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Rhodotorula toruloides Single Cell Oil Production Using *Eucalyptus urograndis* Hemicellulose Hydrolysate as a Carbon Source

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Abstract: Microbial oil is a potential substitute for vegetable oils in the biodiesel industry. Efforts to obtain cheap carbon sources for the cultivation of lipid-producing microorganisms comprise an active research area. This work aimed to extract the hemicellulose fraction from *Eucalyptus uograndis* and to use its hydrolysate as a carbon source for *Rhodotorula toruloides* (an oleaginous yeast) cultivation for microbial oil production. Hemicellulose hydrothermal extractions were performed at different temperatures, times, and ratios of solid to liquid (S/L). Temperature and time showed a stronger effect on the solubilization of hemicellulose. Hemicellulose extraction at 155 °C, 195 min, and an S/L ratio of 1/2 resulted in a hydrolysate with a xylose content of 37.0 g/L. *R. toruloides* cultivation in this hydrolysate showed that initial pH had a strong influence on cell growth. At an initial pH of 6.2, cells grew to 6.0 g/l of biomass with a lipid content of 50%. Therefore, we believe that *E. urograndis* hemicellulose hydrolysate could be a potential substrate for *R. toruloides* for lipid production based on the biorefinery concept.

Keywords: *Eucalyptus urograndis*; *Rhodotorula toruloides*; hemicellulose; hydrolysate; acetic acid; single cell oil

1. Introduction

The International Energy Outlook/2017 projected that world energy consumption could increase by 28% from 2015 to 2040, which would lead to higher fossil fuel utilization and consequently intensify problems linked to its use, especially environmental ones [1]. Development of renewable energy sources, such as biodiesel, is considered to be key to overcoming these issues.

Biodiesel is obtained after the transesterification of triacylglycerol, generating glycerol as a byproduct, and its production is projected to reach more than 40 billion liters in 2025 [2]. However, the main drawback is using edible vegetable oils as feedstock, taking up 70–95% of biodiesel operational costs [3]. Microbial oils (single cell oil, SCO) are a potential alternative feedstock as they do not compete with the edible sectors and their production can be done in bioreactors (in areas an order of magnitude smaller than the area required for crop cultivation) and regardless of weather and geopolitical conditions [4].

Oleaginous microorganisms are those that can accumulate more than 20% of their dry mass in the form of lipids. *Rhodotorula toruloides* (previously called *Rhodosporidium toruloides* [5]) is a promising oleaginous yeast for the biodiesel industry, since it can accumulate more than 67% of its cell mass in the form of lipids [6]. However, its relatively high costs of cultivation are a barrier to making its SCO an economically feasible raw material for biodiesel production. When *R. toruloides* is cultivated with conventional sugars like glucose, the substrate can represent up to 70% of the operational cost [7].

An alternative source of cheap carbon is hemicellulose hydrolysate, a byproduct of many agro-industrial sectors as ethanol, pulp and paper, and beverages. Since cellulose is targeted at second-generation ethanol production in sugarcane biorefineries and in the pulp and paper industry, it is the most valuable wood fraction, and hemicellulose has, as yet, no direct noble utilization in these industries. Moreover, hemicellulose is the second most abundant carbohydrate in nature, composing one-quarter of dry vegetable biomass [8], which allows this fraction to be considered an abundant feedstock for industrial purposes.

In the kraft pulping process, wood chips are treated with a white liquor at high temperatures (145–170 °C) and pressure, aiming at delignifying the biomass and obtaining the raw cellulose. After the cooking process, the solid phase (mainly cellulose) is washed and a black liquor containing 65% solids composed mainly of solubilized lignin and hemicellulose is generated [9]. This liquor is commonly burned in boilers to generate heat for plant operation [10,11]. Lignin has a relatively high specific heat (around 27 MJ/kg), while the specific heat of hemicellulose is only around half of this value. Therefore, hemicellulose contributes to lowering the heat generation efficiency of the boiler. Hence, there is an opportunity to develop new and more valuable strategies for hemicellulose utilization, thereby adding more value to this wood fraction. One possibility involves using hemicellulose hydrolysates as a carbon source for biologic processes to obtain high-value products [11] such as SCO for biodiesel production.

There are few reports in the literature regarding SCO production using wood hemicellulose fractions: Osório-Gonzáles et al. [12] reported the use of a hemicellulose fraction without mentioning its source, Brandenburg et al. [13] used birch, and Matsakas et al. [14] used *Betula pendula*. To our knowledge, there is no report of eucalyptus hemicellulose hydrolysate (EHH) utilization for SCO production, although there are only a few works that used EHH as a carbon source for xylitol production [15–18]. Brazil is the world's second largest cellulose pulp producer and one of the largest *Eucalyptus* ssp. producers, with 5.7 million hectares planted in 2018 [19]. Taking this into consideration, and because *E. urograndis* is one of the major subspecies cropped [20,21], Brazil has a promising future in the utilization of hemicellulose from woody biomass for microbial oil production in the biodiesel industry. Therefore, this work evaluated the use of hemicellulose hydrolysate from *E. urograndis* as a carbon source for microbial oil production. A set of experiments determined conditions for hemicellulose extraction using the hydrothermal method and a hydrolysate with high xylose concentration obtained based on these first experiments was used for the cultivation of *Rhodotorula toruloides*, an oleaginous yeast considered a workhorse for biotechnology applications [5].

2. Materials and Methods

2.1. Wood Samples

E. urograndis chips, donated by Fibria (Jacareí, Brazil), a pulp and paper company, were milled with a knife mill. Eighty-two percent of the particles in the ground chips used for hemicellulose extraction ranged in size from 65 to 32 mesh. According to Fibria, the woody biomass was composed of the sugars (%, m/m) glucose (47%), xylose (13%), and mannose (1.14%), in addition to other compounds. This xylose content of 13% (m/m) was used as the basis for calculating the xylose yields in the present work.

2.2. Microorganism

The *R. toruloides* used in this work was a strain that had been adapted to grow in sugarcane bagasse hemicellulosic hydrolysate (the adapted strain was named *Rhodosporidium toruloides* CCT 7815) [22]. The strain was kept at −80 °C in glycerol solution (10% v/v).

2.3. Reactor for Hemicellulose Extraction

Hemicellulose extraction was carried out in a model TE-028 oven (Tecnal, Piracicaba, Brazil) in which stainless steel cylindrical reactors (100 mL of working volume) were agitated by tumbling by being fixed to a shaft rotating at 60 rpm.

2.4. Other Materials

All reagents used in this work were of analytical grade. All solutions were prepared using MilliQ ultra-pure water (Millipore, Bedford, MA, USA). Cultivations were done in 250 ml Erlenmayer flasks using 20% of the total volume, then incubated in a model TE-4200 orbital shaker (Tecnal, Piracicaba, Brazil) at 28 °C and 200 rpm. A model 5804R centrifuge (Millipore, Bedford, MA, USA) was operated at 4 °C and 8228 *g* for 15 min for cell recovery. Lipid determination required amber Schott flasks (100 mL), a model ME 1 vacuum pump (Vaccubrand, Wertheim, Germany), a model LR-271C oven (Grieve, IL, USA), and a model TE-211 rotary evaporator (Tecnal, Piracicaba, Brasil). All material was sterilized at 121 °C for 15 min using an autoclave. All solutions for cultivations were sterilized using a 0.22 µm JetBiofil filter (Guangzhou Jet Bio-Filtration, Guangzhou, China). Statistica® 7.0 software was used for experiment design and statistical calculations.

2.5. Hemicellulose Extraction from Ground Wood Chips

Water was added to 10 g of ground wood to achieve different solid-to-liquid (S/L) ratios in the cylindrical reactors. The reactors were then closed and kept rotating in the extraction oven for specific durations and at specific temperatures. Next, the material was filtered using filter paper (Unifil, Germany), and the solubilized hemicellulose in the liquid phase was hydrolyzed at 121 °C for 60 min with addition of 0.5% (v/v) H_2SO_4 accordingly [23]. Hydrolysates were pH-adjusted to a specific value with CaO (5.0 or 6.2) and were filtered using quantitative paper, then sterilized as described in Section 2.4.

2.6. Microorganism Cultivation

The *R. toruloides* inoculum was prepared starting with cell reactivation in a Falcon-type tube containing 10 mL of YPD (yeast extract–peptone–dextrose) medium and 1 mL of cells for 24 h at 200 rpm and 28 °C. Then, reactivated cells were transferred to a 250 mL Erlenmeyer flask containing YPD medium and were kept in an orbital shaker for 24 h at 28 °C and 200 rpm. Finally, cells were separated by centrifugation and suspended in 0.9% NaCl solution to a OD (absorbance at 600 nm) of 20.

Cultivation was done in triplicate in a medium composed of 44 mL of EHH, 5 mL of inoculum, and 1 mL of mineral solution [24]. For the first cultivation study, KH_2PO_4 and yeast extract (3.8% ammoniacal nitrogen and 10.5% total nitrogen) were used to achieve the desired carbon-to-phosphorus (C/P) and carbon-to-nitrogen (C/N) molar ratios, respectively. Flasks were incubated for 96 h for the first set of cultivations. The second set of cultivations—with concentrated EHH (cEHH)—took 120 h, 24 h longer than the first set due to a higher concentration of the carbon source. In both sets, sampling for dry cell mass and lipid content determination occurred only at the end of cultivation.

2.7. Analytical Methods

The total sugar content was quantified as reducing sugar utilizing the method proposed by Miller [25], using xylose as the standard. The xylose, acetic acid, glucose, hydroxymethylfurfural (HMF), and furfural concentrations were determined using HPLC equipment (Dionex Ultimate model 3000, ThermoScientific, Sunnyvale, CA, USA) with an HPX-87H column (Biorad, Hercules, CA, USA) at 50 °C, with 5 mmol/L H_2SO_4 at 0.6 mL/min as the mobile phase.

Cell growth was monitored via OD at intervals of 24 h. The dry cell mass was quantified gravimetrically: cells were collected by centrifugation at 1790 *g* at 4 °C for 15 min, washed twice with water, and dried in an oven for 24 h at 100 °C. Lipid quantification of dried cells was also performed gravimetrically after extraction with chloroform and methanol as described in Folch et al. [26].

3. Results

3.1. Hemicellulose Extraction

A first set of extraction runs was completed in accordance with a 2^2 factorial design to investigate how three crucial factors in the process—S/L ratio, time, and temperature—could affect hemicellulose solubilization (Table 1). In the temperature range studied, only time and temperature affected xylose yield determined as the total mass of xylose present in hydrolysate (Table 2). As reported for other lignocellulosic biomass types, the S/L ratio might have a stronger effect on xylose yields in higher temperature ranges (160–190 °C) [27].

Table 1. Factors and levels of the 2^2 factorial design for the first set of extraction runs.

Factor	Level		
	−1	**0**	**+1**
T (°C)	120	140	160
t (min)	60	105	150
S/L ratio	1/4	1/6	1/8

Table 2. Results from the 2^2 factorial design of hemicellulose extraction.

T (°C)	S/L Ratio (g/mL)	Time (min)	Total Reducing Sugar (g)
120	1/4	60	0.04
120	1/8	60	0.03
120	1/4	150	0.11
120	1/8	150	0.08
140	1/6	105	0.19
140	1/6	105	0.19
140	1/6	105	0.16
160	1/4	60	0.13
160	1/8	60	0.25
160	1/4	150	1.60
160	1/8	150	1.56

A maximum for the reducing sugar concentration was not achieved. Based on these results, for the extraction time range studied, the global maximum for hemicellulose extraction/reducing sugar yield would be achieved at higher temperatures above 160 °C, the maximum operational temperature of the rotating oven. Therefore, the results indicated that a higher concentration of solubilized hemicellulose could be achieved with longer times and higher temperatures.

The second extraction study was conducted at temperatures ranging from 145 to 160 °C and time ranging from 60 to 240 min with the S/L ratio fixed at 1/8 (Figure 1, Table 3) in a one-factor-at-a-time experimental design. Extractions at 160 °C for 195 or 240 min resulted in near-absolute removal of hemicellulose, and at 155 °C and 195 min, in 95% removal. The concentrations of glucose, arabinose, acetic acid, HMF, and furfural also increased with longer extraction times, in most cases achieving maximum content within 195 min of extraction (Table 3).

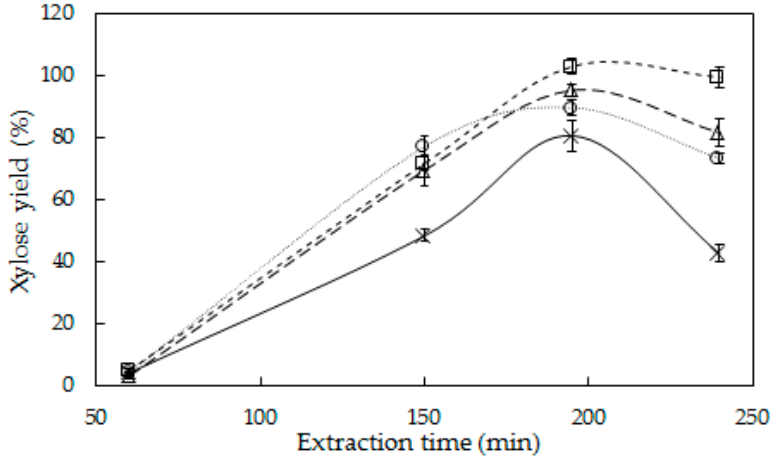

Figure 1. Xylose yields for hemicellulose extraction at an S/L ratio of 1:8 with different times and temperatures: (X) 145 °C, (○) 150 °C, (Δ) 155 °C, and (□) 160 °C. Lines are a guide to the eye.

Table 3. Eucalyptus hemicellulose hydrolysate (EHH) partial composition for extractions at an S/L of 1:8.

T (°C)	t (min)	Volume (L)	Compound (g/L)					
			Glucose	Xylose	Arabinose	Acetic Acid	HMF	Furfural
145	60	0.115	0.215	0.472	0.052	0.094	0.001	0.013
	150	0.135	0.326	4.668	0.073	1.137	0.017	0.249
	195	0.135	0.387	7.781	n.d. [1]	1.885	0.021	0.424
	240	0.13	0.293	4.264	0.081	1.075	0.000	0.212
150	60	0.105	0.153	0.541	0.045	0.094	0.000	0.017
	150	0.135	0.372	7.447	0.054	1.767	0.022	0.371
	195	0.135	0.419	8.656	n.d. [1]	2.129	0.023	0.516
	240	0.137	0.348	6.963	n.d. [1]	1.727	0.020	0.341
155	60	0.115	0.177	0.357	0.059	0.071	0.003	0.007
	150	0.133	0.344	6.809	0.052	1.649	0.017	0.334
	195	0.132	0.532	9.552	n.d. [1]	2.337	0.012	0.652
	240	0.130	0.390	8.177	n.d. [1]	2.023	0.007	0.452
160	60	0.112	0.187	0.573	0.002	1.750	0.003	0.410
	150	0.130	0.320	7.790	0.001	1.840	0.018	0.492
	195	0.115	0.421	12.196	n.d. [1]	2.412	0.025	0.587
	240	0.110	0.489	12.352	n.d. [1]	2.348	0.021	0.644

[1] not detected.

3.2. R. toruloides *Cultivation and Lipid Production*

To verify the best cultivation condition in terms of molar C/P and C/N ratios, experiments based on a central composite design (Table 4) were carried out using EHH obtained from extraction at 160 °C and 195 min.

Table 4. Central composite design for cultivations with EHH at different C/N and C/P ratios.

Factor	Level				
	−1.41	**−1**	**0**	**1**	**+1.41**
C/N	38.5	100	250	400	461.5
C/P	50.8	100	220	340	389.2

The best results were obtained with cultivations at the initial C/N ratio of 100: a lipid content of 26.42% for Condition 1 and biomass and productivity of 6.52 g/L and 15.00 g/(L·h) for Condition 2 (Table 5). The lipid content at this initial C/N ratio was slightly higher than that observed at C/N ratios

of 400 and 250. In terms of lipid productivity, the best result obtained at the C/N ratio of 100 was slightly higher than the 11.00 g/(L·h) achieved at C/N and C/P ratios of 250 and 50.8, respectively. Regarding biomass production, lower C/N ratios produced higher values. According to the Pareto chart at a 95% confidence level, the effect of the C/N ratio was expressively more significant than that of the C/P ratio (Figure 2).

Table 5. Results for biomass and lipid production by *R. toruloides* after 96 h of cultivation in EHH at different C/N and C/P ratios.

Sample	C/N	C/P	Biomass (g/L)	Lipid * (g/L)	Lipid ** (%)	Productivity [mg/(L·h)]
1	100	100	5.11	1.35	26.42	14.00
2	100	340	6.52	1.45	22.24	15.00
3	400	100	3.05	0.69	22.62	7.00
4	400	340	3.02	0.39	12.91	4.00
5	38.5	220	5.96	0.81	13.59	8.00
6	461.5	220	3.16	0.64	20.25	7.00
7	250	50.8	4.81	1.04	21.62	11.00
8	250	389.2	3.88	0.84	21.65	9.00
9	250	220	5.08	0.76	14.96	8.00
10	250	220	3.25	0.66	20.31	7.00
11	250	220	3.75	0.65	17.33	7.00

* Lipid concentration: mass of lipids per volume of cultivated broth. ** Lipid content: mass of lipids per dry cell mass.

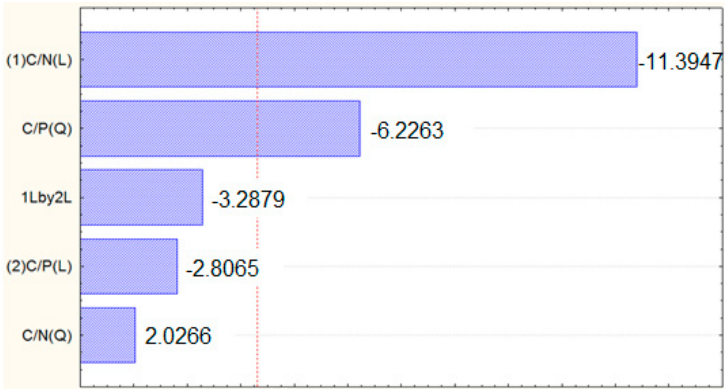

Figure 2. Pareto chart for cultivations with *R. toruloides* at different C/N and C/P ratios. Standardized effect estimate (absolute values, p = 0.05).

The relatively low lipid content in all the cultivations was probably due to the low carbon content in the hydrolysate (12.196 g/L of xylose). To overcome this, a concentrated EHH was prepared via hemicelullose extraction at a higher S/L ratio based on data that indicated that the profile of sugar extraction did not change at higher S/L ratios: extractions at the S/L ratio of 1/3 showed that 155 °C and 195 min remained the best conditions for hemicellulose extraction (Figure 3). Then, a hydrolysate (named cEHH) was prepared with an S/L of 1:2 at 155 °C for 195 min (Table 6). After liming, it contained 3 times more xylose (37.399 g/L) than the highest content previously obtained (12.196 g/L) (Table 3).

No growth was detected in the first cultivations with cEHH (initial pH of 5.0), even with water-diluted hydrolysate at 30%, 50%, and 70%. However, cultivation at an initial pH of 6.2 was successful (Figure 4). This cultivation resulted in a biomass of 6.0 g/L and a lipid content of 50%. At the beginning of fermentation, xylose was at 32 g/L, and 30% of this sugar was consumed after 120 h of cultivation. Acetic acid was completely consumed after 96 h of cultivation, increasing the broth pH to 7.8. Glucose was rapidly depleted in 24 h.

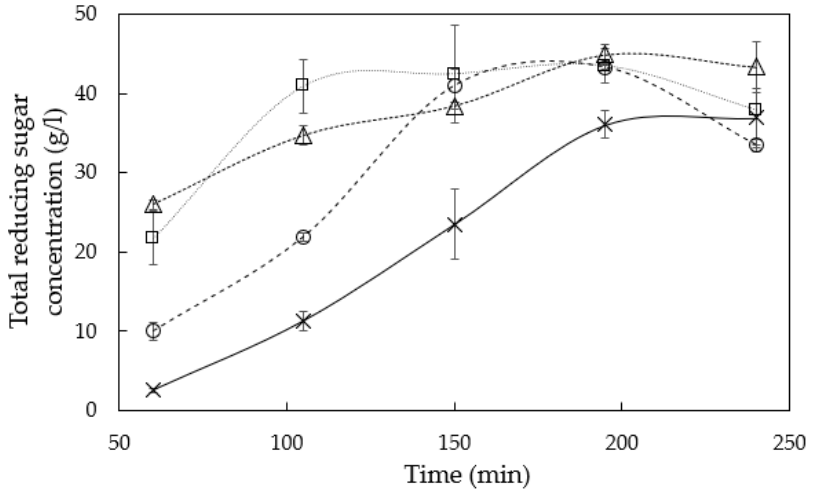

Figure 3. Kinetics of total reducing sugar for EHH obtained at 145 °C (×), 150°C (O), 155°C (□), and 160 °C (Δ) over different durations and at an S/L of 1:3. Lines are a guide to the eye.

Table 6. cEHH obtained from extraction at 155 °C, 195 min, and an S/L of 1:2 before and after liming.

Overliming	Concentration (g/L)				
	Xylose	**Acetic Acid**	**Glucose**	**Arabinose**	**HMF**
Before	47.810	14.092	1.965	1.458	0.160
After *	37.399	9.884	1.810	0.950	0.119

* pH of 6.2.

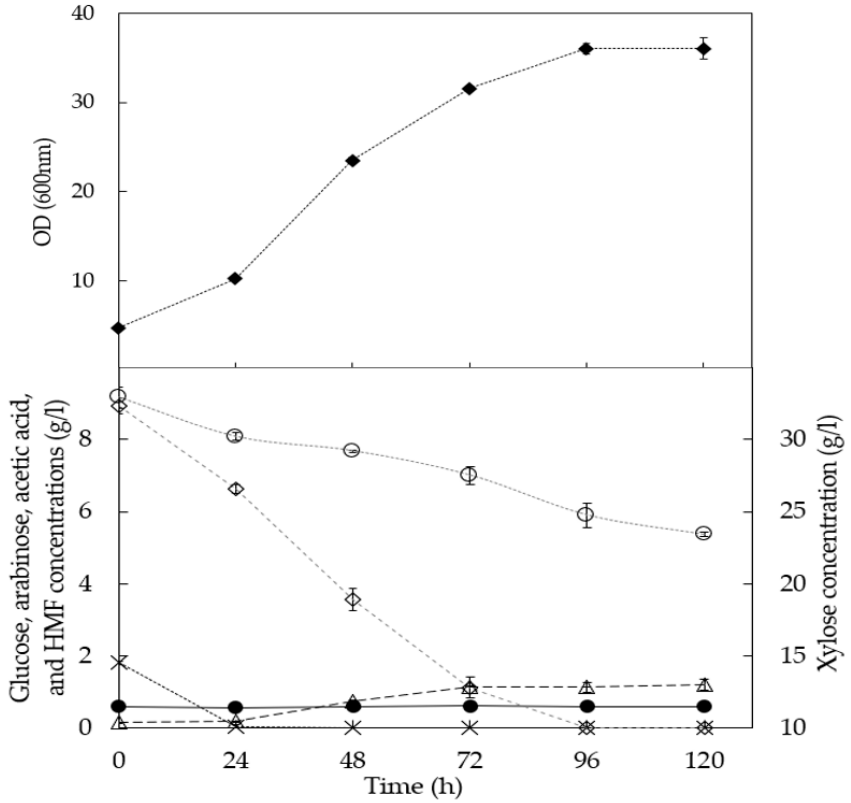

Figure 4. Kinectics of *R. toruloides* growth and carbon source consumption. Cultivation time 120 h. Symbols: xylose (o), glucose (×), acetic acid (◊), arabinose (•), and HMF (Δ) concentrations; OD at 600 nm. Lines are a guide to the eye.

4. Discussion

4.1. Hemicellulose Extraction

Extractions for durations of 195 and 240 min resulted in hydrolysates with the highest sugar content. However, the extraction at 155 °C and 195 min was considered the most efficient, since 95% hemicellulose was achieved in less time and with less energy than with extractions at 240 min and 160 °C. The reducing sugar concentration obtained in 195 min of extraction was slightly higher than those concentrations obtained in 240 min. This is possibly a result of monomer degradation due to pH drop and autohydrolysis. This drop in pH during hydrothermal hemicellulose extraction at high pressure and temperature is a result of water autoionization, producing a higher concentration of H_3O^+ [28]. The formation of acetic acid after cleavage of acetyl groups from hemicellulose also contributes to this drop in pH [29]. This phenomenon represents the release of acetic acid from acetyl groups and secondary reactions that produces reducing groups such as levulinic and formic acids from the monomers (sugars) [30,31].

Selective extraction of hemicellulose is paramount to the idea of adding value to biomass utilization and keeping cellulose characteristics intact (not interfering in pulp processing). It is possible that cellulose was not attacked during the hydrothermal extraction since xylose accounted for 96% of the total sugar in the hydrolysate obtained with extractions at 195° and 155 °C (Table 3). The glucose present in the hydrolysate possibly originated from the hemicellulose, since the hemicellulose fraction of eucalyptus is composed of xylose, glucose, mannose, arabinose, rhamnose, and galactose [32].

The best conditions for xylose extraction (96% of sugars in the hydrolysate as xylose) in the present work, even under relatively mild conditions, are considerably better than those reported in the specific literature. Gallina et al. [33] achieved 82% as the maximum global yield of pentose during continuous hemicellulose extraction at 285 °C. Rafiqul and Sakinah [34] studied the effect of time, acid concentration, and S/L ratio during wood sawdust hemicellulose extraction and hydrolysis. Those authors reported a maximum yield of 86% xylose after extraction/hydrolysis at 125 °C, for 60 min, with an S/L ratio of 8, and with 4% (v/v) H_2SO_4 [29].

4.2. R. toruloides *Cultivation and Lipid Production*

The SCO content (26.42%) obtained with *R. toruloides* cultivation in EHH extracted at an S/L ratio of 1/8 is much lower than those found in the literature, where values of up to 67.5% of lipid content were reported [6]. A possible reason for this low lipid content might be the low xylose concentration—12.196 g/L, less than half of the sugar content reported in related work [14,22]. Therefore, the next step to enhance biomass and lipid production was to use a hydrolysate with a higher concentration of xylose. However, the conditions to increase the sugar concentration in the hydrolysate result in higher concentrations of secondary compounds such as HMF, furfural, and acetic acid that can inhibit growth. This was observed in the first cultivation trial using cEHH reported in this work.

Since the hydrolysate pH after liming was 5.0, only slightly higher than the pKa (4.75) of acetic acid, the undissociated form of acetic acid may have diffused into intracellular cytosol, producing a pH drop in the cytoplasm and inhibiting cellular growth [35]. Adjusting the cEHH pH to 6.2 solved this problem. At a pH of 5.0, 3.56 g/L of acetic acid was in an undissociated form, while at a pH of 6.2, the concentration of undissociated acetic acid was almost 10 times lower. Acetic acid inhibition in cultivations at low pH values was also observed by Brandenburg et al. [13]. Those authors found that acetic acid at 3.8 g/L and pH 4.9 inhibited *Lipomyces starkeyi* growth. However, at this same pH value and 2.11 g/L of acetic acid, they observed yeast growth. These authors also observed *L. starkeyi* growth at an acetic acid concentration of 3.9 g/L, but at pH 6.0. Comparing this case with cultivation using cEHH in the present work, we can conclude that when this weak organic acid is present, the pH level interferes more with yeast development than does the organic acid concentration.

Even with more xylose in the cEHH, the biomass content (6.00 g/L) was almost the same as that observed with EHH cultivations (6.54 g/L). However, the lipid content increased almost 100% in

cultivations with cEHH, achieving 50%. The higher concentration of acetic acid in cEHH possibly enhanced lipid production, since this carbon source is linked to lipid production and accumulation. Acetic acid has a shorter pathway than glucose and xylose when used in cell maintenance [36]. This acid is converted into acetyl-CoA and acyl-CoA in sequence. Therefore, when the system is under nutrient limitation (e.g., nitrogen depletion), acetyl-CoA in excess is driven towards lipogenesis [36]. Interestingly, arabinose was not assimilated by the yeast. The effect of catabolic repression is not likely to be the answer, since during glucose consumption, acetic acid and xylose were simultaneously consumed (Figure 4). The growth stopped in parallel with acetic acid depletion. Possibly, acetic acid consumption resulted in a pH (7.8) that inhibited adequate cell development. Moreover, metabolites formed during cultivation might also contribute to this reduction in growth.

Acetic acid was consumed faster than xylose, with consumption rates of 93.00 mg/(L·h) and 79.00 mg/(L·h), respectively (Figure 4). Acetic acid consumption by *R. toruloides* is an important characteristic of this yeast, since this carbon source is commonly present in hemicellulosic hydrolysates. Moreover, studies have tested the potential use of acetic acid co-generated during anaerobic treatment of wastewater as a carbon source for lipid production by yeasts [37,38]. Therefore, this acid is a potential source of cheap carbon for lipid production, since wastewater treatment by anaerobic bacteria is widely used by many industries [39].

Brandenburg et al. [13] studied pH-stat fed-batch cultivation of *R. toruloides* using birch wood hemicellulosic hydrolysate as a carbon source and reported 15.63 g/L of biomass and 51.3% lipid content. These authors used hydrolysate with a xylose content similar to that used in this work, achieving better results, probably due to their fed-batch cultivation process. In this operational mode it is possible to control variables such as pH, aeration, and carbon source feeding throughout the cultivation, differently from experiments done with shake flasks. Matsakas et al. [14] observed biomass and lipid production of 7.1 g/L and 2.8 g/L, respectively (39% lipid content), during the cultivation of *R. toruloides* in a bioreactor with agitation and aeration control using *Betula pendula* hemicellulosic hydrolysate as a carbon source. However, in that experiment, the authors pretreated the hydrolysate to remove acetic acid with *Bacillus* sp. and furfural and phenols by treatment with activated carbon.

5. Conclusions

R. toruloides was able to grow and produce SCO when cultivated using eucalyptus hemicellulose hydrolysate, showing that it has the potential to be a source of low-cost raw material for the biodiesel chain of production. The results obtained in the present work showed that concentrated hemicellulose hydrolysates obtained from extraction at higher S/L ratios enhance lipid production by *R. toruloides*. Moreover, working with cEHH (with a high acetic acid concentration) requires setting the pH above the acetic acid pKa value to enable microbial growth and lipid production. Under this condition, acetic acid can be assimiliated by *R. toruloides* and take part directly in lipogenesis, resulting in a higher lipid content. Therefore, the hemicellulose fraction from industrial wood species can be used as a carbon source for large-scale cultivation of oleaginous yeasts and contribute to the production of biodiesel based on microbial oil.

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