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Abstract

The bioethanol industry expects a huge expansion and new technologies are being implemented with the aim of optimizing the fermentation process. The behavior of cells of *Saccharomyces cerevisiae* immobilized in PVA-LentiKats, during the production of bioethanol in two reactor systems, was studied. The entrapped cell in LentiKats lenses showed a different profile using stirred tank reactor (STR) and packed column reactor (PCR). Low free cells accumulation in the medium was observed for the STR after 72 h of fermentation. On the other hand, no free cells accumulation was observed, probably due to the absence of mechanical agitation in PCR configuration. Better fermentation results were obtained working with STR (final cellular concentration = 13 g.L⁻¹, Pf = 28 g.L⁻¹, Qp = 1.17 g.L⁻¹.h⁻¹, and Yp/s = 0.3 g.g⁻¹) in comparison to PCR (final cellular concentration = 11.4 g.L⁻¹, Pf = 20 g.L⁻¹, Qp = 0.83 g.L⁻¹.h⁻¹, and Yp/s = 0.25 g.g⁻¹). Such results are probably due to the mechanical agitation of the medium provided by STR configuration, which permitted a better heat and mass transference.

**KEYWORDS:** ethanol fermentation, immobilization in PVA-LentiKats, stirred tank reactor, packed column reactor

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1. Introduction

Ethanol is an important molecule used as an intermediary for the production of numerous chemical products. Industrial production of ethanol is experimenting a considerable expansion due to the increasing market of biofuels. Traditional bioethanol production includes the utilization of free cells of *Saccharomyces cerevisiae* in a process that require onerous step for cell recuperation and re-utilization. The immobilization of cells in an inert support could constitute an important alternative for the optimization of bioethanol production (Goksungur and Zorlu, 2001). Entrapped yeast cells present numerous advantages over free cells, such as easiness of bioethanol separation from used medium, reutilization of entrapped cell without any additional treatment, reduction of vulnerability of cells to contamination, since low direct surface contact with the environment, improving the overall process control (Cunha et al., 2006). Nowadays, natural and synthetic polymers are being used to entrap microbial cells used in different fermentation processes. Such classical immobilization supports are: cellulose, alginate, chitosan, agarose, polyacrylamida, polurethane and polycrylate (Goksungur and Zorlu, 2001; Cunha et al., 2006; Carvalho et al., 2004; Qi et al., 2006). Hydro-gels of polyvinyl alcohol (PVA) were considered recently as a promising polymeric matrix used for cells immobilization (Cunha et al., 2006; Najafpour et al., 2004; Sarrouh et al., 2007). A commercially available PVA-LentiKats® support is considered as a low cost material and accepted as GRAS (Generally Recognized as Safe), thus can be utilized in fermented beverages production (Durieux et al., 2000; Bezbradic et al., 2007). Recent works of Cunha et al. (2006) and Parascandola et al. (2006) had shown the potential application of LentiKats® as an immobilization support for yeast cell during the production of value-added products as heterologous protein and xylitol by fermentation processes.

PVA- LentiKats® is considered as a “less stressing” immobilization support for microbial growth. Such fact is due to the preparation process of the gel pellets (PVA-Lentikats), where the pellets are dried at room temperature for their hardening (gelation) without the need to use extreme process conditions (temperatures lower than 4 °C), usually necessary for other polymers.

According to Goksungur and Zorlu (2001) several studies have described ethanol production using Ca-alginate immobilized yeasts with different bioreactor configurations, such as the continuous flow stirred tank bioreactor, fluidized-bed bioreactor and packed-bed bioreactor (Bravo and Gonzales, 1991; Roukas, 1994). Packed-bed bioreactors have become very popular in recent years due to their low manufacturing and operating costs and also due to the ease of process automation in these reactors (Badalo-Santoyo, 1999). Stirred tank reactors (STR) have been used to cultivate entrapped cells even though the catalysts are thus exposed to a rather high degree of shear. Natural polymers used to immobilize the cells include...
pectin (Kesava and Panda, 1996), carrageenan (Lamboley et al., 1997) and alginate (Lamboley et al., 1997; Qureshi and Manderson, 1991; Sarrouh et al., 2007; Hernández et al., 2001). The STR has the advantage of effective agitation system (promoted by the use of an impeller), an easy control of temperature and pH and its continuous operation may be useful in the case of substrate inhibition (Fukuda, 1994).

According to Dias et al., (2001) in the practical utilization of living cells entrapped in synthetic gel, diffusion of essential nutrients, oxygen transfer, physical and chemical properties of the gel and immobilization procedure are the important factors affecting microbial metabolism and the efficiency of the system. Although immobilized cells have received a lot of attention, literature results are not uniform, but vary according to the type of microorganism, of immobilizing matrix and of productive system. This work studies the performance of the yeast Saccharomyces cerevisiae immobilized in PVA-LentiKats® support, for bioethanol production in packed column and stirred tank reactor. Also in this paper, an evaluation of the influence of two different bioreactor configurations on the immobilized cellular growth as well as the process yield and productivity, will be presented.

2. Material and Methods

2.1 Microorganism and inoculum cultivation

Commercial yeast of Saccharomyces cerevisiae was maintained at 4 °C on malt-extract agar slants. A loopfuls of cell was transferred on 125-mL Erlenmeyer flasks containing 50-mL basal medium consisted of glucose, 15 g L⁻¹; ammonium sulphate, 18 g L⁻¹; ammonium phosphate, 10 g L⁻¹; potassium phosphate, 5 g L⁻¹; magnesium sulphate, 5 g L⁻¹ and yeast extract 1 g L⁻¹. The initial pH of the medium was adjusted at 5.0 with convenient volume of phosphoric acid. The inoculum was incubated on a rotary shaker at 200 rpm and 30 °C for 24 h. Afterwards, the cells were collected by centrifugation (2000 x g, 15 min), washed twice with sterile distilled water, centrifuged and re-suspended on sterile water to obtain a suspension of high cell concentration.

2.2 Cell immobilization procedure

The yeast cells were immobilized by using a hydro-gel based on polyvinyl alcohol (PVA). A volume of 160-mL of solidified “LentiKats® liquid” (LentiKats®, geniaLab®, Braunschweig, Germany) was melted in microwave oven (3 cycles, 30 sec/cycle), cooled to room temperature and mixed, by mean of a magnetic stirrer; with 40-mL of a suspension of high cell concentration of Saccharomyces cerevisiae in order to achieve a final concentration of 6 g cell L⁻¹. Under sterile
conditions, in a laminar airflow cabinet, the wires of a LentiKats® Printer (400 wires per 143 cm²) were submerged on the LentiKats® liquid/cell suspension, lifting to permit drops formation and finally printed on an empty and sterile Petri plate. After gelation of LentiKats®/cell drops by water evaporation, the lenses formed were stabilized by a stabilizing solution (GeniaLab®, Braunschweig, Germany) for 2 hours. Then, the produced LentiKat®/cell lenses, with approximately 4 mm of diameter and thickness between 300 and 400 µm, were used for the different fermentations.

2.3 Bioreactor systems

Two different bioreactors configuration were utilized independently for the production of bioethanol by yeast cell entrapped on PVA gel. A packed column reactor (PCR) (working volume, 150-mL; internal diameter, 50 mm) was manufactured in our laboratories and coupled to a thermostatized bath in order to maintain the fermentation temperature under control. An additional 2.0 L stirred tank reactor (STR) (New Brunswick Scientific Co.) with a working volume of 1 L was also utilized. The medium composition for both bioreactors consisted of glucose 100 g L⁻¹, ammonium sulphate, 5.2 g L⁻¹; potassium phosphate, 1.56 g L⁻¹ and magnesium sulphate, 0.6 g L⁻¹. The bioreactors were operated as batch processes, under non-agitated regime (packed column) and under mechanical agitation (stirred tank reactor, 200 rpm) in order to compare the performance of the LentiKats® lenses in different bioreactors for bioethanol production. The fermentation conditions were controlled at 30°C with initial pH adjusted to 5.0 and maintained constant by addition of aqueous solution of NaOH 2M. Both bioreactors were completed until 10 % of their working volume with LentiKats® lenses.

3. Analytical Methods

Free cells concentration was determined by optical density (OD) measurements at 600 nm, in spectrophotometer (Beckman DU 640B). The OD values were correlated with the cell concentration (g L⁻¹) by means of a calibration curve previously established. The immobilized-cell was estimated by the same method after dissolution of lenses in distilled water by heating at 70°C. Glucose and ethanol concentrations were determined by HPLC with a refractive index (IR) detector and Bio-Rad HPX-87-H (300 x 7.8 mm) column at 45°C, using 0.005 M sulphuric acid as the eluent, flow rate of 0.6 ml min⁻¹ and sample volume of 20 µl.
3.1 Calculation of bioprocess parameters

The yield of glucose consumption (\(Y_S\)) was considered as the ratio of glucose consumption at the end of each run (\(S_O - S_F\)) to starting glucose level (\(S_O\)); the ethanol yield (\(\frac{Y_P}{S}\)) was considered as the ratio of final ethanol concentration (\(P_F\)) to (\(S_O - S_F\)); the bioconversion efficiency (n (%)) was calculated by the ratio between the practical yield factor (\(\frac{Y_P}{S}\)) and the theoretical value of this parameter (0.56 g ethanol.g\(^{-1}\)glucose). Ethanol production rate (\(Q_P\)) was defined as the amount of ethanol produced in the overall fermentation time per unit reactor volume and the ethanol volumetric productivity \(Q_P\) (g.L\(^{-1}\)h\(^{-1}\)) was calculated by the ratio between ethanol production (g L\(^{-1}\)) and fermentation time (h). All experiments were done in triplicate.

4. Results and Discussion

Comparing ethanol production between both reactor configurations, the yeast entrapped in LentiKats yielded 28.1 g.L\(^{-1}\) in the stirred tank reactor, almost 28 % higher than that of packed column reactor (non-agitated) with 20.2 g.L\(^{-1}\) of bioethanol. For both reactor systems a stationary state for product concentration was reached after 24 hours of fermentation, without any considerable increase during the next hours. Figure 1 shows the amount of glucose converted in ethanol during the fermentation time. Substrate was almost totally converted to ethanol within the first 24 hours of fermentation.
Considering the process yield and productivity presented in Table 1, for both bioreactor systems for ethanol production, the complete mixing of solid and liquid face in the stirred reactor permitted a better heat and mass transference than the packed column reactor. The fermentation process in the STR showed best ethanol yield (0.31 g. g\(^{-1}\)) and volumetric productivity 1.17 g. L\(^{-1}.\)h\(^{-1}\), which is almost 30% higher than the productivity under non-agitated conditions present in PCR. Najafpour et al., (2004) obtained a value of ethanol productivity of 0.29 g.L\(^{-1}\) using *Saccharomyces cerevisiae* immobilized in Ca-alginate with fermentation conducted in batch reactor. The productivity reported is lower than the observed in this work with PCR (0.83 g.L\(^{-1}.\)h\(^{-1}\)). However, by using a continuous reactor with immobilized cell, the same authors achieved a productivity of 1.3 g.L\(^{-1}.\)h\(^{-1}\), it value is similar with the value observed for STR in the present work.
Table 1. Bioconversion parameters after 24 h of fermentation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Packed column reactor (PCR)</th>
<th>Stirred tank reactor (STR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( P_F ) (g.L(^{-1}))</td>
<td>20.20±1.8</td>
<td>28.10±2.2</td>
</tr>
<tr>
<td>( Y_{P/S} ) (g.g(^{-1}))</td>
<td>0.25±0.02</td>
<td>0.31±0.03</td>
</tr>
<tr>
<td>( Q_P ) (g.L(^{-1}) h(^{-1}))</td>
<td>0.83±0.04</td>
<td>1.17±0.02</td>
</tr>
<tr>
<td>( Y_s ) (%)</td>
<td>79.70±1.1</td>
<td>90.40±1.6</td>
</tr>
<tr>
<td>( n ) (%)</td>
<td>49.70±0.8</td>
<td>60.93±0.6</td>
</tr>
</tbody>
</table>

*\( P_F =\) ethanol final concentration, \( Y_{P/S} = \) ethanol yield, \( Q_P = \) ethanol productivity, and \( n \) (%) = efficiency of substrate bioconversion on ethanol

The immobilized and free cells behavior was further evaluated. For the immobilized cells, the cellular growth was almost similar for both reactor systems using LentiKats\(^{\circledR} \) as inert matrix, as shown in Figure 2.

**Figure 2** - Biomass behavior during fermentation in PCR (open symbols) and STR (full symbols). Immobilized cell per volume of lenses (circles) and free cell (squares)

Using STR, the concentration of entrapped cells increased from 6.0 g. L\(^{-1}\) (at 0 h of fermentation) to 13 g. L\(^{-1}\) at the end of 24 hours of fermentation. On the other hand, in PCR the immobilized biomass concentration achieved a maximum
of 11.4 g L\(^{-1}\). The difference of approximately 12% in immobilized cells concentrations found in the two bioreactors could be associated to the effective mixing supplied by the stirred tank reactor. Such better performance in fermentation medium mixing observed in the STR could have lead to an improvement in the mass transfer between the medium (substrate and nutrients) and the immobilized cells. According to Dias et al., (2001) the mass-transfer resistance offered by the matrix and growth of the entrapped cells determine a gradient of nutrients throughout the gel, which is responsible for both a lower specific growth rate of immobilized cells with respect to that of free ones. Efficient mixing, like that offered by STR, can lead to the reduction of the diffusion coefficient of different substances towards the interior of the gel capsules; such phenomenon has been clearly observed in this paper. Where, better fermentation results has been clearly observed in this paper. Where, better fermentation results has been obtained working with STR (final cellular concentration = 13 g L\(^{-1}\), \(P_F = 28 \text{ g.L}^{-1}\), \(Q_P = 1.17 \text{ g.L}^{-1}.h^{-1}\), and \(Y_{P/S} = 0.3 \text{ g.g}^{-1}\)) in comparison to PCR (final cellular concentration =11.4 g L\(^{-1}\), \(P_F = 20 \text{ g.L}^{-1}\), \(Q_P = 0.83 \text{ g.L}^{-1}.h^{-1}\), and \(Y_{P/S} = 0.25 \text{ g.g}^{-1}\)).

For both reactor configurations, immobilized cellular growth and final concentration corresponded to what was previously reported for *Candida guilliermondii* cells in Ca-alginate and LentiKats\(^{\circledR}\) for the production of xilitol (Cunha et al., 2006).

After 24 h of fermentation for both bioreactor configurations, immobilized cells did not present any significant increase in their concentrations. Such fact is attributed to the saturation of PVA- LentiKats\(^{\circledR}\) support with cells, thus leading to a state of cellular stationary growth inside the immobilization matrix. The stability of the PVA matrix was evidenced by no appreciable mechanical rupture of the lenses by de stirrer under agitated regime. In fact, lenses of LentiKats\(^{\circledR}\) conserved its shape and size after 96 h of continuous agitation. Optical microscopic analysis (images not shown) demonstrated a uniform distribution of cells in the lenses in both reactor systems.

Outside the immobilization support the accumulation of free cells in the fermentation medium was different for both reactor systems. The free cell concentration in STR achieved a maximum of 1.05 g L\(^{-1}\) after 72 h. The free cell phenomenon is associated to the liberation of cells from inside the LentiKats\(^{\circledR}\) lenses due to immobilization support disruption probably caused by two factors: the shearing effect on the lenses resulted from the mechanical agitation in the STR, and the natural release of cells due to the complete saturation of the polymeric immobilization matrix (Cunha et al., 2006; Sarrouh et al., 2007; Carvalho et al., 2004; Beckers et al., 2001). On the other hand, no free cells accumulation was observed in the PCR at the end of 72 h of fermentation. Such behavior is due to the absence of mechanical agitation in PCR configuration. Such fact lead to the absence of shearing stress effect on the LentiKats\(^{\circledR}\) lenses,
resulting in a complete preservation of the immobilization matrix. By comparing the results obtained in this paper for the two different reactor configurations, it can be concluded that the use of stirred tank reactor with cells immobilized in PVA-Lentikats for bioethanol production resulted in higher ethanol concentration and better process yield and productivity than in packed column reactor. Such improvement in process results was caused mainly by the efficient agitation offered by the STR, which permitted a better heat and mass transference as has been discussed earlier.

**Nomenclature**

- \( S_o \) Initial glucose concentration (g glucose. L\(^{-1}\))
- \( S_f \) Final glucose concentration (g glucose. L\(^{-1}\))
- \( P_f \) Final ethanol concentration (g ethanol. L\(^{-1}\))
- \( Y_{P/S} \) Ethanol yield based on glucose consumption (g ethanol. glucose g\(^{-1}\))
- \( Q_P \) Ethanol productivity (g ethanol. L\(^{-1}\).h\(^{-1}\))
- \( n \) Conversion efficiency of glucose to ethanol (%)
- \( Y_S \) Percentage of glucose consumption (%)

**5. References**


