National Technical University of Athens
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THESSALONIKI 2021

Bioethanol production from municipal source- separated biowaste

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WaysTUP!

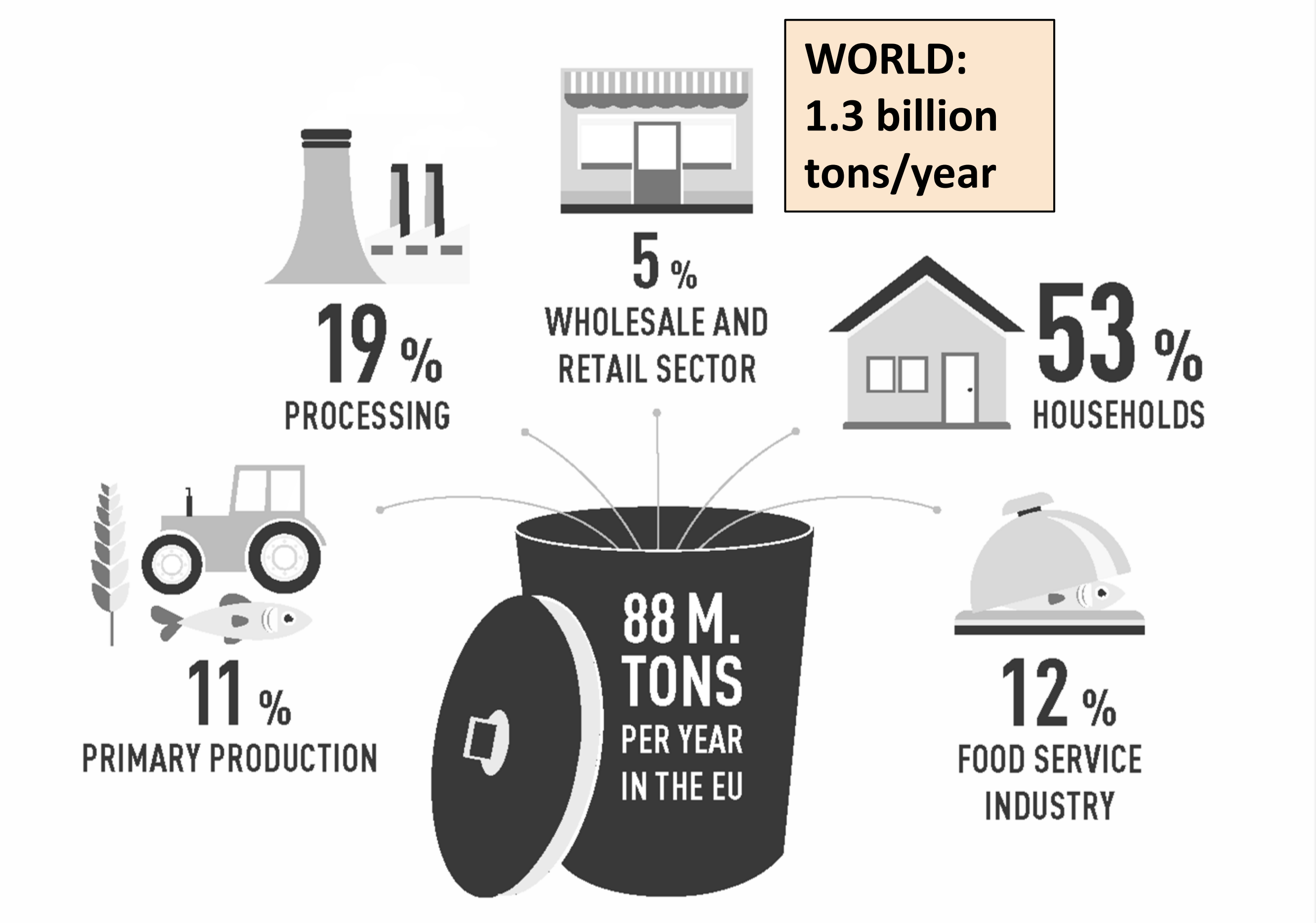
8th International Conference on Sustainable Solid Waste Management

Biowaste

the biodegradable garden and park waste, **food and kitchen** waste from households, restaurants, caterers and retail premises.

- Food Waste (FW) any food and inedible parts of food, removed from the food supply chain to be recovered or disposed (FUSIONS, 2016).

WORLD: 1.3 billion tons/year



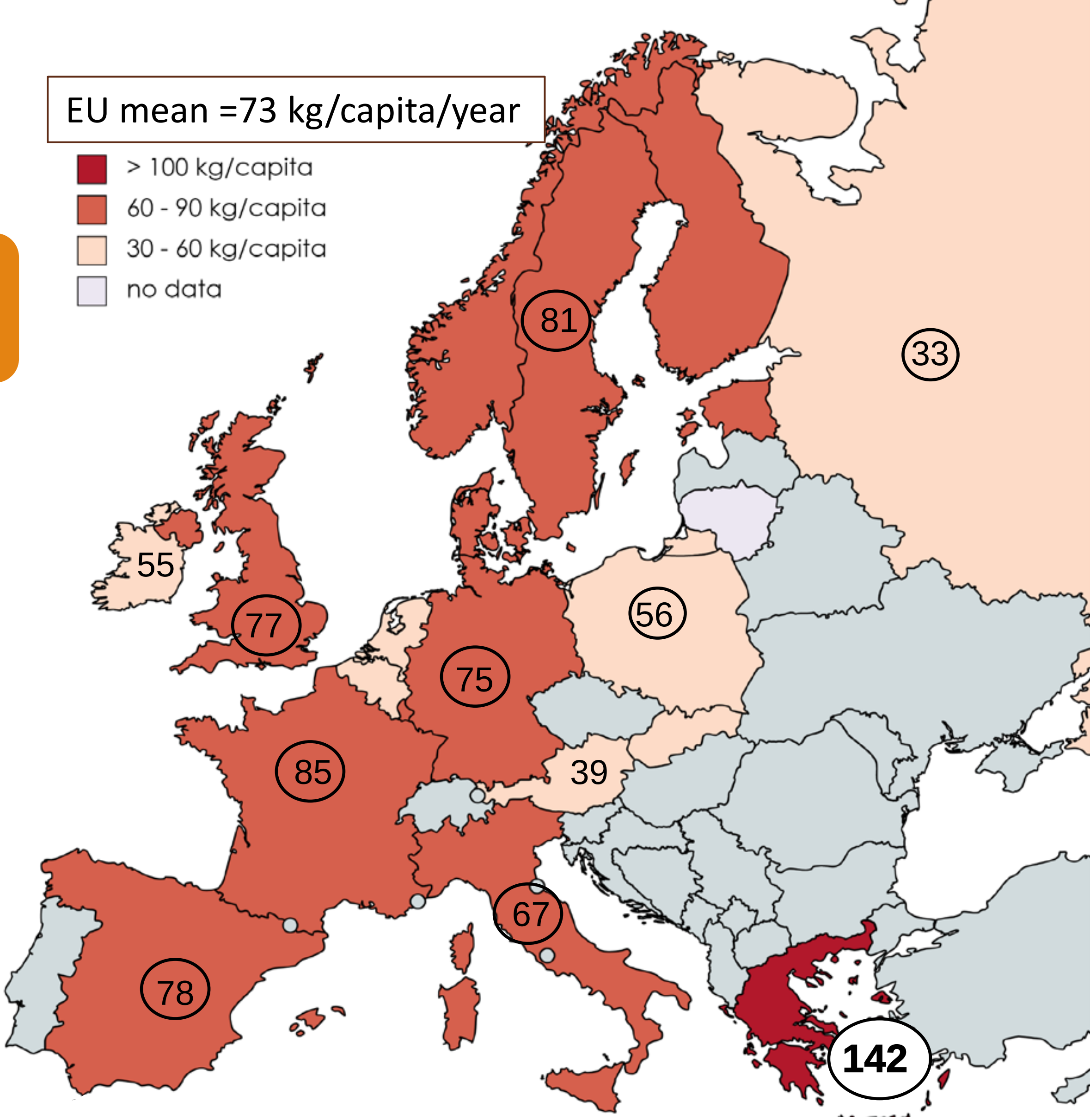
Source: FUSIONS 2016, Estimates of European food waste levels. http://www.eu-fusions.org/

UN sustainable targets 2030

- ✓ Halve per capita FW (retail, consumers)
- ✓ Reduce food loss (production, supply)
- ✓ Encourage revalorization of by-products from food processing & food waste

Renewable Energy Directive 2018/2001

- ✓ sustainability criteria of bioenergy
- ✓ 32% target for renewable energy (14% transport)
- ✓ Biofuels < biomass.
- ✓ Alternative sources than crops?



Source: UNEP Food Waste Index Report 2021, Household food waste in Europe.

Aim of the study

To assess the efficiency of **enzymatic formulations** (cellulase, amylase) on the hydrolysis of **Food Waste** and to **optimize bioethanol** production.

- ❑ How process parameters affect hydrolysis and fermentation?
- ❑ Which parameters and interactions have major effect?
- ❑ Possibility of readjusting process conditions to increase ethanol yield?

Materials & Methods (I)

Raw Material

- Food Waste (FW) from Vari- Voula- Vouliagmeni Municipality, Attica, Greece
- Moisture 9%, Physicochemical characterization

Parameter	Composition % w/w dry basis
Cellulose	18.05 ± 4.17
Hemicellulose	8.76 ± 2.97
Starch	7.86 ± 2.49
Acid insoluble residue	18.24 ± 4.07
Lignin acid soluble	1.37 ± 0.30
Lipids	10.64 ± 0.86
Ash	4.81 ± 1.62

Materials & Methods (II)

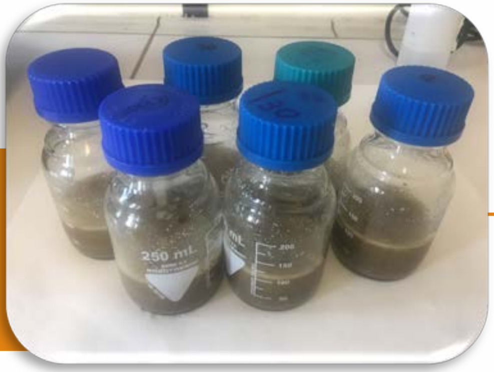
Physicochemical characterization

Centrifugation 3000 rpm

- ❑ Solid fraction
  - ▪ Total Solids (TRS)
  - ▪ Water Soluble Solids (WSS)
  - ▪ Volatile Solids (VS)
  - ▪ Cellulose
  - ▪ Hemicellulose
  - ▪ Starch
  - ▪ Soluble lignin (ASL)
  - ▪ Acid insoluble lignin (AIL)

- ❑ Liquid fraction
  - ▪ Sugars (Glucose, TRS)
  - ▪ Total Organic Carbon (TOC)
  - ▪ Total Nitrogen (TN)
  - ▪ Volatile Fatty Acids (VFAs)
  - ▪ Phenols
  - ▪ Ethanol

Enzymatic Hydrolysis-Saccharification



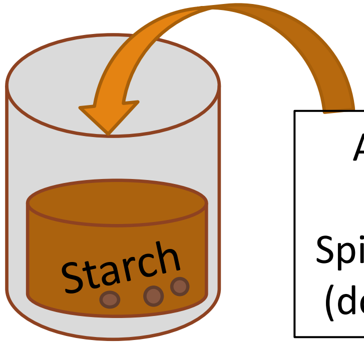
- Cellulase NS 87014 (Novozymes, DK) 333 FPU/ml
- Amylase Spirizyme Excel (Novozymes, DK) 2337 U/ml
- 10% w/w solids loading
- Incubation based on factorial design (T, time, enzyme dosage)

Fermentation

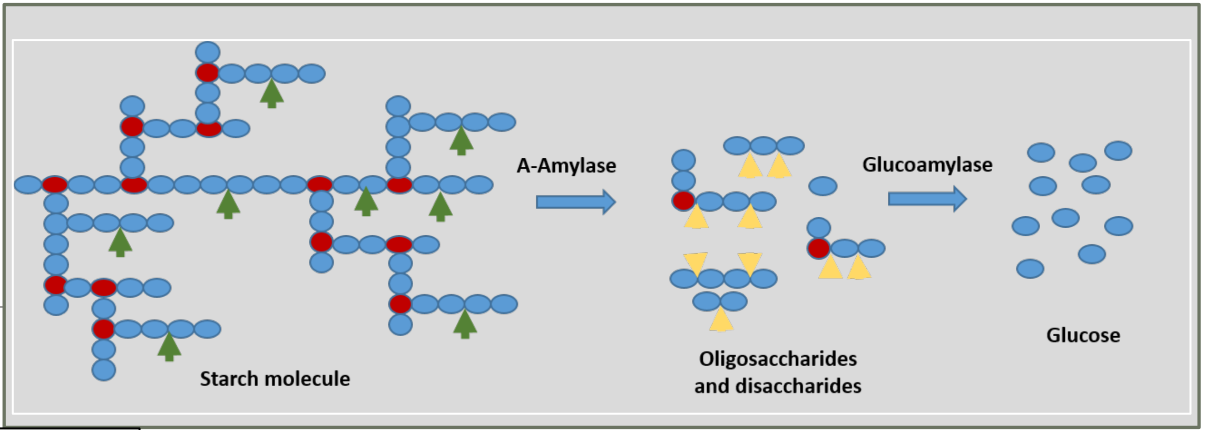
- 2% Saccharomyces cerevisiae (baker's yeast), 24h, 30^{o}C

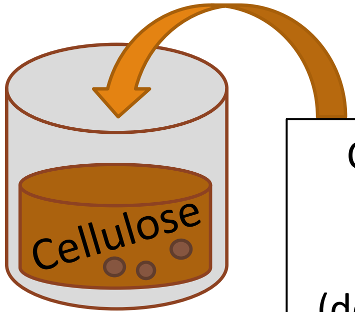
Materials & Methods (III)

Food Waste

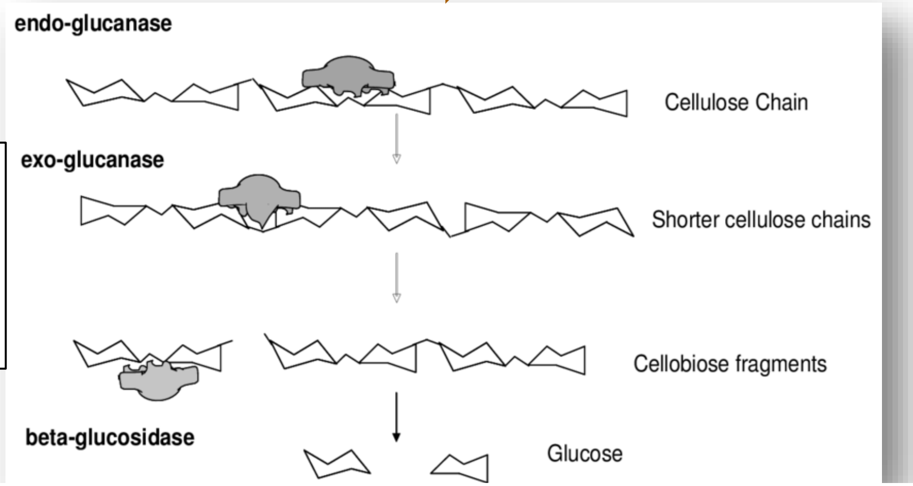


Amylolytic enzyme Spirizyme Excel (dose ,time, T)





Cellulolytic enzyme NS 87014 (dose, time, T)



Fermentation → Bioethanol

Materials & Methods (IV)

Factorial experiment 2^3

Optimization parameter: Ethanol Yield (%Y_E)

$$Y_E\% = \frac{EtOH\ (mg)}{Theoretical\ EtOH\ from\ the\ total\ conversion\ of\ carbohydartes} *100\%$$

Controlling parameters:

- ❑ Enzymatic hydrolysis time (h)
- ❑ Temperature of hydrolysis (°C)
- ❑ Enzyme dosage (μL/ g carbohydrate)

SET 1

Controlling Parameter	Variation intervals		
	level (-1)	level (0)	level (+1)
Time (h)	3	5	7
Temperature (°C)	35	50	65
NS 87014 (μL/g cellulose)	150	220	290

SET 2

Controlling Parameter	Variation intervals		
	level (-1)	level (0)	level (+1)
Time (h)	1	2	3
Temperature (°C)	35	50	65
Spyrizyme Excel (μL/g starch)	20	40	60

Results & Discussion

Set 1: Cellulase hydrolysis

	Enzyme (μL/g cellulose)	T_{hydr} (h)	T (°C)	%TS hydrolysis			% Cellulose degradation			% Hemicellulose degradation			% AIL degradation		
A1	220	5	50	48.50	±	0.65	62.91	±	11.37	10.96	±	2.47	34.20	±	4.08
A2	290	7	65	47.04	±	0.67	70.21	±	5.68	6.38	±	16.91	34.11	±	4.37
A3	290	7	35	52.20	±	0.62	74.00	±	1.85	36.54	±	16.55	33.25	±	16.79
A4	150	7	65	43.91	±	1.77	63.92	±	8.02	50.87	±	15.56	26.38	±	6.66
A5	150	7	35	48.04	±	1.02	58.99	±	7.32	72.67	±	16.17	33.58	±	0.86
A6	290	3	65	44.25	±	1.31	78.19	±	3.26	45.17	±	13.87	16.68	±	0.51
A7	290	3	35	43.43	±	1.15	62.41	±	0.78	48.32	±	5.99	9.35	±	12.34
A8	150	3	65	46.33	±	0.93	77.38	±	4.14	56.91	±	20.84	10.96	±	13.31
A9	150	3	35	45.38	±	1.83	67.27	±	16.20	65.05	±	9.89	12.53	±	16.32

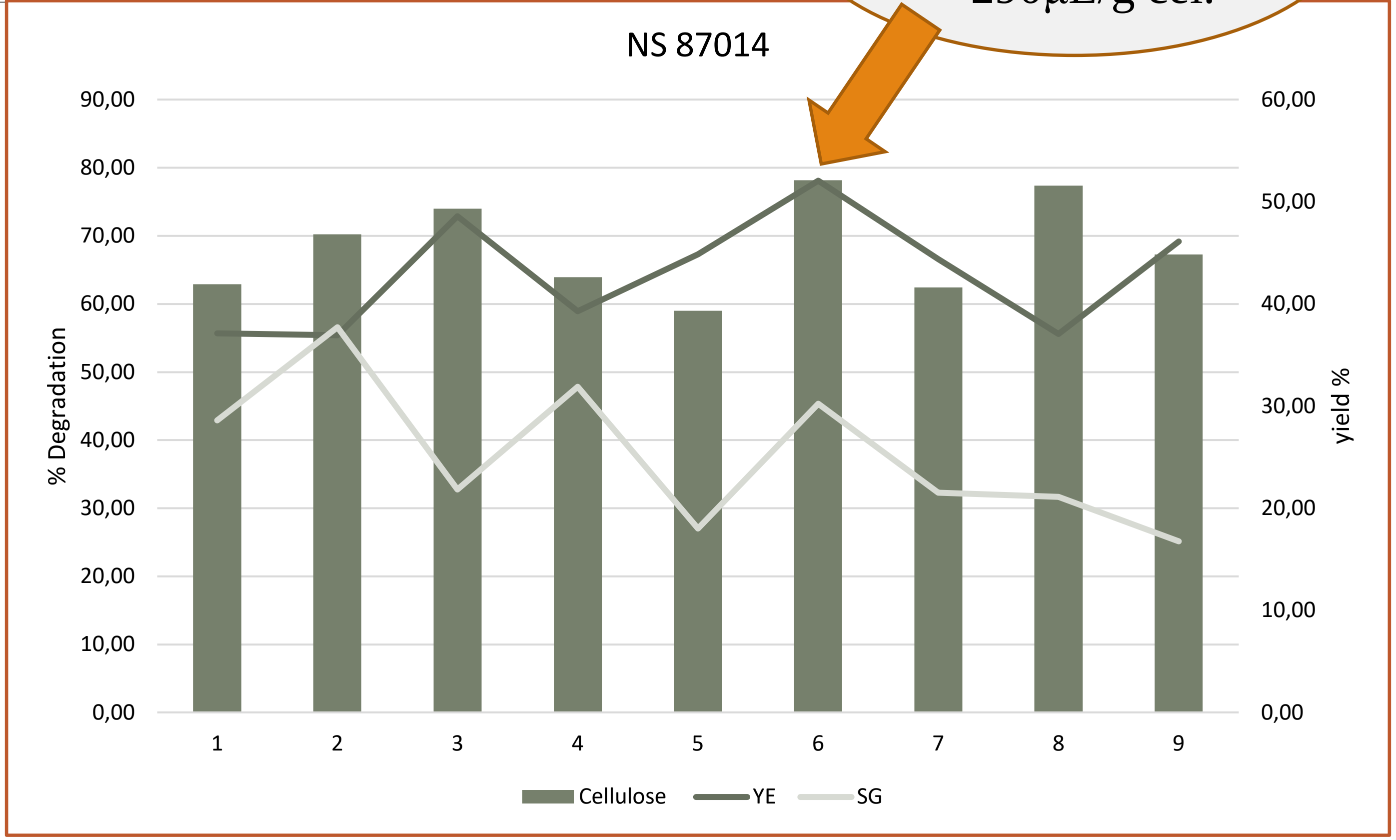
- ❑ Volatile fatty acids: 28.47-52.54 mg/g FW
- ❑ Phenolic compounds 0.48-0.87 mg/g FW

Set 1: Cellulase hydrolysis

	Enzyme (μL/g cellulose)	Thydr (h)	T (°C)
A1	220	5	50
A2	290	7	65
A3	290	7	35
A4	150	7	65
A5	150	7	35
A6	290	3	65
A7	290	3	35
A8	150	3	65
A9	150	3	35

- Glucose residual: 0.041-0.085 g/g
- Ethanol detected: 0.047- 0.066 g/g

Y_{EtOH} max= 52.05% at 3h, 65oC, 290μL/g cel.



Set 1: Cellulase hydrolysis

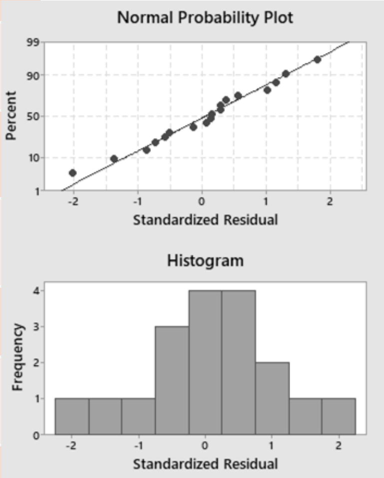
Y_{EtOH} = 108.3 + 0.323*enzyme – 9.89*t-1.594*T + 0,0571*enz*t +0.00804*enz*T +0.232*t*T – 0.0014 enz*t*

Statistical important parameters:

- Enzyme dosage ↑ ↑
- Temperature of hydrolysis ↑ EtOH yield ↓
- Time of hydrolysis ↑ ↓
- Interaction among 3 parameters significant

Optimum conditions: 290 μL/g cel, at **65°C, 3h**

Coefficient	Value	P-value (95%, a=0.05)
b0	44.29	0.00
b1 (enz)	1.84	0.117
b2 (time)	-1.24	0.274
b3 (Temp)	-2.33	0.045
b12	-1.49	0.195
b13	1.33	0.243
b23	-1.98	0.094
b123	**-2.85**	0.024



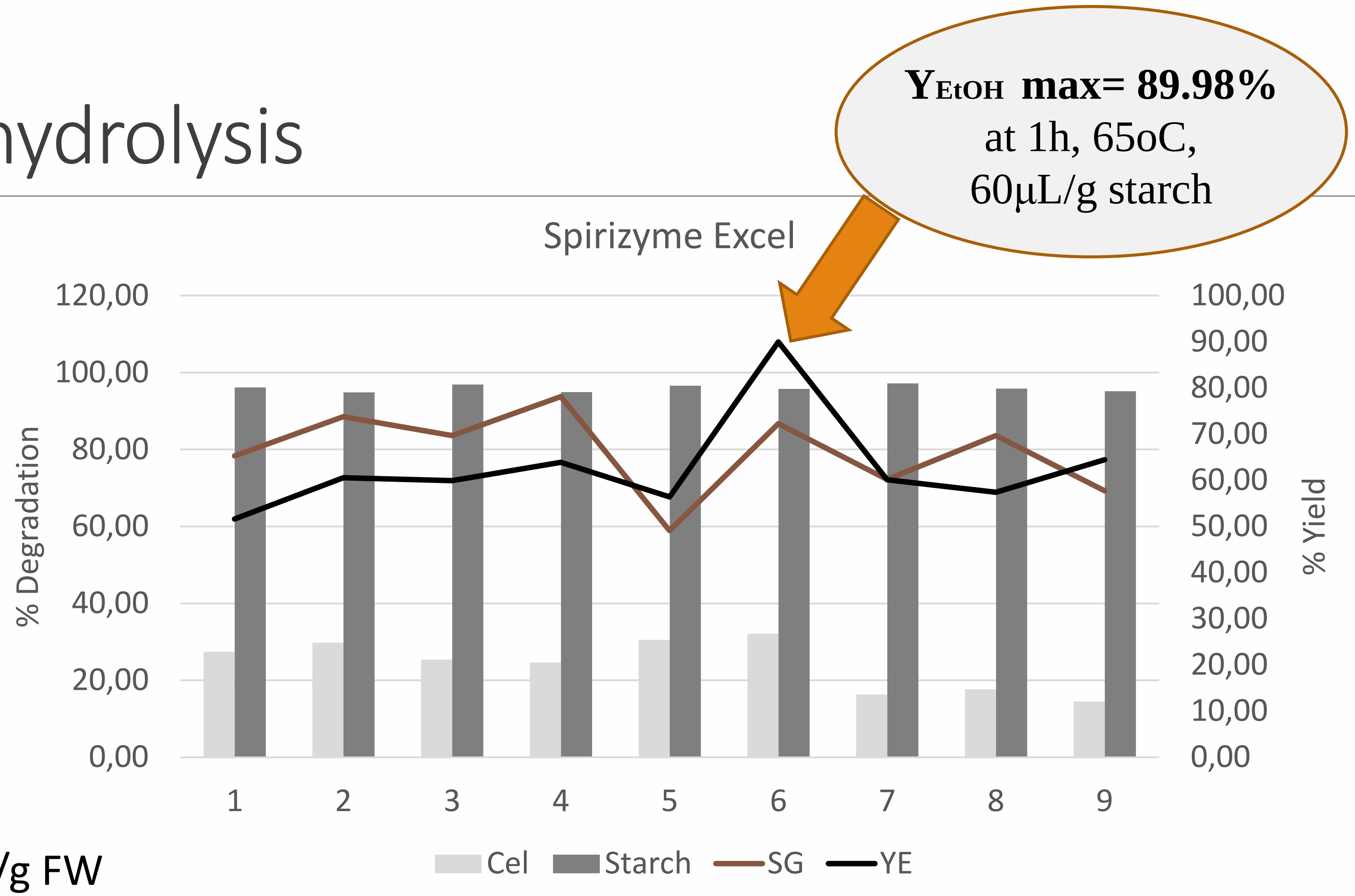
Set 2: Amylase hydrolysis

	Enzyme (μL/g cellulose)	T_{hydr} (h)	T (°C)	%TS hydrolysis			% Starch degradation			% Cellulose degradation			% AIL degradation		
A1	40	2	50	53.98	±	0.59	96.11	±	1.80	27.40	±	3.69	34.20	±	4.08
A2	60	3	65	56.45	±	1.32	94.86	±	0.89	29.73	±	1.17	34.11	±	4.37
A3	60	3	35	53.43	±	0.29	96.86	±	0.78	25.41	±	1.07	33.25	±	16.79
A4	20	3	65	54.98	±	1.74	94.89	±	1.20	24.61	±	0.28	26.38	±	6.66
A5	20	3	35	54.50	±	0.75	96.60	±	0.93	30.49	±	3.02	33.58	±	0.86
A6	60	1	65	56.65	±	1.74	95.74	±	0.17	32.11	±	8.95	16.68	±	0.51
A7	60	1	35	54.41	±	0.71	97.15	±	0.02	16.31	±	0.93	9.35	±	12.34
A8	20	1	65	53.58	±	3.11	95.86	±	0.67	17.68	±	1.35	10.96	±	13.31
A9	20	1	35	54.01	±	0.89	95.13	±	0.71	14.51	±	2.13	12.53	±	16.32

- Volatile fatty acids: 18.09-39.41 mg/g
- Phenolic compounds 0.64-1.13 mg/g FW

Set 2: Amylase hydrolysis

	Enzyme (μL/g cellulose)	Thydr (h)	T (°C)
A1	40	2	50
A2	60	3	65
A3	60	3	35
A4	20	3	65
A5	20	3	35
A6	60	1	65
A7	60	1	35
A8	20	1	65
A9	20	1	35

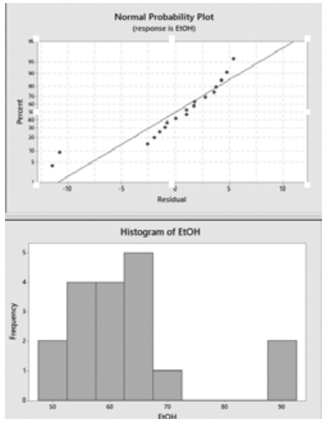


- Glucose residual: 0.151-0.229 g/g FW
- Ethanol detected: 0.076-0.136 g/g FW

Set 2: Amylase hydrolysis

Y_{EtOH} = 135.79 – 1.919*enz – 33.3*time – 1.7*T1 + 0.735*enz*time + 0.048*enz*T – 0.0182 *enz*time*T (a=0.05)

- Enzyme dosage ⇧ EtOH yield ⇧
- Time of hydrolysis ⇧ ⇩
- Temperature ⇧ ⇩
- Interactions significant



Optimum conditions: 60 μL/g starch, at **65°C, 1h**

Coefficient	Value	P-value (95%, a=0.05)
b0	64.09	0.00
b1	3.56	0.042
b2	-3.91	0.028
b3	-3.87	0.030
b12	-3.50	0.045
b13	3.77	0.033
b23	-1.82	0.261
b123	**-5.471**	0.005

b1–b13: <0.05

b123: <0.05

Conclusions

<u>Cellulase - NS 87014</u>

- ❑ Moderate cellulose hydrolysis (64.48% ± 8.53%)
- ❑ Ethanol yield = 42.97% ± 5.59%
  5-8 g/L
- ❑ Optimum conditions
  290μL /g cellulose, 3h, $65^{o}C$.
  Y_{EtOH} max= 52.05%
- ❑ Good performance compared to conventional cellulase mixtures

<u>Amylase - Spirizyme Excel</u>

- ❑ High starch hydrolysis (93.85% ± 3.57%).
- ❑ Additional cellulose degradation (23.71% ± 11.94)
- ❑ Ethanol yield (theoretical) 62.70% ± 10.95%
  9-16 g/L
- ❑ Optimum conditions 60μL /g starch, 1h, $65^{o}C$
  Y_{EtOH} max= 92.36%
- ❑ High efficiency at all conditions examined

- ✓ **Amylase** hydrolysis & fermentation proved <u>in all cases</u> much **better** performance on Y_{EtOH} than cellulase hydrolysis alone.
- ✓ Possibility of adapting amylase hydrolysis to optimize the overall process
- ✓ **FW** bioconversion to ethanol is a **viable valorization scheme**
- ✓ Steps towards **tackling food loss & compliance with sustainability criteria**

Thank you!