XVII^th World Congress of the International Commission of Agricultural and Biosystems Engineering (CIGR)

Hosted by the Canadian Society for Bioengineering (CSBE/SCGAB)
Québec City, Canada June 13-17, 2010

CULTIVATION AND UTILIZATION OF SPECIFIC WOOD BIOMASS FOR SYNTHESIS OF CELLULOSE BASED BIOETHANOL

LAURENTIU FARA[1], CORNELIU CINCU[2], GHEORGHE HUBCA[2], MIHAI FILAT[3], DANUT CHIRA[3], CORNELIA NUTESCU[4], SILVIAN FARA[5], CATALIN ZAHARIA[2], AUREL DIACON[2], DRAGOS COMANECI[1]

[1] Polytechnic University of Bucharest, Faculty of Applied Sciences, Splaiul Independentei 313, Bucharest, Romania, lfara@renerg.pub.ro
[2] Polytechnic University of Bucharest, Faculty of Applied Chemistry, Str. Polizu 1-7, Bucharest, Romania
[3] Forest Research and Management Institute, Sos. Stefanesti 128, Voluntari, Ilfov, Romania
[4] National Wood Institute, Str. Fabrica de Glucoza nr.7, Sector 2, Bucharest, Romania
[5] Institute for Research and Design of Automation, Str. Calea Floreasca 169, Bucharest, Romania

CSBE101283 – Presented at Section IV: Rural Electricity and Alternative Energy Sources Conference

ABSTRACT The determination of energetic characteristics of six types of poplar clones was achieved, four developed by Alasia New Clones - Italy, (AF-2, AF-6, AF-8, Monviso) and two by ICAS - Romania (Turcoaia, Sacrau-79); they were cultivated for different pedoclimatic conditions in Romania. The plant survival rate and biomass production rate were analyzed in five experimental cultures. The main results obtained after two years of studies were as follows: Very good adaptability of Italian clones to the pedoclimatic conditions in Romania in comparison with local clones (better growth speeds and biomass production capabilities); the Italian clones Monviso and AF-6 registered the most substantial growths and the highest resistance to disease. The synthesis of bioethanol was achieved by acidic hydrolysis of the cellulose following two methods. In the first approach the lingo-cellulosic raw material was hydrolyzed with diluted sulfuric acid (4.5%) at 50°C for 24h. After filtration, the solid residue was treated with 30% H_2SO_4 at 100° for 6h. Following again filtration, the obtained solutions were neutralized with $Ca(OH)_2$ and the resulted solution (pH 6.5) was subjected to fermentation with Saccharomices Cerevisiae. In the second approach the lingo-cellulosic raw material was subjected to hydrolysis with 10% H_2SO_4 at 100° for 4h. After filtration, the solid residue was hydrolyzed with 30% H_2SO_4 at 100° for 6h. The resulted solution were neutralized with $Ca(OH)_2$ and then subjected to alcoholic fermentation with Saccharomices Cerevisiae. The fermentation took place at 25°C for 72h. The results show no significant difference between the two methods.

Keywords: poplar clones, short-cycle crop production, bioethanol, cellulose biomass, hydrolysis, enzymes

INTRODUCTION Three Romanian research institutes (ICAS, IPA, INL) and one Romanian university (UPB) together with a scientific consultant from Italy (Alasia New Clones) have assessed the possibilities of introducing specialized poplar plantations in the energy industry, having considered the fact that the wood of rapidly-growing species, with a high capacity of vegetative multiplication, has become of foremost importance, due to its becoming accessible for various uses. This approach was considered for a research national project to be developed in the period 2008-2011 (PLEN Research National Project Proposal, 2008).

Easy vegetative multiplication and very active growth especially in the juvenile stage have made the hybrid poplars to be increasingly used in short cycle crop production (Benea V., 1986). In a national research project five experimental plots have been planted in different site conditions from Romania (Table 1). Six hybrid poplar clones have been used to determine the proper conditions and culture technique to produce increased quantities of woody biomass.

Table 1. Location and the main site characteristics of experimental plots for biomass production

No	Name	Geographic location		Altitude (m)	Relief	Climate and soil characteristics
		Latitude	Longitude			
1	Boianu	+ 44° 11' 35"	+ 27° 18' 25"	9	river meadow (Danube Holm)	Steppe continental climate; T = 11,1°C; P = 550 mm; fluvisol*, pH= 8,2
2	Urleasca	+ 45° 08' 55"	+ 27° 37' 28"	19	plain (Plain –Bărăgan)	Steppe continental climate; T = 11,0°C; P = 440 mm; chernozem*, pH = 7,8
3	Râşeşti	+ 46° 44' 47"	+ 28° 10' 07"	21	river meadow (Prut Holm)	Steppe continental climate; T = 9,5°C; P = 530 mm; fluvisol*, pH = 7,5
4	Zăval	+ 43° 50' 00"	+ 23° 52' 52"	36	river meadow (Jiu Holm)	Steppe continental climate; with slight Mediterranean influence; T = 12°C; P = 500 mm; fluvisol*, pH = 7,2
5	Nufăru	+ 45° 09' 07"	+ 28° 54' 32"	2	river meadow (Danube Delta)	Steppe continental climate; with slight maritime (Black Sea) influence; T = 11,2°C; P = 400 mm; fluvisol*, pH = 7,7
Note: T – average annual temperature; P – average annual rainfall; pH – soil reaction in water, *WRB-SR-1998						

The state forest administration (RNP Romsilva) and many farmers are interested to introduce the most efficient poplar clones to produce wood biomass in Danube Delta & Danube Valley, inland river holms and lower wet sites of the Danube and Pannonian Plains.

EXPERIMENTAL CULTURE OF POPLAR CLONES The experimental plots have been planted in the spring of 2008 (Nufăru) (Fara L. et al., 2009) and 2009 (Boianu, Urleasca, Râşeşti, Zăval) using four Italian clones, selected for energetic biomass (http://www.alasiafranco.it), tested for the first time in Romania, and two clones widely used in the region (Filat and Chira , 2004) (Table 2).

Table 2. Origin of poplar clones used in experimental plantations installed for the production of energetic biomass

No	Clone	Provenance
1	AF 2	Italy - Alasia New Clones
2	AF 6	Italy - Alasia New Clones
3	AF 8	Italy - Alasia New Clones
4	Monviso	Italy - Alasia New Clones
5	Turcoaia	Romania – Institutul de Cercetări şi Amenajări Silvice
6	Sacrau 79	Romania – Institutul de Cercetări şi Amenajări Silvice

Experimental cultures consist of randomized blocks with three repetitions with each 12-28 plants per basis unit, in three densities:
Variant A - 5500 plants / ha;
Variant B - 6600 plants / ha;
Variant C - 9500 plants / ha.
Plant spacing varies function of culture techniques (soil mobilization machines), providing in the same time the necessary density for each clone.
Cuttings of 30 cm length and 15-25 mm diameter with at least three viable buds have been used. Cuttings have been planted after autumn land ploughing and spring land discing, leaving 5 cm above the ground.
Between starting of vegetation (April) and the end of August 3-4 soil mobilization (mechanized between rows and handy on plant rows) in order to destroy weeds soil loosening. In the same time, depending on the rainfall, crops have been irrigated once or twice a month.
During the growing season several culture features have been noticed: plant survival (the ratio between the number of viable plants found growing in the end of the season and number of cuttings planted), growth rate and susceptibility to foliar diseases.
Plant diameter have been measured at end of vegetation (first decade of October), at 30 cm above the ground. Plant height has been measured in the beginning of each month, from June to October.
Evaluation of resistance to foliar diseases was made on sick leaves, studied uredial stage of disease (first decade of October). Scale used for intensity of infection was: 0 (immune) - no infection, 1 (very poor) - some uredospors / plant, 2 (weak) - some uredospors / leaf, 3 (moderate) - many uredospors on most leaves (threshold level of sensitivity), 4 (strong) - leaves are covered with uredospors, most leaves are browning; 5 (very strong) - total defoliation (Chira D. et al., 2004).
After the vegetation season three plants from each clone have been cut from the soil surface for preliminary laboratory determinations.
THE POPLAR CLONES CULTURE RESULTS In three cultures (Boianu, Urleasca, Nufăru) the survival rate was very high (> 95%) in all clones. In the other two crops, clone Turcoaia had the higher survival rate (96%), AF 6, AF 8, and Monviso had better survival rates (> 90%), but Sacrau 79 and AF 2 had poor survival (<75%).

Table 3. Percentage of maintenance, the average diameter and height of clones tested in experimental cultures, in the first growing season

Plantation	Clone	Plants survival (%)	Biometric characteristics	
			Height (cm)	Diameter (mm)
Boianu	AF2	97	239.2	24.5
	AF6	99	266.3	26.4
	AF8	99	287.2	30.9
	Turcoaia	98	243.3	23.9
	Monviso	99	309.1	32.8
	Sacrau 79	98	224.3	24.0
	Average	**98.3**	**261.6**	**27.1**
Urleasca	AF2	96	205.9	19.7
	AF6	98	206.9	21.0
	AF8	100	217.5	21.9
	Turcoaia	100	202.8	21.1
	Monviso	97	212.6	22.1
	Sacrau 79	97	160.0	13.5
	Average	**98.0**	**200.9**	**19.8**
Rasesti	AF2	69	138.3	13.9
	AF6	93	139.2	14.4
	AF8	92	147.8	15.2
	Turcoaia	96	135.2	13.5
	Monviso	82	132.1	13.9
	Sacrau 79	57	11.4	9.5
	Average	**81.4**	**134.0**	**13.4**
Zaval	AF2	75	197.2	19.1
	AF6	90	202.4	19.6
	AF8	80	206.9	20.1
	Turcoaia	96	196.6	18.6
	Monviso	90	211.5	19.6
	Sacrau 79	61	157.3	15.9
	Average	**82.2**	**195.3**	**18.8**
Nufaru	AF2	99	328.0	28.7
	AF6	98	303.9	25.9
	AF8	95	312.2	25.6
	Turcoaia	98	261.9	21.0
	Monviso	99	324.5	26.3
	Sacrau 79	99	293.4	21.7
	Average	**97.7**	**304.0**	**24.9**

Height increase, achieved during the first growing season, was sustained in all clones, from June until late September, Italian clones being more efficient compared with local ones. The highest value of average height was 328 cm (AF 2) and the largest average diameter was 32.8 mm (Monviso). Graph of height is suggestive in terms of differentiation both among cultures and clones (Fig. 1).

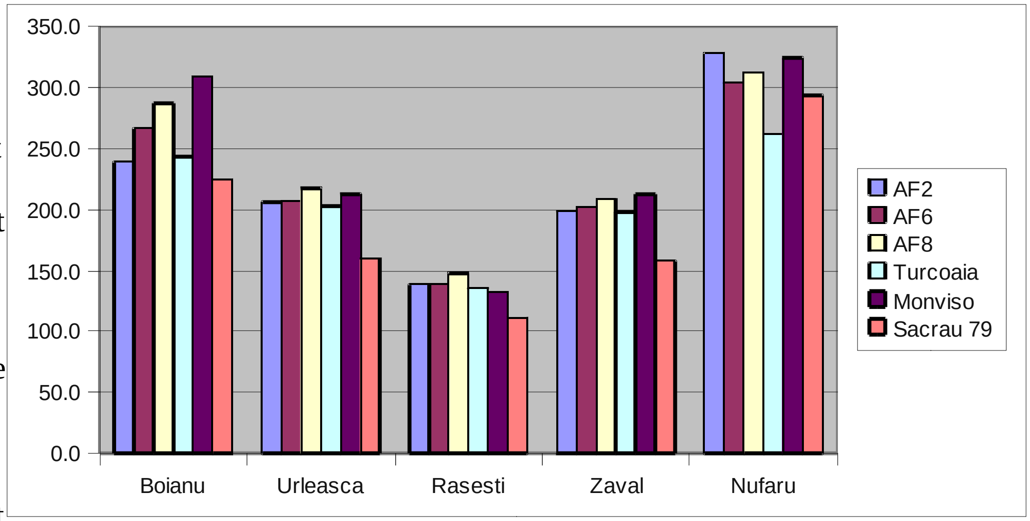

Figure 1. Height of energetic poplar clone recorded after the first vegetation season

Clone resistance / susceptibility to foliar diseases (*Mellampsora* ssp. rust and *Marssonina brunea* leaf spot) varied, as follows (Table 4):

- Monviso and AF6 were relatively immune to rust and relatively resistant to leaf spot;
- AF2 and AF 8 were relatively resistant to rust and virtually immune to leaf spot;
- Control clones have had the same moderately sensitivity.

Table 4. Poplar clones resistance to *Mellampsora ssp*. leaf rust and *Marssonina brunea* leaf spot

Clone	***Mellampsora* ssp.**				***Marssonina brunea***	
	S	**I**	**Med**	**Max**	**Med**	**Max**
AF 2	1.5	2	1.5	3.5	0	0
AF 6	0	0	0	0	2.2	3.5
AF 8	2	2.5	2.5	3	0	0
Monviso	0	0	0	0	2.2	2.5
Turcoaia	3	2	2.5	3.5	?	?
Sacrau 79	3	3.5	3	3.5	?	?

Clone resistance: 0 - immune, 1 - resistent, 2 - relative resistent, 3 - moderate sensitive, 4 - sensitive, 5 - very sensitive; S – values at the sprout superior part; I - values at the inferior part of sprout; Med – average values, Max – maximum per plant

BIOETHANOL OBTAINMENT A series of experiments were carried out for obtaining bioethanol from cellulose biomass of fast growing poplar clones: A-F8, Turcoaia (3 years) and Sacrau-79. Cellulose hydrolysis was done with sulfuric acid and soluble sugar fermentation with *Saccharomyces Cerevisiae* in an anaerobic environment.

For hydrolysis, the following experimental methods were used (Hubca G. et al., 2008):

In the first method, A-F8 poplar wood flour was treated with a H_2SO_4 4.5 % solution for a period of 24 hours at a temperature of 50 ºC. After filtration, the solid residue was treated with a H_2SO_4 30 % solution for 6 hours at 100 ºC.

After the mixing and neutralization (pH = 6.5) of the two solutions with $Ca(OH)_2$, fermentation was carried out using *Saccharomyces Cerevisiae* in and anaerobic environment.

After fermentation, a fraction of the liquid mixture was separated through distillation. The fraction has a boiling point situated between 75 and 80 ºC which, through further fractioning and distillation leads to ethylic alcohol of 95.5 % concentration.

In the second method, the cellulose biomass was subjected to hydrolysis in two distinct stages. In the first stage an H_2SO_4 10 % solution was used for 4 hours at a temperature of 100 ºC. After filtration, the solid produce was further hydrolyzed with a H_2SO_4 30 % solution for 6 hours at a temperature of 100 ºC. The solutions obtained in these two stages were mixed, neutralized to pH = 6.5 with $Ca(OH)_2$ and then subject to fermentation using Saccharomyces Cerevisiae in an anaerobic environment. After distillation and rectification a bioethanol solution with 95.5% purity was obtained.

These experiments were carried out on cellulose biomass collected from the three types of poplar clones considered for study. Comparative results between the three types are presented in Table 5 (250g of wood flour was used in each case).

Table 5. Laboratory experimental results

Method / **Clone**	**Bioethanol Quantity**								
	A-F8			**Turcoaia 3ani**			**Sacrau-79**		
	mL	g	g/100*	mL	g	g/100*	mL	g	g/100*
	70.7	56.5	22.6	64.7	51.8	20.72	65.5	52.4	20.96
	65.2	52.2	21.28	67.6	54.1	21.64	73.2	58.6	23.44

g/100* = grams of bioethanol/ 100g wood flour

The results presented in Table 5 show that there are no significant differences between the poplar wood flour samples used in the experiments.

BIOETHANOL CHARACTERIZATION After distillation and rectification, the following bioethanol chracteristics were determined: concentration, distillation interval, refraction index, density and Raman spectral charts.

Regardless of the type of wood flour used and hydrolysis method (I or II), the characteristics presented in Table 6 are practically the same for all of the bioethanol obtained.

Table 6. Bioethanol characteristics

Characteristics	**Value**
Refraction index, n_D^{20}	1.36
Concentration, %	95.5
Distillation interval, ºC	79-79.5
Density, g/ mL la 20 ºC	0.81

Figure 2 presents the Raman spectra for the bioethanol obtained from the A-F8 poplar clone using the two hydrolysis methods (I and II) in comparison with a similar product from Chimopar S.A. currently in production. The three spectra are practically the same.

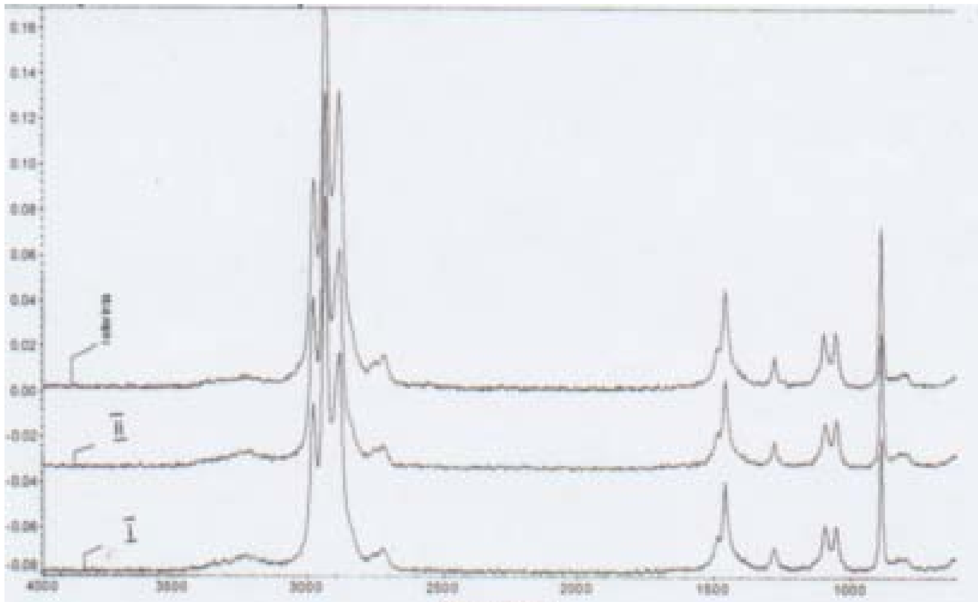

Figure 2. Raman spectra for A-F8 poplar clone sample hydrolysis method I (bottom chart) and II (middle chart) in comparison with a commercial product from Chimopar S.A. (top chart).

CONCLUSIONS AND PERSPECTIVES In the first vegetation season Italian energetic clones have adapted very well to the pedo-climatic conditions of south-eastern Romania, being more competitive than control clones both concerning growth rate and higher biomass production.

Danube Valley and Delta offer better conditions for development of energetic clones in comparison with other locations.

Italian clones Monviso and AF8 have registered the most active growth and better resistance to local abiotic factors. The results obtained from using cellulose biomass from A-F8, Turcoaia (3 years) and Sacrau-79 poplar clones are promising both as efficiency and as bioethanol purity.

Taking into account that sulfuric acid hydrolysis is uneconomical and doesn't satisfy the ecological requirements, research was focused on using enzymes both for hydrolysis and for fermentation. Using biocatalytic enzimes presents multiple advantages (Lynd L. R. et al., 2002; Ingram L. O. et. al,1991):

- high product efficiency
- high product purity
- co-fermentation of multiple sugar types
- reduced sensibility to inhibitors

From the biological alternatives proposed in the literature we have chosen the transformation of biomass cellulose directly into bioethanol in a single stage, called “Bioproces Consolidat” (CBP), the results being in the course of patenting.

ACKNOWLEDGEMENTS The paper is based on a research national project contract No. 22-092/01.10.2008, Reasearch regarding cultivation and energetic valorification of some rapid growth short cycle poplar clones, funded by the Romanian Ministry of Reserach.

REFERENCES

Benea, V., 1986: Selecţia speciilor de plop şi salcie pentru producerea de fitomasă energetică în culturi specializate. Dezvolt. cercet. şt. din Silvicultură, 50 ani de la crearea ICAS (1933-1983).

Chira, D., Filat, M., Chira, F., Colin, J., Mantale, C., 2004: Testări privind rezistenţa la rugini foliare (Melampsora spp.) a speciilor / clonelor de plop. Revista de Silvicultură şi Cinegetică nr. 19-20, 41-45.

Fara, L., Filat, M., Chira, D., Fara, S., Nutescu, C., 2009: Preliminary Research on Short Cycle Poplar Clones for Bioenergy Production. Proceedings of the International Conference RIO 9. World Climate & Energy Event, Rio de Janeiro, Brazil, 127-132.

Filat, M., Chira, D., 2004: Researches concerning introduction in culture of the poplars and willows species / clones with superior forest productive potential and increased resistance to adversities. Anale ICAS, vol. 47, 83-99.

http://www.alasiafranco.it

Hubca, Gh., Lupu, A., Cociaşu, A.C., Biocombustibile: biodiesel, bioetanol, sun diesel, Ed. MATRIX ROM, Bucureşti, 2008.

Cellulose Enzyme Research, http://www.1.eere.energy.gov/biomass/cellulose_enzyme.html

Lynd, L.R., ş.a., Microbiology and Molecular Biology Reviews, 2002, 66(3), 506-577.

Ingram, L.O., and Clark, D.P., 215. Pat. 5028539/1991.