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The new flow system approach in packed bed reactor applicable for immobilized enzyme

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ABSTRACT

Several studies have been directed to development of reactors for chemical–biological reactions have been carried out successfully. "Dead zones" are areas where the substrate has non-contact with catalyst, and this situation in reactors has been seen as inconvenience in relation to productivity. Moreover, a catalyst immobilized in porous support also must be difficult. We have been obtained preliminary results in batch system of invertase immobilized on glass–ceramic support (GCS) (1000 °C/3 h), were the optimum pH and temperature of free and immobilized enzyme was similar, 5.0 and 45 °C. The apparent K_m for free invertase was 13.53 times higher than the free invertase. Aiming the work using packed bed reactors to study the activity of invertase in different flow systems, was obtained sigmoid curves using reactors 1 and 2, with the best result 4.75 U/g GCS and 3.09 U/g GCS, respectively. The lab-scale setups from flow system continuous, down-flow, up-flow and alternate-flow were studied. Alternate-flow is the alternation of down-flow and up-flow in previous time mentioned. This innovative flow system has facilitated contact among sucrose solution and immobilized enzyme to obtain the invert sugar production 24 h faster, due elimination of "dead zones" in the reactor in comparison to others flow systems.

1. Introduction

Several studies have been directed to the development of reactors for catalysis to provide an efficient operation and significant increase of productivity process. These inventions respect to multidisciplinary areas such as biotechnology and hydrodynamic processes. The development of new types of reactors is widely interconnected mass transfer of the substrate to provide heterogeneous catalyst and consequently a decrease in diffusion effect. A significant number of studies designed to eliminate "dead zones" has been performed. In appropriate words, they are the areas where the substrate has not contacted the catalyst. Due to the complicated geometry and high mechanical strength of the ceramic supports, a reactor was proposed. The ceramics were used as a basis for design of a novel type of vortex reactor. It was used to improve substantially the mass transfer of substrate to the immobilized enzyme and eliminate "dead zones" and jet stream in the reactor [1]. Some authors reported problems of flow characterization in a reactor. For example, a high density commercial modules and high area per volume offered by hollow fiber modules is one of their main advantages. The tendency is thereby to minimize the fiber diameter and maximize their number in the bundle. For this reason, the modules offering the highest theoretical area per volume are also the tightest. The consequence is the occurrence of dead zones, back mixing, by passing and channeling, especially on the shell side, causing irregular mass transfer along the module [2]. But, specific experiments to evaluate sludge removal rates from the hollow fiber bundle showed the high capacity of aeration to remove sludge of this device without the occurrence of "dead zones" [3].

Other solution was presented to avoid dead from the bed is supported by a glass-sintered plate placed between the bottom head and the main part of the reactor. The bottom head is packed with glass spheres in order to improve feed distribution and avoid "dead

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volumes" [4]. Other reactor was investigated palm oil hydrolysis by lipase from *Candida rugosa* immobilized in a biphasic oil/aqueous hollow-fiber membrane, in order to optimize the flow regime in the membrane reactor at different flow rates in lumen and shell zones of the reactor [5].

It was mentioned that the up-flow circulates for sucrose hydrolysis in the reactor using immobilized invertase in compost from chitosan and sodium alginate as the standard for their experiments. Other parameters were studied for using in packed-bed reactors sophisticated with down-flow or up-flow due the differences in hydrodynamic and heat and mass transfer factors [6].

However, packed bed operation has some disadvantages, including "dead zones", channeling, and high pressure drop across the column. Glucoamylase was immobilized on ceramic support from carbonized mesostructured with porosity high to the reaction in packed bed reactor. It was obtained good results due this physical characteristic when the flow velocity was inferior [7]. When the density of the particulate material (e.g., silica aerogels) is less than the density the liquid, inverse fluidization can be applied to disperse the solid particles in liquid [8]. Results were found with these two types of flows in full-bed reactor, due to possible relationship between the chemical composition of support and the activity of the immobilized enzyme due to the formation of "dead zones" [9].

In present literature, there are no studies about the relation between porosity of support and flow system in packed bed reactor as important factor for enzymatic reactions. We have been used a coal fly ashes (CFA) to produce a glass–ceramic support (GCS) at high temperature, it is a nontoxic, compatible with adequate mechanical strength and 3-aminopropyltriethoxysilanefuctionalization and well-established glutaraldehyde coupling process for most biotechnological applications [10]. And the novelty of our work was using a flow to facilitate of the sucrose solution circulation in contact with the enzyme (β-D-fructofuranidase EC 3.2.1.26) immobilized on the glass ceramic support inside the reactor in order to homogenize sucrose solution. In addition, batch system was carried out further studies on physic-chemical and kinetics for free and immobilized enzyme.

2. Material and methods

2.1. Material

Invertase (EC 3.2.1.26) was donated by Novozymes Latin America Ltda. (Araucaria, PR, Brazil). 3-Aminopropyltriethoxysilane (3-APTES), polyvinyl alcohol (PVA (MM 85,000)) and 3,5-dinitrosalicylicacid (DNS) were obtained from Sigma–Aldrich Chemicals Inc. (St. Louis, MO, USA). Glasses bead size 50–100 μm was obtained from Polysciences, Inc., USA. The glutaraldehyde, sucrose, toluene, sodium citrate, citric acid, monobasic sodium phosphate, bibasic sodium phosphate were obtained from Vetec Fine Chemistry Ltda. (Duque de Caxias, RJ, Brazil). Coal fly ashes (CFA) were obtained from the combustion of coal mineral of Thermal Power Plant President Medici (Candiota, RS, Brazil).

2.2. Chemical characterization CFA and investigation of GCS macro–microstructures

The coal fly ashes (CFA) were analyzed for chemical composition after dried at 110 °C followed by heating at 1000 °C/2 h, using a Rigaku model RIX 3000, X-ray fluorescence spectroscopy (XRF) unit, according to Maheshwari et al. [11]. The main crystalline phases present in GCS were measured by Miniflex X-ray Diffractometer (model RIX 3000, Rigaku Americas Corporation). The detector was scanned over a range of 2θ angles from 19° to 28°, at a step size of 0.02° and a time of 2 s/step.

2.3. Preparation of glass–ceramic support (GCS)

Samples (65%) of CFA were previously treated using a sieve mesh opening of 3.0 mm and 1.5 mm, followed the standardized samples were dried at 70 ± 2 °C for 12 h under air circulation. These samples were mixed with glass bead (25%) and PVA (0.1 M) using distilled water, homogenized and dry (2 h) at 70 °C for 12 h under air circulation. After cooling, the mixture was used to obtain green bodies in mold, followed the dimensions: 5.5 mm of height and 6 mm of diameter, and dry at 70 ± 2 °C for 12 h under air circulation. Then, the green bodies were submitted to the treatments of sintering: 1100 °C/3 h. The furnace was programmed with heating rate of 6 ± 2 °C/min to reach each sintering temperature. After, cooling (25 ± 2 °C) the glass–ceramic supports were stored in airtight containers.

2.4. Physical characterizations of macro–microstructures of GCS

The physical properties were estimated from apparent values of porosity (*Ap*), water absorption (*Awa*) and density (*Ad*) [12]. They were obtained in the five conditions of sintering. They were determined by measuring the dry mass (dry*m*), immersed mass (imm*m*) and 24 h saturated surface-dry mass (sat*m*). The *Ap* (%) Eq. (1), *Awa* (%) Eq. (2) and *Ad* (g/cm^3) Eq. (3) were obtained respectively by the following relations: using Archimedes' principle individual samples were calculated from:

$$Ap = \frac{\text{sat}m - \text{dry}m}{\text{sat}m - \text{imm}m} \times 100 \quad (1)$$

$$Awa = \frac{\text{sat}m - \text{dry}m}{\text{dry}m} \times 100 \quad (2)$$

$$Ad = \frac{\text{dry}m}{\text{sat}m - \text{imm}m} \quad (3)$$

Studies were done (10 samples) about the resistance to uniaxial compressive strength (2.13–21.28 MPa) of GCS samples in mechanical press (Ronald TOP Ltd. – USA) with application speed of 0.77 mm/min.

Other studies of microstructures on the surfaces of sintered GCS sample were performed with scanning electron microscope (SEM) (JEOL Model JSM-5600 LV) at setting of 10 and 15 kV.

2.5. Immobilization of invertase on GCS samples

Invertase was immobilized as described by Suzuki et al. [13]. The sintered GCS samples at 1100 °C for 3 h (0.20 g, 0.60 cm height, 0.65 cm diameter) was washed with distilled water and sterilized in an autoclave at 121 °C for 20 min to ensure support which is uncontaminated. After, they were submerged in 5 ml of toluene with 3-APTES (0.42 mM) to the period reaction at 85 °C for 6 h under agitation. Posteriorly, the treated samples were washed 3 times distilled water and dried at 100 °C for 1 h. After cooling, the samples were placed in 1 ml sodium phosphate buffer (10 mM, pH 7.5) with glutaraldehyde (0.38 mM) under vertical agitation (3 rpm) at 4° for 24 h. Then, the GCS*C* samples activated surface were washed with distilled water and submerged in 1 ml of phosphate buffer containing invertase (7.17 mg) at 4 °C for 36 h under vertical agitation. After covalent coupling period, the immobilized derivatives (GCS-invertase) were washed 3 times with NaCl (10 mM) and stored in 100 mM sodium citrate buffer pH 4.5 at 4 °C.

The amount of immobilized proteins on the surface of activated supports (μg proteins/g GCS) was estimated by measurement of free protein concentration [14] in the supernatant and washing solutions.

Table 1
Types of flows.

Time (h)	Up-flow	Down-flow	Alternate-flow
1	A	B	A
2	A	B	B
3	A	B	A
4	A	B	B
8	A	B	A
16	A	B	B
24	A	B	A
32	A	B	
40	A	B	
48	A	B	

2.6. Activity of free and immobilized invertase in batch system

The free enzyme (5 μl containing 36.10 μg of proteins) or immobilized invertase (0.2 g) was incubated in batch reactor containing 15% (w/v) sucrose solution prepared in 0.1 M sodium citrate buffer pH 5.0 at 25 ± 2 °C and under horizontal agitation (150 rpm). The final volume of reaction enzymatic mixture was 5 ml for both preparations, using polypropylene tubes (25 mm diameter and 50 mm height) with a conical bottom. The reducing sugars produced were analyzed by DNS method [15]. A unit of activity (U) was defined as being the amount of enzyme necessary to produce 1 mmol of reducing sugar per min in pH 5.0 at 25 ± 2 °C. The activities were expressed for U/μg proteins and U/μg proteins/g GCS, free and immobilized enzymes, respectively.

2.7. Determination of optimum pH and temperature

The effect of pH was studied at 25 ± 2 °C by varying the pH of the reaction mixture in the range of 3.0–6.0 and 7.0–9.0, 0.1 M sodium citrate and sodium phosphate buffer, respectively. The optimum temperature was determined by performing the reaction in the temperature range of 25–85 °C. The enzyme activity was represented as a percentage relative to initial activity, called relative activity.

2.8. Kinetic parameters for the invertase enzyme

In order to determine maximum velocity (V_{max}) of reaction and Michaelis–Menten constant (K_m) for free and immobilized invertase system, activity assay was applied for different concentrations of sucrose (29–584 mmol/dm^3). The apparent Michaelis–Menten constant (K_m) was calculated from Lineweaver–Burk plot, according to under condition optimum pH and temperature.

2.9. Packed bed reactor

The packed bed reactors in glass columns have the following dimensions, 63 cm height, 1 cm diameter and 7 cm height, 1 cm diameter, reactors 1 and 2, respectively. The experiments were carried out with the sucrose solution (500 ml, 15% (w/v) in sodium citrate buffer (0.1 M pH 5.0) with immobilized invertase (400 samples = 80 g) at 25 ± 2 °C in each experimental run).

It was carried out two experiments with reactors cited above. The first was studied different flow rates in relation to the enzymatic activity (0.03, 0.06, 0.12, 0.18, 0.24 and 0.30 l/h) using the flow system up-flow. Then the activity of invertase immobilized on GCS seen in better speed and reactor (1 or 2) was chosen to perform the last experiment. It was studied using the types of feeding of reactor the following: down-flow, up-flow and a novel type of feeding called alternate-flow. This type of feeding was used by alternating the two other types of flows in time determined as mentioned below (Fig. 1 and Table 1). The last experiment carried out in reactor observed the best result. The sample for measurement reducing sugars was collected in the container containing the sucrose solution which leaves for reactor (Fig. 1b).

All experiments were performed in triplicate.

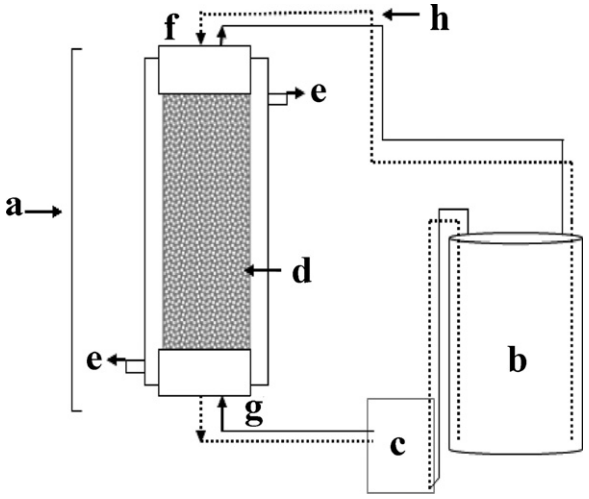

Fig. 1. Scheme of reactor: (a) reactor; (b) container with the solution of sucrose; (c) peristaltic pump, (e) circulation of water for temperature control; (f) GCS-invertase, (h) down-flow; (g) up-flow.

Table 2
Physical properties of GCS.

Analyses	Treatment 1200 °C/3 h
Mass green body (g)	0.29 ± 0.00
Mass dried (g)	0.20 ± 0.00
Shrinkage (%)	31.03 ± 0.00
Height (cm)	0.65 ± 0.02
Volume (cm^3)	0.19 ± 0.02
Diameter (cm)	0.60 ± 0.00
Apparent porosity (%)	32.15 ± 0.03
Apparent water absorption (%)	23.90 ± 0.01
Apparent density (g/cm^3)	1.47 ± 0.02
Uniaxial compressive strength (MPa)	8.30 ± 0.02

3. Results and discussion

3.1. Physical properties of GCS

In previous studies, GCS samples were obtained from CFA and glass beads with different formulations using organic additives (PVA, carboxymethyl-cellulose) uniaxially pressed (255 MPa) or molded. However, the physical and mechanical properties of formulation were chosen with PVA and the green body (molded) obtained a greater preservation of glass beads added and cenospheres, with a regular size and distribution of pores compared to others samples. This initial result in present work was essential to its continuation due these characteristics. Thus the respective summary of physical properties and chemical composition glass–ceramic support is presented in Tables 2 and 3. The parameters such as porosity and water absorption apparent are much more studied when compared with other studies [8,16].

Table 3
Chemical composition of glass–ceramic support from CFA by XRF (%).

SiO_2	FeO_3	Al_2O_3	CaO	K_2O	TiO_2	SO_3	P_2O_5	MgO	SrO
58.2	16.2	10.2	7.5	3.2	1.4	0.1	1.1	0.4	0.1

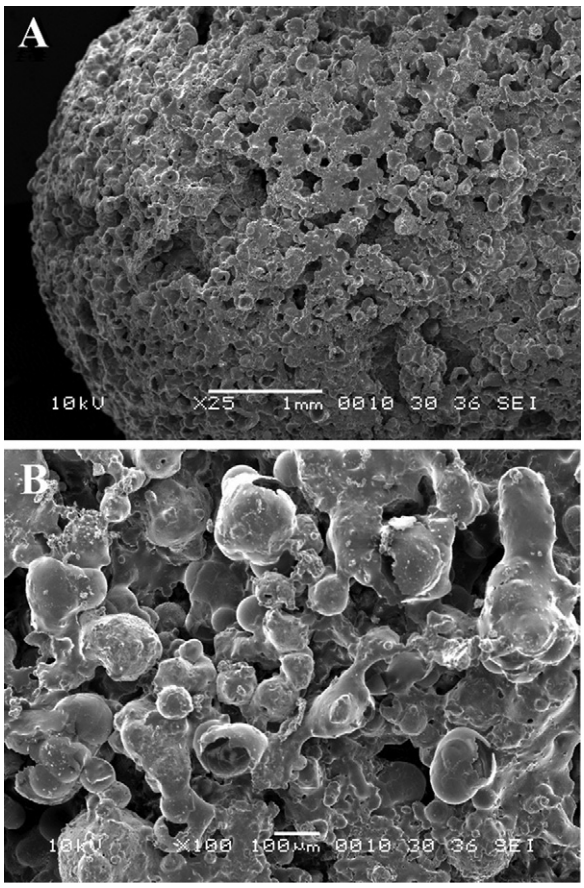

Fig. 2. Microphotographs of the ceramics (GCS) from coal fly ash (CFA) sinterization.

3.2. SEM and XRD

The microstructural studies of CGS samples were performed using SEM and they are shown in Fig. 2. The samples present qualitatively characteristics that may explain the dense glassy phase formation. There are larger pores and cracks with evident micro and macropores. The dispersion of crystal and amorphous materials, used as addictive (PVA and glass bead), caused some melting regions increasing the nucleation and cenospheres deformation, which influenced in the physical properties. The binders commonly used in spray-drying are water-soluble polymers such as polyvinyl alcohol (PVA), cellulose, polyethylene glycol (PEG), and polyacrylate. Thus the selection criteria for the binder are based on its ability to form granules that readily deform during compaction, to burnout cleanly before sintering, and to give a high compact density and strength [17]. In addition, small particles of PVA may be joined to other products in CFA and formed small clusters, which in turn is interconnected to other cores to form true internal channels in the ceramic. But, Fig. 2A shows tridimensional structures with characteristics such as cenospheres and glass, probably because of the drying of excipients (additives), cited above, and sintering time/temperature used. And in Fig. 2B cracks are shown in both the glass spheres and cenospheres because of the effect provided by the treatment in the green bodies.

Analyses of X-ray diffraction for the samples of GCS to study crystalline phases are shown in Fig. 3. This result concerns to the GCS (black-line). However, the sample glass–ceramics for the peak at (2θ) 20.89°, 26.13° and 26.91° corresponding to the reflection of quartz (SiO_2), 25.02° to mullite ($Al_6Si_2O_8$) and uncommon result is found to large phase called alunogen ($Al_2(SO_4)_3$ in 21.81° and 24.02°. They were similarly found in the same intensity

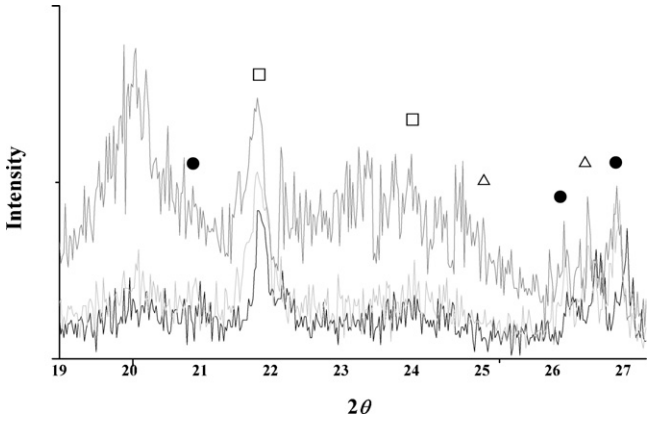

Fig. 3. X-ray diffraction (XRD) to demonstrate the crystalline phases, glass–ceramic support sample from coal fly ashes (CFA) sintering for the immobilization of invertase process. (Black-line) GCS: mullite (△), quartz (●), aluminum sulfate (□); (light gray-line) GCS functionalized with 3-APTES and glutaraldehyde; (gray-line) 7.17 mg immobilized protein/g GCS.

corresponding stages of the analysis in alunogen found in clay [18] when the acid leaching of malachite in synthetic mixtures of clay and zeolite-rich gangue. According to methodology employed (1100 °C/3 h) and principal material resource (coal fly ashes) must not a high temperature, for this, they are evidence about crystallization due the alunogen presence [19].

3.3. Immobilization of invertase

The development of methodology for enzyme immobilization is conceived as one of the most important experiments to be performed. Usually, a simple test has been performed to verify the enzyme coupling – analyses the "wash solution" of derivative. Therefore, analyses of X-ray diffraction for the derivatives still are shown in Fig. 3. There is an interference level very accentuated due to intercalation to the enzyme immobilization into GCS between 19° and 27°. The order to follow is: GCS-functionalized by 3-APTES and glutaraldehyde (light gray-line), GCS-functionalized by 3-APTES and glutaraldehyde with enzyme coupling (gray-line).

The initial experiments were tested with concentrations of glutaraldehyde, 3-APTES with some non-aqueous solvents. Then, it have been tested the concentration of 3-APTES and glutaraldehyde–enzyme to bind to the ceramic (not shown). The efficiency of immobilization of this enzyme was reached under conditions where the silanization reaction was performed in an aqueous solution and cross-linking with the bi-functional agent (glutaraldehyde), used in at 2%. Activity of enzymatic has been done in wash solution samples containing enzyme non-covalent. And when there is more activity, the coupling process covalent was completed successfully. The amount of immobilized enzyme was 9.76 μg/g GCS. This amount was determined according to the quantification of protein solution in relation to the main protein solution that did not bind to the support and the effluent obtained from washing in the derivative. This small quantity of proteins bond can be partially attributed to the incomplete activation of the amino groups of glutaraldehyde in support, due to diffusion limitations during the activation step [20]. A study of protocols in respect of chemicals (glutaraldehyde and 3-APTES) and biological (proteins) for greater binding of proteins in different sizes of media in the future should be done. Thus, a greater surface area will be implemented in this ceramic. However, after the tenth reuse immobilized invertase activity was around 57%, and its half-life in relation to complete inactivation rate was compromised from one year after the coupling process of the enzyme.

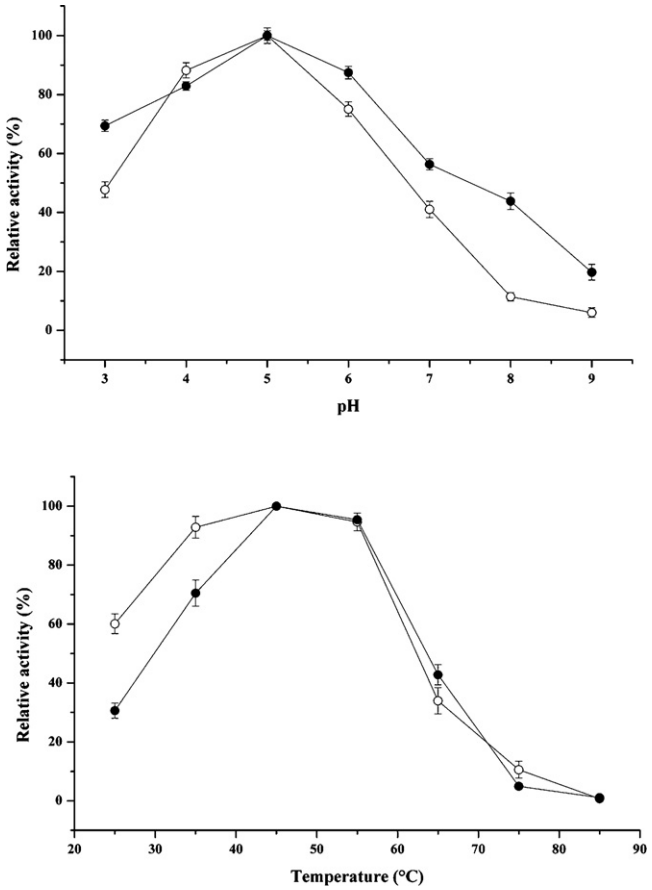

Fig. 4. Effect of pH and temperature for free enzyme (○) and immobilized (●) in GCS.

However, some studies have been showed good results as Sanjay and Sugunan [21] used montmorillonite (10 mg proteins/g support), Amaya-Delgado et al. [22] used nylon-6 micro beads (4.95 mg proteins/g support) and Akgöl et al. [23] used magnetic microspheres of PVA (7.18 mg proteins/g support). However, the number of reuses of the immobilized invertase in GCS to hydrolysis of solution sucrose and other studies suggest checking for further understand the relation between amount of protein and support.

3.4. Effect of temperature and pH

Proteins are very unstable when exposed to environmental conditions significantly different from those found in natural conditions. The effect of temperature on the situation of the catalytic activity of free and immobilized invertase was studied in 0.1 M sodium citrate buffer pH 4.5 (25 ± 2 °C) at 25–85 °C (Fig. 4A). The result of the curve shows maximum activity at 45 °C for both free and immobilized invertase. However, the activity of invertase immobilized showed strong temperature dependence at temperatures below with decreased activity showed critical temperature dependence at temperatures above the optimum temperature. Some authors observed temperature dependence in the activity of invertase immobilized due to varying physical and chemical properties of the enzyme when covalently bound to inorganic supports [23–25].

Moreover, high temperatures can be an additional cost to achieve industrial-scale or optimum activity may not be the best alternative because this also allows the natural enzyme denaturation. Bayramoğlu et al. [26] cited the process of immobilization via amino groups cannot be deteriorative to the structure and conformational flexibility of the enzyme and its own organization to connect to the substrate and impending natural causes due to denaturation temperature employed. Often, enzymes are tested in their optimal pH for appreciable rate of reaction to happen. The pH effect of both free and immobilized invertase was estimated in 3.0 to 9.0 (Fig. 4B).

The result of the curve also was obtained as the optimum value for free and immobilized invertase at pH 5.0. This result supports the evidence that the system of detention was not as detrimental to the enzyme. The dependence pH activity is to profile the immobilized invertase is expansive to 4.0–6.0 pH. Then, this expansion is possible due to stabilization of invertase molecules as a consequence of multipoint connections on the surface of the ceramic due to the immobilization process [25].

3.5. Kinetic properties

The kinetic parameters of enzymatic reaction can be estimated by direct line of Lineweaver–Burk of the initial rate of hydrolysis of sucrose of the experiment. The result is given by two straight lines as the Michaelis–Menten equation for the reaction. The apparent Michaelis constant (K_m) and V_{max} for free invertase and invertase immobilized invertase were $5.90 \pm 0.00 \times 10^{-3}$ mM and $4.11 \pm 0.00 \times 10^{-3}$ mmol/min and $7.99 \pm 0.01 \times 10^{-2}$ mM and $6.34 \pm 0.13 \times 10^{-1}$ mmol/min, respectively. The apparent K_m for free invertase was 13.53 times higher than the free invertase.

However, V_{max} for immobilized invertase is 150 times higher than V_{max} for free invertase. This is an uncommon result, because normally a decreasing on V_{max} for an immobilized enzyme would be expected. According to Tomotani and Vitolo [27], probably, the extra stabilization of supra-molecular aggregates (hexamers and octamers, mainly) acquired through the coupling covalent on GCS, favored the V_{max}. Or greater distribution throughout the ceramic even with the small amount bound proteins. The results obtained can be an indicating to use in packed bed reactor according to circulation of sucrose solution concentration.

For hydrolysis of sucrose with invertase immobilized K_m and V_{max} values were not increased and decreased, respectively. Generally, K_m and V_{max} values of invertase are free are minor and major, respectively. This may be an indication that there was a structural modification of the enzyme after the immobilization process. Amaya-Delgado et al. [22] used nylon-6 microbeads showed an apparent K_m of 1.2 times greater than V_{max} of invertase immobilized similar results were obtained free invertase. The formation of enzyme–substrate complex is more difficult with the invertase immobilized due to porous structure of the support [10,28]. Alternatively, the irregular porosity of ceramics must proportionate an uncommon contact to substrate–enzyme complex. At present, the complex becomes easier to led to the increase in affinity for the substrate and therefore a low K_m value compared with other studies [7,21,23,29].

3.6. Operational stability of invertase immobilized in packed bed reactors, in different flow systems

The proposal to compare the effect of flow rate on invertase activity using the reactors 1 and 2, to verify the best sucrose hydrolysis with the change of flow rate runs with two sizes of reactors. It was obtained sigmoid curves and they are shown in Fig. 5. But, all flow rate, the activities of immobilized invertase were superior using reactor 2.

The physical properties of glass–ceramic support as water absorption and porosity apparent must has influence to obtain this result in the relation to the parameter reactors height/diameter

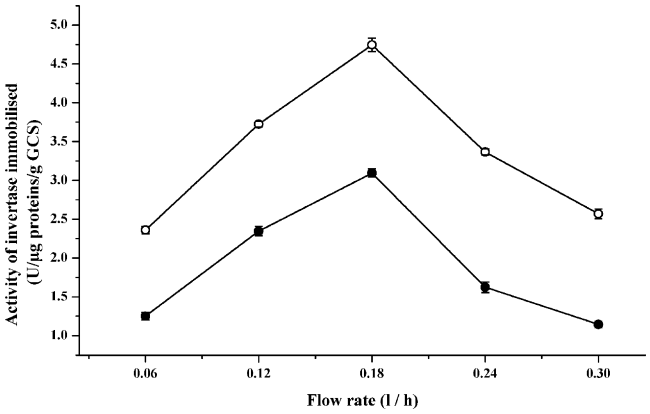

Fig. 5. Dependence of sucrose hydrolysis rate on the flow different rates for the reactors 1 (○) and 2 (●). Experimental conditions are described in Section 2.8.

(reactor 1 equivalent to 21:1 and reactor 2 equivalent to 7:1). This may be an indication that gravity must have been an important factor for improved performance in the productivity of invert sugar has been using the reactor 2.

One way the pressure of the flow to enter interior has been facilitated to the sucrose solution to circulate derivative (ceramic/enzyme). The GCS sample obtained a porous structure the enzyme apparently does not suffer influence in the reaction, meaning that the substrate is converted only to a small extent on the support surface. However, the value of the activity was higher in the reactor, probably due to a higher pressure at the entrance of a sucrose solution in a smaller diameter. In addition, the fraction of the sucrose inversion was relatively higher in the long residence time and high effective mass transfer inside the system. This phenomenon is consistent with other results [20]. Other result can be cited in relation this effect when glucoamylase was immobilized in a ceramic support from carbonized mesostructured with porosity high to the reaction in a particular reaction residence time. In other words, the higher velocity flow, the sucrose solution penetrates inside the support, but nonetheless the reaction is reduced [7].

For economic proposition for industrial scale production invert sugar, it is usually preferable to implement the continuous feed flow system. An innovative flow type that we tested type of flow, which is used both in alternate times. This type of flow is called alternate-flow due the alternation (down-flow and up-flow). Using this flow system may have been a destabilization or elimination of "dead zones". The results are shown in Fig. 6. Sucrose hydrolysis

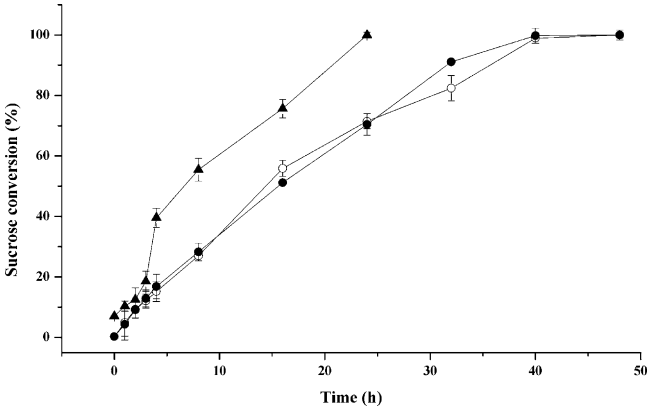

Fig. 6. Operational stability of immobilized invertase in packed bed reactors, in different flows system such as: up-flow (○), down-flow (●), and alternate-flow (▲). Experimental conditions are described in Section 2.8.

total (100%) was measured in the alternate-flow 24 h in operational stability, was 16 h later when compared with other type of flow studied (down-flow and up-flow). This can be explained in terms of diffusional resistance and inhibition of by-products or inefficiency in the internal distribution of sucrose solution in reactor using only one type of flow. Then, the innovative flow system facilities the contact among solution sucrose and enzyme immobilized through improved efficiency to obtain inverted sugar for the "dead zones" elimination in reactor. However, this elimination also can be obtained the "micro-dead zones" elimination in interior of ceramics due their irregular porosity. The alternate-flow allows a substrate circulation through areas which offer greater resistance, and the liquid tends to flow along the wall reactor (lower resistance) and not completely through in packed bed reactor [9]. These observations should also be applied to ceramic due their physical characteristics.

A research carried out involving continuous flow rotary circular or spiral of the solution inside the reactor-vortex reactor. The first named rotor inertial (disposal of the reactor in order horizontal), and the other reactor is immersed (disposal of the reactor in order vertical – relation height/diameter was 10/1). Both reactors were respectively 1.2 and 1.5 times better than the reactor of the fixed bed reactor. The use of these reactors were to eliminate "dead zones" and thus obtain better performance, and the relation to the size of molecular weight of the substrate or porosity of the support [1].

4. Conclusion

First, in relation the GCS, obtained using coal fly ashes with PVA, was successfully used as a support for immobilization of invertase by covalent linkage. This can be a viable alternative to reduce environmental pollution by coal fly ashes.

Second, according to the result obtained with the innovative type of flow, the production of invert sugar maybe performed on larger scales in relation to what was used in this study because without the need for greater investments in reactor technology. Another aspect to this technology is to add value to process sugar inverted production from sugar cane juice, and we still can highlight the possibility of using other enzymes or microorganisms of industrial interest.

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