Impact of Wheat Straw Pretreatment Technologies on Sugar Yield and Succinic Acid Fermentation

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Aim

The study examined the effects of inhibitors, such as acetic acid and furfural, released with fermentative sugars from hemicellulose-lignin matrix after an acid catalyzed steam explosion (SE), on the production of succinic acid by *Actinobacillus succinogenes* through batch fermentation. Moreover, a comparative investigation was carried out on the effectiveness of pretreatment technologies (diluted acid (DA), organosolv (OS), and green solvent (GS) - specifically γ-valerolactone) on wheat straw biomass, focusing on cellulose recovery, hydrolysis efficiency, lignin removal, and preliminary lignin characterization. By comparing these methods, the study assessed the selective extraction efficiency and quantified monomeric, oligomeric, and degradation by-products. Additionally, this research is part of the Agritech, a project aimed to valorizing green wastes, and it provides insights into optimizing pretreatment strategies for improved biomass conversion and valorization of wheat straw.

Process set-up

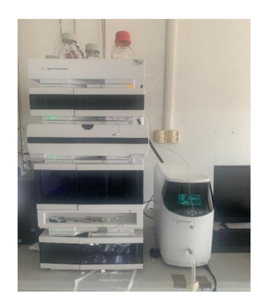
PRELIMINARY FERMENTATION SET-UP

- These tests were conducted on a hydrolysate of wheat straw treated by catalyzed steam explosion

- Inoculum 25% (v/v)
- Temperature 37°C
- 180 rpm (in the dark)
- TSB growth medium (CTL and IN tests)

- **Strain growth (OD value 600 nm)**

- **Sugars concentration (uHPLC-ELSD)*(Agilent p/n 685775-924)***
- **Organic acids concentration (uHPLC-DAD) *(Agilent p/n PL1170-6830)***



Tab. 1. Concentration of strain, sugars, acetic acid and furfural for fermentation tests

	Concentration (mg/l)									
	CTL-G-L	CTL-G/X-L	IN-AA-L	IN-AA/F-L	WS-L	CTL-G-H	CTL-G/X-H	IN-AA-H	IN-AA/F-H	WS-H
A. succinogenes	370	370	370	370	370	1100	1100	1100	1100	1100
D-(+)-Glucose	367	367	367	367	367	1130	1130	1130	1130	1130
D-(+)-Xylose	0	171	171	171	171	0	428	428	428	428
Acetic acid	0	0	21	21	21	0	0	52.5	52.5	52.5
Furfural	0	0	0	6	6	0	0	0	15	15

PRETREATMENT SET-UP

- Further there were done optimization of pretreatment steps by comparing several pre-treatments:

- Diluted acid pretreatment H_2SO_4 - concentration:
  - ❑ 0.2%
  - ❑ 0.6%
  - ❑ 1%
- Organosolv (H_2SO_4 1%/EtOH) 1:1- time:
  - ❑ 1h
  - ❑ 2h
  - ❑ 3h
- Green solvent (GVL/H_2O) - ratio:
  - ❑ 0.2
  - ❑ 0.5
  - ❑ 0.8

Set-up: steel vessel 50mL - 5% solids - 150°C - 100rpm

Steam explosion - 203°C 5' H_2SO_4 0,05M

Results

Tab. 2. Performance of fermentation process: inbihition rate of the strain growth, sugars consumption, concentration of succinic acid (SA), yield and productivity

	Growth inhibition rate (%)	Glucose consumption (%)	Xylose consumption (%)	SA concentration (g/l)	SA concentration Reduction (%)	Yield ($gr_{SA}/gr_{glucose}$)	Productivity at 24h (mg/(l*h))
CTL-G-L		31.9		0.20		55.6%	4.5
CTL/X-L		30.5	9.3	0.20		53.5%	4.3
IN-AA-L	29.5	23.7	-	0.10	53	25.7%	3.1
IN-AA/F-L	32.4	21.0	-	0.07	67	17.8%	2.1
WS-L	61.6	11.4	-	0.01	95	2.7%	0.3
CTL-G-H		51.3	-	0.52		47,3%	16.0
CTL-G/X-L		51.1	6.1	0.51		46,4%	16.3
IN-AA-H	33.5	25.2	4.2	0.36	30	32,9%	11.5
IN-AA/F-H	41.2	23.9	5.4	0.20	62	17,8%	6.7
WS-H	58.7	19.5	3.7	0.09	83	8,2%	3.4

- ❖ The effect of the inhibitory compounds both individually for acetic acid and together with furfural was evident at all concentrations tested;
- ❖ Better results in terms of succinic acid concentration were obtained from a higher initial strain and sugar concentration;
- ❖ The higher initial strain concentration seemed to have better counteracted the presence of inhibitor when straw hydrolysate was used as the sole source of sugars

Tab. 3 Analysis of hemicellulose liquid fraction and determination of internal lignocellulosic composition of pretreated wheat straw

	Liquid fraction				Solid residue		
Wheat straw treatment	Xylose recovery [a] %	xylooligomer [b] %	acetic/ xylose %	Furans/ xylose %	Cellulose content (%)	Xylan content (%)	Acid insoluble lignin content (%)
Raw Material	/	/	/	/	34.2	18.9	23.5
ACSE	87.5	5.7	13.6	6.2	53.2	2.1	32.5
DA 0.2%	51.1	20.1	19.0	4.5	40.8	11.1	28.5
DA 0.6%	62.7	0.8	19.6	3.5	39.2	9.3	34.0
DA 1%	42.8	0	41.6	7.2	45.7	1.6	33.4
OS EtOH 1h	44.7	61.0	27.3	5.2	53.3	6.3	21.2
OS EtOH 2h	53.8	65.1	23.3	4.2	53.7	6.8	19.9
OS EtOH 3h	44.1	71.2	25.6	5.3	57.8	6.2	22.3
GVL 20%	nd	nd	nd	nd	35.2	16.1	22.9
GVL 50%	nd	nd	nd	nd	40.9	18.8	21.3
GVL 80%	nd	nd	nd	nd	39.1	18.9	20.5

(a) with respect to xylose in the raw material; (b) with respect to the total xylose recovery

ENZYMATIC HYDROLYSIS PROCESS

2% WIS; 50°C; pH 4.8; Cellic® Ctec2 15FPU/g; 150rpm; 72h

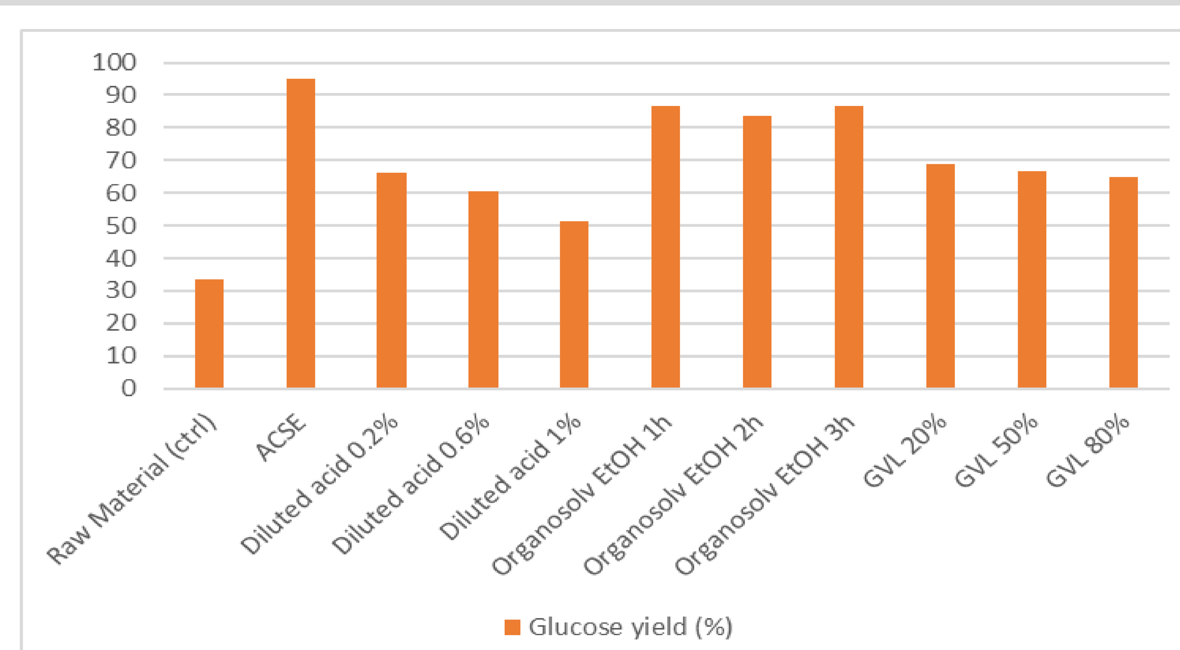


Fig. 1. Determination of saccharification yields at the end of processes

Conclusion

- ✓ The synergistic effect of acetic acid and furfural has been shown to inhibit bacterial growth and succinic acid production. Despite the poor fermentability of wheat straw hydrolysate, succinic acid was obtained at $9*10^{-2} \pm 7*10^{-3}$ g/L from glucose and xylose in hydrolysate at concentrations of 1.1 g/L and 0.4 g/L.
- ✓ The highest xylose recovery in the liquid fraction was observed with the ACSE treatment (87.5%), followed by DA treatments, with the highest recovery at 0.6% DA (62.7%). OS-EtOH treatments achieved moderate xylose recovery, primarily in oligomeric form. GVL eluted with sugars, and residual xylan analysis indicated poor recovery, with increased degradation byproducts observed due to the DA 1% treatment. In terms of solid residue, the highest cellulose content was found after the OS-2h treatment (57.8%). SE steam explosion achieved nearly quantitative enzymatic hydrolysis yield, whereas the DA treatment demonstrated poorer performance.

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