Co-production of ethanol and xylitol from sugarcane biomass hemicellulosic hydrolysate by yeasts isolated from the Atlantic Forest and the Brazilian Amazon Forest

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Introduction

Zero-waste

Changes in the energy matrix approaching renewable energy sources

The use of sugarcane bagasse as raw material in biorefineries is one strategy that has been intensively applied, because of its availability and its potential to meet current energy consumption

The cellulose and hemicellulose of sugarcane biomass are important sources of fermentable sugars for biorefineries:

Compositon of Sugarcane Biomass

Sugarcane bagasse

33 %	28 %	20 %
Cellulose	Hemicellulose	Lignin

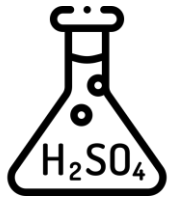
Sugarcane straw

30 %	23 %	20 %
Cellulose	Hemicellulose	Lignin

- Dilute acid hydrolysis is one of the more efficient pre-treatment strategies for releasing fermentable sugars
- Xylose fermenting by yeast would decrease the cost of producing ethanol from lignocellulosic materials
- Other products, such as xylitol can be obtained by fermenting media derived from xylose

Dilute acid hydrolysis

Release fermentable sugars



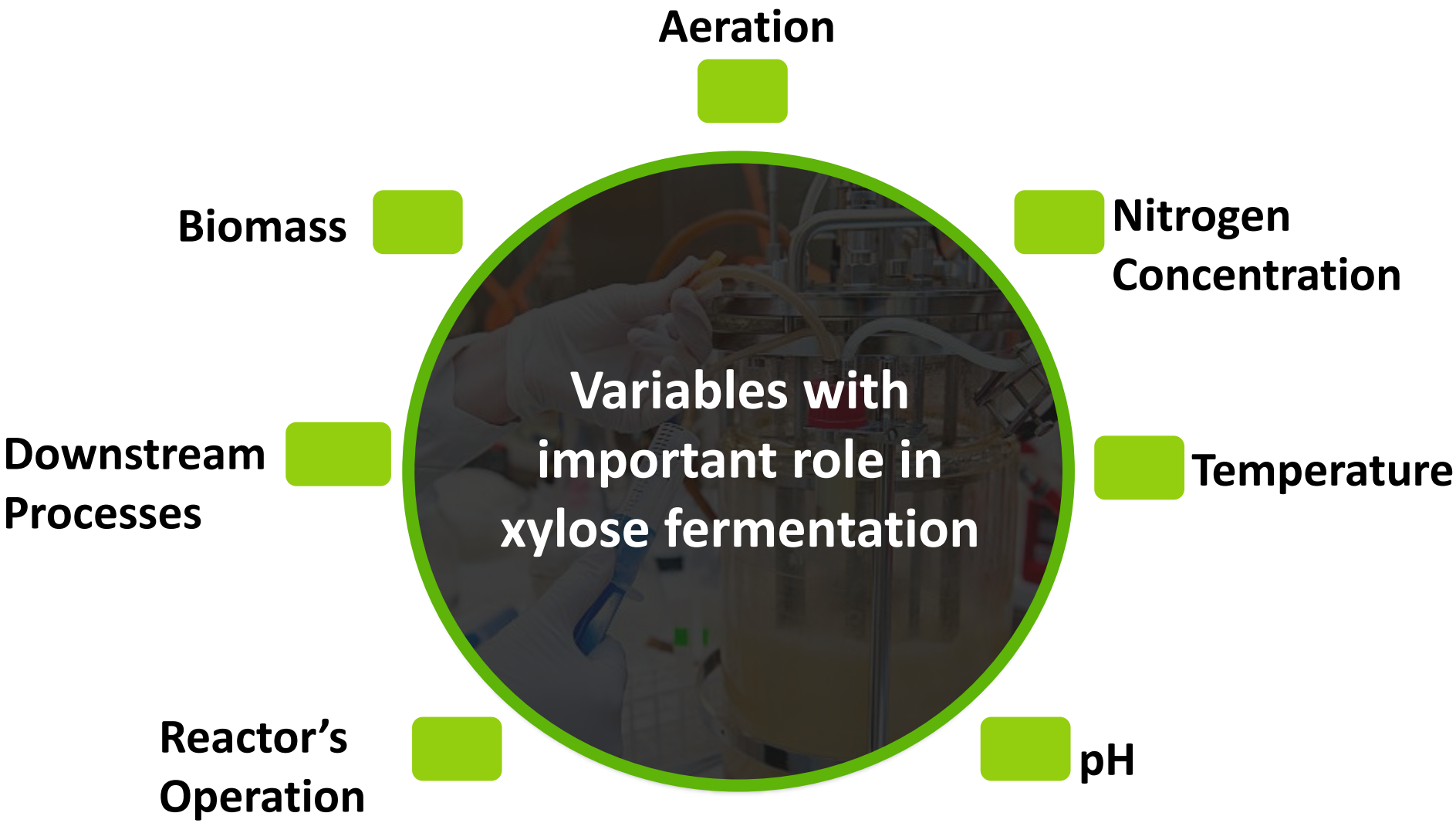
Xylose

is the most common hemicellulosic sugar

Bioprocess

Xylitol

Ethanol


Aeration
Biomass
Nitrogen Concentration
Variables with important role in xylose fermentation
Downstream Processes
Temperature
Reactor's Operation
pH

Efforts have been made in recent years to identify xylose-fermenting-yeasts

Isolated from decaying wood

Spathaspora brasiliensis

Atlantic Forest

Spathaspora boniae

Amazon Forest

There are no reports on the behavior of these yeasts using sugarcane biomass hemicellulosic hydrolysate as fermentation medium, especially optimizing nitrogen sources for the best fermentation performance.

The main objective

Investigating the fermentative performance of the yeasts *Spathaspora boniae* and *Spathaspora brasiliensis* to produce xylitol and/or bioethanol from sugarcane biomass hemicellulose hydrolysate by evaluating different organic and inorganic nitrogen sources

Spathaspora brasiliensis
Atlantic Forest

Spathaspora boniae
Amazon Forest

Materials and Methods

1 Microorganism

Spathaspora boniae (UFMG-CM-Y306) and *Spathaspora brasiliensis* (UFMG-HMD19.3) were kindly donated from the Microorganisms Collection of the Federal University of Minas Gerais

2 Sugarcane biomass hemicellulosic hydrolysate

pre-treatment: 1:10 solid:liquid ratio, 0.5% H_2SO_4 (w/v); 140 °C, 15 min

The composition of the hemicellulose hydrolysate was ($g.L^{-1}$): 26.29 xylose, 3.79 glucose, 3.58 arabinose, 3.77 acetic acid, 0.008 furfural and 0.42 total phenols

3 Detoxification of sugarcane biomass hemicellulosic hydrolysate

The pH was adjusted with calcium oxide (CaO) until pH 7.0, then with phosphoric acid (H_3PO_4) to pH 2.5, followed by the addition of 1.0% (w/v) activated charcoal and incubated in a shaking incubator at 60 °C, 100 rpm for 30 minutes

4 Fermentation

in 250mL Erlenmeyer flasks with 100mL of hydrolysate, initial pH= 5.5, inoculum concentration 1.0 $g.L^{-1}$, 30°C, 200 rpm for 72 hours

Nitrogen sources in different amounts (urea, yeast extract, peptone, and ammonium sulphate) were added to the hydrolysate for each experiment using a face-centered central composite design

5 Statistical analysis

A face-centered central composite design was proposed to optimize the independent variables (urea, yeast extract, peptone, and ammonium sulphate)

Results and Discussion

Responses to the face-centered central composite design for evaluating N sources on the production of ethanol and xylitol by *S. boniae*

	Nitrogen sources				Ethanol			Xylitol		
Assay	Urea ($g.L^{-1}$)	Yeast extract ($g.L^{-1}$)	Peptone ($g.L^{-1}$)	$(NH_4)_2SO_4$ ($g.L^{-1}$)	$Y_{p/s}$ (gg^{-1})	Q_p ($gL^{-1}h^{-1}$)	Ethanol (gL^{-1})	$Y_{p/s}$ (gg^{-1})	Q_p ($gL^{-1}h^{-1}$)	Xylitol (gL^{-1})
1	0.05	0.23	0.19	0.11	0.123	0.010	0.74	0.00	0.00	0.00
2	1.3	0.23	0.19	2.9	0.158	0.018	1.27	0.00	0.00	0.00
3	0.05	6.1	0.19	2.9	0.173	0.055	3.99	0.330	0.106	7.63
4	1.3	6.1	0.19	0.11	0.182	0.053	3.82	0.356	0.103	7.44
5	0.05	0.23	4.8	2.9	0.202	0.032	2.28	0.030	0.005	0.33
6	1.3	0.23	4.8	0.11	0.211	0.028	2.03	0.033	0.004	0.32
7	0.05	6.1	4.8	0.11	0.173	0.058	4.15	0.348	0.116	8.37
8	1.3	6.1	4.8	2.9	0.186	0.059	4.25	0.239	0.076	5.47
9	0.7	3.1	2.5	1.5	0.162	0,046	3.31	0.457	0.114	8.20
10	0.7	3.1	2.5	1.5	0.192	0,050	3.57	0.513	0.115	8.31
11	0.7	3.1	2.5	1.5	0,243	0,063	4.56	0.451	0.103	7.41
12	0.05	3.1	2.5	1.5	0.152	0.039	2.83	0.386	0.100	7.20
13	1.3	3.1	2.5	1.5	0.130	0.046	3.34	0.367	0.131	9.40
14	0.7	0.23	2.5	1.5	0.200	0.037	2.67	0.078	0.015	1.05
15	0.7	6.1	2.5	1.5	0.157	0.054	3.86	0.437	0.149	10.74
16	0.7	3.1	0.19	1.5	0.101	0.049	3.49	0.180	0.086	6.23
17	0.7	3.1	4.8	1.5	0.154	0.057	4.10	0.328	0.121	8.71
18	0.7	3.1	2.5	0.11	0.161	0.053	3.79	0.338	0.111	7.96
19	0.7	3.1	2.5	2.9	0.171	0.062	4.44	0.301	0.108	7.81
20	0.7	3.1	2.5	1.5	0.180	0.065	4.66	0.295	0.106	7.66

Assay 15 (yeast extract at the top level): the **highest xylitol** formation

Comparing assays **15** and **14**: **confirmation** that **increasing** the **yeast extract**, while with other N sources remain fixed at level 0 ⇒ **increasing xylitol** production by tenfold

The yeast extract has a significant impact on the fermentation of sugarcane biomass hemicellulose hydrolysate by *S. boniae*

Responses to the face-centered central composite design for evaluating N sources production of ethanol and xylitol by *S. brasiliensis*

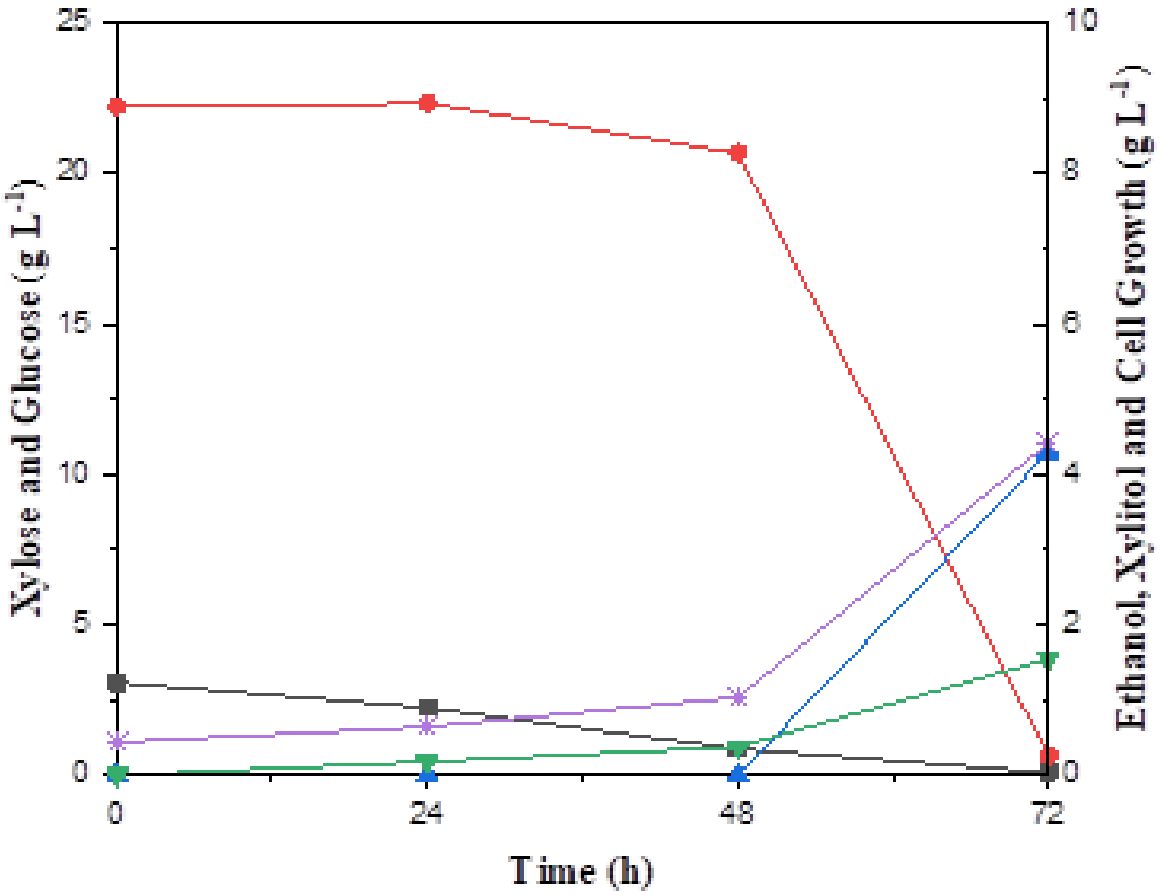
	Nitrogen sources				Ethanol			Xylitol		
Assay	Urea (gL^{-1})	Yeast extract (gL^{-1})	Peptone (gL^{-1})	$(NH_4)_2SO_4$ (gL^{-1})	$Y_{p/s}$ (gg^{-1})	Q_p ($gL^{-1}h^{-1}$)	Ethanol (gL^{-1})	$Y_{p/s}$ (gg^{-1})	Q_p ($gL^{-1}h^{-1}$)	Xylitol (gL^{-1})
1	0.05	0.23	0.19	0.11	0.077	0.023	1.65	0.135	0.034	2.44
2	1.3	0.23	0.19	2.9	0.102	0.032	2.32	0.432	0.118	8.46
3	0.05	6.1	0.19	2.9	0.072	0.025	1.78	0.507	0.154	11.09
4	1.3	6.1	0.19	0.11	0.054	0.019	1.40	0.495	0.156	11.22
5	0.05	0.23	4.8	2.9	0.062	0.021	1.55	0.399	0.120	8.65
6	1.3	0.23	4.8	0.11	0.063	0.024	1.70	0.342	0.112	8.09
7	0.05	6.1	4.8	0.11	0.053	0.020	1.41	0.451	0.147	10.58
8	1.3	6.1	4.8	2.9	0.096	0.029	2.09	0.494	0.029	9.34
9	0.7	3.1	2.5	1.5	0.092	0.029	2.11	0.393	0.102	7.31
10	0.7	3.1	2.5	1.5	0.153	0.046	3.29	0.416	0.105	7.53
11	0.7	3.1	2.5	1.5	0.055	0.017	1.22	0.368	0.098	7.04
12	0.05	3.1	2.5	1.5	0.101	0.035	2.51	0.339	0.103	7.43
13	1.3	3.1	2.5	1.5	0.093	0.037	2.68	0.328	0.114	8.18
14	0.7	0.23	2.5	1.5	0.104	0.036	2.63	0.204	0.057	4.12
15	0.7	6.1	2.5	1.5	0.120	0.042	3.04	0.354	0.111	7.96
16	0.7	3.1	0.19	1.5	0.129	0.044	3.16	0.327	0.098	7.05
17	0.7	3.1	4.8	1.5	0.096	0.034	2.42	0.437	0.135	9.69
18	0.7	3.1	2.5	0.11	0.119	0.041	2.94	0.363	0.105	7.59
19	0.7	3.1	2.5	2.9	0.121	0.039	2.81	0.407	0.111	7.99
20	0.7	3.1	2.5	1.5	0.122	0.036	2.57	0.405	0.102	7.35

Assay 4: the **highest xylitol production**

Assays 14 and **15**: the **yeast extract** has a **positive influence** on **xylitol production** than the other variables

Fermentative performance of *S. boniae* in sugarcane biomass hemicellulosic hydrolysate

- Glucose was preferably consumed in the first 48 hours fermentation
- The highest amount of xylose consumed after 48 hours
- This result suggests that the presence of glucose can inhibit or delay the consumption of xylose by *S. boniae* (catabolic repression ?!)

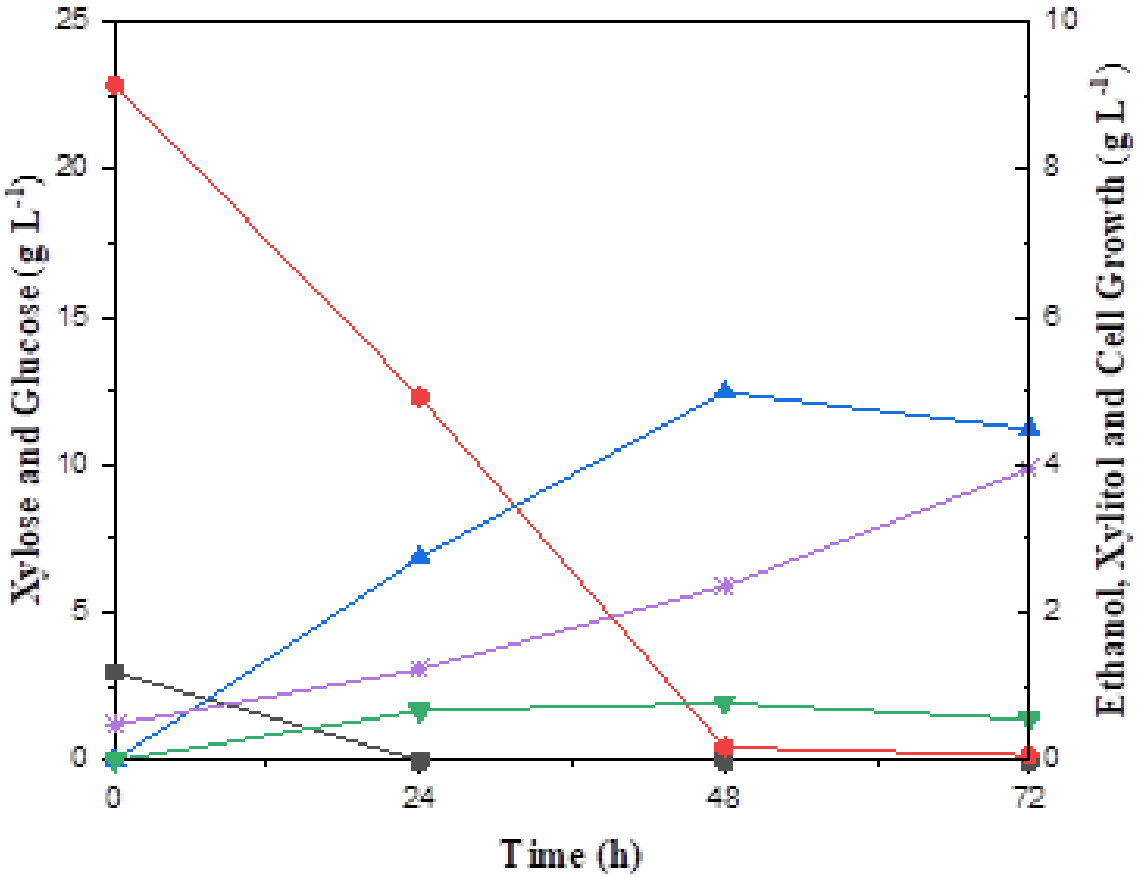


Consumption of glucose (**black**) and xylose (**red**); formation of xylitol (**blue**) and ethanol (**green**); cell growth (**purple**) after 72h fermentation (**assay 15**)

Fermentative performance of *S. brasiliensis* in sugarcane biomass hemicellulosic hydrolysate

- *S. brasiliensis* presented a simultaneous consumption of glucose and xylose
- This demonstrates that the presence of glucose did not lead to a diauxic growth or carbon catabolic repression in *S. brasilienis*

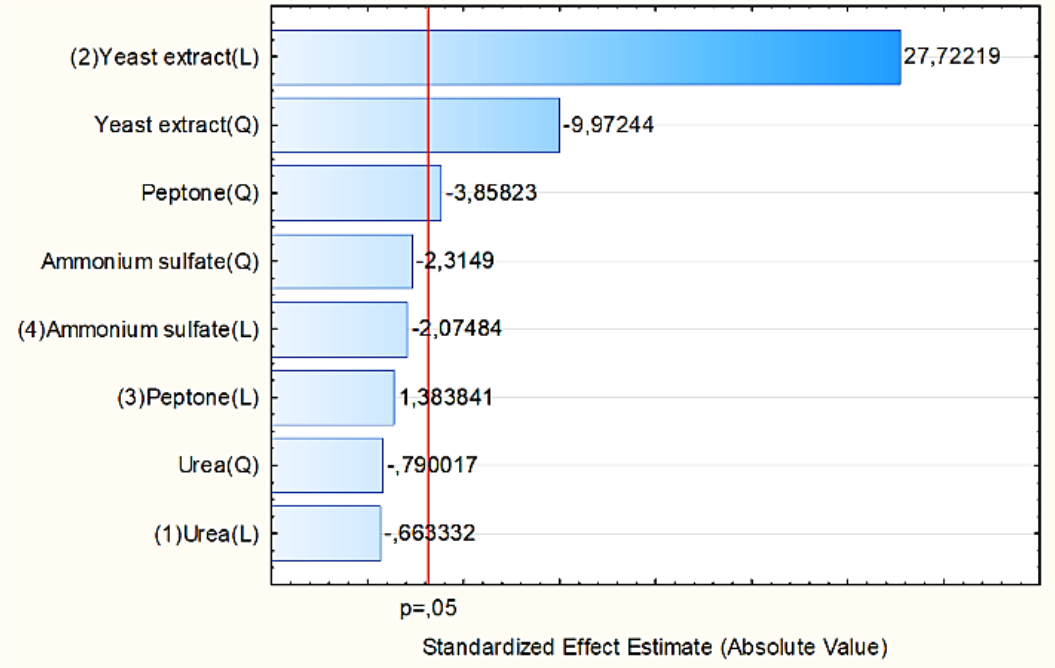
Also, it is important to highlight that both yeast-species could assimilate arabinose and acetic acid present in the hydrolysate



Consumption of glucose (**black**) and xylose (**red**); formation of xylitol (**blue**) and ethanol (**green**); cell growth (**purple**) after 72h fermentation (**assay 4**)

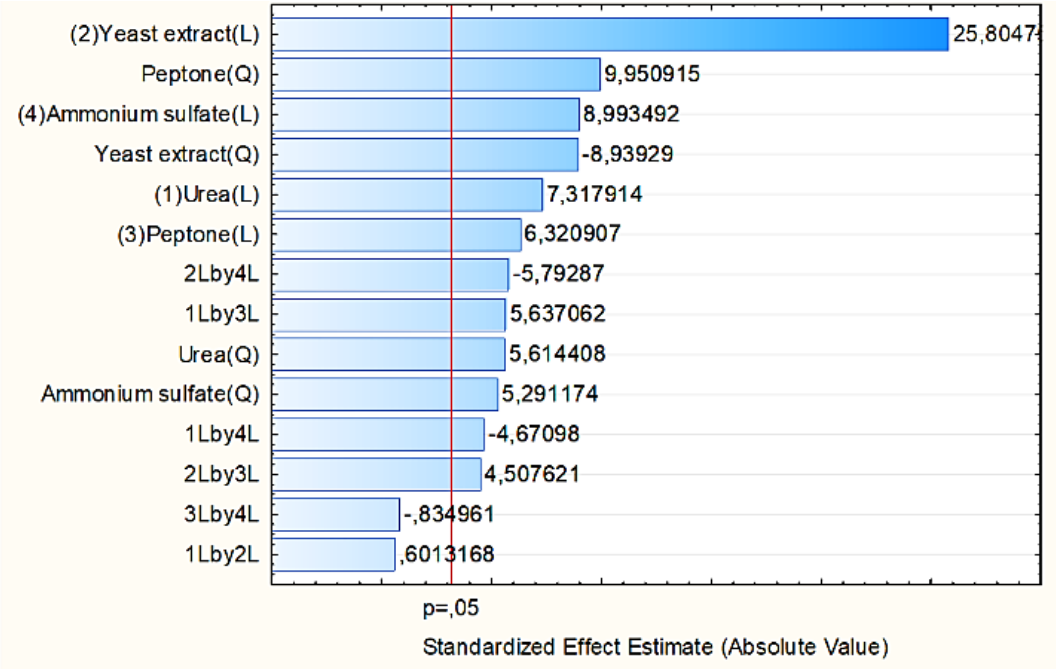
S. boniae - Statistical analysis

The variable concentrations of yeast extract and peptone had a greater influence on the xylitol production response (g L^{-1})



S. Brasiliensis - Statistical analysis

The significant variables are yeast extract, ammonium sulfate, urea, peptone and the interactions for linear terms 2 and 4, 1 and 3, 1 and 4, 2 and 3



Pareto charts indicating the effects of the variables (X_1) urea, (X_2) yeast extract, (X_3) peptone, and (X_4) ammonium sulfate, as well as their interactions with the fermentation parameter of xylitol concentration (g L^{-1}) during sugarcane bagasse hemicelluloses hydrolysate fermentation

The correlation coefficient for *S. boniae* (R^2 = 0.9432)

The correlation coefficient for *S. brasiliensis* (R^2 = 0.9945)

Analysis of variance (ANOVA) of the adjusted model from xylitol production (g L^{-1}) by *S. boniae* and *S. brasiliensis*

Factor	SQ	GL	MQ	F	p-Valor
Regression	222.9985	8	27.8748	22.92754014	8.222E-06
Residues	13.3736	11	1.2158		
Lack of Fit	12.8116	8	1.6014	8.549098946	0.05238
Pure Error	0.5620	3	0.1873		
Total SS	236.3720	19			

Factor	SQ	GL	MQ	F	p-Valor
Regression	80.76159	14	5.7687	65.2459	0.000106501
Residues	0.44207	5	0.0884		
Lack of Fit	0.32082	2	0.1604	3.9687	0.143652556
Pure Error	0.12126	3	0.0404		
Total SS	81.20366	19			

$$Xylitol\ (g\ L^{-1}) = -0.89 + 0.60 \times x1 - 0.55 \times x1^2 + 3{,}28 \times x2 - 0.31 \times x2^2 + 1.07 \times x3 - 0.20 \times x3^2 + 0.77 \times x4 - 0.32 \times x4^2$$

The model can explain 94.32 % of the response variability

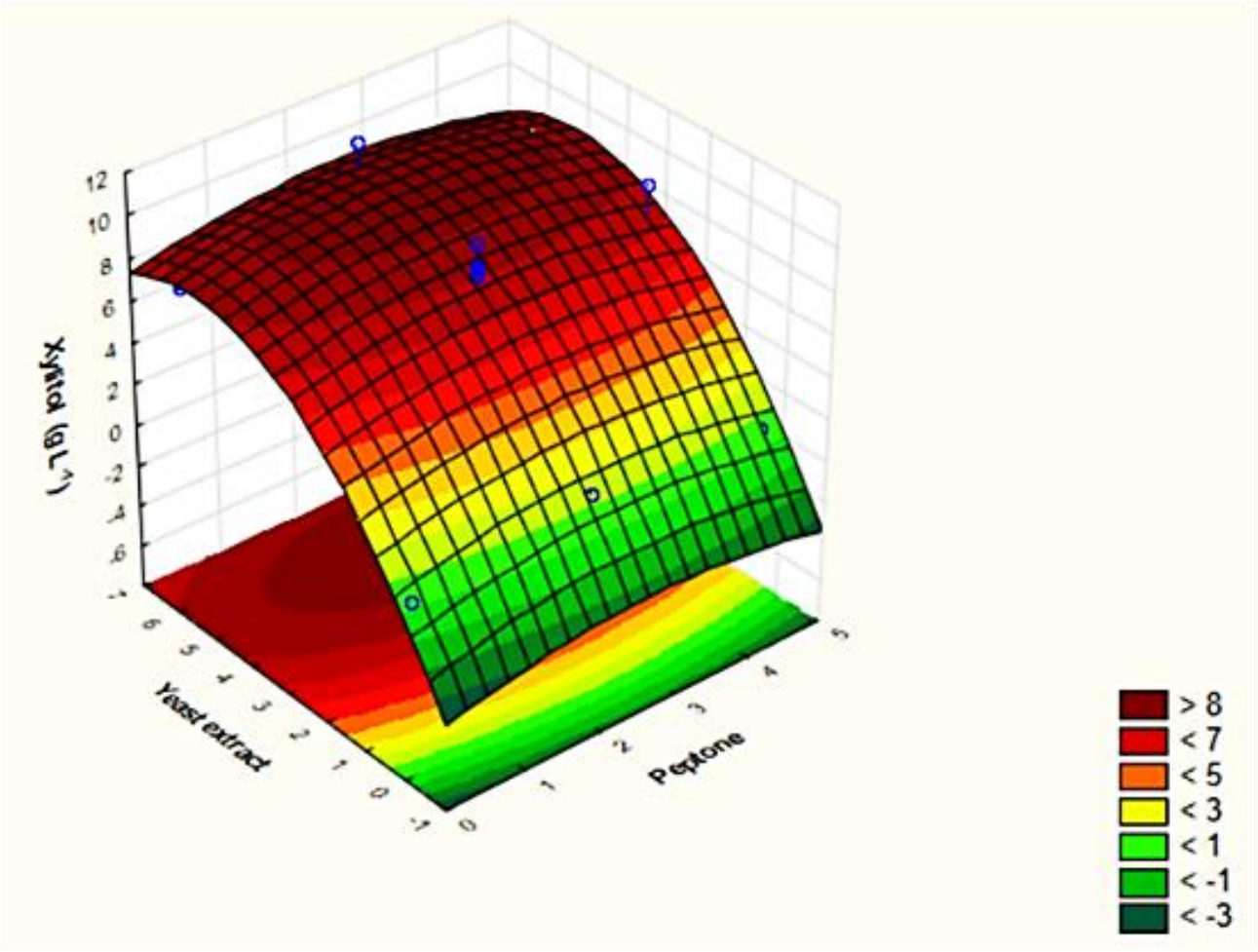
$$Xylitol\ (g\ L^{-1}) = 2.30 - 16.31 \times x1 + 1.81 \times x1^2 + 3{,}66 \times x2 - 0.13 \times x2^2 - 29.69 \times x3 + 0.24 \times x3^2 + 52.96 \times x4 + 0.34 \times x4^2 + 1.15 \times x1 \times x2 + 24.82 \times x1 \times x3 - 33.85 \times x1 \times x4 + 4.21 \times x2 \times x3 - 8.98 \times x2 \times x4 - 0.91 \times x3 \times x4$$

The model can explain 99.45% of the response variability

The proposed models were significant, and the lack of fit test was not statistically significant (p-value >0.05), indicating the fitted model's capacity for predicting the response

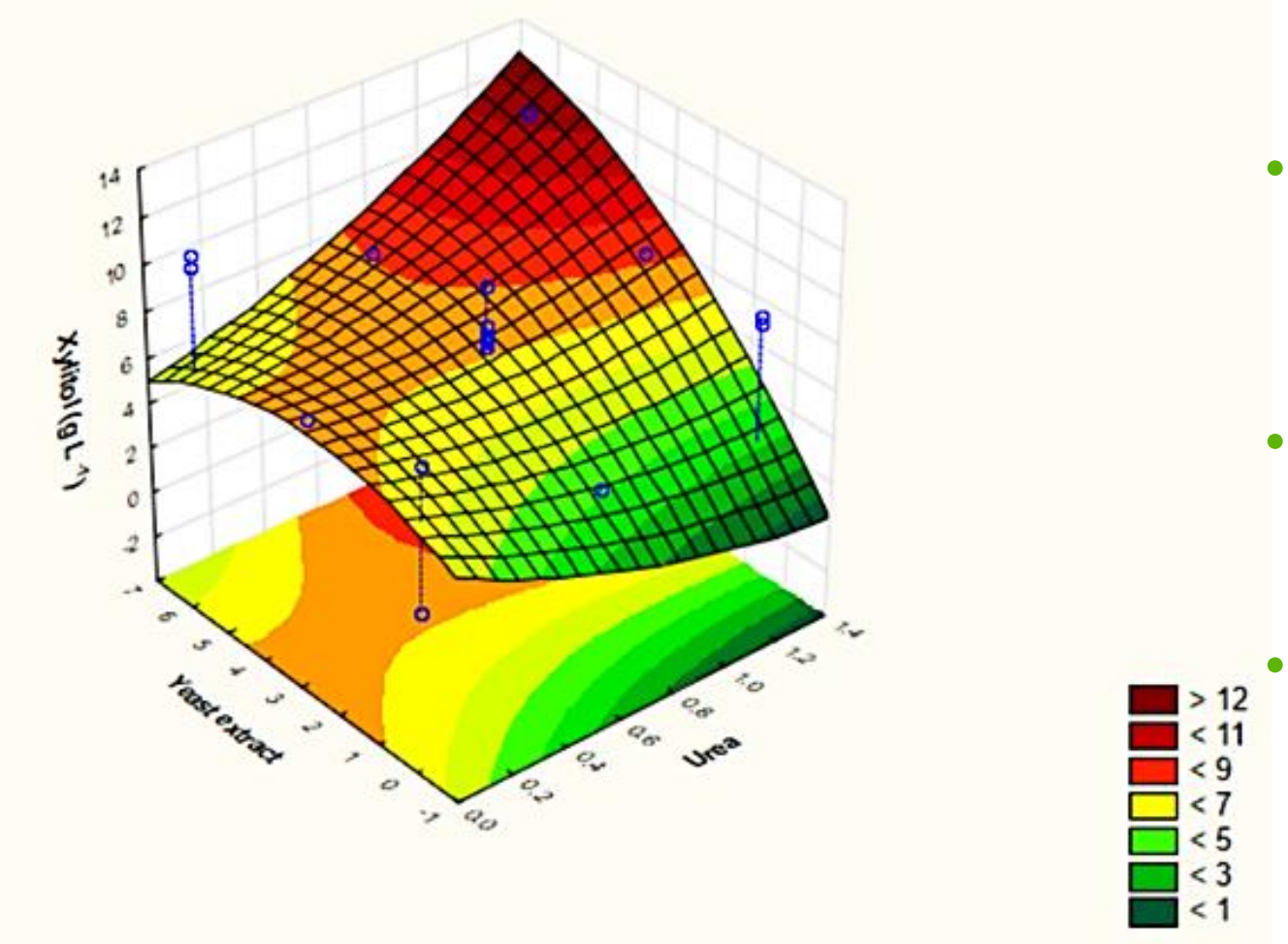
S. boniae

- The optimal concentration of yeast extract is between 5 and 6 g. L^{-1}, peptone is between 2 and 3 g. L^{-1}
- The desirability function predicted that the highest xylitol production was 9.72 g L^{-1}
- The optimal concentrations of the variables were estimated to be 0.58 g L^{-1} urea, 5.26 g L^{-1} yeast extract, 2.82 g L^{-1} peptone, and 1.3 g L^{-1} ammonium sulfate



Response surface for xylitol (g L^{-1}) production by *Spathaspora boniae* UFMG-CM-Y306

S. brasiliensis



Response surface for xylitol (g L^{-1}) production by *Spathaspora brasiliensis* UFMG-HMD19.3(b)

- The optimal concentration of yeast extract is between 4 and 5 g. L^{-1}, urea is between 1.2 and 1.4 g. L^{-1}
- The desirability function predicted that the highest xylitol production was 12.64 g L^{-1}
- The optimal concentrations of the variables were estimated to be 1.3 g L^{-1} urea, 4.42 g L^{-1} yeast extract, 4.8 g L^{-1} peptone, and 2.9 g L^{-1} ammonium sulfate

Conclusions

- The two new yeasts isolated from Brazilian biomes were able to ferment sugars present in the sugarcane biomass hemicellulosic hydrolysate
- *Spathaspora brasiliensis* showed more ability to assimilated sugars and produce xylitol
- *Spathaspora boniae* was able to growth in hydrolysate non-detoxificated, showing more resistance to the inhibitors
- It were also determined the best conditions for nitrogen source supplementation of hydrolysate for *S. boniae* and for *S. brasiliensis*, verifying that the decrease in the yeast extract concentration leads to maximum xylitol production by yeasts
- These yeasts are promising to be used in biotechnological xylitol production from sugarcane biomass hemicellulosic hydrolysate and could be applied in a biorefinery

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