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Bioprocess intensification: a potential aqueous two-phase process for the primary recovery of B-phycoerythrin from *Porphyridium cruentum*

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Abstract

A process for the primary recovery of B-phycoerythrin from *Porphyridium cruentum* exploiting aqueous two-phase systems (ATPS) was developed in order to reduce the number of unit operations and benefit from an increased yield of the protein product. The evaluation of system parameters such as poly(ethylene glycol) (PEG) molecular mass, concentration of PEG as well as salt, system pH and volume ratio was carried out to determine under which conditions the B-phycoerythrin and contaminants concentrate to opposite phases. PEG 1450-phosphate ATPS proved to be suitable for the recovery of B-phycoerythrin because the target protein concentrated to the top phase whilst the protein contaminants and cell debris concentrated in the bottom phase. An extraction ATPS stage comprising volume ratio (Vr) equal to 1.0, PEG 1450 24.9% (w/w), phosphate 12.6% (w/w) and system pH of 8.0 allowed B-phycoerythrin recovery with a purity of 2.9 (estimated as the relation of the 545–280 nm absorbances). The use of ATPS resulted in a primary recovery process that produced a protein purity of 2.9 ± 0.2 and an overall product yield of 77.0% (w/w). The results reported demonstrated the practical implementation of ATPS for the design of a primary recovery process as a first step for the commercial purification of B-phycoerythrin produced by *P. cruentum*.

Keywords: Bioprocess intensification; Aqueous two-phase systems; *Porphyridium cruentum*; B-phycoerythrin

1. Introduction

The increasing need to rapidly and economically bring new biotechnological products to market using scaleable and efficient technology has encouraged manufacturers to seek competitive advantages through bioprocess intensification. In this context, colouring compounds used in food, cosmetic, detergent and molecular genetic industries are products of great commercial significance [1,2]. The particular production of B-phycoerythrin (a red-coloured protein) by *Porphyridium cruentum* represents a very interesting case, because the industrial and commercial value of this product is considerable. The commercial value of highly purified B-phycoerythrin (purity greater than 4, defined as the relationship of 545–280 nm absorbances) for pharmaceutical or fluorescent uses can be more than US$ 50/mg [3,4]. B-phycoerythrin is the most valuable of the three main classes of phycoerythrins (B, R and C) from the photosynthetic systems of *P. cruentum*, due mainly to its wide range of potential commercial applications, such as natural dyes in foods, cosmetics and in the development of biosensors [2,5]. It is formed by three sub-units, α, β and γ (in a relative molar ratio of 6:6:1) of 18.0, 18.0 and 29 kDa molecular weight, respectively [6].

The recovery of B-phycoerythrin from *P. cruentum* has been attempted previously [5,7–10]. However, the resulting protocols have been characterised by an excessive number of unit operations (more than 10 steps), mainly in the primary recovery part of the downstream process. Consequently, affecting product yield and potential scaling up of these procedures at commercial scale. To overcome some of the disadvantages attributed to the established B-phycoerythrin purification protocols, the use of aqueous two-phase systems (ATPS) has been suggested as an attractive alternative. This technique has several advantages including bio-compatibility, ease of scale-up, low cost, etc. [11]. The use of ATPS for the recovery of protein products from fermentation broth has been addressed before [12–16]. However, no scientific reports, known to the authors, exist on the primary recovery and purification of B-phycoerythrin from *P. cruentum* cultures using ATPS.

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The research presented here aims to generate knowledge on the partition behaviour of B-phycoerythrin on ATPS to benefit the production of such colouring compounds. A practical approach which exploits the known effect of systems parameters such as the concentration of poly(ethylene glycol) (PEG) and salt (i.e. phosphate), phase volume ratio (Vr), molecular weight of PEG and system pH upon protein partition and purity was used to establish a greatly simplified primary recovery process for the purification of B-phycoerythrin from cell homogenate from *P. cruentum*. The way in which the developed process greatly simplifies the primary recovery of the protein product, potentially defines the first step for the development of a commercial process for the purification of B-phycoerythrin produced by *P. cruentum*.

2. Materials and methods

2.1. Characterisation of aqueous two-phase systems

The binodal curves were estimated by the cloud point method [17] using poly(ethylene glycol) (Sigma, St. Louis, MO, USA) of nominal molecular mass of 1000, 1450, 3350 and 8000 g/gmol (50% (w/w) stock solution) and di-potassium hydrogen orthophosphate/potassium di-hydrogen orthophosphate (Sigma) (30%, w/w). Fine adjustment of pH was made by addition of orthophosphoric acid or sodium hydroxide.

2.2. Culture medium and cultivation conditions

P. cruentum was cultivated in the culture medium described by Bermejo et al. [5]. The algae were grown in a batch culture (500 ml Erlenmeyer flasks) at 22–25 °C under natural light conditions, agitation and aeration was provided with an air flow rate of 3.2 cm^3/seg using a peristaltic pump (ELITE 799, Mexico). The cells were allowed to grow for 30 days and were harvested by centrifugation at 3500 rpm for 5 min (Eppendorf 5415C). After harvesting, cellular fragmentation was performed manually in a ceramic pot using glass beads and de-ionised water (4.3 cm^3/g wet biomass) for 15 min. Temperature was controlled with a dry ice bath. Complete cellular fragmentation was verified using an optical microscope (Olympus CK2). Cell debris removal was achieved by centrifugation at 3500 rpm for 5 min (Eppendorf 5415C) and the supernatant was processed with a solution of ammonium sulphate (0.47 g/cm^3 supernatant) containing 0.01% of sodium azide. This precipitation step was introduced just to concentrate the protein content in the cell homogenate in order to facilitate the estimation of protein concentration in the ATPS experiments. The precipitate was re-suspended in a potassium phosphate buffer (50 mM, pH 7.0) with sodium azide (5 mM), the resulting solution (referred to as crude extract) was introduced into the aqueous two-phase system previously selected as described further.

2.3. Influence of system parameters upon partition behaviour of B-phycoerythrin in PEG–salt systems

All experimental systems used to establish the operating conditions for the ATPS process were prepared for convenience on a fixed mass basis. Predetermined quantities of stock solutions of PEG and potassium phosphate were mixed with either, a single model system (containing purified B-phycoerythrin obtained from a commercial supplier, Sigma) or a complex model system (containing 20% (w/v) wet homogenate from *P. cruentum* fermentation; referred earlier as crude extract) to give a final weight of 1.0 g. The stock solution (PEG or salts) were mixed and phases dispersed by gentle mixing for 30 min at 25 °C. Adjustment of pH was made by addition of orthophosphoric acid or sodium hydroxide. Complete phase separation was achieved by low speed batch centrifugation at 1500 × g for 20 min at 25 °C. Visual estimates of the volumes of top and bottom phases and solids, were made in graduated tubes. The volumes of the phases were then used to estimate the volume ratio (volume of the top phase/volume of the bottom phase (Vr)). Samples were carefully extracted from the phases and diluted for biochemical analysis and subsequent estimation of B-phycoerythrin partition coefficient (K = concentration of solute in the top phase/concentration of solute in the bottom phase). The systems tie-line length (TLL), which represents the length of the line that connects the composition of the top and bottom phase of a defined ATPS was calculated as described by Albertsson [11]. The top phase recovery was estimated as the amount of protein presents in the upper phase (volume of the phase × protein concentration in the phase) and expressed relative to the original amount loaded into the system. Bottom phase and interface protein recovery was not estimated due problems associated to the very low amount of total protein presents in such phases. Results reported are the average of two independent experiments and errors were estimated to be a maximum of ±10% of the mean value.

2.4. Analytical procedures

Protein concentration in the samples was estimated by the method of Bradford [18]. The purity of B-phycoerythrin was determined as the relation of the 545–280 nm absorbance (i.e. purity of B-phycoerythrin = $Abs_{545\,nm}/Abs_{280\,nm}$). Bermejo et al. [5] reported the use of the 545–280 nm absorbance relation as an estimation of B-phycoerythrin purity, since the absorption spectrum of this protein exhibits a peak at 545 nm. Under this circumstances, a ratio greater than four corresponds to a highly purify B-phycoerythrin (defined as pure commercial B-phycoerythrin; Sigma).

3. Results and discussion

Predictive design of extraction processes exploiting aqueous two-phase systems depend upon the understanding of

the mechanism governing the behaviour of proteins in ATPS. However, the lack of accumulative knowledge of such phenomena demands a practical approach for the design of these type of processes. In this paper, for the design of an aqueous two-phase primary recovery process, the influence of systems parameters on the partition behaviour of B-phycoerythrin was studied using single model systems. Such systems were characterised by the presence of purified (commercially available) B-phycoerythrin only in the ATPS. These systems took no account of the influence upon the performance of ATPS of the whole range of proteins, contaminants and cell debris which may be present in the fermentation broth of the *P. cruentum*.

A practical approach which exploits the known effect of system parameters such as tie-line length, phase volume ratio, system pH and molecular weight of PEG on the protein partition behaviour can reduce the extent of the necessary empirical experiments to determine the process conditions for the development of an ATPS extraction process. For the fractionation of the cell homogenate from *P. cruentum*, the concentration of PEG and phosphate, system pH, the phase volume ratio (Vr) and the molecular weight of PEG were manipulated to maximise B-phycoerythrin recovery from the top PEG-rich phase. Initially, the effect of increasing TLL upon partition behaviour of B-phycoerythrin was evaluated. Changes in the TLL affect the free volume [19] available for a defined solute to accommodate in the phase an as a consequence in the partition behaviour of such solute in the ATPS.

The impact of increasing TLL upon B-phycoerythrin purity from model and complex ATPS, when PEG of four different molecular weight (i.e. 1000, 1450, 3350 and 8000 g/gmol) were used, is illustrated in Table 1. For all these systems, volume ratio and system pH were kept constant at 1.0 and 7.0, respectively. The partition experiments that used purified B-phycoerythrin in ATPS revealed that this protein exhibited a strong top-phase preference (data not shown), which imply that the majority of the target protein concentrated in the top phase. The top-phase preference of the B-phycoerythrin resulted in partition coefficients greater than 100.0 and with great variations (i.e. from 100 to 200) for all the systems studied. Such behaviour was explained by problems associated with the detection of the presence of B-phycoerythrin in the bottom phase, caused by the very low amount of the protein concentrated in this phase. As a consequence, it was very difficult to evaluate the impact of system parameters upon the partition behaviour of B-phycoerythrin, by monitoring the protein partition coefficient (K). As a result, it was decided to use the purity of B-phycoerythrin (expressed as the relation of the 545–280 nm absorbance) from the top PEG-rich phase as the response variable to evaluated the effect of system parameters on the behaviour of the protein in ATPS. The results of Table 1 showed that for both experimental systems: model (with purified B-phycoerythrin) and complex (crude extract from *P. cruentum*), increasing TLL caused the purity of B-phycoerythrin from the top PEG-rich phase of the different molecular weight of PEG (1000, 1450 3350 and 8000 g/gmol) used to remain relatively constant. Such behaviour may be explained by the minimum effect of the possible increase or decrease of the contaminant proteins in the top phase caused by the rise in the TLL. It has been reported that the free volume in the bottom phase decreases when the TLL is increased [19] and, as a result, the solutes in the lower phase may be

Table 1
Influence of increasing TLL upon the purity of B-phycoerythrin from PEG/phosphate ATPS

System	Molecular weight of PEG (g/gmol)	PEG (%, w/w)	Phosphate (%, w/w)	TLL (%, w/w)	Purity of B-phycoerythrin	
					Model system	Complex system
1	1000	15.6	12.6	28.3	4.2 ± 0.4	2.6 ± 0.1
2		17.6	13.6	36.1	4.6 ± 0.4	2.5 ± 0.1
3		19.8	14.8	38.0	4.7 ± 0.4	2.5 ± 0.1
4		22.2	16.0	49.4	5.1 ± 0.5	2.8 ± 0.3
5	1450	17.6	10.9	34.3	3.8 ± 0.3	1.8 ± 0.1
6		22.2	12.1	47.0	4.3 ± 0.4	2.3 ± 0.1
7		24.9	12.6	53.2	3.8 ± 0.3	2.6 ± 0.1
8		26.1	13.0	55.0	4.2 ± 0.4	2.6 ± 0.1
9	3350	16.9	10.1	33.6	3.0 ± 0.3	2.2 ± 0.1
10		18.7	11.2	39.6	2.5 ± 0.2	2.4 ± 0.1
11		21.0	12.9	45.0	3.0 ± 0.3	2.2 ± 0.1
12		22.1	14.0	48.1	3.6 ± 0.3	2.3 ± 0.1
13	8000	16.1	8.1	27.1	1.7 ± 0.1	1.0 ± 0.1
14		19.0	9.1	40.2	1.6 ± 0.1	1.1 ± 0.1
15		20.0	9.5	45.0	1.5 ± 0.1	1.0 ± 0.1
16		22.9	10.3	49.4	1.5 ± 0.1	1.0 ± 0.1

The tie-line lengths of the systems were estimated from the composition of PEG and phosphate as described in Section 2. The purity of B-phycoerythrin is expressed as the relation of the 545–280 nm absorbances. For all systems, volume ratio (estimated from non-biological experimental systems) and the system pH were kept constant at 1.0 and 7.0, respectively.

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 target product may be negatively affected. However, it seems that in this case the purity of B-phycoerythrin was not significantly affected by the increased concentration of the contaminant protein in the top phase, due probably to the increase in the concentration of B-phycoerythrin in the top phase with increasing TLL. The differences observed in the purity from top PEG-rich phases from the model and complex systems (see Table 1) is explained by the nature of the experimental vehicles (the purity from the starting material for the model system was 1.6, whilst that from the crude extract was approximately equal to 0.9). In the case of the model systems, the sole presence of the target protein, resulted in a high purity from the top phase. In contrast, for the complex system, the presence of contaminants from the homogenate from *P. cruentum* caused an effect in the partition behaviour and purity of B-phycoerythrin.

For all the system studied using cell homogenate from *P. cruentum* (or crude extract), the purity of B-phycoerythrin increased in ATPS compared with that from the crude extract (i.e. purity from the crude extract was approximately equal to 0.9). PEG 1000/phosphate ATPS characterised by TLLs of 49.4% (w/w) (PEG 22.2% (w/w) and phosphate 16.0% (w/w), Vr = 1.0 and system pH of 7.0) resulted in the maximal purity (2.8 ± 0.3) from these experiments. Once the impact of increasing TLL upon the purity of B-phycoerythrin from the top phase was evaluated, the effect of system pH on the purity of the protein was investigated using cell homogenate from *P. cruentum*.

Several authors [14–16,20,21] have discussed the influence of system pH on protein partition behaviour. In general, these reports concluded that increasing the pH (e.g. from 6.5 to 9.0) caused an increase in the protein concentration in the top phase and a decrease in the bottom phase. Such behaviour of proteins has been attributed to free-volume effects [16]. An alternative explanation may be associated to the speciation of the phosphate salts over the pH range and to conformational changes in the structural integrity of proteins [14]. Table 2 shows the influence of pH on the purity of B-phycoerythrin in PEG/phosphate ATPS, when four different molecular weights (i.e. 1000, 1450, 3350 and 8000 g/gmol) of PEG was used. ATPS from each molecular weight of PEG were selected base upon the best purity of the target protein from the results of Table 1. Purity of B-phycoerythrin decreased at the highest system pH evaluated (i.e. pH 9.0) regardless of molecular weight of PEG, except for the case of PEG 8000 in which a slight increase in the purity was observed (from 1.1 to 1.4; see Table 2). The decrease of purity can be associated with an increased in the contaminant proteins migration to the top phase with the increase in pH. Although, increasing the system pH from 7.0 to 9.0 resulted in changes in the purity of B-phycoerythrin from the ATPS studied, it is clear that no great differences to the previous protein purity obtained (see Table 1) was achieved. In the case of top phase recovery of B-phycoerythrin, it is evident that ATPS characterised by low molecular weight of PEG (i.e. 1000 and 1450 g/gmol) exhibited the highest recovery (>70%), compared with that from the ATPS of higher molecular weight of PEG (3350 and 8000 g/gmol; recovery less than 57%, see Table 2). Such behaviour in protein recovery can be associated to the decrease in purity (and top phase concentration of B-phycoerythrin) in these ATPS. Although, the small differences in purity and protein recovery obtained from the ATPS of PEG 1000 and 1450 g/gmol, ATPS with TLL of 49.4% (w/w) (PEG 1000 22.2% (w/w), phosphate 16.0% (w/w)), and 53.2% (w/w) (PEG 1450 24.9% (w/w), phosphate 12.6% (w/w)) with Vr = 1.0 at pH of 8.0 were selected as those that provided the best conditions to satisfy the needs of maximal protein purity (i.e. 2.8 ± 0.2) and top phase protein recovery (i.e. 73.0 ± 3.0 and 77.0 ± 3.0, respectively).

Table 2
Influence of changing system pH upon the purity and top phase recovery of B-phycoerythrin from PEG/phosphate ATPS

System	Molecular weight of PEG (g/gmol)	PEG (%, w/w)	Phosphate (%, w/w)	TLL (%, w/w)	System pH	Purity of B-phycoerythrin	Top phase recovery of B-phycoerythrin (%)
a	1000	22.2	16.0	49.4	7.0	2.8 ± 0.3	73.0 ± 3.0
					8.0	2.8 ± 0.2	73.0 ± 3.0
					9.0	2.5 ± 0.2	72.0 ± 3.0
b	1450	24.9	12.6	53.2	7.0	2.6 ± 0.1	76.0 ± 3.0
					8.0	2.9 ± 0.2	77.0 ± 3.0
					9.0	2.2 ± 0.2	76.0 ± 3.0
c	3350	18.7	11.2	39.6	7.0	2.4 ± 0.1	50.0 ± 2.0
					8.0	1.6 ± 0.1	55.8 ± 2.0
					9.0	1.7 ± 0.1	55.8 ± 2.0
d	8000	19.0	9.1	40.2	7.0	1.1 ± 0.1	20.0 ± 2.0
					8.0	1.3 ± 0.1	56.0 ± 2.0
					9.0	1.4 ± 0.1	45.0 ± 2.0

System pH was adjusted as described in Section 2. The purity of B-phycoerythrin is expressed as the relation of the 545–280 nm absorbance. For all systems, volume ratio (estimated from non-biological experimental systems) was kept constant at 1.0. The top phase recovery is expressed relative to the original amount of B-phycoerythrin loaded into the system.

Table 3
Influence of changing system Vr upon the purity and top phase recovery of B-phycoerythrin PEG/phosphate ATPS

System	Volume ratio	Molecular weight of PEG (g/gmol)	PEG (%, w/w)	Phosphate (%, w/w)	Purity of B-phycoerythrin	Top phase recovery of B-phycoerythrin (%)
I	2.4	1000	29.5	9.0	2.7 ± 0.2	66.4 ± 3.0
II	1.8		24.0	12.0	2.5 ± 0.1	70.2 ± 3.0
III	0.9		18.0	15.5	2.6 ± 0.1	74.8 ± 3.0
IV	0.4		12.5	18.5	2.5 ± 0.1	61.3 ± 3.0
V	0.2		7.5	21.7	2.2 ± 0.1	32.0 ± 3.0
VI	2.4	1450	29.0	8.0	2.6 ± 0.1	64.3 ± 3.0
VII	1.8		23.5	11.0	2.2 ± 0.2	65.2 ± 3.0
VIII	0.9		17.7	14.0	1.8 ± 0.1	66.0 ± 3.0
IX	0.4		12.0	7.0	1.7 ± 0.1	55.0 ± 2.5
X	0.2		7.0	20.0	1.7 ± 0.1	31.5 ± 3.0

The volume ratio (Vr) in non-biological experimental systems along a single tie-line length (49.4 and 53.2% (w/w), for PEG 1000 and PEG 1450, respectively) was estimated after phase separation in graduate centrifuge tubes. The purity of B-phycoerythrin is expressed as the relation of the 545–280 nm absorbance. For all ATPS, system pH was kept constant at 8.0. The top phase recovery is expressed relative to the original amount of B-phycoerythrin loaded into the system.

In the selected ATPS (TLL of 49.4% (w/w), PEG 1000 22.2% (w/w), phosphate 16.0% (w/w), Vr = 1.0 at pH of 8.0 and TLL of 53.2% (w/w), PEG 1450 24.9% (w/w), phosphate 12.6% (w/w), Vr = 1.0 at pH of 8.0), a decrease in the Vr caused the purity of B-phycoerythrin to slightly decline (see Table 3). Hustedt et al. [22] proposed that the protein partition behaviour remains constant for systems along the same tie-line. Such proposal may be extended for the behaviour of B-phycoerythrin in ATPS along the same tie-line. Changes in the protein purity with Vr can be attributed to a concentration effect. A decrease in the Vr imply a reduction of the volume of the top phase. Consequently, the contaminant in this phase will concentrate further and as a result a decrease in the protein purity from that phase is possible. Although, a reduction in the volume of the top phase will also have a concentration effect on the target protein (B-phycoerythrin), it seems that such effect was either smaller than that of the contaminants or caused a possible precipitation of B-phycoerythrin, which in both cases resulted in a reduction on protein purity. From the results of this experiments, it was observed that a change in system volume ratio (different from Vr = 1.0) caused no benefits in the purity and top phase recovery of phycoerythrin, thus ATPS with Vr = 1.0 were preferred for the potential development of a primary recovery process.

In a further comparison of the effect of PEG molecular weight on the purity and top phase recovery of B-phycoerythrin from ATPS, the purity and recovery of B-phycoerythrin from ATPS decreases when high molecular weights of PEG were used (see Table 4). The effect of increasing molecular weight of PEG upon protein partition behaviour has been explained based upon the protein hydrophobicity [23,24] and phase excluded volume [12,25,26]. In the case of B-phycoerythrin, the decrease in protein purity when high molecular weights of PEG were used, may be explained by a migration of contaminant proteins from the bottom phase or interface to the top phase. An alternative explanation involves B-phycoerythrin migration from the top to the bottom phase or the interface. ATPS with low molecular weight of PEG (i.e. PEG 1000 and PEG 1450) exhibited the best protein purity and recovery. ATPS using PEG 1450-phosphate (instead of PEG 1000-phosphate) was selected for the extraction stage, since the cell debris concentrated in the bottom phase (data not shown). In contrast, in ATPS with PEG 1000 cell debris accumulated at the interface. Such a situation may cause contamination problems when the top PEG-rich phase is removed for further processing.

From the studies of the influence of system parameters upon the purity and top phase recovery of B-phycoerythrin from ATPS, process conditions (i.e. Vr = 1.0, PEG 1450 24.9% (w/w), phosphate 12.6% (w/w), TLL of 53.2% (w/w) and system pH of 8.0) were selected for the ATPS extraction stage. Such extraction conditions resulted in a protein product with a purity of 2.8 and top phase recovery of

Table 4
Influence of molecular weight of PEG upon the purity and recovery of B-phycoerythrin from PEG/phosphate ATPS

System	PEG (%, w/w)	Phosphate (%, w/w)	TLL (%, w/w)	Molecular weight of PEG (g/gmol)	Purity of B-phycoerythrin	Top phase recovery of B-phycoerythrin (%)
A	22.2	16.0	49.2	1000	2.8 ± 0.3	73.0 ± 3.0
B	24.9	12.6	53.2	1450	2.8 ± 0.1	77.0 ± 3.0
C	18.7	11.2	39.6	3350	1.6 ± 0.1	55.8 ± 2.0
D	19.0	9.1	40.2	8000	1.3 ± 0.1	56.0 ± 2.0

The purity of B-phycoerythrin is expressed as the relation of the 545–280 nm absorbance. For all systems, volume ratio (estimated from non-biological experimental systems) and the system pH were kept constant at 1.0 and 8.0, respectively. The top phase recovery is expressed relative to the original amount of B-phycoerythrin loaded into the system.

77.0%. The general process proposed for the potential primary recovery of B-phycoerythrin produced by *P. cruentum* is characterised by three unit operation involving one ATPS extraction stage for the downstream processing to produce a B-phycoerythrin with a purity of 2.8, suitable for the purification steps. In contrast, the process reported by Bermejo et al. [5], involves a minimum of eight unit operation for the primary recovery stage to obtain a product with similar characteristics. Therefore, a direct comparison of the new proposed strategy with the existing process, highlights the superiority of the current approach. The research presented here, resulted in a primary recovery process that produced an overall B-phycoerythrin recovery of 77%. The recovery process proposed here increase the protein purity up to 2.8 in ATPS extraction stage, which raise the potential commercial application of this process as an alternative for the practical purification of B-phycoerythrin produced by *P. cruentum*.

4. Conclusions

This study reports for the first time the fractionation of cell homogenate of *P. cruentum* in aqueous two-phase systems for the development of a process for the potential primary recovery of B-phycoerythrin. It has been shown that tie-line length, volume ratio, molecular weight of PEG and system pH influence the purity of B-phycoerythrin from the top PEG-rich phase. PEG 8000-phosphate ATPS proved to be unsuitable for the primary recovery of B-phycoerythrin since top phase recovery and protein purity resulted in low values (i.e. $<$56% and 1.4, respectively). The operating conditions established for the PEG 1450-phosphate ATPS extraction resulted in a one-stage process for the potential recovery of B-phycoerythrin from *P. cruentum*, that concentrated the protein preferentially to the top phase and the contaminants to the opposite phase. Overall, the results reported here demonstrated the potential application of ATPS for the primary recovery of B-phycoerythrin as a first step for the development of a downstream process with commercial application.

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