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Journal of Chromatography B, 835 (2006) 77–83
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Direct comparison between ion-exchange chromatography and aqueous two-phase processes for the partial purification of penicillin acylase produced by *E. coli*☆

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Received 5 January 2006; accepted 6 March 2006
Available online 29 March 2006

Abstract

A direct comparison of a chromatography and an aqueous two-phase system (ATPS) processes for the partial purification of penicillin acylase (PA) produced by a recombinant strain of *E. coli*, was performed. An established chromatography process for the recovery of PA was selected as a model system and characterised for comparison with a developed ATPS prototype process. PEG-phosphate systems were selected for the recovery of PA over PEG-citrate systems, since higher enzyme recovery and increased purity was obtained. ATPS proved to be suitable to process highly concentrated disrupted extract (35%, w/w) and maintain a high top phase enzyme recovery. In the direct comparison of the processes, the superiority of the ATPS approach was highlighted since a reduction of the number of unit operations from 7 to 4 was achieved. An outline economic analysis based on the cost of separation agent of the processes favour the ATPS process, in which a gross cost reduction of 37% (from $0.47 to $0.30 USD) was achieved. Such result was obtained considering a potential re-use of up to 100 times of the resin used in the chromatography process. Additionally, by assuming that all the unit operations are equivalent in investment and operating cost, further reduction of approximately 43% of the respectively involved cost can be obtained when the ATPS process is used. Overall, the proposed ATPS process comprising of PEG1450-phosphate, tie-line length (TLL) of 48.5% (w/w), volume ratio (Vr) of 1.0, pH of 7.0 and 35% (w/w) PA crude extract loaded into the system proved to be more efficient, recovering 97% of PA at the top phase (PEG rich phase) with a purity factor of 3.5. It is clear that the results reported herein raise the consideration for the potential substitution of the chromatography process for PA recovery from *E. coli* as a first step for the development of an optimised and economic process with evident commercial application.
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Keywords: Penicillin acylase recovery; Process comparison; Aqueous two-phase systems

1. Introduction

The widespread interest to rapidly and economically bring new biotechnological products to market using scaleable and efficient technology is evident. The large number of biopharmaceuticals approaching the end of their patent protection period will encourage manufacturers to seek competitive advantages through bioprocess technologies that have not been extensively exploited at commercial scale [1]. A typical process route for the primary recovery of intracellular protein products involves the use of conventional operations of cell disruption, centrifugation and ultrafiltration [2]. Purification is then frequently achieved by multiple chromatography steps [3]. The multi-step nature of conventional purification processes for biotechnological products together with the large amount of stainless steel involved is viewed as a significant disadvantage. To overcome some of the disadvantages attributed to the established biotechnological processes for protein production, different approaches have been proposed. Such approaches involved the reduction of the chromatography steps [4] or potential substitution of these steps by alternative recovery techniques such as aqueous two-phase systems (ATPS) [5]. The success of process intensification

☆ Presented at the International Conference on Biopartitioning and Purification, The Netherlands, 20–24 June 2005.

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doi:10.1016/j.jchromb.2006.03.016

achieved, at prototype processes scale, for the recovery of proteins in ATPS support the consideration of this technology as an alternative to well-established methodologies [5]. In a formal comparison of two different technologies, technical and economical aspect needs to be considered. However, detailed economic analyses of biotechnological processes are not common [1,6,7]. In particular, the need of reports addressing the direct comparison of established and alternative processes is evident [8].

In the present research the recovery of penicillin acylase (PA), produced by a recombinant strain of *E. coli*, was selected as an experimental model system to address the direct comparison – from the technical and economical point of view – of a chromatography based process with an ATPS process in order to evaluate the potential benefits of each process. A well-established chromatography process for the recovery of PA [9] was characterised for comparative purposes. For the case of the ATPS, the recovery of PA using this technique has been addressed before [10–13]. In this research, the successful development of a prototype process for the recovery of PA produced by recombinant *E. coli* was achieved based upon these previous studies [10–13]. Also, potential process intensification was attempted by evaluating the effect of increasing concentration of PA crude extract from *E. coli* cultures upon ATPS performance. Furthermore, a direct comparison between the two types of processes was performed taking into account enzyme purity and overall process recovery as selection criteria. Besides, economic analysis involved the costs of each unit operation and the costs of separating agents are presented here. It is clear that this type of reports is needed to establish the potential benefits of alternative technology over established one for the recovery of biological products, in particular the recovery of PA from *E. coli*.

2. Materials and methods

2.1. Production of penicillin acylase extract

Production of penicillin acylase extract was obtained by fermentation as described before [14]. Briefly, *E. coli* ATCC 9637 with penicillin acylase activity was grown in a 14 L fermenter with 10 L of BSG supplemented medium at pH 7, 370 rpm and 29 °C for 10–12 h. After a maturation time of 8 h [15], cells were concentrated by centrifugation at 12,000 × *g* for 10 min. Cells were resuspended in a pH 7 phosphate buffer (0.1 M) then disrupted in a Malton Gaulin mill at 1.0 L/min (500–580 kg/cm^2) for 15 min to obtain enzymatic crude extract and then stored at 4 °C.

2.2. Enzyme purification

The crude enzymatic extract was further purified by precipitation and ion-exchange chromatography following the protocols reported before by Kutzbach and Rauenbusch [16] and Rodriguez et al. [9], respectively. Briefly, the enzyme was recovered from the crude extract by ammonium sulphate precipitation. The 40–60% fraction was dissolved in potassium phosphate buffer (pH 5.8, 0.015 M) and dialysed against the same buffer. The enzyme was then applied to a CM Sepharose column previously equilibrated with the same potassium phosphate buffer (pH 5.8, 0.015 M). Enzyme was washed with the same buffer and desorbed with potassium phosphate buffer (pH 7.8, 0.05 M). Samples after each process step (i.e. cell disruption, precipitation and chromatography) were carefully taken for biochemical analysis. For the alternative purification process, aqueous two-phase systems were used. ATPS were prepared for convenience on a fixed mass basis. Predetermined quantities of stock solutions of polyethylene glycol (PEG; Sigma, St. Louis, MO, USA) and potassium phosphate or sodium citrate were mixed with different concentrations of crude enzymatic extract (from 10% to 40%, w/w) to give a final determined weight of the systems (i.e. 10 and 50 g). The system parameters for PEG-phosphate and PEG-citrate ATPS were selected based upon previous reports [11,17]. The stock solutions (PEG, salt and crude enzymatic extract) were mixed and phases dispersed by gentle mixing for 10 min. Adjustment of pH was made by addition of orthophosphoric acid or sodium hydroxide when needed. Complete phase separation was achieved by low speed batch centrifugation at 1500 × *g* for 10 min. Measurement of the volumes of top and bottom phases were visually made in graduated tubes. The volumes of the phases were then used to estimate the experimental volume ratio (Vr, defined as the ratio between the volume of the top phase and the bottom phase). The system tie-line length (TLL), which represents the length of the line that connects the composition of the top and bottom phases of a defined ATPS, was estimated as described by Albertsson [18]. Samples were carefully extracted from the phases for biochemical analysis. The top phase recovery was estimated as the amount of enzyme present in the phase (volume of the phase × enzyme activity in the phase) and expressed relative to the original amount loaded into the system. Results reported are the average of three independent experiments and standard errors were judged to be ±10% of the mean value.

2.3. Analytical procedures

Protein concentration was determined by the method of Bradford [19]. Penicillin acylase activity was estimated by the *p*-dimethylaminobenzaldehyde method reported by Balasingham et al. [20]. One unit of activity is defined as the amount of enzyme producing 1 μmol of 6-aminopenicillanic acid (6-APA) per minute.

3. Results and discussion

In order to define the best ATPS extraction conditions for the recovery and purification of PA, a practical approach which exploited previous reports concerning the partial purification of PA was used. These reports [10,11] described the potential recovery and partial purification of PA exploiting PEG-citrate aqueous two-phase systems. ATPS characterised by the use of two molecular weight of PEG (e.g. 1000 and 3350 g/mol) were identified as potential candidates for the purification of PA. In these systems several parameters were evaluated that included; system pH, increase in TLL, molecular weight of PEG, sys-

Table 1
Influence of increasing volume ratio upon the recovery of penicillin acylase (PA) from PEG/citrate ATPS

System	Molecular weight of PEG (g/mol)	System volume ratio (Vr)	Top phase PA purification factor	Top phase PA recovery (%)
I		0.38 ± 0.04	1.5 ± 0.10	90 ± 5.0
II	1000	0.96 ± 0.1	1.4 ± 0.10	95 ± 5.0
III		2.1 ± 0.21	1.0 ± 0.10	97 ± 5.0
IV		0.38 ± 0.04	0.8 ± 0.08	15 ± 1.5
V	3350	0.68 ± 0.07	0.5 ± 0.05	40 ± 4.0
VI		1.63 ± 0.16	0.6 ± 0.06	20 ± 2.0

Volume ratio (Vr) estimated from non-biological experimental systems was determined after phase separation in graduated tubes. Top phase purification factor for penicillin acylase (PA) is the ratio between specific enzyme activity obtained at the top phase of the system and the one from the crude extract of *E. coli* homogenate. The top phase PA recovery is expressed relative to the original amount of PA from crude extract loaded into the systems. For all PEG-sodium citrate systems of 10 g, the pH was kept constant at 7.0.

tem volume ratio and the addition of sodium chloride (NaCl). Partition behaviour of PA under these systems parameters was discussed [10,11]. However, the report exploiting the change in system volume ratio upon PA partition behaviour [10] did not fully addressed the impact of Vr on product purity and the establishment of optimal process conditions were not achieved. Thus, it was decided to further evaluate the impact of system Vr upon the partition behaviour of PA in PEG-citrate ATPS from a practical perspective that considers the enzyme recovery and purity. Furthermore, it was also decided for the purpose of the objective of this work to characterise a process based upon ATPS extraction in our laboratories.

In the selected ATPS (e.g. PEG1000-citrate and PEG3350-citrate), an increase in the system Vr caused a significant decline of the purification factor of PA (see Table 1). It has been proposed [17,21] that the protein partition behaviour remains constant for systems along the same TLL. Such proposal may be extended for the behaviour of PA in ATPS along the same TLL. Changes in the enzyme partition behaviour and purity (expressed as purification factor) with Vr can be attributed to a concentration effect. An increment in Vr implies an increase of the volume of the top phase. Consequently, the PA in this phase will dilute further and as a result a decrease in the top phase specific enzyme activity (and enzyme purification factor) is possible. Although, an increase in the volume of the top phase will also have a dilution effect on the contaminant proteins, it seems that such effect was either smaller than that of the target product (i.e. PA) or caused a possible partial inactivation of the enzyme, which in both cases resulted in a reduction on the enzyme purification factor. In the case of top phase PA recovery, it is evident that PEG-citrate ATPS characterised by low molecular weight of PEG (i.e. 1000 g/mol) exhibited the highest recovery (>90%), compared with that from the ATPS of higher molecular weight of PEG (i.e. 3350 g/mol; recovery less than 40%, see Table 1). Such low enzyme recovery obtained may be caused by protein aggregation or PA partition in the bottom phase which could lead to mass loss of PA in the upper phase. As a result purification factor less than one were obtained. Although, enzyme recovery above 90% were obtained from the ATPS of PEG1000-citrate as in the case of the previous results reported by Marcos et al. [10,11], it was observed that changes in volume ratio caused no benefits in the purity of PA (purification factors equal and less than 1.5 were obtained; Table 1), thus it was decided to explore alternative ATPS to further optimise the process for PA recovery.

The PEG-phosphate systems are better studied than the PEG-citrate. Consequently, the potential use of this type of system for the recovery and purification of PA was explored. Previously, the potential application of PEG-phosphate ATPS for the recovery of PA has been reported exploiting modified and unmodified PEG [12,13]. These studies concluded that the use of ATPS with modified PEG together with ultrafiltration provided a good method of purifying PA [12,13]. For the purpose of the present study, the impact of increasing TLL upon PA top phase recovery, when PEG of four different molecular weight (i.e. 600, 1000, 1450 and 3350 g/mol) were used, is illustrated in Table 2. For all these systems, volume ratio and system pH were kept constant at 1.0 and 7.0, respectively. It is clear that increasing TLL caused the PA purification factor from the top PEG rich phase of PEG600 to remain relatively constant or slightly decrease (i.e. from 2.2 to 2.0). In contrast, ATPS of PEG1000, PEG1450 and PEG3350 exhibited an increase in the top phase PA purification factor when TLL increased. In this case purification factors equal to and above 4.0 were obtained for PEG1000 and PEG1450 systems (see Table 2). Such behaviour may be explained by the effect of the possible increase or decrease of the contaminant proteins in the top phase caused by the rise in the TLL. The free volume in the bottom phase decreases when the TLL is increased [22] and as a result, the solutes in the lower phase may be promoted to partition to the top phase. Consequently, the increase of contaminant proteins that concentrate in the top phase with increasing TLL is possible and as a result the purity of the target product may be negatively affected. However, top phase purification factor of PA (and specific enzyme activity) was not affected by the potential increase in the contaminant proteins in the top phase, probably due to a higher increase in the enzyme activity in the upper phase with increasing TLL. From each molecular weight of PEG used, it was possible to obtain systems that resulted in a top phase PA recovery equal to or greater than 95%. Particularly, for ATPS of PEG600 and PEG1000, the top phase PA recovery remained relatively constant and above 95% when TLL increased. In contrast, ATPS of PEG1450 and PEG3350, top phase PA recovery increased from 76% to 97% and from 63% to 96%, respectively with increasing TLL.

Table 2
Influence of increasing TLL upon the purification factor and top phase recovery of penicillin acylase (PA) from PEG/phosphate ATPS

System	Molecular weight of PEG (g/mol)	TLL (%, w/w)	Top phase specific activity (U/mg)	Top phase PA purification factor	Top phase PA recovery (%)
1	600	32.8	10.9 ± 1.0	2.2 ± 0.1	95 ± 1.3
2		37.0	12.3 ± 1.0	2.4 ± 0.1	95 ± 1.3
3		41.2	8.4 ± 0.8	1.7 ± 0.1	98 ± 1.5
4		45.0	10.2 ± 1.0	2.0 ± 0.2	97 ± 1.0
5	1000	41.1	17.4 ± 1.3	3.4 ± 0.2	98 ± 1.0
6		44.2	16.3 ± 1.6	3.2 ± 0.2	97 ± 1.3
7		49.2	15.8 ± 1.6	3.1 ± 0.2	98 ± 1.0
8		62.0	20.3 ± 2.0	4.0 ± 0.2	98 ± 1.0
9	1450	40.5	14.4 ± 1.3	2.9 ± 0.2	76 ± 2.0
10		41.3	13.7 ± 1.2	2.7 ± 0.1	88 ± 2.4
11		48.5	21.3 ± 2.2	4.2 ± 0.2	97 ± 1.3
12		60.0	16.6 ± 1.3	3.3 ± 0.2	97 ± 1.0
13	3350	39.4	14.1 ± 1.4	2.8 ± 0.2	63 ± 5.3
14		46.6	16.7 ± 1.6	3.3 ± 0.3	73 ± 1.5
15		51.1	17.6 ± 2.1	3.4 ± 0.2	80 ± 1.0
16		54.8	18.0 ± 1.8	3.6 ± 0.2	96 ± 1.3

The tie-line lengths (TLL) of the systems were estimated from the composition of PEG and phosphate as described in Section 2. Top phase specific activity is expressed as the ratio of total enzyme activity (U) and total protein (mg) in the upper phase. The top phase PA recovery is expressed relative to the original amount of PA from crude extract loaded into the systems. Top phase purification factor for PA is the ratio between specific enzyme activity obtained at the top phase of the system and the one from the crude extract of *E. coli* homogenate. For all systems of 10 g, volume ratio (estimated from non-biological experimental systems) and the system pH were kept constant at 1.0 and 7.0, respectively.

PEG1000-phosphate and PEG1450-phosphate ATPSs characterised by TLLs of 62% (w/w) and 48.5% (w/w), respectively (systems 8 and 11 in Table 2) resulted in the maximum increase in the purity of PA. After ATPS, the specific enzyme activity of PA increased to 4.0 and 4.2 from systems 8 and 11, respectively. However, PEG1450-phosphate with a TLL of 48.5% (w/w) required a reduced amount of chemical forming phases compared with that from PEG1000-phosphate with a TLL of 62% (w/w). Thus, it was decided to select the PEG1450-phosphate ATPS for further evaluation. Furthermore, the potential economic benefits associated with a reduction in the cost of the chemicals, justified the selection of an ATPS with a reduced TLL. It is important to consider that the increase in purity achieved from the PEG1450-phosphate ATPS was superior compared to that reported for PEG4000-phosphate systems by Guan et al. [12,13]. Once the impact of increasing TLL upon PA purity and recovery from the top phase was evaluated, the effect of high concentration of the PA crude extract upon ATPS was investigated. The potential to fractionate heavily loaded biological systems by ATPS has been proved [23,24]. Such biological systems include cells, cell debris, RNA, virus-like particles and proteins. An increment in the level of PA crude extract concentration fractionated via ATPS may benefit the potential intensification of the proposed ATPS process.

The effect of the concentration of PA crude extract upon the enzyme top phase recovery and purification factor is shown in Table 3. Top phase recovery remained relatively constant and over 95%, regardless of the concentration of PA crude extract loaded to the ATPS. Such robust behaviour can be explained in terms of the excluded volume of the top phase of the system [22]. Partition of PA to the top-PEG rich phase in ATPS is favoured by using PEG of low molecular weight. Such behaviour has been attributed to the fact that the interaction net of short polymer chains generates a lower excluded volume than the one created by long chains. PEG of low molecular weight (for example, 1450 g/mol) helps to overcome saturations problems at the top phase, and therefore PA is able to migrate at the top phase. However, an increase in the concentration of PA crude extract in the system caused PA purification factor to decline (see Table 3). This can be explained in terms of the increase in the contaminant proteins associated with the increase in the concentration of the crude extract loaded to the ATPS. When the concentration of PA crude extract increments (from 10% to 40%, w/w), the amount of contaminants proteins presents in the system also increases. The proteins with a higher affinity for the top phase (because of their size, electrical charge, hydrophobicity, etc) occupied the free volume available at the top phase [22]. Once the particles with

Table 3
Influence of the concentration of penicillin acylase (PA) crude extract upon enzyme recovery and purification factor

System	Concentration of crude extract (%, w/w)	Top phase PA recovery (%)	Top phase PA purification factor
A	10	97 ± 2	4.2 ± 0.3
B	20	96 ± 3	3.9 ± 0.3
C	30	96 ± 3	3.8 ± 0.3
D	35	97 ± 2	3.5 ± 0.2
E	40	95 ± 3	2.8 ± 0.2

The top phase PA recovery is expressed relative to the original amount of PA from crude extract loaded into the systems. Top phase purification factor for PA is the ratio between specific enzyme activity obtained at the top phase of the system and the one from the crude extract of *E. coli* homogenate. ATPS of 10 g were used comprising PEG 1450-phospahte, TLL 48.5% (w/w), Vr = 1.0 and pH 7.0.

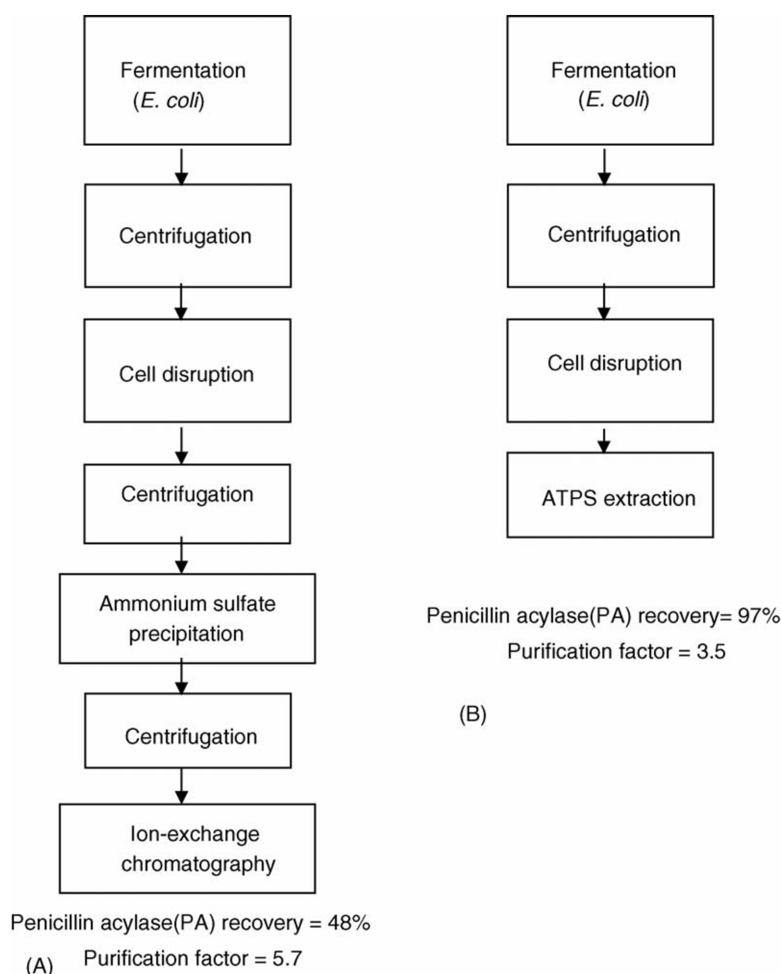

Fig. 1. Simplified comparison between ion-exchange chromatography (A) and aqueous two-phase processes (B) for the purification of penicillin acylase (PA) produced by *E. coli*. In the ion-exchange chromatography process, after cell harvesting, mechanical cell disruption and cell debris removal by centrifugation, a precipitation stage is included. Prior to the ion-exchange stage, the stream from centrifugation was dialysed to reduce the salt content. In contrast, in the ATPS process, after cell harvesting and mechanical cell disruption, the ATPS extraction stage was considered.

high affinity for the PEG rich phase have partitioned, molecules with lower affinity can migrate to the remaining free volume. The increment in the amount of contaminants proteins loaded to the systems causes a decrease in the free volume available for the target product. Thus, the PA purification factor at the top phase decreases from 4.2 to 2.8 (Table 3). In order to exploit the potential benefit of the increment in the level of PA crude extract concentration fractionated via ATPS over the intensification of the proposed ATPS process, system D (35%, w/w crude extract concentration in ATPS) from Table 3 was selected for a direct comparison with an ion-exchange chromatography process. The selected ATPS comprised a PEG molecular weight of 1450 g/mol, TLL of 48.5%, Vr = 1.0, system pH of 7.0 and produced a PA recovery of 97% and an increase in purity of 3.5-fold.

The chromatography process for the purification of PA has been well characterised and used frequently at the pilot plant facilities of one of the laboratories involve in this research (Institute of Biotechnology, Mexico). Thus, process conditions previously defined were used without further optimization in this work. This purification process involves a total of seven unit operations that include the use of ammonium sulphate precipitation and ion-exchange chromatography after cell disruption (see diagram A in Fig. 1). In order to characterise the chromatography process, the complete purification sequence was carried out. In our laboratory, an overall yield of 48% and a purification factor of 5.7 were obtained when the PA crude extract was applied to the ammonium sulphate fractionation step and to the chromatography column (Fig. 1). In contrast, the ATPS process that include a total of four unit operations (see diagram B in Fig. 1) produced an overall PA recovery of 97% with a purification factor of 3.5 when PA crude extract from the cell homogenate was applied to the PEG-phosphate extraction stage. A simplified comparison of the processes assuming that all the unit operations are equivalent in cost support the fact that ATPS process is superior to the ion-exchange chromatography process. A reduction of approximately 43% of the cost of the purifications steps of the chromatography process can be obtained when the ATPS process is used. Furthermore, a higher enzyme recovery (i.e. 97%) is obtained from this latest process as compared with that from the multi-step chromatography process (i.e. 48%). However, ATPS produce a less favourable increase in the purification of PA (i.e.

Table 4
Direct comparison between ion-exchange chromatography and aqueous two-phase processes for the purification of penicillin acylase (PA) produced by *E. coli*

Parameter	Ion-exchange chromatography process	ATPS process
Separating factor	Ionic interactions	Protein size and charge Affinity to PEG rich phase
Mode of operation	Semi-continuous	Batch
Working volume processed	50 mL	Equivalent to 50 g ATPS
Extract inlet concentration	10% (w/v)	35% (w/w)
Overall process yield	48%	97%
Purification factor	5.7	3.5
Final presentation of the target product	Potassium phosphate buffer	PEG solution
Cost of separating agents	$1.4 USD/mL CM sepharose resin	$1.35 USD/kg PEG $0.60 USD/kg salt
Cost of the operation	$0.47 USD/column	$0.30 USD/system

The chromatography process considers a column with a volume of 34 mL and an inlet flow of 3.3 mL/min and the ATPS comprised a 50 g batch system. In both processes a base of 15 min of operation time was defined to estimate the volume processed of inlet material. The overall process yield is expressed relative to the starting material for each process. In the case of the chromatography process the enzyme recovery of each unit operation involved was considered. The enzyme recovery of ATPS process is that obtained from this single extraction unit. Purification factor for penicillin acylase (PA) is the ratio between specific enzyme activity obtained after and before the chromatography or the ATPS stage, respectively. The cost of the operation for the chromatography process was estimated by considering that the resin can be re-used up to 100 times. In contrast, for the ATPS process, the potential savings for the recycling of PEG and phosphate were not considered.

3.5-fold) compared with that produced after ion-exchange chromatography step (i.e. 5.7-fold).

A further direct comparison between the processes highlights the superiority of the ATPS approach (see Table 4 and Fig. 1). Implementation of ATPS extraction after cell disruption resulted in a reduction (from 7 to 4) of the number of unit operations involved in the chromatography protocol. Consequently a significant economic benefit (i.e. reduction in the cost of unit operations of 43%) can be obtained. Additionally, further bioprocess intensification was achieved by loading with 35% (w/w) PA crude extract (in comparison with the 10% (w/w) PA extract load used in process A of Fig. 1) into the ATPS (Table 4). This implies that the ATPS process (diagram B in Fig. 1) can potentially process 3.5 times the amount of PA extract with the same working volume when compared with that of the previous process, with a PA top phase recovery of 97%. A simplified outline economic analysis based solely upon the cost of separation agent of the chromatography and ATPS steps was undertaken for the processes operated to process a volume of 50 mL (Table 4). In this analysis, a potential re-use of up to 100 times of the resin used for the chromatography process was considered and savings for the re-use of PEG and phosphate were not considered. Under this scenario, the ATPS process implemented here achieved a gross cost reduction of 37% (from $0.47 to $0.30 USD). Furthermore, by assuming that all the unit operations are equivalent in operating cost, an additional reduction of approximately 43% of the running cost can be obtained when the ATPS process is used. Such analysis supports the fact that ATPS process is superior to the ion-exchange chromatography process for the recovery of PA produced by *E. coli*. One cost saving adaptation that can be used with the ATPS process is the recycling of phase forming chemicals. This has been successfully used for diverse experimental vehicles [23,25]. It is clear that, for PA this process opens the way to further bioprocess scale up and enhancement.

4. Conclusions

A direct comparison between an ion-exchange chromatography and an ATPS processes for the recovery of penicillin acylase produced by recombinant *E. coli* was performed. PEG-phosphate systems were preferred for the recovery of PA over PEG-citrate systems, since higher enzyme recovery and increase in purity was obtained. An increase in PA crude extract loaded to the ATPS (from 10% to 40%, w/w), although slightly affecting the increase in enzyme purity (expressed as purification factor), proved to be suitable to benefit the processing of highly concentrated disrupted extract and maintain a high top phase enzyme recovery. In the direct comparison of the processes, the superiority of the ATPS approach was highlighted since a reduction of the number of unit operations (from 7 to 4) was achieved. The potential economic benefits were also demonstrated based upon the significant reduction in the purification steps and operation costs. Overall, the proposed ATPS process comprising of PEG1450-phosphate, TLL 48.5% (w/w), Vr = 1.0, pH of 7.0 and 35% (w/w) PA crude extract loaded into the system proved to be more efficient, recovering above 97% of PA at the top phase (PEG rich phase) with a purity factor of 3.5. It is clear that the results reported herein raise the consideration for the potential substitution of the chromatography process for PA recovery from *E. coli* as a first step for the development of an optimised and economic process with evident commercial application.

Acknowledgements

The authors wish to acknowledge the financial support of the ITESM research chair (Grant CAT005). We thank the technical assistance of Mario Caro and Myriam Ortiz (Pilot Plant Unit, IBT-UNAM).

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