

Selection and Fermentative Cultures In Synthetic Medium With Microorganisms Isolated From Dairy For Use Of Industrial Waste And Ethanol Production

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Working with conventional microorganisms for bioconversion of industrial substrates is a challenge. In this sense, has been studied the possibility of replacing the conventional microorganisms with new strains prospected from specific environments. In the case of the dairy, the cheese whey is considered the main by-product, with lactose being its highest concentration constituent (about 90 % of the organic load). This carbohydrate is produced at a high and constant rate by the dairy industry each year. In this sense, is important to have viable alternatives to convert the lactose, available as a residue form, into value added products. The objective of this study was to select and evaluate the fermentative potential of strains prospected from the dairy industry, evaluating their kinetic parameters of growth, specific substrate consumption and formation of metabolites. The strains were cultivated in mineral medium with and without supplementation for the biochemical test. And The selected strains were grew in 250 mL Erlenmeyer flasks, pH 6.0 and lactose, glucose or galactose as the sole carbon source (10 g L^{-1}) at $30 \text{ }^\circ\text{C}$. The isolated were able to grow and produce ethanol in cultivation with lactose, glucose and galactose. In addition, less expressive growth was observed when galactose was used as carbon source. It was observed that when compared to glucose, some strains shows grown in lactose presented specific maximum growth rates (μ_{max}) equal to or greater than those presented with glucose. This allows affirming that these strains are promising in the use of lactose as carbon source, since it efficiently metabolizes a disaccharide as much as it does with a monosaccharide. The characteristic of the studied strains of convert lactose efficiently reveals their potential for residue treatment applications.

1. Introduction

The availability of substrates in agricultural and industrial waste triggers research that values these products. Thus, different microorganisms are used to convert a wide variety of available substrates into value-added products. According to Gonzalez-Siso et al. (2015), lactose is a potential substrate, since its significant availability, as a form of residue in cheesemaking processes is known. Lactose direct elimination into the environment can cause serious problems due to high chemical and biological oxygen demand. Therefore, the use of lactose as a substrate for the production of biofuels offers an economic production of value-added products. Yeasts are unicellular fungi, capable of consuming different substrates. Although many yeasts that assimilate lactose in aerobiosis, lactose-fermenting yeasts are quite rare, including species of *Kluyveromyces lactis* (Fonseca et al. 2008b) and *Candida pseudotropicalis* (Guimarães et al., 2010). The ability of the microorganism to grow in lactose as the only source of carbon depends on the hydrolysis of lactose and on the metabolism of the resulting glucose and galactose (Fonseca et al., 2008a). The physiological tests commonly used are the fermentation of seven to eight carbohydrates and also growth in several sources of carbon and nitrogen (Kurtzman et al., 2011). The microorganisms that bioconvert the lactose can generate products such as biogas, organic acids, amino acids, vitamins, polysaccharides, lipids and enzymes

(Guimarães et al., 2010). In addition, lactose fermentation may provide products such as ethanol, microbial biomass and volatile aromatic compounds, especially alcohols and esters responsible for pleasant aromas and flavors in cheese (Andrade et al., 2016). The objective of the study was to select the strains of wild microorganisms with potential for lactose consumption from biochemical tests and then to evaluate their kinetic parameters for growth, consumption and product generation in cultures grown in synthetic medium containing lactose as the only source of carbon.

2. Material and methods

2.1 Microorganisms and maintenance

Eleven microorganisms were isolated from butter (M1), parmesan cheese (P1, PI1, PI2, PI3 and P3), residual whey of ricotta cheese production (SR), whey tank intended for rural producers (STFR and STFB) and first effluent tank (1TE), from a dairy industry located in the city of Guaraçai, São Paulo, Brazil. They were kept in a medium YEPL solid (in g L⁻¹: yeast extract, 20.0; bacterial peptone, 20.0; agar, 15.0; lactose, 20.0) at 4 °C.

2.2 Biochemical test for assimilation and fermentation of lactose, glucose and galactose in mineral medium (MM) and supplemented mineral medium (MMS)

Microorganisms isolated from dairy were tested for their ability to assimilate and ferment lactose, glucose and galactose as the sole source of carbon. Before inoculating the tubes containing MM or MMS, the cells were grown in YEPL medium (g L⁻¹: yeast extract, 20.0; bacterial peptone, 20.0, substrate, 20.0). Thus, the microorganisms were inoculated at the concentration of 1:10. The MM tests were run in test tubes with 10 mL culture medium, which contained in g L⁻¹ distilled water: (NH₄)₂SO₄, 5.0; KH₂PO₄, 3.0; MgSO₄.7H₂O, 0.5 (Verduyn et al., 1992); substrate, 20.0. Samples were considered positive for assimilation in which the medium became cloudy after incubation, which evidences cellular multiplication. While for fermentation, samples containing the Durham tube filled with gas were considered positive. Whereas, the tests performed with MMS contained in g L⁻¹ distilled water: (NH₄)₂SO₄, 5.0; KH₂PO₄, 3.0; MgSO₄.7H₂O, 0.5 (Verduyn et al., 1992); yeast extract, 4.5; bacteriological peptone, 7.5; substrate, 20.0. As described by Kutzman et al. (2011) was added to the medium a filter-sterilized (0.45 µm) bromothymol blue indicator solution. When sugars are consumed, the indicator changes the color of the medium from green to yellow. However, if the sugars are not consumed and the amino acids present are used as the carbon source, the medium becomes blue. In both (MM and MMS), the pH of the medium was adjusted to 6.0 with 1 M NaOH. Invert Durham's tube was added to each assay, then autoclaved (121 °C, 15 min). A test tube without substrate was used as a negative control. The tubes were incubated (New Lab, Brazil) at 30 °C for 168 h and inspected at 24 h intervals.

2.3 Synthetic medium and culture conditions

The mineral medium used for the precultures and for the main cultures were of the same composition, containing in g L⁻¹ distilled water: (NH₄)₂SO₄, 5.0; KH₂PO₄, 3.0; MgSO₄.7H₂O, 0.5; 1 mL of trace element solution and 1 mL of a solution of vitamins (Verduyn et al., 1992). The mineral medium was supplemented with yeast extract and bacteriological peptone (in g L⁻¹: 4.5 and 7.5, respectively). The pH of the medium was adjusted to 6.0 with 1 M NaOH, and then autoclaved. A lactose (10 g L⁻¹) was sterilized (121 °C, 15 min) separately and added to the culture medium at the time of the inoculum. Precultures were performed by transferring a sample of the yeast strain contained in solid YEPL medium into an Erlenmeyer flask containing 200 mL of MMS and 50 mL of the lactose. They were then incubated under aerobic conditions on orbital shaker (New Lab 2201, Brazil) at 30 °C, 200 rpm, for 12 h. Cellular multiplication was observed by the optical density (OD₆₀₀) measured at the wavelength of 600 nm in a spectrophotometer (Hach DR 2800, Brazil). In order to evaluate the growth profile of the prospective microorganisms, the main cultures started with 0.1 OD₆₀₀, so the inoculum of the main cultures was carried out from a determined amount of preculture. The aerobic cultures, performed in duplicate, were incubated in an orbital shaker at 30 °C and 200 rpm. Samples were collected at the initial time and at 2 h intervals, for determination of dry cell mass and extracellular metabolites.

2.4 Determination of substrate, concentration of extracellular metabolites and microbial biomass

Samples were centrifuged for 20 minutes, filtered (0.22 µm) and the filtrate obtained was used for the determination of extracellular substrate and metabolites using HPLC (Varian 920-LC) equipped with refractive index detector and a Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA). The analyzes were carried out in a mobile phase of 5 mM H₂SO₄, at 60 °C, with flow rate of 0.6 mL min⁻¹, for 25 min. The biomass sediment was oven dried (105 °C) to constant mass. The dry cell mass (g L⁻¹) was obtained by the quotient of the difference in mass per volume of centrifuged medium. The OD₆₀₀ was also measured to determine biomass

indirectly. To this end, the measured absorbance values were converted to mass values using a linear relationship determined in each experiment. All values are measured of two separate experiments.

2.5 Determination of kinetic parameters

The exponential growth phase (EGP) was identified as the linear region of the growth kinetic curve by time. And the maximum specific growth rate (μ_{max}) was determined as the slope of this line. The substrate to cell ($Y_{X/S}$) conversion factor was determined as the slope of the line obtained from the cell concentration curve (X) as a function of substrate (S) concentration, necessarily including EPG points. The substrate to ethanol conversion factor ($Y_{Eth/S}$) was determined from the concentration of the product in relation to substrate (S). The specific rate of substrate consumption (μ_S) was calculated according to Equation 1:

$$\mu_S = \frac{\mu_{max}}{Y_{X/S}} \quad (1)$$

Where, μ_{max} = maximum specific growth rate (h^{-1}); $Y_{X/S}$ = substrate conversion factor in cells ($g\ g^{-1}$).

The productivities (ethanol and biomass) were calculated according to Equations 3:

$$P_{Eth\ or\ X} = \frac{P_f - P_i}{t} \quad (3)$$

Where, P_f = final product (ethanol or biomass) ($g\ L^{-1}$); P_i = starting product (ethanol or biomass) ($g\ L^{-1}$); t = final time (h).

3. Results and discussion

3.1 Assimilation and fermentation of lactose, glucose and galactose in MM and MMS

The results of the behavior of the isolated yeast strains in lactose, glucose and galactose assimilation and fermentation test as the sole carbon source in MM, are presented in Table 1.

Table 1: Assimilation and fermentation of lactose, glucose and galactose in MM with eleven strains

Strains	After 24 h						After 168 h					
	Lactose		Glucose		Galactose		Lactose		Glucose		Galactose	
	A	F	A	F	A	F	A	F	A	F	A	F
P1	-	-	-	-	+	-	+	-	+	-	+	+ ^{1/2}
PI1	-	-	-	-	-	-	+	+ ^{1/3}	+	-	+	-
PI3	+	-	+	-	+	-	+	+ ^{1/3}	+	+ ^{1/2}	+	+ ^{1/2}
SR	+	-	+	-	+	-	+	+ ^{1/3}	+	+ ^{1/3}	+	+ ^{1/2}
1TE	+	-	+	-	+	-	+	+ ^{1/3}	+	+ ^{1/2}	+	+ ^{1/3}
STFB	-	-	-	-	-	-	+	+ ^{1/3}	+	-	+	-
STFR	-	-	-	-	-	-	+	-	+	-	+	-
PI2	-	-	-	-	-	-	+	-	+	-	+	-
M1	-	-	-	-	-	-	+	-	+	-	+	-
R2	-	-	-	-	-	-	+	-	+	-	+	-
P3	-	-	-	-	-	-	+	-	+	-	+	-

Where, A: assimilation; F: fermentation; -: negative, did not assimilate or fermented; + °: Durham's tube filled with a small bubble of gas; +1/3: 1/3 of the Durham tube filled with gas; +1/2: half of the Durham tube filled with gas; +: Durham tube fully (or more than 1/2) filled with gas.

It was observed that in 24 h of cultivation, only the PI3, SR and 1TE strains were able to assimilate lactose, glucose and galactose. In addition, P1 strain was able to assimilate galactose. The other strains cultivated in MM did not reach positive result of assimilation. For fermentation, the eleven isolated yeast strains when grown in MM did not present positive results in the first 24 h of culture. After 168 h of cultivation, the PI3, SR and 1TE strains, besides assimilating the three carbon sources, also fermented. The PI3 strain cultured in MM was shown to be faster by fermenting glucose and galactose (half of the gas filled Durham tube) than lactose (1/3 of the gas filled Durham tube). Although some strains show signs of fermentation in MM, when considering the total time it is observed that the bioconversion of the substrates by the yeasts was not efficient in MM. As there is no single standardized method for many of these physiological and biochemical tests, the results sometimes depend on the techniques employed (Kurtzman et al., 2011). In this sense, we investigated the influence of MM supplementation with yeast extract and bacteriological peptone on the performance of yeast strains by assimilating and fermenting different carbon sources. For this purpose, we chose to work with the six strains that were highlighted in the test using MM (Table 1). The results of the cultures with the six strains cultivated on different substrates, after 24 h and 168 h of incubation, are presented in Table 2.

Table 2: Assimilation and fermentation of lactose, glucose and galactose in MMS with six strains

Strains	After 24 h						After 168 h					
	Lactose		Glucose		Galactose		Lactose		Glucose		Galactose	
	A	F	A	F	A	F	A	F	A	F	A	F
P1	+	+ ^o	+	+ ^o	+	+ ^o	+	+	+	+	+	+
PI1	+	-	+	+ ^o	+	+ ^o	+	+	+	+ ^{1/2}	+	+ ^{1/2}
PI3	+	+ ^o	+	+ ^o	+	+ ^o	+	+	+	+ ^{1/2}	+	+
SR	+	+ ^o	+	+ ^{1/3}	+	+ ^o	+	+	+	+	+	+ ^{1/2}
1TE	+	+ ^o	+	+ ^o	+	+ ^o	+	+	+	+	+	+
STFB	+	-	+	-	+	-	+	+ ^{1/3}	+	+ ^{1/3}	+	+ ^{1/3}

Where, A: assimilation; F: fermentation; -: negative, did not assimilate or fermented; +^o: Durham's tube filled with a small bubble of gas; +^{1/3}: 1/3 of the Durham tube filled with gas; +^{1/2}: half of the Durham tube filled with gas; +: Durham tube fully (or more than 1/2) filled with gas.

Evaluating the assimilation and fermentation capacity of six yeast strains, this time cultivated in MMS, a significant difference was observed in the results. In terms of the ability of these strains to ferment, it was observed that most of the strains evaluated presented fermentation (Durham tube filled with a small gas bubble) in the first 24 h. This confirms that the composition of the medium directly affected the microorganism's consumption of the substrate. At the end of the test in MMS, it was observed that the strains P1, PI1, PI3, SR and 1TE presented vigorous fermentation in cultures with lactose, since the tubes of Durham were totally filled with gas. Alves-Jr et al. (2007) also noted a better fermentative performance by yeast cells when grown in medium supplemented with yeast extract and peptone, producing significantly higher levels of ethanol. They further complement that since yeast cells could obtain nutrients needed for growth from the added extra compounds (peptone or yeast extract), more of the carbon source could possibly have been channeled into the production of ethanol.

3.2 Kinetic parameters of growth, consumption and generation of products

The results of the kinetic parameters of growth, substrate consumption and ethanol and biomass yields obtained in the aerobic cultures of six yeast strains in lactose, glucose and galactose are presented in Table 3.

Table 3: Parameters obtained in the independent cultures with lactose, glucose and galactose

Strains	S	μ_{max} (h ⁻¹)	μ_s (h ⁻¹)	$Y_{X/S}$ (g g ⁻¹)	$Y_{ETOH/S}$ (g g ⁻¹)	P_{Eth} (g L ⁻¹ h ⁻¹)	P_x (g L ⁻¹ h ⁻¹)	T_{s0} (h)
P1	LAC	0.20±0.02	1.91±0.40	0.11±0.00	0.07±0.00	0.06±0.00	0.11±0.00	10
PI1		0.32±0.01	1.95±0.16	0.16±0.00	0.19±0.00	0.11±0.01	0.15±0.00	10
PI3		0.32±0.00	2.58±0.15	0.12±0.00	0.22±0.00	0.14±0.00	0.08±0.00	10
SR		0.34±0.02	0.76±0.14	0.46±0.05	0.18±0.01	0.11±0.01	0.40±0.01	10
1TE		0.25±0.01	0.92±0.00	0.28±0.01	0.25±0.02	0.13±0.00	0.29±0.00	10
STFB		0.34±0.03	1.51±0.17	0.22±0.00	0.20±0.00	0.11±0.00	0.32±0.04	10
P1	GLU	0.23±0.00	1.79±0.02	0.13±0.00	0.07±0.00	0.05±0.00	0.14±0.00	10
PI1		0.25±0.03	1.22±0.07	0.20±0.01	0.09±0.01	0.07±0.01	0.20±0.04	10
PI3		0.38±0.01	1.68±0.03	0.22±0.00	0.14±0.01	0.19±0.00	0.29±0.04	08
SR		0.34±0.01	1.31±0.03	0.26±0.01	0.18±0.01	0.18±0.02	0.22±0.04	08
1TE		0.25±0.00	0.82±0.08	0.30±0.02	0.17±0.00	0.18±0.02	0.31±0.00	08
STFB		0.23±0.00	1.12±0.07	0.21±0.00	0.24±0.00	0.11±0.00	0.16±0.02	10
P1	GAL	0.23±0.01	1.17±0.02	0.20±0.01	0.12±0.00	0.07±0.00	0.14±0.01	12
PI1		0.21±0.00	0.66±0.00	0.31±0.00	0.17±0.01	0.06±0.02	0.15±0.02	14
PI3		0.13±0.01	0.64±0.07	0.20±0.00	0.11±0.01	0.08±0.00	0.16±0.01	12
SR		0.23±0.03	0.92±0.11	0.24±0.01	0.10±0.00	0.09±0.01	0.21±0.01	12
1TE		0.13±0.00	0.60±0.01	0.21±0.00	0.15±0.01	0.07±0.00	0.16±0.00	14
STFB		0.16±0.00	2.79±0.03	0.06±0.00	0.11±0.01	0.09±0.00	0.23±0.01	10

S: substrate; LAC: lactose; GLU: glucose, GAL: galactose; μ_{max} : maximum specific growth rate; μ_s : specific rate of substrate consumption; $Y_{X/S}$: substrate conversion factor in cell; $Y_{ETOH/S}$: substrate conversion factor to ethanol; MCS: dry cell mass, P_x : yield in cells; P_{Eth} productivity in ethanol; T_{s0} : time for substrate depletion. Data presented with mean ± standard deviation.

In relation to the cultures with lactose, during the EPG, the SR and STFB strains stood out, presenting μ_{max} of 0.34 h⁻¹ for both. The $Y_{X/S}$ for this strains were 0.46 and 0.22 g g⁻¹, respectively. And μ_s were calculated as 0.76 and 1.51 h⁻¹, respectively. PI1 and PI3 strains presented values of μ_{max} (0.32 h⁻¹, for both) close to those obtained by SR and STFB strains. In addition, the specific μ_s of PI1 and PI3 were higher (1.95 and 2.58 h⁻¹, respectively) than those obtained by the other strains in this substrate. It was observed that, when compared to glucose, the PI1, SR, 1TE and STFB strains being cultured in lactose presented μ_{max} equal to or greater than those presented with glucose. This allows affirming that these strains are promising in the use of lactose

as carbon source, since it efficiently metabolizes a disaccharide as much as it does with a monosaccharide. In addition, the consumption of lactose indicates that these strains are alternatives to be applied in ethanol fermentation processes, which apply a previous enzymatic hydrolysis of lactose by β -galactosidase for subsequent fermentation, since the main disadvantages of this type of pretreatment, are the price of the enzyme and slow growth. With glucose, some cultures showed shorter times (8 h). Glucose is reported as a simple sugar and easily assimilated by the metabolic pathways. The strains that stood out for the fast glucose consumption were PI3 and SR, reaching μ_{\max} of 0.38 and 0.34 h⁻¹, respectively. PI3 and SR showed $Y_{x/s}$ of 0.22 and 0.26 g g⁻¹, respectively. In relation μ_s , these strains reached 1.68 and 1.31 h⁻¹, respectively. Galactose cultures were the ones that had the longest time for substrate depletion, showing that, although it is a simpler molecule (when compared to lactose), it is not necessarily metabolized in shorter times. In galactose, PI1, PI3, SR, 1TE and STFB produced the lowest values of μ_{\max} . Fonseca et al. (2013) characterizing the physiology of the yeast *Kluyveromyces marxianus* CBS 6556, in terms of μ_{\max} , verified that the cultivation of this strain in galactose at 37 °C was what obtained showed growth when compared to the other sugars investigated (lactose, sucrose, fructose, glucose). In this sense, they suggested that the absorption of galactose by the yeast *K. marxianus* CBS 6556 is less efficient than the other sugars, corroborating with the present work.

As for the ethanol, yield data (Table 3), the PI3 strain excelled in cultures with both lactose (0.14 g L⁻¹ h⁻¹) and glucose (0.19 g L⁻¹ h⁻¹). In relation to the biomass productivity, the SR strain in lactose (0.40 g L⁻¹ h⁻¹) and 1TE in glucose (0.31 g L⁻¹ h⁻¹) was highlighted. Formation of ethanol and succinate was observed in all cultures in the different sugars. Table 4 shows the maximum biomass formation and extracellular metabolites of six yeast strains grown on different substrates.

Table 4: Maximum formation of biomass and extracellular metabolites during cultures

Strains	S	Biomass (g L ⁻¹)	Succinic Acid (g L ⁻¹)	Acetic Acid (g L ⁻¹)	Ethanol (g L ⁻¹)	Total Metabolites (g L ⁻¹)
P1	LAC	1.53±0.20	0.38±0.03	0±0	0.91±0.03	1.29
PI1		2.27±0.12	0.54±0.05	0.37±0.01	1.77±0.02	2.68
PI3		1.33±0.04	0.50±0.01	0.34±0.02	1.78±0.01	2.62
SR		4.99±0.24	0.53±0.03	0.18±0.17	1.61±0.15	2.32
1TE		2.96±0.06	0.63±0.04	0.28±0.02	1.79±0.00	2.70
STFB		3.93±0.50	0.56±0.04	0.30±0.00	1.56±0.06	2.42
P1	GLU	1.48±0.07	0.17±0.01	0±0	0.68±0.07	0.85
PI1		2.14±0.09	0.37±0.07	0±0	0.93±0.22	1.30
PI3		3.30±0.12	0.38±0.00	0±0	1.54±0.07	1.92
SR		2.73±0.49	0.42±0.01	0±0	1.60±0.03	2.02
1TE		3.32±0.19	0.45±0.04	0±0	1.42±0.11	1.87
STFB		1.84±0.00	0.40±0.02	0±0	1.65±0.00	2.05
P1	GAL	1.87±0.13	0.55±0.04	0.44±0.03	0.94±0.05	1.93
PI1		1.75±0.10	0.35±0.09	0.32±0.02	0.62±0.08	1.29
PI3		0.34±0.18	0.56±0.00	0.23±0.02	1.10±0.09	1.89
SR		3.10±0.23	0.56±0.01	0.30±0.07	1.27±0.15	2.13
1TE		2.36±0.05	0.54±0.00	0.36±0.01	0.98±0.02	1.88
STFB		3.09±0.18	0.59±0.00	0.29±0.03	1.13±0.11	2.01

S: substrate; LAC: lactose; GLU: glucose, GAL: galactose. Data presented with mean ± standard deviation.

In relation to ethanol, the metabolite with the highest concentration, its generation varied from 0.62 g L⁻¹ to the PI1 strain in galactose to 1.79 g L⁻¹ with the 1TE strain in lactose (Table 4). In addition, it was observed that in lactose most of the strains presented their maximum ethanol formation, being in decreasing order the strains 1TE (1.79 g L⁻¹), PI3 (1.78 g L⁻¹), PI1 (1.77 g L⁻¹) and SR (1.61 g L⁻¹). In general, the strains of yeasts cultivated with glucose presented maximum ethanol formation, smaller when compared to the same cultures grown with lactose (Table 4), except for the STFB strains that showed a slightly higher ethanol formation in glucose (1.65 g L⁻¹).

In cultures with galactose as carbon source, the maximum ethanol formation (Table 4) was obtained by the SR strain (1.27 g L⁻¹), however, this value is lower than those presented by the same strains in lactose and glucose. In this sense, it was observed that for the yeasts studied in this work, the use of galactose as the only source of carbon is not preferable in relation to the other substrates tested. It was observed (Table 4) that the maximum biomass formation by the different yeast strains varied according to the substrate to be assimilated, so the strains that presented the highest biomass formation in lactose, glucose and galactose were SR (4.99 g L⁻¹), 1TE (3.32 g L⁻¹) and SR (3.10 g L⁻¹), respectively. Fonseca et al. (2013) in their study with the strain *K. marxianus* CBS 6556, also evaluated the maximum biomass formation, in that sense, the strains referred to

when grown at 30 °C with lactose, presented maximum biomass formation of 5.00 g L⁻¹, similar to that presented by the SR strain also at 30 °C in the present work. This shows that, just as *K. marxianus* has a strong tendency to convert substrates into biomass, the isolated strain SR also presented this profile.

Succinic acid production was observed in all cultures, and its maximum formation ranged from 0.17 g L⁻¹ to 0.63 g L⁻¹. Acetic acid was produced by yeast strains only in cultures with lactose and galactose, with the exception of the P1 strain, which produced only in galactose (Table 4). The formation of this extracellular metabolite was observed, mainly, from the EPG phase until a certain time of culture, where it was noticed that the concentration of this organic acid gradually decreased until zero final concentration at the end of the cultures. Colombo et al. (2016) studying the production of PHA polymers from fermented cheese whey with mixed microbial cultures have found that the organic acid composition of the fermented cheese serum has been inferred in the composition of the polymer produced since some organic acids are precursors of certain polymers. In addition, the authors have also evaluated the influence of serum enrichment with organic acids (such as acetate), where it was observed that supplementation with acetate favored the production of polymers. In this context, it is suggested that the strains evaluated in this work used acetate as an alternative substrate as the concentration of the main carbon source decreased, so no residual acetate concentration was observed at the end of the crops.

4. Conclusions

From the results, it is noticed that the supplementation of synthetic medium is important for the performance of the studied microorganisms independent of the carbon source used. Most strains cultivated in lactose presented higher ethanol formation than the other substrates (the strains P13 and 1TE stood out). This shows that lactose is metabolized and converted to ethanol efficiently. Biomass and ethanol were the main products. The selected strains fermented lactose, glucose and galactose in the synthetic medium, being possible to be used in processes where lactose is available as a substrate (also in the residue form, as in cheese whey).

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