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Ethanol production from cheese whey permeate by *Kluyveromyces marxianus* UFV-3: A flux analysis of oxido-reductive metabolism as a function of lactose concentration and oxygen levels

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Abstract

In order to investigate the effect of lactose concentration and oxygen level on the growth and metabolism of *Kluyveromyces marxianus* UFV-3 in cheese whey permeate, batch cultures were conducted under aerobic, hypoxic, and anoxic conditions, with lactose at initial concentration ranging from 1 to 240 g L^{-1}. The increase in lactose concentration increased ethanol yield and ethanol volumetric productivity, and has reduced cell yield. When lactose concentration was equal or above 50 g L^{-1} and the oxygen levels were low, the ethanol yield was close to its theoretical value. Maximum ethanol concentrations attained in this study were 76 and 80 g L^{-1} in hipoxia and anoxia, respectively. The lactose consumption rate in anoxia was greater than in aerobiosis and hipoxia. However, under anoxia, the lactose consumption rate of *K. marxianus* followed a saturation kinetics, which was not observed in hypoxia and aerobiosis. All oxygen levels investigated, showed a tendency for saturation of the ethanol production rate above 65 g L^{-1} lactose. Ethanol production rate was also higher on anoxia.

Keywords: *Kluyveromyces marxianus*; Whey; Ethanol; Lactose concentration; Oxygen level; Oxido-reductive metabolism

1. Introduction

The dairy industry generates approximately 10 L of cheese whey for each kilogram of cheese produced, and this effluent can have a major environmental impact if released as such [1]. The ultrafiltration of cheese whey has allowed the recovery of nutritionally important whey proteins, but the milk lactose left in permeate still contributes to the high Demand Biochemistry Oxygen (DBO) of the effluent in the dairy industry [2]. The bioconversion of lactose to ethanol is a promising alternative that would not only reduce the environmental impact of cheese whey [3], but also present an alternative way of production of ethanol as a valuable fuel resource [4]. Some distillers are producing alcohol from cheese whey on commercial scale in Ireland, in the United States and especially in New Zealand, where one-fifth of the cheese whey has been converted to ethanol [5]. Because of the diluted character of lactose in cheese whey, its conversion to ethanol has been admitted to be economically unfeasible [6]. The concentration of permeate could be a solution to reduce cost, eliminating in advance, part of the water that later would require energy to be removed by distillation [5]. The well-known fermentative potential of *Saccharomyces cerevisiae* cannot be exploited to produce ethanol from cheese whey, because that yeast lacks assimilatory mechanisms for lactose [7]. In contrast, dairy yeasts such as *Kluyveromyces* are able to assimilate lactose, although they have a more respiration-oriented metabolism than *S. cerevisiae* [8]. In *S. cerevisiae*, sugar concentration in the growth media determines the choice of respiratory or fermentative pathways, while in *Kluyveromyces*, it has been suggested that fermentative utilization of sugar is limited by sugar transport capacity. In this latter case, the oxygen level can be critical in the control of the fermentative metabolic flow [9]. In addition to their capacity of lactose assimilation, some yeast from *Kluyveromyces* genera (*K. marxianus* and *K.*

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lactis) markedly differ from *S. cerevisiae* in the regulation of sugar metabolism, especially by their low sensibility to glucose repression. The genes for respiratory metabolism in *S. cerevisiae* are subject to glucose repression, while in *K. lactis*, the respiration is not glucose repressed and fermentative, and oxidative metabolism can take place simultaneously [10]. The difference exhibited by *K. lactis* strains to glucose repression seems to be associated to glucose uptake capacity and is strain dependent [11]. Therefore, the sugar concentration and the oxygen level appear to be the major parameters that determine the metabolic preference of *K. lactis* strains, and consequently their capacity of ethanol production [8]. Optimization of these parameters is the object of the present study.

Substantial information has been published about lactose fermentation from cheese whey, but available information is limited on the effects of a wide range of lactose concentration and oxygen level in the fermentation of permeate. The physiological conditions to maximize oxido-reductive metabolic flow remain to be established for those respiro-fermentative yeasts.

Recently, a yeast strain from a Brazilian regional dairy factory has been selected for its high β-galactosidase activity (manuscript in preparation). This strain has been identified as *K. marxianus*, and named UFV-3. It was thought that this high β-galactosidase activity might also be accompanied with a high lactose permeate activity, because the two genes *LAC4* and *LAC12*, encoding β-galactosidase and lactose permease, respectively, are contiguously placed on the chromosome and controlled by a common bi-directional promoter that responds to a same activator protein [12]. This is a possible condition that may ensure high flow substrate necessary to support the fermentation. It is on this basis that we investigated the potential of *K. marxianus* UFV-3 to convert lactose from cheese whey into ethanol. The study focused on the lactose concentration and the oxygen level that would maximize ethanol yield.

2. Materials and methods

2.1. Yeast strain studied

The yeast strain used in this study was isolated from Brazilian regional dairy environments and selected for its high β-galactosidase activity. This yeast was identified by the Institute of Yeasts Identification, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, as *Candida Kefir* (Beijerinck) van Uden & Buckley (synonym: *K. marxianus* (E.C. Hansen) van der Walt, non-ascospore forming state. In this work, it is designated as *K. marxianus* UFV-3. It has been maintained frozen in 20% glycerol at −80 °C.

2.2. Substrate and fermentation media

Whey permeate (permeate) was obtained from a regional Dairy industry (Maroca & Russo Industry, Cotochés, Brazil). Permeate was spray-dried at the pilot plant of the Food Technology Department of Universidade Federal de Viçosa. From this powder form, permeate was reconstituted by solubilization in distilled water at lactose concentrations from 1 to 240 g L^{-1}. Permeate was then filter-sterilized (pore size 0.2 μm) before use as fermentation media. To evaluate the possible inhibitory effects of ethanol, permeate with lactose concentrations of 85 g L^{-1} (for aerobiosis and hyoxia) or 65 g L^{-1} (for anoxia) was supplemented with ethanol at 5, 10, 20, 30, 50 or 80 g L^{-1}. The inhibition (μ_E/μ) was expressed as the ratio of the specific-growth rate in media with ethanol (μ_E) over the specific-growth rate in the absence of ethanol (μ).

2.3. Culture conditions and analysis of time-course experiments

Batch cultures both in aerobiosis and hypoxia were conducted in 250 mL Erlenmeyer flasks filled at its one-fifth capacity. The cultures were incubated at 30 °C in a rotary shaker. For the process defined as aerobiosis, the rotation was 250 rpm and for the process defined as hypoxia, the rotation was 40 rpm. In hypoxic condition, nitrogen gas (99.9%, v/v, purity) was purged into the culture medium for 15 min after inoculation. Batch cultures in anoxic condition were performed in a 1000-mL jacketed flask (Wheaton®, CA, USA) also filled with media at its one-fifth capacity and magnetically stirred at 30 °C. In this case, the 99.9% nitrogen gas was continuously injected during all course of the process. In anoxic condition, permeate was supplemented with Tween at 80 g L^{-1} and ergosterol at 20 μg mL^{-1}.

2.4. Determination of dry weight and specific-growth rate

The culture was initiated with an A_{600} between 0.08 and 0.1. The fermentation was monitored by measuring cell density, substrate, and product concentration on samples collected at indicated time intervals. The cell mass was calculated from the A_{600} value at the range of linear relationship of A_{600} versus dry weight (g L^{-1}). One A_{600} unit corresponded to 0.493 g L^{-1} of cell mass. The specific-growth rate was determined by linear regression of the plot ln A_{600} unit versus time (h), at the exponential growth phase.

2.5. Analysis of primary metabolites

Quantitative determination of lactose, ethanol, and glycerol was carried out by high-performance liquid chromatography (HPLC), using an ion exclusion column Aminex HPX-87H (Bio-Rad), kept at 60 °C. The eluant for separation was 5 mM H_2SO_4, applied at an elution rate of 0.7 mL min^{-1}. The column was coupled to the refractive index detector HP 1047 A.

2.6. Determination of fermentation parameters

Once the maintenance coefficient and maintenance yield was fixed at zero, as suggested by data fitting on preliminary results (data not shown), three yield parameters were determined:

$$Y_{X/S} = \frac{X_f - X_i}{S_i - S_f} \ (g\,g^{-1})$$

$$Y_{P/X} = \frac{P_f - P_i}{X_f - X_i} \ (g\,g^{-1})$$

and

$$Y_{P/S} = \frac{P_f - P_i}{S_i - S_f} \ (g\,g^{-1})$$

where X_f is the final biomass concentration ($g\,L^{-1}$); X_i the initial biomass concentration ($g\,L^{-1}$); S_f the final lactose concentration ($g\,L^{-1}$); S_i the initial lactose concentration ($g\,L^{-1}$); P_f the final ethanol concentration ($g\,L^{-1}$); P_i the initial ethanol concentration ($g\,L^{-1}$).

The theoretical yield of ethanol was 0.538 g for 1 g of consumed lactose. The ethanol volumetric productivity (Q_P) was determined as:

$$Q_P = \frac{P_f - P_i}{t} \ (g\,L^{-1}\,h^{-1})$$

where t (in h) is the time interval.

The lactose consumption rate (Q_L) was determined as:

$$Q_L = \frac{(S_i - S_f)}{X\Delta t} \ (g\,g^{-1}h^{-1})$$

where X is the average biomass at time intervals and Δt is the time interval of successive measurements during the exponential growth phase.

The ethanol production rate (Q_E) was determined as:

$$Q_E = \frac{P_f - P_i}{X\Delta t} \ (g\,g^{-1}\,h^{-1})$$

during the exponential growth phase.

3. Results

We investigated the effect of concentration of lactose and oxygen level on the growth and the production of ethanol by *K. marxianus* UFV-3 from permeate of cheese whey. Table 1 shows that the specific-growth rates (μ) and the cell mass yield were higher in aerobic than in hypoxic conditions, always higher than in anoxic conditions. Growth of *K. marxianus* UFV-3 in lactose under anoxic conditions indicated that this yeast was Kluyver effect negative for lactose. Under aerobic conditions, the increases in lactose concentration on whey permeate from 1 to 50 g L^{-1} was accompanied by a linear increase of specific-growth rate from 0.299 to 0.445 h^{-1}.

Further increase in lactose concentration from 50 to 130 g L^{-1} did not seem to result in further increase of specific-growth rate. Lactose concentrations above 130 g L^{-1} clearly affected negatively the specific-growth rate. In hypoxia also, increase of lactose concentration from 1 to 50 g L^{-1} led to a linear increase of specific-growth rate from 0.251 to 0.343 h^{-1}, and again as it was observed in aerobiosis, the increase on lactose concentration from 50 to 130 g L^{-1} did not change the specific-growth rate of the cultures and above 130 g L^{-1}, the growth rate was negatively affected in hypoxia. Interestingly, in anoxia, the negative effect occurred at lower concentration of lactose, i.e., above 65 g L^{-1} instead of 130 g L^{-1}. It is to be noted that the high concentration of substrate did not severally affect the growth of *K. marxianus* UFV-3. This strain seems to be able to maintain an osmotic homeostasis to grow until substrate concentrations as high as 240 g L^{-1} (Table 1).

Table 1
Effect of substrate concentration and oxygen level on the growth kinetics and cell mass yields of *Kluyveromyces marxianus* UFV-3

So[a]	Aerobic growth			Hypoxic growth			Anoxic growth		
	μ (h^{-1})[b]	$Y_{X/S}$ maximum[c]	Cell mass maximum (g L^{-1})	μ (h^{-1})[b]	$Y_{X/S}$ maximum[c]	Cell mass maximum (g L^{-1})	μ (h^{-1})[b]	$Y_{X/S}$ maximum[c]	Cell mass maximum (g L^{-1})
1.00	0.299	0.418	0.49	0.251	0.358	0.45	[d]	[d]	[d]
5.00	0.387	0.310	1.03	0.317	0.178	0.85	[d]	[d]	[d]
10.0	0.414	0.259	1.56	0.325	0.189	1.18	0.251	0.081	0.84
25.0	0.429	0.255	3.64	0.339	0.086	2.38	0.253	0.074	1.52
50.0	0.445	0.229	5.16	0.343	0.087	3.09	0.260	0.046	2.27
65.0	0.443	0.167	8.11	0.344	0.061	4.00	0.260	0.050	2.16
85.0	0.450	0.108	9.17	0.356	0.063	4.05	0.250	0.043	2.77
130.0	0.440	0.134	10.33	0.340	0.114	4.06	0.240	0.083	5.17
170.0	0.435	0.080	13.37	0.290	0.066	4.42	0.210	0.046	3.25
240.0	0.393	0.074	12.06	0.200	0.069	3.79	0.200	0.035	3.92

[a] Initial lactose concentration (g L^{-1}).
[b] Specific-growth rate (h^{-1}).
[c] Cell yield maximum (g cell g^{-1} lactose).
[d] Not examined.

Table 2
Effect of substrate concentration and oxygen level on the maximum ethanol yield and maximum volumetric ethanol productivity of *Kluyveromyces marxianus* UFV-3 on whey permeate

So[a]	Aerobic growth			Hypoxic growth			Anoxic growth		
	$Y_{E/S}$ maximum[b]	$Y_{E/X}$ maximum[c]	Q_P maximum[d]	$Y_{E/S}$ maximum[b]	$Y_{E/X}$ maximum[c]	Q_P maximum[d]	$Y_{E/S}$ maximum[b]	$Y_{E/X}$ maximum[c]	Q_P maximum[d]
1.00	n.d.	n.d.	n.d.	0.202	0.65	0.014	[e]	[e]	[e]
5.00	0.104	0.63	0.020	0.373	2.74	0.152	[e]	[e]	[e]
10.0	0.102	0.67	0.034	0.354	4.01	0.159	0.424	7.18	0.168
25.0	0.105	0.73	0.070	0.406	6.55	0.620	0.453	9.67	0.377
50.0	0.273	2.23	0.300	0.510	9.89	0.920	0.520	14.2	0.680
65.0	0.271	1.96	0.400	0.520	11.0	1.200	0.530	18.6	0.930
85.0	0.343	3.37	0.740	0.535	13.50	1.170	0.510	16.8	1.300
130.0	0.390	3.71	0.980	0.530	19.00	1.410	0.520	26.3	1.150
170.0	0.380	4.90	1.330	0.530	17.30	1.490	0.510	24.9	1.050
240.0	0.348	5.46	1.100	0.534	15.90	0.860	0.530	35.9	0.860

n.d.: Not detectable.
[a] Initial lactose concentration.
[b] Maximum ethanol yield per substrate ($g\,g^{-1}$).
[c] Maximum ethanol yield per cell mass ($g\,g^{-1}$).
[d] Maximum volumetric ethanol productivity ($g\,L^{-1}\,h^{-1}$).
[e] Not examined.

In all conditions of oxygen availability tested, high lactose concentration tended to increase the total cell mass production, while cell mass yield per substrate consumed decreased (Table 1). This result reveals that, as lactose concentration increases, smaller proportion of sugar is channeled to cell mass production.

The maximum values for ethanol yields ($Y_{E/S}$) in hypoxic and anoxic conditions were similar and higher than those observed for aerobic conditions, as shown in Table 2. The maximum ethanol yields per cell mass ($Y_{E/X}$) were higher in anoxic followed by hypoxic then aerobic conditions. In general, the maximum volumetric ethanol productivity (Q_P) was higher in hypoxic, followed by anoxic and then aerobic conditions. These results indicate that low oxygen levels favor fermentative metabolism in *K. marxianus* UFV-3. For all oxygen levels tested, $Y_{E/S}$, $Y_{E/X}$, and Q_P exhibited a tendency to increase as the lactose concentration increased (Table 2).

According to chemical stoichiometry, 1 mole of lactose consumed by the yeast will produce a maximum of 4 moles of ethanol, or 342 g of lactose will produce 184 g of ethanol, a theoretical yield of $0.538\,g\,g^{-1}$. It was observed that lower lactose concentrations (up to $25\,g\,L^{-1}$), either in hypoxic or anoxic conditions, have resulted on ethanol yields ($Y_{E/S}$) which deviates from the theoretical value (Table 2). However, lactose concentrations equal or above $50\,g\,L^{-1}$, give an $Y_{E/S}$ much closer to theoretical value (95% or higher).

The maximum values for ethanol and glycerol production were higher in anoxic and hypoxic conditions than in aerobic conditions (Table 3). Highest levels of glycerol were found on anoxic cultures. Cultivation in anoxic condition thus favors glycerol formation, or a high level of ethanol production is accompanied with the appearance of glycerol.

Fermentations high lactose concentrations generally produced high levels of ethanol. But under hypoxic and anoxic

Table 3
Effect of substrate concentration and oxygen level on the maximal production of ethanol and glycerol by *Kluyveromyces marxianus* UFV-3 on whey permeate

So[a]	Aerobic condition		Hypoxic condition		Anoxic condition	
	Ethanol[b]	Glycerol[c]	Ethanol[b]	Glycerol[c]	Ethanol[b]	Glycerol[c]
1.00	n.d.	0.12	0.26	0.11	[d]	[d]
5.00	0.48	0.30	1.57	0.31	[d]	[d]
10.0	0.81	0.21	3.87	0.29	2.36	0.60
25.0	1.84	0.42	9.47	0.72	9.52	2.30
50.0	7.20	1.10	23.5	1.62	24.44	1.98
65.0	13.40	1.04	35.7	2.70	30.60	4.04
85.0	23.30	1.15	46.0	3.67	43.70	7.73
130.0	33.10	2.40	66.7	4.45	68.70	6.40
170.0	45.77	2.51	76.0	6.30	80.04	12.40
240.0	57.00	2.42	59.8	3.50	64.17	9.90

n.d.: Not detectable.
[a] Initial lactose concentration.
[b] Maximum ethanol concentration ($g\,L^{-1}$).
[c] Maximum glycerol concentration ($g\,L^{-1}$).
[d] Not examined.

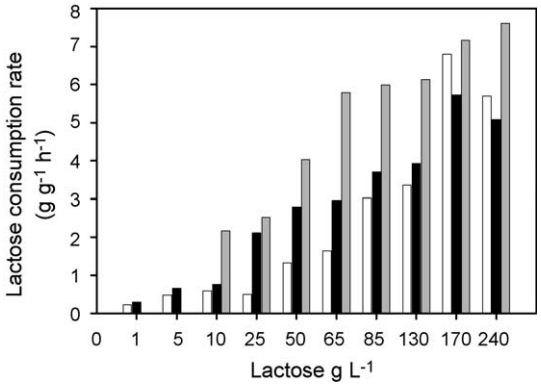

Fig. 1. Effect of substrate concentration and oxygen level on the lactose consumption rate ($g\,g^{-1}\,h^{-1}$) by *Kluyveromyces marxianus* UFV-3. Aerobiosis (□), hypoxia (■), and anoxia (▩).

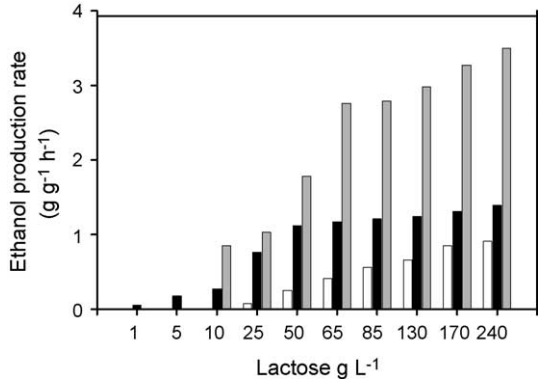

Fig. 2. Effect of substrate concentration and oxygen level on the ethanol production rate ($g\,g^{-1}\,h^{-1}$) by *Kluyveromyces marxianus* UFV-3, under aerobiosis (□), hypoxia (■), and anoxia (▩).

conditions, the ethanol production began to decrease at the lactose concentration of about $240\,g\,L^{-1}$ (Table 3). At this concentration of lactose, it was observed that only about one half of the sugar was consumed under hypoxia and anoxia, while at lactose concentration of $170\,g\,L^{-1}$ practically all lactose was depleted from the media, i.e., 170 and $155\,g\,L^{-1}$ for hypoxic and anoxic conditions, respectively. At the lactose concentration of $170\,g\,L^{-1}$, the maximum ethanol production was attained at $76\,g\,L^{-1}$ in hypoxic conditions and at $80\,g\,L^{-1}$ in anoxic conditions (Table 3).

Lactose consumption rate was higher in anoxia than both in aerobiosis and hypoxia for all concentrations of lactose (Fig. 1). In hypoxia, the lactose consumption rate was higher than in aerobiosis except at the highest lactose concentrations, 170 and $240\,g\,L^{-1}$. The increase on lactose concentration up to $50\,g\,L^{-1}$ led to an increase in lactose consumption rate for all growth conditions. In anoxia, above the lactose concentration of $65\,g\,L^{-1}$, the lactose consumption rate seems to reach saturation whilst in aerobiosis and hypoxia this saturation was not observed. In the latter cases, the lactose consumption rate increased 45.8% in hypoxia and 102% in aerobiosis, when lactose concentration increased from 130 to $170\,g\,L^{-1}$.

As shown in Fig. 2, the ethanol production rates increased as the oxygen levels decreased. Above the lactose concentration of $65\,g\,L^{-1}$, it is observed a saturation kinetics of the ethanol production rate, as ethanol production rate remains constant.

The inhibitory effect of ethanol on cell growth is presented in Fig. 3. Ethanol concentrations up to $10\,g\,L^{-1}$ does not change the specific-growth rate compared to the control, except in aerobic growth where an inhibition ratio was 0.9 at the ethanol concentration of $10\,g\,L^{-1}$. In anoxia, the culture was less sensitive to ethanol inhibition. Ethanol concentration of $50\,g\,L^{-1}$ under anoxia, allowed the growth with an inhibition ratio of 0.80 compared to the control, while under aerobiosis and hypoxia, the specific-growth rate was 60% of the control value. At the ethanol concentrations of $80\,g\,L^{-1}$, the specific-growth rate was only about 15% of the control in aerobiosis and 25% in hypoxia and anoxia.

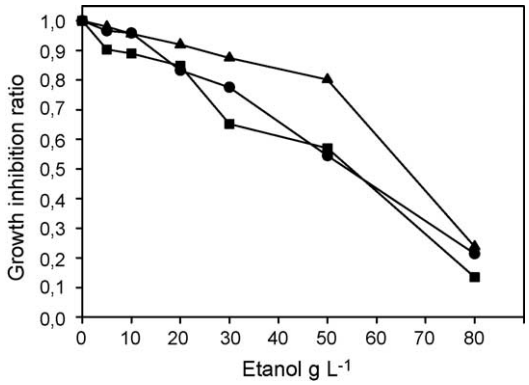

Fig. 3. Effect of ethanol on growth of *Kluyveromyces marxianus* UFV-3 in whey permeate with lactose concentrations of $85\,g\,L^{-1}$ under aerobiosis (■), hypoxia (●), and $65\,g\,L^{-1}$ under anoxia (▲).

4. Discussion

The increase of substrate concentration was considered here as the increase of lactose concentration (major component of permeate). However, others components nutritionally important which have been concentrated together with lactose, also affect cell growth [13]. The decrease of specific-growth rate at high substrate concentration is well documented and appears as substrate-inhibition kinetics. The decrease of cell yield with the increase of lactose concentration indicates that high substrate concentrations favor the metabolic flux through fermentative pathway. This was confirmed by an increase in maximal ethanol yield both per substrate ($Y_{E/S}$) and per cell mass ($Y_{E/X}$), as well as by the maximal ethanol volumetric productivity and maximal ethanol production obtained by *K. marxianus* UFV-3. High substrate concentration as well as low oxygen levels allowed obtaining an ethanol yield ($Y_{E/S}$) approaching the theoretical value. The strain *K. marxianus* UFV-3 was found to be capable of fermenting both glucose and galactose. The ability to ferment galactose is mandatory in order to completely exploit permeate potential resource for alcohol production.

Certain sugars, often disaccharides, that support vigorous growth under oxidative conditions, cannot be assimilated fermentatively by some yeast. Such yeasts are called Kluyver effect positive on those sugars. *K. marxianus* UFV-3 was found to be able to grow on lactose in anoxic conditions. Thus, *K. marxianus* UFV-3 is "Kluyver effect negative" on lactose. In other words, it is able to grow on lactose in absence of respiration. In some cases with *Kluyveromyces*, the Kluyver effect could be attributed to a limitation of the sugar transport capacity [14]. That mean, the limited transport system for lactose, and consequently the limited intracellular lactose concentration on *Kluyveromyces* cells could be responsible for the complete oxidation of the sugar through the respiratory metabolism.

The regulation of sugar metabolism in *S. cerevisiae* has been extensively described. In high sugar concentration, this yeast adopts a fermentative metabolism in highly aerated conditions. This behavior has been explained by the repression of gene expression related to respiratory metabolism and has been called Crabtree effect. When *S. cerevisiae* is cultured under glucose-limited conditions, oxygen plays a central role on metabolic flux. Anoxic conditions are essential for ethanol formation. Some species of the genera *Kluyveromyces* including *K. marxianus* and *K. lactis*, have been identified as Crabtree negative yeasts. In these cases, the catabolic repression is relaxed and fermentation is not the major pathway of sugar metabolism. Although *K. lactis* have been recognized as "Crabtree negative", previous work has suggested that this yeast is respiro-fermentative [15].

In contrast to the *S. cerevisiae*, the respiro-fermentative yeast *K. lactis* does not fully exploit its glucose uptake capacity during oxidative growth [9]. In fact, it is verified in this work that in *K. marxianus* UFV-3, the lactose consumption rate was higher in anoxic condition than in hypoxic and aerobic conditions. This supports the idea that sugar uptake may be primarily controlled by the availability of oxygen [11]. Although the lactose consumption rate is higher in anoxic condition, it tends to saturate in higher substrate concentration. This saturation could be due to changing in affinity of sugar carriers for the substrate [16]. The saturation of lactose consumption but not of ethanol production rates on aerobiosis and hypoxia suggests that fermentative flow may be limited by glycolytic flow.

Besides ethanol, another primary metabolite produced during fermentation of permeate in anoxic condition was glycerol. This is expected given the physiological role of glycerol to maintain the redox balance, by regenerating NAD^+ from the NADH produced by biosynthesis of cellular components [17].

An important question in the fermentation process is the capacity of yeast to support the toxic effects of the produced ethanol. The growth of *K. marxianus* UFV-3 was practically not affected by relatively high ethanol concentrations. Even in presence of higher ethanol concentrations, this yeast was able of grow indicating its adaptation capacity. Adaptation of *K. lactis* to ethanol concentration has been associated to changes on membrane fatty acids. In contrast to *S. cerevisiae*, *K. lactis* decrease fluidity of membrane by reducing the insaturations of fatty acids in membrane [18].

The fermentative capacity of *K. marxianus* UFV-3 was confirmed in terms of the ethanol yields close to the theoretical value. These yield were reached on permeate with initial lactose concentration equal or above 50 g L^{-1} under hypoxic and anoxic conditions. To conclude, a high concentration of permeate lactose under low oxygen levels allows maximal conversion of lactose to ethanol by the use of the *K. marxianus* UFV-3 for cheese whey fermentation process.

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