# Tobacco stalk as promising feedstock for second generation ethanol production

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**Resumo**

A composição química e as propriedades morfológicas das fibras, de materiais lignocelulósicos, são fatores-chave que afetam a eficiência da produção de biocombustíveis durante os processos de conversão. Assim, a composição química de plantas de tabaco foi examinada para avaliar seu potencial para produção de bioetanol. Após hidrólise de tecidos (xilema, medula, folhas e raízes) com ácido trifluoroacético e ácido sulfúrico, os hidrolisados foram analisados através de sua composição de carboidratos utilizando cromatografia de troca iônica (HPAE-PAD). Na haste de tabaco (tecido do xilema), os teores de carboidratos e lignina foram 62,4% e 20,8% de matéria seca (MS), respectivamente. Esses valores são muito próximos dos encontrados em madeira (eucalipto) e em bagaço de cana. As propriedades morfológicas das fibras do tecido xilemático e cortes histológicos efetuados na base do caule foram também avaliados e os resultados mostraram que as suas características são semelhantes aos de biomassa utilizada para a produção de biocombustíveis. A fibra no xilema de plantas de tabaco apresentaram valores de comprimento, diâmetro, espessura da parede celular e lumem de 0,95 mM, 30,7 uM, 20,8 uM, e 4,9 uM, respectivamente. Portanto, através da produção de plantas de tabaco no Sul do Brasil, este trabalho identificou cerca de 2 milhões de toneladas secas / ano de biomassa como caules de tabaco com grande potencial para produção de bioetanol de segunda geração ou biopolímeros. Assim, de acordo com a composição química dos caules de tabaco, a produção de etanol pode chegar a 108 litros por tonelada de peso seco.

**Palavras chave:** carboidratos, parede celular, HPAE-PAD, *Nicotiana tabacum*, tecido xilemático.

**Abstract**

Chemical composition and fiber morphological properties of lignocellulosic materials are key factors that affect efficiency of biofuel production during conversion processes. Thus, chemical composition of tobacco plant was examined to evaluate if it has potential for bioethanol production. After tissue hydrolyzation (xylem, pith, leaf and root) with trifluoroacetic acid and sulfuric acid, the hydrolyzates were analyzed for their carbohydrates composition using ion exchange chromatography (HPAE-PAD). In the tobacco stalk (xylem tissue), the carbohydrates and lignin content were 62.4% and 20.8% of dry matter (DM), respectively. These values are very close as found in hardwood (eucalyptus) and common non wood materials (sugarcane bagasse). The morphological properties of xylematic fibers tissue and histological cuts through the base of stalk were also evaluated and the results showed that their characteristics are similar to those of biomass used for biofuel production. The fiber in the xylem of tobacco plants presented values of length, diameter, lumem and cell wall thickness of 0.95 mm, 30.7 μm, 20.8 μm, and 4.9 μm, respectively. Therefore, through the

tobacco plant production in the South of Brazil, this work identified around 2 million dry tons/year of biomass as tobacco stalks with great potential for second generation bioethanol or biopolymers production. Thus, according to chemical composition of tobacco stalks, the ethanol production could reach 108 gallons per tons of dry weight.

**Keywords:** carbohydrates, cell wall, HPAE-PAD, *Nicotiana tabacum*, xylem tissue

**Resumen**

La composición química y las propiedades morfológicas de las fibras, de materiales lignocelulósicos, son factores clave que afectan a la eficiencia de la producción de biocombustibles durante el proceso de conversión. Por lo tanto, la composición química de plantas de tabaco fue examinado para evaluar su potencial para la producción de bioetanol. Después de la hidrólisis de tejidos (xilema, medula, hojas y raíces) con ácido trifluoroacético y ácido sulfúrico, los hidrolizados fueron analizados a través de su composición de carbohidratos usando cromatografía de intercambio de iones (HPAE-PAD). En el tallo de tabaco (tejido xilema), el contenido de carbohidratos y lignina fueron 62,4% y 20,8% de materia seca (MS), respectivamente. Estos valores son muy cercanos a los encontrados en la madera (de eucalipto) y bagazo de caña de azúcar. Las propiedades morfológicas de las fibras del tejido de xilema y los cortes histológicos realizados en la base del tallo también fueron evaluados y los resultados mostraron que sus características son similares a las de la biomasa usada para la producción de biocombustibles. La fibra del xilema de plantas de tabaco muestra los valores de la longitud, diámetro, espesor de la pared celular y del lumen de 0,95 mM, 30,7 uM, 20.8 uM y 4,9 uM, respectivamente. Por lo tanto, mediante la producción de plantas de tabaco en el sur de Brasil, este trabajo ha identificado alrededor de 2 millones de toneladas secas / año de biomasa como tallos de tabaco con un gran potencial para la producción de bioetanol de segunda generación o biopolímeros. Así, de acuerdo con la composición química de los tallos de tabaco, la producción de etanol podría llegar a 108 galones por tonelada de peso en seco.

**Palabras clave:** hidratos de carbono, la pared celular, HPAEC-PAD, Nicotiana tabacum, tejidos de xilema

## INTRODUCTION

The national energy security and global climate change will require large-scale substitution of petroleum-based fuels by other sources of energy. Thus, researches to find new lignocellulosic biomass for biofuels have intensified considerably around the world (LYND *et al.*, 2008). Nowadays, the biofuels are produced mainly from corn, oil plant and, sugarcane. Although, the first-generation of biofuels are dependent on starches, sugars and vegetable oils and the requirement to increase the biofuels production at lower cost without compromise the food source has been focused in new alternative raw materials (PU *et al.*, 2008). In addition, the use of large amount of food source to produce biofuels would naturally lead to a raise in the cost of food items (ROSILLO-CALLE; TSCHIRLEY, 2010). Hence, it is fundamental to find new raw material for biofuels production. In this context, South of Brazil presents special condition, considering the lignocellulosic residues, mainly tobacco plant from the agricultural sector.

The tobacco plant (*Nicotiana tabacum* L.), originated from South America, is an annual plant that shows a good plasticity, rapid growth rate (170 ton/ha – fresh weight) (SCHILLBERG *et al.*, 2003), and can be coppiced to stimulate re-sprouting from the stump after cutting; thus, multiple biomass harvests are possible in a single year. The tobacco plant is cultivated in more than 125 countries worldwide, with an area of over 4 million hectares. Currently, the world production is about 6 million tons of leaves (mainly to smoking), and the largest producers in the world are China followed by Brazil, India, and USA (FAOSTAT, 2012).

The tobacco plant belongs to a group of plants that produce agricultural wastes. Besides leaves, which are used to produce cigarettes, tobacco plants show a great potential to become a biofuel alternative source with main focus in the production of bioethanol from stalks, biodiesel from seeds and also chemical blocks (multi-purpose). For biodiesel fuel, tobacco plant has potent oil biosynthesis machinery in the seeds, which contain over 40% of oil DM. Nowadays, tobacco seed oil has been successfully tested for its potential as a fuel for diesel engines. However, the tobacco oil yield is modest, significantly lower than the traditional biofuel oil producer, such as soybean. Recently Andrianov and co-workers (2009), modified genetically tobacco plant, and this alteration resulted in significant increase of oil concentration per dry weight in the leaves. Seed contains high amount of protein 35-44%, and can be used as livestock feed (PESEVSKI *et al.*, 2010). Moreover, among the advantages of using this biomass, mainly in biotechnological process, this kind of plant is frequently used as a model system plant, because its DNA has been sequenced and extensively

studied in the last years and, also has short generation periods. This model system also can substitute woody species in genetic engineer researches (TEULIÈRES *et al*., 1994).

The numbers of the national production volumes are impressive, in the season 2010/2011, the Brazil produced about 0.83 million tons of leaves (dry weight), for cigarette manufacturing purpose, with a cultivated area of approximately 373 thousand hectares (BRAZILIAN TOBACCO YEARBOOK, 2011). Hence, the tobacco yield (leaves dry matter) is about 2.3 tons per hectare.

The Brazilian South region is main producer (98%), the tobacco industry has been the agriculture backbone with strong economic importance, because it raises income, as well as utilizes low fertility soils, unproductive for other crops. In addition, over 1.0 million direct jobs are generated by the crop at field level. (Brazilian Tobacco YEARBOOK, 2011). Currently, tobacco leaves are harvested manually and the stalks are burned or left in the field to be incorporated into the soil (SHAKHES *et al*., 2011).

According to Tobacco Yearbook (2011), some Brazilian companies are testing mechanical harvesters. Therefore, the mechanized harvesting will increase the biomass collected in the field, increasing possibility to become these residue in biofuel (mainly ethanol from biomass). Although, to produce biofuels (biodiesel and bioethanol) from tobacco plants economically viable, the concept of biorefinery is needed. Thus, the biorefinery can be understood as a set of processes that enable the use of each fraction of lignocellulosic biomass at the highest value for energy and chemicals compounds production (KAMM *et al*., 2006). Indeed, the great interest in the development of viable biorefining strategies to convert lignocellulosic material into new sources of energy is driven by environmental concerns and petroleum sources depletion (KOUTINAS *et al*., 2007; FITZPATRICK *et al*., 2010). Therefore, a specific knowledge of fibrous raw material, especially of nonwood plants, is useful to predict its behavior during transformation processes. Thus, in this work, tobacco stalks were studied as feedstock for biorefineries in Brazil. The aim of this work was to analyze chemical composition of different tobacco plant tissues (xylem, pith, leaf and root) using chromatographic methods, as well as morphological evaluations of fibers, xylem tissue and histological stem cuts.

## METHODS

### *Plant material and growth conditions*

Tobacco plants (*Nicotiana tabacum* L. cv. Petit havana), were germinated in growth chambers (Conviron E15) under controlled conditions: 500 $\mu mol.m^{-2}.s^{-1}$ irradiance using a 16/8 hours light/dark photoperiod and 24/18 ºC day/night temperature regime. After that, tobacco plants were cultivated in green house for 90 days in compost-vermiculite (1:1) and irrigated daily with a complete nutrient solution containing 10 mM nitrate and 2 mM ammonium (HOAGLAND; ARNON, 1950). All analyses were carried out 100 days after germination, with five replicates.

### *Harvesting plant material*

To determine the chemical composition of tobacco plants, tissues such as xylem, pith, leaves, roots and stem parts (base, middle and top) were collected and hydrolyzed with different acids, as shown in Figure 1. In the chemical analysis of the stem, it was sectioned into base, middle, and top. The xylem tissue (secondary xylem) was manually separated from pith and the roots were washed to remove soil. All samples fraction were frozen in liquid nitrogen, lyophilized for 48 hours and stored in plastic bags at room temperature until further processing.

### *Hidrolysis with sulfuric acid ($H_2SO_4$):*

Tobacco samples (xylem and root tissues) were milled in Willey mill using a 40-60 mesh screen as described in the standard protocol (ASTM E 1757-01, 2001). Samples, extractives free, were prepared from the resulting powder as described in the standard protocol (TAPPI T264 om-88, 1999; ASTM E 1690-01, 2001) and used for acid hydrolysis. A representative 100 mg sample of powder tissue (extractives-free) was hydrolyzed with sulphuric acid in two steps (TAPPI T249 cm-85, 1991; ASTM E 1758-01, 2001). The primary hydrolysis was carried out with 72% $H_2SO_4$ at 30 ºC for 1 hour, diluted to 2.5% and autoclaved at 121 ºC for 1 hour. The samples were cooled on ice and filtered through a crucible porous plate. The amount of acid-soluble and acid-insoluble lignin (Klason) were determined spectrophotometrically (215 and 280 nm) and gravimetrically, respectively (TAPPI T222 om-98, 1998; ASTM E 1721-01, 2001). High performance anion exchange-pulsed

amperometric detection (HPAE-PAD) was carried out using a Dionex ICS 2500 instrument with a CarboPac PA1 column (4 x 250 mm) and CarboPac PA1 guard column (4 x 50mm) to determine carbohydrates and uronic acids (ASTM E 1758-01, 2001). Monosaccharides and uronic acids peak areas in HPAE-PAD were used for calibration and samples values were adjusted based on standard recovery rates (L-arabinose, L-rhamnose, D-galactose, D-glucose, D-xylose D-manose, D-glucuronic acid and D-galacturonic acid - Sigma-Aldrich).

***Hydrolysis with trifluoroacetic acid (TFA):***

A moderated acid hydrolysis method (TFA) was described for hemicelluloses determination (xylose, arabinose, galactose, and glucose) (FENGEL; WEGENER, 1979). This method is often used to analyze samples with low lignin content, such as leave and pith tissues of tobacco. Therefore, leave, pith and stem (base, middle and top) were milled in Willey mill using a 40-60 mesh screen (ASTM E 1757-01, 2001). Samples (~500 mg) were analyzed sequentially for soluble sugars and starch. Soluble sugars were removed with 80% ethanol (v/v) at 60 °C in a water bath. The remaining alcohol insoluble solids (AIS) were subjected to DMSO (dimetilsulfóxido) 90% (v/v) for starch removal (SELVENDRAN; O'NEILL, 1987). Hemicelluloses and celullose were extracted by TFA (2mol/L) (FENGEL; WEGENER, 1979) and 72% $H_2SO_4$ (TAPPI T249 cm-85, 1991; ASTM E 1758-01, 2001), respectively. AIS without starch (~50mg) were hydrolyzed with 2M TFA for 2 h at 121 °C for hemicellulose determination. The remaining TFA of supernatants were evaporated under vacuum at 35 °C and resuspended in 1 mL water. For cellulose (glucose) determination, pellets were dried and hydrolysed with 72% $H_2SO_4$. All analyses for carbohydrates were determined with a Dionex HPAE-PAD

***Histochemical staining and morphological fibers:***

The lower part of tobacco stems (3cm above root intersection) were transverse sectioned with a Leica SM 200R microcuter (60-80 μm) and arranged on glass slides. The sections were observed with optical microscope (Zeiss Axios Kop) under bright field after iodine green or congo red stain, for lignin and cellulose analysis, respectively (DOP; GAUTIÉ, 1928). Images were obtained with a Media Cybernetics PL A662 camera. The fibers of tobacco xylem tissue were measured. For fibers analysis, stalks without their barks were cut into match sticks and treated with a

mixture of same volume of glacial acetic acid and 29% hydrogen peroxide (FRANKLIN, 1937), mixed well and incubated at 60 $^{o}$C for 48 hours. The resulted macerated fibers were washed at least six times in distilled water and the resulting fibers were stained with safranin (1%). Cell wall thickness were calculated as the difference of fiber diameter and lumen width, with the results divided in half.

## RESULTS AND DISCUSSION

Since polysaccharides are the major constituents of the plant cell wall and these chemical blocks are essential for biofuel production, it is required methods to precise determine the structure and composition of cell wall and its polysaccharides. Many methods which are commonly used for analysis of lignocellulosic rich samples, such as tobacco, requires a prior hydrolyze step. Usually, acid hydrolysis are performed using either sulphuric or trifluoroacetic acids. Both acids cause saccharification of polymeric (hemi-cellulose) chains and low degradation of monosaccharides (FENGEL; WEGENER, 1979; ANTAL *et al.*, 1990). Subsequent analysis can be efficiently performed using separation methods, such as ion chromatography.

High performance anion exchange (HPAE), coupled with pulsed amperometric detection (PAD), permits direct quantification of nonderivatized carbohydrates at low levels (picomole) with minimal clean-up and sample preparation. Nowadays, this is the most powerful analytical technique to analyze type and concentration of mono and oligosaccharides in most plants. Thus, cell wall matrix polysaccharides are acid-hydrolyzed and monosaccharides analyzed by HPAE-PAD. Chemical composition of each tissue of tobacco are shown in Table 1. In fact the amount of carbohydrates varied among tissues showing a xylose concentration in xylem with magnitude of tenfold higher than that observed in pith. Xylem tissue has more glucose (420.95 $mg.g^{-1}$/dry weigth) and xylose (197.96 $mg.g^{-1}$/dry weigth) than pith tissue. The hexose/pentose ratio had no difference for xylem (2.15) and root (2.22) tissues, but was higher in pith tissue (7.53).

**Table 1. Carbohydrates, uronics acids and lignins contents of different tobacco tissues (mg.g$^{-1}$ dry weight), means of five replicates ± standard error.**

| Component | Tissues from tobacco | | | |
|---|---|---|---|---|
| | xylem[A] | root[A] | pith[B] | leaf[B] |
| **Arabinose** | 5.37 ± 0.34 | 29.69 ± 0.79 | 26.39 ± 1.03 | 22.85 ± 3.47 |
| **Xylose** | 197.96 ± 3.10 | 77.94 ± 6.19 | 21.46 ± 1.50 | 22.03 ± 4.07 |
| **Rhamnose** | nd | 3.25 ± 0.40 | 19.47 ± 2.05 | 11.01 ± 0.42 |
| **Galactose** | 5.95 ± 0.43 | 25.88 ± 0.6 | 39.76 ± 5.99 | 52.09 ± 7.88 |
| **Manose** | 10.99 ± 0.94 | 8.73 ± 0.72 | 13.59 ± 1.06 | 8.06 ± 1.66 |
| **Glucose** (including cellulose) | 420.95 ± 5.80 | 201.34 ± 4.27 | 286.90 ± 21.60 | 181.07 ± 13.27 |
| **Pentoses** | 203.33 ± 3.18 | 107.64 ± 6.22 | 47.84 ± 2.72 | 44.89 ± 7.23 |
| **Hexoses** | 437.89 ± 5.81 | 239.21 ± 5.18 | 359.74 ± 29.40 | 252.23 ± 20.97 |
| **Hexoses/Pentoses** | 2.15 ± 0.32 | 2.22 ± 0.10 | 7.53 ± 0.64 | 5.70 ± 0.50 |
| **Galacturonic Acid** | 6.02 ± 0.96 | nd | 156.12 ± 8.12 | 111.98 ± 9.57 |
| **Glucuronic Acid** | 0.68 ± 0.15 | nd | 2.26 ± 0.38 | 8.74 ± 0.47 |
| **Total Uronic Acid** | 6.68 ± 1.09 | nd | 158.38 ± 8.34 | 120.72 ± 9.95 |
| **Total Carbohydrates** | 641.22 ± 7.90 | 346.85 ± 10.47 | 407.59 ± 30.15 | 297.13 ± 27.07 |
| **Insoluble Lignin** | 172.67 ± 8.45 | 252.33 ± 12.80 | nd | nd |
| **Soluble Lignin** | 35.48 ± 3.45 | 50.00 ± 3.66 | nd | nd |
| **Total Lignin** | 208.16 ± 8.30 | 302.32 ± 12.60 | nd | nd |

**A** - carbohydrates extracted with sulphuric acid (72%)

**B** - carbohydrates extracted with trifluoroacetic acid (2 mol L$^{-1}$)

**nd** - not detected

In many cell types, primary cell wall (found mainly in pith and leaf tissues) consists of structurally independent but interacting networks: cellulose microfibrils coated with branched non-cellulosic polysaccharides (>50% dry weight) and embedded in a pectin matrix (25-40% dry weight), locked into shape by glycoproteins (1-10% dry weight) (CARPITA; GIBEAUT, 1993). After cell growth break off, cross-linking may occur between cell wall constituents and a secondary wall is formed (mainly in xylem tissue). The transition form of primary to secondary wall synthesis is marked by interruption of pectin deposition and a notable increase in the synthesis of cellulose, hemicellulose and lignin (RAVEN *et al.*, 2001). Lignin, a major component of secondary cell wall (xylem tissue), is a heterogeneous tridimensional phenolic polymer resulting from oxidative

polymerization of at least two of the following compounds: cinnamyl alcohols, also known as monolignols: p-coumaryl, coniferyl and sinapyl alcohols, giving rise to hydroxyphenyl (H), guaiacyl (G) or syringyl (S) lignin types, respectively. Cell wall deposition occurs in certain specialized cells such as tracheary elements, leading to dramatic variation in cell wall properties, providing additional strength and water impermeability. Lignin is thought to be bound to polysaccharides, by both covalent and non-covalent interactions to form a lignin-polysaccharide complex (SARKANEN, 1998). This leads to a maximum wall strength and rigidity for the plant body.

Chemical composition of tobacco stalks (xylem tissue) and its comparison with other fibrous materials are presented in Table 2. The holocellulose content of tobacco stalks was 62.4% of dry material. Thus, the carbohydrates content in the tobacco stalks was very close to the other biomasses which are used in biorefinery process. The lignin content in tobacco stalks also is in satisfactory amount (minus of 21%) in dry material. Lignin cannot be used in fermentation processes; however, it may be useful for other purposes (e.g. to burn). Cellulose and hemicellulose, which typically make up two-thirds of cell wall dry matter, are polysaccharides that can be hydrolyzed to sugars and then fermented to ethanol. The fermentation process is directly related to cellulose, hemicellulose, and soluble sugar concentration in the biomass. Therefore, considering the chemical composition of biomass (Table 2) and using the *Theoretical Ethanol Yield Calculator* of US Department of Energy (DOE US, 2012), the ethanol yields for tobacco stalks, eucalyptus wood, and sugarcane bagasse were predicted to be 108.7, 112.6, and 111.9 gallons/tons of dry feedstock, respectively, consideringbrazilian, lignocellulose agricultural wastes in form of tobacco stalks in 2010 (FAOSTAT, 2012). The estimation was made on the basis of the average number of plants of tobacco per hectare (over 20.000) and 100 grams of dry biomass per plant. Taking these considerations, we identified 1-2 million dry tons of biomass generated per year only from tobacco stalks which shows a great potential to second generation ethanol production.

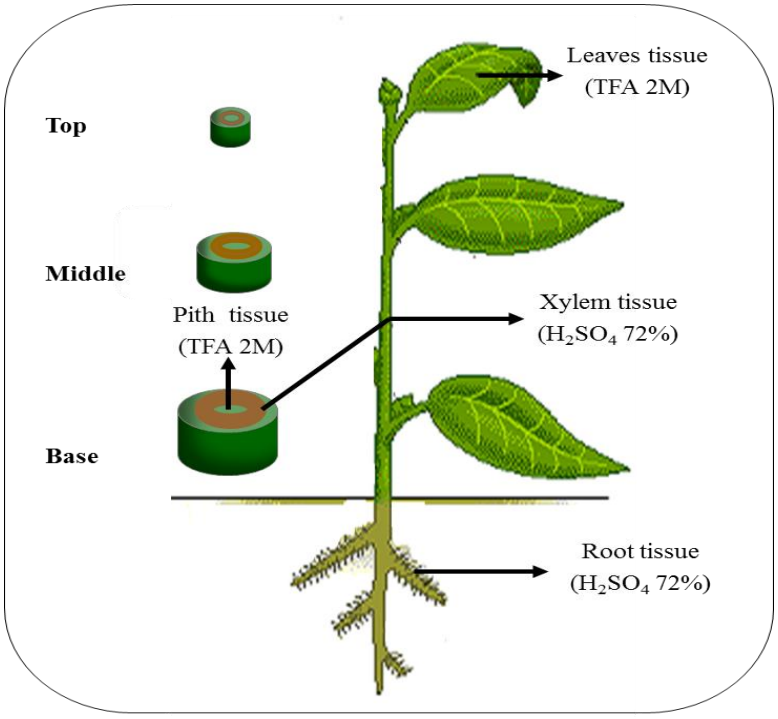
**Table 2. The mean value of chemical composition and fiber dimensions for tobacco stalk comparison with common biomass for biofuel.**

| | Tobacco stalk | Corn stalk [a, b] | Eucalyptus wood [c, d] | Sugacane bagasse [d, e] |
|---|---|---|---|---|
| **Length (mm)** | **0.95** | **1.3** | **1.04** | **1.5** |
| **Diameter (µm)** | **30.7** | **24.3** | **19.4** | **21.4** |
| **Lumen width (µm)** | **20.7** | **10.7** | **11.6** | **6.3** |
| **Cell wall thickness (µm)** | **4.9** | **6.8** | **3.9** | **7.7** |
| **Cellulose (% DM)** | **42.1** | **35.0** | **48.1** | **36.0** |
| **Hemicellulose (% DM)** | **20.3** | **23.0** | **16.7** | **28.0** |
| **Lignin (% DM)** | **20.8** | **19.0** | **26.7** | **20.0** |

**a**: (Usta et al., 1990), **b**: (SunGrant initiative, 2007), **c**: (Belini et al., 2008), **d**: (Carroll and Somerville, 2009), **e**: (Rocha, 2012)

The chemical composition was also determined in different parts of tobacco stalks as shown in Figure 2. In fact the differences in the structure of xylem tissue (stiffness) increase by about 30% from the base to the top of the plant as shows the Figure 1 (HEPWORTH *et al*, 1998). The xylose content (main hemicellulose in the cell wall) decreased from the base to top and other hemicelluloses (sum of arabinose and galactose) increased from the base to top.

**Figure 1. The structural scheme of tobacco plant and the regions utilized for cell wall analysis.**



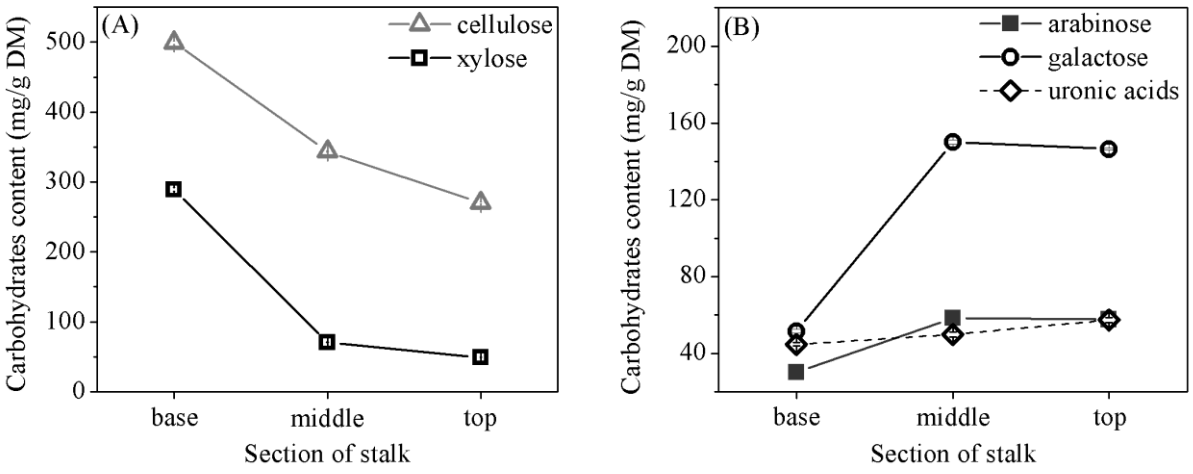




**Figure 2. Carbohydrates contents in different parts of tobacco stalks**

Histochemical staining of the tobacco stem cuts with iodine green and congo red were efficient to show the different tissues of tobacco plants as shown in Figure 3.

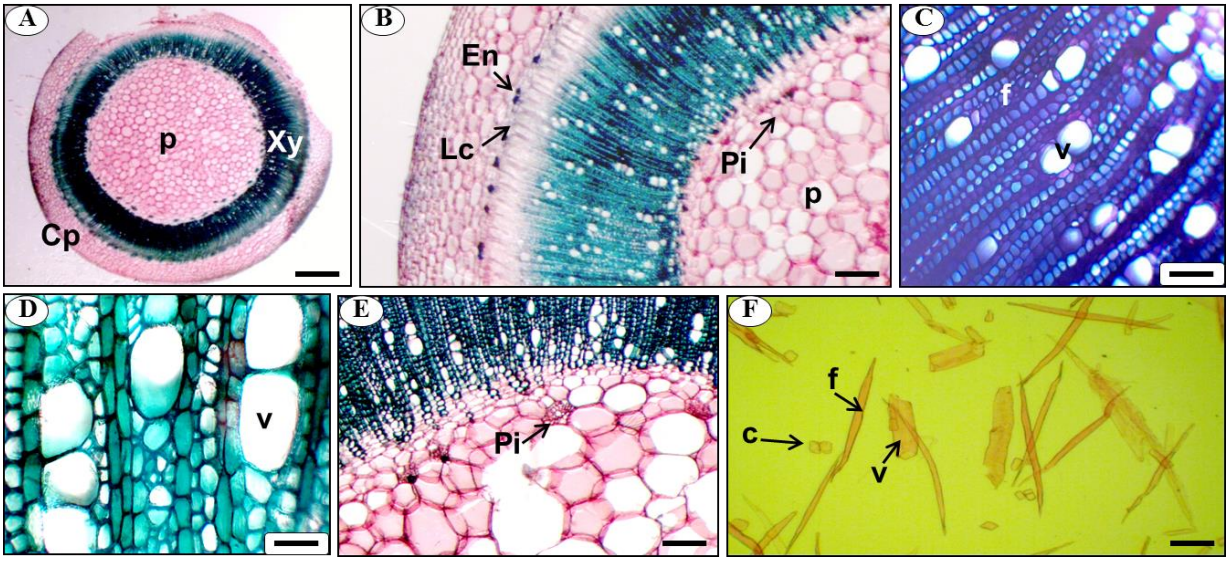



**Figure 3. (A), (B), (C), (D), and (E) are transversal cuts. (F) fibers macerate. p-pith tissue; Xy-xylem tissue; Cp-cortex parenchyma tissue; En- endodermis; Pi-Inner phloem; f-fiber; v-vessels; Pc- parenchyma cell; Lc – layer cambium**

**Bar = (A) 4mm; (B) 500µm; (C) 100µm; (D) 50µm; (E-F) 500µm**

Secondary wall or xylem tissue of tobacco plants are composed of fibers, vessel and parenchyma. Fibers are the most abundant component cells, comprising 70% of the tissue. This property has been proposed to be useful for others industrial purpose (AGRUPIS *et al.*, 2000; PESEVSKI *et al.*, 2010; SHAKHES *et al.*, 2011). The fiber cells of xylem tissue have only two distinguishable layers in the cell wall: primary wall which includes middle lamella and secondary wall (HEPWORTH *et al*, 1998). In trees, xylem tissue cells have several distinct layers within the secondary wall, which are usually called S1, S2 and S3 layers. Secondary xylem comprises several types of lignified cells particularly in wood dicotyledons: water conducting tracheary elements (vessel members and tracheids) and mechanically supporting elements (fiber-tracheids, libriforms fibers and sclereids) (LEV-YADUN, 2000). In herbaceous plants, such as tobacco, vessels and fibers are the main lignified components of xylem and generally assumed that the compositions of lignin differ according to cell type; generally, vessels are enriched wich guaiacyl (G) units and fibers in syringyl (S) units (FERGUS; GORING, 1970). The fiber dimensions from xylem tissue of tobacco plants were obtained by peroxide-HAc and their comparison with other fibrous materials are summarized in

Table 2. The average fiber length of tobacco stalks was 0.95 mm, which are shorter than eucalyptus wood and sugarcane bagasse. The fiber diameter and lumen width results were 30.7 μm and 20.7 μm, respectively. These morphological properties were higher in comparison to the other fibrous materials (Table2). The thickness of the fiber (4.9 μm), was in normal range when compared to other biomass.

## CONCLUSION

Based on the data obtained in this work, tobacco represents an attractive and promising energy plant platform, and could also serve as a model for the utilization of other high-biomass plants for biofuel production. The agricultural wastes (mainly stalks) from the harvesting of tobacco farms are useful biomass which can be used as raw material in biotechnological processess. The chemical composition mainly holocellulose in the xylem tissue has potential for ethanol production. The results of morphological fibers showed that tobacco stalks is included in a group known as a short fiber, and that properties are very similar to those of the common biomass used for biofuels production. Therefore, regarding the mentioned in this work, it can be noted that the future of tobacco is promising not only in conventional use for cigarettes production.

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