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Study of Biocatalyst to Produce Ethanol from Starch

Coimmobilization of Glucoamylase and Yeast in Gel

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Abstract

This article presents a detailed study on the conditions for achieving a stable biocatalyst to be used in the production of ethanol from starch. Different pellets were used depending on which characteristic of the biocatalyst was being studied: (a) *Saccharomyces cerevisiae* entrapped in pectin or calcium alginate gel particles; (b) silica containing immobilized glucoamylase entrapped in pectin gel particles; or (c) pectin gel particles, with the silica-enzyme derivative and yeast coimmobilized. The influence of several variables on the mechanical resistance of the particle, on the viability of the microorganism, and on the rate of substrate hydrolysis was studied with biocatalyst. The best conditions found were 6% pectin gel, 2-mm particle diameter, and cure in 0.2 *M* $CaCl_2 \cdot 2H_2O$/60 m*M* acetate buffer, pH 4.2, for gel preparation; and 6.0 g/L of $CaCl_2 \cdot 2H_2O$ in the fermentation medium. Biocatalyst (c) was successfully tested for the production of ethanol from liquefied manioc flour syrup.

Index Entries: Ethanol; biocatalyst; glucoamylase; yeast; coimmobilization; starch.

Introduction

The production of ethanol from starch may be an attractive alternative in some specific situations. In the United States, hydrolyzed cornstarch is already an important substrate for alcoholic fermentation. Ethyl alcohol is

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still an important component of Brazil's energy matrix. The use of anhydrous ethanol offers several advantages. One is its smaller environmental impact when used as an additive in fuel gasoline. The 1996 Habitat II Agenda "encourages countries, in particular developing countries, to cooperate in exchanging knowledge, experience and know-how in the phasing out of lead gasoline, through, *inter alia*, the use of biomass ethanol as an environmentally sound substitute" *(1)*.

Therefore, studies continue to be carried out to improve the economics of biomass ethanol, using different sources of carbon for alcoholic fermentation. The process of ethanol production from starch has been developed in Brazil *(2–4)* as a complementary alternative to the fermentation of sugar cane molasses. An enzyme (glucoamylase) and a microorganism *(Saccharomyces cerevisiae)* are coimmobilized in pectin gel. In this way, hydrolysis and fermentation are carried out simultaneously, at 30°C (optimum fermentation temperature). The process is economically feasible owing to the immobilization of high loads of enzyme, allowing for acceptable rates of hydrolysis in the reactor, even at such a low temperature. This strategy counterbalances the fact that the optimal operating temperature for glucoamylase is 60°C. This approach would also avoid the operational difficulties that may appear if the microorganism is genetically modified to metabolize starch. The preservation of the engineered microorganism's stability during long-term runs may be a difficult task.

Pectin pellets are formed by the action of bivalent cations, such as calcium, that form crossed bonds between the polymeric pectin precursors. The presence of calcium in the medium is essential to avoid its leaching and, therefore, to maintain gel integrity. Operation with 2.0 g/L of $CaCl_2$ in the medium *(2,3)* preserved pellet integrity during 25-d runs in continuous, fixed-bed reactors, although for high flow rates gel disruption was observed. A solution for this problem might be to raise the calcium concentration in the medium, to decrease its leaching and prevent gel disruption. However, yeast fermentation activity is reduced in the presence of an excessive concentration of calcium *(5)*. Calcium ion is an inhibitor of phosphofructokinase-1, a regulatory enzyme in the glycolytic pathway, which transforms glucose into pyruvate *(6)*. If the microorganism is not genetically modified to metabolize starch, the substrate hydrolysis is essential. Glucoamylase and a-amylase are currently used to catalyze this reaction, and inhibition of glucoamylase by calcium has been reported *(7)*.

This study focuses on the preparation of a stable biocatalyst to produce ethanol from liquefied manioc syrup, in long-duration runs. The influence of several variables on the integrity of gel particles after glucose fermentation was studied. Gel containing entrapped yeast, biocatalyst (a), tested to determine whether it could produce ethanol from glucose, and the effects of calcium chloride and the acetate buffer concentrations on the fermentative rate and yeast viability were studied. The effects of these variables on the enzymatic activity was analyzed with the aid of biocatalyst (b), the derivative silica-glucoamylase entrapped in pectin gel. Finally,

biocatalyst (c), consisting of the silica-enzyme derivative entrapped with the yeast into the pectin gel particles, was studied to determine ethanol from liquefied manioc flour syrup.

Materials and Methods

Microorganism

Commercial, pressed *S. cerevisiae* with 70% moisture (dry basis) (Itaiquara, Brazil) was used. Supports were sodium alginate (BHW) (Aubytex, Brazil); citric pectin (8002), donated by Braspectina S/A; and controlled porosity silica (CPS) (code 691952; Corning Glass Works) with a 37.5-nm porous diameter and a 200-μm particle diameter.

Enzymes

α-Amylase (EC 3.2.1.1) (500 bacteria α-amylase units/mL) and glucoamylase (190 U/mL of activity; 1 U is the quantity of enzyme that produces 1 g of glucose/h, from 4% soluble starch at 60°C and pH 4.2) were donated by Novo Industri do Brasil. Manioc flour was purchased from Ricieri Pechat & Filho (Araras, SP, Brazil) and soluble starch from Merck. EDTA and all other reagents used were of laboratory grade, from different commercial sources.

Biocatalyst (a)

Pectin (5 or 6% weight) or sodium alginate (1.5, 2.0, or 2.5 % weight) was dissolved in buffer acetate solution (1000, 500, or 60 m*M*). Wet yeast (10% weight) was then added to the solution. This suspension was dropped into a calcium chloride solution (with different concentrations), using a needle. Gel particles with a 2 mm diameter were obtained using airflow parallel to the needle. The pellets thus formed were cured for a period of 2 or 18–24 h in a refrigerator. $CaCl_2 \cdot 2H_2O$ concentrations in the cure solution were between 0.01 and 0.5 *M*.

Biocatalyst (b)

Pectin (6% weight) was dissolved in 60 m*M* acetate buffer (pH 4.2) and 1.5 g of wet silica containing the enzyme previously immobilized (162 U/g dry silica)/20 mL of gel was added to the solution. The silica support was activated with γ-aminopropyltriethoxysilane (γ-APTS)/glutaraldehyde *(2)*.

This suspension was dropped into a 200 m*M* calcium chloride solution, using a needle, and cured for 24 h in a refrigerator. Silica was suspended in 0.5% γ-APTS (pH 3.3) at 75°C, (3 mL of solution/g of silica) during 3 h of gentle agitation. The suspension was filtered and washed with distilled water. The silica was then dried at 60°C, and the beads were treated with 5% glutaraldehyde, in phosphate buffer (pH 7.0) for 1 h, followed by exhaustive rinsing with distilled water. The enzyme solution (in 60 m*M* acetate buffer, pH 4.2) was added to the activated wet silica at a ratio of 10 mL of

solution/g of dry silica, at 25°C for 36 h. The suspension was filtered and washed with distilled water and acetate buffer.

Biocatalyst (c)

Pectin (6% weight) was dissolved in 60 m*M* acetate buffer (pH 4.2) and 1.5 g of wet silica (50% of moisture on dry basis) containing the enzyme previously immobilized and wet yeast (10% weight) was added to each 20 mL of pectin solution. This suspension was dropped into a 0.2 *M* calcium chloride solution and cured for 24 h in refrigerator.

Pellet Dissolution

One gram of cured pellets was dissolved in 20 mL of 5% EDTA solution at constant agitation.

Fermentation Medium for Biocatalyst (a)

The medium consisted of 100 g/L of glucose; 1.0 g/L of yeast extract (Difco), 1.0 g/L of ammonium sulfate; 0.5 g/L of $MgSO_4 \cdot 7H_2O$; 1.0 g/L of potassium diacid phosphate; and 0.0, 2.0, 4.0, or 6.0 g/L of calcium chloride.

Liquefied Manioc Flour Syrup (used with biocatalyst [c])

Flour (380 g) was suspended in 0.01 *M* NaOH/0.01 *M* $CaCl_2$, pH 6.0. The suspension was heated under agitation. At 55°C, α-amylase (0.3 g/L) was added. The temperature was kept at 90°C for 10 min and then raised until ebullition. After 5 min the solution was filtered (using filter paper) under vacuum.

Fermentation Runs (biocatalyst [a] and [c])

Twenty-milliliter Erlenmeyers with sterilized medium at pH 4.2 were inoculated with 10 g of biocatalyst (a) or 17 g of biocatalyst (c). The fermentation was carried out in a reciprocal shaker at 31°C and 150 rpm. In the repeated batch assays, after each run (10 h with biocatalyst [a]), pellets were washed with 0.01 *M* calcium chloride solution, slightly dried on filter paper, and reused with an additional 20 mL of medium.

Maltose and Soluble Starch Hydrolysis (biocatalyst [b])

Forty grams/liter of soluble starch or maltose was dissolved in 60 m*M* acetate buffer at pH 4.2. Erlenmeyer flasks containing 50 mL of the solution were put into a bath at 30°C under agitation, and 4.3 g of biocatalyst (b) was added.

Analytical Methods

Cell Concentration and Viability

A sample of 5% EDTA solution containing either free cells or immobilized cells after pellet dissolution was added to a methylene blue solu-

tion, at a ratio of 1:10. An aliquot was taken and microorganisms were counted in a 4×10^6 mL Neubauer cell. Viable cells were not colored and dead cells were blue. In the free-cell viability experiments, commercial yeast was consecutively suspended in a $CaCl_2$/acetate buffer or EDTA solutions during the same time used to cure and dissolve biocatalyst (a). After exposure to $CaCl_2$/ acetate buffer solution, the free cells were filtered, washed, and suspended in EDTA solution.

Maltose and glucose produced after the hydrolysis of soluble starch were analyzed using the glucose-oxidase enzymatic method *(8)*. In fermentation runs, glucose was analyzed by the Somogyi *(9)* method. Total reducing sugar (TRS) (g of glucose/L) in liquefied manioc flour syrup was determined after total enzymatic hydrolysis of the substrate using glucoamylase *(10)*. TRS = 0.977 (*Gm* – *Gi*)/0.9) + *Gi*, in which *Gm* is the glucose concentration in the sample after hydrolysis (g/L); 0.977 is the starch to glucose conversion factor *(10)*; and 0.9 is the stoichiometric factor for the conversion of starch to glucose.

Ethanol Analysis

Distilled ethanol was oxidized with $K_2Cr_2O_7$ *(11)*.

Results and Discussion

Gel Selection

During preliminary tests in discontinuous fermentation experiments with yeast immobilized in calcium alginate and pectin, a considerable degree of gel rupture was observed. Different gels were prepared and tested under several conditions; Table 1 presents the results. It can be observed that pectin gel displayed a better performance when compared to calcium alginate, with a slight increase in gel resistance for higher concentrations of polymer. Pectin, 6% weight basis, was chosen because solutions that were more viscous clogged the needle orifice. The longer the cure time, the more calcium bridges are made. However, if yeast is entrapped into the gel, 24 h is the maximum recommended time of cure, and 2 h is sufficient when the calcium chloride concentration is above 0.1 *M (6)*.

The results also showed that particle diameter is the most significant variable to reduce the number of broken gel particles. A considerable decrease in the percentage of broken particles was achieved when the average diameter of the gel was 2 mm. The main cause for particle disruption was vaporization of CO_2, released during the fermentation. In a study by Ogbonna et al. *(12)*, they reported that a low initial load of yeast was immobilized and allowed to grow inside the gel. They observed that the yeast grew close to the surface of the gel particle. There were no cells in the particle center. The performance of their biocatalyst during alcoholic fermentation was compared to the observed when a high load of cells was used. No rupture was observed when the microorganism had grown inside gel, whereas many broken gel particles were detected when high loads of cells were immobilized during preparation of the biocatalyst. These results agree with ours.

Table 1
Rupture of Pectin and Calcium Particles with Immobilized Yeast, Prepared Under Different Conditions, After Alcohol Fermentation for Different Initial Concentrations of Glucose[a]

Polymer	C_{pol} (% wt)	Cure time (h)	*dp* (mm)	C_I (*M*)	Glucose (g/L)	*Rupt* (%)
Alginate	1.5	24	3–4	0.10	100	100
	1.5	24	3–4	0.10	150	100
	2.0	24	3–4	0.01	180	100
	2.0	24	3–4	0.10	180	75
	2.0	24	3–4	0.10	150	10
	2.5	24	3–4	0.01	180	23
	2.5	24	3–4	0.10	180	43
	2.5	24	<2	0.10	180	0
Pectin	5.0	24	3–4	0.20	180	27
	6.0	24	3–4	0.20	180	25
	5.0	24	≈2	0.20	180	7
	6.0	24	≈2	0.20	180	0

[a]C_{pol}, polymer concentration; *dp*, particle diameter; C_I, $CaCl_2$ concentration into cure medium; *Rupt*, percentage of disrupted particles.

Decreasing the intraparticle diffusion path of the dissolved CO_2 would reduce the probability of building up gas bubbles inside the gel. Of course, reducing the load of immobilized cells and/or initial glucose concentration would also decrease gel rupture, owing to the smaller yield of CO_2. However, whenever high fermentation velocities are required, the particle diameter must be small.

Influence of $CaCl_2$ Concentration in the Cure Solution on Pellet Dissolution Time and on the Viability of Free and Immobilized S. cerevisiae

For higher concentrations of calcium in the cure solution, more bonds are expected to occur between adjoining polymeric chains. This will lead to an increase in gel resistance and, consequently, dissolution time. In continuous runs, calcium would leach from the gel into the medium, and higher dissolution times would imply on better operational stability during long-term runs. Nevertheless, calcium may act as either an activator or as an inhibitor of different enzymes. Its negative effects on the fermentation activity of yeast have been reported elsewhere *(13,14)*. Therefore, it is necessary to evaluate the influence of calcium concentration on the dissolution time of the gel particle and on cell viability. Table 2 shows the results of our experiments.

As expected, higher concentrations of calcium in the cure solution led to an increase on dissolution time (and consequently in gel resistance). Immobilized yeast viability was not affected by $CaCl_2$ concentration in the cure solution up to 0.2 *M*. At 0.5 *M*, cell viability decreased significantly. Therefore, the highest calcium concentration that may be used without

Table 2
Influence of $CaCl_2$ Concentration Used in Cure Solution on Immobilized Yeast Viability (*Vic*), Free Yeast Viability (*Vfc*), and Dissolution Time of Pectin Gel in 5% EDT (*t*)[a]

$CaCl_2 \cdot 2H_2O$ (*M*)	pH	V_{iv} (%)	V_{fc} (%)	*t* (h)
0.01	5.3	98.0	—	0.45
0.05	5.1	99.0	98.0	0.68
0.1	5.1	97.3	—	0.93
0.2	5.9	97.9	—	1.83
0.5	5.5	85.6	23.5	>4[b]

[a]Initial cell viability was 99.3%. V_{ic}, immobilized yeast viability; V_{fc}, free yeast viability.
[b]Ten percent EDT solution, 1 g of pellet/40 g of EDTA solution.

affecting yeast viability is 0.2 *M*, a trade-off between cell viability and gel integrity. From the results of Table 2, it also can be observed that free cells are more sensible than immobilized ones with respect to calcium concentration. At 0.5 *M*, the viability of immobilized yeast was 85.6%, compared to 23.5% for the free cells. One can conclude that immobilization protects the cells against the deleterious effects of calcium.

One aspect that might mask these results is a possible influence of the 5% EDTA solution used to dissolve the gel on cell viability. Because dissolution times increased with $CaCl_2$ concentration, a 10% EDTA solution had to be used when gel particles were cured in a 0.5 *M* $CaCl_2$ solution. Therefore, before accepting the results in Table 2 as conclusive, the influence of the experimental methodology had to be checked. To do so, the influence of the time of exposure of the immobilized yeast to a 5% EDTA solution was investigated, using 0.1 *M* $CaCl_2$ in the cure solution. The data in Table 3 show that EDTA did not affect viability. Therefore, it is reasonable to assume that the decrease in viability presented in the data in Table 2 was owing to the increase of $CaCl_2$ in the cure solution; EDTA did not affect the results.

Effect of Acetate Buffer Concentration Used in Cure Solutions on Free and Immobilized Yeast Viability

Enzyme immobilization should be carried out under optimal conditions, particularly pH, to ensure that its active site is not altered during the process. For glucoamylase, acetate buffer was used because its pH range was the same as the enzyme's optimal pH. Table 4 shows the effect of buffer concentration on immobilized and free yeast viability.

A strong decrease in yeast viability was observed when 400 and 500 m*M* of acetate buffer was used. The decrease in viability was more accentuated for free cells than for immobilized ones. These results confirm that immobilization protects the cells against changes in the external medium.

Table 3
Effect of Time of Exposure
to a 5% EDTA Solution
on Viability of Immobilized Yeast[a]

Time (h)	Viability (%)
0.5	99.0
1.0	98.0
2.0	98.0
3.0	98.0
4.0	99.0

[a]Initial yeast viability was 99.0%.

Table 4
Influence of Acetate Buffer Concentration on Immobilized (V_{ic})
and Average Free Yeast av(V_{fc}) Viability[a]

Acetate buffer, pH 4.2 (m*M*)	pH	V_{ic} (%)	av(V_{fc})	σ (%)	*n*
60	4.05	99.0	97.5	0.33	4
200	4.0	97.0	—	—	—
400	3.9	73.0	—	—	—
500	4.0	42.0	21.3	0.57	3

[a]σ, standard deviation of average = $[\Sigma(V_{fc} - \text{av}V_{fc})^2/n]^{1/2}$; *n*, number of replicates. Initial immobilized cell viability was 100%. Initial free cell viability was 98%.

To estimate the consistency of these results, free-cell experiments were conducted in replicate.

Table 4 shows that the replicated results of cell viability had small standard deviations. Free-cell experiments were also performed, with and without $CaCl_2$, in order to compare the combined effects of acetate buffer and $CaCl_2$. The presence of calcium did not modify the results. In view of these results, the recommended acetate buffer concentration in the cure solution is 200 m*M*.

Influence of $CaCl_2$ Concentration in Fermentation Medium on Yeast Viability

Figure 1 illustrates glucose and ethanol concentrations during fermentation assays in the presence of different concentrations of $CaCl_2$ in the fermentation medium. Between 0.0 and 4.0 g/L, an increase in $CaCl_2$ concentration leads to a decrease in ethanol production and glucose consumption rates. For 6 g/L of calcium chloride, an unexpected behavior appears. A significant decrease in the initial velocity of ethanol production was accompanied by a higher consumption of glucose—what seems to be a stoichiometric contradiction. After 10 h, all curves present a similar behavior. Cell viability remained at about 98%. Apparently, an intermedi-

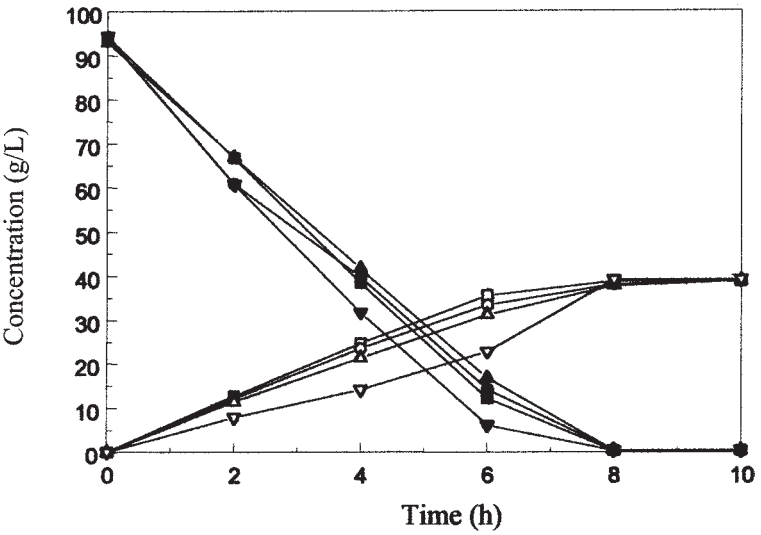

Fig. 1. Glucose and ethanol concentration as a function of time for different $CaCl_2$ concentrations in the reaction medium. For ethanol: (—□—), 0.0 g/L $CaCl_2 \cdot 2H_2O$; (—○—), 2.0 g/L $CaCl_2 \cdot 2H_2O$; (—△—), 4.0 g/L $CaCl_2 \cdot 2H_2O$; (—▽—), 6.0 g/L $CaCl_2 \cdot 2H_2O$. For glucose: (—■—), 0.0 g/L $CaCl_2 \cdot 2H_2O$; (—●—), 2.0 g/L $CaCl_2 \cdot 2H_2O$; (—▲—), 4.0 g/L $CaCl_2 \cdot 2H_2O$; (—▼—), 6.0 g/L $CaCl_2 \cdot 2H_2O$.

ate was being accumulated in the glycolytic pathway, delaying ethanol formation, or, in other words, delaying energy production for cellular growth. Cell concentrations in the supernatant of the fermentation medium were correlated to the $CaCl_2$ contents. Increasing amounts of calcium ions in the medium implied a smaller number of cells escaping from the gel. This was probably owing to lower leaching rates in the presence of calcium ions that may have helped keep the particle structure intact, decreasing the escape of cells. Another run was performed with 2.0 g/L of calcium concentration in the medium, and a repeated batch assay was performed in the presence of 6 g/L of $CaCl_2$, to keep the same gel particles operating for longer periods. The results of this experiment showed the same pattern as the first one.

The purpose of this assay was to verify whether the cell metabolism adapts to calcium concentrations higher than 2.0 g/L. This experiment showed that the second batch run, with 6 g/L of $CaCl_2$, presented results quite similar to those of the run with 2 g/L. Adaptation of the microorganism in the repeat batch was evident. Therefore, to prevent leaching of the cation, calcium concentrations in the medium as high as 6.0 g/L can be used in continuous runs.

Effect of $CaCl_2$ Concentration on Activity of Glucoamylase

Calcium is a known cofactor of many enzymes. However, this ion may have an inhibitory effect on glucoamylase action *(7)*. Figures 2 and 3 show

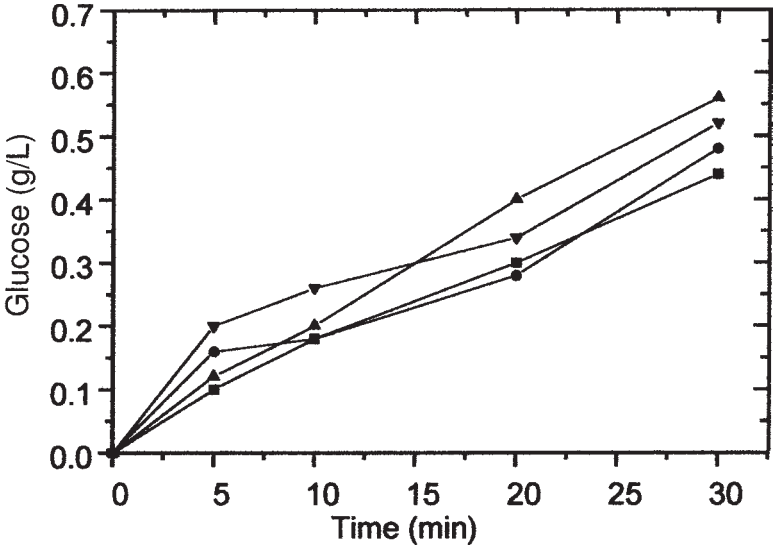

Fig. 2. Glucose concentration as a function of time for different degrees of $CaCl_2$ in the cure solution. Medium: 40 g/L soluble starch; 2 g/L $CaCl_2$ (—▼—), 0.05 *M* (cure); (—●—), 0.10 *M* (cure); (—▲—), 0.20 *M* (cure); (—■—), 0.50 *M* (cure).

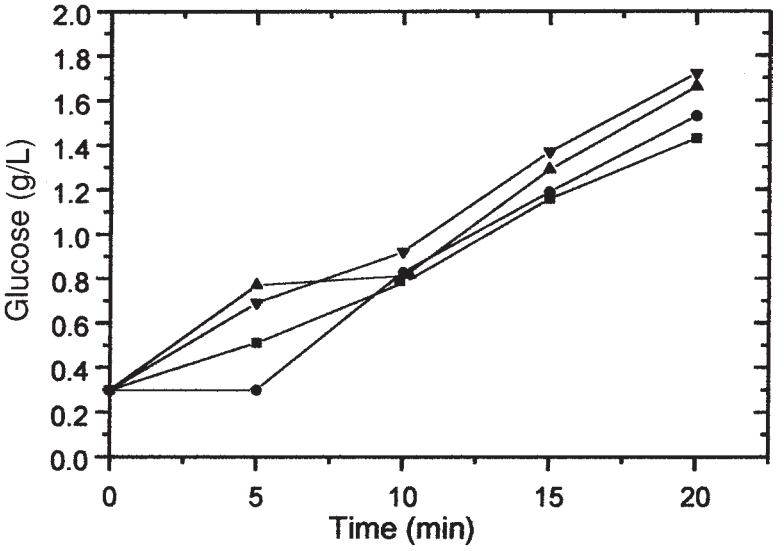

Fig. 3. Glucose concentration as a function of time. Pellets were cured in 0.2 and 0.5 *M* $CaCl_2$. Medium concentrations of $CaCl_2$: 2 and 6 g/L. Substrate: 40 g/L maltose (—■—), 0.5M (cure), 2 g/L (medium); (—●—), 0.5 *M* (cure), 6 g/L (medium); (—▲—), 0.2 *M* (cure), 2 g/L (medium); (—▼—), 0.2 *M* (cure), 6 g/L (medium).

the effect of this cation on the activity of the enzyme (immobilized in silica and wrapped in pectin gel) for two different experiments.

Figure 2 shows the inhibitory effect of calcium. After cure in the presence of different calcium concentrations, the particles of gel had the same

Table 5
Results of Three Consecutive Runs to Produce Ethanol from Liquefied Manioc Flour Syrup Using Ciocatalyst (c) (yeast and derivative silica-glucoamylase coimmobilized in pectin gel)[a]

Run	Time (h)	Glucose (g/L)	TRS (g/L)	Ethanol (g/L)	*Em* (U)
1	17.5	0.0	0.0	31.5	0.0
2	23.0	0.0	0.0	37.5	0.0
3	19.5	0.0	0.0	37.5	0.0

[a]*Em*, enzyme present in the medium. Initial total reducing sugar in the substrate was 149.0 g/L. Initial glucose in the substrate was 6.0 g/L. Useful reactor volume = medium volume + particles volume. Enzyme load, 0.55 U/mL of reactor; yeast load, 0.05 g of wet yeast/mL of reactor.

amount of enzyme but different levels of calcium. The intragel calcium concentration was about the same as the calcium concentration in the cure solution (2.0 L of cure solution are used to cure 100 g of gel). In the beginning of the hydrolysis reaction, an inhibitory effect of calcium was observed. However, as the high intragel concentrations of the cation decreased toward the lower $CaCl_2$ concentration in the reaction medium (2 g/L or 13.6 m*M*), the inhibitory effect was reversed. This indicates that the level of calcium present during the preparation of the biocatalyst (cured in 200 m*M* of $CaCl_2 \cdot 2H_2O$) may inhibit glucoamylase. However, the decrease in enzymatic activity is reversible. Figure 3 shows that increasing $CaCl_2$ concentrations in the medium from 2 to 6 g/L (40.8 m*M*) does not affect the rate of hydrolysis. Therefore, calcium concentrations in the medium as high as 6.0 g/L can be used in continuous runs, to maintain gel integrity.

Simultaneous Saccharification and Fermentation Runs

The performance of the biocatalyst (c)—coimmobilized glucoamylase and *S. cerevisiae* in pectin gel—was tested in repeated batch runs; Table 5 presents the results. After 17.5 h, all dextrin present in the substrate was hydrolyzed by glucoamylase and converted to ethanol by the immobilized *S. cerevisiae*. Biocatalyst (c) is very stable, since no enzymatic activity was detected in the supernatant. It displayed similar performances in the three consecutive runs.

Ethanol concentrations in the second and third runs were higher than those observed in the first run owing to the dilution of the medium by the water present in the gel at the beginning of the experiment.

Conclusion

Pectin (6%) provides a gel matrix that is more resistant than the one made of calcium alginate. Decreasing of the particle gel diameter, even for high ethanol yields, may attenuate gel disruption. In this study no rupture of gel particles was observed for average diameters of 2 mm. When prepar-

ing the biocatalyst, buffer acetate concentration in the cure solution up to 200 m*M* may be used to keep the optimum pH for glucoamylase, without affecting yeast viability. $CaCl_2$ concentrations as high as 200 m*M* in the cure solution and 6.0 g/L in the fermentation medium may be used to diminish the leaching of this cation without affecting either the viability of immobilized *S. cerevisiae* or glucoamylase activity. The biocatalyst (yeast and silica-glucoamylase coimmobilized in pectin gel particles) was stable, maintaining similar performance after three consecutive runs, producing ethanol from liquefied manioc flour syrup.

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