TREE TOBACCO (NICOTIANA GLAUCA R. GRAH.) STEMS AS A BIOETHANOL FEEDSTOCK

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ABSTRACT: The aim of the work is to assess the potential of tree tobacco stems as a bioethanol feedstock. In order to reach this objective a carbohydrate characterization of whole and fractionated stems was done. Different fermentation trials based on first generation bioethanol production were also carried out in order to determine the best procedure for a maximum ethanol concentration in the fermenting media. Higher heating values of different fractions and fermentation bagasses were determined in order to discuss different approaches to a comprehensive energetic use of tree tobacco stems.
Keywords: bioethanol, calorific value, fermentation, fibre, nicotiana, sugar crops.

1 INTRODUCTION

Tree tobacco is a woody plant native from South America that has spread out to several warm, semiarid, or even arid regions around the world, being considered in occasions as invasive specie [1],[2]. It is a shrubby or arboreal plant, well adapted to water shortage conditions [3] and extremely poor soils (it can be seen growing in such oligotrophic substrates as wall cracks).

Although one of its Spanish name is "gandul" (the Spanish word for *idler*) because it cannot be used as forage (as it is a poisoness plant) and it also has a very low-quality wood, the fact is that nicotiana has several uses. Traditional ones include medicinal applications and phytosanitary products, [4], [5], [6], [7]. Nowadays, tree tobacco is also investigated as a promising plant for soil phytoremediation [8], [9], [10], [11], [12] and there is, at least, one proposal to use it as rootstock for the "tree tomato" plant (*Cyphomandra betacea* Sendt) [13] as it is tolerant to root-knot nematode (*Meloidogyne spp*).

Literature review performed on this matter have shown that few attempts have been made to investigate tree tobacco managed as a crop, including the one conducted by members of our investigation group in the middle eighties [14], [3]. According to this latest one, yields of above-ground biomass of about 3.9 t/ha (d.m.b.) can be harvested from young tree tobacco plants.

Tree tobacco productivity and their stems content in non-structural carbohydrates, which can reach up to 20% of its dry matter, suggest that Tree Tobacco stems could be a potential bioethanol feedstock for semiarid regions

2 MATERIALS AND METHODS

A selection of season stems was picked up in September of 2009 in Almeria (Spain). During spring and summer, sprouts have a characteristic grey-greenish colour that turns into dark purple along the autumn, as tissues get more lignified. Shoots, between 25 and 55 cm long, were collected just before this change was observable. After this, leaves and twigs were removed and shoots were preserved at 4ºC.

2.1 Characterization of plant material

Once at laboratory, shoots were categorised into two groups according diameters: category I = mean diameters ≤ 15; category II = mean diameters > 15 mm.

Shoots in Category I were measured, noting down their lengths and mean diameters (diameters at half length). Afterwards they were cut into small pieces (of about 3 cm) with an axe, crushed with a hammer, and frozen until analysis.

Shoots in Category II were measured, noting down the following data: length, diameters at both extremes of the shoot (D1, D2), and pith diameters at both extremes of the shoot (PD1, PD2). After this, they were cut longitudinally and their main tissues separated; one fraction made up of pith and the other, of the outer tissues. Pith fraction includes pith itself and primary xylem, while outer tissues fraction includes those from secondary xylem to epidermis.

Tissues separation was carefully made with a set of sharp-edged copper cylinders with different diameters. Although this fact, it was unavoidable that small fractions of tissues remain in the unchosen fraction.

Length was measured with a measuring tape and with a precision of ±1 cm. As the criterion for picking up shoots was based on diameter size (between 5 and 25 mm), lengths noted down here do not represent real length of plant stems or branches and are only shown in order to give a wider description of the plant material used in this work.

Diameters were measured using a digital calliper with precision up to 0.01 mm.

Dry matter contents were determined by drying samples at a temperature of 103 – 105 ºC until constant weight. Dry matter content of entire shoots was determined from samples in both categories.

2.2 Sugar content

Determination of non-structural carbohydrates of the different fractions and whole shoots was performed following Nelson-Somogy's method as reported in [15].

This method only determines reducing sugars, so sucrose and polysaccharides must be previously hydrolyzed. Considering this and the fact that sugar concentration in the extract should be between 0 and 100 ppm in order to apply the method, the complete process is described below.

About 3 grams of sample are boiled in 100 ml of distilled water for 30 min. After this, they are filtered through a 73 g/m^2 laboratory filter paper and the liquid fraction led to 500 ml with distilled water. The content of reducing sugars **(RS)** in the sample is then determined in this extract by Nelson-Somogy's method.

In order to determine the total soluble sugars content (↔monosaccharides+sucrose) 112.5 ml of the previous extract is boiled together with 12.5 ml of HCl 0.1 M in order to hydrolyze the sucrose. Afterwards it is led to pH=7 with NaOH 2.5 M, and to 500 ml with distilled water. Finally, its sugar content is determined by Nelson-Somogy's method, and it would represent the total content in soluble sugars **(SS).**

Solid residue is, afterwards, boiled in 100 ml of HCl 1M for 30 min, so insoluble but non-structural carbohydrates (↔starch) are hydrolyzed. Once led to pH=7 with NaOH 2.5 M and diluted with distilled water (1:33), sugars are determined by Nelson-Somogy's method. Results would represent the total amount of insoluble sugars **(IS)**.

According to this process, the total amount of monosaccharides (↔glucose+fructose) would be equivalent to **RS,** the total content of sucrose would be **SS – RS,** and the total amount of starch, **IS.**

Determinations were done thrice for each sample.

2.3 Fibre content

Fibre was determined according to Van Soest method adapted from the one described in [16] using a *Fibertec M6 1020* device (Foss). Samples were previously dried at 103-105 ºC and milled to dust. Determinations were done twice for each sample.

2.4 Higher Heating Value

Higher Heating Value was determined using an AC-350 calorimeter (Leco Instruments) and according to the UNE 164001 EX method.

Samples were previously dried until its dry matter reach at least 85% and milled to dust. Determinations were done twice for each sample. Results were corrected to HHVo.

2.5 Total contents in carbon and nitrogen

Total contents in C and N were determined using a NA 2000 analyzer (Fison Instruments). Samples were previously dried at 103-105 ºC and milled to dust. Determinations were done twice for each sample.

2.6 Fermentable substrates

Three kinds of different fermentable substrates were prepared. First of them with shoots of category I, following the next process:

An amount of about 750 g of crushed shoots is mixed with enough distilled water to ensure a good extraction of soluble sugars during the next step, which consists in an autoclaving process (20 min, 121ºC). The boiled mixture is afterwards pressed in a manual press; two fractions – solid (S1) and liquid (L1) - are obtained. Solid fraction is then mixed with concentrated sulphuric acid (96%) at about 1ml for each 4.5 g of dry matter, and enough water to ensure a homogeneous hydrolyzation of the substrate. Later, it is autoclaved again and then pressed in a manual press, so two new fractions are separated. Liquid fraction (L2) is then led to a pH between 5 and 5.5 (recommended for the yeast strain used in this experiment) with KOH 3.61 M. Liquid fraction L1 is supposed to contain most soluble sugars from shoots, while L2 presumably contains the glucose from starch hydrolysis. Liquid fractions are afterwards mixed and led to pH= 5-5.5 if needed, and total reducing and total soluble sugars in the mixture are determined as described in 2.2. This first fermentable substrate is what we called "whole shoot substrate" **(W**).

Dry matter of final acid bagasses is determined as described in 2.1. Afterwards a portion of them is washed with deionizated water until its pH reach values above 3 (what means about 4 litres for each 75 g of fresh weight), and dry at 103-105ºC to determine the dry matter content of the washed bagasse. This dry sample is finally milled to dust and its contents in fibre, total carbon and total nitrogen are determined, as well as its Higher Heating Value (as described in 2.3, 2.4, and 2.5)

Total amounts of whole shoots used in W trials are shown in Table I.

Table I: Total amounts of whole shoots used in W trials

Trial	Initial weight (f.m.b., g)
W1	750.41
W2	750.78
W3	751.32

f.m.b. = fresh matter basis

The second substrate is obtained from pith tissues. An amount of about 300 g is mixed with enough distilled water to ensure a good extraction of soluble sugars during the next step, which consists in an autoclaving process (20 min, 121ºC). The boiled mixture is afterwards pressed in a manual press; two fractions – solid and liquid - are obtained. Liquid fraction is– if needed- led to pH between 5 and 5.5 with citric acid 0.1M and total reducing and soluble sugars in the mix are determined as described in 2.2. This second fermentable substrate is what we called "pith substrate" **(P)**. Bagasses dry matter is determined as described in 2.1. A dry sample is milled to dust and its contents in fibre, total carbon and total nitrogen are determined, as well as its Higher Heating Value (as described in 2.3, 2.4, and 2.5)

Total amounts of pith and added water used in P trials are shown in Table II.

Table II: Total amounts of pith and added water used in P trials.

Trial	Initial weight (f.m.b., g)	Water added (ml)
P1	313.12	20
P2	296.11	20
P3	299.71	20

f.m.b. = fresh matter basis

The third substrate is obtained from the outer tissues fraction. An amount of about 400 g is mixed with concentrated sulphuric acid (96%) at about 1ml for each 4.5 g of dry matter, and enough water to ensure a homogeneous hydrolyzation of the substrate. Later, it is autoclaved and then pressed in a manual press. Liquid fraction is then led to a pH between 5 and 5.5 with KOH 3.61 M. This third fermentable substrate is what we called "external tissues substrate" **(**ET).

Dry matter of acid bagasses is determined as described in 2.1. Afterwards a portion of then is washed with deionizated water until its pH reach values above 3 (what means about 4 litres for each 40 g of fresh weight), and dry at 103-105ºC to determine the dry matter content of the washed bagasses. This dry portion is finally milled

to dust and its contents in fibre, total carbon and total nitrogen are determined, as well as its Higher Heating Value (as described in 2.3, 2.4, and 2.5)

Total amounts of external tissues, added water, and added sulphuric acid used in ET trials are shown in Table III.

Table III: Total amounts of external tissues, added water, and added sulphuric acid used in ET trials

Trial	Initial weight (f.m.b., g)	Water added (ml)	Sulphuric acid added (ml)
ET1	417.90	468	32.8
ET2	410.77	400	32.2
ET3	401.44	420	32.1

f.m.b. = fresh matter basis

2.7 Fermentations

Concerning fermentations, a high-yield ethanol selected strain of *Saccharomyces cerevisiae* (*Ethanol Red*, Fermentis) was kindly supply by the Biomass Unit of CIEMAT (Spain). Two days before each fermentation 0.1 grams of lyophilized yeast were introduced in a sterile Erlenmeyer flask with 100 ml of a medium containing: yeast extract (5 g/l), $(NH_4)_2SO_4$ (2 g/l), KH_2PO_4 (1 g/l), $MgSO_4.7H_20$ (3 g/l), sucrose (30 g/l) and distilled water, and led to pH=5 with citric acid 0.1M. Afterwards yeasts were cultivated during 48 h at a temperature of 34±2 ºC. An air compressor supplied air into the medium through a gas filter with a 0.2 µm pore membrane, and kept the medium mixed. The final product obtained by this process is what we called "inoculum" or "starter".

Ammonium sulphate was added at a rate of 1g/l to every substrate just before autoclaving again in order to ensure their sterility. Once they were cooled, starter was inoculated into substrates using a sterile glass syringe at 20 ml/trial. Dry matter in starter was 0.41±0.018 g/20 ml.

Fermentations with W as substrate were carried out in a "Minifor Laboratory Fermentor" (Lambda Instruments) at 35ºC during 4 days. About 950 ml of W substrate was fermented in each one of the three trials performed.

Fermentations with P as substrate were carried out in Erlenmeyer flasks with water-repellent cotton-plugs at 34.5±3ºC during 4 days. A magnetic stirring unit was use to kept the substrate mixed. About 200 ml of P substrate was fermented in each one of the three trials done.

Fermentations with ET as substrate were carried out in Erlenmeyer flasks with water-repellent cotton-plugs at 34.5±3ºC during 4 days. A magnetic stirring unit was use to kept the substrate mixed. About 650 ml of ET substrate was fermented in each one of the three trials done.

A blank fermentation trial was also carried out with 1000 ml of distilled water (led to pH = 5 with citric acid 0.1 M), ammonium sulphate (1 g) and yeast inoculum (20 ml), in the same conditions as fermentations with P as substrate.

2.8 Ethanol determinations

Once fermentations were over, samples of fermented substrates were cooled in a fridge and frozen at -26ºC until ethanol determinations.

Samples were then placed in microtubes and centrifuged (7000 rpm, 10 min) to separate solid particles. Fermented liquid was afterwards filtered through a 0.45 µm pore membrane. Finally, ethanol concentration was determined using a gas chromatograph (GC 8000, CE Instruments)

3 RESULTS

3.1 Characterization of plant material

Characteristics of shoots of category I are shown in Table IV.

Table IV: Characteristics of shoots of category I

	Mean	Standard Desviation
Number of shoots	158	
Length (cm)	41	9.1
Mean Diameter (mm)	10.55	2.791
Mean Weight (g)	38.78	-

Characteristics of shoots of category II are shown in Table V.

Table V: Characteristics of shoots of category II.

	Mean	Standard Desviation
Number of shoots	21	
Weight (g)	110.94	22.365
Length (cm)	44	7.2
Diameter 1(mm)	19.48	2.125
Diameter 2 (mm)	15.75	2.420
Pith Diameter 1 (mm)	11.40	2.193
Pith Diameter 2 (mm)	9.71	2.341
Pith (%, f.m.b.)	37.09	14.188

f.m.b.= fresh matter basis

Dry matter contents of shoots of both categories are shown in Table VI.

Table VI: Dry matter contents (%)

	Mean	Standard Desviation
Whole shoot	25.31	1.234
Pith Fraction	10.76	0.854
Outer Tissues Fraction	35.28	2.413

3.2 Sugar content

Content in sugars was determined from shoots randomly selected among those of category II. Their characteristics are shown in Table VII.

Table VII: Characteristics of shoots used in sugars determination.

	Mean	Standard Desviation
Number of shoots	4	
Weight (g)	119.86	21.573
Length (cm)	44	5.0
Diameter 1(mm)	20.11	1.458
Diameter 2 (mm)	16.55	1.142
Pith Diameter 1 (mm)	11.12	2.364
Pith Diameter 2 (mm)	11.07	1.148
Pith (%, f.m.b.)	42.00	10.797

f.m.b.= fresh matter basis

Results of shoot content in non-structural carbohydrates are shown in Table VIII.

Table VIII: Non-structural carbohydrates in shoots (%, d.m.b.)

		Whole Shoot	Pith Fraction	Outer Tissues Fraction
Reducing sugars (RS)	Mean Value	**3.85**	**19.76**	**0.68**
	Standard Desv.	0.607	1.574	0.389
Sucrose (SS-RS)	Mean Value	**4.51**	**15.87**	**2.33**
	Standard Desv.	0.509	4.266	0.398
Insoluble sugars (IS)	Mean Value	**10.68**	**4.57**	**14.40**
	Standard Desv.	0.918	0.681	0.977
Total fermentable sugars		**19.04**	**40.20**	**17.41**

d.m.b.= dry matter basis

3.3 Fibre content

Shoots fibre composition is shown in Table IX.

Table IX: Fibre composition of shoots

	Hemicellulose (%, d.m.b.)	Cellulose (%, d.m.b.)	Lignin (%, d.m.b.)
Pith fraction	3.46	3.93	9.00
External tissues	19.47	44.47	10.90
Whole shoot	10.85	52.89	7.60

d.m.b.= dry matter basis

Bagasses fibre composition is shown in Table X

Table X: Fibre composition of bagasses.

Trial	Hemicellulose (%, d.m.b.)	Cellulose (%, d.m.b.)	Lignin (%, d.m.b.)
W1	6.94	62.93	19.88
W2	6.05	62.16	20.33
W3	8.40	58.46	20.92
P1	17.03	36.39	6.85
P2	15.56	37.31	9.12
P3	14.49	31.52	10.62
ET1	3.14	63.22	23.89
ET2	4.78	61.74	22.14
ET3	4.96	62.21	20.31

d.m.b.= dry matter basis

3.4 Higher Heating Value

Higher Heating Values from whole and fractionated stems are shown in Table XI.

Table XI: Higher Heating Values from shoots

	HHV (Kcal/Kg d.m.b.)
Pith fraction	3600.3
External Tissues fraction	4470.7
Whole shoot	4540.2

d.m.b.= dry matter basis

Higher Heating Values from bagasses are shown in Table XII.

Table XII: Higher Heating Values from bagasses

Trial	HHV (Kcal/Kg d.m.b.)
W1	4764.2
W2	4745.7
W3	4733.5
P1	4005.1
P2	4055.4
P3	4071.2
ET1	4801.3
ET2	4751.6
ET3	4706.9

3.5 Contents in carbon and nitrogen

Contents in total C and total N in shoots, as well as their C/N relations are shown in Table XIII.

Table XIII: Contents in total C and total N, and C: N ratio in shoots

	Total N (%)	Total C (%)	C:N Ratio
Pith fraction	4.18	35.88	8.59
External Tissues fraction	1.29	45.10	34.87
Whole shoot(1)	1.73	43.69	25.22

(1) As calculated from separated fractions considering their amounts and dry matters percentages.

Contents in total C and total N in bagasses, as well as their C/N relations are shown in Table XIV.

Table XIV: Contents in total C and total N, and C: N ratio in bagasses.

Trial	Total N (%)	Total C (%)	C:N Ratio
W1	0.41	43.81	106.6
W2	0.44	45.46	102.3
W3	0.48	43.50	90.4
P1	1.57	40.45	25.8
P2	1.59	41.62	26.1
P3	2.27	41.02	18.0
ET1	0.34	47.74	5304.7
ET2	0.35	47.70	5299.5
ET3	0.37	56.90	6322.0

3.6 Fermentable substrates

Amounts of liquids and bagasses resulting from sugar extraction processes described in 2.6 are shown in Table XV.

Table XV: Amounts of liquids and bagasses resulting from sugar extraction processes.

Trial	Bagasses weight (g)	Bagasses dry matter (%)	Total Liquid (ml)
W1	472.52	18.66	1025
W2	616.86	24.15	1040
W3	483.72	21.29	1115
P1	41.85	35.41	210
P2	46.59	38.16	220
P3	34.91	34.95	230
ET1	348.66	25.49	450
ET2	295.41	25.29	410
ET3	369.76	24.09	370

Table XVI shows amounts of liquid actually used as fermentations broths after samples for sugar determination were taken and pH was led to 5-5.5. Fermentable sugars concentrations are also shown.

Table XVI: Fermentable broth volume and sugar concentration

Trial	Fermentable broth (pH 5-5.5) (ml)	Fermentable sugars concentration in broth (ppm)
W1	938	15364
W2	927	20550
W3	1050	20802
P1	200	46519
P2	213	44963
P3	223	55109
ET1	650	20166
ET2	635	18988
ET3	600	20269

3.7 Fermentations

Volumes of fermented substrates as well as their ethanol concentrations are shown in Table XVII.

Table XVII: Volumes of fermented substrates and ethanol concentration

Trial	Volume of fermented substrates (ml)	Ethanol concentration (ppm)
W1	895	1376
W2	895	5313
W3	1000	4132
P1	195	15695
P2	195	16786
P3	214	22890
ET1	605	3546
ET2	605	2678
ET3	545	3186

Ethanol concentration in blank fermentation was less than 2 ppm, and thus, negligible.

From data shown in Table XVI and Table XVII, fermentation yields can be obtained as shown in Table XVIII.

Table XVIII:. Available sugar in broths, amounts of ethanol in fermented broths, and fermentation yields.

Trial	Total available sugar (mg)	Total ethanol (mg)	Fermentation yield (% w/w)	Fermentation energy yield (%)
W1	14411	1232	8.5	16.2
W2	19050	4755	25.0	47.2
W3	21842	4132	18.9	35.8
P1	9304	3061	32.9	62.2
P2	9577	3273	34.2	64.6
P3	12289	4898	39.9	75.4
ET1	13108	2145	16.4	30.9
ET2	12057	1620	13.4	25.4
ET3	12161	1736	14.3	27.0

4 DISCUSSION

Specifications from *Ethanol Red* yeast developers state that fermentation yields of 48% (w/w) can be reached at 35ºC. Maximum fermentation yield in our experiment was 39.9%, i.e., much lower. Some discussion is needed to explain that.

In order to evaluate if chosen materials and fermentation parameters were adequate to reach as high yields as possible, two new fermentations (hereinafter referred as Y trials) were carried out. Broths in both of them had the same composition as starter medium (see 2.7), but with a sucrose concentration of 50g/l, and so, a sugar concentration similar to the one that P trials presumably contain. An amount of about 200 ml of medium was used, so total sugar content was slightly above 10000 mg (ca. 10030 mg, as yeast extract contains a small amount of sugar itself).

Water used in first broth (Y1) was distilled water (as used in all previous trials), but “water” in second broth

(Y2) was obtained after dropping distilled water original's pH (around 5.5) to 0.16 with sulphuric acid. Afterwards pH was increased to 5.25 with KOH 3.61 M. The aim of this process was to achieve a concentration of K^+ in the medium similar to those existing in ET trials, so possible inhibitory consequences of its high concentration could be found

Inoculation of starter was carried out as previously described and fermentation processes take place in same conditions as P trials (see 2.7).

Results from both fermentations revealed ethanol yields of almost 100% of the theoretical yield (51.1% w/w) for trial Y1 and 93.8% for trial Y2 (48.7% w/w). Although the number of trials does not allow us to reach conclusions about possible significant differences between results from both fermentations, it seems clear that no inhibitory consequences of the studied ion at the present concentration can drop yield values to those obtained in most trials. Besides, chosen materials and fermentation parameters have been proved to be adequate at obtaining high ethanol yields.

According to the above exposition, low fermentation yields cannot be attributed to experimental conditions, so there are, to our knowledge, four main reasons to explain them:

I. *Inaccuracies due to the chosen method performed to obtain the initial sugar concentration in fermentable broths*. Nelson-Somogy's method as applied (see 2.2) is only suitable to determine small concentrations of reducing sugars, so great dilutions (ca. 1/500) must be done to determine sugar concentration in broths. Small inaccuracies in the diluted broth (for example 1 ppm) became great deviations (500 ppm) in actual fermentable broths, and affect fermentation yields. Obviously, performing determinations in triplicate decreases the final error. On the other hand, inaccuracies could also lead to an underestimation of the sugar concentration (what means an overestimation of fermentation yields) so, although this first reason should not be ignored, it probably could not explain by itself the low yields of all fermentations.

II. *Presence of pentoses in the fermentable broth*. Nelson-Somogy´s method determines all reducing sugars (in fact it determines all reducing compounds in the sample), and this includes pentoses that are not fermentable by the yeast strain used in these experiments. Hemicellulose hydrolysis is expected to be the main source of pentoses in our experiments. Although degradation of hemicellulose was not a target of the acid hydrolysis applied, fibre analysis of original plant material and final bagasses (see 3.3) show a significant decrease in the amount of this component during the sugar extraction process (up to 90,3% in ET1 trial). Pentoses could, consequently, represent an important amount of reducing sugars in fermentable broth.

III. *Presence of inhibitory compounds in the fermentable broth*. Furfural, 5-hydroxymethylfurfural, acetic acid, and aromatic inhibitory compounds, are common inhibitory compounds originated by degradation of carbohydrates and lignin.

A method to estimate the nature of inhibitory compounds originated from lignocellulosic substrates is by calculating the *Combine Severity* (CS) parameter of the acid hydrolysis [17]. According to Larsson, [18] CS could be obtained from the expression:

$$CS = \log (t \cdot e^{(T-100)/14.75}) - pH$$

Where:

t : Hydrolysis time (min)
T: Hydrolysis temperature (ºC)
pH: pH of the acid solution.

Calculating CS parameter of our hydrolysis processes faces at least one problem; autoclaving process has three steps. During first of them, initial temperature increases its value from about 20ºC to 121ºC, second step is the autoclaving one *sensu stricto*, at 121ºC, and third step is required to cool down the samples, usually to the initial temperature. In order to determine the CS parameter in our hydrolysis, only second step (the only one properly characterized in terms of temperature and duration) has been considered, as most of the transformation processes of the substrate probably take place during this step. Despite this, real CS is supposed to be slightly higher than the calculated one.

Highest CS in our trials correspond to ET ones (with values about 2.3) and thus, to the lowest fermentations yields of the experience (a exception must be considered for W1, which does not fit with the general trend)

According to experiments carried out by Larsson *et al* with spruce wood as substrate [18], processes with a CS around 2.5 can lead to high concentrations of acetic acid in the media. About 4.8g/l of acetic acid were obtained in a hydrolysate of 200 g (d.m.b) of wood. Despite this fact, no fermentation yield decreases were found when fermentations with this concentration of acetic acid in the media were carried out.

Nevertheless, they concluded that this CS of 2.5 completely hydrolyzes hemicellulose, and this would be consistent with the second reason previously explained, even though differences between gymnosperm and angiosperm hemicelluloses must be considered.

It also must be taken into account that in a process capable of hydrolyzing hemicellulose, is very possible that free hexoses, and those originated by the hydrolysis of sucrose and starch, could be dehydrated to 5-hydroxymethylfurfural. Besides its inhibitory properties, this compound is also a reducing one, so it can be considered as a reducing sugar by Nelson – Somogy method.

IV. *Lack of nutrients in the fermentation broth*. Fermentations are provided with large quantities of nitrogen as well as with potassium in W and ET trials, but phosphorous, magnesium, and microelements contained in the original substrates may not be enough for a maximum fermentation yield. Unlike what happens in broths of Y fermentations. Further research concerning this issue would be needed.

Considering average dry matter contents and best fermentation yield for each group of trials, 1000 kg of fresh tree tobacco stems could be processed to obtain between 12,05 kg of ethanol (in case of whole shoots) and 13.09 kg (in case of following the fractionating process). If we take into account deviations of each mean value considered (dry matter content, fermentable sugars content), this two values are not significantly different, so previous separation of pith and external tissues does not seem to be interesting in a process aiming to obtain ethanol from non-structural carbohydrates from complete stems.

Related to this conclusion is the fact that ethanol concentration in fermented broths of W trials (as well as of ET trials) is far below the economically feasible limit

(commonly assumed as 5%), whereas fermented P trials, although below the limit themselves, have ethanol concentrations that can reach 2.3 % (in case of P3), what seems more promising in order to be applied to industrial scale in a short time.

From the results of this work (carbohydrate composition) and from literature values (hydrolysis yields for cellulose and hemicellulose proposed by [19] and [20] respectively, and theoretical fermentation yields), the total amount of energy that can be obtained by the transformation of 1000 kg of fresh tree tobacco biomass in bioethanol is shown in Figure 1.

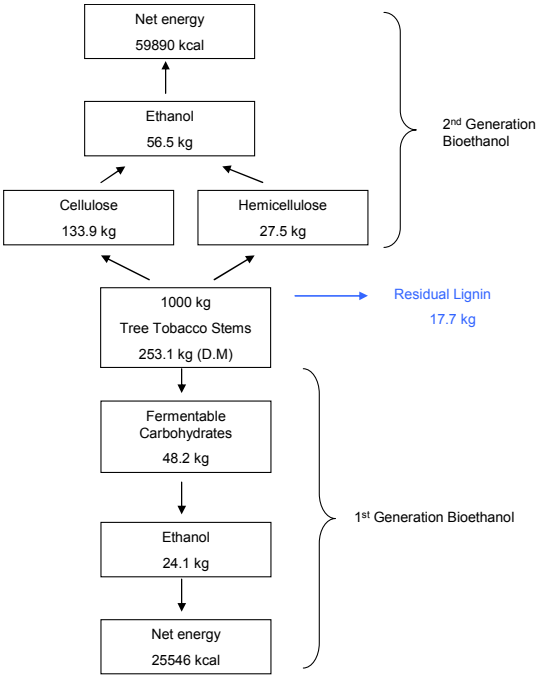

Figure 1: Bioethanol conversion of tree tobacco biomass.

According to results in this work (dry matter content, HHV), amounts of energy that can be obtained from 1000 kg of fresh stems used as solid biofuel are shown in Figure 2.

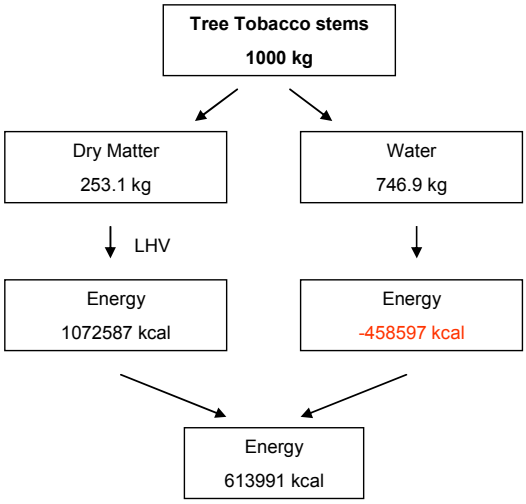

Figure 2: Energy from tree tobacco stems used as solid biofuel.

Some considerations could be made to both diagrams: *Net energy* in Figure 1 takes into account the energy consumption in distillation process. On the other hand, no energy consumption from needed pretreatments (chopping or similar) or hydrolysis has been considered. Similar pretreatments are necessary in both processes; in ethanol production they facilitate a homogeneous hydrolysis of substrate, whereas in case of solid biofuel production they facilitate drying the substrate (and subsequent processes like pelletize –if done). Hydrolysis energy consumption is only necessary in case of bioethanol production, so net energy from the process would be less than the total 85436 kcal.

As it can be seen, and from a strictly energetic point of view, ethanol production is not as efficient as solid biofuel production from tobacco tree stems. Nevertheless, this comparison should not lead us to non-extrapolatable conclusions; ethanol uses are not the same as those of solid biofuels and so happens with their prices. In Spain, and to final users, solid biofuels (pellets, firewood) have a price of 0.1 – 0.17 euro/kg, while gasoline price is about 1.1 euro/ litre.

Finally, Figure 3 shows a combined energy production (*first generation* bioethanol and solid biofuel) according to data of whole stems in this work (dry matters, carbohydrate composition, amounts of bagasses, and bagasses dry matters) and theoretical fermentation yields.

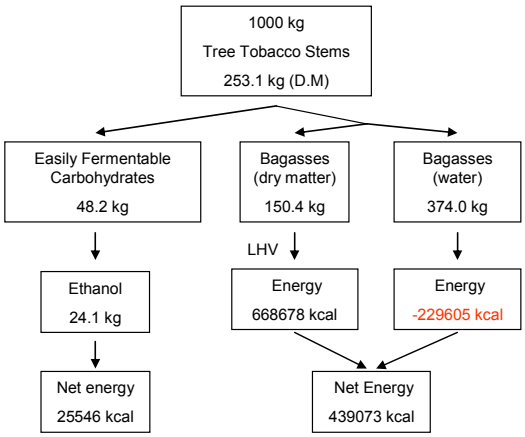

Figure 3: Combined energy production from tree tobacco

5 CONCLUSIONS

Tree Tobacco, due to its adaptability to water shortage and oligotrophic soils, is a potential crop for semiarid - or even arid - marginalized lands, in a context of agroenergetic purposes. Its composition suggests that two pathways can be considered to obtain interesting amounts of energy; one based on *first generation* ethanol technologies (with thermal use of bagasses) and the other based on *second generation* ethanol production. Further research is needed in order to optimize hydrolysis and fermentation parameters.

Finally, an approach to tree tobacco managed as an energy crop could achieve certain economic profitability from some of the most marginalized lands existing: those chemically polluted.

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7 ACKNOWLEDGEMENTS

This work is framed within the agreement "*Estudio del cultivo de chumbera (Opuntia ficus-indica (L.) Miller) y tabaco arbóreo (Nicotiana glauca Graham) para la producción de bioetanol*" between *Universidad Politécnica de Madrid* and *Fundación CAJAMAR*. Financial support from *Fundación CAJAMAR* through the project CENIT I+DEA "*Investigación y Desarrollo del Etanol para Automoción*", *(Ministerio de Ciencia e Innovación)* is gratefully acknowledged.