Detoxification strategy of wheat straw hemicellulosic hydrolysate an approach for cultivating *Trichoderma reesei*

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15.06.2022

Outline

Introduction

Methodology

Detoxification stages

Determination of toxicity thresholds

Cultivation of *T. reesei*

Results

Conclusions

Wheat production 2021:
778.6 Millions metric tons

Wheat straw
Approx. 1.13 ton/ton grain

Hemicellulose

Hydrolysate
Liquid Hot Water

Recalcitrance!

Pretreatment
Detoxification

Proof of concept

Trichoderma reesei

Methodology

1. Detoxification stages

Finding conditions

Simulation software Aspen Plus V10

Evaporation synthetic hydrolysate (SH) / wheat straw hydrolysate (WSH)

Removal of acetic acid with pH modification (SH and WSH) + evaporation

Evaporation of WSH with pH modification

2. Determination of toxicity thresholds for *T. reesei*

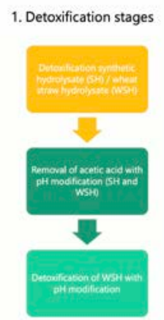
Culture medium:
Macro and micronutrients
Glucose as carbon source (70 g/L)

+
Acetic acid
Furfural
HMF
(individual analysis)

% reduction in produced biomass compared to the control

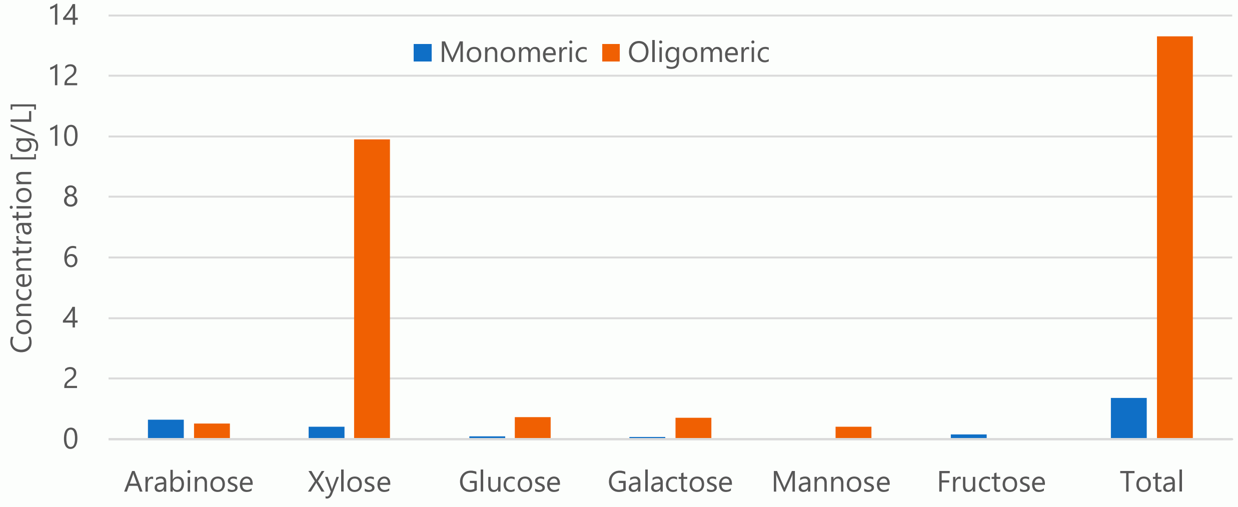
3. *T. reesei* cultivation in detoxified media

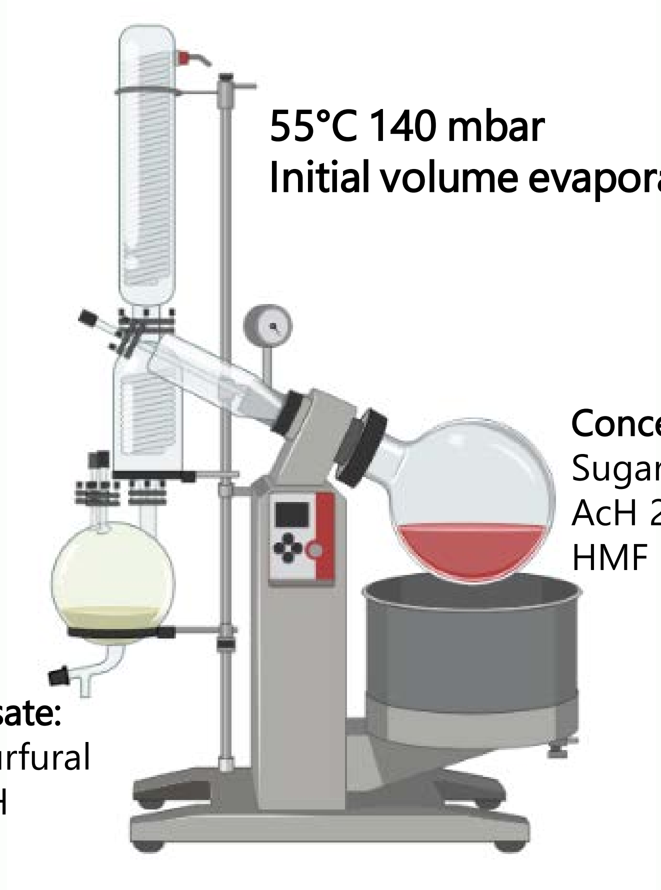
Carbon sources (approx. 30 g/L):
Glucose (control)
Pre-treated SH and WSH



Biomass yield (g/g total sugars at the beginning)

Fig 2. Monomeric and oligomeric sugars in wheat straw hydrolysate





55°C 140 mbar
Initial volume evaporated: 66.7%

Concentration factors:
Sugars 3.1 times
AcH 2.5 times
HMF 5 times

Condensate:
100 % furfural
30% AcH

Table 1. Detoxification of synthetic hydrolysate without pH modification

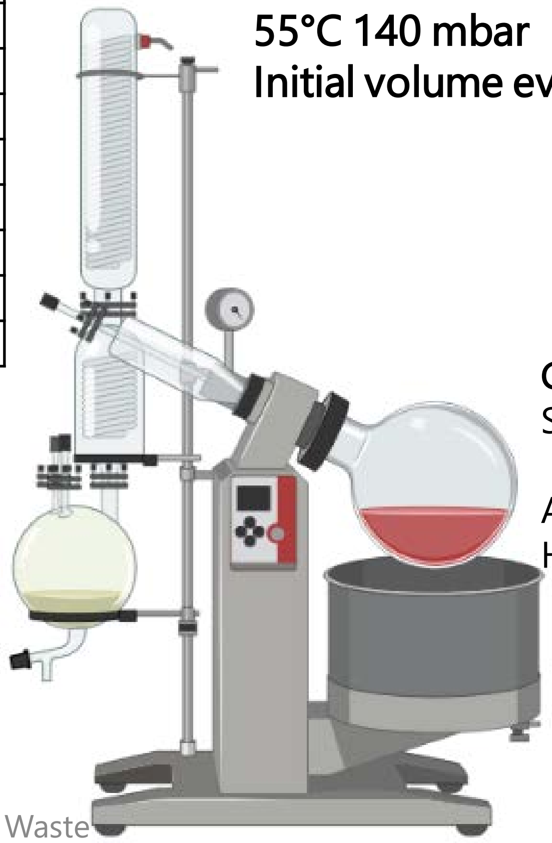
Compounds	**Concentration [g/L]**		
	Synthetic Hydrolysate	**Concentrate**	**Condensate**
Glucose	0.24	7.40±0.39	N.D
Xylose	0.85	26.64±1.43	N.D
Acetic acid (AcH)	1.1	2.71±0.12	0.58 ±0.048
Furfural	0.15	N.D	0.27 ± 0.022
HMF	0.006	0.030±0.002	N.D

 Stage 1: preliminary detoxifications using wheat straw hydrolysate (WSH)

Table 2. Detoxification of wheat straw hydrolysate without pH modification

Compounds	Concentration [g/L]		
	Wheat straw Hydrolysate	Concentrate	Condensate
Total glucose	1.74	6.22 ± 0.47	N.D
Total galactose	0.62	2.78 ± 0.13	N.D
Total mannose	0.27	1.95 ± 0.14	N.D
Total fructose	0.20	1.25 ±0.039	N.D
Total xylose	8.97	31.80 ± 2.33	N.D
Total arabinose	0.97	3.99 ± 0.29	N.D
Acetic acid	1.40	3.50 ± 0.10	0.46 ± 0.11
Furfural	0.41	N.D	0.44 ± 0.09
HMF	0.021	0.086 ± 0.004.5	N.D

55°C 140 mbar
Initial volume evaporated: 70.6%



Concentration factors:
Sugars 3.5 times
47.9 g/L total
AcH 2.5 times
HMF 4 times

- Similar trend than synthetic hydrolysate
- Fructose is degraded
- Limited removal of acetic acid

Condensate:
100 % furfural
25% AcH

Results detoxification. Stage 2: removal of acetic acid from SH with pH modification

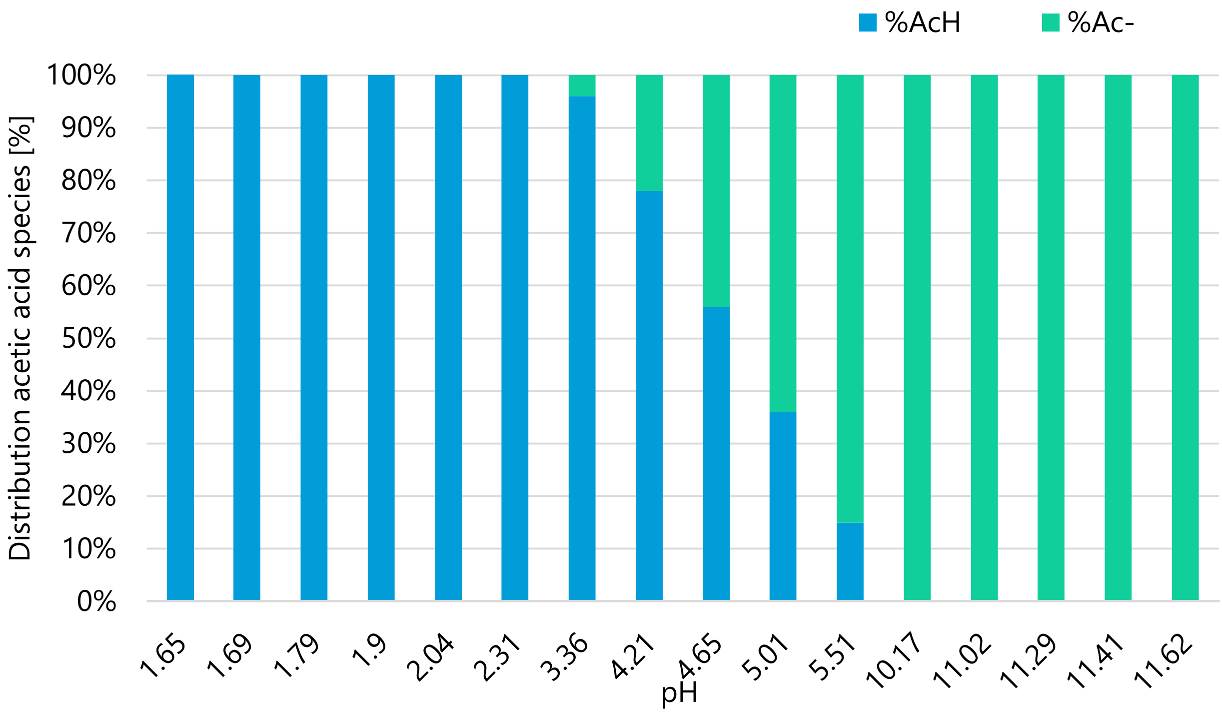


Fig 3. Acetic acid (AcH) and acetate (Ac-) equilibrium in a synthetic hydrolysate.

Table 3. Detoxification of synthetic hydrolysate with alkaline and acid addition

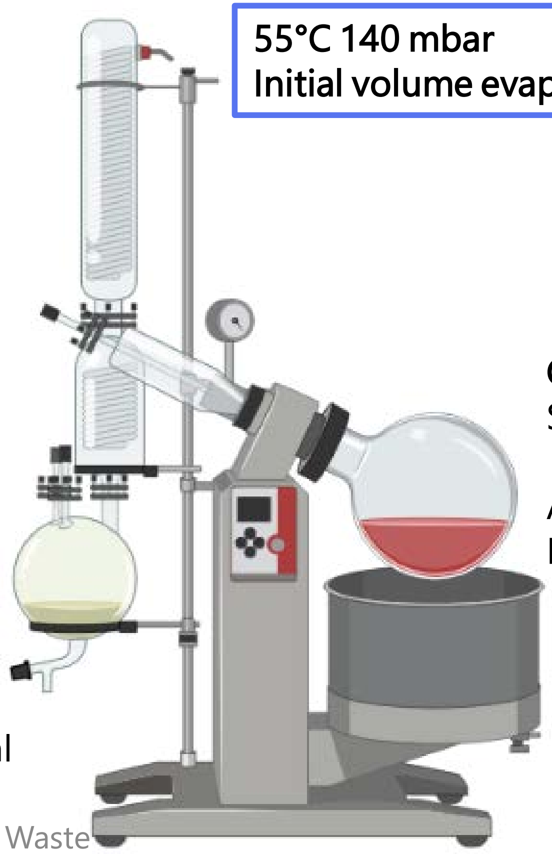
Feed				
Feature	**Units**	**SH + H_2SO_4**	**SH + NaOH $_{(1)}$**	**SH + NaOH $_{(2)}$**
pH	-	2.00	4.66	5.46
Total AcH	[mg/L]	1.46	1.46	1.46
AcH	[mg/L]	1.44	0.81	0.24
Ac^-	[mg/L]	0.02	0.65	1.22
% AcH	%	100	56	17
Concentrate				
Total volume evaporated	%	71.2	63.7	73.0
Final pH	-	1.60	4.93	5.74
Concentration total AcH	[mg/L]	2.79	3.54	5.06
AcH	[mg/L]	2.78	1.43	0.48
Ac^-	[mg/L]	0.01	2.11	4.58
Acetic acid removed	%	44.8	22.2	6.5
Concentration factor AcH	times	1.9	2.4	3.5
Concentration factor HMF	times	3.7	3.4	4.0

Results detoxification. Stage 3: detoxification of WSH with pH modification

Table 4. Detoxification of acidified wheat straw hydrolysate

Compounds	Concentration [mg/L]			Mass distribution after treatment [%]	
	Wheat straw Hydrolysate	Concentrate	Condensate	Concentrate	Condensate
Total glucose	1.89	20.01 ± 7.1	N.D	100	0
Total galactose	0.91	9.83 ± 3.6	0.017± 0.010	98.2	1.8
Total mannose	0.53	5.58 ± 1.88	N.D	100*	0
Total fructose	0.11	0.68 ± 0.08	N.D	66.2	0
Total xylose	11.72	117.1 ± 3.64	N.D	100	0
Total arabinose	1.36	14.44 ± 4.80	N.D	100	0
Acetic acid	1.74	7.45 ± 1.79	1.20 ± 0.014	44.1	55.9
Furfural	0.41	0.012 ± 0.006	0.38 ± 0.08	0.3	99.7
HMF	0.03	0.34 ± 0.14	N.D	100	0

55°C 140 mbar
Initial volume evaporated: 87.7 %



Concentration factors:
Sugars 10 times
167 g/L
AcH 4.2 times
HMF 13 times

Condensate:
99.7 % furfural
55.9 % AcH

Results. Determination of toxicity thresholds for *T. reesei*

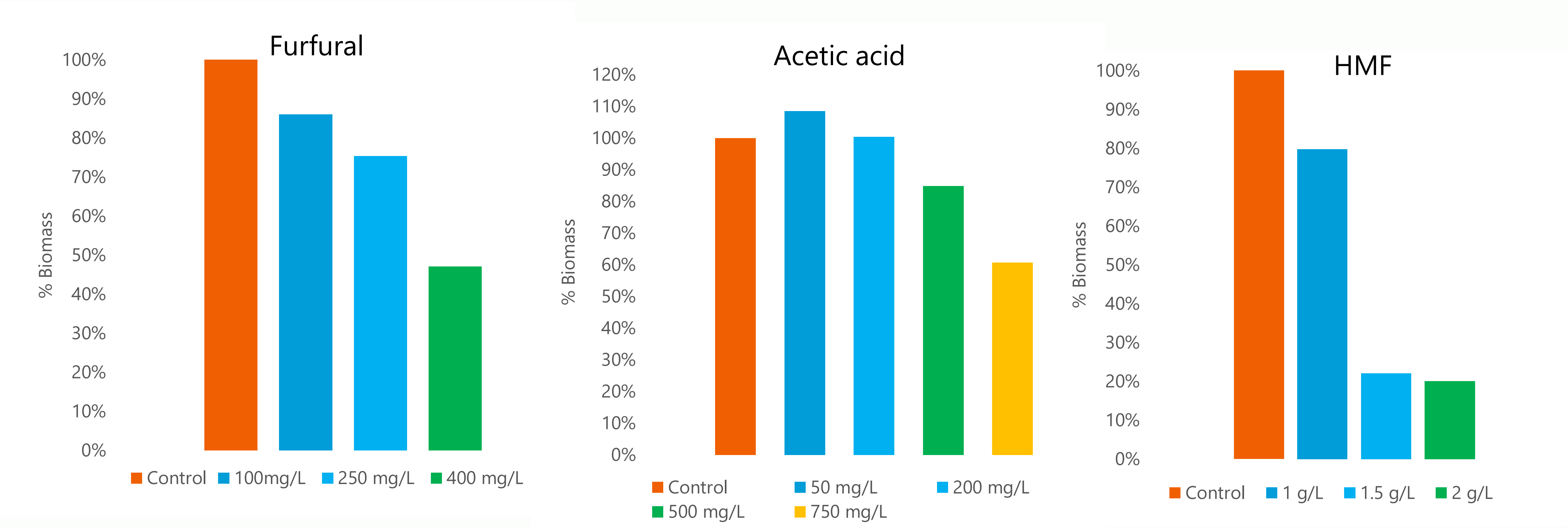


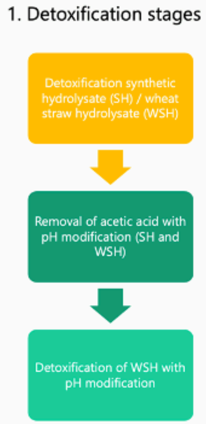
Fig 4. Toxicity thresholds of a) furfural, b) acetic acid, and c) HMF.

- Furfural is the most harmful degradation product
- Acetic acid in low concentrations can be metabolized
- Detoxified wheat straw hydrolysate contain safe concentrations of furans.

Results. *T. reesei* cultivation in detoxified media

3. *T. reesei* cultivation in detoxified media

Carbon sources (approx. 30 g/L):
Glucose (control)
Pre-treated SH and WSH



Biomass yield (g/g total sugars at the beginning)

Table 5. Bioma

Culture media	HMF [g/L]		Biomass	
	T_0	T_f	T_f [g]	Yield [g/g]*
Control glucose	0	0	0.297	0.430
SH	026	0.01	0.014	0.017
SH + NaOH (2)	041	0	0.032	0.050
SH + H_2SO_4	045	0	0.045	0.078
WSH	043	0	0.028	0.035
WSH + H_2SO_4	**036**	**0**	**0.230**	**0.420**

Table 5. Biomass cultivation in different culture media

Culture media	Evap Vol. %	Initial sugars [g/L]	Sugars consumption %	Acetic acid [g/L]		HMF [g/L]		Biomass	
				T_0	T_f	T_0	T_f	T_f [g]	Yield [g/g]*
Control glucose	0	28	98.1%	0	0	0	0	0.297	0.430
SH	approx. 63-73	32	0.0%	2.4	2.0	0.026	0.01	0.014	0.017
SH + NaOH (2)		26	4.4%	2.6	0.1	0.041	0	0.032	0.050
SH + H_2SO_4		23	34.0%	2.4	1.7	0.045	0	0.045	0.078
WSH		32	2.1%	2.2	1.5	0.043	0	0.028	0.035
WSH + H_2SO_4	**approx. 88**	**22**	**60.3%**	**0.9**	**0**	**0.036**	**0**	**0.230**	**0.420**

AcH effect (AcH and Ac^-) over biomass production

Conclusions

•Wheat straw hydrolysate was detoxified, removing 99.7% furfural and 55.9% of acetic acid without sugar losses.

•Furfural in concentrations above 250 mg/L can cause severe inhibition in the cultivation of *T. reesei.*

•Acetic acid is produced in wheat straw hydrolysate at levels capable of impairing the production of *T. reesei* biomass.

•The acidification of wheat straw hydrolysis improved the removal of acetic acid and increased the production of biomass, reaching up to 98% of the yields in control.

Outlook...
Detoxified wheat straw hydrolysate can be used as a substrate for cultivating *T. reesei.* Further research is required towards the identification of possible products like enzymes.

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Thank you for you attention!

Questions, comments?

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