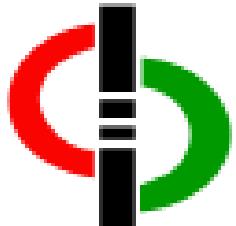


Bio-delignification and consolidated bioprocessing of Napier grass (*Pennisetum purpureum*) for bioethanol production by filamentous fungi

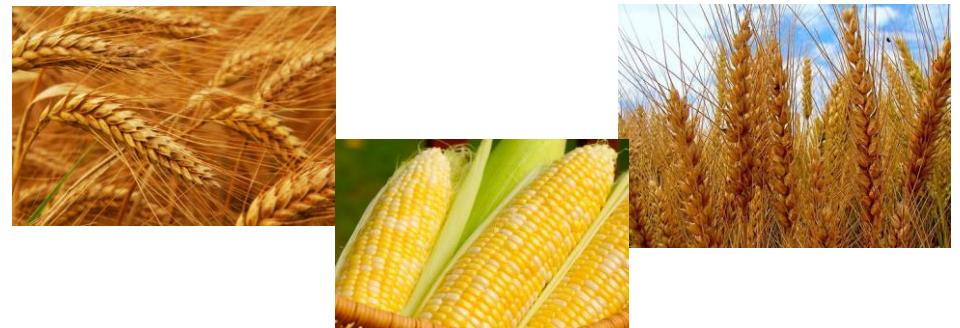


Marco Lao
MS Energy Engineering

BACKGROUND

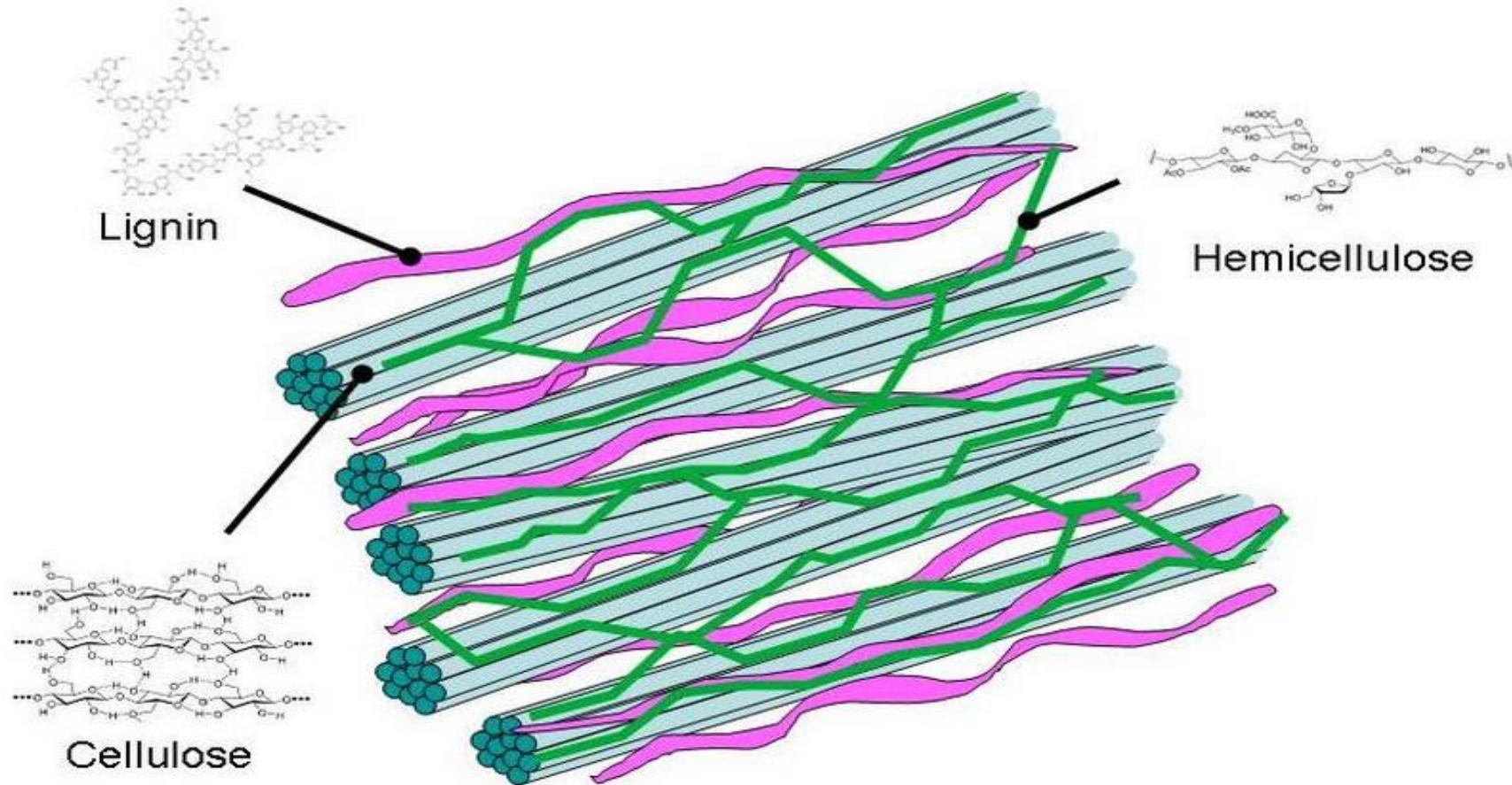
TYPES OF FEEDSTOCK

- Sucrose-containing
- Starchy materials
- Lignocellulosics



<http://www.juntadeandalucia.es/agriculturaypesca/raif/reglamentos/reglamentoPI.html>
<http://auto.howstuffworks.com/fuel-efficiency/biofuels/sweet-sorghum-fuel.htm>
<http://www.livestrong.com/article/506695-side-effects-of-sugar-cane-extract/>
<http://www.nebraskacom.org/issues-initiatives/your-food/field-corn-vs-food-corn/>
<http://www.weightzonefactor.com/blog/why-whole-wheat-bread-is-not-healthier-than-white/>
<http://www.foodify.com/barley.html>
[http://rachel.golearn.us/modules/en-infonet/export/default\\$ct295\\$biodiversity.html](http://rachel.golearn.us/modules/en-infonet/export/default$ct295$biodiversity.html)
<http://www.mtnsideview.com/howto/h2index.php>
<http://biothekeologic.com/energy-crops-for-biofuels/?lang=en>
<http://cellulose.org/GreenestInsulation/2015/08/25/cellulose-insulation-can-help-epa-landfill-reduction-plan/>

LIGNOCELLULOSE



Typical Biomass Composition

Table 1

The contents of cellulose, hemicellulose, and lignin in common agricultural residues and wastes^a

Lignocellulosic materials	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hardwoods stems	40–55	24–40	18–25
Softwood stems	45–50	25–35	25–35
Nut shells	25–30	25–30	30–40
Corn cobs	45	35	15
Grasses	25–40	35–50	10–30
Paper	85–99	0	0–15
Wheat straw	30	50	15
Sorted refuse	60	20	20
Leaves	15–20	80–85	0
Cotton seed hairs	80–95	5–20	0
Newspaper	40–55	25–40	18–30
Waste papers from chemical pulps	60–70	10–20	5–10
Primary wastewater solids	8–15	NA ^b	24–29
Swine waste	6.0	28	NA ^b
Solid cattle manure	1.6–4.7	1.4–3.3	2.7–5.7
Coastal Bermuda grass	25	35.7	6.4
Switch grass	45	31.4	12.0

^a Source: Reshamwala et al. (1995), Cheung and Anderson (1997), Boopathy (1998) and Dewes and Hünsche (1998).

^b NA – not available.

NAPIER GRASS

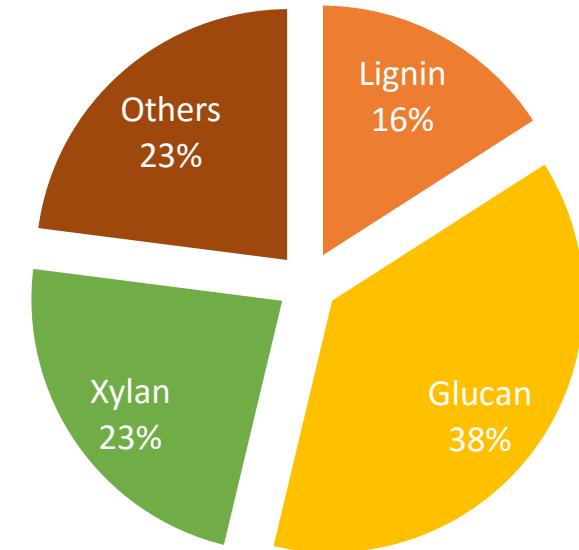
- Perennial tropical grass
- Low water and nutrient requirements
- High dry mass yield (45 tons/ha-yr)*



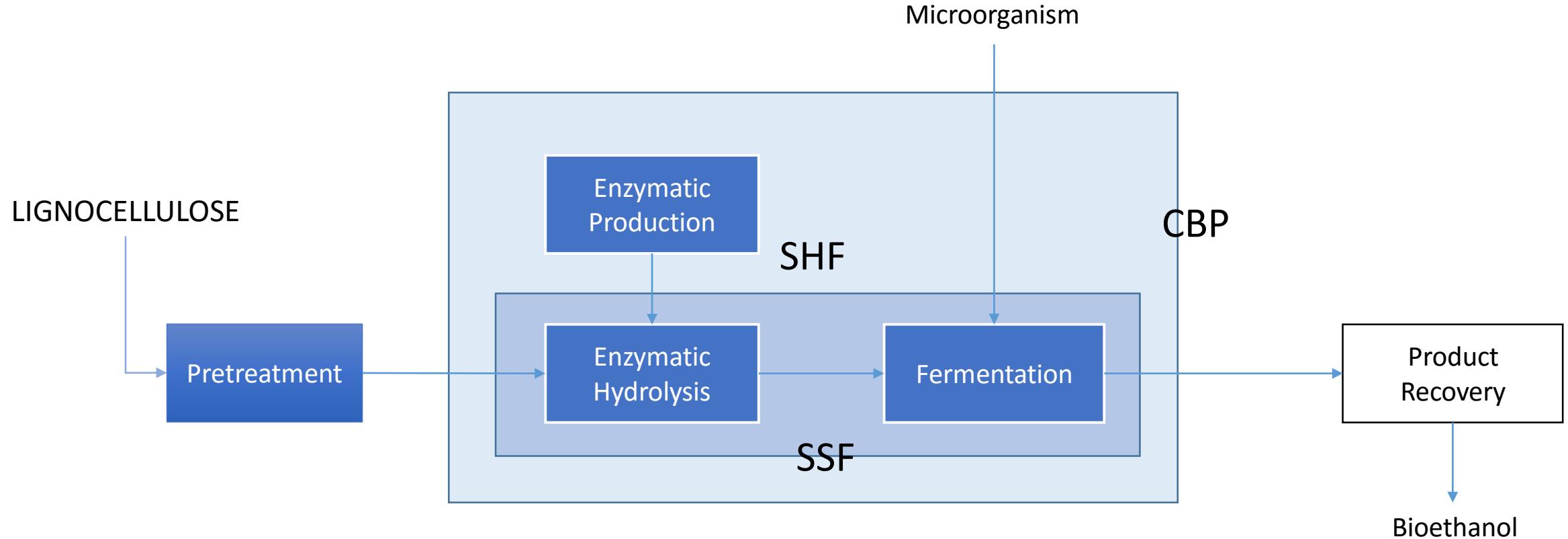
<http://www.bbsrc.ac.uk/engagement/exhibitions/gb-bioscience-festival/food-environment-energy-grass-secures-future/>



% Composition of extractive-free Napier grass



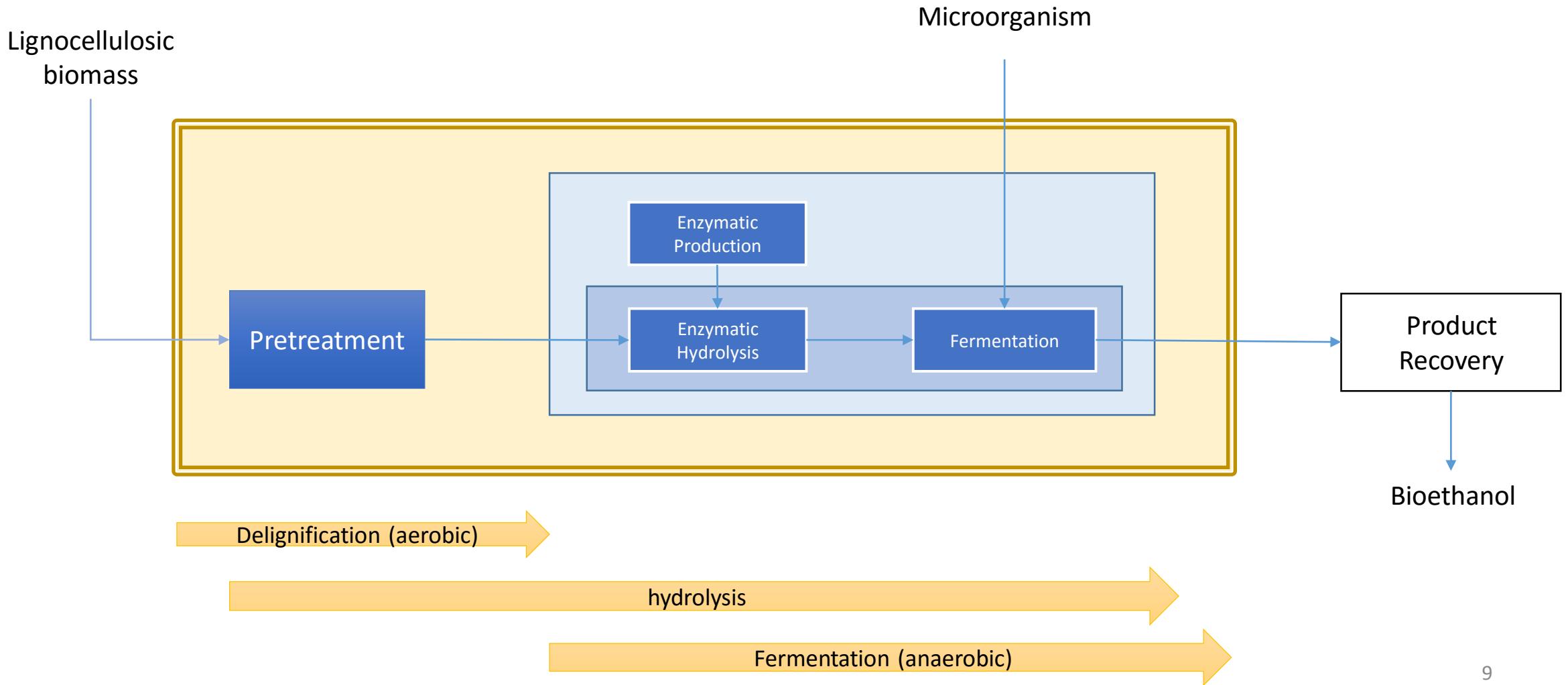
Ethanol Production from Lignocellulose



Consolidated Bioprocessing (CBP)

- Low cost
- No need for ex-situ enzyme production
- Reduced diversion of substrate
- More compatible enzyme and fermentation system
- Candidates: *Fusarium*, *Trichoderma*, *Aspergillus*, *Rhizopus*, *Neurospora*,
Monilia, White rot fungi

Bio-delignification and CBP



OBJECTIVES

Produce ethanol directly from Napier grass
(Pennisetum purpureum) via bio-delignification and
consolidated bioprocessing

1

Screen locally available
filamentous fungi



2

Investigate what are the
necessary conditions for
the process



3

Produce ethanol at high
productivities



METHODOLOGY

Research Roadmap

Screening of Filamentous Fungi

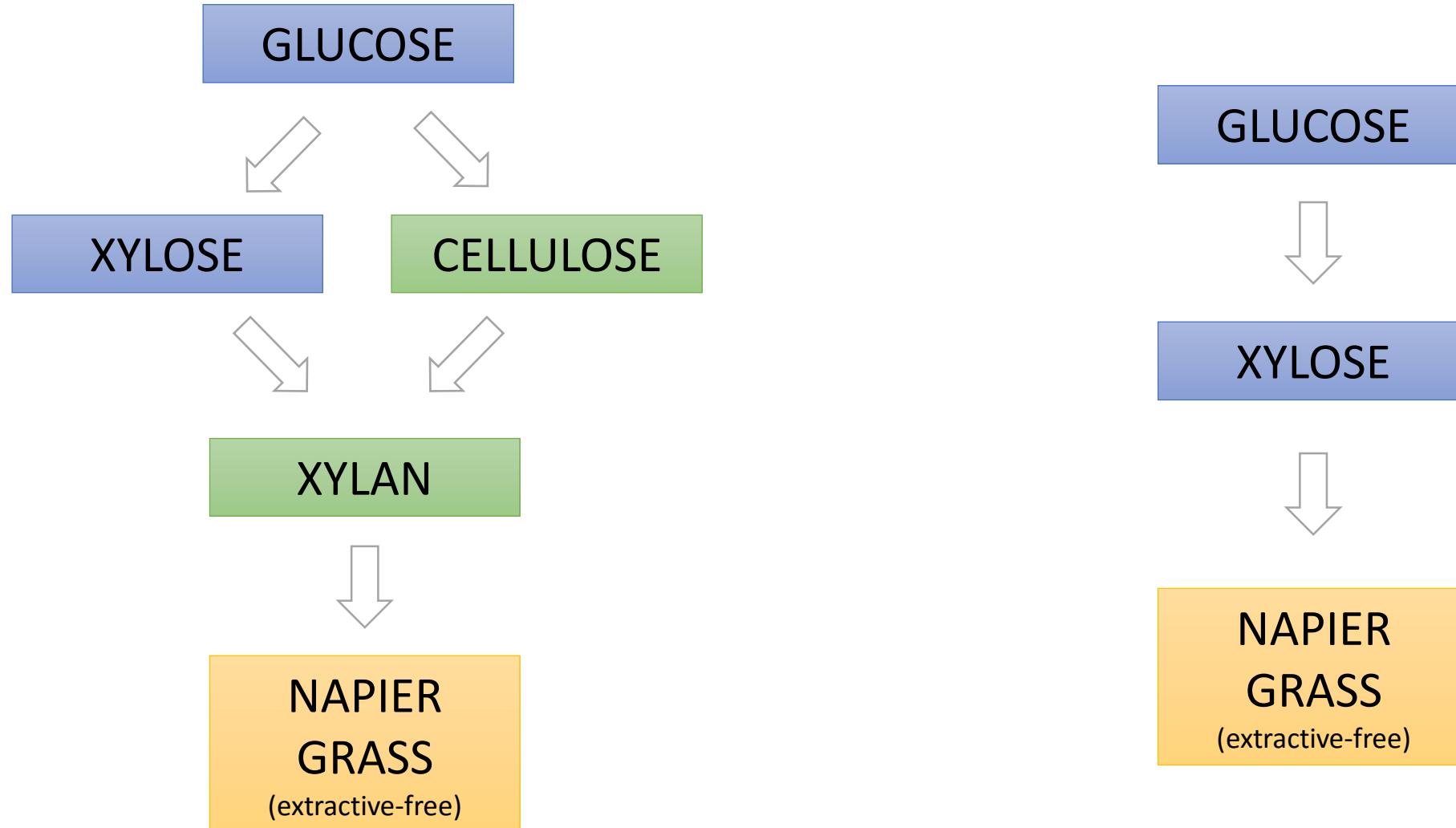


Time Profile Bio-delignification & CBP

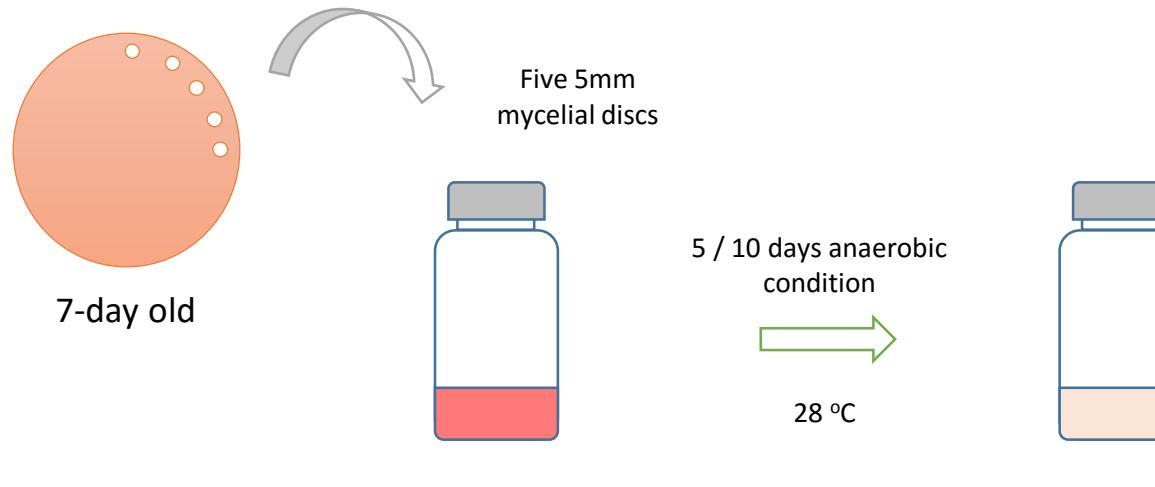


Process Intensification

Screening of ethanol-producing filamentous fungi

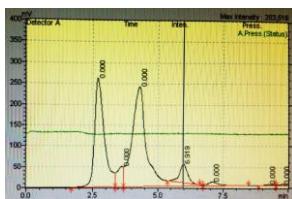


Screening using glucose and xylose



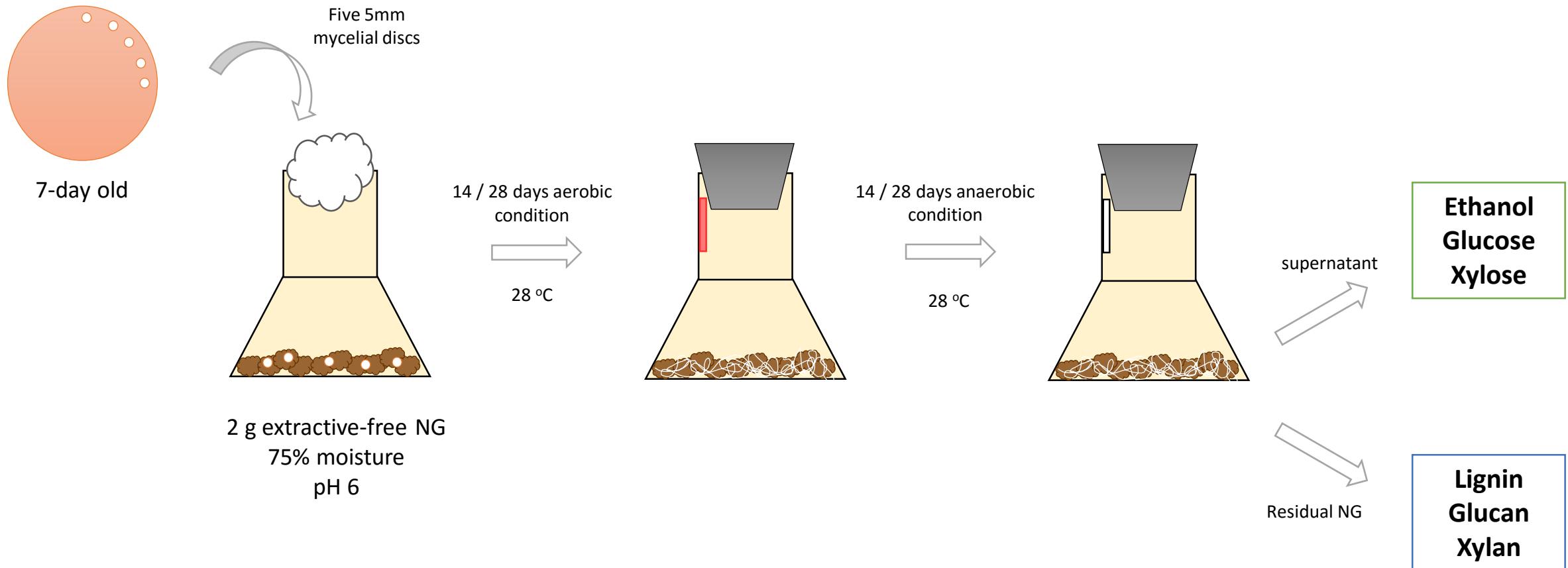
Component	Amount (g/L)
Glucose or Xylose	20
Yeast Extract	5
KH_2PO_4	10
$\text{Mg}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$	0.5
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.5
$\text{Fe}_2\text{SO}_4 \cdot \text{H}_2\text{O}$	0.005
CoCl_2	0.005
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.005
CuSO_4	0.005
MnSO_4	0.005
Tween 80	1
Resazurin	0.010

MacroNutrients MicroNutrients Surfactant Indicator



Ethanol Analysis

Bio-delignification and CDP of Napier grass

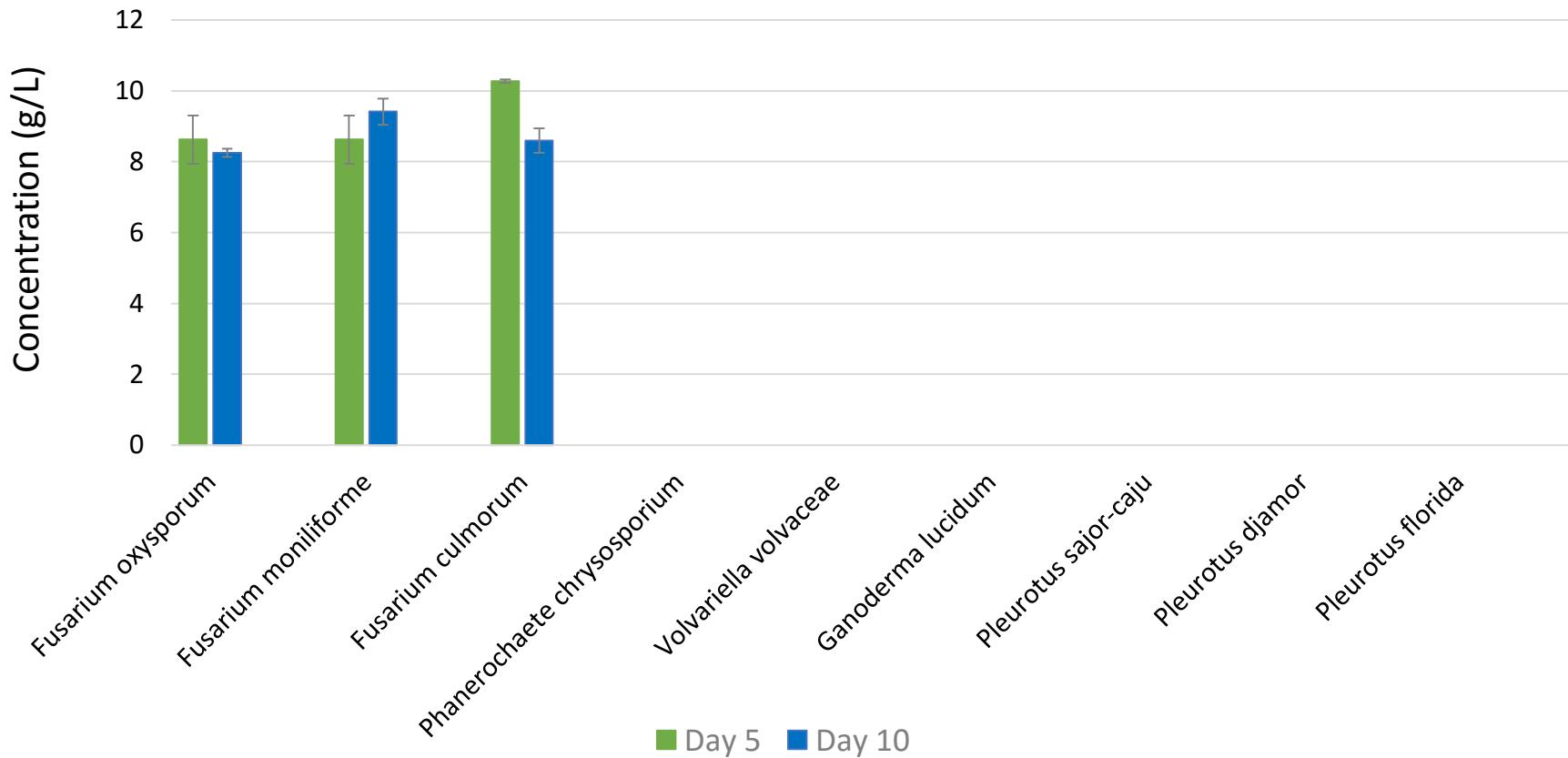


RESULTS & DISCUSSION

**Screening of ethanol-producing filamentous fungi
from glucose and xylose**

Screening from Glucose

Ethanol production by different fungi from 20 g/L glucose.



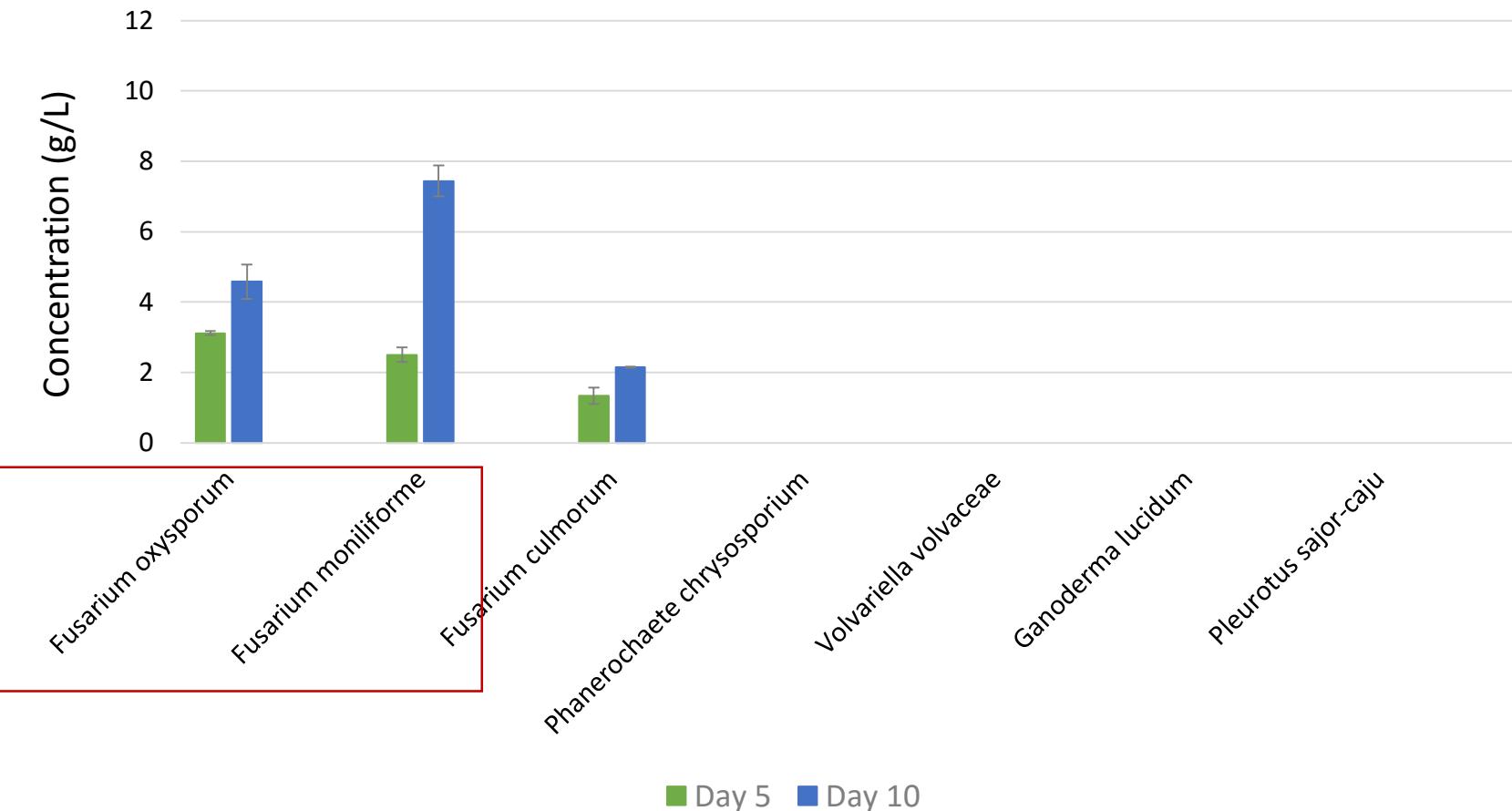
Day 0



Day 5

Screening from Xylose

Ethanol production by different fungi from 20 g/L xylose.



Day 0

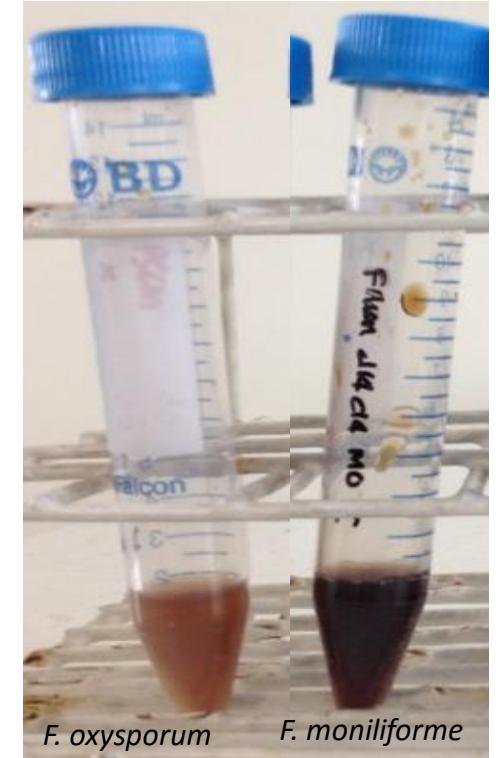
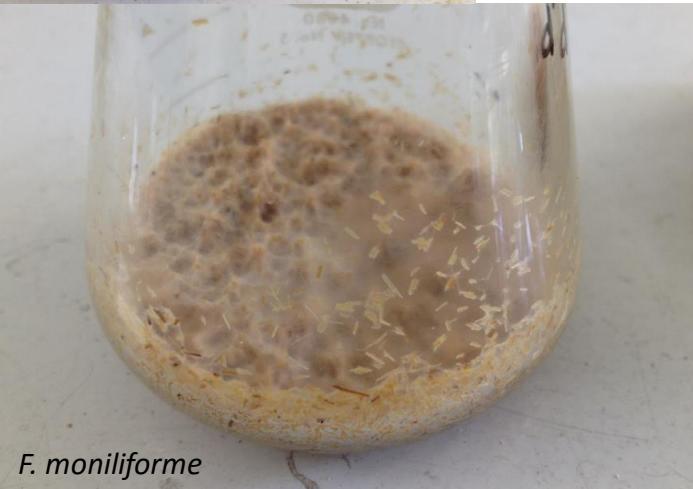
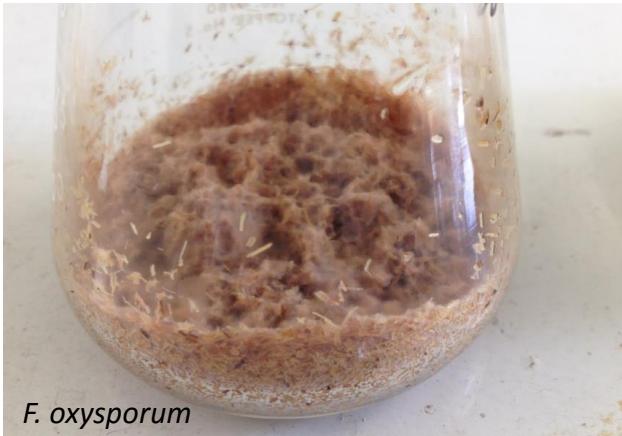


Day 5

RESULTS and DISCUSSION

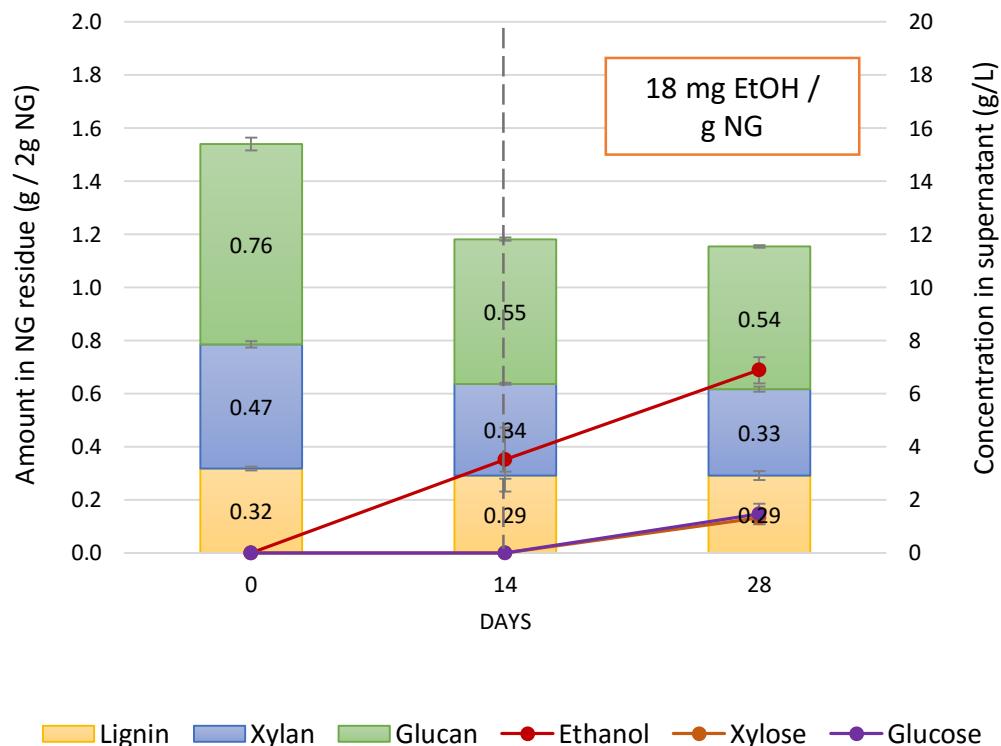
**Bio-delignification and CBP by
F. oxysporum and *F. moniliforme***

Bio-delignification and CBP by *F. oxysporum* and *F. moniliforme*

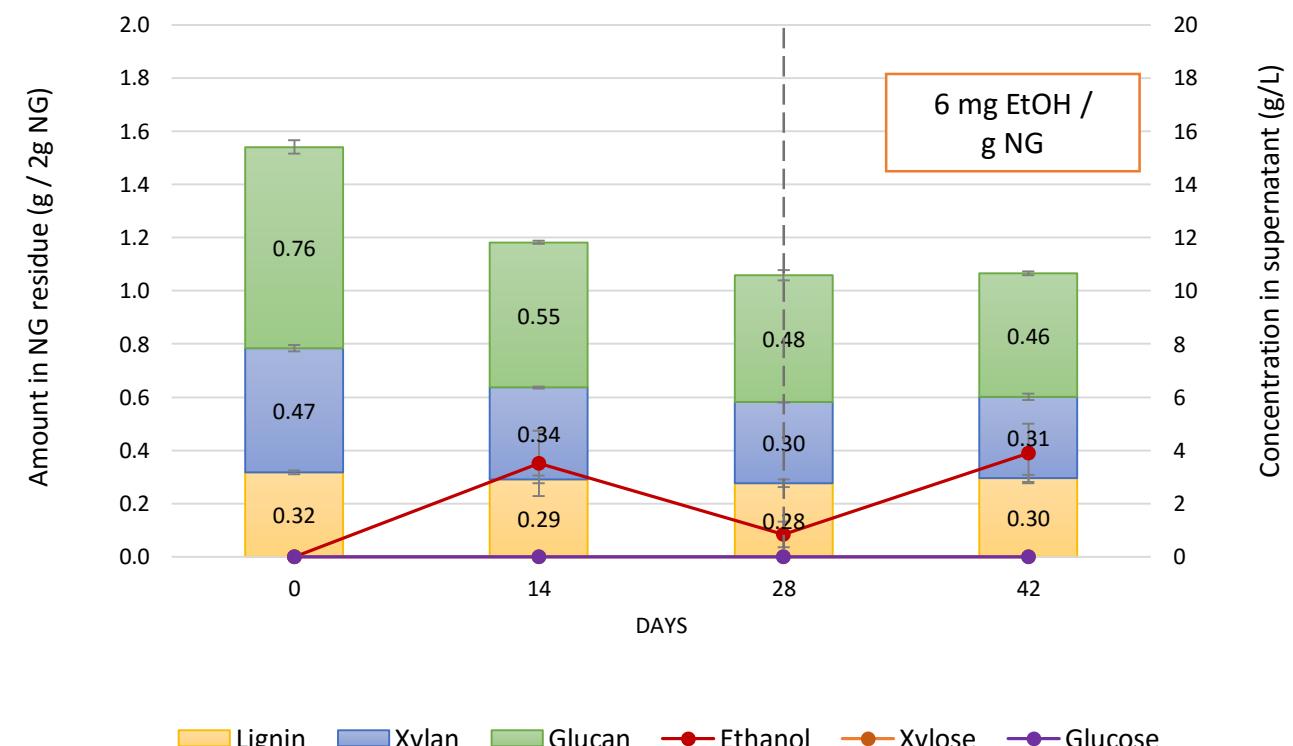


Bio-delignification and CBP by *F. oxysporum*

Composition profile for 14 days bio-delignification and 14 days CBP of NG by *F. oxysporum*.

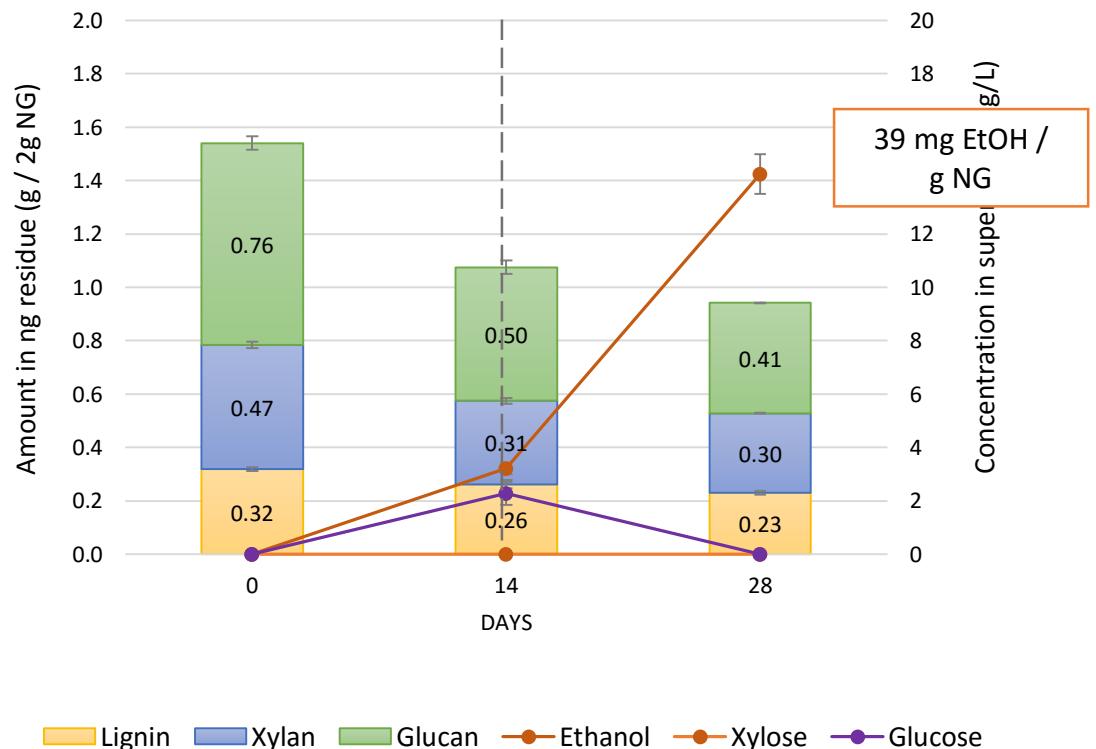


Composition profile for 28 days bio-delignification and 14 days CBP of NG by *F. oxysporum*.

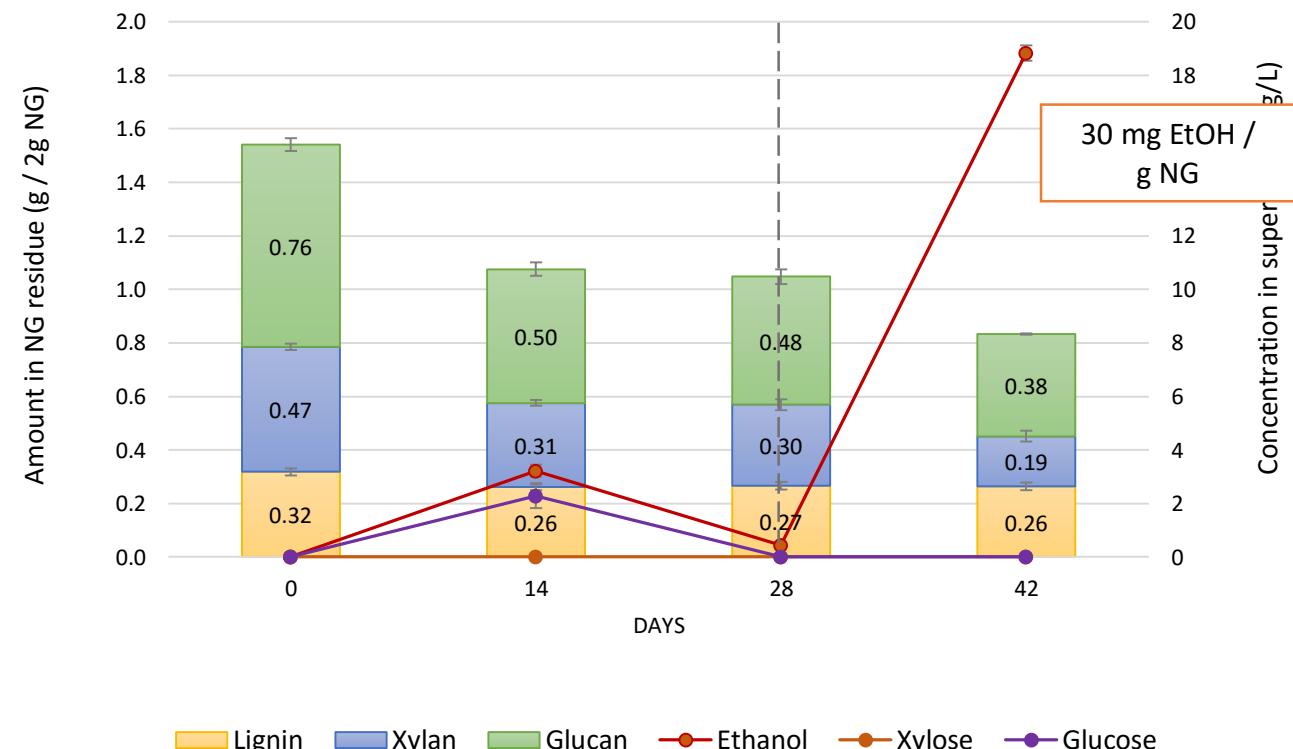


Bio-delignification and CBP by *F. moniliforme*

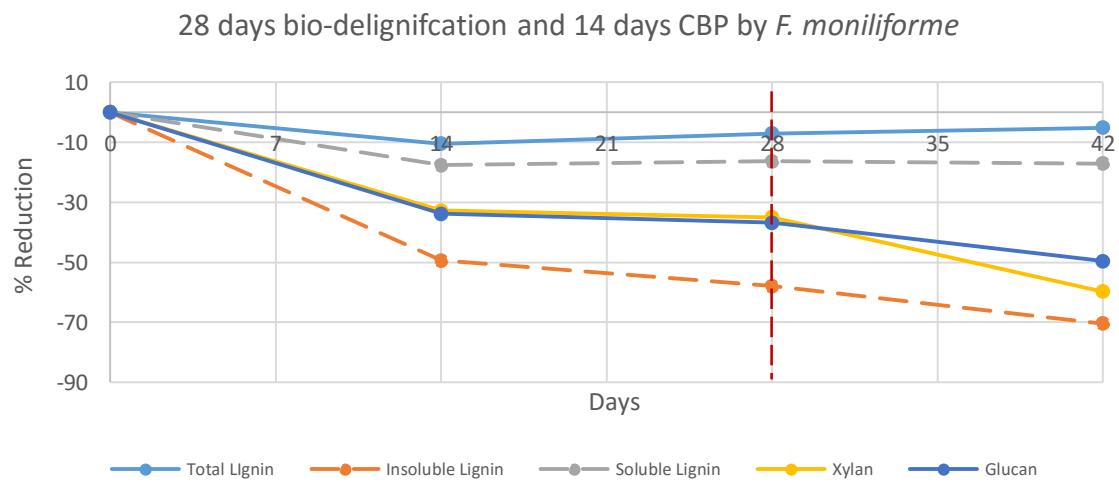
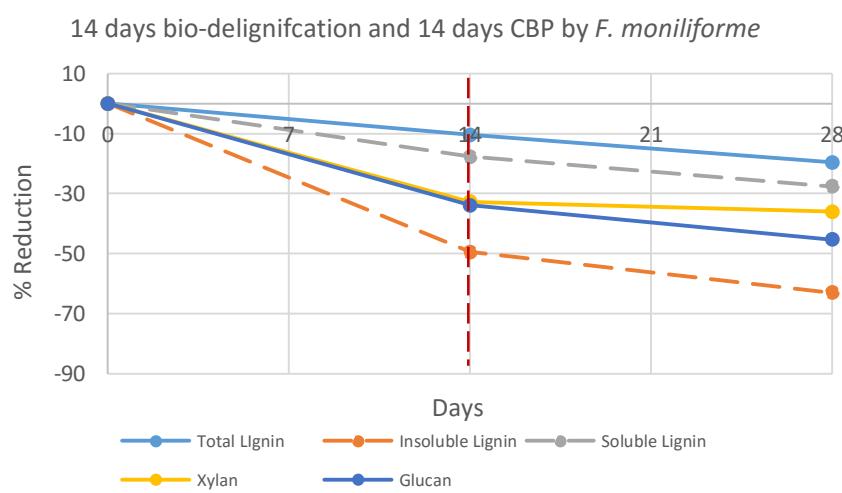
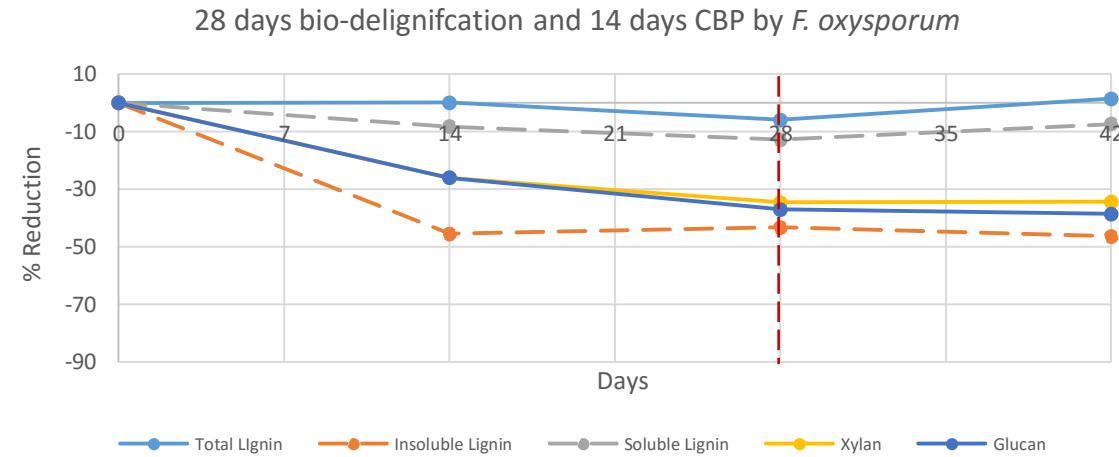
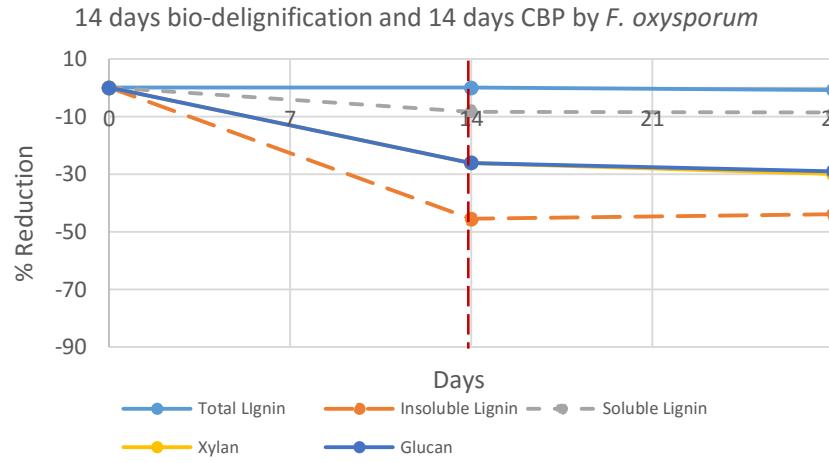
Composition profile for 14 days bio-delignification and 14 days CBP of NG by *F. moniliforme*.



Composition profile for 28 days bio-delignification and 14 days CBP of NG by *F. moniliforme*.



% Reduction of NG components



% Reduction of NG components

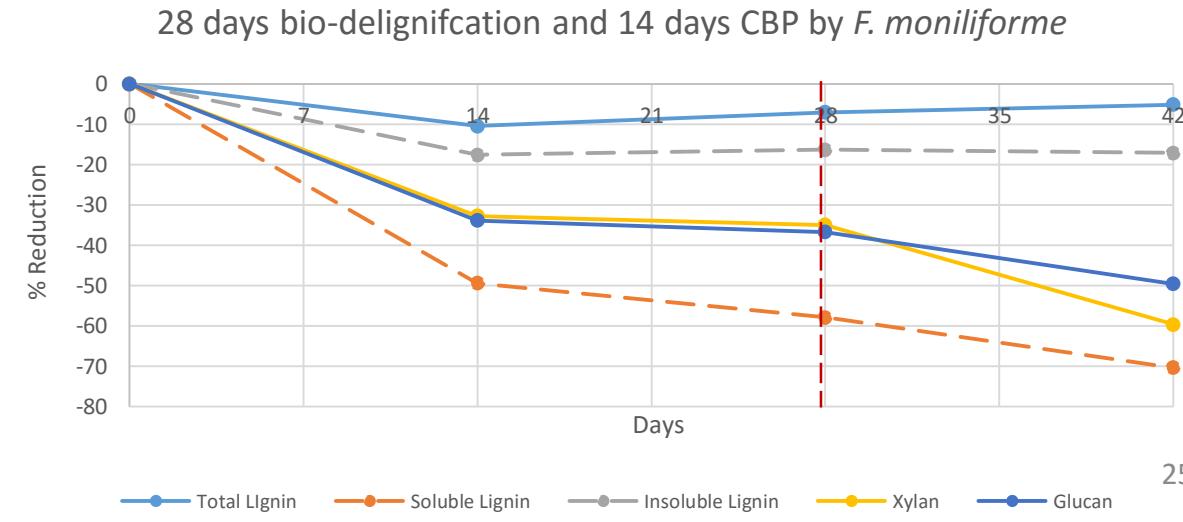
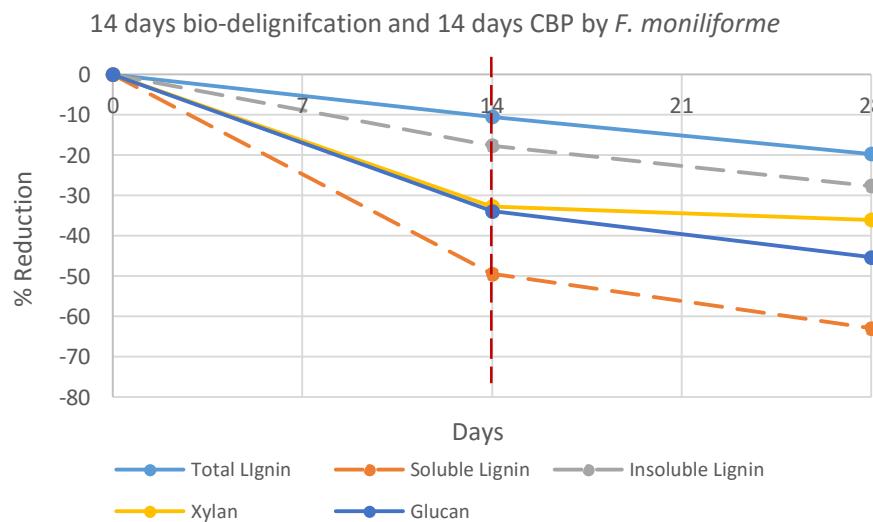
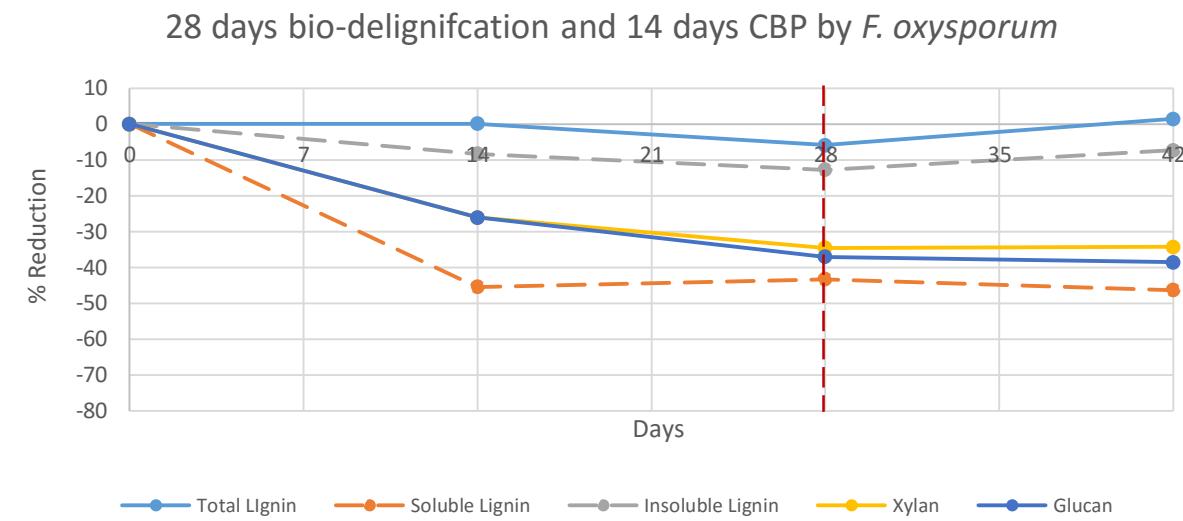
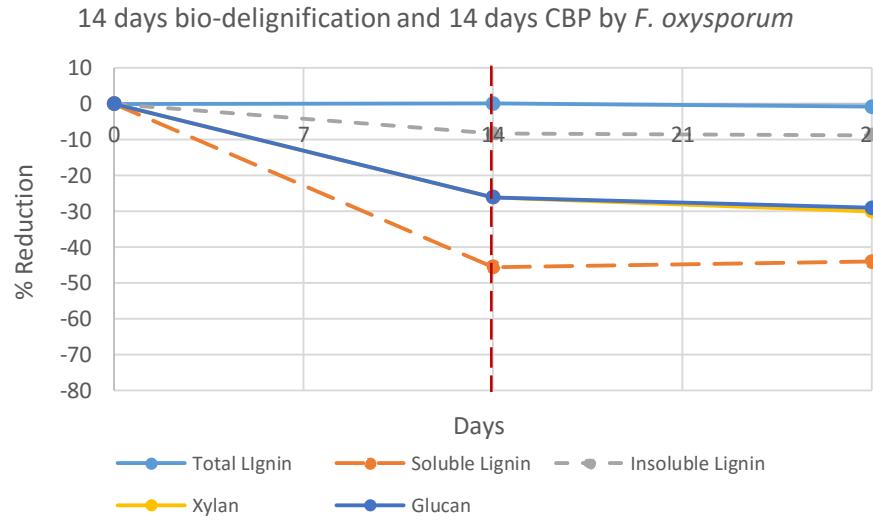


TABLE 3. Weight loss, lignin and carbohydrate losses, and crude protein content of wheat-straw lignocellulose decomposed by *Fusarium* species^a

Strain	Weight loss (%)	Lignin loss ^b (%)	Carbohydrate loss ^b (%)	Crude protein ^b (%)
<i>F. episphaeria</i> 1079	13.6	3.6	22.8	3.9
<i>F. lateritium</i> L-80	6.8	10.5	3.2	4.0
<i>F. moniliforme</i> 279	24.7	10.9	30.2	4.1
<i>F. moniliforme</i> var. <i>subglutinans</i>				
M-1122	16.6	2.8	24.0	5.1
<i>F. nivale</i> 5080	17.9	12.4	22.9	3.7
<i>F. oxysporum</i> f. sp. <i>conglutinans</i>				
4580	11.3	3.1	16.8	4.0
<i>F. oxysporum</i> f. sp. <i>pini</i> 2380	21.8	7.0	32.8	5.0
<i>F. oxysporum</i> f. sp. <i>pisi</i> 5780	21.2	6.2	31.7	3.2
<i>F. rigidiusculum</i> 379	12.0	6.1	15.6	4.1
<i>F. roseum</i> 'Crookwell' 1080	15.8	6.5	23.2	4.2
<i>F. roseum</i> 'Culmorum' 2879	17.0	2.2	20.5	4.7
<i>F. roseum</i> 'Equiseti' 4980	25.3	7.4	30.2	4.9
<i>F. roseum</i> 'Graminearum' 5480	15.4	4.5	21.4	4.2
<i>F. roseum</i> 'Sambucinum' 1180	14.1	6.7	17.5	4.7
<i>F. solani</i> 3179	10.3	17.5	13.5	4.7
<i>F. solani</i> 4A-1	16.4	11.8	19.1	4.7
<i>F. solani</i> f. sp. <i>pisi</i> P-A	19.9	5.2	33.1	4.2
<i>F. tricinctum</i> 179	16.1	7.3	24.0	5.0
Control	2.3	0.5	9.2	3.3

^aCultures were grown for 60 days at 25°C in 1-L réagent bottles with 5 g of lignocellulose and 42 mL of an acid-hydrolyzed casein – salts solution. Before inoculation, the lignocellulose contained 32.6% acid-insoluble lignin, 38.3% carbohydrate, and 3.2% crude protein.

^bTriplicate samples were assayed; average standard deviation was less than 2%.

Sutherland, J. B., Pometto III, A. L., & Crawford, D. L. (1983). Lignocellulose degradation by *Fusarium* species. *Botany*, 61(4), 1194–1198. doi:10.1139/b83-126

Table 1Evaluation of selected fungal isolates^a after 10-day cultivation.

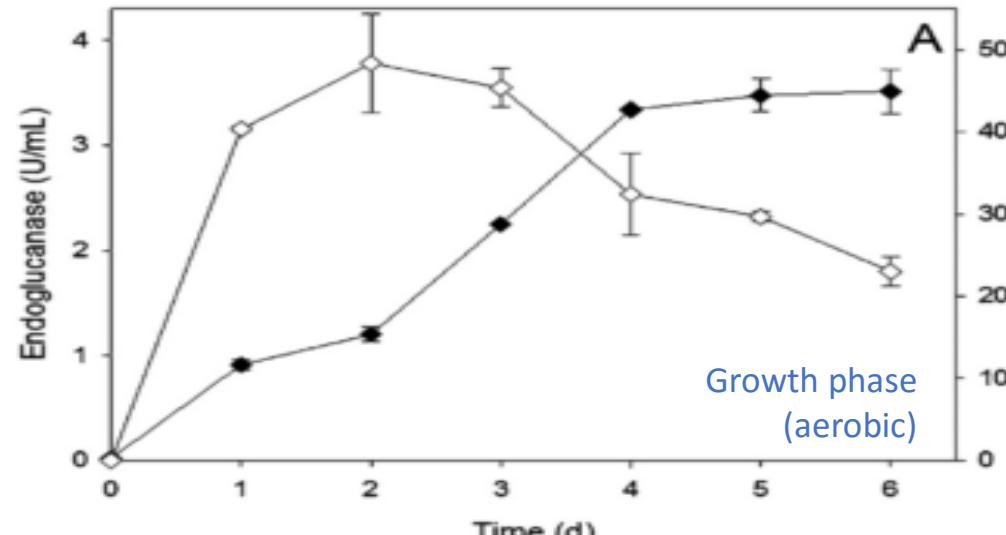
Strain	Species	Radial growth ^c (mm day ⁻¹)	Lignin degraded ^c (per)	Holocellulose degraded ^c (per)	Selectivity ^d
H ^b	<i>Phanerochaete chrysosporium</i>	16.6	28.3	28.4	1.0
82	<i>Fusarium</i> sp.	13.4	29.3	4.8	6.2
89	<i>Fusarium</i> sp.	13.5	33.5	9.9	3.4
812	<i>Fusarium moniliforme</i>	15.6	34.7	2.1	16.5

^a The three isolates represent the three highest lignin degradation out of the 57 samples.^b Used as a reference control.^c Values represent the average of two samples per strain.^d Selectivity based on lignin degradation (%) over holocellulose degradation (per).

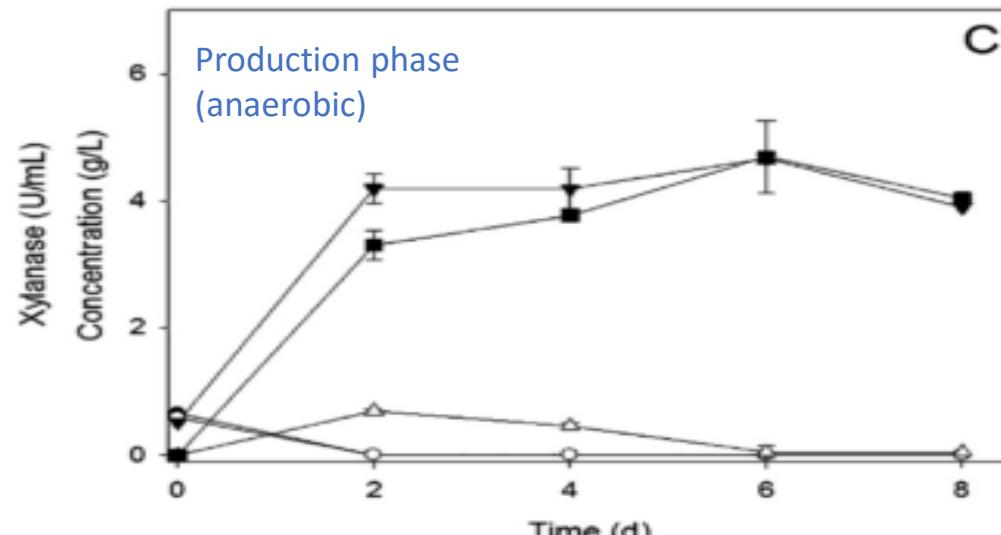
Chang, A. J., Fan, J., & Wen, X. (2012). Screening of fungi capable of highly selective degradation of lignin in rice straw. *International Biodegradation & Biodegradation*, 72(June), 26–30.
doi:10.1016/j.ibiod.2012.04.013

Present Work		
Fungi	Treatment	% Lignin Loss
<i>F. oxysporum</i>	d14 c0	8.29
	d14 c14	8.67
	d28 c0	12.77
	d28 c14	7.36
<i>F. moniliforme</i>	d14 c0	17.65
	d14 c14	27.63
	d28 c0	16.39
	d28 c14	17.13

Enzyme production and products formation by *Fusarium verticillioides* from 40 g/L pretreated sugarcane bagasse (de Almeida, 2013).



endoglucanase (♦) and xylanase (◊) activities during growth phase



Glucose (●); xylose (○); ethanol (▼); xylitol (△); and acetic acid (■).

Composition of Pretreated Sugarcane Bagasse:
Glucan = 51.8%;
Xylan = 25.9%;
Arabinan = 2.3%;
Lignin = 7.7%

Ethanol Yield =
150 mg EtOH / g pretreated wheat straw

Table 4

Fermentation performance of diverse microorganisms using lignocellulosic biomasses.

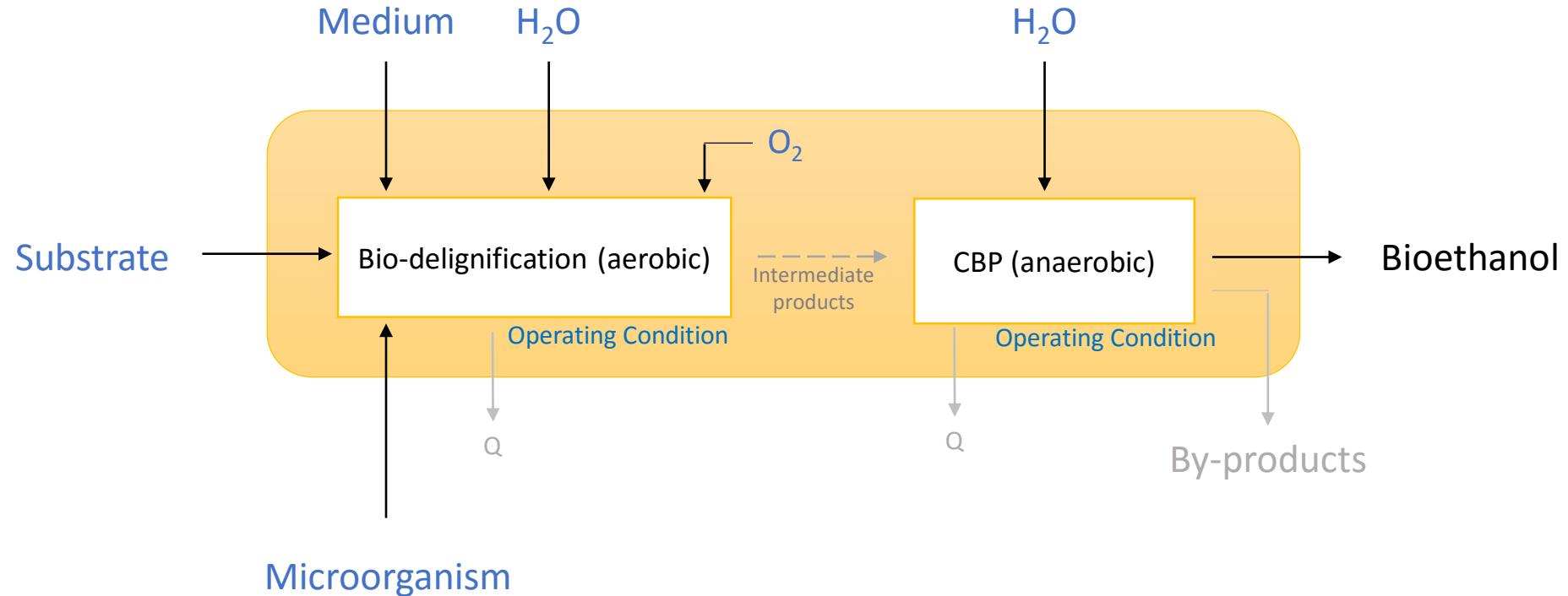
Microorganism	Biomass	S (g/L)	Main sugars	Y_E (g/g)	Reference
<i>Fusarium verticillioides</i>	PSB	40	Glucose, xylose	0.15	de Almeida (2013)
<i>Acremonium zeae</i>	PSB	40	Glucose, xylose	0.13	
<i>Fusarium oxysporum</i>	Corn cobs	40	Glucose, xylose	0.05	Anasontzis et al. (2011)
<i>Fusarium oxysporum</i>	Wheat bran	40	Glucose, xylose, arabinose	0.14	Anasontzis et al. (2011)
<i>Fusarium oxysporum</i> ^b	Corn cobs	40	Glucose, xylose	0.07	Anasontzis et al. (2011)
<i>Fusarium oxysporum</i> ^b	Wheat bran	40	Glucose, xylose, arabinose	0.22	Anasontzis et al. (2011)
<i>Trametes hirsute</i>	Wheat bran	20	Glucose, xylose, arabinose	0.21	Okamoto et al. (2011a)
<i>Trametes hirsute</i>	Rice straw	20	Glucose, xylose, galactose, mannose	0.15	Okamoto et al. (2011a)
<i>Clostridium cellulolyticum</i> ^b	Switchgrass	10	Glucose; xylose	0.13	Li et al. (2012)
<i>Fusarium oxysporum</i>	Cellulose	20	Glucose	0.35	Panagiotou et al. (2005b)
<i>Fusarium oxysporum</i>	Cellulose	20	Glucose	0.17	Panagiotou et al. (2005b)
<i>Phlebia</i> sp.	Kraft pulp	20	Glucose	0.42	Kamei et al. (2012)
<i>Phlebia</i> sp.	Newspaper	20	Glucose	0.20	Kamei et al. (2012)
<i>Clostridium phytofermentans</i>	Filter paper	10	Glucose	0.35 ^a	Tolonen et al. (2011)
<i>Kluyveromyces marxianus</i> ^b	β -Glucan	10	Glucose	0.42	Yanase et al. (2010)
<i>Saccharomyces cerevisiae</i> ^b	β -Glucan	20	Glucose	0.46	Jeon et al. (2009)
<i>Clostridium cellulolyticum</i> ^b	Avicel	10	Glucose	0.27	Li et al. (2012)

S: substrate concentration; Y_E : ethanol yield considering sugar available; PSB: pre-treated sugarcane bagasse.^a Adapted results.^b Genetically modified microorganism.

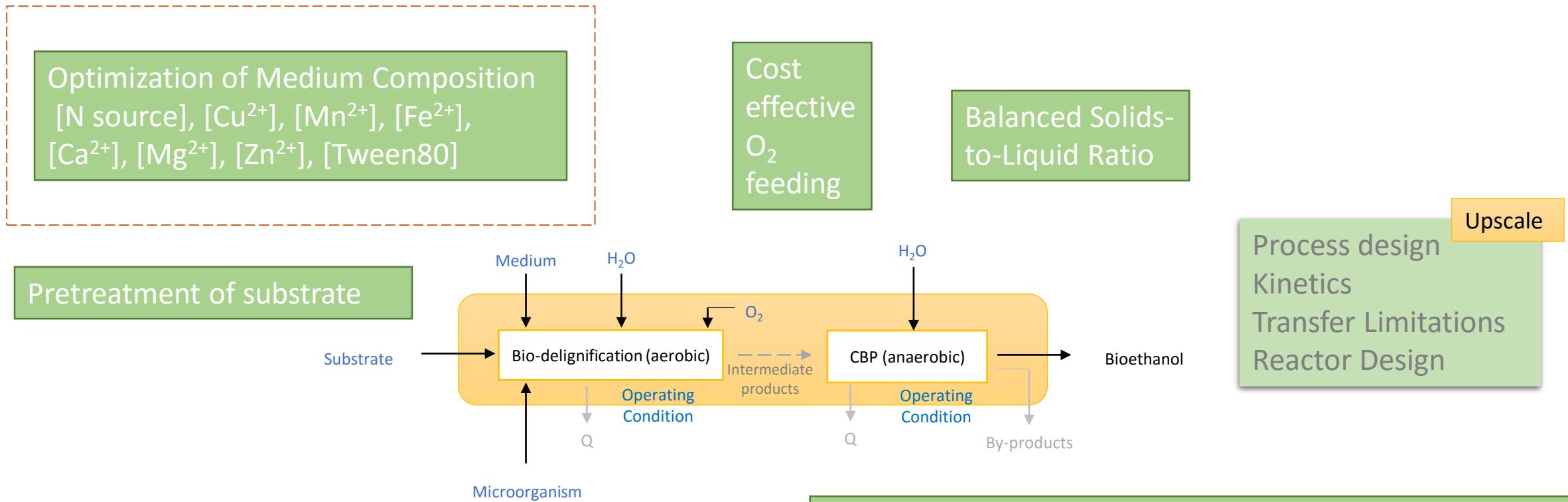
<i>F. oxysporum</i>	Napier grass	75% MC (SSF)	Glucose, xylose	0.02	Present work
<i>F. moniliforme</i>	Napier grass	75% MC (SSF)	Glucose, xylose	0.04	Present work

Maximum Theoretical Yield = 0.336 g EtOH / g biomass
 % Yield = 11.73 %

Strategies for productivity improvement / process intensification



Strategies for productivity improvement / process intensification



1. Screening of other microorganisms
2. Co-culturing
3. Genetic Engineering

1. Optimization of temperature for enzyme production, delignification, enzyme hydrolysis and fermentation
2. Optimized time shift from aerobic to anaerobic conditions

Thank you!

