Growth characterization of adapted *Rhodosporidium toruloides* in sugarcane biomass hemicellulosic hydrolysate

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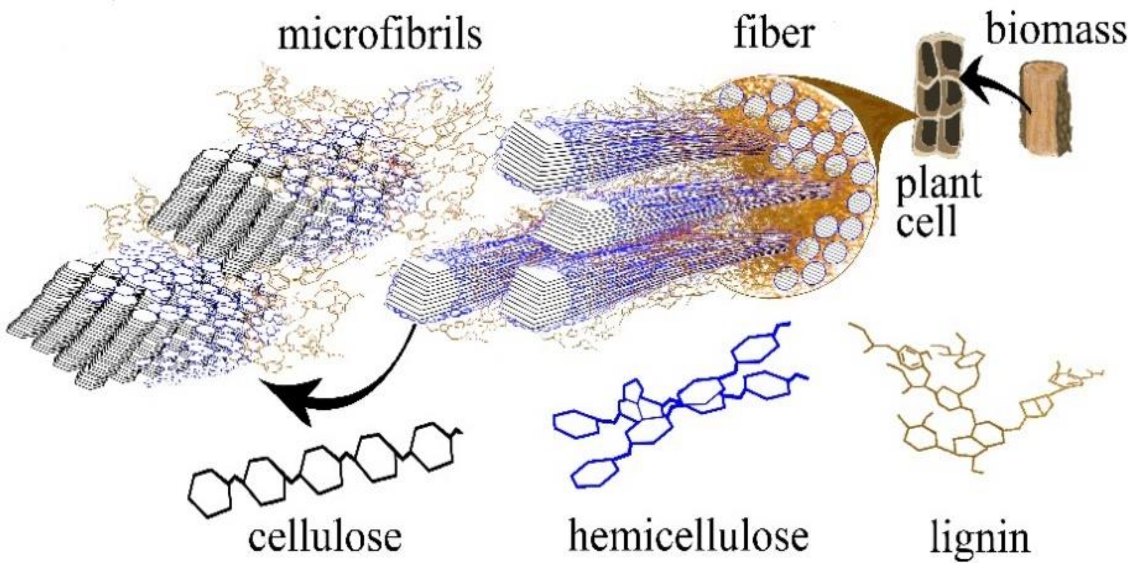
Araraquara

Introduction

❖ Biomass

Scientific efforts have been demanded focus on to the diversification of the energy matrix, the obtainment of biomolecules and building blocks from renewable sources.



The sugars, present in the cellulosic and hemicellulosic portions of the biomass, represent more than **60%** of the biomass composition and the others **40%** being composed mainly of lignin, a polyphenolic biomolecule.

de Almeida, Sâmilla GC *et al*. 2022. Chapter: Methods for hemicellulose deconstruction aiming to xylose recovery: Recent progress and future perspectives

Introduction

❖ Biomass

- Valorization of agro-industrial waste, and lignocellulosic biomass has emerged as a potential raw material because of its **availability**, and **capacity to be processed** to obtain value-added products.

Biofuels

Functional Ingredients

Building blocks

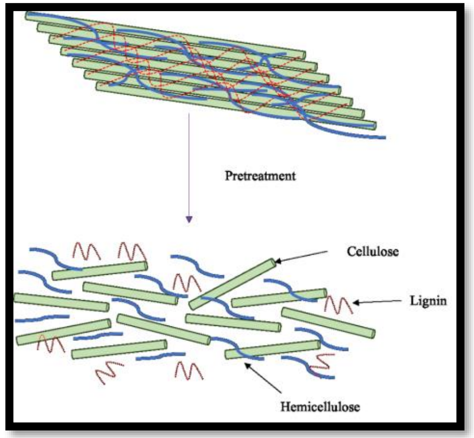
Introduction

❖ Chemical Pretreatment

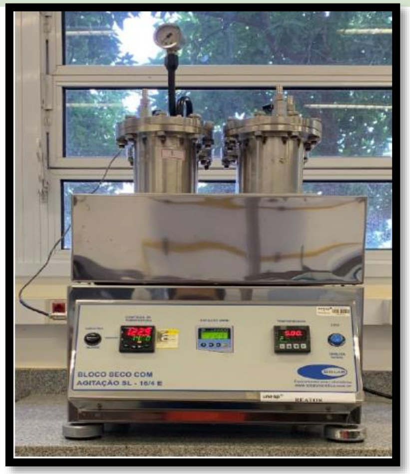
Nonspecific processes

Formation of a variety of **undesirable subproducts**

Furanic derivative, weak acids and phenolic compounds.



Biomass chemical pretreatment



Inhibitors of fermentative process

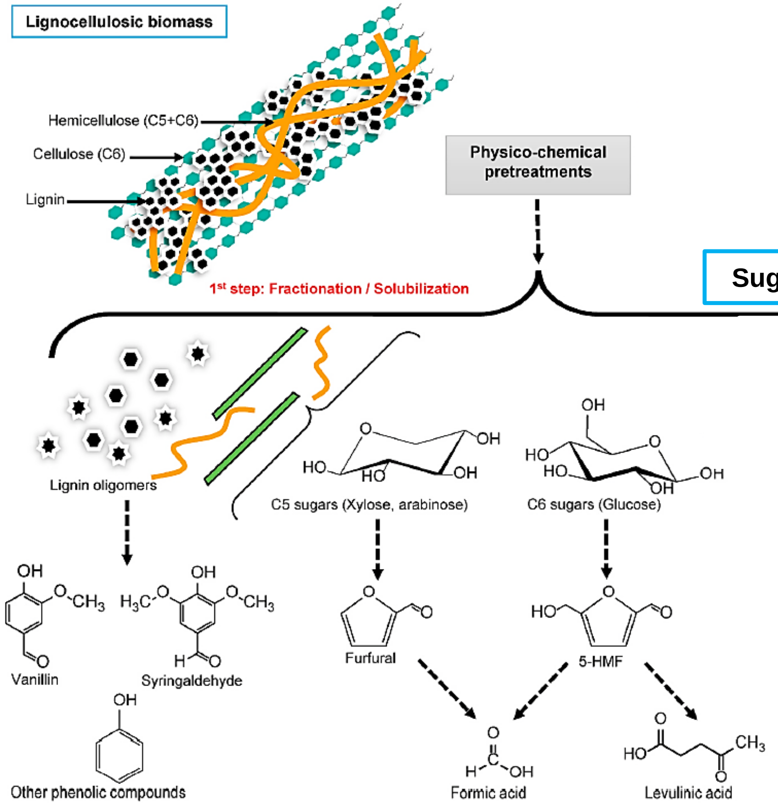
- **Adaptation:** alternative to replace the detoxification process and consists in the **microorganism acclimation** into gradually increased concentrations of the hydrolyzed in the presence of the toxic compounds, inducing the microorganism to adapt itself in the growth medium through its innate capability, which is the result of a selective pressure by the medium and the development of a metabolic apparatus.

Latif *et al.*, Ammonia-based pretreatment for ligno-cellulosic biomass conversion – an overview, 2018.

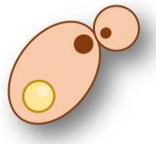
Introduction

❖ Bioprocess

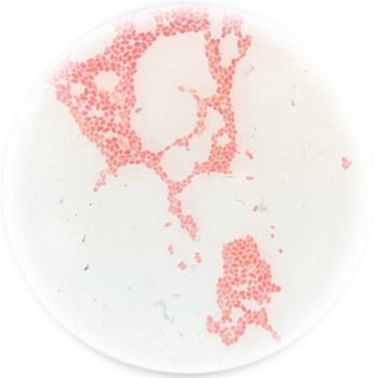
- The **oleaginous yeasts** can consume the xylose from a wide range of materials ⇒ they are able to grow in the presence of compounds that breaks down the biomass, being able to metabolize these compounds present in the hemicellulosic hydrolysate ⇒ favoring the implementation of these types of bioprocesses.



Sugarcane Biomass



Lipids Accumulation



Basak *et al.,* Dark fermentative hydrogen production from pretreated lignocellulosic biomass: Effects of inhibitory byproducts and recent trends in mitigation strategies, 2020.

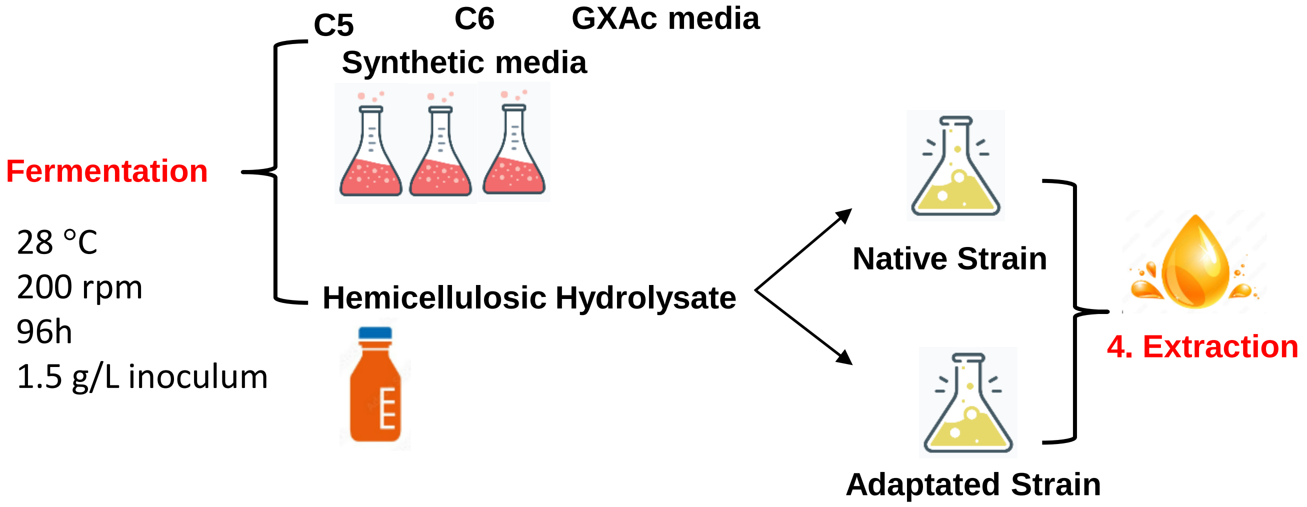
Goals

Exploitation of sugarcane straw and bagasse to produce lipid via yeast by exploring the:

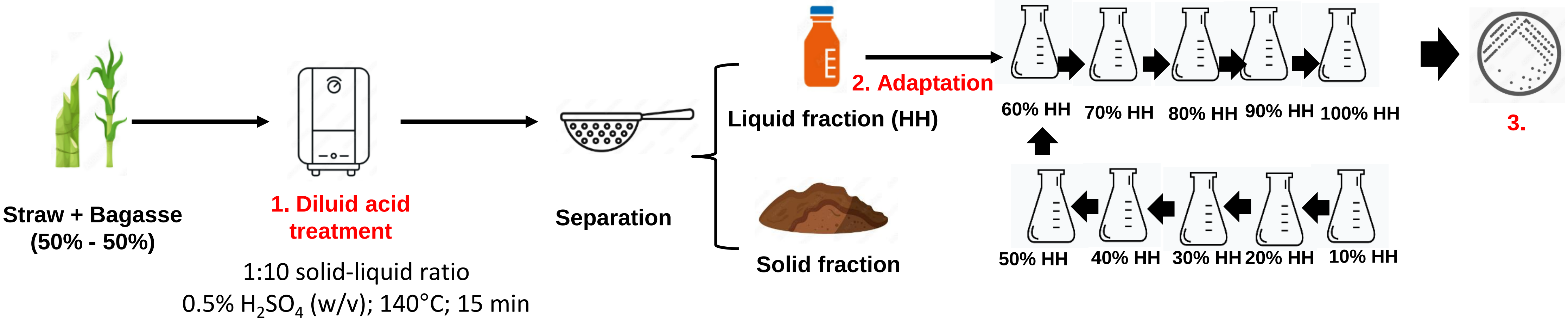
- ✓ Three strains were cultivated in synthetic media to assess the pattern of consumption of carbon sources and strain growth
- ✓ Adaptative strategy in hemicellulosic hydrolysate
- ✓ The growth profile, lipid production of adapted and no-adapted cultivated in the non-detoxified hemicellulosic hydrolysate were compared for microbial lipid production

Approach

❖ *Fermentation*



1. Sugarcane biomass pre-treatment
2. For adaptation of strain and lipids production, the sugarcane hydrolysate was used as culture media, pH=5.5 (**incremented hydrolysate concentrations**)
3. At the end of the cultivation, 100 µL of broth were spread on agar plate YMA and incubated, obtaining the **adapted strain** from *R. toruloides*
4. Extraction and quantified gravimetrically: mixture of chloroform:methanol (2:1) and incubated in a thermostatic bath at 20 °C for 24 h (Folch et al. (1957) modified). Samples were incubated in an ultrasonic bath for 10 minutes, vortexed for 1 minute, and 2 mL of NaCl (1M) was added to each tube. The resulting mixture was centrifuged at 3000 rpm for 5 min to separate the two phases: **aqueous and organic phases**.



Results and Discussion

Table 1. Comparison of lipid production by *R. toruloides* on synthetic media

Yeast	pH_i	pH_f	Biomass ($g.L^{-1}$)	Consumption of C (%)	Lipid accumulation % ($m.m^{-1}$)	Yield biomass on carbohydrate $Y_{X/S}$ ($g.g^{-1}$)	Yield lipid on carbohydrate $Y_{P/S}$ ($g\ g^{-1}$)	Lipid production rate QP ($mg.\ L^{-1}.h^{-1}$)
C5								
2781	4.68	3.03	7.09	30.74	3.92%	0.366	0.0168	2.89
2882	5.03	3.54	7.30	26.54	4.87%±0.770%	0.428	0.0248	3.706±0.5840
2896	5.16	4.46	11.44	41.26	4.69%±0.350%	0.492	0.0260	5.595±0.4169
C6								
2781	4.99	2.57	10.86	34.91	6.16%±0.590%	0.493	0.000294	0.059±0.0057
2882	5.27	3.03	10.92	32.81	7.42%±0.506%	0.571	0.000396	0.071±0.0048
2896	5.22	2.69	9.74	36.69	6.70%±0.177%	0.432	0.000359	0.072±0.0019

- The three strains of *R. toruloides* were cultivated in synthetic media to compare the ability of the strains to metabolize different sources of sugars
- The only strain that showed greater growth capacity in xylose (**C5**) was *R. toruloides* 2896
- *R. toruloides* 2781 and *R. toruloides* 2882 showed more expressive growth in glucose (**C6**)

Results and Discussion

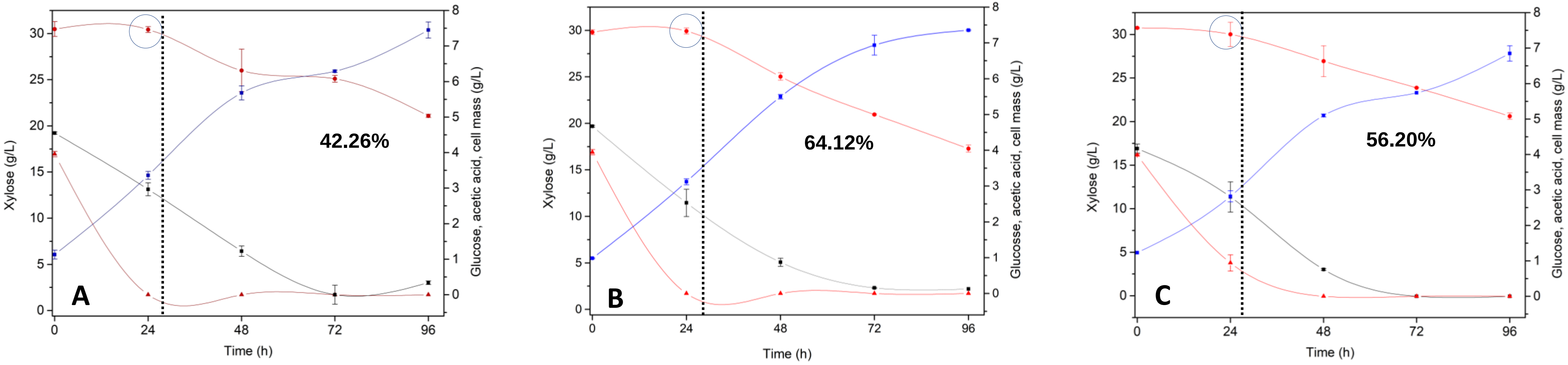


Fig. 1. Fermentation of synthetic medium with glucose, xylose and acetic acid (MGXAc) by (**A**) *R. toruloides* 2882, (**B**) *R. toruloides* 2896 and (**C**) *R. toruloides* 2781 at 28°C and 200 rpm. -▲- Glucose, -●-Xylose, -■- acetic acid, -■- cell mass

- In synthetic medium containing acetic acid (GXAc media): simultaneous consumption of glucose and xylose was not observed.
- Xylose consumption only started after 24 hours, with the exhaustion of glucose.

Results and Discussion

❖ *Adaptation yeast: increasing resistance to growth*

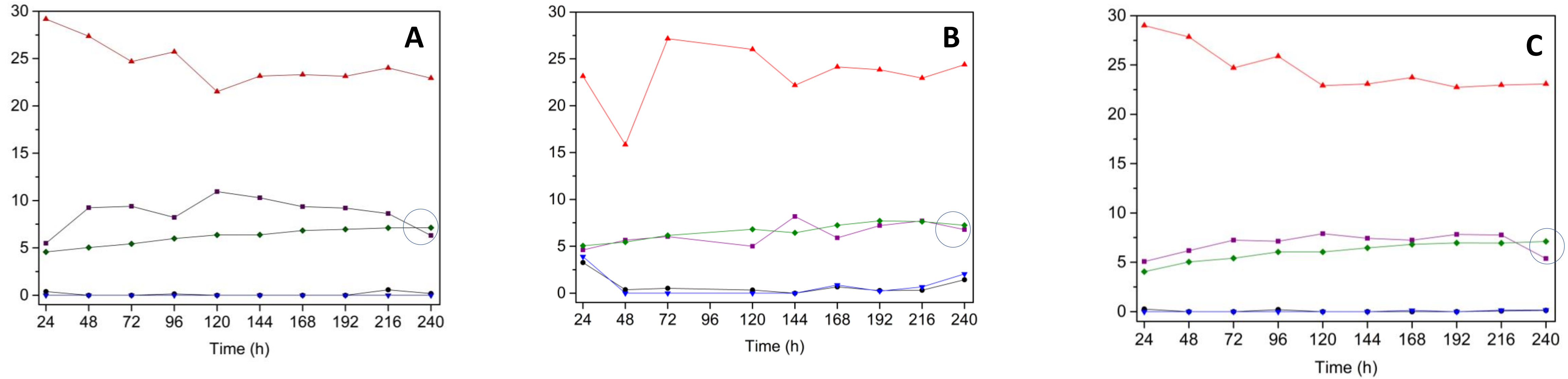
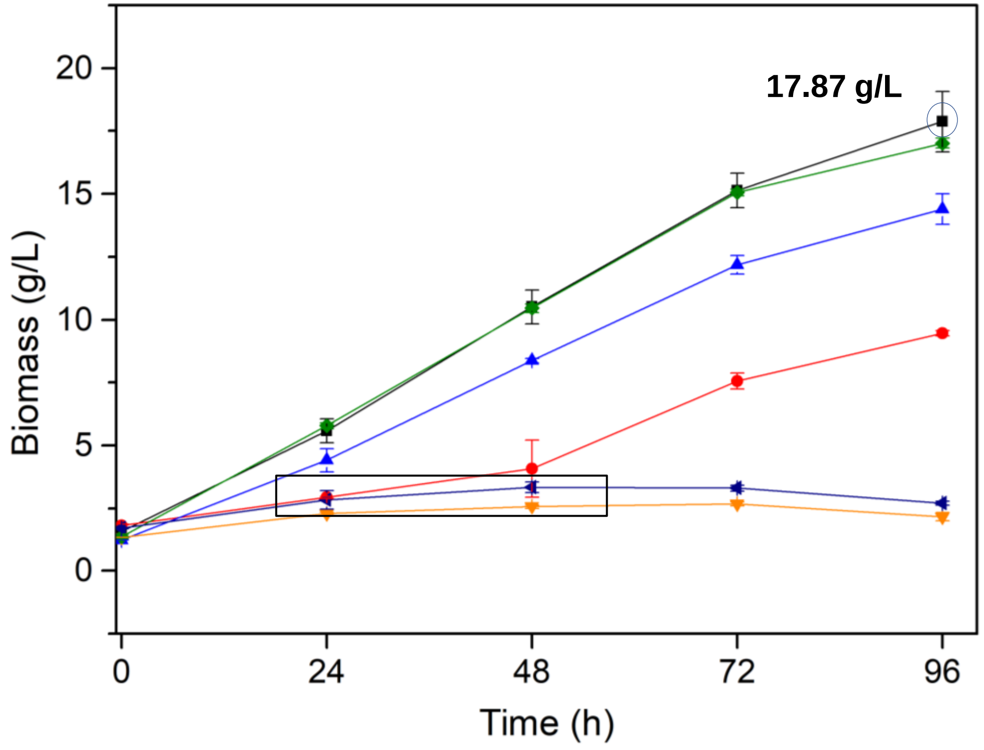


Fig. 2. Temporal profile of biomass accumulation (-■-), pH (-◆-), consumption of glucose (-●-), xylose (-▲-) and acetic acid (-▼-) by *R. toruloides* 2882 (**A**), *R. toruloides 2896* (**B**) and *R. toruloides* 2781 (**C**) during the adaptation phase in HHB

- All strains were able to grow in 100% supplemented hydrolysate
- The cell concentration reached at the 10th level of adaptation (100% HHB) was higher to *R. toruloides* 2896, followed by *R. toruloides* 2882 and *R. toruloides* 2781
- In each adaptation cycle (lasting 24 hour) glucose was depleted as a preferred carbon source, for all strains

Results and Discussion



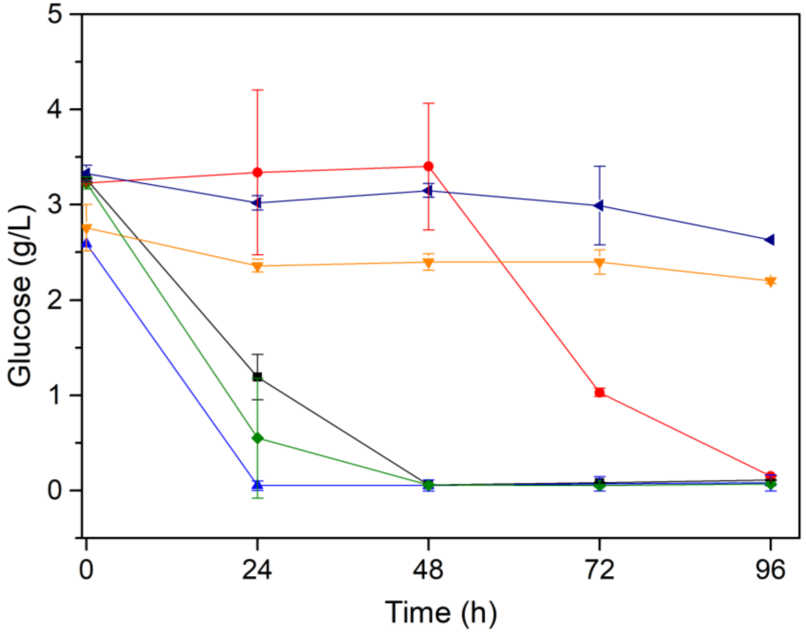
Fermentative performance of non-adapted and adapted strains of *Rhodosporidium toruloides* in non-detoxified HHS supplemented:

- non-adapted strains: showed a lag phase up to at least 48 hours
- adapted strains: showed an exponential growth phase
- *R. toruloides* 2882: presented exponential phase, with higher accumulation of cell biomass in both cases (adapted and non-adapted)

Fig. 3. Cell growth of *R. toruloides* in HHS non-detoxified supplemented
non-adapted cells: -●- *R. toruloides* 2882, -▼- *R. toruloides* 2781, -◀- *R. toruloides* 2896
adapted cells: -■- *R. toruloides* 2882, -▲- *R. toruloides* 2781, -◆- *R. toruloides* 2896

Results and Discussion

❖ *Comparison between R. toruloides adapted and no adapted in HHS*



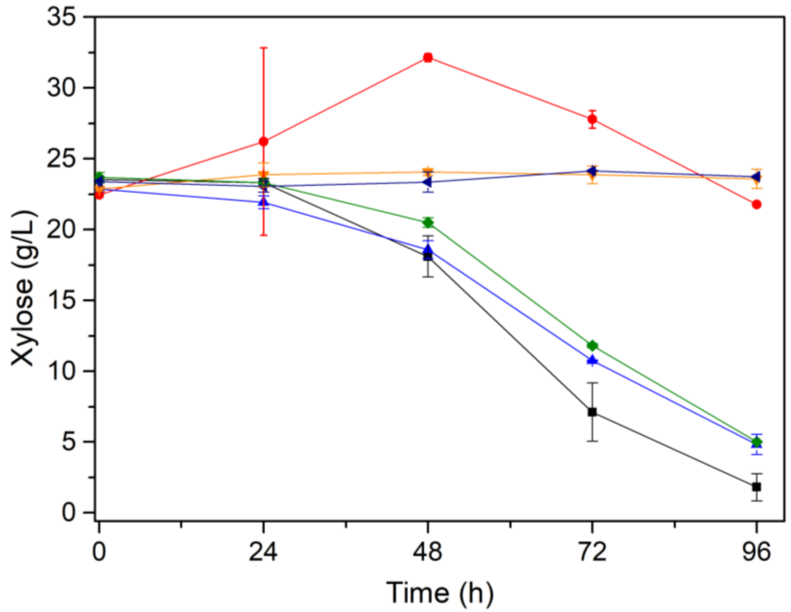


Fig. 4. Glucose and xylose consumption by *R. toruloides* grown in HHS non-detoxified supplemented non-adapted cells: -●- *R. toruloides* 2882, -▼- *R. toruloides* 2781, -◀- *R. toruloides* 2896
Adapted cells: -■- *R. toruloides* 2882 -▲- *R. toruloides* 2781 -◆- *R. toruloides* 2896

- Consumption of sugars was improved in the adapted cells: glucose consumption occurred in the first 24 hours; xylose consumption occurred after glucose depletion, observing its exhaustion in 96 hours of cultivation
- The consumption of sugars (glucose and xylose) by non-adapted cells was **10%, 24% e 8.18%** for strains 2781, 2882 e 2896, respectively. For adapted cells, the consumption of total sugars was **83%, 93.31% e 82%** for strains 2781, 2882 and 2896

Results and Discussion

❖ *Comparison between R. toruloides adapted and no adapted in HHS*

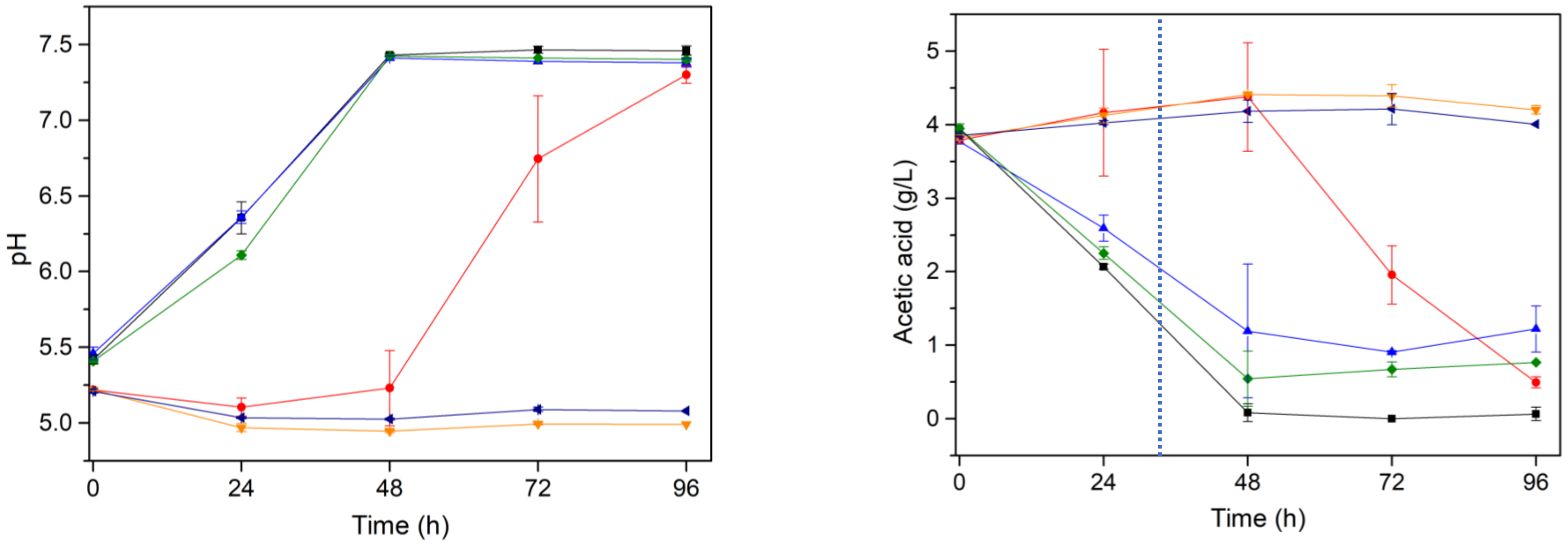


Fig. 5. Acetic acid consumption and pH variation during growth of *R. toruloides* in HHS non-detoxified supplemented non-adapted cells: -●- *R. toruloides* 2882, -▼- *R. toruloides* 2781, -◀- *R. toruloides* 2896
adapted cells: -■- *R. toruloides* 2882, -▲- *R. toruloides* 2781, -♦- *R. toruloides* 2896

- With the exception of the non-adapted strains 2781 and 2896, the ability of *R. toruloides* to assimilate the acetic acid presented in the hydrolysate (as a carbon source) was observed. The decrease in acid concentration promoted the neutralization of broth, pH was increased from **5.5** up to a value of **7.5**

Results and Discussion

❖ *Phenolic compounds degradation and inhibitor effect*

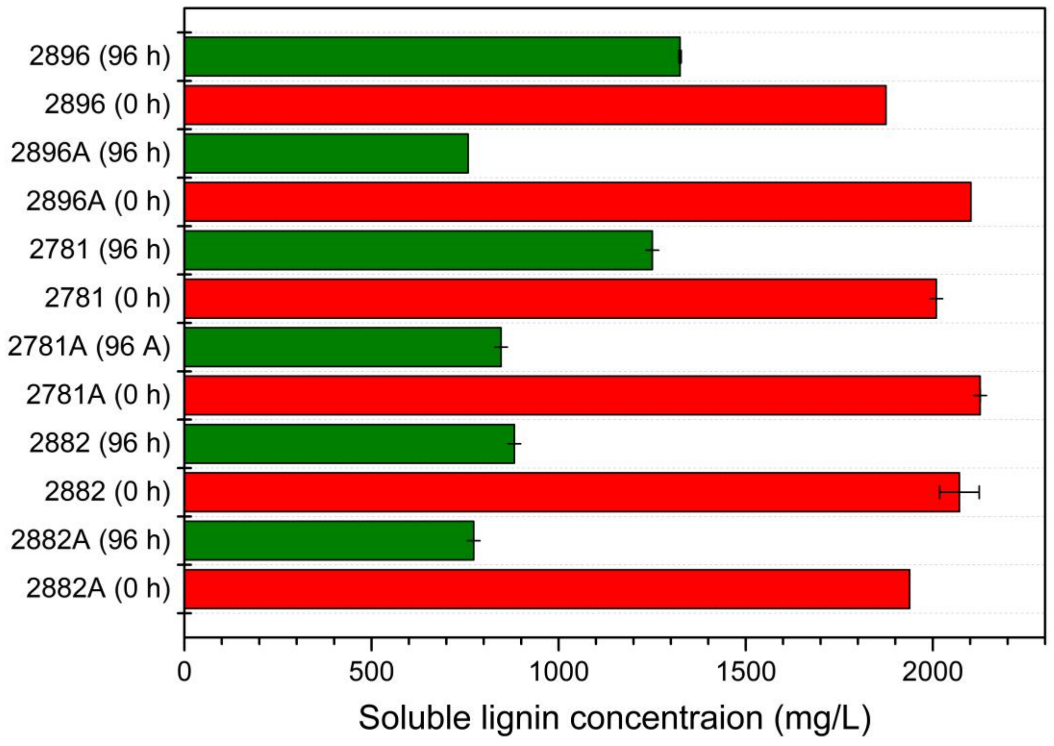


Fig. 6. Phenolics compounds degradation by adapted and non-adapted *R. toruloides* in hemicellulosic hydrolysate
Red: Initial lignin soluble concentration
Green: soluble lignin concentration at 96 h fermentation

- A decrease in phenolic compounds was observed in all tested conditions. A reduction in acid-soluble lignin was noted, being **more expressive for the adapted strains** of **63.93%**, **60.11%**, and **63.93%** while for the non-adapted strains it was noted a reduction of **29.38%**, **57.41%,** and **37.77%** for *R. toruloides* 2896, *R. toruloides* 2882 and *R. toruloides* 2781; respectively.
- These components are strong inhibitors of the fermentation process and, due to the variety available, it is difficult to determine the mechanism of inhibitory action provoked, since they can act alone or synergistically.

Results and Discussion

❖ *Lipid production by R. toruloides adapted and no-adapted grown in HHS*

Table 2. Comparison of lipid production by *R. toruloides* in hemicellulosic hydrolysate

Yeast	pH_i	pH_f	Biomass ($g.L^{-1}$)	Consumption of C (%)	Lipid accumulation % ($m.m^{-1}$)	Yield biomass on carbohydrate $Y_{X/S}$ ($g.g^{-1}$)	Yield lipid on carbohydrate $Y_{P/S}$ ($g\ g^{-1}$)	Lipid production rate QP ($mg.\ L^{-1}.h^{-1}$)
Non-adapted								
2781	5.22	4.99	2.16	10.00	4.6%±1,04%	0,284	0.034±0,0077	1.0±0.2344
2882	5.22	7.30	9.45	24.00	3.5%±0.87%	1.126	0.048±0.0120	3.4±0,8520
2896	5.21	5.08	2.70	8.18	3.27%±0.17%	0.428	0.0377±0.2573	0.919±0,0486
Adapted								
2781	5.46	7.38	14.39	83	7.8%±0.22%	0.553	0.047±0.0017	11.6±0.42
2882	5,42	7.48	17.87	93.31	8.8%±0.68%	0.609	0.059±0.0045	16.3±1.26
2896	5.41	7.40	17.03	82	9.3%±0.36%	0.663	0.067±0.0026	16.6±0.63

- The higher lipid accumulation was found in *R. toruloides* 2896 (9.3%), followed by *R. toruloides* 2882 (8.8%), and *R. toruloides* 2781 (7.8%) ⇒ evidencing the positive effect of cell-adaptation

Highlights

- The **adaptation** process for strains of ***R. toruloides*** in non-detoxified sugarcane biomass hydrolysate was **successful** resulting in strains capable of **growing** and **accumulating lipids** in mixed sugars even in presence of microbial inhibitory compounds

- *R. toruloides* 2896 strain shows strong evidence that its lipid accumulation can be improved by optimizing the process conditions compared to the other strains. This finding is quite significant due to (1) **high tolerance and degradation of inhibitory compounds present in the hydrolysate**, (2) **high accumulation of cellular biomass** and (3) **high yield of lipids**

- The **production** of **lipids** by ***R. toruloides*** is a promising **alternative** for the **use** of **fermentable sugars from lignocellulosic biomass**

ACKNOWLEDGEMENTS