Using *Leiotrametes menziesii* and *Abortiporus biennis* for biological pretreatment of willow sawdust for enhancing biogas production.

Maria Alexandropoulou [1,2], **Georgia Antonopoulou** [1], **Ioanna Ntaikou** [1], **Gerasimos Lyberatos** [1,2]

[1] Institute of Chemical Engineering Sciences, Stadiou, Platani, Patras,GR26504, Greece
[2] School of Chemical Engineering, National Technical University of Athens, GR 15780 Athens, Greece

3rd International Conference on Sustainable Solid Waste Management
2-4 July 2015, Tinos island, Greece

Renewable Energy Resources

- Growing energy demands
- Decreasing reserves of fossil fuel resources
- Environmental problems

➡ Renewable Energy Resources

BIOMASS : Biological (organic) material that can be converted into ***biofuels*** (CH_4, H_2, bio-ethanol, bio-diesel).

⬇

energy crops
agricultural residues
livestock residues

forest residues

Willow

hardwood type biomass

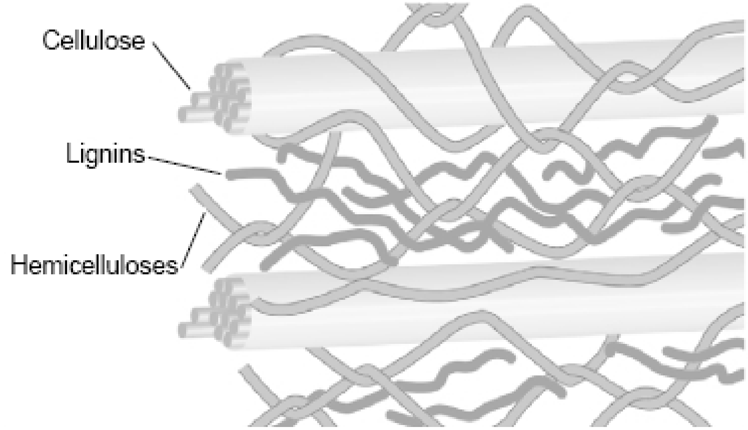
Lignocellulosic material

willow

promising feedstock for 2nd generation biofuels

Lignocellulosics

Lignocellulosics' structure



Cellulose
Hemicellulose → target substrates to be bio-converted

Lignin → barrier for the efficient exploitation of lignocellulosic feedstocks

Pretreatment methods

- Mechanical
- Physicochemical
- Biological (white rot fungi)
- Combination of them

Biological pretreatment

- Promising technology due to : low environmental impact
  less energy demands
  mild reaction conditions

- Bio-delignification also avoids the formation of degradation compounds that inhibit the subsequent steps.

- Different microorganisms including bacteria and fungi can be involved in lignin degradation

- Only the ''White – Rot'' fungi are able to depolymerize lignin efficiently.

- These fungi have developed an extracellular and unspecific oxidative enzymatic system for lignin degradation.

  - Laccase (Lac)
  - Manganese peroxidase (MnP)
  - Lignin peroxidase (LiP)

Microorganisms

- In this study, two different white rot fungi were used :

  Leiotrametes menziesii and *Abortiporus biennis*

- *Leiotrametes menziesii*

  - Belongs to the genus *Trametes* which includes some of the most efficient lignin degrading species
  - Their ligninocellulolytic enzyme system is comprised of laccase (Lac), manganese peroxidase (MnP) and lignin peroxidase (LiP)

- *Abortiporus biennis*

  - Reported to produce Lac and MnP with the Lac activity being actually quite high

Anaerobic Digestion

Organic material, is converted into CH_4 and CO_2 (biogas) in dark conditions, from various types of anaerobic microorganisms

A.D. Stages		**Metabolic products**
Hydrolysis	→	Aminoacids, sugars, fatty acids, alcohols
Acidogenesis	→	Propionic acid, butyric acid, **H_2, etc**
Acetogenesis	→	acetic acid, H_2, CO_2
Methanogenesis	→	CH_4, CO_2

Aim of this study

The assessment of the effect of biological pretreatment and of the combination of biological and chemical pretreatment of willow sawdust (WSD) on anaerobic digestion efficiency, using two different white rot fungi i.e. *Leiotrametes menziesii* and *Abortiporus biennis.*

Materials and methods

Solid state fermentations (SSF) – Biological pretreatment

- (SSF) experiments were carried out in erlenmeyer flasks for 30 days
- Without any addition of nutrient
- Samples taken at days : 5, 14, 22 and 30

Inoculation procedure

Leiotrametes menziesii

Aquatic solution

6 g willow

Abortiporus biennis

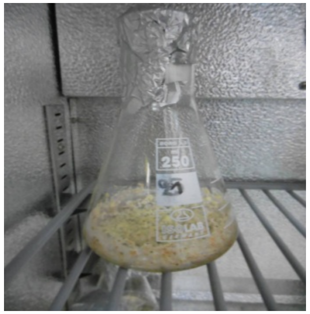
Aquatic solution

6 g willow

Conditions :

Humidity = 80 %

T =27 °C



Materials and methods

Combination of biological and alkaline pretreatment

Biologically pretreated samples (30d)

↓

Alkaline solution: NaOH 1% w/v

Conditions: 80^{o}C for 24 h

Loading: 20 g NaOH/100 g TS

Mass/Volume ratio: 5:100 (solids load 5% w/v)

- The same pretreatment was carried out in raw willow sawdust for comparison purposes

Materials and methods

BMP tests

headspace

Biogas measurement

CH_4 content

- **Inoculum:** Anaerobic sludge from anaerobic digester of a wastewater treatment plant of Athens

- **BMP assays**: In 160 mL serum bottles, at 35^{o}C+ 20 % (v/v) inoculum +**2 g VS /L** +trace elements

- **Were performed for**: a) biologically pretreated samples after 14 and 30 d

  b) combined alkaline/biologically pretreated samples after 30 d

  c) alkaline pretreated willow sawdust samples

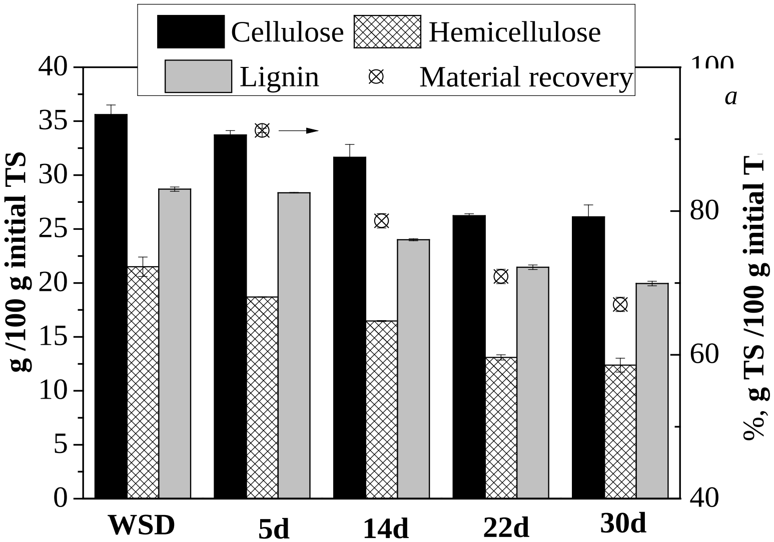
Results

The main characteristics of WSD used in this study

Characteristic	Value
TS (%)	94.5 ± 1.1
VS (g/100gTS)	94.1 ± 1.2
Cellulose (g/100gTS)	35.6 ± 0.9
Hemicellulose (g/100gTS)	21.5 ± 0.9
Lignin (g/100gTS)	28.7 ± 0.2
Extractives (g/100gTS)	3.0 ± 0.1
Ash (g/100gTS)	5.9 ± 1.6
Proteins (g/100gTS)	0.7 ± 0.1

Results

The effect of biological pretreatment on solid material recovery and on fractionation of WSD



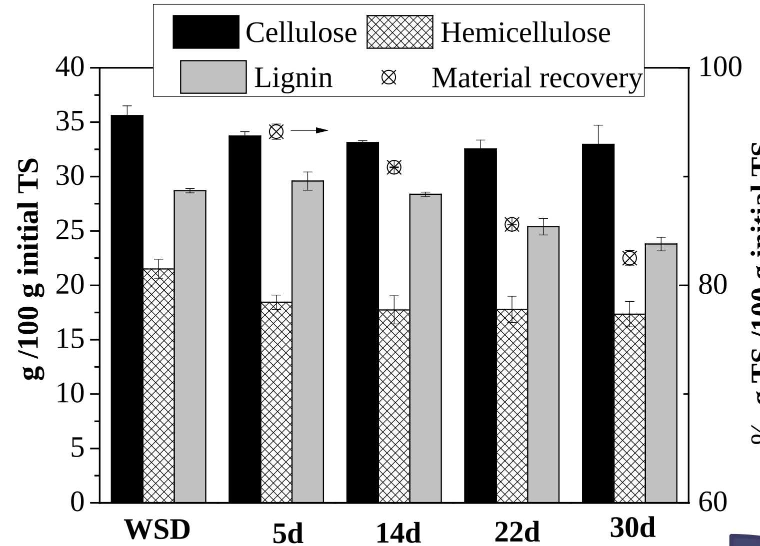
Leiotrametes menziesii

removal

Lignin: 30.5 %

Cellulose: 26.6 %

Hemicellulose: 42.3 %



Abortiporus biennis

removal

Lignin: 17 %

Cellulose: 7.6 %

Hemicellulose: 19.5 %

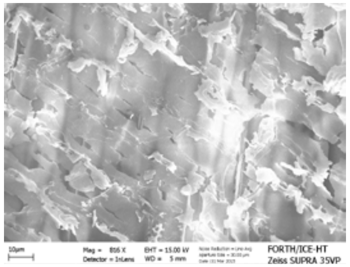
A. biennis seems to be more efficient for biological pretreatment of WSD

- Optimum pretreatment time: 22 d

Results

SEM

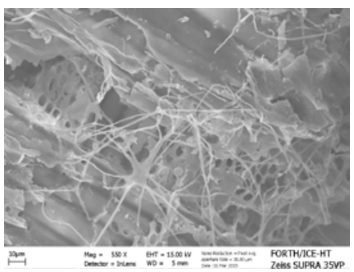
Raw WSD



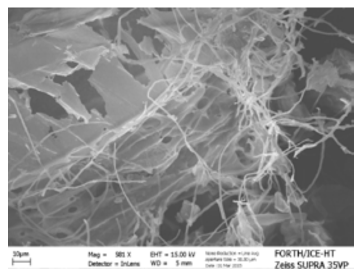
Intact surface structure, with acute edges due to the trimming process

Leiotrametes menziesii

Abortiporus biennis

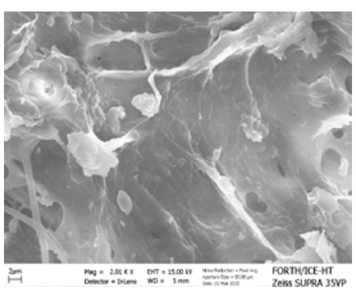


14 d

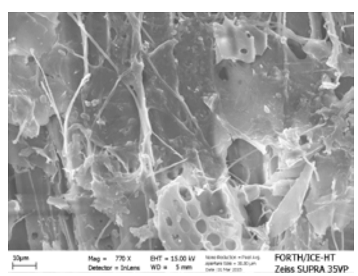


14 d

Apparent mycelium growth, with hyphae starting to colonize on the substrate



30 d



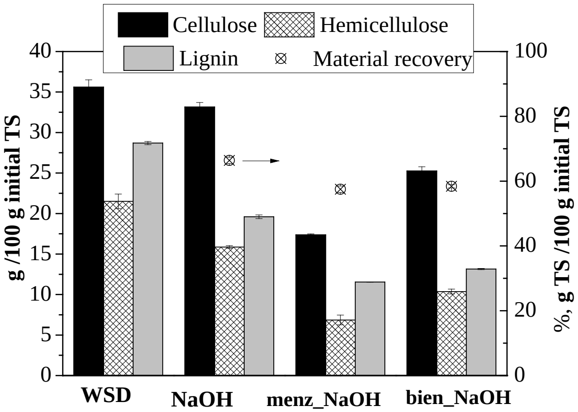
30 d

Almost the whole surface was covered by the mycelia

Results

The effect of alkaline pretreatment on solid material recovery and on fractionationt of WSD

- SSF at 30 d and raw WSD
- NaOH 1%, at 80ºC for 24 h



Leiotrametes menziesii + NaOH removal

Lignin: 59.5 %
Cellulose: 73.5 %
Hemicellulose: 51.5 %

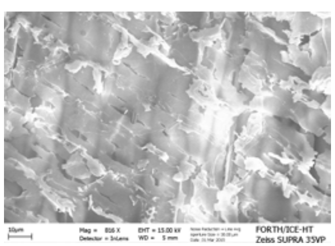
Abortiporus biennis + NaOH removal

Lignin: 54 %
Cellulose: 49.8 %
Hemicellulose: 29.2 %

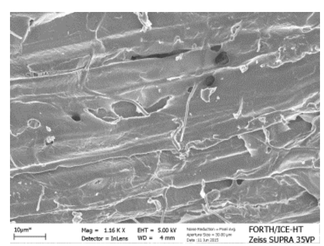
Lignin and holocelluloses removal efficiency was higher for *L. menziesii* and NaOH due to higher lignin degradation and cellulose and hemicellulose uptake efficiencies for *L. menziesii.*

SEM

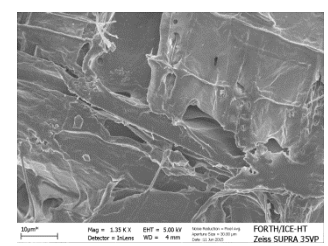
Raw WSD



Leiotrametes menziesii + NaOH



Abortiporus biennis + NaOH

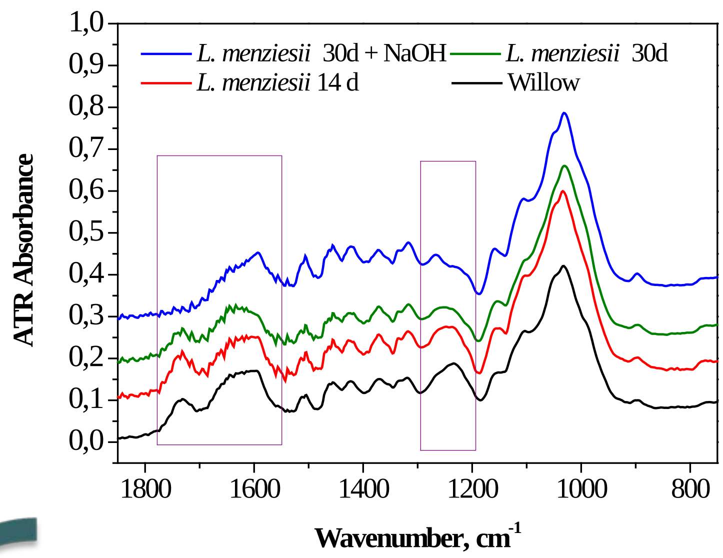


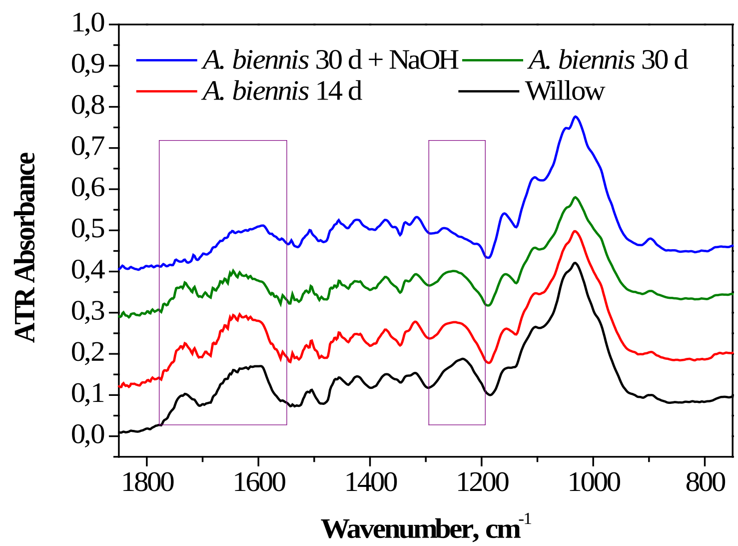
- The initial structure seems to have been altered
- Pinholes and gaps are visible, leading to the speculation that the surface area and the porosity increased

Results

IR spectra

- Spectral range of 800 to 1800 cm^{-1}
- Raw WSD
- Biologically pretreated WSD after 14 and 30 d
- Combination of alkaline with biological treatments after 30 d



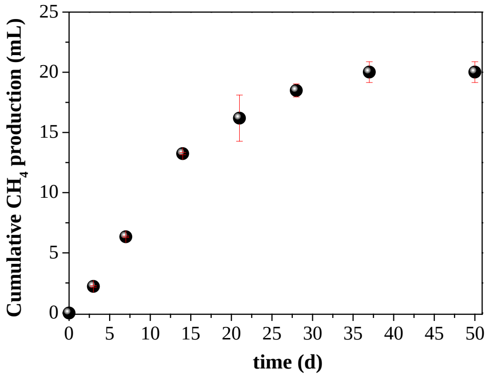


Decrease of the wide Raman bands in the region 1180-1300 and 1550 -1700 cm^{-1} corresponding to aromatic skeletal vibrations in lignin

Decrease of the Raman peak centered at ~1735 cm^{-1} related to carbonyl C=O stretching in lignin is observable.

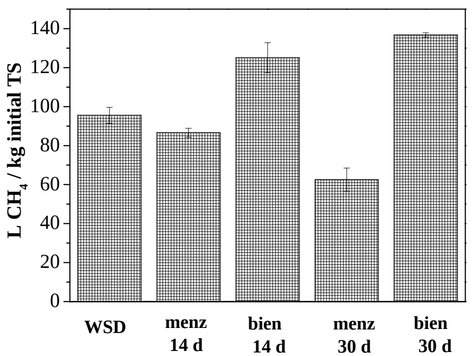
Results

The effect of pretreatment on BMP experiments with WSD



Cumulative methane production of raw WSD

BMP = 95.5 ± 4.3 L CH4/kg TS



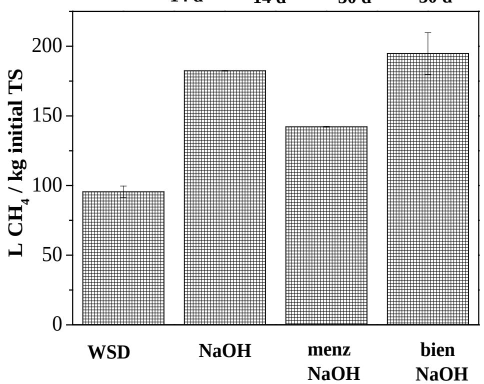
Methane yields

Leiotrametes menziesii 14 d : 8.9 % reduction in comparison with raw WSD

Leiotrametes menziesii 30 d : 34.6 % reduction → **holocelluloses uptake**

Abortiporus biennis 14 d : 31 % increase

Abortiporus biennis 30 d : 43 % increase



NaOH : 91 % increase in comparison with raw WSD

Leiotrametes menziesii 30 d + NaOH : 49 % increase

Abortiporus biennis 30 d + NaOH : **108 %** increase

Conclusions

➢In this study, the effect of biological pretreatment of willow sawdust on anaerobic digestion efficiency, using two different white rot fungi (*Leiotrametes menziesii* and *Abortiporus biennis)* was investigated.

➢Biological pretreatment results revealed that *L. menziesii* resulted to higher lignin degradation with high cellulose and hemicellulose uptake than the respective of *A. biennis,* implying that the second microorganism seems to be more efficient for biological pretreatment of WSD.

➢Observation of the SEM images and IR spectra, verified these arguments.

➢ Combination of alkaline with fungal pretreatment, after 30 d of cultivation resulted to a very high lignin and holocellulose removal especially for combined alkaline and biological pretreatment with *L. menziesii.*

➢The BMP of raw WSD used in this study was 95.5 ± 4.3 L CH_4/ kg TS . Biological pretreatment with *A. biennis*, resulted in a BMP increase by 31 and 43%, for 14 and 30 d cultivation, respectively.

➢ Combination of alkaline treatment with the biological pretreatment enhanced the BMP of WSD and the maximum BMP was observed for the combined alkaline pretreatment with biological (with *A. biennis*) and was 108% and 9% higher than the respective of raw and alkaline treated WSD.

Thank you for your attention !!