National Technical University of Athens
School of Chemical Engineering
*Biotechnology Laboratory*

IWWATV

Industrial Waste & Wastewater Treatment & Valorization

# Enzymatic Bioconversion and Fermentation of Corn Stover at High-solids Content for Efficient Ethanol Production

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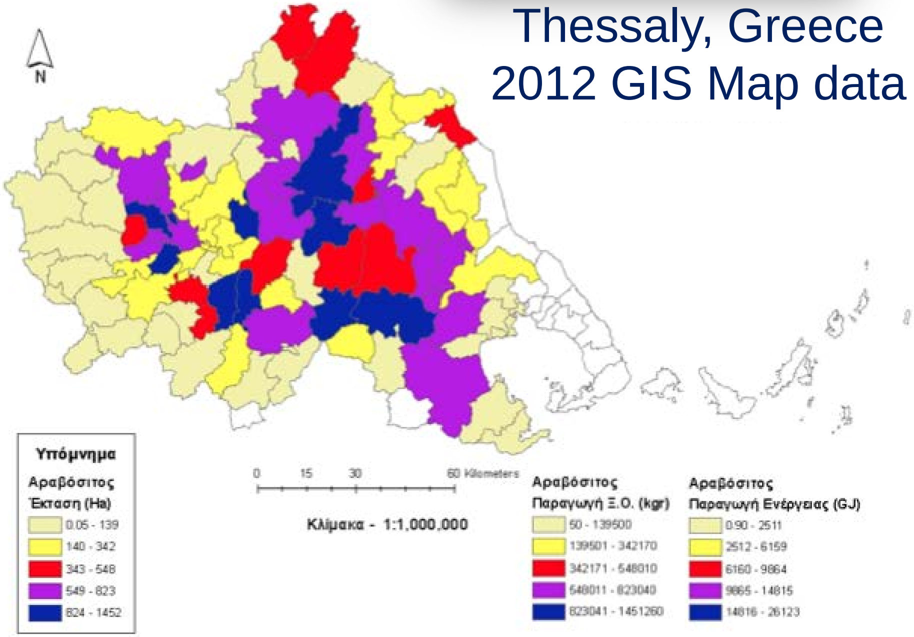
# Potential of Greek agroindustrial residues

| Culture | Type of culture | Biomass production (kg/acre) |
|---|---|---|
| Cereals | *Wheat* | 217.5 |
| | *Oats* | 120 |
| | *Rye* | 150 |
| | *Barley* | 150 |
| **Corn** | | **1000** |
| Cotton | | 314 |




Thessaly, Greece
2012 GIS Map data

*Data kindly provided by Professor Gemtos*
*Farm Mechanization, University of Thessaly*

UNIVERSITY OF THESSALY

# Preparation of corn stover samples

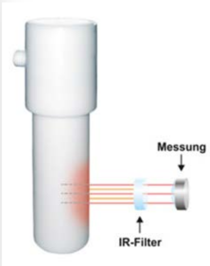
2.0 mm

0.75 mm

| Corn Stover | |
|---|---|
| **Fraction** | **% (w/w)** |
| Cellulose | 38.8 |
| Hemicellulose | 23.5 |
| Lignin | 20.2 |
| Ash | 5.3 |

# Hydrothermal pretreatment (laboratory scale)

- Speed wave ™ MWS-2, Berghof Instruments GmBH, Germany
  - ✓ DAP-60K (60 mL, 40 bar, 260 º C)
  - ✓ DIRC (temperature sensor)


- ✓ 10 % w/v
- ✓ 0.3 % v/v $CH_3COOH$
- ✓ 9-51 min
- ✓ 170-230 $^oC$

- ✓ 3 % w/v
- ✓ 10 FPU/g DM
- ✓ pH 5.0
- ✓ 50 $^oC$ & 700 rpm
- ✓ 72 h

✓ Response surface methodology had been employed in order to optimize the pretreatment step (temperature, duration)

✓ Pretreated samples were evaluated through enzymatic hydrolysis using the benchmark commercial cellulase preparation Celluclast 1.5L together with β-glucosidase Novozyme 188

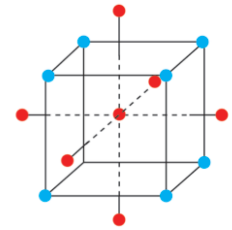
# Hydrothermal pretreatment optimization

- Response surface methodology (RSM) → group of mathematical and statistical techniques

  → $3^2$ central composite design (CCD)

  2 factors: Temperature ($^{o}$C) & Time (min)

  4 factorial points, 5 central points, 4 axial points



- This design is represented by a second order polynomial regression model:

$$G=a_0+a_1\,x_1+a_2\,x_2+a_3\,x_1^2+a_4\,x_2^2+a_5\,x_1\,x_2$$

Range of each independent variable in the central composite design

| Variables | | |
|---|---|---|
| **Coded level of factors** | **Time (min)** | **Temperature (°C)** |
| **Axial point (-1.414)** | 9 | 170 |
| **Low level (-1)** | 15 | 180 |
| **Central level (0)** | 30 | 200 |
| **High level (+1)** | 45 | 220 |
| **Axial point (1.414)** | 51 | 230 |

- **Design-Expert®**
  - ✓Design of Experiments
  - ✓Analysis of CCD
  - ✓ANalysis Of VAriance (ANOVA)
  - ✓Contour & Surface plots

# Hydrothermal pretreatment optimization

## ➢ Pretreated samples evaluation

- ✓ Compositional analysis (NREL)
- ✓ Enzymatic hydrolysis (glucose release (g/L) after 8h)

| Run | Factors | | Responses | | |
|---|---|---|---|---|---|
| | Temperature (ºC) | Time (min) | Cellulose (%w/w) | Glucose release (g/L) | Predicted glucose release (g/L) |
| 1 | 200 | 30 | 58.8 | 6.59 | 6.54 |
| 2 | 220 | 15 | 61.7 | 7.55 | 7.57 |
| 3 | 200 | 51 | 60.0 | 4.82 | 4.69 |
| 4 | 200 | 9 | 56.1 | 6.42 | 6.24 |
| 5 | 200 | 30 | 58.5 | 6.54 | 6.54 |
| 6 | 170 | 30 | 46.8 | 4.21 | 3.77 |
| 7 | 180 | 45 | 51.7 | 4.08 | 4.05 |
| 8 | 220 | 45 | 63.4 | 5.73 | 5.81 |
| 9 | 200 | 30 | 59.2 | 6.46 | 6.54 |
| 10 | 200 | 30 | 58.5 | 6.58 | 6.54 |
| 11 | 180 | 15 | 48.5 | 4.42 | 4.49 |
| 12 | 200 | 30 | 59.1 | 6.33 | 6.54 |
| 13 | 230 | 30 | 64.2 | 7.32 | 7.22 |

# Hydrothermal pretreatment optimization

- The second order polynomial regression model that was obtained in our study is described as:

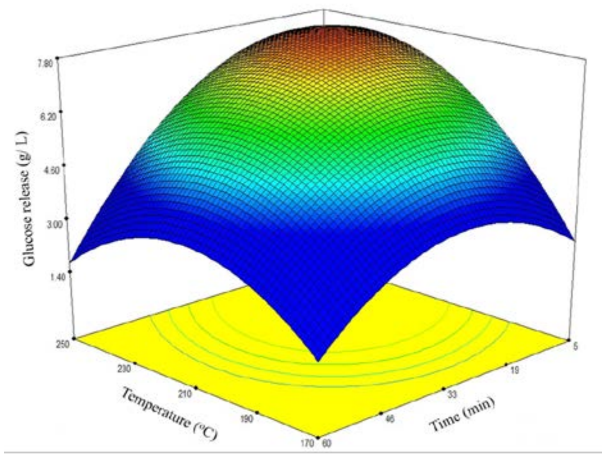
$$C_g=-58.63+0.552x_1+0.318x_2-0.001158x_1^2-0.002432x_2^2-0.001044x_1\,x_2$$

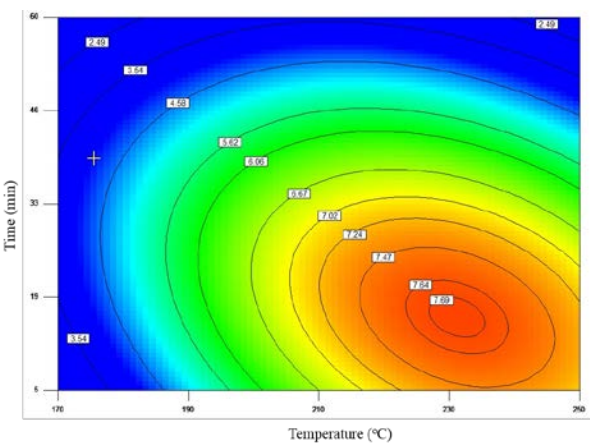
✓ Low probability p-value ($p<0.0001$)
✓ Coefficient of variation $R^2=0.92$

Optimum pair of pretreatment temperature and duration for maximum glucose release → **230 °C** & **15 min**

Maximum predicted glucose release → **7.71 g/L** after 8h

Experimental value →7.96 g/L



# Enzymatic hydrolysis at high solids loading

## Why high solids loading?

Higher sugars concentration → Higher ethanol concentration

Ability to achieve EtOH conc. above 4% (w/w)

## Why free fall mixer?

High solids content → Viscous slurry

Conventional stirring techniques → Poor mass-transfer

Gravimetric mixing → Increased enzymatic hydrolysis @ shorter time

# Liquefaction step

Solids loading → **24% (w/w)**

Enzyme solution → Cellic® CTec2 at **9 mg/g DM**

Conditions → **50$^{o}$C, pH: 5.0, 7 rpm**

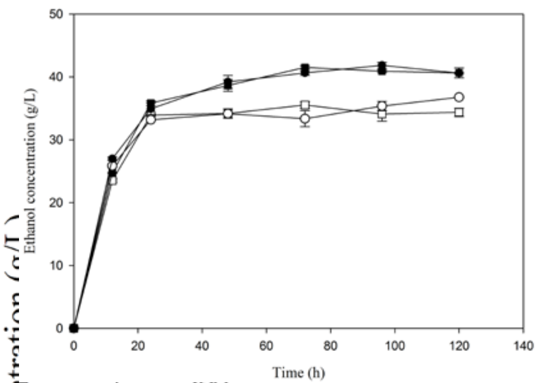
Duration → **12 or 24 h**




# Simultaneous Saccharification & Fermentation

In Erlenmeyer flasks

- 25 g liquefacted Corn Stover
- 15 mg/g DM yeast Ethanol Red® @ 35℃, 80 rpm




Effect of different liquefaction-saccharification conditions on ethanol production

Time course of ethanol production after 12 h of liquefaction without (o) and with (•) the addition of extra enzymes, and 24 h of liquefaction without (□) and with (□) the addition of extra enzymes.

| Saccharification conditions | | Ethanol production | | |
|---|---|---|---|---|
| **Duration (h)** | **Addition of extra enzymes** | **Concentration (g/L)** | **Yield (g/ 100 g DM)** | **Relative ethanol yield** |
| 12 | - | 35.54 | 11.25 | 43.6 |
| 12 | + | 41.53 | 13.15 | 50.9 |
| 24 | - | 36.77 | 11.64 | 45.1 |
| 24 | + | 41.85 | 13.25 | 51.3 |

# Conclusions

- ✓ Hydrothermal pretreatment with dilute acetic acid of corn stover was optimized by RSM.
- ✓ At optimal conditions (230 $^{o}$C, 15 min) 7.96 g/L of glucose were released after 8 h of hydrolysis which is very close to the predicted value of 7.71 g/L
- ✓ Gravimetric mixing enabled Corn stover's proper liquefaction at 24% (w/w) solids loading
- ✓ A 12 h liquefaction step was adequate for the efficient cellulose hydrolysis
- ✓ Liquefacted Corn Stover permitted submerged fermentation
- ✓ High ethanol concentration was achieved ( > 4% )

\+ Extra work need to be done towards a more efficient enzymatic hydrolysis, studying the effect of the addition of auxiliary enzymes, such as xylanases, ferulic acid esterases and lacasses.

# *Thank you for your attention!*

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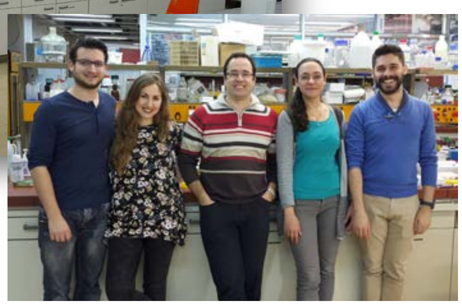

Ευρωπαϊκή Ένωση
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Περιφερειακής Ανάπτυξης

ΑΝΤΑΓΩΝΙΣΤΙΚΟΤΗΤΑ
ΕΠΙΧΕΙΡΗΜΑΤΙΚΟΤΗΤΑ
ΕΠΑΝ II
η περιφέρεια στο επίκεντρο της ανάπτυξης

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