Enzymatic hydrolysis and fermentation of pretreated Tunisian lif of date palm “*Phoenix dactylifera* L.” for cellulosic bioethanol production

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Introduction

In the last decades, petro-chemistry is being replaced by biorefineries. Lignocellulose biomass represents an abundant feedstock with a great potential for bioethanol 2G production. The lif is the waste of date palm, being the fibrous part that covers the trunk of the tree and ensures its protection. In Tunisia, its annual quantity is about 30460 t/year. This by-product has not been thermochemically and biochemically exploited. Thus, the target of this study is to produce ethanol from pretreated Tunisian lif of date palm. Lignocellulose biomass is a complex structure composed by lignin, cellulose and hemicellulose that must be pretreated to reduce carbohydrates into monomeric sugars C_5 and C_6 to make easier the action of enzymatic hydrolysis catalyzed by cellulases. After that, D-glucose will be converted to ethanol using microorganisms such as *Saccharomyces cerevisiae*, *Pachysolen tannophilus* and *Candida shehatae.* Ethanol solution obtained should be distillated to achieve anhydrous ethanol.

Objective

Cellulose ethanol production from both young and old pretreated lif of date palm *Phoenix dactylifera* L. by the mean of enzymatic saccharification and fermentation

Lif of date palm: Potential lignocellulosic feedstock for bioenergy recovery

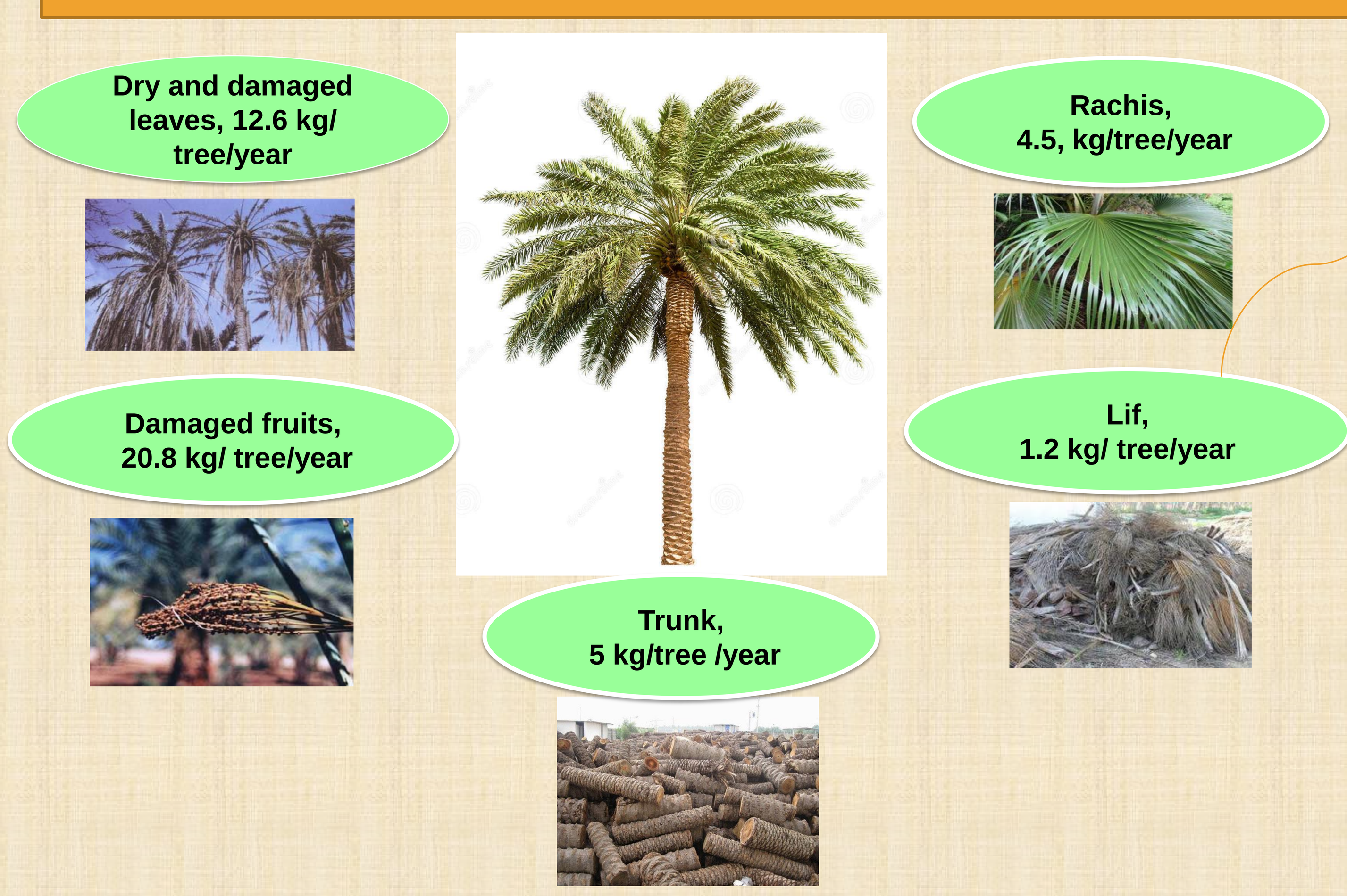

Fig 1. Estimation of date palm byproducts [1]

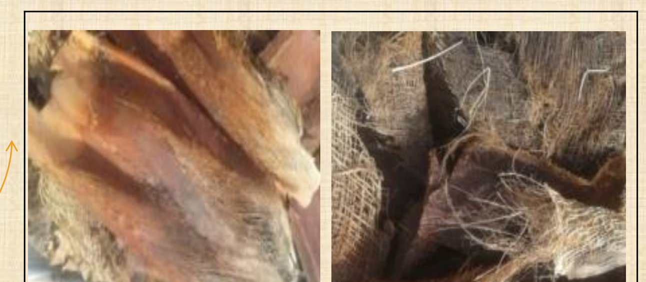

Fig. 2. Crude fibers of *P. dactylifera*, (a) young lif and (b) old lif

Crushed in a hammer mill ϕ=0.425-0.6 mm

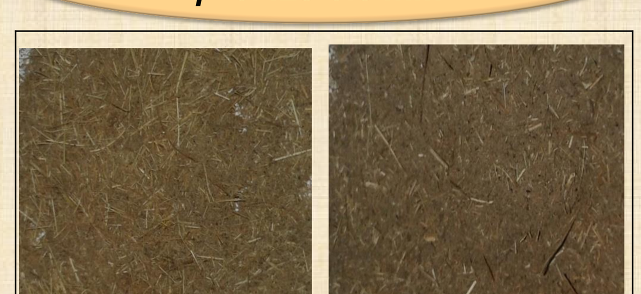

Fig. 3. Crushed fibers of *P. dactylifera*, (a) young lif and)b) old lif

Experimental

Hyrdothermal pretreatment

Pretreament temperature 220°C

Solid fraction + Liquid fraction

Enzymatic hydrolysis

- Reaction volume: 250 ml
- Time: 72 h / T=55°C / Agitation: 250 rpm
- Enzymes: Cellulases and β-glucosidases

Liquid fraction

Analytical methods
- Acetic acid concentration referring to the standard of Bergmeyer & Möllering [2]
- Reducing sugars (RS) following colorimetric method of Miller [3]
- D-glucose concentration following Trinder’s method [4]

Fermentation

- Reaction volume in fermentation : 100 ml
- T=30°C / pH= 4.5 / Agitation: 250 rpm
- Yeast: *Pachysolen tannophilus* ATCC32691

Liquid fraction

- Ethanol concentration referring to (Beutler & Michal, 1977) [5].

Results and Discussion

Table 1. Total reducing sugars (TRS) and D-glucose concentrations after pretreatment and enzymatic hydrolysis of both lifs

	Young Lif		Old Lif	
	After Pretreatment	After Enzymatic hydrolysis	After Pretreatment	After Enzymatic hydrolysis
TRS (g/dm³)	3.2	16.4	2.1	23.9
D-glucose (g/dm³)	0.2	20.0	0.1	25.9

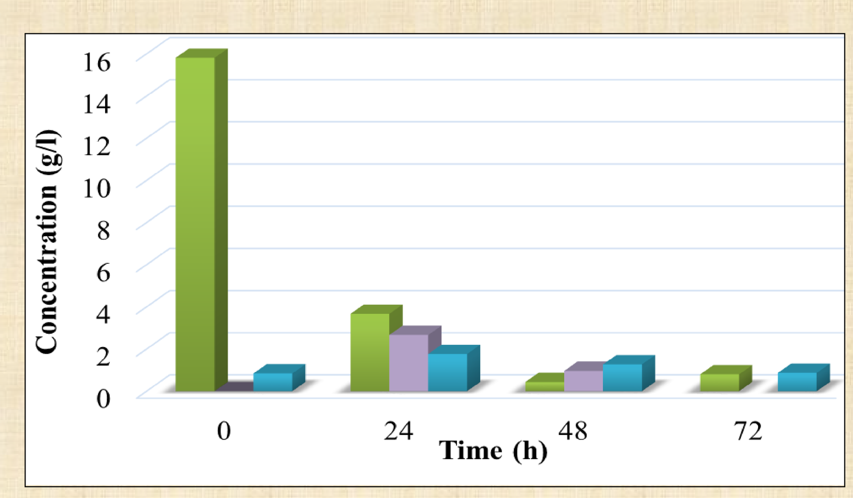

Fig 4. Concentration of D-glucose (■), ethanol (■) and acetic acid (■) during fermentation process of liquid hydrolysates of young lif.

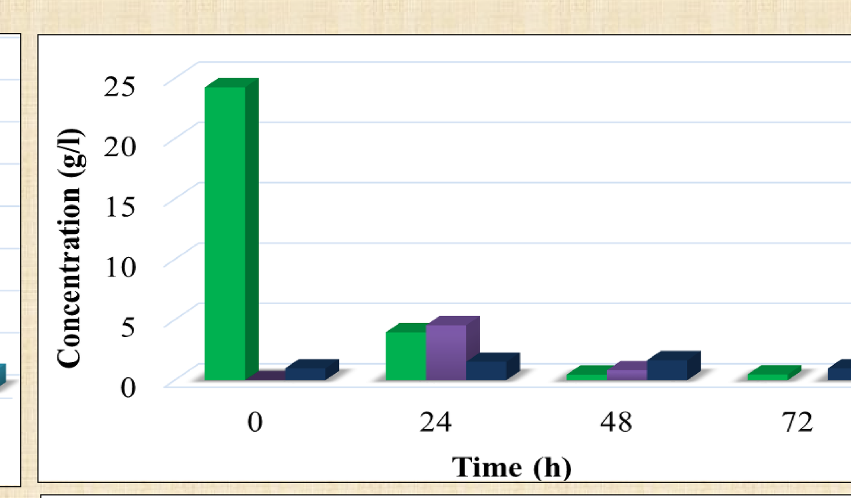

Fig 5. Concentration of D-glucose (■), ethanol (■) and acetic acid (■) during fermentation process of liquid hydrolysates of old lif.

- During the enzymatic hydrolysis, D-glucose liberation is increasing during reaction time. Therefore, it proves that enzymatic hydrolysis has an important influence on D-glucose production. Maximum D-glucose concentration is about 25.9 for the pretreated old lif hydrolysates.
- Maximum concentration of ethanol was around 3.2 g/dm³ for young lif and 4.6 g/dm³ for old lif after 24 h and the D-glucose concentration was decreasing simultaneously. After that, ethanol concentration was decreasing, which could be explained both evaporation of this product and it also could be consumed by the yeast [6].

Conclusions

- ❖ Lignocellulosic fibers of lif are a promising feedstock for energy recovery.
- ❖ This research have shown that producing alternative energy from the date palm tree *P. dactylifera* waste is a good strategy to valorize this abundant by-product considering the concentrations of ethanol liberated.
- ❖ After 72 h of enzymatic hydrolysis, TRS yield was 0.35 kg/kg pretreated fibers for young lif and 0.24 kg/kg pretreated fibers for old lif.
- ❖ Ethanol was produced during the fermentation by *P. tannophilus* from enzymatic hydrolysates, reaching a maximum instantaneous yield of 0.21 kg ethanol/kg D-glucose for young lif and 0.19 kg ethanol/kg D-glucose for old lif

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