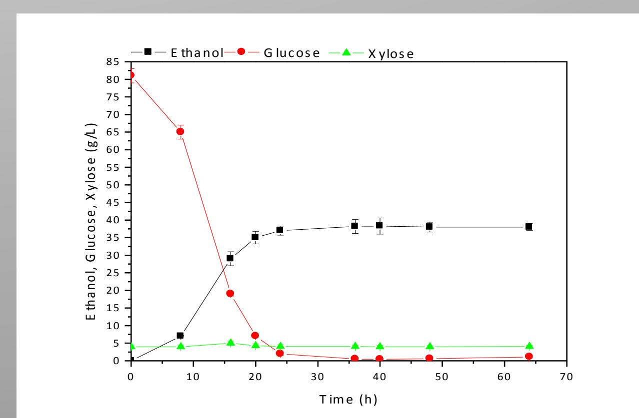
Separate Fermentation of DLF-PEOTP (C5 sugars) and PWWIF-PEOTP(C6 sugars)



DLF-PEOTP



PWWIF-PEOTP



DLF-PEOTP:

No glucose at 6 h. Xylose was consumed in a linear rate.

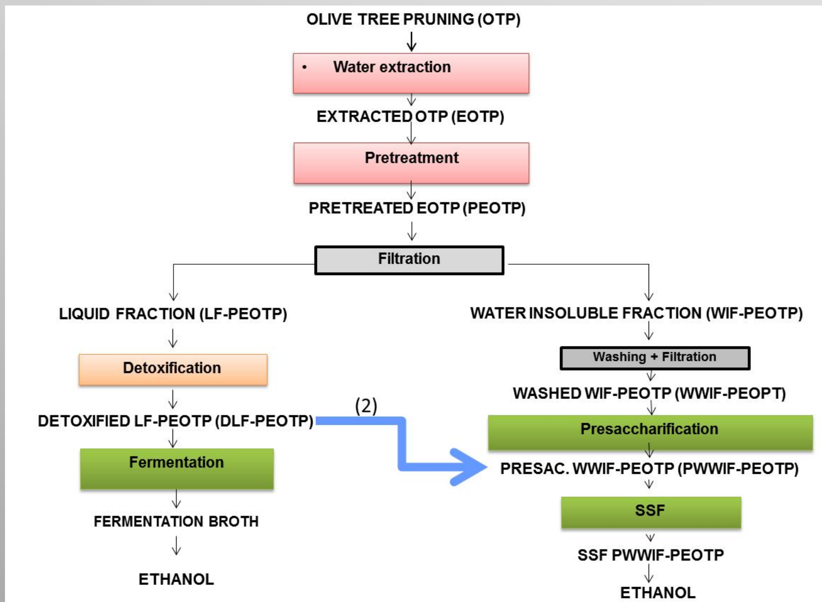
9 g/L (77% theoretical yield)

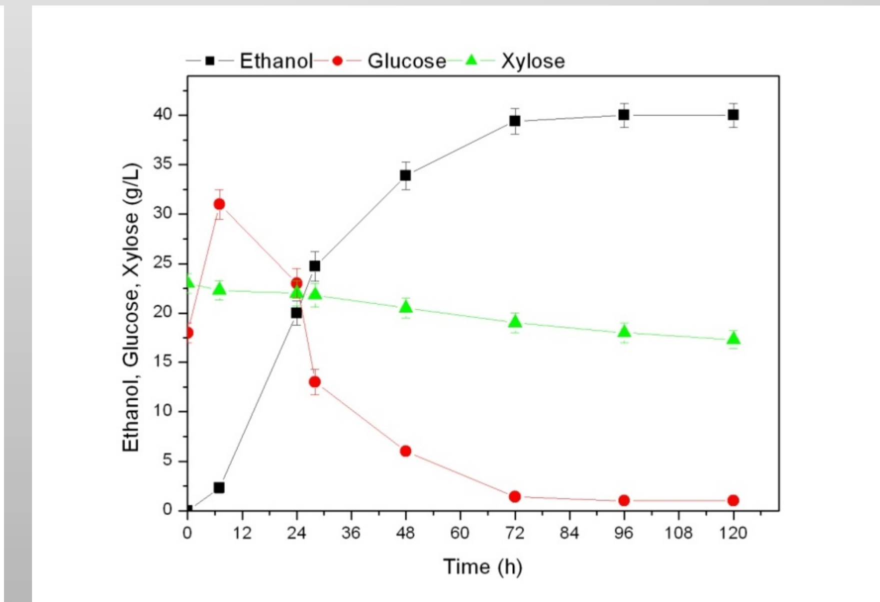
PWWIF-PEOTP

80 g/L of glucose in Presacc. step

After 24 h from fermentation 37 g/L ethanol (85% of SSF theoretical yield)

Combined Fermentation of DLF-PEOTP (C5 sugars) and PWWIF-PEOTP(C6 sugars)

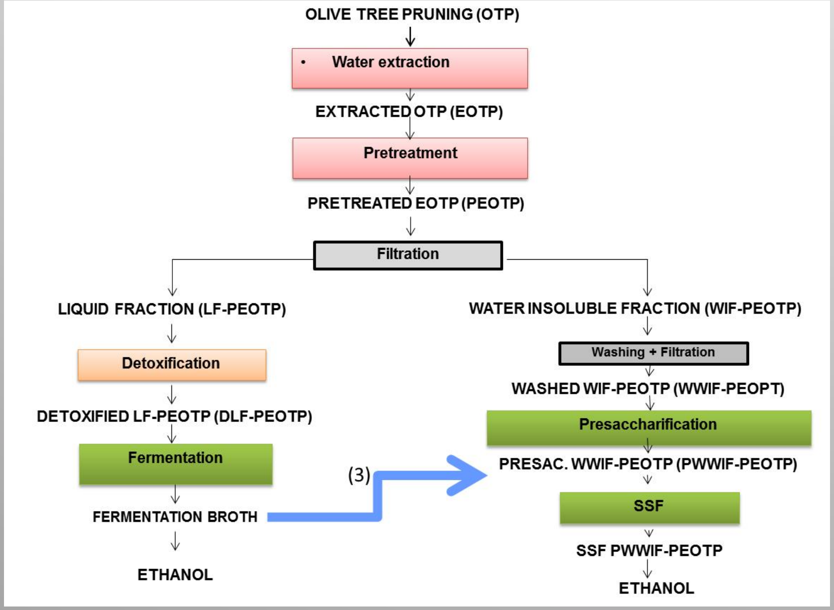


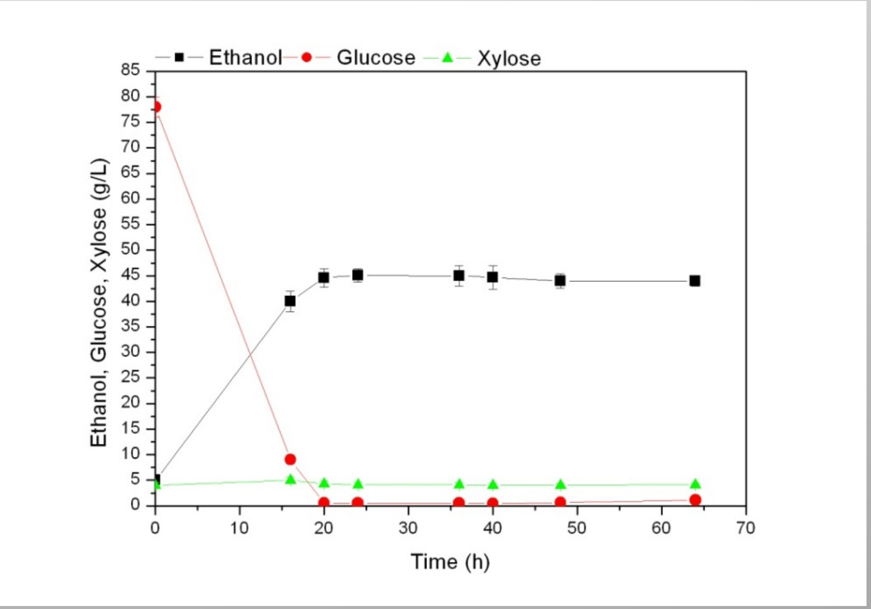


Glucose was completely assimilated after 72 h. (40 g/L)
No xylose consumption even when glucose is completely exhausted.
73% of theoretical yield

Sequential Fermentation of DLF-PEOTP (C5 sugars) and PWWIF-PEOTP(C6 sugars)

Prehydrolyzate was used for cell propagation and then was added to PWWIF-PEOTP





All xylose was consumed to produce more cells and ethanol (8 g/L)
Sequential configuration permits to shorten fermentation time to 20 h
45 g/L (82% of SSF theoretical yield)

Conclusions

Sequential fermentation permits:

- To transform into ethanol all sugars contained in the OTP biomass.
- To preadapt the cells to hydrolyzate , leading to higher ethanol yields and productivities.
- To obtain an overall process yield of 192 L per ton of EOTP biomass.

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¡¡¡Thank you for your attention¡¡¡

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