REPEATED-BATCH FERMENTATION OF SUGARCANE BAGASSE HEMICELLULOSIC HYDROLYSATE TO ETHANOL USING TWO XYLOSE-FERMENTING YEASTS

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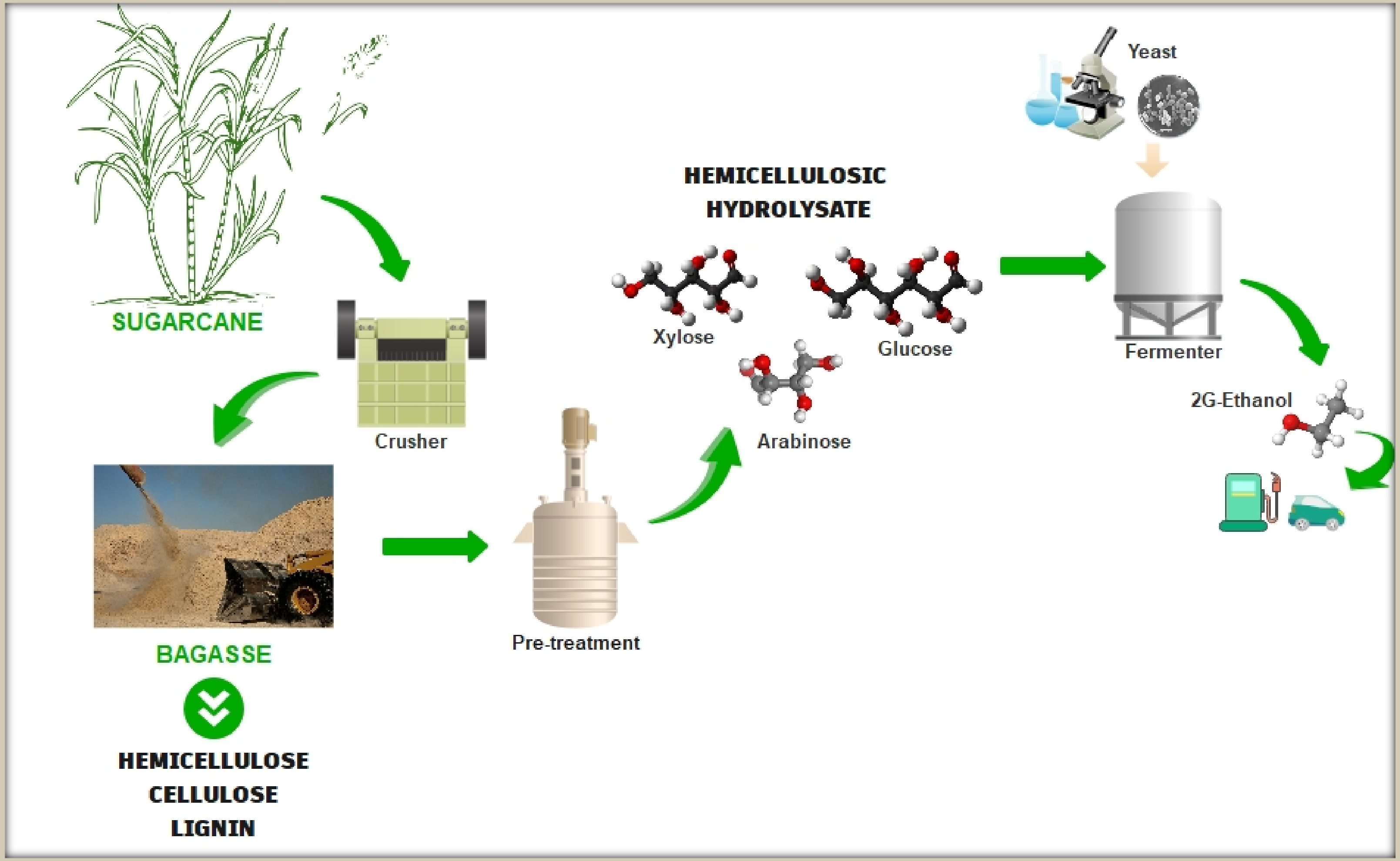
INTRODUCTION



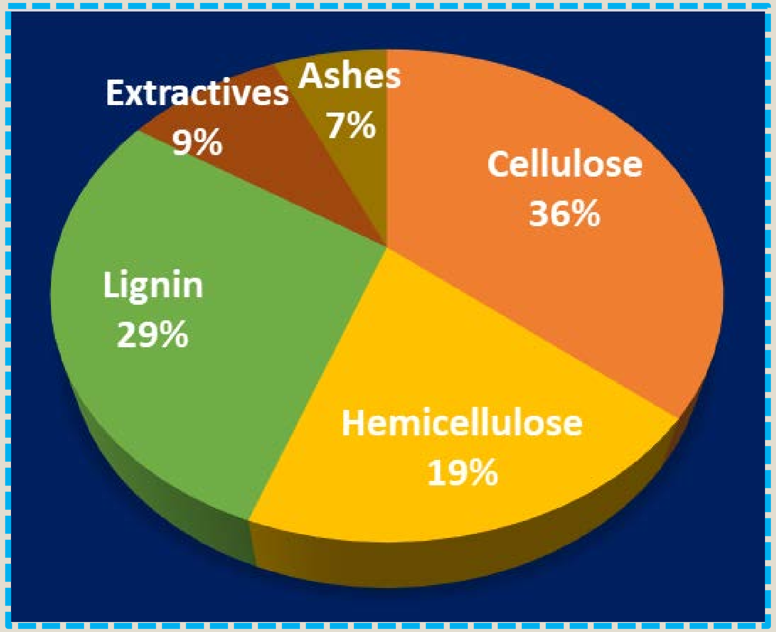
Evaluation of pretreatment of **sugarcane bagasse** to optimize the obtaining of **hemicellulosic hydrolysate**

Repeated batch process fermentation of hemicellulosic hydrolysate by ***S. stipitis*** and ***S. shehatae***, comparing the **efficiency** of **ethanol production** as a **strategy** to be applied **to large-scale commercial ethanol production**.

MATERIAL AND METHODS

9844+6V Piracicaba, São Paulo

Sugarcane Bagasse



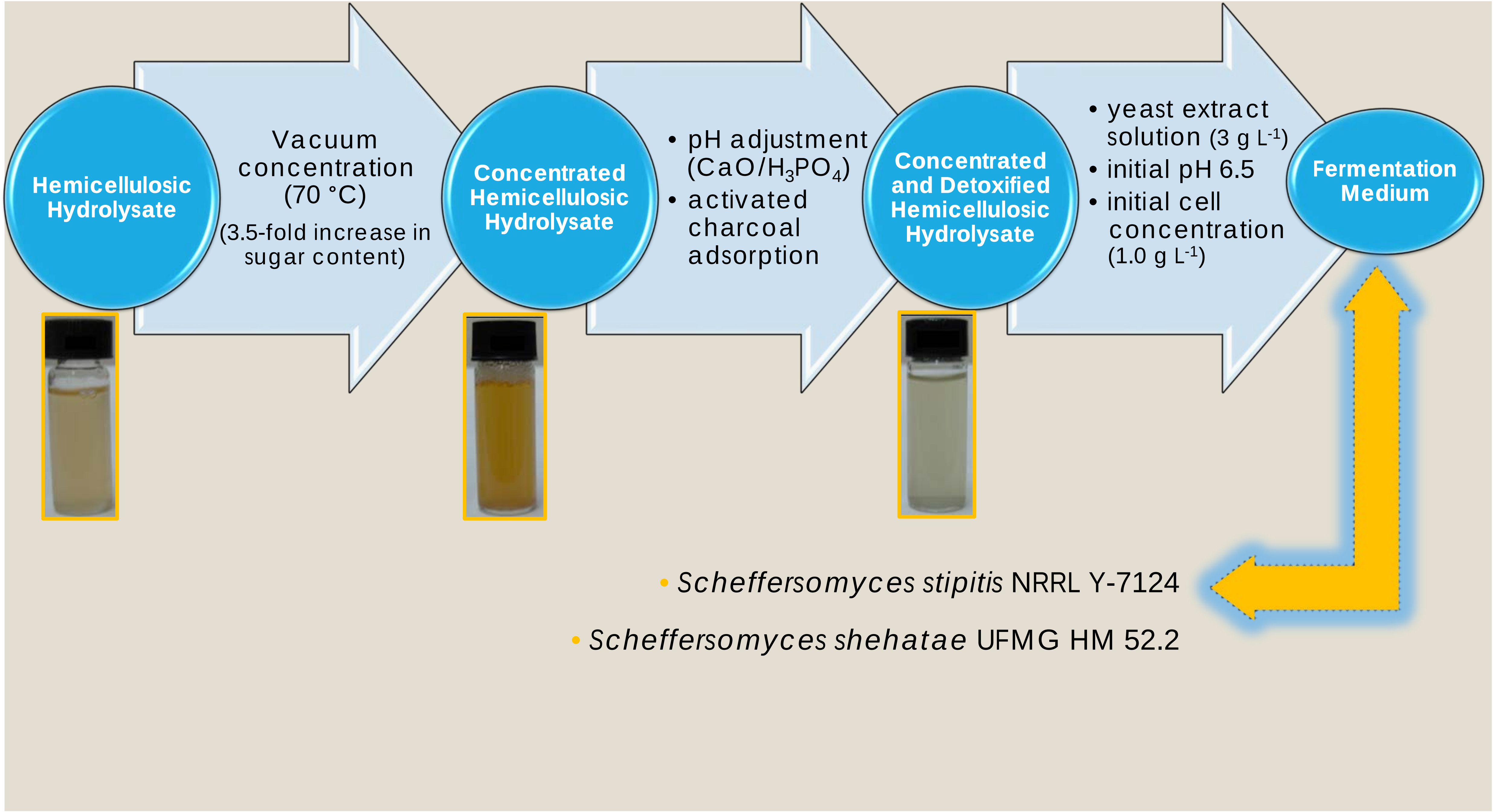
pretreatment 50-L rotary reactor

❖ The morphology of raw and pretreated bagasse was analyzed by scanning electron microscopy (SEM) - LEO 440 equipment with an Oxford detector operating at 20 kV, 2.82 A and 950 pA.

Table 1. 2^3 face-centered full factorial design of pre-treatment efficiency of sugarcane bagasse

Assays	Code variables			Real variables		
	X_1	X_2	X_3	Temperature (°C)	mg H_2SO_4/g dry bagasse	Time (min)
1	-1.0	-1.0	-1.0	100	50	10
2	-1.0	-1.0	1.0	100	50	30
3	-1.0	1.0	-1.0	100	250	10
4	-1.0	1.0	1.0	100	250	30
5	1.0	-1.0	-1.0	140	50	10
6	1.0	-1.0	1.0	140	50	30
7	1.0	1.0	-1.0	140	250	10
8	1.0	1.0	1.0	140	250	30
9 (FC)	-1.0	0.0	0.0	100	150	20
10 (FC)	1.0	0.0	0.0	140	150	20
11 (FC)	0.0	-1.0	0.0	120	50	20
12 (FC)	0.0	1.0	0.0	120	250	20
13 (FC)	0.0	0.0	-1.0	120	150	10
14 (FC)	0.0	0.0	1.0	120	150	30
15 (PC)	0.0	0.0	0.0	120	150	20
16 (PC)	0.0	0.0	0.0	120	150	20
17 (PC)	0.0	0.0	0.0	120	150	20
18 (PC)	0.0	0.0	0.0	120	150	20

*PC= central point; FC = face-centered


Hemicellulosic Hydrolysate
Vacuum concentration (70 °C)
(3.5-fold increase in sugar content)
Concentrated Hemicellulosic Hydrolysate
• pH adjustment (CaO/H_3PO_4)
• activated charcoal adsorption
Concentrated and Detoxified Hemicellulosic Hydrolysate
• yeast extract solution (3 g L^{-1})
• initial pH 6.5
• initial cell concentration (1.0 g L^{-1})
Fermentation Medium
• *Scheffersomyces stipitis* NRRL Y-7124
• *Scheffersomyces shehatae* UFMG HM 52.2


2.4 L Bioengineering KLF 2000

S. shehatae: 100 rpm, 0.10 vvm, 0.1 h^{-1} k_La

S. stipitis: 100 rpm, 0.70 vvm, 3 h^{-1} k_La

30 °C
72h
Samples every 24h

- Repeated-batch fermentation without recycling cells: after 72 h 2/3 (800 mL) of fermented broth were removed and 800 mL of fresh supplemented hydrolysate was added.
- Batch fermentation was repeated sequentially for 3 cycles: 0, 1 and 2.
- Cycle 0: initial batch culture

- Xylose, glucose, arabinose, xylitol, ethanol, acetic acid, furfural, 5-HMF concentrations were determined using a high-performance liquid chromatography (HPLC).

- The total phenolic compounds concentration was estimated through ultraviolet spectroscopy at 280 nm and cell concentration at 600 nm.

RESULTS

Assays	Efficiency (%)	Xylose (g/L)	HMF (g/L)	Furfural (g/L)	Phenols Total (g/L)
1	31.46	6.02	0.0026	0.0088	0.94
2	24.09	4.61	0.0020	0.0762	2.03
3	57.33	10.97	0.0026	0.0727	1.41
4	39.72	7.60	0.0032	0.0079	1.25
5	59.36	11.36	0.0650	0.1909	11.67
6	38.51	7.37	0.0490	0.1876	11.03
7	37.83	7.24	0.0347	0.1966	15.71
8	22.78	4.36	0.0417	0.2124	19.60
9 (FC)	53.46	10.23	0.0040	0.0595	1.40
10 (FC)	57.43	10.99	0.0374	0.2120	12.06
11 (FC)	42.79	8.19	0.0038	0.0481	1.55
12 (FC)	56.39	10.79	0.0082	0.1624	4.62
13 (FC)	72.69	13.91	0.0029	0.1490	3.47
14 (FC)	58.79	11.25	0.0043	0.1284	2.33
15 (PC)	69.24	13.25	0.0170	0.1492	4.63
16 (PC)	63.54	12.16	0.0151	0.1522	6.25
17 (PC)	72.84	13.94	0.0145	0.1425	4.19
18 (PC)	61.04	11.68	0.0139	0.1371	4.16

Table 2.

Xylose extraction efficiency and concentration of xylose, HMF, furfural and phenols after pre-treatment of sugarcane bagasse according 2^3 face-centered full factorial design

Fig. 1. Pareto chart of standardized effects for a dependent variable:
a) % Efficiency and **b)** xylose concentration in hemicellulosic hydrolysate (g/L)

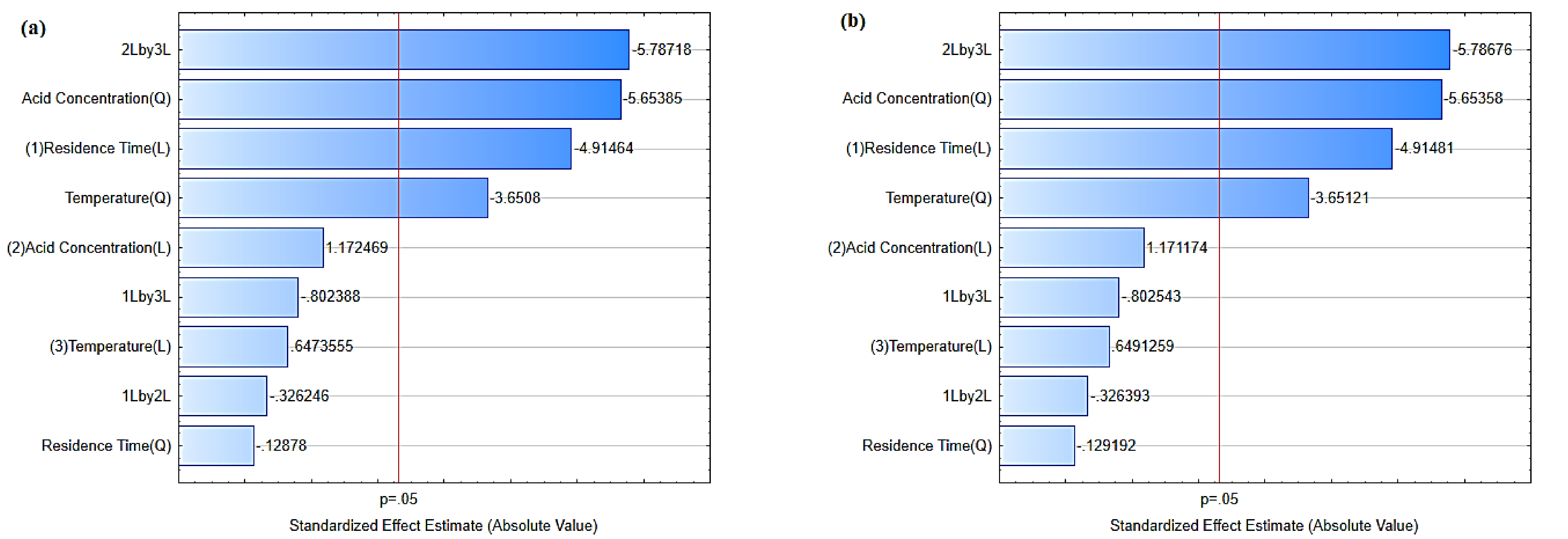


Table 3. ANOVA for dependent variable **% efficiency** for 2^3 face-centered full factorial design

Factor	SQ	GL	MQ	F	p-value
Regression	4026.40	9	447.38	19.32	0.00017
Residues	185.22	8	23.15		
Lack of Fit	99.05	5	19.81	0.69	0.666
Pure error	86.17	3	28.72		
Total	4211.61	17			

$R^2 = 0.956$; $R^2_{adj} = 0.907$; $p<0.05$ significant

Table 4. ANOVA for dependent variable **xylose concentration** in hemicellulosic hydrolysate 2^3 face-centered full factorial design

Factor	SQ	GL	MQ	F	p-value
Regression	147.44	9	16.38	19.32	0.00017
Residues	6.78	8	0.85		
Lack of Fit	3.62	5	0.72	0.69	0.667
Pure error	3.16	3	1.05		
Total	154.22	17			

$R^2 = 0.956$; $R^2_{adj} = 0.907$; $p<0.05$ significant

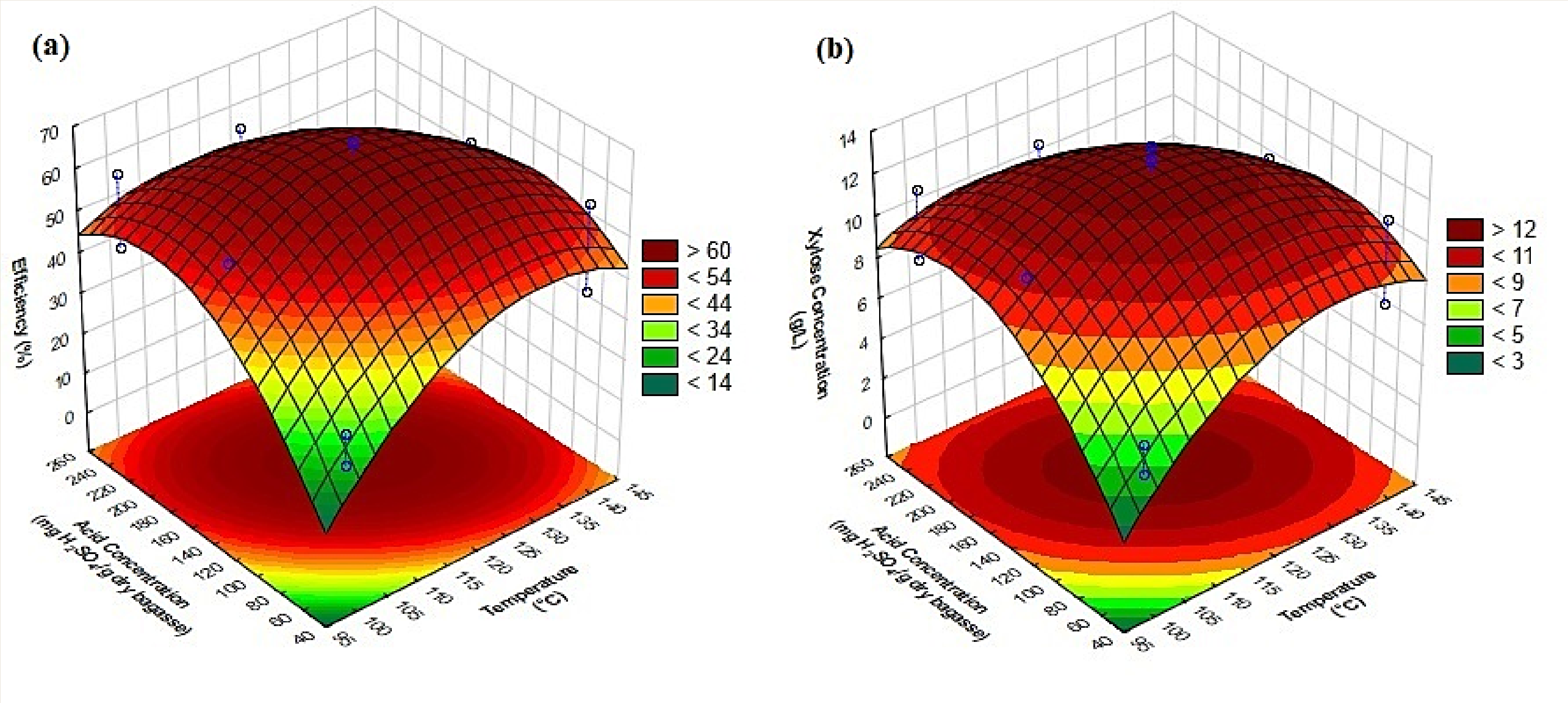
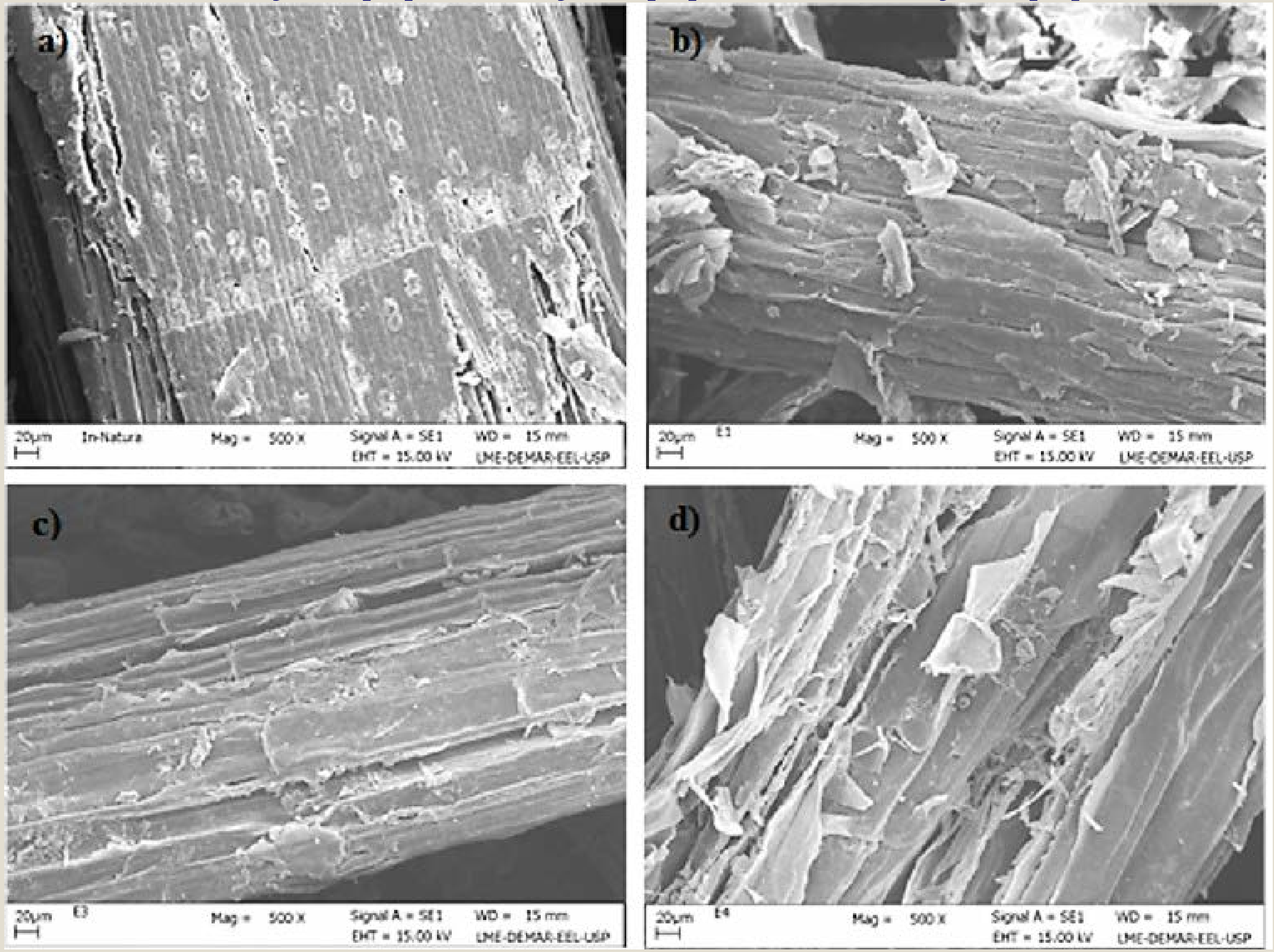


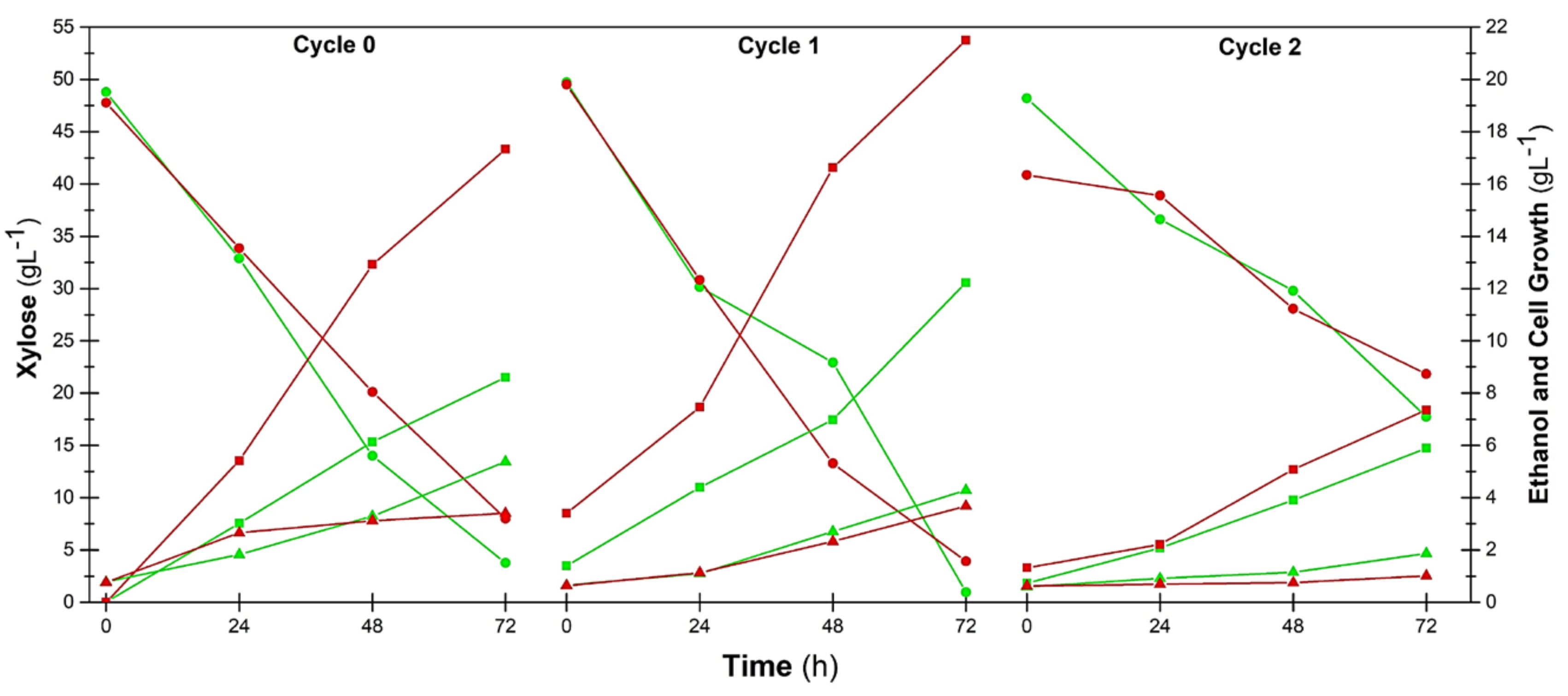
Fig. 2. Response surface plots showing influence of variables on response:

a) % Efficiency

b) Xylose concentration in hemicellulosic hydrolysate (g/L)

Fig. 3. Surface images obtained by SEM of the untreated sugarcane bagasse **(a)** and pre-treated bagasse under different operation variables: assay 1 **(b)**; assay 3 **(c)** and assay 4 **(d)**.





S. stipitis *S. shehatae*

● Xylose ■ Ethanol ▲ Cell growth

Fig. 4.

Time course profiles of ethanol production, cell growth and xylose concentration by 3 cycles of repeated-batch fermentation of *S. stipitis* and *S. shehatae* in sugarcane bagasse hemicellulosic hydrolysate.

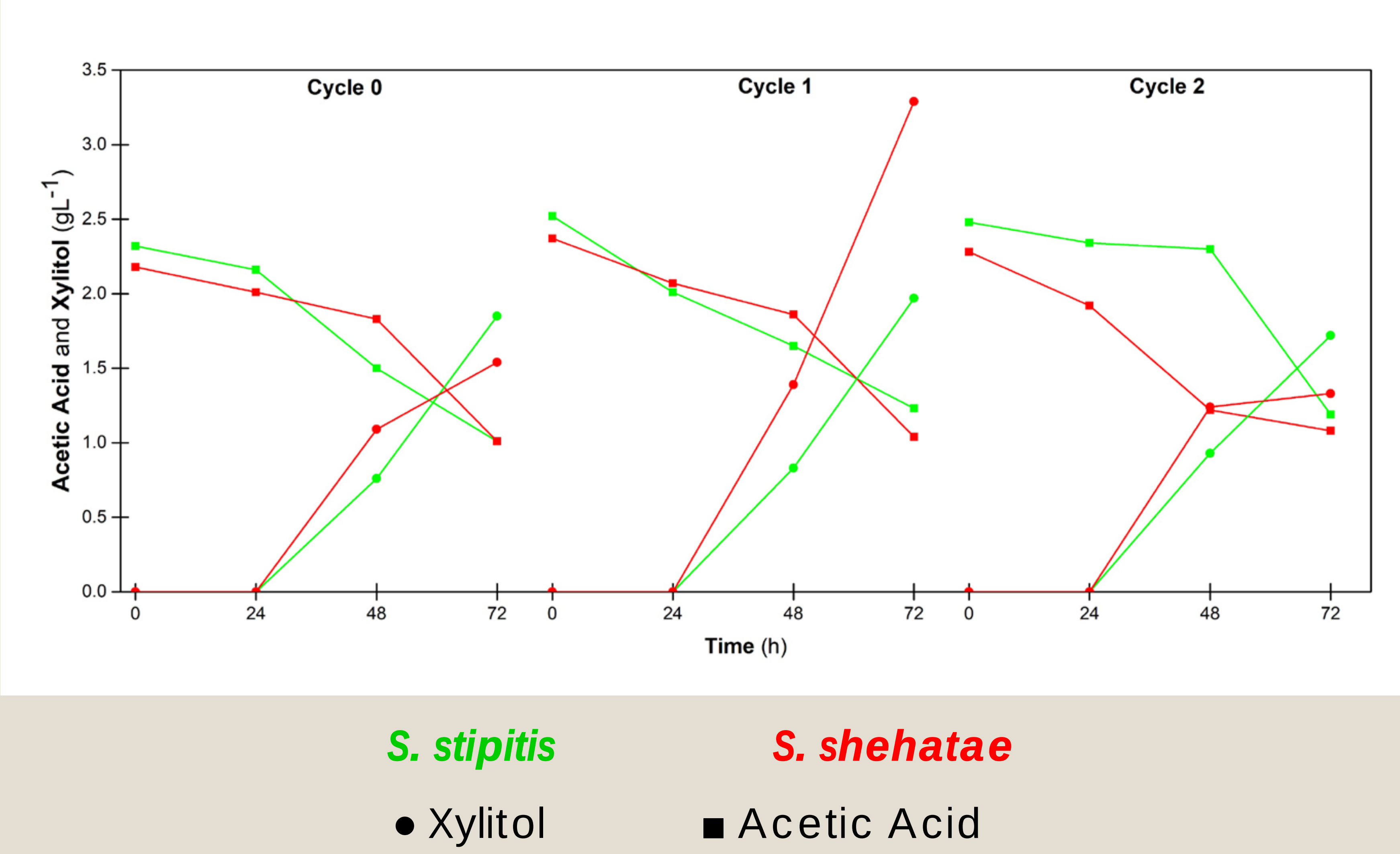
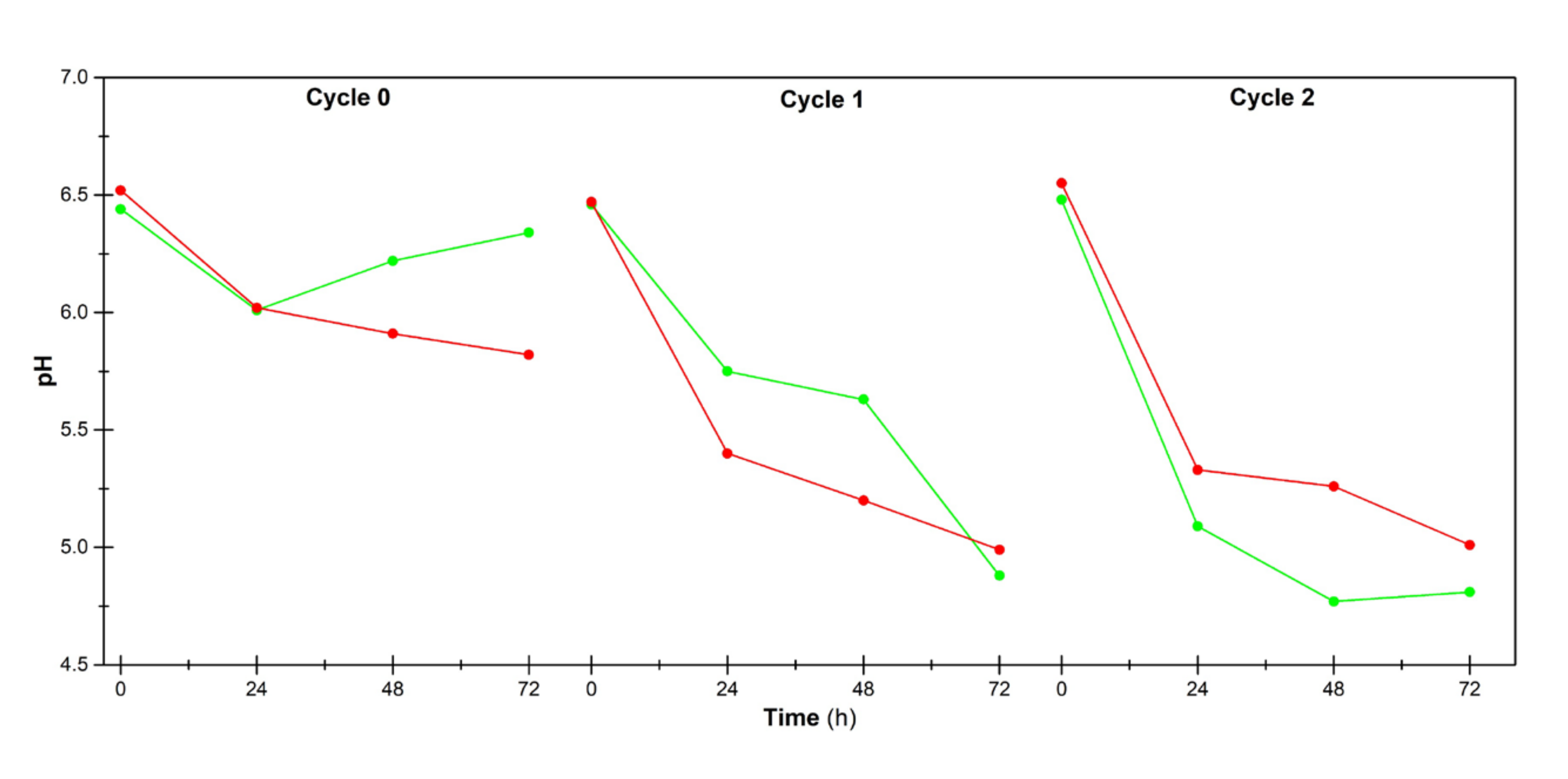


Fig. 5.

Time course profiles of xylitol and acetic acid concentration by 3 cycles of repeated-batch fermentation of *S. stipitis* and *S. shehatae* in sugarcane bagasse hemicellulosic hydrolysate.



S. stipitis *S. shehatae*

Fig. 6.

Time course profiles pH during 3 cycles of repeated-batch fermentation of *S. stipitis* and *S. shehatae* in sugarcane bagasse hemicellulosic hydrolysate.

Table 5. Evaluation of repeated-batch fermentation parameters for ethanol production using *S. stipitis* and *S. shehatae* in sugarcane bagasse hemicellulosic hydrolysate, after 216h total fermentation (72h for each cycle).

Batch Cycles	*Scheffersomyces stipitis*				*Scheffersomyces shehatae*			
	Ethanol (g)	$Y_{P/S}$ (g g^{-1})	Q_P (g L^{-1} h^{-1})	η (%)	Ethanol (g)	$Y_{P/S}$ (g g^{-1})	Q_P (g L^{-1} h^{-1})	η (%)
0	6.89	0.175	0.120	34.28	13.86	0.383	0.241	75.13
1	9.78	0.234	0.170	45.90	17.20	0.436	0.299	85.44
2	7.07	0.168	0.082	32.88	8.82	0.237	0.102	46.40
Total	23.74	0.192	0.040	37.70	39.88	0.398	0.066	77.98

$Y_{P/S}$: ethanol yield
Q_P: volumetric ethanol productivity
η: efficiency of sugars (xylose + glucose) conversion to ethanol

CONCLUSIONS

- **Pre-treatment** of sugarcane bagasse with **150 mg H_2SO_4/g dry bagasse**, at **127 °C** for **10 min** was **efficient** to obtain a **hemicellulosic hydrolysate** used for production of ethanol by *S. stipitis* and *S. shehatae* using the repeated-batch mode of fermentation.

- Both yeasts produced ethanol, however this production decreased after two-cycle repeated batch. ***S. shehatae* highlighted** for having **better ability** to **convert sugars** in **ethanol** than *S. stipitis*.

- The results, despite preliminaries, clearly indicate that the **combination** of **repeated-batch** operation and ***S. shehatae***, a yeast from Brazilian ecosystems, can be used for **bioethanol production** from **sugarcane bagasse** and have **potential** to be used for **industrial production** of this bioproduct, from others **lignocellulosic biomass**.

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