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Design Strategies for Integrated β-Galactosidase Purification Processes

The operational conditions for an aqueous two-phase system (ATPS) for β-galactosidase purification were optimized and applied to the design of a purification strategy as an alternative to the primary purification steps. The ATPS proved to be suitable for the recovery and primary enzyme purification. The purification process design developed by ATPS, diafiltration, ion exchange, and diafiltration/ultrafiltration was successful, yielding a more than tenfold purification. The purification strategy design resulted in a powerful integrated purification and recovery process, an evidence of the potential for a scale-up of the β-galactosidase purification process.

Keywords: β-Galactosidase, Purification, Separation techniques, Two-phase system

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1 Introduction

Numerous techniques have been applied for the purification and recovery of various bioproducts. However, in order to obtain a purified bioproduct, besides its intrinsic characteristics, the choice of unit operations suitable for each process step must be taken into consideration. Besides, there are feasible strategies which lead to integration in one or more techniques [1]. In the case unit operations are not well employed, the development of the process and the resulting byproduct may be affected. The total costs of downstream processing are around 50–90 % of total process costs [2]. The industrial manufacturing of bioproducts is a complex task that requires considerable effort in process development. Thus, it is necessary to investigate purification strategies which use low-cost technologies. Traditional methods for isolation and purification of proteins generally involve several steps: ammonium sulfate precipitation, ion and affinity chromatography, dialysis, and the final concentration of the product [3]. Therefore, there is a need to study the application and optimization of alternative techniques that may increase the effectiveness of the purification process and, consequently, develop a low-cost separation process [4].

The possibility of applying aqueous two-phase systems (ATPS) to the purification and recovery of biomolecules represents an alternative to substitute conventional purification techniques and to develop hybrid processes coupling ATPS to classical separation and concentration techniques. Advantages of this technique include scale-up potential, continuous operation, ease of process integration, low toxicity of phase-forming chemicals, and biocompatibility [5]. This technique proved to be a powerful method to extract and separate soluble proteins from cell homogenates or whole broth [6]. In addition, excellent results have been obtained in the purification and recovery of many bioproducts, such as proteins, enzymes, and dyes [7–9]. ATPS can, therefore, be applied as a potential method in the early stages of the purification process of the enzyme β-galactosidase.

β-Galactosidase (EC 3.2.1.23), an enzyme that catalyzes the hydrolysis of lactose into glucose and galactose, is fundamental in the reduction of the lactose content of dairy products so that lactose-intolerant people can consume such products. Lactose hydrolysis also contributes to improvement in the technological and sensory characteristics of foods and in the formation of galactooligosaccharides, which are favorable to the human organism [10, 11]. However, the effective use of an enzyme requires recovery and purification techniques that do not compromise its viability.

Several procedures and techniques for the purification of β-galactosidase have been published. However, most available studies in the literature employ and evaluate a single sequence of purification and do not consider the peculiarities of the techniques compromising the efficiency of the process and hindering the scale-up procedure [12–15]. Furthermore, it is necessary to take into account that an enzyme extracted from different sources results in enzymatic extracts with various characteristics, such as different concentrations and a variety of proteins and contaminants. Conseqeuntly, it is necessary to develop specific procedures for each source of the desired enzyme.

Considering these aspects, the design of a β-galactosidase purification process is investigated by means of different strategies based on the combination of an optimized ATPS condition and classic purification techniques, a low-cost alternative to maximize the purity and recovery of the target enzyme.

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2 Material and Methods

2.1 Chemicals

Polyethylene glycol (PEG, molecular mass 4000, 6000, and 8000 g mol^{-1}), potassium phosphate, ammonium sulfate, and yeast extract were purchased from Synth (São Paulo, Brazil). The Nessler reagent was supplied by Merck (Darmstadt, Germany). The polyethersulfone membranes were from Millipore (Billerica, USA). Glucose, yeast extract, agar, and lactose were obtained from Vetec (Rio de Janeiro, Brazil). Peptone and malt extract were purchased from Himedia (Mumbai, India). The anion-exchange resin (Q Sepharose Fast Flow) was from GE Helthcare (Uppsala, Sweden). All reagents were of analytical grade.

2.2 Microorganism

The microorganism *Kluyveromyces marxianus* CCT 7082 was obtained from the Laboratory of Bioprocess Engineering, State University of Campinas (UNICAMP, Campinas, SP, Brazil) and maintained at 4 °C in YM agar slants containing (in g L^{-1}): malt extract (3.0), yeast extract (3.0), peptone (5.0), glucose (10.0), and agar (20.0) [16].

2.3 Inoculum

The inoculum was prepared in 500-mL Erlenmeyer flasks with 150 mL culture medium containing (in g L^{-1}): yeast extract (1.0), $(NH_4)_2SO_4$ (1.2), KH_2PO_4 (5.0), $MgSO_4{\cdot}7H_2O$ (0.4), and lactose (10.0) in 0.2 mol L^{-1} potassium phosphate pH 5.5. The culture conditions were 30 °C and 180 rpm for 24 h [17].

2.4 Submerged Cultivation

The inoculum was added to the cultivation medium in a 10 vol % proportion. The medium contained (in g L^{-1}): yeast extract (17.0), $(NH_4)_2SO_4$ (8.8), KH_2PO_4 (5.0), $MgSO_4{\cdot}7H_2O$ (0.4), and lactose (28.2) in 0.2 M potassium phosphate buffer pH 6.0. Fermentation was carried out at 30 °C and 180 rpm for 96 h [16].

2.5 Enzyme Extraction

The enzyme was extracted by adding 1.1 g glass beads with 0.6–0.8 mm diameter to each mL of cell suspension and stirred in a vortex mixer for 40 min. The cell extract was clarified by centrifugation at 4700 × *g* and 4 °C for 10 min [18] and suspended in a 50 mmol L^{-1} potassium phosphate buffer pH 6.6 [3].

2.6 Aqueous Two-Phase Systems

2.6.1 Screening of Variables

The ATPS was prepared in graduated centrifuge tubes by mixing suitable amounts of PEG (molar mass 4000, 6000, and 8000 g mol^{-1}), stock solution of potassium phosphate (pH 6.0, 7.0, and 8.0), and deionized water. The tube contents were homogenized and the system was maintained at 4 °C. The enzyme extract was added at a final concentration of 20 % to the pH of each specific system by weight and the system homogenized and centrifuged for 10 min at 4700 × *g* and 4 °C. The stock solution of potassium phosphate and the enzyme extract had a specific pH for each system to ensure the desired pH. After phase equilibrium, the phase volumes were measured and the phases were separated. The top and bottom phases were assayed for enzyme activity and total protein, and the purification factor and the enzyme recovery were monitored as the responses. The selection of compositions of the systems (Tab. 1) was based on Silva's previous study [19].

2.6.2 Optimization of ATPS

After screening the variables, the composition of the ATPS was optimized by a 2^2 central composite rotatable design (CCRD) with eleven runs, i.e., four factorial, four axial, and three central points. Two independent factors, namely potassium phosphate concentration (X_1) and PEG 4000 concentration (X_2), were investigated. The range under study (Tab. 2) was based on the screening results and on the phase diagrams (Fig. 1). The purification factor and the enzyme recovery were monitored as responses. The statistical significance of the regression coefficients was 95 %. Statistica 6.0 software (StatSoft Inc., USA) was used for the regression and graphical analysis of the data obtained by the experimental design. The validation of the results was assessed by analysis of variance (ANOVA).

2.7 Design of a Purification Strategy by ATPS

The optimized ATPS was compared to classic purification techniques. It also included different purification strategies, such as ammonium sulfate, ion-exchange chromatography, ultrafiltration, and diafiltration.

2.7.1 Precipitation

The enzyme was precipitated by adding sufficient ammonium sulfate so that concentration equivalent to 70 % saturation could be reached at 4 °C. After precipitation, the sample was maintained at 4 °C for 14 h and then centrifuged at 4700 × *g*, 4 °C, for 10 min. The precipitate was resuspended in 50-mmol L^{-1} potassium phosphate pH 6.6 containing 0.1 mol L^{-1} manganese chloride [20].

2.7.2 Ion-Exchange Chromatography

Ion-exchange chromatography was carried out at 4 °C by a C10/20 column (GEHelthcare) packed with a Q Sepharose Fast Flow anion-exchange resin. The resin was equilibrated with 25-mmol L^{-1} potassium phosphate buffer pH 7.5 and the column was fed with the enzyme extract. The system was operated under

Table 1. β-Galactosidase purification factor (*PF*), recovery (% *R*), partition coefficient (*K*), and volume ratio (*V*) for the systems using PEG 4000, 6000, and 8000 at pH 6.0, 7.0, and 8.0.

PEG	pH	System	Composition [wt %]		Results of bottom phase			
			PEG	Phosphate	*PF*	% *R*	*K*	*V*
4000	6.0	1	12	12	1.3	110.9	0.01	1.3
		2	19	12	1.7	90.9	0.01	1.7
	7.0	3	12	12	1.8	117.7	0.01	1.0
		4	19	12	3.3	92.6	0.01	1.4
	8.0	5	12	12	1.4	93.0	0.01	0.8
		6	19	12	1.8	70.0	0.02	1.2
6000	6.0	7	12	12	1.3	114.7	0.01	1.1
		8	19	12	1.8	95.5	0.01	1.7
	7.0	9	12	12	2.1	119.8	0.01	0.9
		10	19	12	2.4	92.2	0.01	1.4
	8.0	11	12	12	1.3	85.9	0.01	0.8
		12	19	12	3.1	89.3	0.02	1.1
8000	6.0	13	14	16	1.6	90.3	0.06	0.8
		14	19	12	2.2	61.0	0.04	1.8
	7.0	15	14	16	2.9	101.3	0.01	0.6
		16	19	12	2.2	90.6	0.01	1.3
	8.0	17	14	16	1.8	68.7	0.06	0.6
		18	19	12	2.2	77.4	0.01	1.2

Table 2. Factors and value levels used in the central composite rotational design (CCRD).

Factors	Coded levels				
	(−1.41)	(−1)	(0)	(+1)	(+1.41)
Potassium phosphate (X_1) [wt %]	12	12.9	15	17.1	18
PEG (X_2) [wt %]	10	11.2	14	16.8	18

the following conditions: feed rate 20 cm h^{-1}, 10 °C, and pH 7.5. The washing solution was 25 mmol L^{-1} potassium phosphate buffer, pH 6.5; pre-elution: 0.1 mol L^{-1} potassium chloride, pH 6.5; elution: potassium chloride, pH 6.5, with a concentration gradient from 0.1 to 1 mol L^{-1} with 30-fold the bed volume.

2.7.3 Ultrafiltration (UF)

The UF runs were carried out in a stirred dead-end cell with 200 mL with a 50-kDa UF membrane (polyethersulfone; Millipore PBQK 15005). The system was operated at 1.5 kgf cm^{-2} and 4 °C with a suitable pH for each step in the purification process. The enzyme activity and the protein content of the feed, retentate, and permeate were assayed for each batch to determine the purification factor and the enzyme recovery.

2.7.4 Diafiltration

Diafiltration was carried out by the same UF apparatus. The diafiltration solution was 50 mmol L^{-1} potassium phosphate buffer at a suitable pH for each case. The end of the diafiltration was determined by following the complete removal of the ammonium sulfate by adding some drops of Nessler reagent to the permeate [20] up to the point that there was no color.

2.8 Determination of the Performance of the Purification Procedure

The performance of the enzyme purification procedure was evaluated by the following parameters: purification factor, recovery, coefficient partition, and volume ration. The purification factor (*PF*)[1)] was calculated by the ratio between the specific activity (U mg^{-1}) of the enzyme in the phase obtained after

1) List of symbols at the end of the paper.

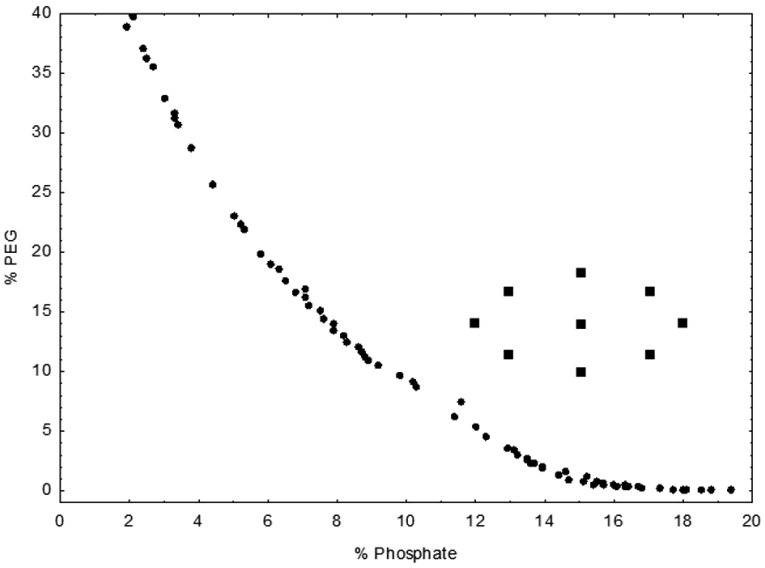

Figure 1. Phase diagram (PEG 4000, pH 7.0) and composition points of the ATPS.

the purification step and the specific activity ($U\,mg^{-1}$) in the feed phase of the purification step. The specific activity is defined as the ration between the activity ($U\,mL^{-1}$) and the total protein concentration ($mg\,mL^{-1}$). The recovery (%*R*) of the process was defined as the ratio between the total enzyme activity (*U*) in the phase obtained after the purification step and the total activity (*U*) fed to the system [9]. The partition coefficient (*K*) is the activity ($U\,mL^{-1}$) of the enzyme found in the top phase by the activity of the enzyme ($U\,mL^{-1}$) in the bottom phase [21]. The volume ratio (*V*) was calculated by the ratio between the volume (mL) of the top phase and the volume (mL) of the bottom phase [22]. The uncertainties of the measured values were below 3 % on average.

2.9 Analytical Methods

2.9.1 Enzyme Activity and Total Protein

The enzyme activity was determined by using *o*-nitrophenyl-galactopyranoside as the substrate, in agreement with the method proposed by Inchaurrondo et al. [23]. One unit of enzyme activity (*U*) is defined as the amount of enzyme necessary to yield 1 µmol of *o*-nitrophenol per minute at 37 °C and pH 6.6. The total protein content was assayed by the method proposed by Bradford [24], who used bovine serum albumin as the standard.

2.10 Statistical Analysis

The Statistica 6.0 software (StatSoft Inc., USA) was applied for the regression and graphical analysis of the data obtained in the experimental design whereas the validation of the results was assessed by ANOVA.

3 Results and Discussion

3.1 ATPS

The effect of the different parameters on the purification and recovery of the enzyme are indicated in Tab. 1. A clear correlation between the molar mass of PEG and the partition coefficient was not evident. The enzyme was always partitioned into the bottom phase, which is rich in potassium phosphate. Thus, all values for *K* were much lower than 1, showing its affinity to the salt-rich phase and suggesting that hydrophilic interactions were dominant in this system [25]. These results could also be a consequence of the use of high molar masses and concentrations of PEG, a fact that could increase the repulsive forces between the PEG and the enzyme, forcing its partition into the bottom phase [26].

The highest purification factors occurred in systems with high PEG concentrations, regardless of its molar mass or the pH value of the system, except for pH 7.0 PEG 8000 kDa. The higher PEG concentration induced an increase in the ratio among the volumes of the phases, resulting, in general, in an increment of the *PF*. However, an inverse trend was observed for enzyme recovery, which decreased as the PEG concentration increased. Typically, when the volume ratios were high, the volume of the bottom phase was low, tending to decrease the partition of the proteins into this phase. However, even a small bottom phase volume did not prevent the partition of the enzyme from happening into the bottom phase. This effect might have influenced the partition of the contaminant proteins, resulting in enhanced purification. The partition of the enzyme into the phase with a lower volume reduced the volume to be treated in the subsequent purification steps, favoring the concentration and purification processes.

High purification factors were found at pH 7.0 and 8.0 (systems 4, 12, and 15) with recoveries ranging from 90 to 120 %. Since this enzyme is more stable between pH 6.6 and 7.0 [27], the latter is of interest for purification by ATPS to avoid enzyme denaturation. The enzyme recoveries above 100 % obtained in systems 1, 3, 7, 9, and 15 can be explained by the removal of inhibitors of the enzyme activity or even due to the presence of salt [28] and PEG, which may help to keep the protein in the active form. This behavior has frequently been reported by other authors who purify enzymes using ATPS [5, 9, 29]. It also justifies the viability and performance of ATPS in the recovery and purification of the enzyme.

The purification factors obtained in these systems were equivalent to those from other authors. Silva et al. [30] studied the purification of β-galactosidase from *Kluyveromyces lactis* by ATPS (PEG-phosphate-dextran) and obtained 3.9 and 83 % for the *PF* and recovery, respectively, adding NaCl to the system and a biospecific ligand, which could have impaired the scaling-up of the process. Silva and Franco [31] described the purification of β-galactosidase from *Kluyveromyces fragilis* using a PEG/dextran system and reached a 2.8-fold *PF* value and 57 % enzyme recovery.

The previous results helped to define the factors and ranges to be investigated in the optimization of the enzyme purification and recovery by ATPS. The experimental design was carried out with PEG 4000 and potassium phosphate at pH 7.0, since this combination yielded the best results in the previous step and is close to the stable pH range of the enzyme. Furthermore, lower molecular weights are easier to work with in separation processes using membranes. Fig. 1 shows the compositions of the ATPS under study in this experimental design [32]. The responses of these experiments were the *PF* and enzyme recovery. Results are summarized in Tab. 3, as well as the purification factors ($PF_{predicted}$) and recoveries ($\%R_{predicted}$) predicted by the model and the respective relative deviations ($D_{relative}$) by comparison with the experimental model.

ANOVA was carried out for the validation of the empirical model obtained for the PF and recovery of the enzyme. Correlation coefficients (R) of 0.94 and 0.93 were determined for the purification factor and recovery, respectively (Tab. 4), showing that the model fitted the data satisfactorily. The ratio $F_{calculated}/F_{listed}$ was 7.1 and 6.5 for the *PF* and enzyme recovery, respectively, validating the coded models shown in Eqs. (1) and (2). The models, linear and quadratic for the recovery and purification factor, respectively, were used to build the contour curves in Fig. 2.

$$PF = 3.8 - 1.16X_1^2 - 1.08X_2^2 \quad (1)$$

$$\%R = 93.3 - 15.2X_1 - 17.5X_2 \quad (2)$$

The contour curves demonstrate that the best condition for enzyme purification was achieved with 14 % PEG and 15 % potassium phosphate, yielding *PF* values ranging from 3.2 to 4.2 and recoveries between 95 and 105 %. Changes in the concentration of PEG and the buffer caused a decrease in the *PF*. The highest recoveries of the enzyme were obtained at the lowest PEG and potassium phosphate concentrations.

Table 3. Optimization of enzyme purification and recovery using PEG 4000/potassium phosphate buffer pH 7.0.

Systems	[wt %]		*PF*	$PF_{predicted}$	$D_{relative}$	% *R*	$\%R_{predicted}$	$D_{relative}$
	PEG	Phosphate						
1	11.2	12.9	1.6	1.7	5.8	128.5	126.1	1.9
2	16.8	12.9	1.5	1.8	20.0	91.4	95.6	5.6
3	11.2	17.1	1.5	1.3	15.3	92.7	90.6	2.3
4	16.8	17.1	1.4	1.4	0.0	55.0	60.5	10.0
5	10	15	1.4	1.4	0.0	111.2	114.7	4.1
6	18	15	1.7	1.5	11.7	77.2	71.8	7.0
7	14	12	2.3	1.9	17.4	107.4	118.0	9.9
8	14	18	1.1	1.3	18.2	59.3	68.5	15.5
9	14	15	4.2	3.8	9.5	105.0	93.3	11.1
10	14	15	3.2	3.8	18.75	94.7	93.3	1.5
11	14	15	4.1	3.8	7.31	105.4	93.3	11.5

The relative error deviation (*RED*%) was calculated by: $RED\% = \left[\frac{Y_{experimental} - Y_{model}}{Y_{experimental}}\right] \times 100$ where Y is the parameter under analysis.

Table 4. Results of ANOVA for the purification and recovery of enzyme.

Source	Sum of squares		Degrees of freedom		Mean squares		*F*-test [a]	
	PF	% *R*	*PF*	% *R*	*PF*	% *R*	*PF*	% *R*
Regression	10.9	4303.5	2	2	5.43	2151.7	31.94	29.2
Residues	1.4	589.2	8	8	0.17	73.65		
Lack of fit	0.7	514.9	6	6				
Pure error	0.7	74.3	2	2				
Total	12.3	4892.7	10	10				

[a] *F*-test (regression/residues).
PF, regression coefficient: $R = 0.94$; $F_{0.95;\,2,8} = 4.46$; *R* %, regression coefficient: $R = 0.93$; $F_{0.95;\,2,9} = 4.46$.

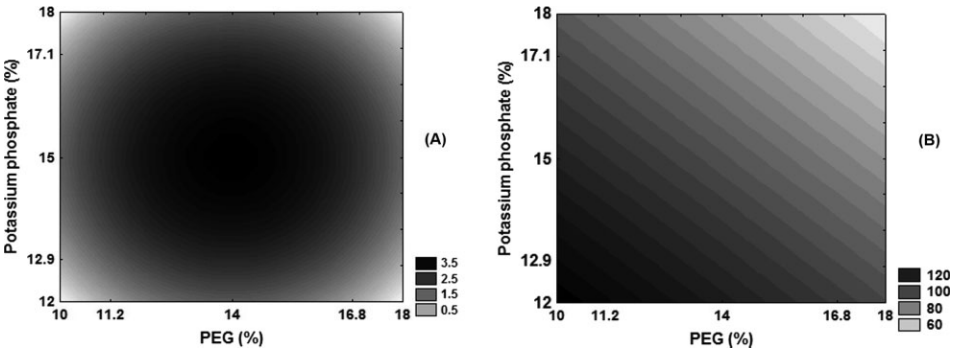

Figure 2. Contour curves obtained for the purification factor (A) and recovery (B) of enzyme.

3.2 Design Strategies for Integrated β-Galactosidase Purification

The possibility of integrating ATPS into the recovery and purification of enzymes is a good opportunity for the industrial use of such a process. The downstream processing strategies are illustrated in Fig. 3 as well as the results of *PF* and % *R* of each step and the results of the overall sequence.

Results showed that the performance of the ATPS, i.e., designs 3 and 4, differed from the previous results with a PF of up to 4.3 and recovery of up to 105.4 %. The enzymatic extracts used in the design were different from those applied for optimization which suggests that the use of enzyme extracts obtained from different cultivation batches might have various compositions and concentrations (mg mL^{-1}) of protein, cells, enzyme activity (U mL^{-1}), and other biological materials. It may explain the divergence in the results since the partition of biomolecules is highly influenced by the composition of the enzyme extract. Thus, the performance of an enzyme purification process is difficult to predict [33], but it does not invalidate the optimization, which was carried out with the same enzyme extract.

The purification techniques have various principles and advantages which make them viable in the β-galactosidase purification. Design 1 involves only precipitation and ultrafiltration. Precipitation occurs by increasing the ionic strength of the system, i.e., the water molecules interact more strongly with ions from the dissociation of salt, thus promoting the dehydration of the proteins. During this process, the inter-protein interaction becomes stronger, decreasing the solubility in an aqueous medium and, consequently, protein precipitation occurs [34]. Ultrafiltration is based on the difference in size of macromolecules; its main advantage is its high product yield. Furthermore, it does not require the use of additives or phase changes and can be done isothermally at low temperature and pressure, with lower energy consumption, thus ensuring minimal denaturation, inactivation, and/or degradation of byproducts that are highly unstable [35–37].

In design 2, ion-exchange chromatography after precipitation and ultrafiltration was employed. Ion-exchange chromatography has the higher purification factor and is a technique suited for separation of charged molecules. Chemically, ion exchange involves the exchange of solutes of like charge from a

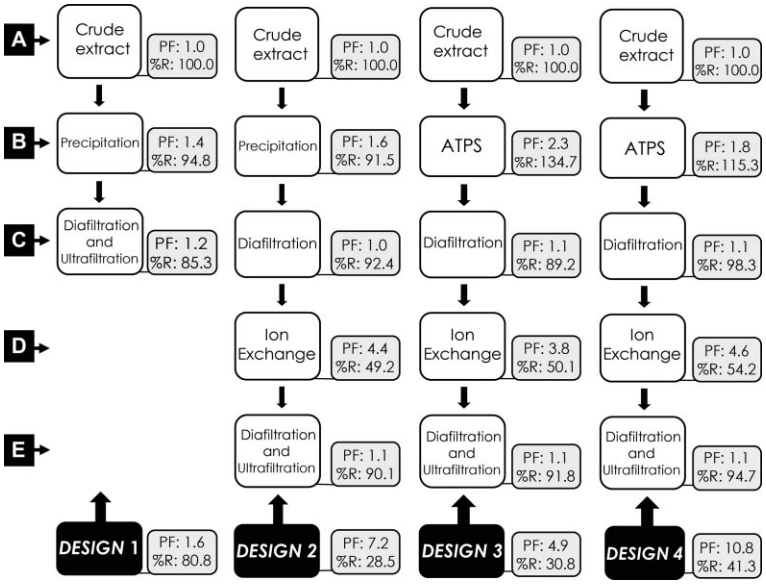

Figure 3. Schematic diagram of the sequences under study for the enzyme purification with four sequences of different processes. The *PF* and the recovery of the enzyme for each step and for the global sequence are also included.

solid support bearing the opposite charge (adsorbent). The mixture enters in the chromatographic column with the mobile phase and each individual component is adsorbed by the system at different rates depending on its interaction with the stationary phase and the mobile phase flow [38].

In design 3 and 4, the APTS was used instead of precipitation. When the application of ATPS in sequences 3 and 4 was compared with that of ammonium sulfate precipitations in sequences 1 and 2 as an initial step in the purification strategy, the use of ATPS proved to be advantageous, considering that (i) it presented higher *PF* and a clearly higher recovery due to the removal of metabolites that inhibited the enzyme activity, and (ii) the processing time was reduced.

The usage of diafiltration and ultrafiltration did not provide any increment in enzyme purity. The *PF* values in this step were 1.0- to 1.2-fold, although the diafiltration/ultrafiltration step led to a small reduction in the recovery of the enzyme. These values could be due to similarities in the molar masses of the enzyme and the contaminant proteins. On the other hand, the contaminants might even be rejected by the dynamic membrane formed by the fouling layer on the membrane surface [39]. However, ultrafiltration was effective in the concentration steps, up to 8.0-fold (data not shown), and was helpful in reducing the working volume and removing the salts using ion exchange in sequence.

Ion-exchange chromatography had the highest enzyme losses, but also the highest *PF* values in the sequences. The reason for the good performance of this technique was related to its high selectivity since the separations were based on the net charge of the biomolecules.

Even though it is fundamental to optimize the purification techniques separately when they are used in a process, it is essential to establish some operational adjustments during the integration of the unit operations under study, when developing a purification strategy for an enzyme. Although the adjustments may represent losses, mainly by not working at optimum pH in each step, they can represent significant overall gains throughout the whole process. Ignoring these adjustments during the development of designs can compromise or even ruin a process, especially regarding the scale-up.

It can be visualized in designs 3 and 4 since they have the same sequences of purification techniques. In design 3, the steps were carried out at the optimum pH value for each separate step. In design 4, the steps were performed at the pH for maximum stability of the enzyme (pH 6.6). This adjustment in the pH value (pH 6.6) of the process caused an increase of about 34 % in enzyme recovery and of 220.4 % in the purification factor, due to the absence of enzyme denaturation. Thus, a reduction in the loss of the target molecule in the purification process is critical to ensure the viability of the process, but it should always be associated with a high purification factor. It was achieved in purification sequence 4, which indicated high overall recovery by application of an adjusted purification method, which reduced the losses in the final purification sequence.

To the best of our knowledge, no previous reports have been published on the design of a purification strategy for β-galactosidase from microorganisms. Former studies on the purification of this enzyme focused on highly selective techniques with the final purpose of enzyme characterization; however, they were not concerned with the final enzyme recovery [12–15]. Therefore, most studies of bioproduct purification evaluate each step separately; besides, they consider enzymes that are not obtained through a biotechnological process. Consequently, the development of a specific purification process for each enzyme should be taken into account, since for each source a different strategy is required in order to achieve the highest purification factor and recovery in a process that enables a scale-up, because contaminants may be different.

4 Conclusions

The feasibility of using ATPS instead of a conventional primary step in the purification strategy and the possibility of its coupling to different purification techniques is demonstrated. This system proved to be suitable for the recovery and primary purification of β-galactosidase since it yields average recoveries of 101.7 % and a PF of 3.8 under optimized conditions.

The purification strategy design resulted in a powerful integrated purification and recovery process, proving its potential for a scale-up of the β-galactosidase purification process. It led to a potential recovery of 41.3 % from a sequence consisting of ATPS, diafiltration, ion exchange, and diafiltration/ultrafiltration and a 10.8-fold increase in purity.

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Symbols used

K	[–]	partition coefficient
PF	[–]	purification factor
R	[%]	recovery
V	[mL]	volume ratio
X_1	[%]	potassium phosphate concentration
X_2	[%]	PEG concentration

Abbreviations

PEG	polyethylene glycol
UF	ultrafiltration

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Research Article: As an alternative to the usual primary purification steps, a strategy for β-galactosidase was established from purification methods amenable to scale-up, allowing the application of the enzyme in enzymatic hydrolysis processes. The proposed process design involving aqueous two-phase system, diafiltration, ion exchange, and diafiltration/ultrafiltration led to a high purification factor and recovery of the enzyme.

Design Strategies for Integrated β-Galactosidase Purification Processes

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