SESSION VI

Waste & Resource *Recovery*

Chair: J. Wang, S. Van Hulle, L. Limousy

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Novel yeast strains for the efficient conversion of steam-exploded lignocellulosic waste streams to bioethanol

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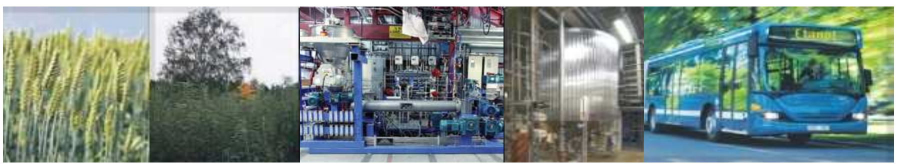
Second generation bioethanol: the bottlenecks

Feedstock

Conversion Technologies

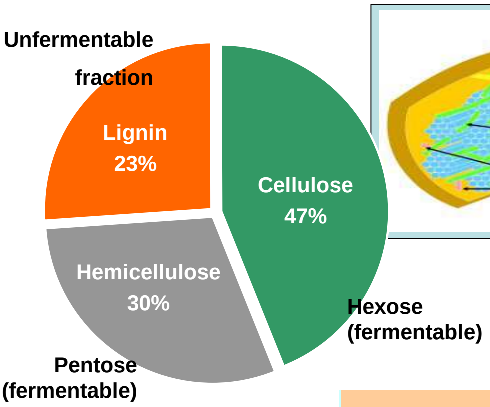


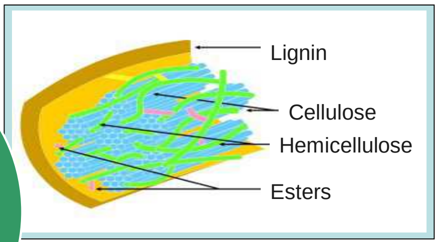
- France: Souston
- Spain: Salamanca
- UK
- Sweden
- Italy

Pre-treatments

Microbes

Lignocellulosic biomass





Rice by-products

4-10% Hemicellulose

20-88% Starch

2-16% Cellulose

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Pre-treatment could greatly affect CBP

Agri-industrial residues

Energy crops

AFEX
(Ammonia Fiber EXpansion)

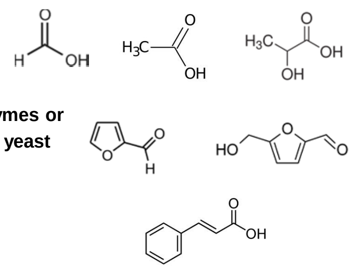
LHW
(Liquid Hot Water)

T→ 160-190 °C
P→ 6-14 ATM
H_2SO_4-SO_2

High temperature and pressure

STEAM EXPLOSION

Enzymes or CBP yeast



High yields:
90% glucose
85% xylose

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Pioneer work on *S. cerevisiae* ATCC24860

Applied and Environmental Microbiology, May 1992, p. 1661–1669 Vol. 58, No. 5
0099-2240/92/051661-09$02.00/0

Isolation and Characterization of Acetic Acid-Tolerant Galactose-Fermenting Strains of *Saccharomyces cerevisiae* from a Spent Sulfite Liquor Fermentation Plant

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Received 15 October 1991/Accepted 22 February 1992

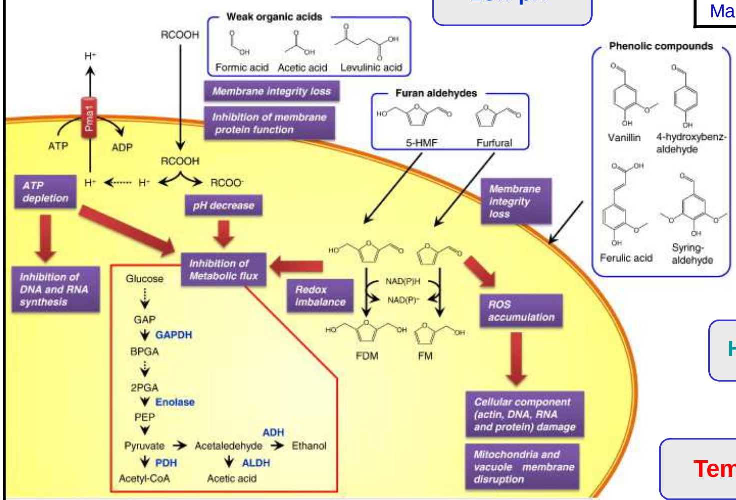
From a continuous spent sulfite liquor fermentation plant, two species of yeast were isolated, *Saccharomyces cerevisiae* and *Pichia membranaefaciens*. One of the isolates of *S. cerevisiae*, no. 3, was heavily flocculating and produced a higher ethanol yield from spent sulfite liquor than did commercial baker's yeast. The greatest difference between isolate 3 and baker's yeast was that of galactose fermentation, even when galactose utilization was induced, i.e., when they were grown in the presence of galactose, prior to fermentation. Without acetic acid present, both baker's yeast and isolate 3 fermented glucose and galactose sequentially. Galactose fermentation with baker's yeast was strongly inhibited by acetic acid at pH values below 6. Isolate 3 fermented galactose, glucose, and mannose without catabolite repression in the presence of acetic acid, even at pH 4.5. The xylose reductase (EC 1.1.1.21) and xylitol dehydrogenase (EC 1.1.1.9) activities were determined in some of the isolates as well as in two strains of *S. cerevisiae* (ATCC 24860 and baker's yeast) and *Pichia stipitis* CBS 6054. The *S. cerevisiae* strains manifested xylose reductase activity that was 2 orders of magnitude less than the corresponding *P. stipitis* value of 890 nmol/min/mg of protein. The xylose dehydrogenase activity was 1 order of magnitude less than the corresponding activity of *P. stipitis* (330 nmol/min/mg of protein).

Which are the industrial stress factors?

Low nutrients availability

Low pH

Pereira et al. (2011)
Albers and Larsson (2009)
Garay-Arroyo et al. (2004)
Martin and Jönsson (2003)



High sugars levels

Temperature

Modified from Hasunuma and Kondo (2012)

Grape marc as trove of biodiversity for bioethanol

- Temperature fluctuations
- Solar radiations
- Ethanol and low pH
- Nutrient limitations

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Looking for robust yeast…

Bioprospecting

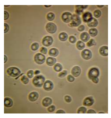
Fermentative abilities at 25, 30, 40, 45 °C

Inhibitors tolerance at 30 and 40 °C

Effects of pH

Effects of high sugars levels

Fermentation of synthetic inhibitors mixtures



Yeast optimizer©

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Looking for robust yeast…

Bioprospecting

Fermentative abilities at 25, 30, 40, 45 °C

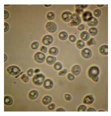
Inhibitors tolerance at 30 and 40 °C

Effects of pH

Effects of high sugars levels

Fermentation of synthetic inhibitors mixtures

Fermentation of real lignocellulosic hydrolysates



Yeast optimizer©

Grape marc as trove of biodiversity for bioethanol

Favaro *et al. Biotechnology for Biofuels* 2013, 6:168
http://www.biotechnologyforbiofuels.com/content/6/1/168

Biotechnology for Biofuels

RESEARCH Open Access

Exploring grape marc as trove for new thermotolerant and inhibitor-tolerant *Saccharomyces cerevisiae* strains for second-generation bioethanol production

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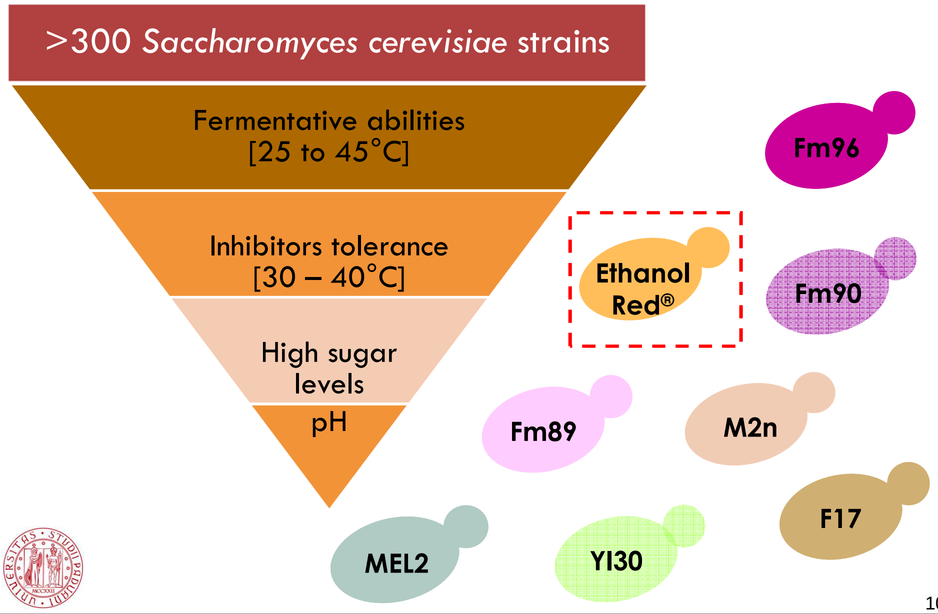
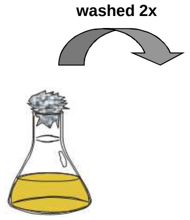
To select for novel industrial yeast strains



Inhibitors tolerance: a short and powerful method



900 μL medium

100 μL cells suspension

YNB (Yeast Nitrogen Base)

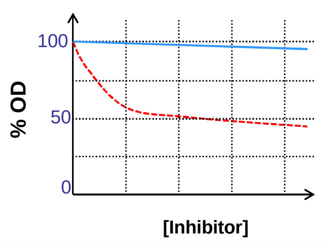
OD_{600nm} = 0.075 (~1*10^6 cell/mL)

Temperature **30 °C**

Incubation at 100 rpm for 40 h

$$\% OD = \frac{OD_{inib.} - OD_{inib.\ N.I.}}{OD_{YNB} - OD_{YNB\ N.I.}} \times 100$$

- >90%
- 71-89%
- 50-70%
- <50%



Elisabetta Bordignon, Master Student

Inhibitors tolerance: synthetic inhibitors mixtures

	Acetic acid	Formic acid	Lactic acid	Furfural	HMF	Coniferyl aldehyde	Cinnamic acid
RC_{25}	1.80	1.70	0.60	0.70	0.90	0.05	0.04
RC_{50}	3.60	3.40	1.20	1.40	1.80	0.09	0.08
RC_{100}	7.20	6.80	2.40	2.80	3.60	0.18	0.15
RC_{200}	14.40	13.60	4.80	5.60	7.20	0.36	0.30

Inhibitors mixtures concentrations (g/L) and composition (HMF stands for 5-hydroxymethyl-2-furaldehyde).

	F17	Fm89	Fm90	Fm96	M2n	MEL2	Y130	Ethanol Red®
RC_{25}	94	81	87	79	55	82	76	65
RC_{50}	71	62	59	53	21	60	63	44
RC_{100}	55	45	42	39	14	28	59	11
RC_{200}	0	0	0	0	0	0	0	0

Relative growth in YNB supplemented with 20 g/L glucose and increasing dosages of inhibitors mixtures.

pH was adjusted to 5.0 with 5M NaOH. Standard error was always less than 7% (not shown)

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Inhibitors tolerance: steam-exploded lignocellulosic materials

Substrates		$LogR_0$	pH	Glucose	Formic acid	Acetic acid	Furfural	HMF
PG1	***P. australis***	3.60	3.75	0.14	0.32	1.00	0.24	0.05
PG2	***P. australis***	4.00	3.29	0.29	0.78	2.18	0.97	0.13
PG3	***P. australis***	4.40	3.23	0.43	1.08	3.50	1.43	0.48
PG4	***C. cardunculus***	3.85	3.86	0.30	2.73	3.15	0.46	0.30
PG5	***C. cardunculus***	4.28	3.79	0.13	4.28	5.80	0.64	0.39
PG6	***C. cardunculus***	4.02	3.93	0.20	2.18	2.76	0.44	0.20
PG7	***C. cardunculus***	4.28	4.10	0.02	0.50	0.71	0.09	0.05
SH	***S. officinarum***	n.a.	3.28	0.50	3.0	11.20	1.70	0.50

Severity factor $LogR_0$ correlates with the harshness of the pre-treatment

P. australis **(common reed)**

C. cardunculus **(cardoon)**

S. Officinarum **(sugarcane)**

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Inhibitors tolerance: steam-exploded lignocellulosic materials

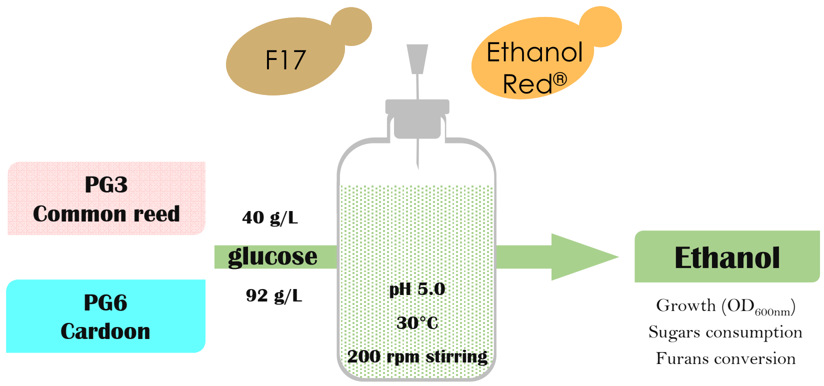
	F17	Fm89	Fm90	Fm96	M2n	MEL2	Y130	Ethanol Red®
PG1	+	+	+	+	+	+	+	+
PG2	+	-	+	+	+	+	+	+
PG3	-	-	-	-	-	-	-	-
PG4	+	+	+	+	+	+	+	+
PG5	-	-	-	-	-	-	-	-
PG6	+	-	+	+	+	+	+	+
PG7	+	+	+	+	+	+	+	+
SH	-	-	-	-	-	-	-	-

Pre-hydrolysates **PG3** from **common reed**, **PG5** from **cardoon**, and **SH** from **sugarcane bagasse** did not support the growth of any yeast indicating that the concentration of toxic chemical species was higher than yeast could tolerate.

The effect of pH on the inhibitors tolerance of yeast strains

	F17	Fm89	Fm90	Fm96	M2n	MEL2	Y130	Ethanol Red®
PG3 pH 3.3	0	0	0	0	0	0	0	0
PG3 pH 5.0	63	68	61	9	16	8	57	27
PG5 pH 3.8	0	0	0	0	0	0	0	0
PG5 pH 5.0	59	63	60	54	60	56	60	63
PG6 pH 3.9	62	48	61	51	53	30	70	49
PG6 pH 5.0	90	71	80	79	73	61	76	78

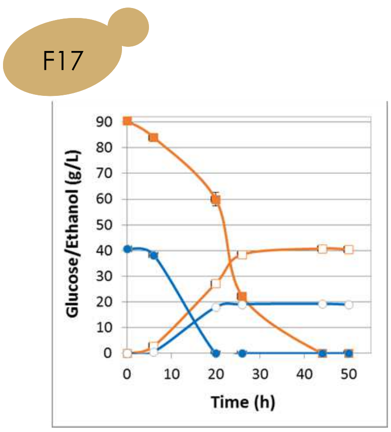
Fermentation studies using lignocellulosic waste

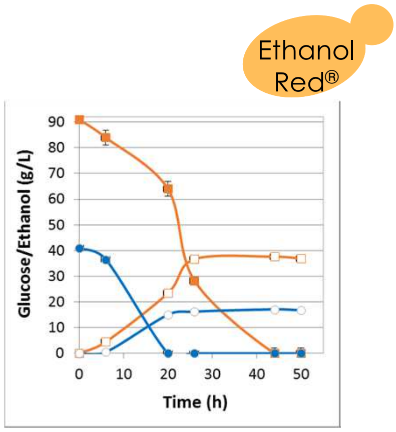


Cotana et al. (2015) Ind. Crops Prod. 69, 424-432

Cotana et al. (2015) Sustainability 7, 12149-12163

Fermentation studies using lignocellulosic waste





Yield: **0.43 (85%)**

Yield: **0.42 (83%)**

Yield: **0.45 (88%)**

Yield: **0.41 (80%)**

Conclusions and future perspectives

- **The search for robust yeast is a strategic approach to maximize ethanol production from lignocellulosic materials**.
- **For the first time two undiluted lignocellulosic pre-hydrolysates were proficiently fermented with yields even higher than those exhibited by *S. cerevisiae* Ethanol Red®**, the most used industrial yeast strain in lignocellulosic ethanol plants.

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Thank you for your attention