Effect of the use of mixtures of lignocelluloses on the production of second generation bioethanol

Alejandro García, Ramón Aravena,
Crístián Rodríguez, René Carmona,
María Lienqueo, Oriana Salazar and
Humam Mugammar,

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Introduction

- Strong dependence on foreign sources of energy.
- This situation is particularly critical on transportation sector.
- Reduced possibilities to produce biofuels using first generation technologies
- It is needed to explore lignocellulosic residues, which are abundant in the country.
- There are particular good yields of grains and other products such as corn, wheat, sunflower, rapeseed, among others.

Theoretical framework

- The current global ethanol industry is based on the transformation of two main raw materials; sugarcane and corn. Both crops are C4 plants and for this reason they have a characteristic carbon isotope composition. Most of the ethanol obtained is based on First Generation Technology (FGT) which implementation is possible in countries like USA and Brazil because of their wide availability of agricultural soils and favourable climate conditions.
- In Chile, FGT is not feasible due to critical scarcity of agricultural soils and subsequent food competition. However the diversity of climate conditions and soil types generate a "mosaic" of agricultural-forestry scenarios which can combine the existence of C3 and C4 plants. For this reason the use of mixed materials to produce bioethanol is a possibility that will be studied in this proposal, using the natural abundance of stable carbon isotopes, 12C and 13C as a novel tool.

- In nature, carbon is mainly present as a mixture of two stable isotopes (carbon 12 or ^{12}C and carbon 13 or ^{13}C), where the lighter one (^{12}C) is more abundant. The isotopic composition of a certain material is defined as the ratio between the heavier isotope (^{13}C) and the lighter one (^{12}C).

- Natural abundances of isotopes are expressed using the symbol $\delta^{13}C$, which represents a value that reflects the ratio of stable isotopes ($^{13}C/^{12}C$) of a sample minus the ($^{13}C/^{12}C$) of a recognized international standard according to:

$$\delta^{13}C\ s\ (‰) = (^{13}C/^{12}C\ s - ^{13}C/^{12}C\ Is) / (^{13}C/^{12}C\ Is) * 10^3$$

- C3 and C4 plants exhibit contrasting $\delta^{13}C$ values. Thus, C3 plants show characteristic $\delta^{13}C$ values from -29 to -24 ‰ and C4 between -13 and -12 ‰, with average values of -26 ‰ and -13‰, respectively.

- The effect of the use of mixed materials (C3 and C4 plants) on ethanol production seems to be unknown, due to the fact that the international bioethanol industry has been based on the use of a single type of raw material.

- Related isotope research has been focused on:
  - Detection of adulterants in commercial liquors [Weber et al, 1997]
  - Characterization of whey ethanol [Masud et al, 1999]
  - Isotope fractionation during distillation step [Baudler et al, 2006], among others.

- The information is still more scarce for fermentation step and few studies have been conducted such as:
  - The use of deuterated labeled glucose for ethanol yield measurement [Smith et al, 2002]
  - Other authors [Rembacz et al, 2007] studied the use of plasma glucose to measure just evolved CO2 as a final product of fermentation but not ethanol yields, even testing mixes of corn glucose (C4 origin) and potato glucose (C3 origin) on control assays.
  - Use of glucose for studying changes in intramolecular distribution on ethanol produced by mixed cultures of *Saccharomyces cerevisiae and S. bayanus* during fermentation [Pionnier et al, 2002], estimating just conversion rates of labelled glucose with deuterium.

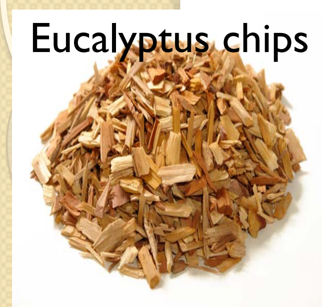
- The application of the isotope approach has a significant contribution to the development of soil science and ecology.

- The effect of the use of mixed materials on ethanol production seems to be unknown, due to the fact that the international bioethanol industry has been based on the use of a single type of raw material.

- The only limitation to use this technique is to have a relatively wide difference among $\delta^{13}C$ values belonging to different sources of carbon. This difference must be at least 5 ‰ to monitor the carbon transfer movement among different pools (Mariotti, 1991).

- If ethanol generation was possible, using a mixture of substrates, it would be interesting to know its global effect on:
  - A) Global yields of ethanol after fermentation of glucose derived from Chilean lignocelluloses
  - B) Estimation of partial contribution of each lignocellulose on ethanol yield. yield and the individual contribution of each original material over the final mixture.

Materials and Methods



Phase 1

Holocellulose isolation

Elemental analysis coupled to Isotope Ratio-Mass Spectrometry

$\delta^{13}C$ values

Phase 2

Biological pretreatment based on WRF bioaugmentation

Enzymatic saccharification

Supernatant dilution

Fermentation of single and mixed media

Ethanol obtainment

Purge trap concentrator-GC-Mass Spectrometry

Gas Chromatography

Materials and Methods

Calculations of $\delta^{13}C$ values:

$$\delta^{13}C(‰) = (R_s / R_{is} - 1) * 1000$$

- Where R is the ratio of $^{13}C/^{12}C$ for each of the samples and international standard, respectively. The international standard is Vienna Pee Dee Belemnite (V PDB).

Partial contributions of each source of glucose on mixed media:

$$x\ (\%) = (\delta_s - \delta_{C4}) / (\delta_{C4} - \delta_{C3}) * 100$$

- Where x is the fraction of ethanol derived from the fermentation of one of the sources of glucose available on mixed media; δ_s is the $\delta^{13}C$ value of bioethanol sample; δ_{C4} and δ_{C3} are $\delta^{13}C$ values of bioethanol produced during the fermentation of single media, from a C4 or C3 glucose source, respectively.

Treatments

Treatment	Material	WRF	Material	WRF	Medium
1	WS	Ga			Single
2	WS	Le			Single
3	CS	Ga			Single
4	CS	Le			Single
5	E	Ga			Single
6	E	Sh			Single
7	CS	Ga	WS	Ga	Mixed
8	CS	Ga	WS	Le	Mixed
9	CS	Le	WS	Ga	Mixed
10	CS	Le	WS	Le	Mixed
11	CS	Ga	E	Ga	Mixed
12	CS	Ga	E	Sh	Mixed
13	CS	Le	E	Ga	Mixed
14	CS	Le	E	Sh	Mixed

Where: WS = wheat straw, CS = Corn stover, E = *Eucalyptus* residues, Ga = *Ganoderma applanatum*, Le = *Lentinus edodes*, Sh = *Stereum hirsutum.*

Statistical analysis

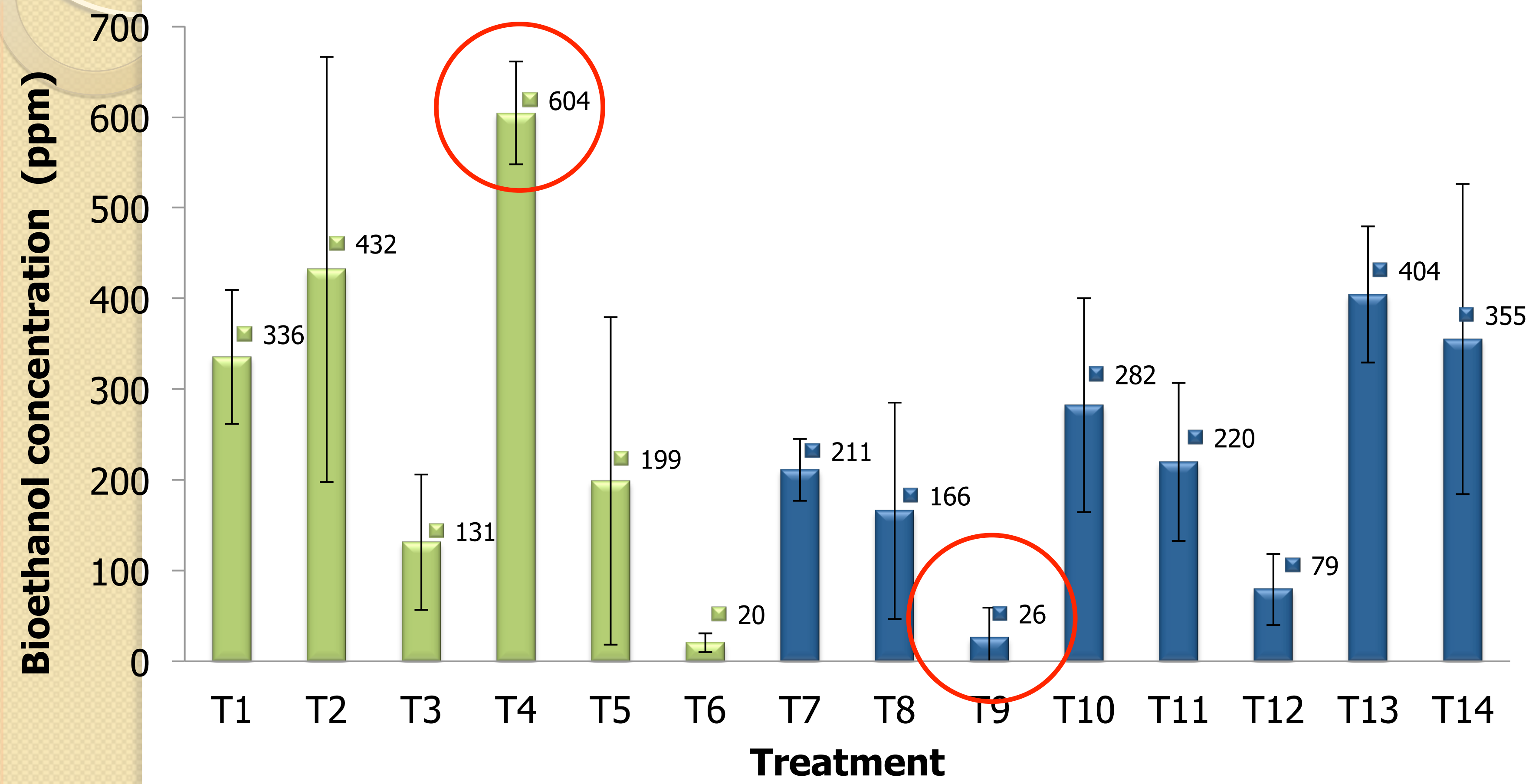
Transformed data of ethanol yields ($Y=\sqrt{yield}$) were subjected to ANOVA analysis and Tukey Kremer test ($\alpha=0.05$), transformed data of $\delta 13C$ and x values according to arcsin $\sqrt{(|\delta 13C|/1000)}$ and arcsin $\sqrt{(\%/100)}$, respectively, were subjected to Kruskal Wallis and Dunn Sidak tests using MATLAB® (Version 7.8.0.247 (R2009), 32 bits).

Results

Natural abundances of ^{13}C on isolated holocellulose fraction

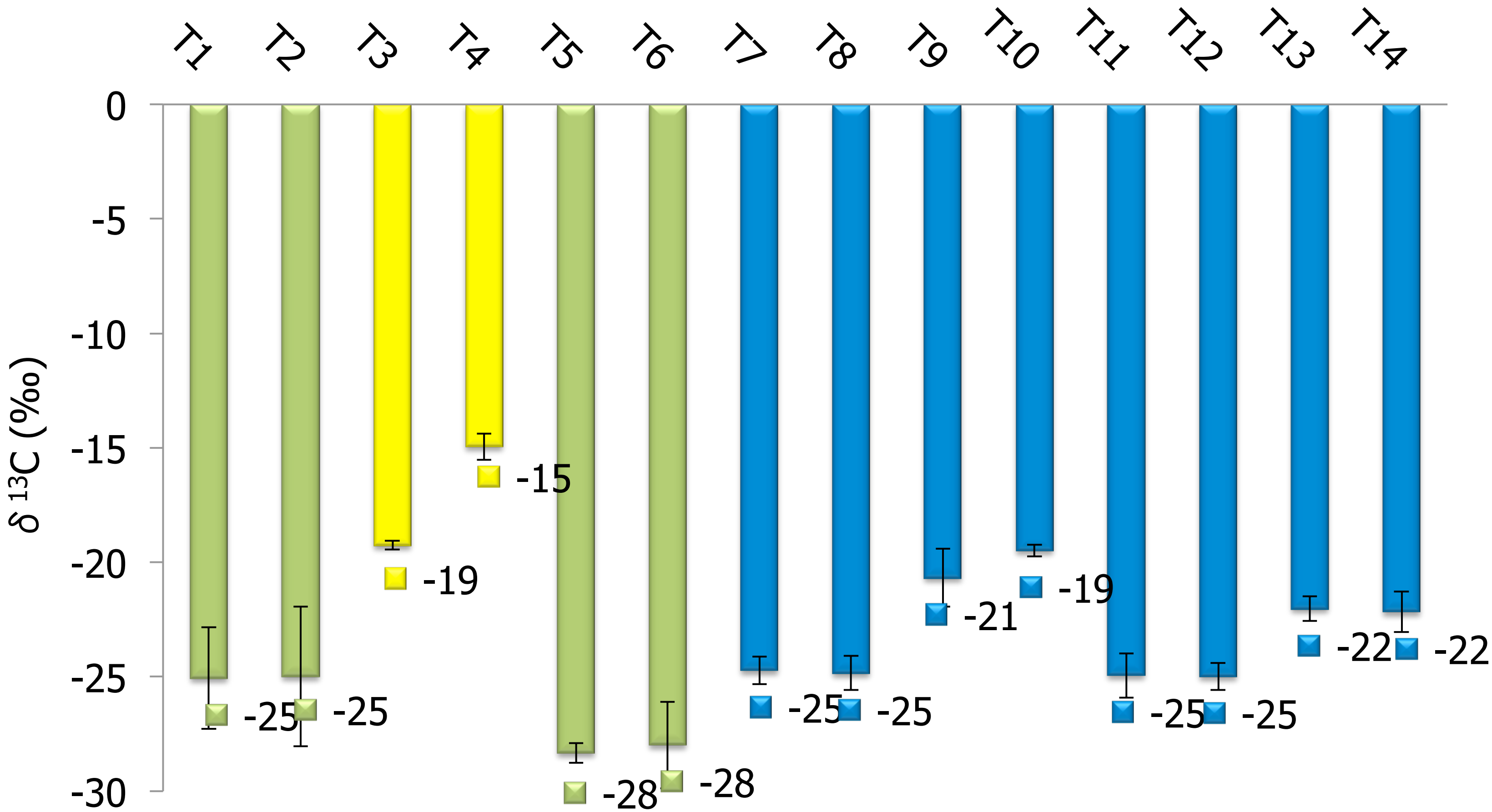
Tipo de residuo	Tipo de metabolismo	Valor $\delta^{13}C$ (promedio, ‰)
Residuos de Lenga (*Nothofagus pumilio*)	C3	-26.23
Residuos de Eucalipto (*Eucalyptus globulus*)	C3	-27,65
Rastrojo de trigo (Triticum aestivum)	C3	-27,19
Rastrojo de maíz (Zea mays)	C4	-17,94

Bioethanol yields



Ethanol yields obtained on fermentations. Vertical bars correspond to standard deviations. Green and blue bars correspond to single and mixed media, respectively.

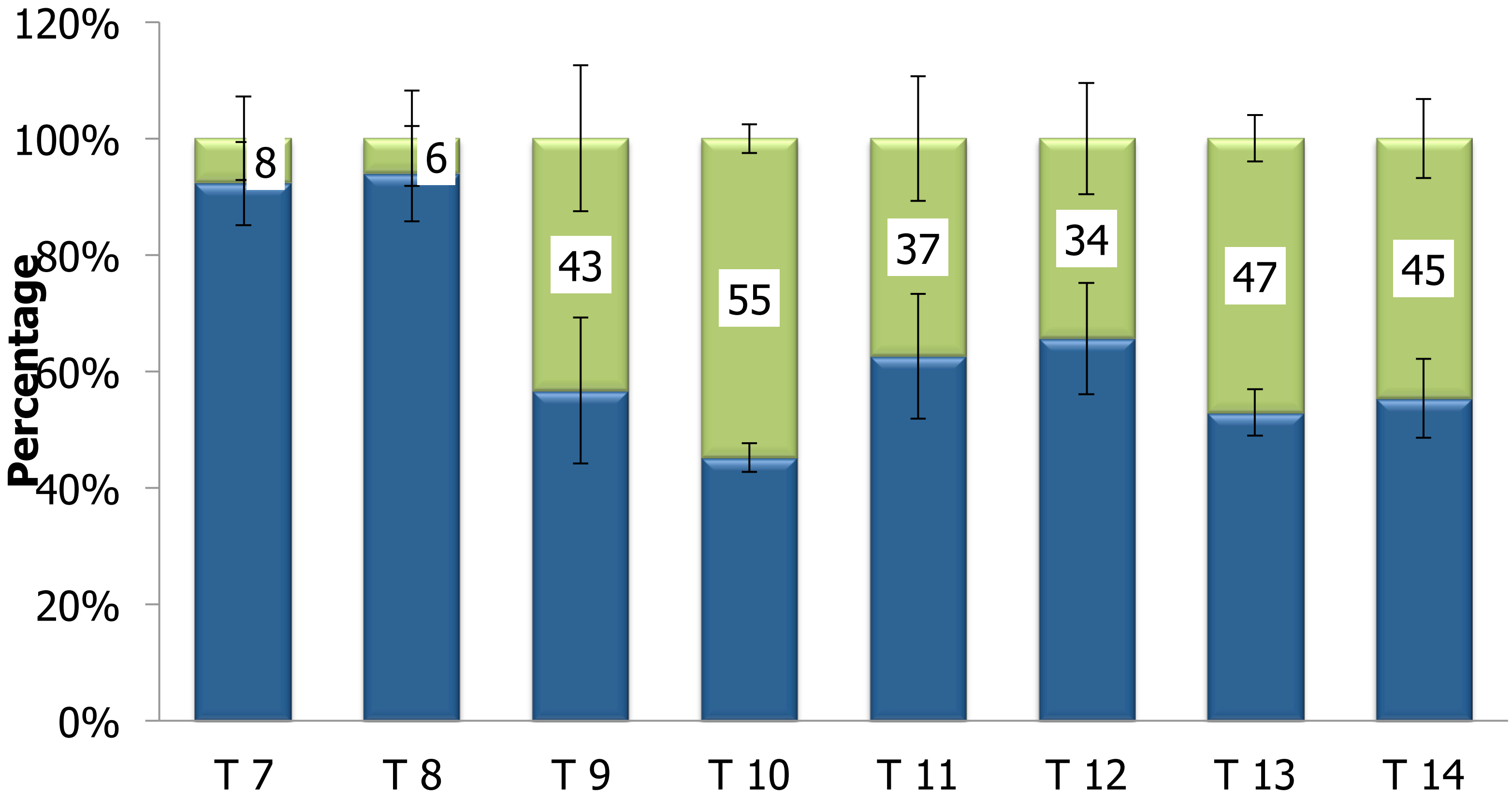
$\delta^{13}C$ values determined on ethanol produced during fermentation



Vertical bars reflect standard deviations for three repetitions.

The first 6 bars correspond to single media and the rest to mixed media, respectively.

x values determined on mixed media.



Vertical bars reflect standard deviations for three repetitions.

Comparisons among fermentations

Fermentation	Component 1	Individual yield (ppm)	Component 2	Individual yield (ppm)	Expected yield (ppm)	Observed yield (ppm)
T 7	T 3	131	T 1	336	234	211
T 8	T 3	131	T 2	432	282	166
T 9	T 4	604	T 1	336	470	26
T 10	T 4	604	T 2	432	518	282
T 11	T 3	131	T 5	199	165	220
T 12	T 3	131	T 6	20	76	79
T 13	T 4	604	T 5	199	402	404
T 14	T 4	604	T 6	20	312	355

Red numbers indicate an inhibitory effect on ethanol yield and blue numbers show a synergistic effect

Conclusions

- Ethanol yields depend on the kind of plants material used in the fermentation and $\delta^{13}C$ values in the produced ethanol clearly reflect the origin of the source material, indicating the effective fermentation of different materials on mixed cultures.

- The contribution of C3 plant residues on final bioethanol yields from mixed media are higher than C4 residues, this could suppose isotopic fractionation during fermentation step. Additional experiments will contribute to clarify antagonistic effect during fermentation step of mixed materials on global ethanol production. Efforts for determining the effect of inhibitors need to be developed.

Ongoing activities

Origin of material	Kind of material	Species	Colection site
Residuo forestal	Restos de manejo silvicultural de leñosa nativa	*Prosopis chilensis* (Algarrobo); *P. tamarugo* (Tamarugo); *Cordia decanta* (Carbonillo)	Norte Grande
Residuo forestal	Residuos del manejo forestal de leñosas exóticas	*Pinus radiata* (Pino insigne); *Eucalyptus nitens* (Eucalipto); *Pseudotsuga menziesii* (Pino oregón)	VI y VII Regiones
Cultivo dendroenergético	Madera de 1 año	C*Eucalyptus camaldulensis* (Eucalipto rojo); *Acacia saligna; Acacia dealbata;* Clones de *Populus* spp.; *Acacia decurrens* (Aromo extranjero).	Ensayos disponibles en Campus Antumapu (RM).
Muestras de especies nativas leñosas	Albura de especies leñosas nativas	*Luma* sp. (Arrayán); *Fitzroya cupressoides* (Alerce); *Nothofagus alpina* (Raulí); *Aristotelia chilensis*. (Maqui); *Nothofagus antartica* (Ñirre); *Laurelia sempervirens* (Laurel); *Drimys winteri* (Canelo); *Podocarpus nubigenus* (Mañío); *Araucaria araucana* (Araucaria); *Persea lingue* (Lingue); *Peumus boldo* (Boldo); *Laureliopsis philippiana* (Tepa); *Eucryphia cordifolia* (Ulmo); *Nothofagus obliqua* (Roble);	IX y X Regiones

Ongoing activities

Origin of material	**Kind of material**	**Species**	**Colection site**
Residuos de especies frutales	Albura	*Gevuina avellana (Avellano); Lomatia dentata (Avellanillo); Vaccinium corymbosum* (Arándano); *Castanea sativa* (Castaño); *Prunus davidiana* (Duraznero portainjerto Nemaguard); Distintas variedades de *Vitis vinífera (Vid); Distintas* especies y variedades de *Prunus* spp.	R.M., VI, VII, VIII, IX y X Regiones
Residuos industriales de origen forestal	Descarte de material	*Populus spp. (residuos de corteza,* astillas, descarte de palitos de helado y fósforos); Aserrín de pino; Astillas; Viruta; Lodo de papel reciclado.	VII Región
Residuos agrícolas	Rastrojos de cultivos anuales	*Triticum aestivum (Trigo); Avena sativa (Avena); Zea mays (Maíz); Oryza sativa (Arroz); Corontas de* choclo; Cascarilla de arroz	VI, VII, VII Regiones
Otras especies	Tallos	*Arundo donax (Pasto elefante); Chusquea quila (Quila); Sequoia sempervirens (Secuoia).*	R.M. y X Regiones

Ongoing activities

- 92 samples have been analized to determined the holocellulose content according to McFarlene et al, 1999, using diglyme-HCl modified method. Average value is 43.95 % and ranges from 21.28 to 69.67 %.

- Samples of isolated holocellulose have been sent to know their natural abundances of carbon 13

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Use of soils in Chile

Use type	Use	Land capability	Surface (ha)	Percentage
Arable soils	Non restrictions	I	90,846	0.1
		II	711,625	0.9
	With restrictions	III	2,195,439	2.9
		IV	2,273,670	3.0
Non arable soils	Cattle	V	2,271,144	3.0
	Cattle-forestry	VI	6,510,613	8.6
	Forests	VII	12,339,882	16.3
	Conservation	VIII	14,200,000	18.8
Non agricultural lands	Unproductive lands		35,144,147	46.4
	TOTAL		75,707,366	100

Chilean balance of energy

Energy source	Production (GJ)	Import (GJ)	Dependence
Crude oil	6,434,992	480,457,088	98,7
Natural gas	85,939,360	237,031,968	73,4
Coal	11,589,048	132,532,384	92,0
Hydroelectricity	111,758,824	0	0,0
Wood and other sources	197,907,384	0	0,0
Biogas	0	0	
TOTAL	413,629,608	850,021,440	

Power matrix for Chilean transportation sector