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

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Renewable Energy Resources

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

**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 2\textsuperscript{nd} generation biofuels
Lignocellululosics

Lignocellululosics’ structure

Cellulose → target substrates to be bio-converted

Hemicellulose

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)
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 CH₄ and CO₂ (biogas) in dark conditions, from various types of anaerobic microorganisms.

<table>
<thead>
<tr>
<th>A.D. Stages</th>
<th>Metabolic products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrolysis</td>
<td>Aminoacids, sugars, fatty acids, alcohols</td>
</tr>
<tr>
<td>Acidogenesis</td>
<td>Propionic acid, butyric acid, H₂, etc</td>
</tr>
<tr>
<td>Acetogenesis</td>
<td>acetic acid, H₂, CO₂</td>
</tr>
<tr>
<td>Methanogenesis</td>
<td>CH₄, CO₂</td>
</tr>
</tbody>
</table>
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*. 

**Aim of this study**
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°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**

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

- **BMP assays:** In 160 mL serum bottles, at 35°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

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

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

14 d

Almost the whole surface was covered by the mycelia

14 d

30 d

30 d
The effect of alkaline pretreatment on solid material recovery and on fractionation of WSD

- SSF at 30 d and raw WSD
- NaOH 1%, at 80°C 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

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 CH₄/ 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 !!