Fermentation medium and oxygen transfer conditions that maximize the xylose conversion to ethanol by *Pichia stipitis*

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ABSTRACT

The xylose conversion to ethanol by *Pichia stipitis* was studied. In a first step, the necessity of supplementing the fermentation medium with urea, $MgSO_4 \times 7H_2O$, and/or yeast extract was evaluated through a 2^3 full factorial design. The simultaneous addition of these three nutritional sources to the fermentation medium, in concentrations of 2.3, 1.0, and 3.0 g/l, respectively, showed to be important to improve the ethanol production in detriment of the substrate conversion to cell. In a second stage, fermentation assays performed in a bioreactor under different K_La (volumetric oxygen transfer coefficient) conditions made possible understanding the influence of the oxygen transfer on yeast performance, as well as to define the most suitable range of values for an efficient ethanol production. The most promising region to perform this bioconversion process was found to be between 2.3 and 4.9 h^{-1}, since it promoted the highest ethanol production results with practically exhaustion of the xylose from the medium. These findings contribute for the development of an economical and efficient technology for large scale production of second generation ethanol.

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1. Introduction

The beginning of this century is marked by the large incentive given to biofuel use in replacement of gasoline, which has been motivated by several reasons, including the rising oil prices and recognizing that the global oil reserves are exhausting fast; the concern about fuel emissions; the requirements of the Kyoto Protocol and the Bali Action Plan on carbon emissions, and the provision of alternative outlets for agricultural producers [1]. Currently ethanol is the main bio-fuel used in the world and the worldwide prospects are the expansion of the production and consumption of ethanol.

Polysaccharides present in lignocellulosic materials, including cellulose and hemicellulose, are of great interest as feedstocks for second generation ethanol production due to their large availability, richness in sugars, and mainly because they do not affect the food provision. However, while technologies to produce ethanol from sugar or starch are well established, the technologies to produce bioethanol from lignocellulosic biomass are still under development all over the world. Hemicellulose is one of the three major components of lignocellulosic biomass together with cellulose and lignin, and can be easily hydrolyzed to monomeric sugars under mild conditions [2]. Xylose is the main sugar obtained by hydrolysis of this fraction, and its bioconversion is an important step in the use of lignocellulosic materials for ethanol production. *Pichia stipitis* is a promising yeast strain for industrial application on ethanol production due to its ability to rapidly convert xylose to ethanol, with high yield [3–5]. Moreover, this yeast is also able to ferment several other sugars besides xylose, including glucose, mannose, galactose and cellobiose along with mannan and xylan oligomers [6], which is an important characteristic considering that hemicellulosic hydrolyzates usually contain these other sugars in mixture with xylose.

As is well know, the efficiency of a fermentation process is affected by the cultivation medium composition and operational conditions used. Different yeast strains require different sources and amounts of nutrients to efficiently convert the sugars in the product of interest. Specific nutrients such as nitrogen, trace elements or vitamins, may be required to obtain rapid fermentation and high ethanol levels, desirable to minimize capital costs and distillation energy [7]. Among the operational conditions, the oxygen availability is considered the most important factor affecting the sugars fermentation by yeasts, since it determines the partitioning of the carbon flux between growth and product formation [8]. The establishment of an adequate oxygen level is thus of great importance to obtain an efficient process with high

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values of conversion and productivity. In this sense, the volumetric oxygen transfer coefficient (K_La) is an important parameter to be observed in fermentations performed in bioreactors. The K_La value depends on several factors such as the agitation speed and aeration flow rate, the geometry of the reactor, the rheological properties of the medium (density and viscosity), and environmental variables (temperature and pressure) [9]. Through the K_La value, the oxygen transfer for different processes can be compared, and the scaling up operations for different geometry of bioreactors are facilitated [10].

Although several studies have been carried out on ethanol production from xylose, much work remains to be done for achieving high ethanol yields and productivities. Aiming to contribute for the development of an efficient technology for large scale production of second generation ethanol, the present study evaluated the influence of medium composition and volumetric oxygen transfer coefficient on ethanol production from xylose by *P. stipitis*. Initially, assays in Erlenmeyer flasks revealed the necessity of supplementing the medium with urea, $MgSO_4 \times 7H_2O$, and/or yeast extract. In a second step, assays in bioreactor under different K_La conditions made possible understanding the influence of the oxygen transfer on yeast performance, as well as to define the most suitable range of values for an efficient ethanol production by this yeast strain.

2. Materials and methods

2.1. Microorganism and inoculum

P. stipitis NRRL Y-7124 was the microorganism used in the experiments. Cultures of this yeast were maintained on malt extract agar slants at 4 °C. For inoculum preparation, cells of the yeast in the maintenance medium were transferred to 500-ml Erlenmeyer flasks containing 100 ml of the medium composed by (g/l): xylose (30.0), glucose (5.0); arabinose (5.0); urea (2.3), $MgSO_4 \times 7H_2O$ (1.0), and yeast extract (3.0). The inoculated flasks were incubated at 30 °C, 200 rpm, during 24 h. After this time, the cells were recovered by centrifugation (2000 $\times$ g, 20 min) and resuspended in the fermentation medium to obtain an initial concentration of 1 g/l.

2.2. Fermentation medium and conditions

To evaluate the influence of medium composition on ethanol production, assays were performed in 125-ml Erlenmeyer flasks containing 50 ml of a 90 g/l xylose solution supplemented or not with nutrients (urea, $MgSO_4 \times 7H_2O$, or yeast extract) according to the experimental design given in Table 1. The flasks were inoculated with an initial cell concentration of 1 g/l and maintained in a rotary shaker at 30 °C, 150 rpm, during 48 h. Fermentation runs were monitored through periodic sampling to determine the cell growth, xylose consumption, and ethanol production.

To evaluate the influence of the volumetric oxygen transfer coefficient (K_La) on ethanol production, assays were performed in a 1.6 L stirred tank bioreactor (Autoclavable Benchtop Fermenter Type R'ALF, Bioengineering AG, Wald, Switzerland), containing 1.2 L of the following fermentation medium (g/l): xylose (90.0), glucose (15.0), arabinose (15.0); urea (2.3), $MgSO_4 \times 7H_2O$ (1.0), and yeast extract (3.0). After inoculated with 1 g/l of cells, the fermentation runs were maintained at 30 °C during 96 h. The K_La values used in each assay are shown in Table 2. During the experiments, samples were taken each 12 h for sugars, ethanol, and cell growth determinations.

Table 1
Experimental conditions for evaluation of the fermentation medium supplementation with nutrients, on ethanol production and xylose consumption by *Pichia stipitis*.

Assay	Variables			Responses	
	Urea g/l	$MgSO_4 \times 7H_2O$ g/l	Yeast extract g/l	Total consumed xylose (%)	Maximum ethanol production (g/l)
1	0.0	0.0	0.0	4.0	0.00
2	2.3	0.0	0.0	5.8	0.00
3	0.0	1.0	0.0	4.8	0.00
4	2.3	1.0	0.0	8.1	0.00
5	0.0	0.0	3.0	47.3	15.70
6	2.3	0.0	3.0	60.3	19.83
7	0.0	1.0	3.0	45.5	15.17
8	2.3	1.0	3.0	74.0	24.17
9	1.15	0.5	1.5	41.0	12.25
10	1.15	0.5	1.5	40.5	12.21
11	1.15	0.5	1.5	42.2	12.20

2.3. K_La determination

For the K_La determination, initially the polarographic oxygen probe was calibrated at atmospheric pressure by setting zero and 100% saturation under nitrogen and air sparging, respectively. Then, the bioreactor was filled with the culture medium not inoculated with cells, and oxygen was stripped from the medium by sparging with nitrogen. In the sequence, the oxygen saturation time course was monitored as the air flow and stirring conditions chosen for use during the fermentation were resumed. The K_La value was calculated by the integrated form of the equation proposed by Stanbury et al. [11], where the value of $-K_L$a is equal to the slope of the resulting straight line representing the ln $(C^* - C_L)$ versus time.

2.4. Analytical methods and determination of fermentation parameters

Cell growth was estimated by measuring the fermentation broth absorbance at 600 nm, which was correlated to a calibration curve (dry weight $\times$ optical density). Glucose, xylose, arabinose, and ethanol concentrations were determined by high performance liquid chromatography (HPLC) in a Jasco chromatograph equipped with a refractive index detector and a Bio-Rad Aminex HPX-87H column (300 $\times$ 7.8 mm) at 60 °C, using 0.005 M sulfuric acid as eluent in a flow rate of 0.7 ml/min.

The ethanol yield factor ($Y_{P/S}$, g/g) was calculated by the ratio between ethanol concentration (g/l) and substrate (glucose + xylose) consumed (g/l), while the cell yield factor ($Y_{X/S}$, g/g) was determined by the ratio between cell concentration (g/l) and substrate consumed

Table 2
Fermentation parameters obtained during the ethanol production from xylose by *Pichia stipitis*, under different K_La values.

K_La (h^{-1})	$Y_{P/S}$(g/g)	$Y_{x/S}$(g/g)	Q_P(g/l h)
0.7	0.33	0.15	0.24
2.3	0.26	0.13	0.27
4.9	0.32	0.15	0.32
11.7	0.30	0.19	0.33
12.1	0.31	0.18	0.34
18.7	0.23	0.21	0.38
58.0	0.13	0.51	0.17
65.8	0.00	0.54	0.00

$Y_{P/S}$: ethanol yield factor; $Y_{x/S}$: cell yield factor; Q_P: ethanol volumetric productivity.

(g/l). The ethanol volumetric productivity (Q_P, g/l h) was defined by the ratio between the maximum ethanol concentration (g/l) and the respective fermentation time (h).

2.5. Statistical analysis

Fermentation assays for evaluation of the influence of medium composition on ethanol production were performed according to a 2^3 full factorial design (Table 1), varying the amounts of urea, $MgSO_4 \times 7H_2O$ and yeast extract added to the fermentation medium. The parameters, $Y_{P/S}$, $Y_{x/S}$, and Q_P were taken as responses of this experimental design. Statistical analysis of the data was carried out using the Design-Expert 5.0 and Statistica 6.0 softwares.

3. Results and discussion

3.1. Influence of the medium composition on xylose-to-ethanol conversion by P. stipitis

Defining the composition of a fermentation medium is considered an important way to increase the productivity of bioconversion processes, and thus, this was the first objective of our study. In addition, it is known that the xylose conversion to ethanol is influenced by nutritional factors including vitamins and nitrogen sources, but that this dependence dramatically varies according to the genus and species of microorganisms utilized, and even by strain within the same genus and species [12]. Such diversity of nutrient requirements implies that optimization to each individual strains is needed to design an appropriate defined medium for experimental applications, or to pursue an economical complex medium for commercial consideration [13]. Moreover, the knowledge about the influence of nutrient sources on ethanol production by P. stipitis will made possible to understand the requirements of this yeast strain for a good bioconversion performance, and will be useful for the development of an efficient technology for the production of second generation ethanol.

Three different nutritional sources namely urea, $MgSO_4 \times 7H_2O$ and yeast extract, where chosen to supplement the xylose medium for ethanol production by P. stipitis, since they are commonly used to compose fermentation media for different microorganisms, and have been proved to be of great importance for a good development of different yeast strains. Table 1 shows the experimental conditions evaluated in this study and the achieved results. As can be seen, significant variations on ethanol production and substrate consumption were found according to the nutrient source added to the fermentation medium. Ethanol production did not occur when the medium was not supplemented with nutrients (assay 1), revealing that P. stipitis needs to assimilate nutrients to produce ethanol. In the absence of these sources, the yeast only consumed some carbon source for cell growth.

Among the three evaluated nutrients, the yeast extract was probably the most important for ethanol production by P. stipitis, since there was not any ethanol production in the assays not supplemented with this nutrient (assays 1 to 4), and the substrate consumption was too low (<10%). Additionally, fermentation medium supplemented only with yeast extract had 47.3% substrate consumption after 48 h fermentation, with an ethanol production of 15.7 g/l. Yeast extract is a mixture of amino acids, vitamins and magnesium [14] that has also been considered an important nutritional source for ethanol production by other yeast strains including Saccharomyces cerevisiae [15] and Kloeckera africana [16]. According to some authors, the yeast extract has protective effects on growth, viability, and fermentation, which stimulate the fermentation rate and ethanol production [17,18]. Supplementation of the medium only with urea or $MgSO_4 \times 7H_2O$, will not made

possible the ethanol production by P. stiptis. However, the addition of urea in presence of yeast extract had a positive influence on ethanol production and substrate consumption, as can be easily observed by comparing the assays 5 and 6, and 7 and 8 (Table 1). Urea has also been reported as a nutrient of great influence on ethanol production by S. cerevisiae [7].

A comparative analysis between the assays 6 and 8 (not-supplemented and supplemented with $MgSO_4 \times 7H_2O$, respectively) revealed also an increase of about 20% on ethanol production due to the $MgSO_4 \times 7H_2O$ addition to the fermentation medium. This result suggests a possible positive interaction effect between urea and $MgSO_4 \times 7H_2O$ since the addition of magnesium sulfate in presence of yeast extract only, did not have the same positive effect on ethanol production (see assays 5 and 7, Table 1). Other authors have also reported improvements on ethanol production by other yeast strains, when supplementing the fermentation medium with magnesium ions [7,19–21]. Magnesium seems to protect yeast cells during fermentation by a mechanism that results in decreased plasma membrane permeability under ethanol stress conditions [20,21].

The fermentation medium supplemented with all the three nutritional sources in the maximum evaluated concentrations (assay 8, Table 1) gave the best ethanol production, which was 2-fold higher than that obtained in the central point assays (9–11) that contained also the three nutrient sources but in concentration values equal to half those of the assay 8. These results allow concluding that not only the presence of the nutrients but also their concentration in the fermentation medium influenced the ethanol production by P. stipitis. This conclusion is valid for the range of concentrations used in the present study, since the excessive nutrient supplementation is reported to cause osmotic inhibition on xylose metabolism [13].

Fig. 1 shows the fermentation parameters values obtained to each experimental assay. Note that some experiments (1–4) gave null values of ethanol yield factor ($Y_{P/S}$) and volumetric productivity (Q_P) since there was not ethanol production in these media. The highest $Y_{P/S}$ values of about 0.40 g/g were obtained for the assays 5–8 (Fig. 1a). The little variation in the $Y_{P/S}$ values in these assays indicates that the yeast extract addition was possibly the factor with larger influence in this response. On the other hand, only the assay 8 gave the highest Q_P value (0.50 g/l h, Fig. 1c), which was significantly different of those obtained in the other fermentation assays. In this experiment, the fermentation medium was supplemented with all the nutrient sources in their maximum concentration values, suggesting thus that the addition of all the nutrients (urea, $MgSO_4 \times 7H_2O$, and yeast extract) in their most elevated concentration, positively and significantly influenced for a faster ethanol formation.

Oppositely to the $Y_{P/S}$ results, the highest values of cell yield factor ($Y_{x/S}$, Fig. 1b) were obtained in the assays 1–3, where the ethanol production did not occur and the medium did not contain nitrogen sources. However, the elevated substrate conversion to cells in these assays may be attributed to the low substrate consumption (less than 5%, Table 1), and not to a high cell growth. For the assays with ethanol production (5–11), the $Y_{x/S}$ values presented few variations, with values between 0.10 and 0.13 g/g.

For a better comprehension about the influence of each nutrient on ethanol production from xylose by P. stipitis, a statistical analysis for the $Y_{P/S}$, $Y_{x/S}$ and Q_P responses was performed. The Pareto charts for the effects estimation revealed that only the variable C (yeast extract) presented a positive and significant effect at 95% confidence level for the $Y_{P/S}$ response (Fig. 2a). For the other responses, $Y_{x/S}$ (Fig. 2b) and Q_P (Fig. 2c), all the independent variables as well as their interactions showed a significant influence at $p < 0.05$.
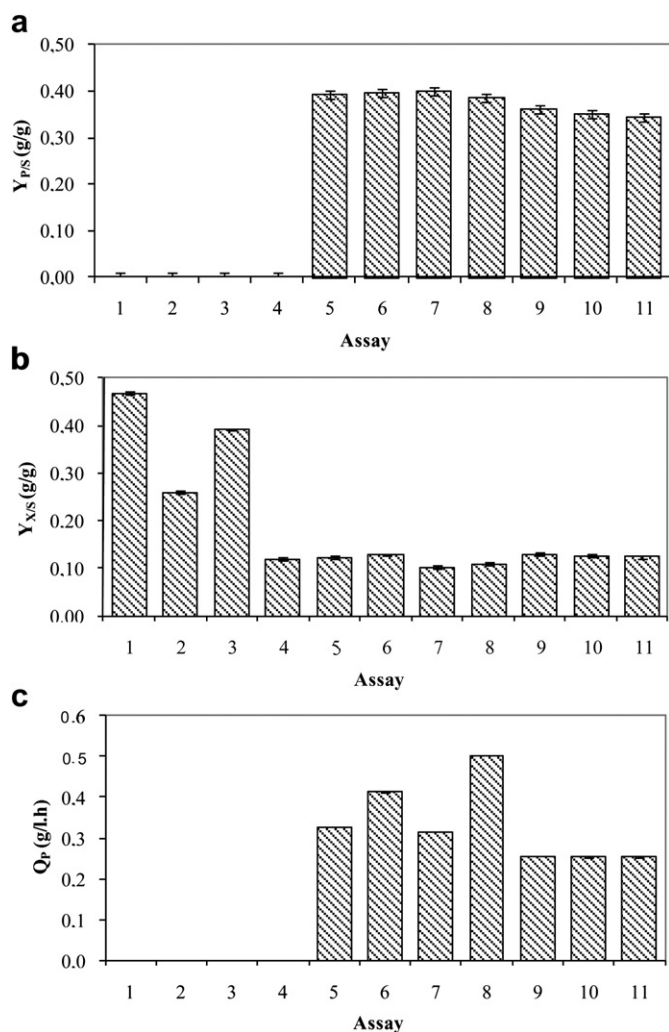
Fig. 1. Values of the fermentation parameters: (a) ethanol yield factor, $Y_{P/S}$, (b) cell yield factor, $Y_{X/S}$, and (c) ethanol volumetric productivity, Q_P, obtained during the ethanol production from xylose by *Pichia stipitis*, from different cultivation media according to a 2^3 experimental design.
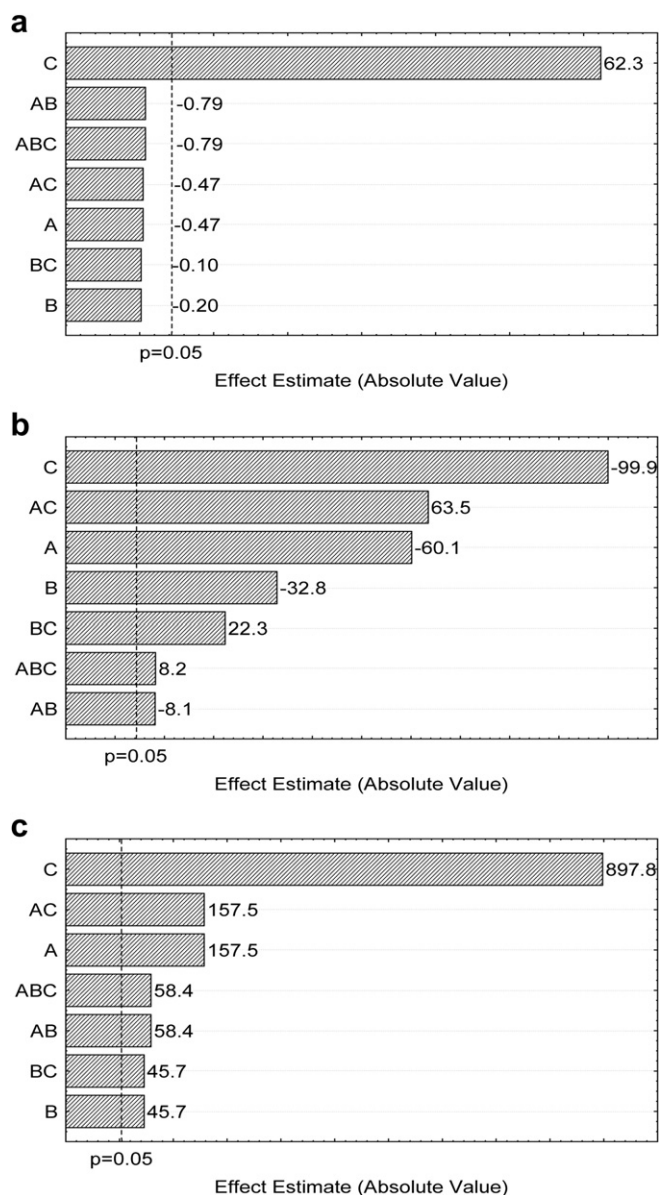

Fig. 2. Pareto charts for the effects of the variables (A) urea, (B) $MgSO_4 \times 7H_2O$, and (C) yeast extract, as well as their interactions, on the fermentation parameters: (a) ethanol yield factor, $Y_{P/S}$, (b) cell yield factor, $Y_{X/S}$, and (c) ethanol volumetric productivity, Q_P, obtained during the xylose fermentation by *Pichia stipitis*. The length of each bar is proportional to the standardized effect. Bars extending beyond the vertical line correspond to effects statistically significant at 95% confidence level.

However, the variables A (urea), B ($MgSO_4 \times 7H_2O$) and C (yeast extract), and the interaction AB showed negative effects for $Y_{X/S}$, while all the variables and interactions showed positive effects for Q_P. This means that the addition of urea (2.3 g/l), $MgSO_4 \times 7H_2O$ (1.0 g/l) and yeast extract (3.0 g/l) favored the ethanol production, improving the ethanol yield and productivity in detriment of the substrate conversion to cell.

3.2. Influence of $K_L a$ on xylose-to-ethanol conversion by P. stipitis

After defined the best fermentation medium composition for ethanol production by *P. stipitis*, assays were performed in bioreactor to define a suitable oxygen transfer level to maximize the results of this bioconversion process. These experiments were performed in a fermentation medium containing glucose and arabinose in mixture with xylose, since these sugars are usually present in hemicellulosic hydrolysates used for the second generation ethanol production. The kinetic behavior of cell growth, sugars consumption (glucose and xylose, since arabinose was not consumed by the yeast during the considered fermentation time), and ethanol production for these assays are shown in Fig. 3. It is clear that in the studied range of values, the $K_L a$ variations (0.7–65.8 h^{-1}) strongly affected the bioprocess. The sugars

consumption was similar for several $K_L a$ conditions (Fig. 3a,d–f), but the kinetics of product formation and cell growth presented very different profiles.

As a whole, the cell growth was favored the higher the $K_L a$ value used, whereas the ethanol production had the opposite behavior. The assays with $K_L a$ values of 11.7 and 12.1 h^{-1} (Fig. 3d and e, respectively), which can be considered repetitions, revealed an interesting behavior of xylose conversion by the yeast. These assays attained similar maximum values of ethanol and cells concentration (about 16 g/l) at the end of the fermentation (96 h), and when compared to the other experiments, this $K_L a$ value close to 12 h^{-1} appeared to be an inflexion point between the cell and ethanol concentrations, since for values lower than 12 h^{-1} (Fig. 3a–c) the resulting ethanol concentration was higher than the cell concentration at 96 h; whereas for values higher than 12 h^{-1}(Fig. 3f–h),
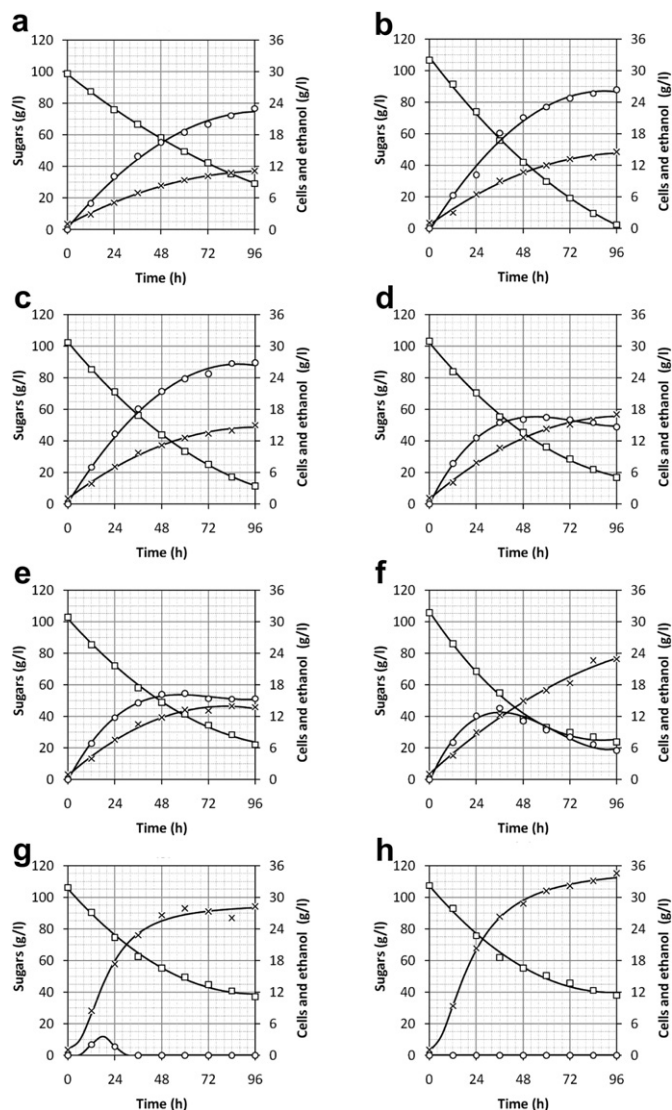
Fig. 3. Kinetic behavior of cell growth (-×-), sugars (xylose + glucose) consumption (-○-), and ethanol production (-□-) by *Pichia stipitis* in a stirred tank bioreactor under different K_La values: (a) 0.7 h^{-1}; (b) 2.3 h^{-1}; (c) 4.9 h^{-1}; (d) 11.7 h^{-1}; (e) 12.1 h^{-1}; (f) 18.7 h^{-1}; (g) 58.0 h^{-1}, and (h) 65.8 h^{-1}.

the final cell concentration was more elevated than the final ethanol concentration.

In fact, the xylose conversion to ethanol by *P. stipitis* was strongly affected for K_La values higher than 18.7 h^{-1}, being observed few (2.1 g/l, K_La = 58 h^{-1}) or none ethanol formation (K_La = 65.8 h^{-1}) under these conditions. In an opposite way, elevated cell concentrations were obtained in these experiments, which indicate that for these oxygen transfer conditions, practically all the consumed sugars were metabolized by the oxidative pathway, reducing the ethanol production and proportioning high cell growth. Similarly, Fiaux et al. [22] reported that *P. stipitis* does not produce ethanol from glucose under aerobic conditions. These results suggest that the ethanol formation by *P. stipitis*, both from xylose or glucose as carbon source, depends on a suitable oxygen supply to the cells. The prerequisite of oxygenation for efficient ethanol formation from glucose or xylose by *P. stipitis* must be ascribed to sugar transport, growth, or an unimpaired mitochondrial function [23].

Besides the low ethanol formation, the assays under the highest K_La values (58 h^{-1} and 65.8 h^{-1}) promoted also slower sugars

consumption when compared to the other experiments, remaining about 40% of the sugars in the medium after 96 h. This fact could be an indicative of inhibition in the sugars transport by oxygen, similar to the effect Pasteur. Yeasts that exhibit this effect present a reduction in the sugars consumption rate, being this effect more pronounced when the respiration is the predominant pathway on the carbohydrates catabolism [24–26].

An interesting fact was observed during the fermentation performed with a K_La value of 18.7 h^{-1} (Fig. 3f). The maximum ethanol production in this experiment (13.5 g/l) occurred at 36 h, and after this time, part of the ethanol produced was assimilated by the cells, even in presence of xylose in the fermentation medium. At the end of the fermentation (96 h) the ethanol concentration had been reduced to half of the maximum value achieved. The ethanol assimilation by the yeast suggests that the oxygen was supplied in excess in this medium since conditions of elevated oxygen availability have been reported to favor the ethanol assimilation by *P. stipitis* [27]. The highest ethanol production (about 26.5 g/l) was obtained when using K_La values of 2.3 or 4.9 h^{-1} (Fig. 3b and c, respectively). These assays presented also the highest substrate consumption, remaining less than 4% of the total sugars after 96 h fermentation, and achieved a cell concentration of about 15 g/l, which is practically half of the value obtained under the conditions of higher oxygen transfer.

Table 2 shows the fermentation parameters values obtained to each K_La condition. Note that the highest $Y_{P/S}$ were found for low K_La values (between 0.7 and 12.1 h^{-1}), while the highest $Y_{X/S}$ were found for high K_La values (0.58 and 65.8 h^{-1}). Differently of $Y_{P/S}$ and $Y_{X/S}$, the highest Q_P values were not found in the extremes of lowest and highest oxygen transfer, but for the intermediate K_La values (4.9–18.7 h^{-1}).

Fig. 4 shows the variations in cell and ethanol concentrations, the percentage of sugars consumed, and the values of the fermentation parameters as a function of the used K_La. For a better visualization of the behavior of these responses, the data were adjusted to second order polynomials, which were well adjusted presenting correlations between 88 and 99%. This figure clearly shows that the K_La increase caused a reduction in the maximum ethanol concentration, as well as in the percentage of sugars consumed, with these two curves presenting similar behaviors, opposite to that observed for the cell growth (Fig. 4a). Similar behavior was also observed during the xylose conversion to ethanol by *Candida shehatae* [28] and during the glucose conversion to ethanol by *P. stipitis* CBS 5774 [29] and *P. stipitis* CBS 6054 [23], where oxygen-limited conditions favored the ethanol production.

Regarding the fermentation parameters (Fig. 4b), the K_La increase caused a reduction in $Y_{P/S}$ with simultaneous increase in $Y_{X/S}$, being the sum of these two parameters maintained near to 0.50 g/g. On the other hand, the maximum Q_P value described by the adjusted model (about 0.40 g/l h) should occur for a K_La value close to 25 h^{-1}, and in fact, for the experimentally obtained results, the maximum ethanol productivity was achieved for a K_La near to 19 h^{-1} (Table 2). However, this productivity value may not be attributed to a high ethanol concentration, but to the fact that due to the oxygen availability, the ethanol produced was quickly assimilated reducing the time where the maximum concentration was obtained, which had a direct influence on the productivity value.

Considering the K_La values evaluated in the present study, the most promising region to perform the xylose conversion to ethanol by *P. stipitis* was found to be between 2.3 and 4.9 h^{-1}, since it promoted the highest ethanol production results with practically exhaustion of the xylose from the medium. If lower K_La values are used, lower productivity values are obtained; while if higher K_La values are used, lower ethanol yield is achieved.
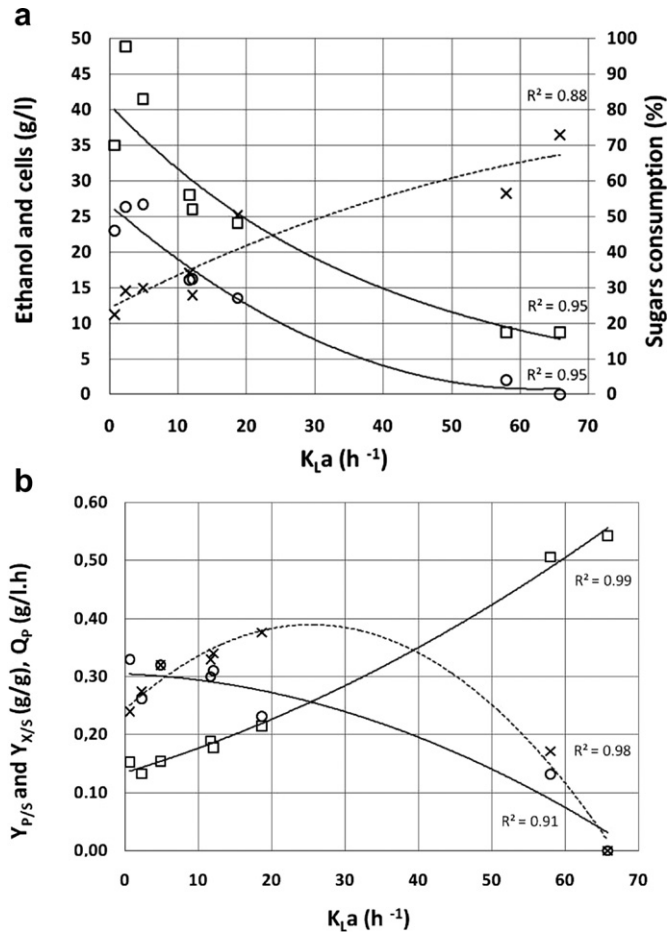
Fig. 4. Second order polynomials representing the variations of: (a) maximum ethanol production (-O-), sugars (xylose + glucose) consumption (-□-) and cell concentration (-×-), and (b) the fermentation parameters ethanol yield factor, $Y_{P/S}$ (-O-), cell yield factor, $Y_{X/S}$ (-□-) and ethanol volumetric productivity, Q_P (-×-) during the xylose conversion to ethanol by *Pichia stipitis*, as a function of the K_La value.

4. Conclusions

This work made possible to understand the nutritional and oxygen requirements to maximize the xylose conversion to ethanol by *P. stipitis*. The ethanol production was strongly dependent on the assimilation of nutritional sources by the yeast. Yeast extract, as a source of amino acids, vitamins and magnesium was the most important nutrient evaluated; however, addition of urea and $MgSO_4 \times 7H_2O$ was also necessary to improve the yeast performance. Since ethanol is a low value product, complex nutrients like yeast extract should not be added to the fermentation medium due to their high cost. However, the knowledge about these yeast requirements allows evaluating, in a next step, other cheaper nutrient sources so that the process may be efficiently and more economically performed. The oxygen availability to the medium was essential to guarantee elevated ethanol production by *P. stipitis*, but this variable must be carefully controlled since the excess of oxygen affect the carbon source metabolism, favoring the cell growth in detrimental to the ethanol formation. Use of suitable oxygen transfer during the fermentation is fundamental for the cells growth and efficient ethanol production. The results here found contribute thus for a better comprehension about the relation among nutrients, respiration, growth and ethanol production by *P. stipitis* from xylose as main carbon source. Based on these

findings, strategies to promote an efficient and economical ethanol production from lignocellulosic resources may be proposed, which is of great interest for the production of second generation ethanol production at industrial level.

Acknowledgments

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