Extractive purification of recombinant thermostable lipase from fermentation broth of *Escherichia coli* using an aqueous polyethylene glycol impregnated resin system

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Abstract

This study aimed at recovery of thermostable lipase from *Escherichia coli* BL21 using porous glass beads grafted with polyethylene glycol (PEG) in aqueous impregnated resins system (AIRS). The influencing parameters such as concentration and pH of extraction solution, concentration of NaCl, size of the beads, and pH of the desorption solution on the partition behaviour of lipase were evaluated. Smaller adsorbent (4 mm) had a 65.5% of recovery yield with approximately two-fold higher purification factor compared to that obtained with the larger adsorbent. Recombinant lipase was purified successfully using AIRS with a purification factor of 7.6 and yield of 78.4% under optimum conditions of 18% (w/w) PEG 4000, 10% (w/w) of potassium citrate at pH 9 with 3% (w/w) of NaCl. Optimum desorption was obtained with 4.0 mm of porous glass beads at pH 9.

Keywords Aqueous impregnated resins system · Aqueous phase · Purification · Recovery · Lipase

Introduction

Lipases are considered of great attention due to their ability to catalyze a wide range of reactions in both aqueous and non-aqueous environments. They gained substantial

interest in different industries, such as food, detergents, bioenergy and biodiesel, and pharmaceuticals (Kumar et al. 2016). The success in lipase purification to homogeneity is largely attributed to the conventional methods of purification such as ammonium sulfate precipitation, gel-filtration, ion-exchange, and affinity chromatography (Tan et al. 2015). Moreover, purification of lipase was also performed using polypropylene matrix of Accurel (Gupta et al. 2005).

Aqueous two-phase extraction (ATPE) is commonly used to separate the biomolecule from the cell culture. It has been used for the purification of lipase from different microorganisms such as *Rhizopus microspores* and *Burkholderia pseudomallei* (Anvari 2015; Ooi et al. 2009). However, this method of extraction has no applications in industry (Iqbal et al. 2016), which is substantially based on very long equilibration time needed for separation of phase and partitioning of protein, causes an increased footprint of the required settler devices or the application of additional equipment such as energy consuming centrifuges (van Winssen et al. 2014b).

A new generation of tunable polymer phase impregnated resins technology (TAPPIR) has been introduced to overcome the diffusional limitation (van Winssen et al. 2014b). TAPPIR technology has been used for recovery of lysozyme and myoglobin (van Winssen et al. 2014a). However, even

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with this technology, immobilizing one aqueous phase of ATPE inside the pores of a solid support did not solve the limitation as ATPE itself, which is one of the steps in TAPPIR. Moreover, pre-equilibration of the two phases in TAPPIR technology is time-consuming and the separation of the phases in industrial scale is difficult to be achieved.

The recovery of biomolecules could also be improved by excluding the ATPE principle in TAPPIR technology to reduce the purification steps and limitation of ATPE. Using the principle of hydrophobicity interaction chromatography, aqueous impregnated resins system (AIRS), which immobilizes the liquid extractant on the inert support and corresponds to the aqueous, stationary phase, was used to surmount the drawbacks present in TAPPIR technology. This technique combines the selectivity of liquid–liquid extraction with the ease of operation of column chromatography. The target biomolecules are adsorbed on the aqueous, stationary phase while the impurities are removed from the flow-through.

The present study aimed to investigate the extraction and purification method for lipase from recombinant *Escherichia coli* BL21 using AIRS. There is no report on the use of AIRS in lipase purification. Since AIRS is a relatively new concept of separation, many factors have to be evaluated with regard to their impact on the performance of the extractive purification system. It is postulated that the maximum recovery of lipase could be achieved by optimizing the influencing factors such as types and concentrations of phase-forming PEG and salt, system pH, crude loading, NaCl concentration, size of the glass beads, and pH of the desorption solution.

Materials and methods

Materials

PEG with various average molecular weights (MW), ranging from 2000 to 6000 (g/mol), ammonium persulfate $[(\text{NH}_4)_2\text{S}_2\text{O}_8]$, trichloroacetic acid (TCA), triton X, tripotassium citrate ($\text{C}_6\text{H}_5\text{K}_3\text{O}_7$), and casein enzyme hydrolysate were obtained from Sigma-Aldrich (St. Louis, MO, USA). *N,N,N',N'*-Tetramethylethylenediamine (TEMED), Tris–HCl, protein assay kit, and albumin standard were supplied by Bio-Rad, USA and Thermo Scientific Pierce, respectively. Sodium citrate ($\text{NaH}_2\text{C}_6\text{H}_5\text{O}_7$) was sourced from SAFC (St. Louis, MO, USA). Porous glass beads (4.0 mm and 8.0 mm, pore size = 60 μm) were purchased from ROBU (Glasfilter-Gerate GmbH, Germany). Luria broth, Luria–Bertani agar, glycine, yeast extract, D (+) glucose anhydrous, and ammonium sulfate were obtained from Merck (Darmstadt, Germany). All chemicals used in this study were of analytical grade.

Preparation of clarified lipase homogenate from *E. coli*

Lipase producing strain, recombinant *E. coli* BL21 (DE3) pLysS harboring the plasmid pET51b-Lip 42, was cultivated according to Hadzir et al. (Hadzir et al. 2016). The culture was centrifuged (Centrifuge Model 5500, Kubota, Japan) at 4000g for 30 min at 4 °C. The cell pellets were resuspended in 20 mM potassium phosphate buffer at pH 7 and then lysed by ultrasonication for 20 min on ice (30 s of pulse-on period and 60 s of pulse-off period). Cell-free supernatant was collected after centrifugation at 4000g for 30 min and used as the crude feedstock throughout the study.

Recovery of lipase using AIRS

Lipase was purified with extraction chromatography using the PEG grafted porous glass beads that were packed into the column (Fig. 1). In this respect, the porous glass beads were washed with distilled water and dried at 60 °C in an oven overnight prior to use. 2 g of porous glass beads were immobilized with PEG phase via wet impregnation and the defined concentration of PEG was added to the porous glass beads. The impregnated porous glass beads were filtered out to remove the unbound PEG and dried in an oven at 60 °C prior to adding into the column. 100 μL of crude feedstock was loaded into the packed column plugged with end cap. The column was then equilibrated with 1.9 mL of extraction solution which consisted of salt and sodium chloride (4%) agitated in the incubator shaker (Infors HT, Switzerland) at 250 rpm, 30 °C for 2 h. After incubation, the end cap was removed to elute the extraction solution. Prior to elution, the desorption process was performed via addition of 2 mL of desorption solution (0.36 M potassium citrate buffer at pH 9) to the column with the capped end tip, and incubated at 30 °C on a shaker agitated at 250 rpm for 2 h. After desorption, the porous glass beads were sonicated at 45 kHz for 30 min and washed with 1 M NaOH solution to regenerate and sterilize.

Optimization of the extraction procedure employing AIR systems

One-factor-at-a-time method (OFAT) experiments were conducted to determine the preliminary range of extraction variables. The concentration of PEGs [10–20% (w/w)] with different molecular weights of 2000, 4000, and 6000 (g/mol) grafted on the porous glass beads, ionic strength of potassium citrate in extraction solution [at the range from 0.1 M (5%, w/w) to 0.67 M (15%, w/w)], pH of extraction solution (5.0, 6.0, 7.0, 8.0, and 9.0), sizes of porous glass beads (8

Fig. 1 Schematic diagram of AIRS. The purification of lipase by AIRS is followed three stages. In first stage, PEG, in excess of volume was impregnated into fixed amount of porous glass beads. Second, the crude feedstock was loaded to the packed column plugged with end cap. The column was then equilibrated with extraction solution which consisted of salt and sodium chloride (4%) agitated in the incubator shaker at 250 rpm, 30 °C for 2 h. After incubation, the end cap at the column was removed to elute the extraction solution. In the last stage, the elution process was performed via addition of desorption solution to the column with the capped end tip and agitated at 250 rpm for 2 h

and 4 mm), NaCl concentration (1, 3, 4, 5, and 7%), and pH of back-extraction solution (5.0, 6.0, 7.0, 8.0, and 9.0) were evaluated for the highest purification factor and yield of lipase.

Analytical methods

Lipase activity was assessed using the *p*-nitrophenyl-laurate (*p*-NPL) assay as described by Tan et al. (2014). One unit (U) of lipase activity was defined as the amount of lipase liberated in 1 μ mol of *p*-NP released per min. The total protein concentration was determined using Bradford assay (Bradford 1976). The protein content of the sample was calculated based on a BSA standard curve.

The recovery performance of lipase in the desorption solution was evaluated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The enzyme sample was precipitated using TCA (Aziz et al. 2017). The samples containing Laemmli buffer were boiled for 5 min and loaded into a polyacrylamide gel made up of 12% (v/v) resolving gel and electrophoresed at 140 V for 60 min. The gel was then stained with PageBlue protein staining solution for 2 h and destained using deionized water for 15 min. The image was captured using gel imaging system (UV Tech Gel Imaging System, Vivantis, Malaysia).

Calculations

The specific activity, defined as the ratio of enzyme activity to the protein content was calculated using Eq. (1):

$$\text{Specific activity (U/mg)} = \frac{\text{Enzyme activity}}{\text{Protein content}} \quad (1)$$

The purification factor (P_{FT}), defined as the ratio of the specific activity of lipase in the desorption solution to the specific activity of crude lipase was calculated using Eq. (2):

$$P_{FT} = \frac{\text{Specific activity of desorbed lipase}}{\text{Specific activity of crude lipase}} \quad (2)$$

The yield, defined as the ratio of the desorbed lipase activity in desorption solution to the activity of crude lipase was calculated using Eq. (3):

$$\text{Yield (\%)} = \frac{\text{Desorbed lipase activity}}{\text{Crude lipase activity}} \times 100 \quad (3)$$

The desorption efficiency, defined as the ratio of the desorbed lipase activity in the desorption solution to activity of the adsorbed lipase in extraction solution was calculated using Eq. (4):

$$\text{Desorption efficiency (\%)} = \frac{\text{Desorbed lipase activity}}{\text{Adsorbed lipase activity}} \times 100. \quad (4)$$

Results and discussion

Influence of molecular weight and concentration of PEG impregnated glass beads on lipase adsorption

Influence of molecular weight and concentration of PEG on P_{FT} and yield of lipase were investigated to evaluate the adsorption performance of the system and the results are as in Table 1. PEG 2000 (g/mol)- and PEG 6000 (g/mol)-based AIRS has low P_{FT} and yield, while the PEG 4000-based AIRS has relative high P_{FT} (2.49) and yield (67.20%) at 18% (w/w). For the PEG 4000 (g/mol)-based AIRS, at a certain temperature (298.15 K), yield increased from 44 to 67% with the increase in PEG amounting from 14% (w/w) to 18% (w/w) and reduced to 64% with further increase of the PEG amount from 18% (w/w) to 21% (w/w), which corresponded to the P_{FT} . The influences of the PEG amount on

Table 1 Purification factor and yield of lipase achieved in AIRS with different molecular weight and concentration of PEGs

PEG molecular weight (g/mol)	PEG concentration (% w/w)	Purification factor (P_{FT})	Yield (%)
2000	14	1.46	38.69
	16	0.72	40.00
	18	0.54	47.23
	21	0.29	25.01
4000	14	0.64	43.97
	16	0.59	43.62
	18	2.49	67.20
	21	2.01	64.10
6000	14	0.34	23.00
	16	0.64	33.99
	18	0.73	57.79
	21	0.38	25.22

0.1 mL of crude feedstock was loaded with extraction solution (1.9 mL of 10% (w/w) potassium citrate and 4% of NaCl at pH 9)

Table 2 Purification factor and yield of lipase achieved in AIRS with different concentration of potassium citrate in extraction solution

PEG 4000 concentration (% w/w)	Potassium citrate concentration (% w/w)/M	Purification factor (P_{FT})	Yield (%)
18	3/0.10	0.49	5.38
	6/0.21	1.28	20.45
	10/0.36	2.49	67.20
	15/0.58	2.02	51.93
	17/0.67	0.81	22.58

0.1 mL of crude feedstock was loaded with extraction solution (1.9 mL) potassium citrate and 4% of NaCl at pH 9

P_{FT} could be interpreted as follows. The molecular mass and concentration of polymer influence the protein extraction by altering the number of polymer–protein interactions. This is usually attributed to hydrophobic interactions between the chains of PEG and the hydrophobic area of protein. This phenomena could be explained as the PEG molecular weight or PEG concentration increases, the compact and hydrophobic structure will be formed and this enhanced the interaction with the active lipase site (Wu et al. 2014). Hydrophobic interaction is due to the presence of hydrophobic surfaces surrounding the active sites of lipases. Low molecular weight and low concentration of PEG are unsuitable for adequate extraction because the volume exclusion effect decreases, and as a result the polymer can attract all proteins to the stationary phase (Ramyadevi et al. 2012). Therefore, the selection of the best molecular weight and concentration of polymer is very important for the extraction of lipase.

Influence of ionic strength of potassium citrate in extraction solution on lipase recovery

Table 2 shows the effect of the ionic strength of potassium citrate extraction solution on the recovery of lipase. The concentration of salt used in the buffer and sample solution influences the ligand–protein interactions and consequently the protein retention (Gedikli et al. 2014). The ionic strength of 0.36 M (10% w/w) potassium citrate extraction solution increases the adsorption capacity in the recovery process of lipase, suggesting that the salt contributes to the recovery of lipase in AIRS. Maximum P_{FT} (2.49) and yield (67.2%) of lipase was achieved with increased ionic strength of potassium citrate from 0.1 to 0.36 M. This could be due to the kosmotropicity of potassium citrate at $T = 298.15$ K which increased the solubility of lipase in water due to salting-in (Zafarani-Moattar and Hamzehzadeh 2011). This is necessary for the adsorption of lipase to the hydrophobic PEG-impregnated porous glass beads. However, increased ionic strength of potassium citrate extraction solution to 0.67 M decreased P_{FT} to 0.81 and yield to 22.58%. As the ionic strength of potassium citrate increases, the solubility of lipase markedly decreased in

the extraction solution due to salting-out. Increased salt concentration may also destabilize the biomolecule due to high water content or the capsid proteins which are twisted into destabilized conformations (Vellekamp 2002).

Influence of pH of extraction solution on lipase recovery

The influence of pH of extraction solution which varied at a range from 5 to 9 on P_{FT} and yield is shown in Fig. 2. Lipase extraction was favored at high pH values. Based on the result, the yield remained at ~65% when the pH was 7 and above, and reduced at a pH below 7. The reduction could be due to the low stability of lipase at low pH (Nandini and Rastogi 2010). The P_{FT} increased with increase of pH and reached to 3.1 at pH 9. This extraction behaviour can be explained as the protein surface charge and isoelectric point (pI) played a huge role on lipase recovery. According to the Hadzir et al. (2016), the pI of lipase was at pH 6.5. Protein extraction to the PEG stationary phase is governed only by hydrophobic interactions at its pI. Lipase becomes negatively charged, when the pH of extraction solution is higher than pI of lipase. This caused the electrostatic repulsions between charged groups of lipase and extraction solution (Ketnawa et al. 2017). Therefore, the pH of the extraction solution may be manipulated to promote selective separation. The use of pH values above the pI of proteins may induce an additional affinity towards the polymer stationary phase (González-Valdez et al. 2011). Herein, hydrophobicity of PEG stationary phase and electrostatic effects of extraction solution may be responsible for the distinct extraction behaviour.

Fig. 2 Influence of pH on lipase partitioning. The pH varied from 5 to 9. Values are given as means $\pm$ SD taken from three trials

Influence of glass bead size on lipase recovery

Influence of two different sizes of glass beads impregnated with 18% (w/w) PEG 4000 (g/mol) is presented in Table 3. It was observed that the smaller size of the porous glass beads had a higher P_{FT} and yield. The P_{FT} and yield obtained in 4.0 mm of porous glass beads were 3.09 and 65.48%, respectively, while the 8.0 mm glass bead had a P_{FT} of 1.45 and yield of 44%, respectively. This phenomena could be due to the increase in the surface contact area of porous glass beads, followed by increase in the interaction of polymer stationary-phase protein, in which the diffusion of protein to the stationary phase is faster at small particles (Babic 2008).

Influence of NaCl concentration in extraction solution on lipase recovery

Influence of NaCl concentration on the purification factor and yield of lipase is presented in Fig. 3. It is found that the interaction of lipase with the stationary phase is apparently selective and the extent of retention of lipase

Table 3 The effect of the glass bead size on lipase purification

Bead size (mm)	Purification factor (P_{FT})	Recovery yield (%)	Desorption efficiency (%)
8.0	1.45 $\pm$ 0.04	43.95 $\pm$ 0.39	99.26 $\pm$ 0.31
4.0	3.09 $\pm$ 0.07	65.48 $\pm$ 0.53	99.63 $\pm$ 0.14

The results reported as a mean of the triplicate

Fig. 3 Influence of addition of NaCl on the purification of lipase. The concentration of NaCl varied from 1 to 5 (% w/w). The purification factor, total recovery yield, and desorption efficiency were calculated and plotted at different NaCl concentrations (%). The result was reported as a mean of triplicate reading

is markedly affected by addition of NaCl. The concentration of salt strongly influences the selectivity in protein adsorption and the effect is different and depends on the stationary phase and the buffer salts (Oscarsson and Kårnsnäs 1998). The highest P_{FT} (5.9) and yield (78%) were achieved at 3% NaCl. This could be due to increase of salt concentration, and can be explain by receding of water molecules from protein's surface which helps the hydrophobic surfaces of protein be exposed to the hydrophobic ligands (Chen and Sun 2003). Therefore, the adsorption of lipase is enriched with the increase of NaCl concentration. However, higher NaCl concentration (> 3%) was not desirable as it led to salting-out occurrence and decreased the lipase activity (Queiroz et al. 2001).

Influence of pH on desorption properties of AIRS

The desorption properties of AIRS, by varying the pH of the desorption solution, are as shown in Fig. 4. The desorption ratio increased with increased pH value from 5 to 9. Desorption ratio of lipase was almost 0 when desorption solution was acidic, in which the pH values were at 5 and 6. In the base environment, lipase in porous glass beads was separated and moved into desorption solution. Up to 99% (v/v) of the adsorbed lipase was desorbed using 0.36 M desorption solution (at pH 9.0) and desorption ratio reached a plateau at pH 8. However, the P_{FT} and yield of lipase increased with increased pH and reached to highest P_{FT} (7.6) and yield (78.4%), at pH 9. Therefore, lipase could be effectively desorbed and purified by extraction solution at pH 9.

Fig. 4 Influence of pH desorption buffer on the purification of lipase. The pH desorption buffer varied from 5 to 9. The purification factor, total recovery yield, and desorption efficiency were calculated and plotted at different pH desorption buffers. The expression of result was reported as a mean of triplicate reading

Evaluation of lipase recovery in AIRS using SDS-PAGE

The SDS-PAGE profiles of the standard protein markers (lane A), crude sample (lane B), and partially purified lipase (lane C, D, and E) are as shown in Fig. 5. The crude sample in lane B showed an intense band which indicated the presence of large quantities of impurities and protein contaminants in the crude enzyme extract as a result from the disruption process of intracellular lipase. The lane C, D, and E verified the success of purification of lipase as the number of bands reduced, and the band obtained lies at the reported range molecular weight of lipase, 43 kDa via SDS-PAGE. It was reported that the lipase 42 from recombinant *E. coli* BL21 has the molecular weight of 43 kDa (Hadzir et al. 2016).

Conclusion

Results from this study revealed that AIRS which is the improved TAPPIR, could be successfully used to purify recombinant lipase from *E. coli* BL21. The molecular weight of PEG, concentration of PEG, type and concentration of salts, pH of salt, crude enzyme loading, bead sizes, NaCl concentration, and pH of the salt for desorption buffer have effect on the purification factor and recovery yield of lipase in AIRS. The maximum recovery yield of 78%, purification factor of 7.6, and desorption efficiency of 95% could be acquired by building the system with the composition of

Fig. 5 SDS-PAGE analysis of the recovery of recombinant lipase from *E. coli* BL. Lane A: standard protein marker with molecular weight of 17–245 kDa. Lane B: crude enzyme lipase; lane C and D, purified lipase

18% (w/w) PEG 4000 (g/mol), 10% (w/w) potassium citrate at pH 9, 10% (w/w) crude loading with the 4.0 mm glass beads and 3% of NaCl concentration. The methodology for the purification of lipase using AIRS system, as presented in this study, remained elusive due to lack of the mechanism and interactive studies between the protein to PEG phase, impregnation stability of PEG phase and mass transfer of PEG phase and extraction solution. For future study, more protein models should be tested to generate more information to enable further improvement of the performance the AIRS. Other parameters such as adsorption and desorption time which affect the AIRS performance should be considered. AIRS performance could be improved by altering the behaviour of the system.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

- Anvari M (2015) Extraction of lipase from *Rhizopus microsporus* fermentation culture by aqueous two-phase partitioning. *Biotechnol Biotechnol Equip* 29(4):723–731
- Aziz NFHA, Abbasiliasi S, Ng HS, Phapugrangkul P, Bakar MHA, Tam YJ, Tan JS (2017) Purification of β -mannanase derived from *Bacillus subtilis* ATCC 11774 using ionic liquid as adjuvant in aqueous two-phase system. *J Chromatogr B* 1055:104–112
- Babic K (2008) Extractant impregnated resins for the recovery of aldehydes and chiral resolution. University of Twente, Enschede
- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72(1–2):248–254
- Chen J, Sun Y (2003) Modeling of the salt effects on hydrophobic adsorption equilibrium of protein. *J Chromatogr A* 992(1):29–40
- Gedikli M, Ceylan S, Erzenin M, Odabaşı M (2014) A novel matrix for hydrophobic interaction chromatography and its application in lysozyme adsorption. *Acta Biochim Pol* 61(4):731–737
- González-Valdez J, Cueto LF, Benavides J, Rito-Palomares M (2011) Potential application of aqueous two-phase systems for the fractionation of RNase A and α -lactalbumin from their PEGylated conjugates. *J Chem Technol Biotechnol* 86(1):26–33
- Gupta N, Rathi P, Singh R, Goswami VK, Gupta R (2005) Single-step purification of lipase from *Burkholderia multivorans* using polypropylene matrix. *Appl Microbiol Biotechnol* 67(5):648–653
- Hadzir MH, Abbasiliasi S, Ariff AB, Yusoff SB, Ng HS, Tan JS (2016) Partitioning behavior of recombinant lipase in *Escherichia coli* by ionic liquid-based aqueous two-phase systems. *RSC Adv* 6(86):82571–82580
- Iqbal M, Tao Y, Xie S, Zhu Y, Chen D, Wang X, Huang L, Peng D, Sattar A, Shabbir MAB (2016) Aqueous two-phase system (ATPS): an overview and advances in its applications. *Biol Proced Online* 18(1):18
- Ketnawa S, Rungraeng N, Rawdkuen S (2017) Phase partitioning for enzyme separation: an overview and recent applications. *Int Food Res J* 24(1):1–24
- Kumar A, Dhar K, Kanwar SS, Arora PK (2016) Lipase catalysis in organic solvents: advantages and applications. *Biol Proced Online* 18(1):2
- Nandini K, Rastogi NK (2010) Separation and purification of lipase using reverse micellar extraction: optimization of conditions by response surface methodology. *Biotechnol Bioprocess Eng* 15(2):349–358
- Ooi CW, Tey BT, Hii SL, Ariff A, Wu HS, Lan JCW, Juang RS, Kamal SMM, Ling TC (2009) Direct purification of *Burkholderia pseudomallei* lipase from fermentation broth using aqueous two-phase systems. *Biotechnol Bioprocess Eng* 14(6):811–818
- Oscarsson S, Kärnsäs P (1998) Salt-promoted adsorption of proteins onto amphiphilic agarose-based adsorbents: II. Effects of salt and salt concentration I. The raw data table can be obtained from the authors upon request. *J Chromatogr A* 803(1):83–93
- Queiroz J, Tomaz C, Cabral J (2001) Hydrophobic interaction chromatography of proteins. *J Biotechnol* 87(2):143–159
- Ramyadevi D, Subathira A, Saravanan S (2012) Aqueous two-phase poly (ethylene glycol)–Maltodextrin system for protein partitioning from shrimp waste: influence of molecular weight and pH. *Int J Environ Sci* 2(4):2462–2469
- Tan JS, Abbasiliasi S, Lin YK, Mohamed MS, Kapri MR, Kadkhodaei S, Tam YJ, Rahman RNZRA, Ariff AB (2014) Primary recovery of thermostable lipase 42 derived from recombinant *Escherichia coli* BL21 in aqueous two-phase flotation. *Sep Purif Technol* 133:328–334
- Tan CH, Show PL, Ooi CW, Ng EP, Lan JCW, Ling TC (2015) Novel lipase purification methods—a review of the latest developments. *Biotechnol J* 10(1):31–44
- van Winssen F, Merz J, Czerwonka L-M, Schembecker G, Dortmund T (2014a) Application of the tunable aqueous polymer-phase impregnated resins-technology for protein purification. *Sep Purif Technol* 136:123–129
- van Winssen F, Merz J, Schembecker G (2014b) Tunable aqueous polymer-phase impregnated resins-technology—a novel approach to aqueous two-phase extraction. *J Chromatogr A* 1329:38–44
- Shabram P, Vellekamp G, Ruan Q, Scandella C (2002) Purification of adenovirus. In: Curiel DT (ed) *Adenoviral vectors for gene therapy*, 2nd edn. Academic Press, San Diego, pp 197–230
- Wu J, Zhao C, Lin W, Hu R, Wang Q, Chen H, Li L, Chen S, Zheng J (2014) Binding characteristics between polyethylene glycol (PEG) and proteins in aqueous solution. *J Mater Chem B* 2(20):2983–2992
- Zafarani-Moattar MT, Hamzehzadeh S (2011) Effect of pH on the phase separation in the ternary aqueous system containing the hydrophilic ionic liquid 1-butyl-3-methylimidazolium bromide and the kosmotropic salt potassium citrate at $T = 298.15$ K. *Fluid Phase Equilib* 304(1):110–120