Optimization of sugars recovery from spoiled date fruits for sustainable bioethanol production via yeasts co-cultures

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INTRODUCTION: The continuously increasing world population and the rapid industrial development have led to the depletion of the global energy reserves and the need to explore alternative energy sources. Biofuels are clean and renewable energy carriers, and among them ethanol produced by fermenting microorganisms is a strong candidate to fill the energy demand gap. Among the most important issues for the industrialization of ethanol production in a sustainable way, is the cost of the raw material and its maximum biotransformation using cost-effective and environmentally friendly methodologies.

The present study investigates the efficiency of bioethanol production via novel yeast strains, using as feedstock the extracted sugars from spoiled date fruits that were discarded as non-edible. The optimization of the extraction process was based on Box–Behnken statistical experimental design with three factors i.e. solids loading, extraction time and extraction temperature, and a relative importance of each independent parameter was assessed in terms of the achieved yield of sugars (g recovered sugars/kg SD)

Approach & Methodology

Aim: To investigate the exploitation of the sugars contained in spoiled date fruits (SD) for ethanol production at high rates and yields.

Composition of SD

Parameter	Value
TS (%)	68.37 ± 1.28
Humidity (%)	31.63 ± 1.81
VS (%TS)	96.71 ± 0.08
Ash (% TS)	2.25 ± 0.11
pH (10% aqueous solution)	6.12 ± 0.02
Soluble carbohydrates (%)	51.32 ± 0.84
Total carbohydrates (%),	66.33 ± 3.67
TKN, (NH3-N %),	0.61 ± 0.04
Proteins (%)	3.75 ± 0.25

Water extraction via Experimental Design (Box –Behnken)

Factors	Coded		
A. Temperature, ^{0}C	-1 ↔ 20,00	0 ↔ 30,00	+1 ↔ 40,00
B. Loading, % w/v	-1 ↔ 20,00	0 ↔ 30,00	+1 ↔ 40,00
C. Time, min	-1 ↔ 10,00	0 ↔ 20,00	+1 ↔ 30,00

2 responses:
Yield, Y_{su}, (g su/g SD) &
Concentration, S_{su} (g/L)

E x t r a c t

Batch fermentation of extracts
S_{in}, ~70g/L (low) & ~140g/L (high)
- ✓ % consumption
- ✓ Rate
- ✓ Yield, Y_{EtOH}

Yeast isolates tested

- S30 *Saccharomyces cerevisiae, YYB205*
- S33 *Saccharomyces cerevisiae, P38*
- S21 *Zygosaccharomyces rouxii, D13*
- S32 *Saccharomyces cerevisiae, 2-7-9*
- S35 *Meyerozyma guilliermondii, LY200*

Fed batch with co-culture of best performing yeasts

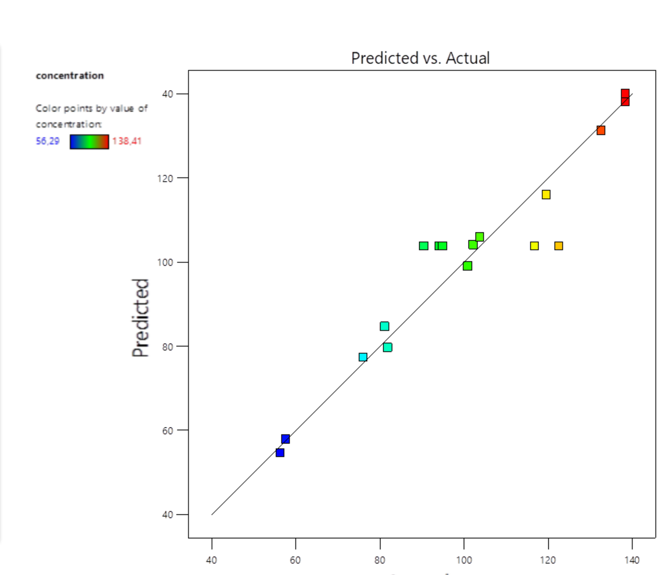
***S. cerevisiae*, YYB205**
+
***S. cerevisiae*, P38**

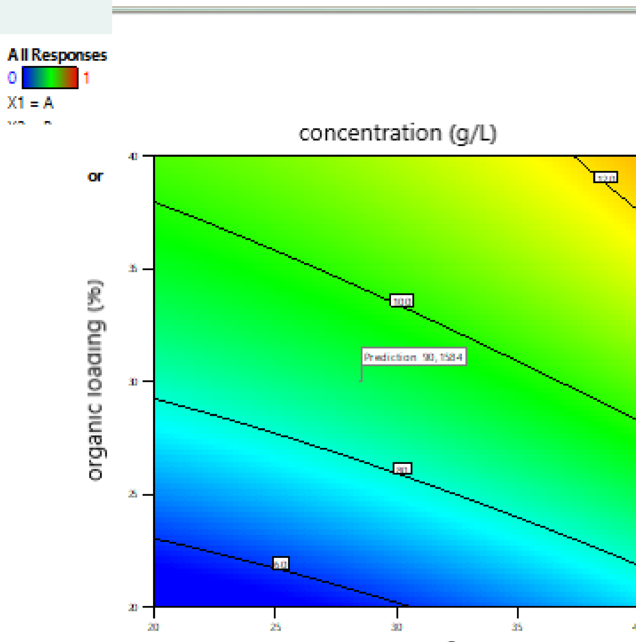
- ✓ V_w: 350 ml
- ✓ Anaerobic conditions, (CO_2 venting via 0.22 μm filters
- ✓ Constant agitation, 150 rpm
- ✓ Incubation, 30 °C
- ✓ S_{in} sugars, 138 g/L
- ✓ Batch 24h, → + feed 75 ml

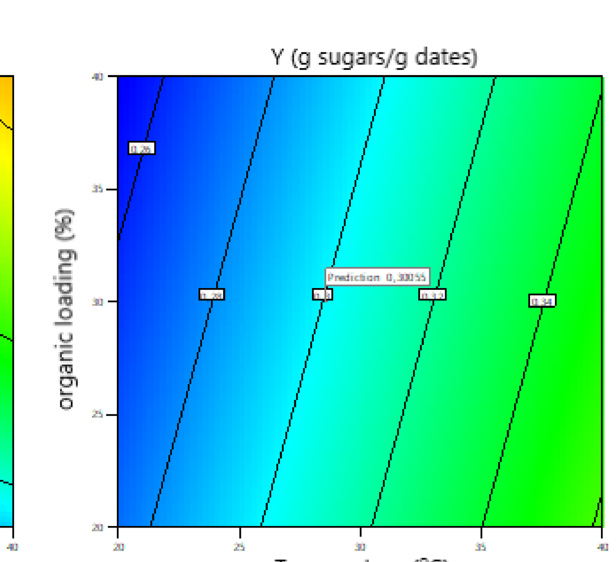
Results I

Optimization of recovery of sugars from dates

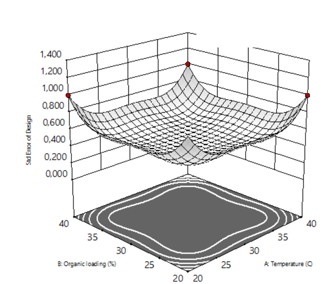
Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	9394,68	9	1043,85	7,92	0,0062	Significant (<0,05)
A-Temperature	1192,31	1	1192,31	9,05	0,0197	
B-Organic loading	6224,49	1	6224,49	47,24	0,0002	
C-Time	1742,42	1	1742,42	13,22	0,0083	
Lack of Fit	50,60	3	16,87	0,0774	0,9689	not significant (>0,05)
Pure Error	871,67	4	217,92			
Cor Total	10316,96	16				







- ✓According to the Model F-value the model is significant
- ✓According to the P-values all parameters tested, A, B and C, are significant model terms.
- ✓The Lack of Fit F-value implies the Lack of Fit is not significant relative to the pure error.

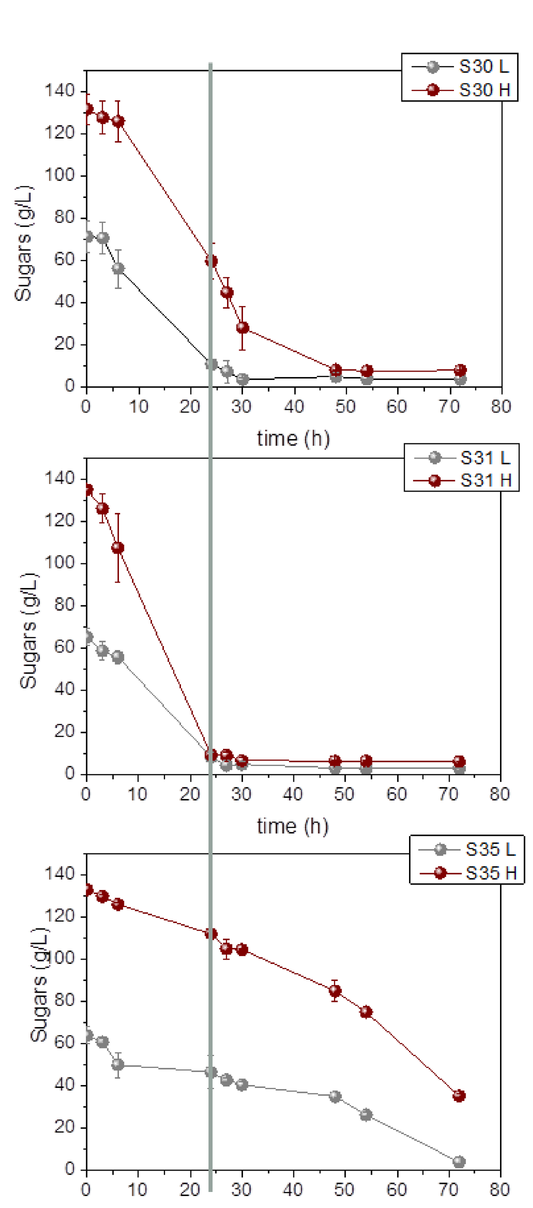


Final Equation in Terms of Coded Factors : **100,453 + 12,2081 * A + 27,8938 * B + 14,7581 * C**

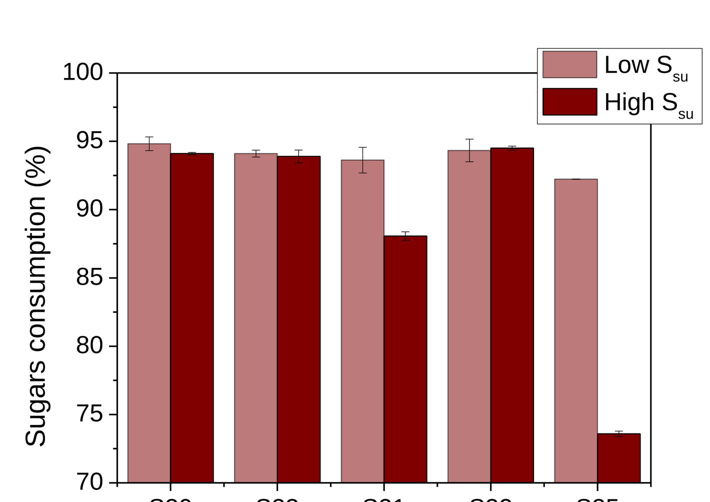
- ➢ Yield was significantly affected by concentration and time, but is was affected by extraction temperature, ranging reaching 0.42 g sugars/g SD
- ➢ Concentration was severely affected by organic loading and also temperature.
- ➢ Maximum concentration of sugars experimentally achieved , 138 g/L in good agreement with the prediction of the model

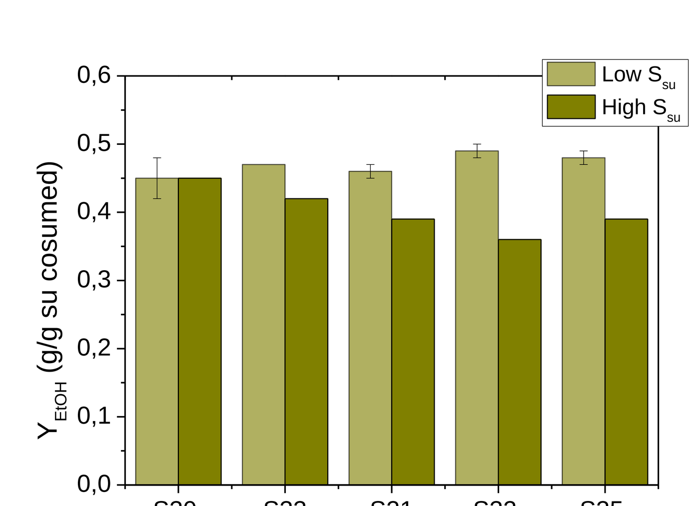
Results II

Alcoholic fermentation of SD extract



Assessment of isolates performance





- The ***S. cerevisiae*** strains P38 and 2-7-9, (isolates **S33** and S32) exhibited
  - ✓highest growth rates compared to the other isolates, and also
  - ✓no substrate inhibition on the consumptions sugars which was proportional for the low and high initial substrate concentration
- ***S. cerevisiae*** strain YYB205 (isolate **S30**) exhibited
  - ✓Relative lower growth rate than strains P38 and 2-7-9, but
  - ✓no substrate inhibition on neither sugars consumption nor ethanol yield for the high initial substrate concentration, being thus advantageous

Co-culture of ***S. cerevisiae*** strains P38 and YYB205, at fed batch mode with the highest substrate concentration

- *✓96% consumption of sugars*
- *✓0.46 g ethanol /g consumed sugars*
- *✓Up to **65g/L** ethanol*

Conclusions

- ➢ *During the optimization of the sugars extraction from the SD via experimental design, the relative severity of each parameter on the recovery yield and concentration was identified*
- ➢ *It was shown that the highest organic loading and temperature were optimal for the extraction efficiency*
- ➢ *All yeast isolates exhibited high ethanol yields from the lower concentration of SD extract, but based on the fermentation rate and substrate inhibition two S. cerevisiae strains were selected for fed batch experiments*
- ➢ *The co-culture in fed batch mode resulted to 20% higher ethanol titter, indicating that the fermentation stategy is crucial for exploiting the potential of the yeasts nd achieving optimal utilization of the substrate*

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