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MICROBIOLOGY

Production of ethanol and xylitol from D-xylose by four strains of *Candida (Spathaspora) materiae*

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Abstract: Xylose is the most abundant pentose sugar in lignocellulosic biomass and can be biologically converted into economically important products by microorganisms such as yeasts. This study aimed to characterize the bioconversion of xylose into xylitol and ethanol using four strains of *Candida materiae* belonging to the *Spathaspora* clade, which includes yeast species with the potential to convert D-xylose to xylitol and ethanol. Fermentation assays were performed in YPX medium (yeast extract, peptone, xylose 5%) under two oxygen-limiting conditions at inoculum concentrations of 1, 5 and 10 g L^{-1}. The best results were obtained using a 5 g L^{-1} inoculum under moderate oxygen-limiting conditions. *C. materiae* UFMG-CM-Y480 produced 32.23 g L^{-1} of xylitol, yielding 0.743 g g^{-1} within 36 h. Different combinations of xylose and glucose (25/25 g L^{-1}, 40/10 g L^{-1}, 10/40 g L^{-1}) were also used to verify the influence of sugars on *C. materiae* metabolism. Xylitol was preferentially produced in medium containing 40 g L^{-1} xylose. *C. materiae* UFMG-CM-Y480 produced 26.20 g L^{-1} of xylitol with a yield of 0.646 g g^{-1} in 36 h. Our results showed that *C. materiae* UFMG-CM-Y480 may convert D-xylose to xylitol with a better fermentative performance than other xylitol-producing yeasts.

Key words: *Candida materiae*, cell concentration, ethanol, oxygen-limiting condition, sugarcane bagasse hemicellulose hydrolyzate, xylitol.

INTRODUCTION

Lignocellulose is an important renewable feedstock for biofuels and other value-added products, since its cellulosic and hemicellulosic fractions contain significant amounts of the fermentable sugars glucose and xylose, respectively (Wijaya et al. 2014, Zhang et al. 2016). For instance, second-generation bioethanol (2G) and xylitol may be generated from these sugars. The first is an alternative to fossil fuels and first-generation (1G) ethanol since it is a renewable energy that does not compete with the food sector (Nosrati-Ghods et al. 2018). Xylitol is a sugar alcohol that is commercially valuable due to its high demand as a sweetener that can replace sucrose; it is tolerated by diabetics and prevents respiratory infections, acute otitis media, and osteoporosis. It also has several interesting applications in the food, pharmaceutical, and chemical industries (Arruda et al. 2017, Mohamad et al. 2015). The ability of microorganisms to ferment pentoses such as xylose is a requirement to implement economically feasible processes of lignocellulosic hydrolyzates for ethanol or xylitol production (Kwak et al. 2019).

Yeasts belonging to the clade *Spathaspora* are known for their ability to bioconvert xylose

into xylitol and ethanol. Among the xylose-fermenting species tested from this clade, Sp. *passalidarum* achieved high conversion yields, which correlate ΔP produced (ΔP ethanol or ΔP xylitol) and ΔS consumed derived from determining the total, initial, and consumed substrate; high productivities (expressing the speed at which ethanol or xylitol is produced, relating its concentration in the substrate by time); and rapid sugar consumption when cultured in media containing xylose under moderate and severe oxygen-limiting conditions (Hou 2012, Cadete et al. 2016).

Under anaerobic conditions, Sp. *passalidarum* has a high ability to convert xylose into ethanol partly because of the NADH-preferring xylose reductase (XR). The enzyme balances cofactors between XR and xylitol dehydrogenase (XDH) enzymes, which convert xylose into xylitol and xylitol into xylulose, respectively (Cadete et al. 2016, Cadete & Rosa 2018). However, most of the xylose-fermenting yeasts contain XR enzymes that preferably use NADPH, thereby unbalancing the cofactors under anaerobic conditions. In such cases, xylose metabolism does not favor ethanol production and there is an accumulation of xylitol in the cell (Sakihama et al. 2015). Some species of the *Spathaspora* clade, such as Sp. *brasiliensis*, Sp. *roraimanensis*, and Sp. *xylofermentans*, are known xylitol producers (Cadete et al. 2013). *Candida materiae*, which was described by Barbosa et al. (2009), is a species belonging to the *Spathaspora* clade. It was reported to preferentially convert D-xylose to xylitol (unpublished data cited by Cadete & Rosa 2018). Based on this information, this study evaluated the production of ethanol and xylitol from D-xylose using four strains of *C. materiae* under different cell concentrations and oxygen-limiting conditions (moderate and severe).

MATERIALS AND METHODS

Yeast strains

Four strains of *C. materiae* UFMG-CM-Y480, UFMG-CM-Y481, UFMG-CM-Y482, and UFMG-CM-Y483 were used in this study. The first three strains were isolated from rotting wood, and the last one was isolated from a wood-boring beetle associated with rotting wood in the Atlantic Rainforest in Brazil as described by Barbosa et al. (2009). The yeast cultures were stored at -80 °C in GYMP broth (glucose, 2%; yeast extract, 0.5%; malt extract, 0.5%; Na_2PO_4, 0.2%) containing 20% glycerol.

Fermentation assays under oxygen-limiting conditions

Each strain of *C. materiae* was streaked onto YM agar plates (YMA, 1% glucose, 0.5% peptone, 0.3% yeast extract, 0.3% malt extract, and 2% agar). After 48 h, a colony was transferred to 50 mL YPX (yeast extract, peptone, xylose) medium (peptone 20 g L^{-1}, yeast extract 10 g L^{-1}, 50 g L^{-1} xylose) and incubated for 24 h. Cells were recovered through centrifugation at 2600 × g for 15 min, washed twice with sterile water, and suspended in the fermentation media with an initial inoculum of 1.0 g L^{-1}, 5.0 g L^{-1}, or 10.0 g L^{-1}. Fermentation experiments were performed on YPX medium under moderate oxygen-limiting conditions in 250 mL cotton-plugged flasks containing 100 mL of medium, and under severe oxygen-limiting conditions in a 100 mL rubber-plugged flask containing 100 mL of medium and equipped with a needle to allow the release of CO_2 (Cadete et al. 2016). The flasks under moderate oxygen-limiting conditions were incubated at 30 °C and 200 rpm for 60 h, and samples were taken at 12 h intervals. The flasks under severe oxygen-limiting conditions were incubated at 30 °C with a rotation speed of 200 rpm for 120 h. Samples were taken at regular 24 h intervals and centrifuged at 13,500 × g for 15 min (Cadete et al.

2016); supernatants were stored at -20 ° C. Cell growth was determined through correlation of the optical density (OD_{600nm}) (Biospectro SP-22) with the dry cell weight using a calibration curve previously built for each strain of *C. materiae*. All experiments were performed in triplicates. Based on the results of the experiments below, the best initial inoculum (5 g L^{-1}) and oxygenation conditions (oxygen-limiting condition) for the conversion of xylose to ethanol or xylitol were selected for subsequent tests.

Glucose and xylose fermentation at different concentrations and combinations

Fermentation assays were performed in YP medium (peptone 20 g L^{-1}, yeast extract 10 g L^{-1}) with the following sugar concentrations: (1) 25 g L^{-1} glucose and 25 g L^{-1} xylose, (2) 10 g L^{-1} glucose and 40 g L^{-1} xylose, and (3) 10 g L^{-1} xylose and 40 g L^{-1} glucose. First, *C. materiae* yeast strains were grown on YM agar for 48 h, and a few colonies were transferred to YPX medium for 24 h. Cells were recovered as described above, and the flasks were incubated at 30 °C and 200 rpm for 60 h. Samples were collected aseptically at 0, 12, 24, 36, 48, and 60 h. Cell growth was determined measuring OD_{600nm} (Cadete et al. 2016). The assays were performed in triplicates.

Analytical methods

Concentrations of glucose, xylose, ethanol, and xylitol were determined using high-performance liquid chromatography (Shimadzu, Kyoto, Japan). The samples resulting from the assays in complex media were filtered using a 0.22 µm cellulose acetate membrane. The aliquots were diluted and analyzed under the following conditions: Supelco Analytical C-610 H column (Sigma-Aldrich, USA) maintained at 45 °C, volume injection of 20 µL, refractive index detector RID 10-A (Shimadzu, Kyoto, Japan), and 5 mM H_2SO_4 mobile phase as eluent at a flow rate of 0.6 mL min^{-1}.

Fermentation parameters $Y_{p/s}^{et}$ (g g^{-1}, ethanol yield), $Y_{p/s}^{xyl}$ (g g^{-1}, xylitol yield), Q_p^{et} (g L^{-1} h^{-1}, volumetric yield in ethanol), Q_p^{xyl} (g L^{-1} h^{-1}, volumetric yield in xylitol), and sugar consumption (%, xylose, and/or glucose) were calculated based on the experimental tests. The sugar conversion factor in ethanol ($Y_{p/s}^{et}$ g g^{-1}) and xylose in xylitol ($Y_{p/s}^{xyl}$ g g^{-1}) was calculated by dividing the produced ΔP (ΔP ethanol or ΔP xylitol) by the consumed ΔS (glucose and/or xylose). Ethanol or xylitol productivity [Q_p^{et} or Q_p^{xyl} (g L^{-1} h^{-1})] was determined using the ratio of the ethanol or xylitol concentration (g L^{-1}) to the maximum production time (h) for the products. The conversion efficiency (η%) was calculated as a percentage of the theoretical maximum conversion factor in ethanol or xylitol (0.51 g ethanol g^{-1} xylose and glucose, and 0.917 g xylitol g^{-1} xylose, respectively), and the xylose or glucose consumption (%) was calculated as a percentage of the sugar consumed from the initial concentration used (Cunha-Pereira et al. 2011, Cadete et al. 2016, Dussán et al. 2016).

RESULTS

Three different inocula (1, 5, and 10 g L^{-1}) were used under moderate and severe oxygen-limiting conditions, with xylose (50 g L^{-1}) as the main carbon source, to evaluate the influence of the inoculum concentration on the production of ethanol and xylitol by *C. materiae* strains. Table I shows the best ethanol and xylitol yields obtained. There is no statistically significant difference between the 5 and 10 g L^{-1} cell inoculums on xylitol production ($p \leq 0.05$) under moderate oxygen-limiting conditions. In this study, the UFMG-CM-Y480 strain was the best xylitol producer, producing 32.23 g L^{-1}, 0.743 g g^{-1} of xylitol, and a volumetric productivity of 0.894

Table I. Fermentation results of the four *C. materiae* strains under the moderate and restricted oxygen conditions, in presence of 50 g L^{-1} xylose.

Strains	Oxygenation	Time (h)	Initial biomass (gL^{-1})	Biomass (gL^{-1})	Ethanol (gL^{-1})	Xylitol (gL^{-1})	Xylose consumption (%)	$Y_{p/s}^{et}$ (g/g)	$Y_{p/s}^{xyl}$ (g/g)	Q_p^{et} ($gL^{-1}.h$)	Q_p^{xyl} ($gL^{-1}.h$)	η^{et} (%)	η^{xyl} (%)
UFMG-CM-Y480	Moderate	48	1	6.10	$6.54\pm0.55^{a,b}$	27.03 ± 0.37^{c}	96.37	0.137	0.555	0.136	0.552	26.85	60.52
UFMG-CM-Y480		36	5	5.40	$5.52\pm0.09^{b,c}$	30.46 ± 0.24^{b}	97.34	0.119	0.655	0.153	0.846	23.28	71.50
UFMG-CM-Y480		36	10	11.31	$5.63\pm0.07^{a,b,c}$	$32.23\pm0.43^{a,b}$	98.85	0.130	0.743	0.156	0.895	25.44	81.01
UFMG-CM-Y481		60	1	9.33	3.42 ± 0.17^{e}	$32.02\pm0.16^{a,b}$	99.44	0.069	0.644	0.057	0.534	13.49	70.22
UFMG-CM-Y481		60	5	9.71	$3.9\pm0.09^{d,e}$	34.26 ± 0.92^{a}	98.49	0.082	0.716	0.065	0.571	15.98	78.09
UFMG-CM-Y481		48	10	10.48	$4.60\pm0.31^{c,d,e}$	$31.78\pm0.66^{a,b}$	97.38	0.100	0.688	0.096	0.662	19.53	75.02
UFMG-CM-Y482		48	1	7.94	7.01 ± 0.18^{a}	19.93 ± 0.54^{e}	99.51	0.156	0.443	0.146	0.415	30.60	48.35
UFMG-CM-Y482		48	5	8.21	$4.47\pm0.26^{c,d,e}$	$26.05\pm0.74^{c,d}$	98.25	0.105	0.610	0.093	0.543	20.52	66.52
UFMG-CM-Y482		48	10	10.01	2.93 ± 0.07^{e}	$26.18\pm0.21^{c,d}$	96.94	0.069	0.614	0.061	0.545	13.48	67.01
UFMG-CM-Y483		48	1	6.82	$5.79\pm0.02^{a,b,c}$	24.14 ± 0.69^{d}	92.58	0.140	0.578	0.121	0.499	27.37	63.06
UFMG-CM-Y483		36	5	5.69	$5.08\pm0.14^{c,d}$	30.79 ± 0.60^{b}	93.84	0.113	0.682	0.141	0.855	22.06	74.44
UFMG-CM-Y483		36	10	11.04	$2.85\pm0.30^{c,d,e}$	$31.25\pm0.10^{a,b}$	94.97	0.059	0.651	0.079	0.868	11.63	71.02
UFMG-CM-Y480	Restricted	120	1	0.87	$0.51\pm0,12^{E,F}$	$2.56\pm0,48^{G,H}$	8.12	0,122	0.616	0.004	0.021	23.88	67.20
UFMG-CM-Y480		120	5	3.37	$1.13\pm0,23^{C,D,E}$	$3.37\pm0.16^{E,F}$	11.12	0.207	0.617	0.009	0.028	40.58	67.24
UFMG-CM-Y480		120	10	6.61	$2.65\pm1,14^{A}$	$4.52\pm0.31^{B,C}$	18.67	0.287	0.490	0.022	0.038	56.33	53.39
UFMG-CM-Y481		120	1	1.03	$0.54\pm0,12^{E,F}$	1.80 ± 0.02^{I}	11.21	0.098	0.328	0.004	0.015	19.22	35.75
UFMG-CM-Y481		120	5	3.64	$1.59\pm0,08^{B,C,D}$	$2.80\pm0.05^{F,G}$	13.32	0.253	0.446	0.013	0.023	49.62	48.64
UFMG-CM-Y481		120	10	7.53	$2.50\pm0,09^{A,B}$	$4.10\pm0.17^{C,D}$	16.93	0.325	0.532	0.021	0.034	63.71	57.98
UFMG-CM-Y482		120	1	0.79	$1.41\pm0,31^{C,D,E}$	1.84 ± 0.08^{I}	4.30	0.644	0.844	0.012	0.015	126.2	92.00
UFMG-CM-Y482		120	5	3.57	$1.32\pm0,02^{A,B,C}$	$2.20\pm0.10^{H,I}$	12.56	0.086	0.330	0.005	0.018	16.94	35.95
UFMG-CM-Y482		120	10	6.98	0.00 ± 0^{F}	$3.37\pm0.21^{E,F}$	23.80	0.000	0.284	0.000	0.028	0.00	31.02
UFMG-CM-Y483		120	1	0.94	$0.91\pm0,01^{D,E,F}$	$3.52\pm0.10^{D,E}$	10.53	0.169	0.654	0.008	0.029	33.15	71.31
UFMG-CM-Y483		120	5	5.37	0.00 ± 0^{F}	5.01 ± 0.05^{A}	19.25	0.000	0.524	0.000	0.042	0.00	57.11
UFMG-CM-Y483		120	10	7.89	0.00 ± 0^{F}	6.65 ± 0.10^{B}	28.21	0.000	0.488	0,.000	0.055	0.00	53.18

$Y_{p/s}^{et}$, $Y_{p/s}^{xyl}$, Q_p^{et}, Q_p^{xy}, η^{et} and η^{xyl} are respectively the conversion, productivity and efficiency factors of ethanol and xylitol . As F calc> F5%, it can be concluded that the means of the treatments differ among themselves at 5% probability ($p\leq0.05$).
The means indicated by the same letter (lowercase letters for moderate and uppercase letters for restricted.) did not differ statistically by the Tukey test ($p\leq0.05$). Values shown are the means of experiments carried out in duplicate.

g $L^{-1}h^{-1}$. The strain consumed 98.9% of the xylose in the 36-h experiment. Further, it produced 4.52 g L^{-1} xylitol under severe oxygen limitation, with a yield of 0.490 g g^{-1} and 0.038 g $L^{-1}h^{-1}$ of volumetric productivity in a-120 h cultivation while consuming only 18.7% of the sugar. However, the lowest xylitol production (19.93 g L^{-1}) was observed for the UFMG-CM-Y482 strain with 1 g L^{-1} of initial inoculum in a 48 h culture.

Under moderate oxygen-limiting conditions, the UFMG-CM-Y481 strain was the best ethanol producer resulting in 7.01 g L^{-1} ethanol in a 48-h experiment with 1 g L^{-1} as the initial inoculum. In the assay using a 10 g L^{-1} cell concentration, this strain produced 2.93 g L^{-1} of ethanol after 48 h of incubation. All strains presented low ethanol and xylitol production under severe oxygen-limiting conditions (Table I).

Four assays were performed using different amounts of sugars and the best oxygen-limitation (moderate) and cell concentration (5 g L^{-1}) conditions to evaluate xylose and glucose fermentation. Table II shows the best ethanol and xylitol yields from different combinations of both sugars. The UFMG-CM-Y480 and UFMG-CM-Y483 strains produced the highest xylitol amounts in 36 h, resulting in 30.46 g L^{-1} ($Y_{p/s}^{xyl}$ 0.655 g g^{-1}, Q_p^{xyl} 0.846 g $L^{-1}h^{-1}$) and 30.79 g L^{-1} ($Y_{p/s}^{xyl}$ 0.682 g g^{-1} and Q_p^{xyl} 0.855 g $L^{-1}h^{-1}$), and they consumed 98% and 93% xylose, respectively. These strains also showed the highest ethanol production at 5.52 g L^{-1} and 5.08 g L^{-1}, respectively. In the experiment using 25 g L^{-1} of glucose and 25 g L^{-1} of xylose (Table II), the UFMG-CM-Y483 strain produced 10.25 g L^{-1} of ethanol in 24 h with 95.3% sugar consumption, 0.265 g g^{-1} yield, 0.377 g L^{-1} h^{-1} of productivity, and 43.1% efficiency. In contrast, the UFMG-CM-Y481 strain produced 16.36 g L^{-1} xylitol, consumed 96.2%, with yield and productivity of 0.798 g g^{-1} and 0.454 g L^{-1} h^{-1}, respectively; the efficiency was 87.1% after 36 h of fermentation. When the culture medium contained 10 g L^{-1} glucose and 40 g L^{-1} xylose (Table II), the UFMG-CM-Y480 strain consumed 95.9% of the sugar, produced 26.2 g L^{-1} xylitol, 0.646 g g^{-1} of yield, 0.727 g $L^{-1}h^{-1}$ of productivity, and 70.5% efficiency in 36 h of fermentation. This strain produced 7.50 g L^{-1} ethanol ($Y_{p/s}^{et}$ 0.164 g g^{-1}, Q_p^{et} 0.185 g $L^{-1}h^{-1}$ productivity) with 32.25 % efficiency in 36 h. According to the assay containing 40 g L^{-1} of glucose and 10 g L^{-1} of xylose, the UFMG-CM-Y482 strain consumed 95.9% of the sugar and reached 14.82 g L^{-1} of ethanol, 0.310 g g^{-1} yield, 0.592 g $L^{-1}h^{-1}$ productivity, and 60.9 % efficiency in 24 h. This strain produced 6.04 g L^{-1} of xylitol ($Y_{p/s}^{xyl}$ 0.131 g g^{-1}, Q_p^{xyl} 0.251 g $L^{-1}h^{-1}$) with an efficiency of 14.84% in 24-h fermentation.

DISCUSSION

Results showed that *C. materiae* strains isolated from decaying wood produced high xylitol yields from xylose. The selection of the best initial inoculum and oxygen-limiting conditions was made according to xylitol production since it was the main product of xylose metabolism by the yeast. *C. materiae* UFMG-CM-Y481 differed statistically in xylitol production under moderate

Table II. Fermentation results from the four *C. materiae* strains. The cultures performed with different concentrations of sugars and initial inoculum of 5 g L^{-1} under moderate oxygen condition.

Medium	Strain	Time (h)	Biomass (gL^{-1})	Ethanol (gL^{-1})	Xylitol (gL^{-1})	Xylose consumption (%)	$Y_{p/s}^{et}$ (g/g)	$Y_{p/s}^{xyl}$ (g/g)	Q_p^{et} (gL^{-1}.h)	Q_p^{xyl} (gL^{-1}.h)	η^{et} (%)	η^{xyl} (%)
25gL^{-1} Glucose/ 25gL^{-1} Xylose	UFMG-CM-Y480	36	8.49	10.04	15.94	96.32	0.211	0.770	0.250	0.442	41.40	83.98
	UFMG-CM-Y481	36	10.20	10.28	16.36	96.18	0.218	0.798	0.260	0.454	42.84	87.09
	UFMG-CM-Y482	36	9.77	11.06	15.53	96.05	0.249	0.781	0.289	0.431	48.99	85.17
	UFMG-CM-Y483	24	9.15	10.25	12.84	95.27	0.265	0.645	0.377	0.535	43.11	70.29
10gL^{-1} Glucose/ 40gL^{-1} Xylose	UFMG-CM-Y480	36	6.86	7.50	26.20	95.93	0.164	0.646	0.185	0.727	32.25	70.48
	UFMG-CM-Y481	36	8.13	6.44	24.88	94.71	0.146	0.641	0.157	0.691	28.66	69.96
	UFMG-CM-Y482	36	7.67	7.74	23.50	93.66	0.176	0.597	0.192	0.652	34.56	65.12
	UFMG-CM-Y483	36	6.66	7.61	22.01	96.49	0.172	0.556	0.188	0.611	33.76	60.73
40gL^{-1} Glucose/ 10gL^{-1} Xylose	UFMG-CM-Y480	24	10.69	14.46	5.44	96.05	0.283	0.113	0.565	0.226	55.67	12.43
	UFMG-CM-Y481	24	13.41	13.90	6.03	96.00	0.274	0.126	0.546	0.251	53.86	13.78
	UFMG-CM-Y482	24	13.12	14.82	6.04	95.82	0.310	0.131	0.592	0.251	60.87	14.38
	UFMG-CM-Y483	24	10.42	15.02	5.42	96.55	0.263	0.102	0.580	0.225	51.67	11.17

$Y_{p/s}^{et}$, $Y_{p/s}^{xyl}$, Q_p^{et}, Q_p^{xy}, η^{et} and η^{xyl} are respectively the conversion, productivities and efficiencies factors of ethanol and xylitol. The total consumption of sugars refers to the sum of the consumption of xylose and glucose. For values of Ypet/s, Ypxil/s, Qpet and Qxil.

oxygen-limiting conditions (34.26 g L^{-1}, $Y_{p/s}^{xyl}$ 0.716 g g^{-1}) with 5 g L^{-1} inoculum in 60 h of culture (Table I). The UFMG-CM-Y480 strain produced 32.23 g L^{-1} xylitol in 36 h and consumed 98.8% of xylose with a conversion factor of 0.743 g g^{-1} and a productivity of 0.895 g $L^{-1}.h^{-1}$ (Table I) under moderate oxygen-limiting conditions. Xylose consumption was high among all the strains under moderate oxygen-limiting conditions, ranging from approximately 92% to 99%. Cadete et al. (2015) used the same conditions as in this study with the yeast *Cyberlindnera xylosilytica* UFMG-CM-Y408 strain. This strain produced 34.46 g L^{-1} xylitol, with a xylose consumption of 94.3%, a conversion factor of 0.720 g g^{-1}, and a productivity of 0.479 g $L^{-1}.h^{-1}$ after 72 h of fermentation. Other studies also investigated the performance of xylitol-producing strains such as *C. tropicalis* (21.30 g L^{-1}, $Y_{p/s}$ 0.53 g g^{-1} in 42 h of fermentation) (Lorliam et al. 2017) and *Cyberlindnera galapagoensis* (17.01 g L^{-1}; $Y_{p/s}$, 0.50 g g^{-1} and Qp, 0.23 g L^{-1} h^{-1}) (Guamán-Burneo et al. 2015). *C. materiae* showed higher productivity by generating a higher amount of xylitol in a shorter time compared to the results obtained for the species reported above. Based on these results, this yeast could be a potential candidate for biological xylitol production from D-xylose.

Lower xylitol production was observed in severe oxygen-limiting conditions compared to other conditions. The 10 g L^{-1} inoculum showed the highest amounts of xylitol for all strains of *C. materiae*, but for the UFMG-CM-Y483 strain, the production was statistically significant at that cell concentration consuming 28.21% of the xylose (Table I). In addition, while the UFMG-CM-Y482 strain showed statistically significant ethanol production, it was deemed the best ethanol producer using a 1 g L^{-1} inoculum in moderate oxygen-limiting conditions with 7.01 g L^{-1} in 48 h of fermentation (Table I). The severe condition compromised xylose conversion with 0.51 to 2.65 g L^{-1} of ethanol among the three inoculums tested (Table I). The UFMG-CM-Y-480 strain was statistically differentiated by the Tukey test ($p \leq 0.05$) with an inoculum of 10 g L^{-1} for ethanol production. While XR from *C. materiae* prefers NADPH, XDH seems to prefer NAD^+. NAD^+ cannot be regenerated in the absence of oxygen, and the imbalance of cofactors generated by that preference leads to cellular xylitol accumulation (Granström & Leisola 2013). The ability of yeasts to assimilate xylose was affected by the reduced oxygen availability, which might be associated to the limited NADPH supply required for XR activity. According to Barbosa et al. (1988), the accumulation and excretion of xylitol is associated to NADPH regeneration. This cofactor may be obtained from the oxidative steps of the pentose phosphate pathway (PPP). In addition, the xylitol flux linearly increases as a function of the dependence of the oxygen limitation and NADH accumulation (Granström et al. 2001). Under high oxygen limitation, the flux from xylose assimilation might be insufficient to promote NADPH regeneration, affecting xylitol production.

We observed a significant increase in cell growth using 1 g L^{-1} inoculum under moderate oxygen-limiting conditions (6.10 g L^{-1} at 9.33 g L^{-1}). The biomass of UFMG-CM-Y481 and UFMG-CM-Y482 strains increased in the 5 g L^{-1} inoculum, while it remained constant during fermentation with the other strains. Under severe oxygen limitation, we did not observe any increase in the biomass in the three inoculums tested (1, 5, and 10 g L^{-1}); instead, a decrease was observed in some cases (Table I). The growth and metabolism profile of *C. materiae* was highly sensitive to oxygenation, as shown in Table I. The oxygen-limiting condition was quite detrimental to this species since there was no increase in biomass, and a rather low yield of ethanol and xylitol was observed for all strains for up to 120 h of

fermentation. More oxygen in the reaction helps Crabtree-negative yeasts to achieve an optimal biomass yield, while its limitation leads to fermentation and the formation of byproducts (de Alteriis et al. 2018). In our studies, there was no biomass production under severe oxygen-limiting conditions. This may be explained by the inhibition of NAD^+ regeneration to XDH activity and xylitol accumulation, which decreases the formation of carbon intermediates and energy production, and affects cell growth (Hernández-Pérez et al. 2016, Veras et al. 2017).

Fermentation experiments were performed to evaluate ethanol and xylitol production (Table II) with 50 g L^{-1} of sugar (xylose: glucose) in different ratios using the best oxygenation (moderate) and cell concentration (5 g L^{-1}) conditions selected in the previous assays. The total assimilation of glucose was achieved after 12 h of fermentation. Xylose consumption began simultaneously, but its uptake was slower until the total consumption of the hexose in the medium reached 25/25 g L^{-1} of sugar (Fig. 1a, b, c and d). In ratios with a high amount of xylose and low amount of glucose (40 g L^{-1}/10 g L^{-1}, respectively), the strains of *C. materiae* started to consume both sugars at the same time and at the same speed (Fig. 2a, b, c and d). When the ratio was 40 g L^{-1} glucose/10 g L^{-1} xylose (Fig. 3a, b, c and d), the pentose was consumed after glucose consumption. Since xylitol was the main product metabolized by *C. materiae* from xylose, it remained even during co-fermentation. Ethanol was the main product from glucose. In the assays containing 25 g L^{-1} of glucose and 25 g L^{-1} of xylose (Table II), only the UFMG-CM-Y483

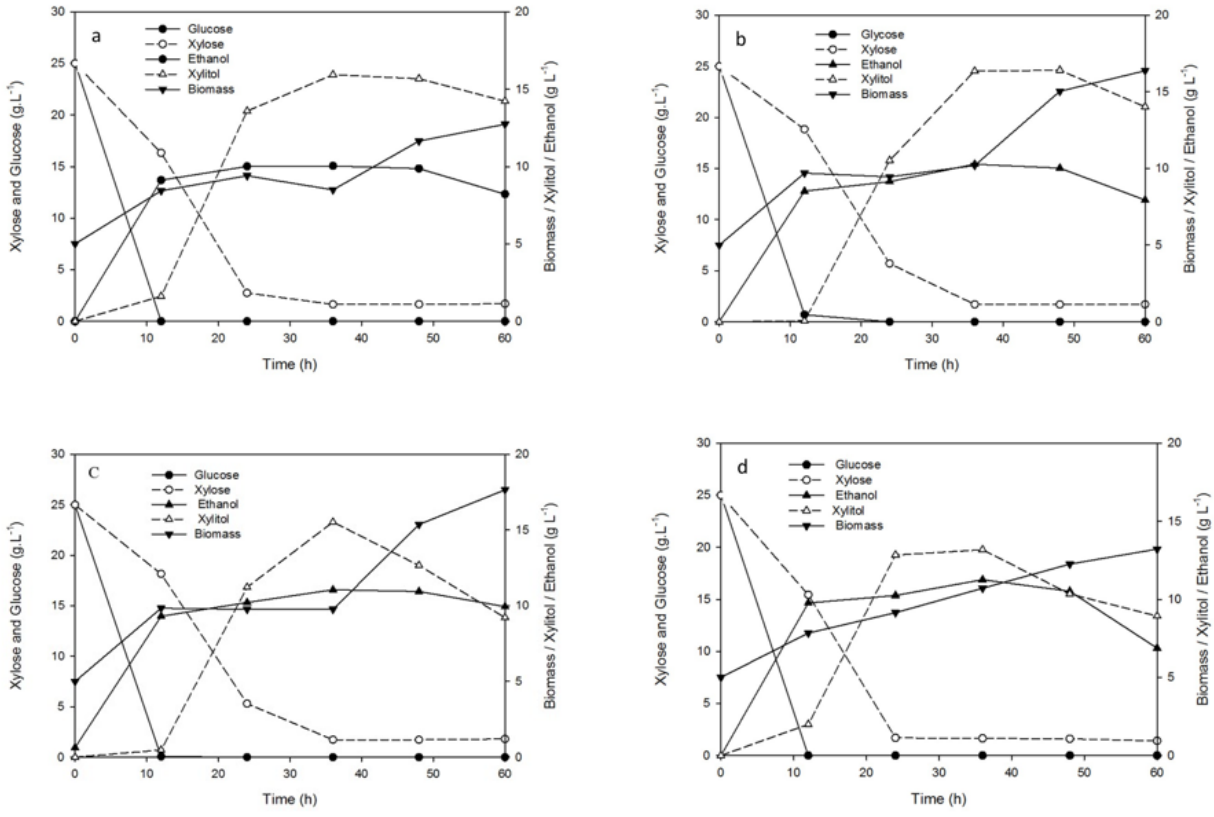

Figure 1. Profiles of xylose and glucose consumption and xylitol, ethanol, and cell biomass production by different strains of *C. materiae*. Fermentation was performed in a medium with 25 g L^{-1} glucose and 25 g L^{-1} xylose (a) UFMG-CM- Y480; (b) UFMG-CM-Y481; (c) UFMG-CM-Y482; (d) UFMG-CM-Y483.

strain produced a lower amount of xylitol (12.84 g L^{-1}) than the other yeasts (~16.0 g L^{-1}) (Table I). Ethanol was produced (~10.0 g L^{-1}) after 10 h of fermentation, and the strains consumed all the glucose. The most xylose-fermenting yeasts have two transport systems for xylose assimilation: low-affinity (facilitated diffusion) and high affinity (proton symporters). Glucose competes with pentose for the first system and inhibits its transport by the high-affinity system in a noncompetitive way (Sharma et al. 2019). *Spathaspora passalidarum* and *Scheffersomyces stipitis* under anaerobic conditions have the same behavior as *C. materiae* since they consume glucose before consuming xylose in the presence of high concentrations of the hexose (Panchal et al. 1988, Cadete et al. 2016). However, under aerobic conditions, Sp. *passalidarum* can take up these sugars simultaneously, indicating that this species uses different transport systems under distinct conditions (Cadete et al. 2016, Hou 2012).

For the results corresponding to 10 g L^{-1} of glucose/40 g L^{-1} of xylose (Table II), the parameters for xylitol production were similar to those with xylose alone. A 20% reduction in xylose led to a 14% reduction in xylitol production. This result can be considered advantageous since the industrial production cost of xylitol is very high (Cheng et al. 2014). Silva et al. (2007) reported the effect of the glucose:xylose ratio on the bioconversion by *C. guilliermondii* using sugarcane bagasse hydrolyzate. These researchers observed that increasing the glucose: xylose ratio improved the assimilation of xylose present in the hydrolyzate by yeast, resulting in biomass increase and xylitol and ethanol formation ($Y_{p/s}$ 0.53 g g^{-1}; Qp 0.53 g g^{-1}). However, the ethanol productivity was similar in experiments with 40

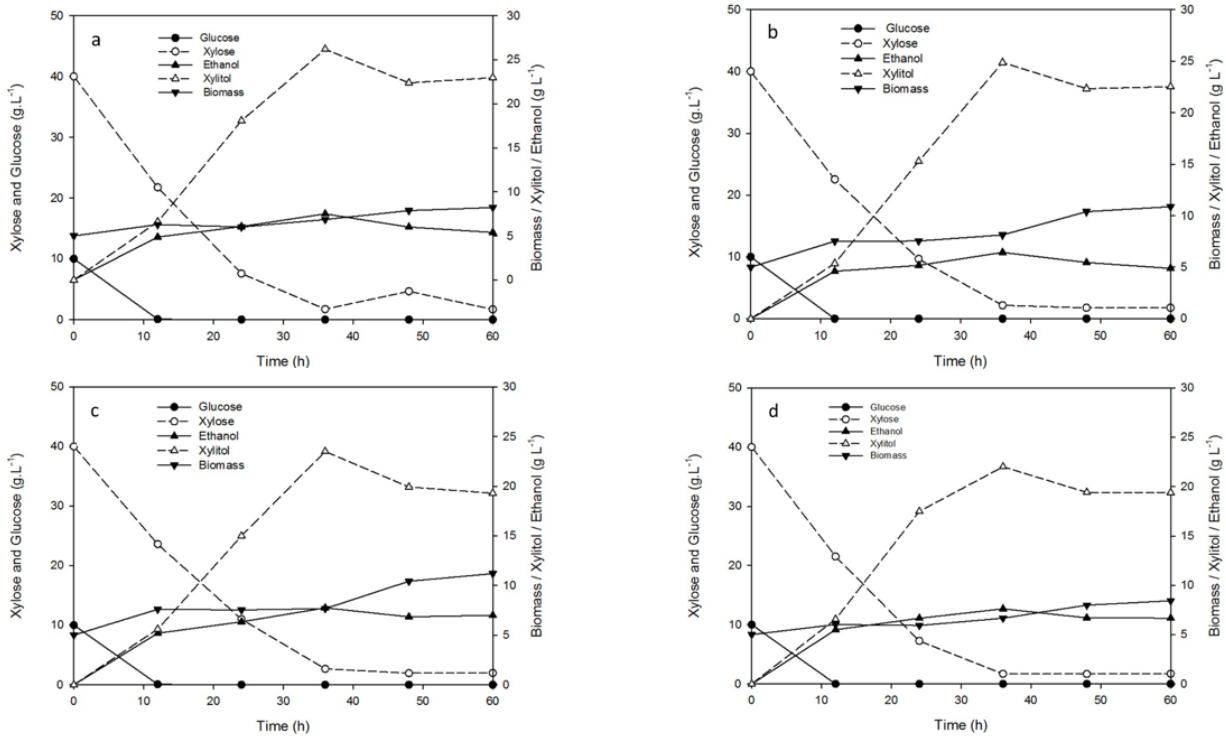

Figure 2. Xylose and glucose consumption and xylitol, ethanol, and cell biomass production profiles for different *C. materiae* strains in a medium with 10 g L^{-1} glucose and 40 g L^{-1} xylose (a) UFMG-CM-Y480; (b) UFMG-CM-Y481; (c) UFMGCM-Y482; (d) UFMG-CM-Y483.

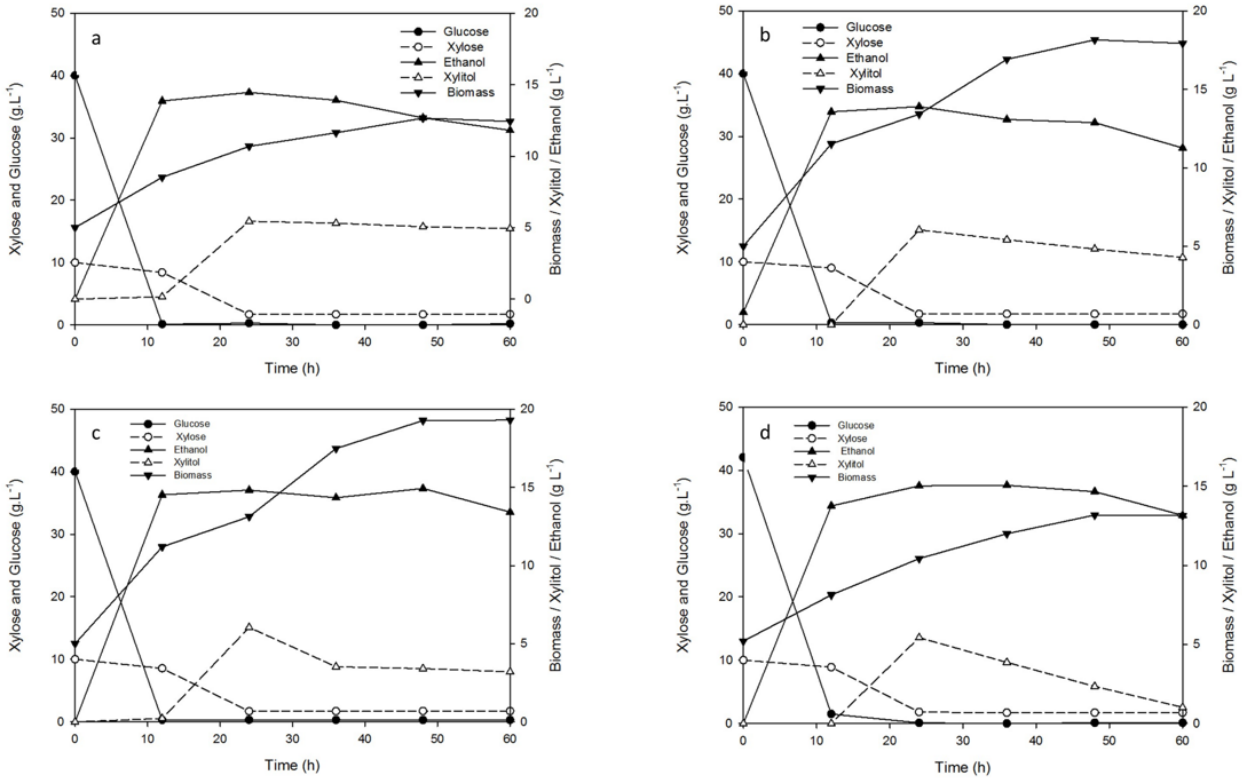

Figure 3. Xylose and glucose consumption and xylitol, ethanol, and cell biomass production profiles for different *C. materiae* strains in a medium with 40 g L^{-1} glucose and 10 g L^{-1} xylose (a) UFMG-CM-Y480; (b) UFMGCM-Y481; (c) UFMG-CM-Y482; (d) UFMG-CM-Y483.

g L^{-1} of glucose and 10 g L^{-1} of xylose. In contrast, xylitol production under this condition occurred until 24 h when the xylose concentration was restricted in the medium. Subsequently, it remained constant or decreased. The yeast-mediated bioconversion of xylose into ethanol and xylitol is more efficient in the presence of a small amount of glucose, since it is used for cell growth and for NADPH regeneration before using xylose (Long et al. 2012). In conclusion, to the best of our knowledge, this is the first study to analyze xylose and glucose metabolism by the yeast *C. materiae*. The use of the initial 5 g L^{-1} inoculum favored xylitol production from xylose by the *C. materiae* strains under moderate oxygen-limiting conditions. The addition of 10 g L^{-1} glucose to xylose fermentation favored xylitol production. In addition, using 10 g L^{-1} of glucose reduced in 20% the cost of xylitol production. Our results showed that the UFMG-CM-Y480 strain was the best producer of xylitol and ethanol in most assays. Therefore, this yeast strain is a promising candidate for converting xylose to xylitol in complex media under similar test conditions.

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