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Recovery of β-Amylase by Hybrid UF Membranes from the Soy Whey Wastewater

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Abstract

Large-scale manufacturing of soybean proteins results in huge volume of “wastewater” which contains substantial amount of β-amylase, and the recovery of β-amylase has potential high value. In order to evaluate the feasibility and effect of novel hybrid Ultrafiltration (UF) method against the normal method, the pretreated effluent was concentrated by a low molecular weight cut off (MWCO) 3 kDa UF membrane firstly, terminated at Volume Concentration Ratio (VCR) of ca.7. To this UF retentate was then precipitated by adding 1.2M $(NH_4)_2SO_4$ to remove other proteins. The supernatant obtained following centrifugation was purified through a further higher 30 kDa MWCO UF membrane, which ended at VCR of ca.10. The prepared was purified 4.8 fold, with an enzyme activity concentration ratio of 61, and is associated with 74% combined recovery for the enzyme activity, giving a final specific activity of 14 U/mg (674 U/mL), of which enzyme activity is fully enough for the industrial applications. Preliminary data of this research indicate that this could be an effective method for treating kinds of related protein wastewaters.

Keywords: β-amylase; Soy whey wastewater; Concentration; Ultrafiltration; Purification

Nomenclature: MWCO: Molecular Weight Cut-Off (Da); VCR: Volume Concentration Ratio; UF: Ultrafiltration; T: Temperature (°C); TMP: Trans-Membrane Pressure (bar); Jv: Permeated volume per unit time and per unit effective filtration area (L m-2 h-1 or LMH); VF: Feed volume (L); VC: Retentate volume (L); VP: Permeate volume (L)

Introduction

Isolated Soy Protein (ISP) is one of the most important and widely used plant proteins, which in 2013 witnessed a production capacity of ca. 600 k tons in China. Manufacturing one ton of ISP results in ca. 40 tons of whey wastewater, which contains up to 8 g L^{-1} protein as well as oligosaccharides [1]. Such soybean whey wastewater possesses high Biochemical Oxygen Demand (BOD) values and therefore by legislation cannot be discharged into the environment directly. Presently, there has been no consensus in this food industry on how to effectively deal with such wastewater. Options explored include anaerobic and/or aerobic digestion approaches [2], but the small gain out of such a treatment (e.g. biogas produced out of anaerobic digestion) has been far from offsetting the hefty investment and operating costs.

A substantial proportion of these whey proteins is β-amylase (α-1,4-glucan maltohydrolase; EC.3.2.1.2), an exo-hydrolase that releases β-maltose from non-reducing ends of α-1,4-linked polyglucans and oligoglucans until encountering the first α-1,6-branching point [3]. While β-amylase mixed with pullulanase has been found to convert starch to high maltose syrup [4].

A sustainable strategy for dealing with such effluent is to regard it as a source of raw materials for further manufacturing processes. The present work intended to develop a proof-of-principle process technology towards manufacturing β-amylase preparation out of the soybean whey wastewater. Whilst the technology developed retained the biological activity of the target enzyme, we have apparently achieved a low-range costing for the conceivable large-volume wastewater. Indeed, sensible use of UF can strike a much sought-after balance between achieving a decent product concentration and purity, handling a large effluent volume, and maintaining a low production cost [5]. For instance, electro-UF has been used successfully for amylase recovery out of food processing wastewater [6] and in isolation of proteases from surimi wash water [7].

Selection of membrane type is crucial to the intended UF centered process application, as well as the membrane chemical properties, configuration and pore size, which affect aspects like protein adsorption on membrane surface and membrane stability [8]. The proteins involved in this application are mostly of hydrophilic nature and include the targeted β-amylase. Our choices for hydrophilic UF membrane included, but not limited to, cellulose acetate, cellulose tri-acetate, polyethersulfone and nylon M. Among these, polyethersulfone membrane possesses high flux, sound chemical stability (including in the alkaline pH range), and outstanding thermal stability (e.g. the intact membrane element should be autoclavable). In consideration of a number of technical and non-technical facts, a type of polyethersulfone membrane from the designated manufacturer was chosen for application.

Typically, in membrane separation processes, the first stage

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membranes should be those with highest capacity, i.e., with higher MWCO, and then the membranes with smaller MWCO are usually used to separate the residue liquid in the subsequent steps. Extraordinary, on the basis of the protein molecular weights distribution and other properties of whey effluent and relevant preliminary data (to be discussed in the results), an effective processing was assembled and tested in this study, involving two sequential UF steps and a $(NH_4)_2SO_4$ salting out step, in which the lower MWCO UF membrane was applied before the one with higher MWCO. The suitable UF membranes were selected, and the befitting operating conditions were selected to recover and purify β-amylase.

Materials and Methods

Pre-treatment of the crude sample

The soybean whey effluent was collected from Shandong Wonderful Industrial Group Co. Ltd (Shandong, China), and then adjusted to pH range from 4 to 9 by 1M HCl or 1M NaOH. After settling for 2 h, a supernatant was obtained following 10-min centrifugation at 3050 g. The procedure of recovery β-amylase from the soy whey wastewater was illustrated in Figure 1.

UF by the centrifuge

For determining the UF operating temperature and optimum pH for preparing Sample 2# (Table 1) for the first UF stage, centrifugal UF experiments were conducted on an Eppendorf 5810R centrifuge at 3050 × g for 20 min at 4°C using Amicon Ultra-15 polyethersulfone membranes of either 3 or 10 kDa MWCO (Millipore, USA), with sample loading volume being 15 mL. The UF operation temperature was adjusted according to the centrifuge set up, and the retentate collected was a counterpart to Sample 2#.

UF by pilot-plant device

Pilot-scale experiments were run with a tangential-flow pilot-plant UF system made of Qiyu Biotechnology Co. Ltd. (Shanghai, China). The set-up contains a 10 L tank and a spiral-wound UF membrane vessel, and the membrane has an effective filtration area of 0.24 m^2 (termed as the "1812" module). Polyethersulfone membranes of 3 kDa, 10 kDa and 30 kDa MWCOs (with catalogue numbers QY-UF-3-T-1812, QY-UF-10-T-1812, and QY-UF-30-T-1812 respectively) from the same supplier were used. The TMP was in the 1-3 bar range, and the cross-flow rate was controlled at 120 L h^{-1}. The UF flux (as L m^{-2} h^{-1} or LMH) is defined as the permeated volume at unit time and for a unit effective filtration area. This permeate flux was measured by timed collection using a graduated cylinder. When needed, samples for permeate and retentate were collected for protein and β-amylase assays. This spiral-wound pilot-plant set-up was used for both the first and the second UF operations. After each batch of experiment, the device should be cleaned with 0.04% (w/w) NaOH, 0.02% (w/w) EDTA, 0.05% (w/w) SDS, and de-ionized water respectively, to recover the UF membrane performance. Volume concentration ratio for the UF is defined as Equation (1):

$$VCR = V_F / V_C = V_F / (V_F - V_P) \quad (1)$$

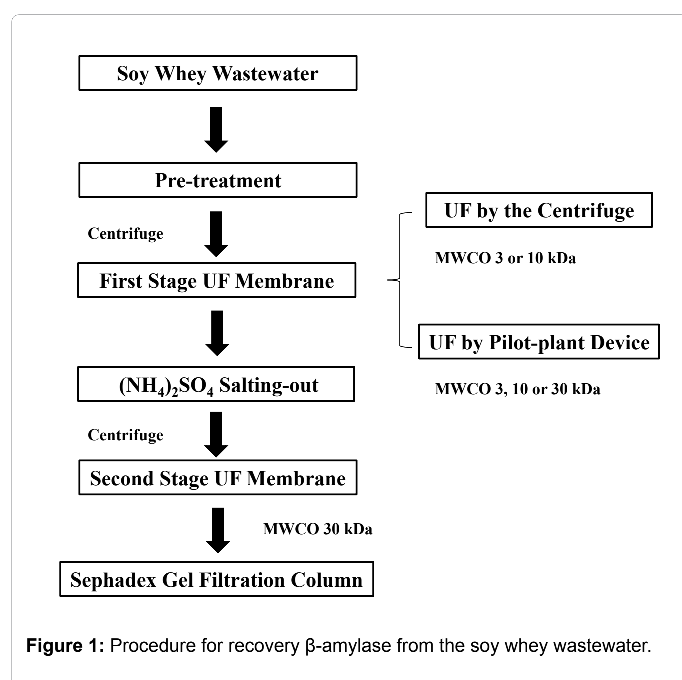

Figure 1: Procedure for recovery β-amylase from the soy whey wastewater.

$(NH_4)_2SO_4$ salting-out

A specified amount of $(NH_4)_2SO_4$ (Sinopharm Chemical Reagent Beijing Co. Ltd, Beijing, China) was added into the first stage UF retentate at a suitably concentration. The pH of this mixture was then adjusted to the range of 4-6.5. After settling for 3 h at 4°C, a supernatant and precipitate were obtained following 10 min centrifugation at ambient temperature and 3050 × g. Volume of the supernatant was then measured in a measuring cylinder following decanting. The precipitate was completely re-dissolved in a suitable volume of deionized water. Enzyme specific activity (as U /mg protein) and the enzyme recovery (in %) in the supernatant were obtained following measuring total protein and the β-amylase activity. This retentate was then adjusted to pH 7 and pumped into the second stage UF.

Further analytical purification

In order to confirm the abundant presence of the target enzyme in the final preparation, further chromatographic purification at analytical scale was operated using a Sephadex G-75 60 × 16 mm gel filtration column (Pharmacia). Sodium acetate buffer (pH 6.0), as mobile phase, was run at 0.5 ml/min flow rate and the eluent monitored at 280 nm for proteins [9].

β-amylase activity and protein concentration measurements

The β-amylase activity was measured following the method described earlier [10], which uses soluble starch as the substrate. One unit of β-amylase activity is defined as the amount of enzyme that liberates 1 μmol min^{-1} of maltose from soluble starch following 3-min incubation in 25 mM sodium acetate buffer at pH 4.8 and at 25°C. For this assay, 3,5-dinitrosalicyclic acid and soluble starch was from Sinopharm Chemical Reagent Beijing Co. Ltd (Beijing, China). Protein content was measured by the Kjeldahl method through nitrogen determination [11].

Sodium dodecyl sulfate-PAGE (SDS-PAGE)

The purity of samples for β-amylase was further assessed using SDS-PAGE on a 12% polyacrylamide gel under the reducing condition in a PhastSystem unit, with the gel being stained using Coomassie brilliant blue (Amersham Pharmacia Biotech). A number of "standard" proteins with molecular weights spanning from 14.3 to 97.2 kDa were used as calibrants [9]. Acrylamide, bis-acrylamide, low molecular weight protein markers, and the Bradford reagent for this assay were

Sample ID	Description of process steps	Exemplified volume (L)	β-amylase activity (U/ml)	Specific enzyme activity (U/mg protein)	Accumulated enzyme recovery (%)	Purification factor
Sample 1#	Soybean whey “wastewater” (pH 4.5, temperature 45°C, β-amylase biological activity 11 U/ml, protein 0.38 (w/w)%, total sugar 1.27%, ash content 12.5%, total solid 2.5%.	20	11	2.9	100	1.0
Sample 2#	Sample 1# adjusted to pH 7 and following centrifugation the supernatant filtered by 3 kDa MWCO UF to obtain retentate.	2	101	3.5	92	1.2
Sample 3#	Sample 2# salting-out using $(NH_4)_2SO_4$, pH adjusted to 4. The obtained centrifugal supernatant is pH further adjusted to 7, followed by 30 kDa MWCO UF to obtain retentate.	0.24	674	14	74	4.8

Table 1: An outline of the developed process towards preparing β-amylase from the soybean whey wastewater.

from BioRad (USA).

Statistical analysis

Throughout this work, all experiments were repeated for at least three times. The data were analyzed using Student’s t-test and the 95% confidence intervals were obtained. SPSS 18.0 for Windows was used in the statistical analysis.

Results and Discussion

An outline for the extraction and purification process developed in the present work can be grasped from the first and the second columns of Table 1. The thrust of this work was not on selecting a particular unit operation for achieving a desired purity, but on achieving an effective process technology using robust and preferably simple techniques.

The crude effluent unfortunately is turbid (Table 1), resulting from causes like, (a) prior to the generation of this wastewater, the soybean protein isolate is precipitated at pH 4.5, a typical PI value for the major soybean proteins, and so is prone to further precipitation for slightly shifted solubility of the same proteins; and (b) the total solid material is as high as 2.5% (Table 1). This situation means that, before feeding such effluent into the first UF device, a low-speed centrifugation was desired for removing solid materials and the UF feed pH is better off to be kept away from the pH 4.5 vicinity (Figure 2). Curve **a** of Figure 2A shows that, the recovered enzyme activity out of this centrifugal supernatant is invariably high irrespective of this effluent pH. So, a befit operation pH depended on the UF performance given below.

Effect of the first stage UF

The starting material (i.e. Sample 1#) effectively is the wastewater, and is a mixture of (a) the depleted soybean whey where the majority of proteins have been precipitated and, to a less extent, of (b) the aqueous washings out of the precedent soybean protein manufacturing process. Overall, components in this effluent are highly diluted. Either for enriching β-amylase or for removing the interfering impurity proteins or both, straightforward use of just one unit operation (such as precipitation) was perceived to be unrealistic. For this reason, our idea was to instate a concentration step for removing a proportionate amount of water, and consequently concentrate both the target enzyme and the impurity proteins.

To follow on introduction part above, UF operation appears to be the most appropriate for this type of applications. Notwithstanding of this, we faced a dilemma in adopting high or low MWCO UF membranes for this, mainly, concentration step. Conceivably, a high MWCO membrane offers advantages including high filtration flux and removal of certain impurity proteins. However, this conventional idea is not suitable for this pretreated clear soybean whey effluent. The target enzyme has a molecular weight ranging from 53-64 kDa [12], as to get exactly what MWCO membrane should be adopted for the first UF, the 3 kDa, 10 kDa and 30 kDa MWCO polyethersulfone membranes were prepared, the 40 kDa membrane was not suit for the retention of β-amylase, also it is less common. Curves **b-d** of Figure 3 show the instantaneous filtration flux with the 3 kDa, 10 kDa, and 30 kDa MWCO membranes in response to varied VCRs over the first UF operation, what surprised us was that the higher MWCO membranes did not offer higher flux but even worse, instead, the flux of 3 kDa membrane was the highest one. The reason is that, the protein molecular weight of the soybean whey effluent ranges from 14 to 97 kDa (Figure 4, lane 5), the main interfering impurity proteins have molecular weight less than 30 kDa, the key of which is Kunitz trypsin inhibitor, with a molecular weight of 21 kDa [1]. Hence, the usage of high MWCO UF membranes (i.e. 10-30 kDa, which were close to the impurity protein molecular weight) by such effluent was doomed to a terrible membrane fouling. For the enzyme activity recovery in the retentate, three sizes of UF membranes all had been invariably more than 90% even the VCR exceeded 9 (data not given), and the 3 kDa membrane showed a better enzyme activity recovery behavior. Obviously, the low MWCO (3 kDa) UF membrane was preferred for the first filtration.

The subsequent 3 kDa UF flux was very much dependent on the pH (Figure 2) [13]. At pH 5, 6, 7, 8 and 9, the stable permeate fluxes were 18, 32, 51, 30 and 29 LMH, respectively. These results unambiguously show that the feed pH for the first UF ought to be kept at a high value and certainly pH 7 was the one. Furthermore, Curve **b** of Figure 2A shows that, within the pH 4.5-9 range, the enzyme recovery in the retentate following the 3 kDa MWCO UF was more than 90% only when pH was around 6-7. Combining all these results, the crude soybean whey wastewater was best adjusted to pH 7 before the centrifugal supernatant was being fed to the first UF device.

Similarly, Figure 2C shows that the temperature impacts enzyme recovery significantly. This outcome verified that the 3 kDa MWCO membrane could be operated at ambient temperature.

$(NH_4)_2SO_4$ salting-out

The main purpose of the first UF operation was to concentrate the crude effluent while the second UF operation was for both concentration and purification. In view of the anticipated scale of the intended process, the membrane fouling problem of the second UF system had to be alleviated by removing the major interfering proteins through imposing an $(NH_4)_2SO_4$ salting-out step between the two UF

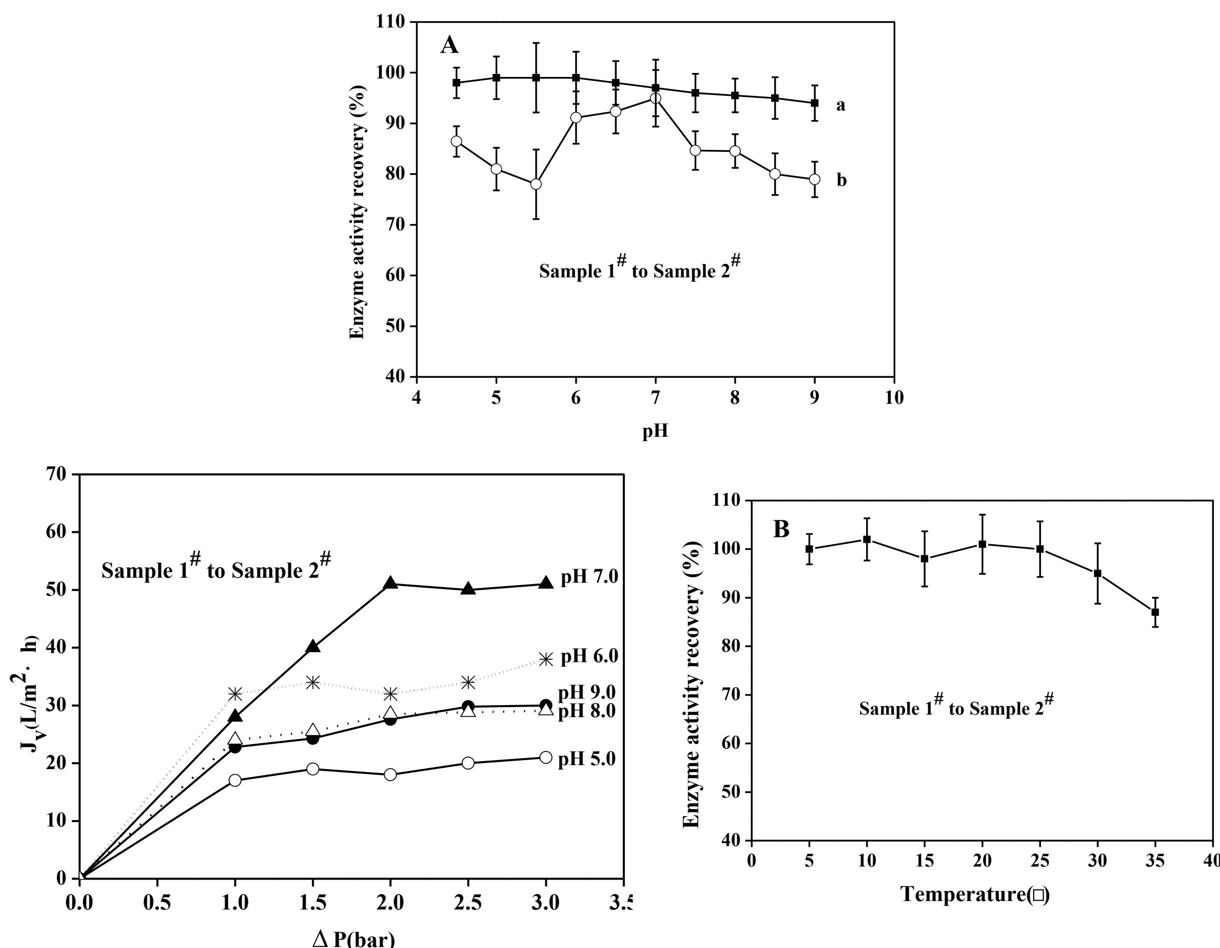

Figure 2: Effects of the crude effluent pH on both β-amylase recovery (as **A**) in the centrifugal supernatant out of the effluent (i.e. Sample 1#) (shown as **Curve a**) and that in the retentate out of the feed to the first 3 kDa MWCO centrifugal UF device (shown as **Curve b**), and the effect of temperature on β-amylase recovery (as **C**) in the retentate of the 3 kDa MWCO centrifugal UF device (the feed to this UF is the centrifugal supernatant of Sample 1# pre-adjusted to pH 7). The centrifuge for **Curve a of A** was at 25°C and 3050 × g for 10 min. With a loading volume of 15 ml, the centrifugal UF (for **Curve b of A**) was conducted at 3050 × g and 4°C for 20 min.Effects of the feed pH and UF operation pressure on the filtration flux at 25°C for 3 kDa MWCO membrane for the spiral-wound device for concentrating the pH adjusted crude soybean whey (i.e. for preparing Sample 2# from Sample 1#, denoted in Table 1).(▲) soybean whey at pH 5.0, (*) soybean whey at pH 6.0, (▲) soybean whey at pH 7.0, (▲) soybean whey at pH 8.0, (●) soybean whey at pH 9.0

operations.

The VCR for the first UF operation had been rationalized to the range of 3.5-10 (Figure 3). Figure 5A further illustrates that this ratio also affects the present $(NH_4)_2SO_4$ salting-out outcome. On one hand, for keeping the β-amylase recovery >90% in the centrifugal supernatant of the $(NH_4)_2SO_4$ precipitation step, the first UF operation should be controlled at VCR <9. On the other hand, an increment of this VCR invariably improved the enzyme purity in the $(NH_4)_2SO_4$-derived supernatant, showing removal of impurity proteins. This improvement is significant with VCR <6 and less significant in the 6-10 VCR range.

Considering compositional variation of the crude effluent, VCR ≅7 for the first UF operation was regarded as being appropriate for maximizing the salting-out efficiency. At this terminal point, the total protein concentration in the first UF retentate was 25 ± 3 g/ L and the β-amylase specific activity ca. 3.53 U/mg protein.

The Salting-out processing consists of, (a) adding $(NH_4)_2SO_4$ up to an appropriate concentration, (b) adjusting the phase system to an appropriate pH, and (c) allowing a settling time period ensued by the low-speed centrifugation. Figure 5B shows that the $(NH_4)_2SO_4$ concentration had two opposite effects: whilst increments of this concentration always reduce the enzyme recovery from the centrifugal supernatant, it also benefits from an improved β-amylase purity to some extent. In detail, when the $(NH_4)_2SO_4$ concentration exceeded 1.2M, the enzyme recovery out of the $(NH_4)_2SO_4$ centrifugal supernatant would drop lower than 90%. The effect for improving the enzyme purity (i.e. the enzyme specific activity) both in the <0.8M and >1.2M $(NH_4)_2SO_4$ concentration ranges are less significant compared to the

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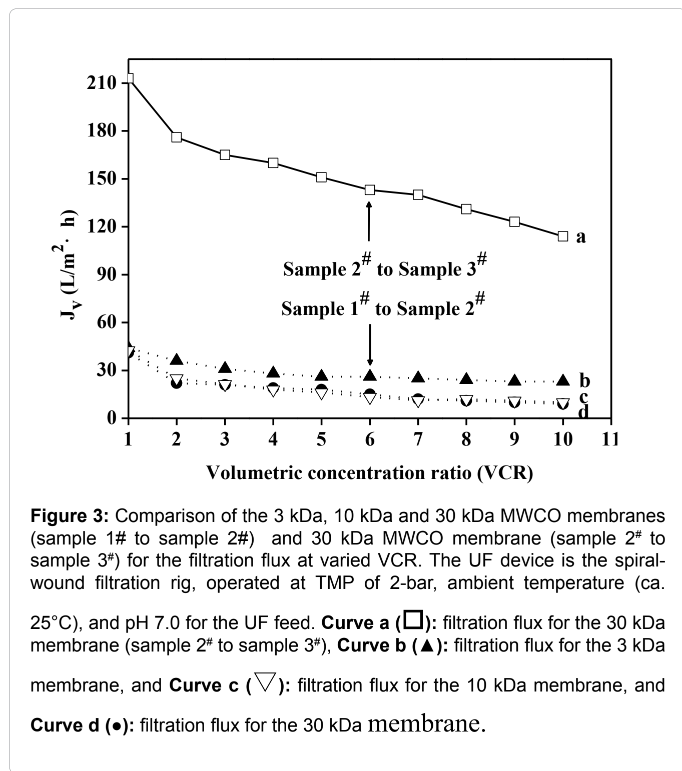

Figure 3: Comparison of the 3 kDa, 10 kDa and 30 kDa MWCO membranes (sample 1# to sample 2#) and 30 kDa MWCO membrane (sample 2# to sample 3#) for the filtration flux at varied VCR. The UF device is the spiral-wound filtration rig, operated at TMP of 2-bar, ambient temperature (ca. 25°C), and pH 7.0 for the UF feed. **Curve a (□):** filtration flux for the 30 kDa membrane (sample 2# to sample 3#), **Curve b (▲):** filtration flux for the 3 kDa membrane, and **Curve c (▽):** filtration flux for the 10 kDa membrane, and **Curve d (●):** filtration flux for the 30 kDa membrane.

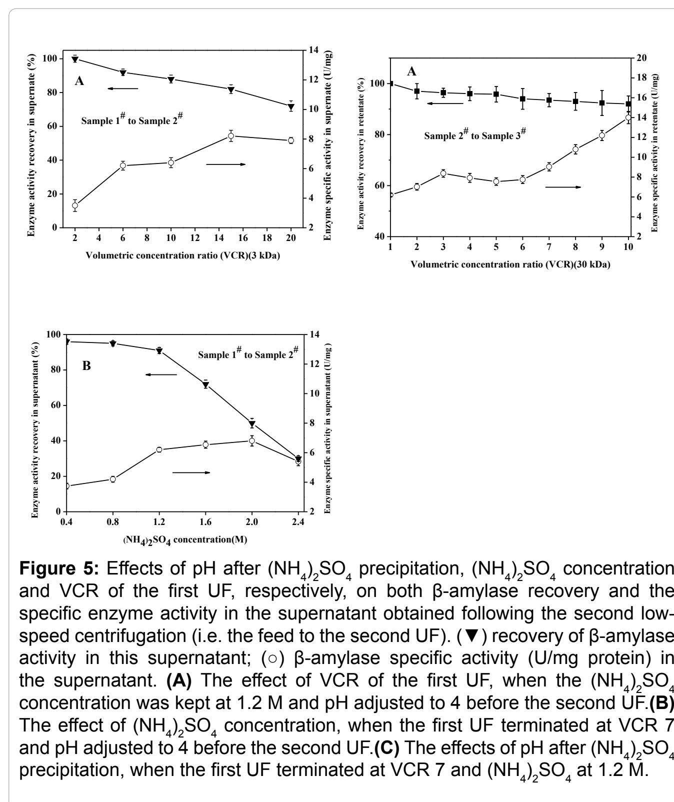

Figure 5: Effects of pH after $(NH_4)_2SO_4$ precipitation, $(NH_4)_2SO_4$ concentration and VCR of the first UF, respectively, on both β-amylase recovery and the specific enzyme activity in the supernatant obtained following the second low-speed centrifugation (i.e. the feed to the second UF). (▼) recovery of β-amylase activity in this supernatant; (○) β-amylase specific activity (U/mg protein) in the supernatant. **(A)** The effect of VCR of the first UF, when the $(NH_4)_2SO_4$ concentration was kept at 1.2 M and pH adjusted to 4 before the second UF.**(B)** The effect of $(NH_4)_2SO_4$ concentration, when the first UF terminated at VCR 7 and pH adjusted to 4 before the second UF.**(C)** The effects of pH after $(NH_4)_2SO_4$ precipitation, when the first UF terminated at VCR 7 and $(NH_4)_2SO_4$ at 1.2 M.

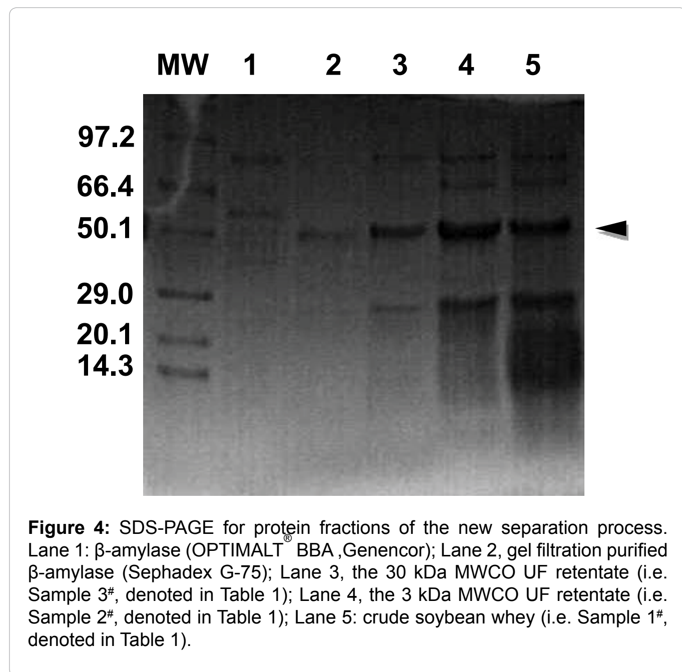

Figure 4: SDS-PAGE for protein fractions of the new separation process. Lane 1: β-amylase (OPTIMALT® BBA ,Genencor); Lane 2, gel filtration purified β-amylase (Sephadex G-75); Lane 3, the 30 kDa MWCO UF retentate (i.e. Sample 3#, denoted in Table 1); Lane 4, the 3 kDa MWCO UF retentate (i.e. Sample 2#, denoted in Table 1); Lane 5: crude soybean whey (i.e. Sample 1#, denoted in Table 1).

0.8-1.2M $(NH_4)_2SO_4$ concentration range. Consequently, selection of the $(NH_4)_2SO_4$ concentration at 1.2M was upheld.

The retentate of the first UF had a value of pH 7 and the subsequent addition of $(NH_4)_2SO_4$ reduced pH value to ca. pH 6. As PI values of the major interfering impurity proteins are in the pH 4-5 range (e.g. PI of Kunitz trypsin inhibitor is at pH 4.5 and that of lipid oxidase at pH 5.4) [1]), selection of an appropriate pH could preferentially precipitate impurity proteins and then enhance this salting-out effect. As shown in Figure 5C, pH had a trade-off effect between removal of impurity proteins and recovery of the enzyme. For instance, a low pH value favored removal of impurity proteins [as shown by increased β-amylase specific activity in Figurer 5C], but brought down the enzyme recovery in the subsequent centrifugal supernatant. Maintaining the enzyme recovery >90% in the supernatant would require controlling pH >4. In the pH 4-6.5 range, its beneficial effect on the enzyme purity can be divided into 2 sub-ranges: reduction of pH had a more marked effect in the pH 4-5 range, as compared with that in the pH 5-6.5 range. As an optimization, the pH of this slurry was adjusted to pH 4.

To summarize, with the first UF being terminated at ca. VCR=7, the 1.2M $(NH_4)_2SO_4$ salting-out step and the subsequent pH adjustment of this suspension to 4, our results gave the β-amylase activity recovery of 91% and the removal of ca. 60% impurity proteins.

The second UF operation

Before the $(NH_4)_2SO_4$ centrifugal supernatant was subjected to the 30 kDa UF (i.e. the second UF operation), its pH needed to be adjusted back from 4 to 7. The reason for this third pH adjustment was at least two-fold. First, the targeted β-amylase is most stable at ca. pH 7 and hence the final preparation ought to be kept at pH 7. Second, there had been a clear trend that UF flux tended to be higher at pH 7 as compared with other pH (Figure 2B).

For the subsequent 30 kDa MWCO membrane (i.e. the second UF), Figure 6 shows that the β-amylase recovery in the retentate had no longer imposed any restrictions on this second VCR termination point. Under this situation, this VCR termination point was determined by considering the effect of the second VCR on the UF flux and the enzyme purity in the second UF retentate. Figure 6 shows that, in the VCR 3-6 range, an increment of this ratio almost had no effect on the

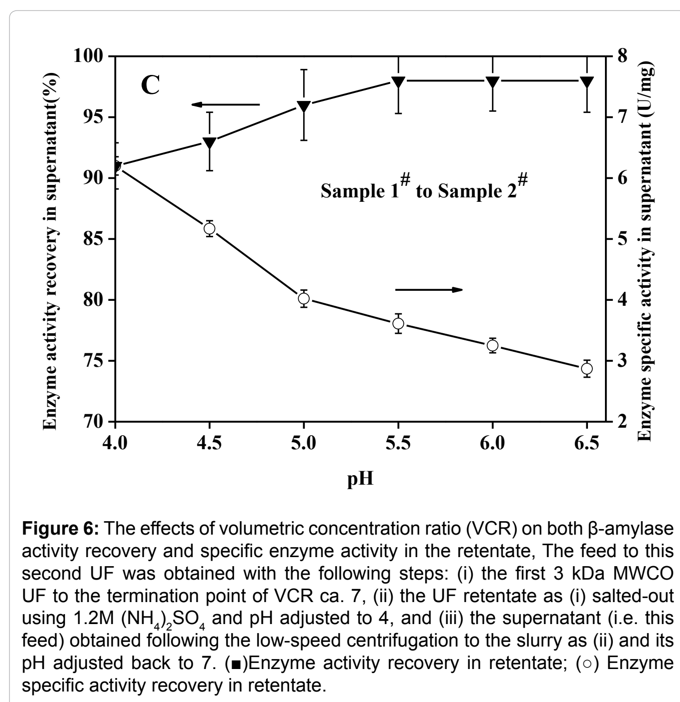

Figure 6: The effects of volumetric concentration ratio (VCR) on both β-amylase activity recovery and specific enzyme activity in the retentate, The feed to this second UF was obtained with the following steps: (i) the first 3 kDa MWCO UF to the termination point of VCR ca. 7, (ii) the UF retentate as (i) salted-out using 1.2M $(NH_4)_2SO_4$ and pH adjusted to 4, and (iii) the supernatant (i.e. this feed) obtained following the low-speed centrifugation to the slurry as (ii) and its pH adjusted back to 7. (■)Enzyme activity recovery in retentate; (○) Enzyme specific activity recovery in retentate.

enzyme purity, but when VCR >6, an increasing of VCR almost linearly augmented the enzyme purity. We had no intention to go over any VCR >10 termination points, as our intended product for this VCR value had already exceeded the specification for such a product, with an enzyme activity of ca. 674 U/mL, the enzyme specific activity of 14 U/mg and recovery for the enzyme activity of 92%. These results compare favorably with a leading product by Genencor, OPTIMALT® BBA, and the measured corresponding results being 237 U/mL for an enzyme activity and 2.75 U/mg for an enzyme specific activity. Using filtration flux of the second UF as a check, from VCR 8 to 10, the flux reduced by only 15 LMH whereas from VCR 10 to 12 this flux declined by 41 LMH (Curve **a** of Figure 3). Combining all these results, selection of VCR 10 for the second UF operation not only exceeded the specification for the intended product, but also was fully justifiable on the bioprocess ground.

The associated biochemical analyses

SDS-PAGE on samples 1, 2 and 3 (Table 1) shows that the soybean whey proteins have molecular weights in the range from 14 to 97 kDa (Figure 6). Cereal β-amylases are monomeric proteins with molecular weights in the range of 53-64 kDa [12]. As illustrated in Figure 4, the single band at about 50 kDa in lane 2 could be confirmed to be the target β-amylase.

The maximal activity of this target β-amylase appears to be at ca. pH 5.5, and this compares consistently with those in the literature [14]. For instance, the maximal activity for a purified soybean amylase is at pH 6.0 [15], that for a β-amylase from the bulbs of *G. klattianus* at pH 5.5 [9], that for a β-amylase from malted African finger millet (*Eleusine coracana*) seed at pH 5.0 [16].

The maximal enzyme activity for the presently target β-amylase appears to be at 70°C and is also broadly in agreement with the published results. A soybean β-amylase displays highest enzyme activity at 70°C [15]. A microbial β-amylase extracted from *Bacillus polymyxa* 26-1 possesses maximal β-amylase activity at 45°C and that from *Clostridium thermocellum* SS 8 at 60°C [17].

Furthermore, the results demonstrate that the presently targeted soybean amylase is stable in the pH range within 5-7.5, and it has a higher enzyme activity at the temperature range of 55-70°C. However, this β-amylase may well be thermally stable up to 60°C, but over this temperature its activity tends to become permanently lost in a rather sharp fashion.

Conclusions

Compared to the present dilemmas on the soybean protein manufacturing wastewater and/or to the reported technologies on separating β-amylase or comparable products from soybean, the presently developed process entails a combination of two UF operations and $(NH_4)_2SO_4$ precipitation to extract and partially purify the β-amylase. This proof-of-principle process technology can potentially turn the troublesome large-volume soybean whey wastewater into a profitable product, β-amylase.

In summarize this novel application of hybrid UF membrane process technology comprises of two UF operations and an $(NH_4)_2SO_4$ salting-out step. Apart from UF, which requires only simple equipment and only low cost chemicals? The arrangement for the two UF operations has been such that membrane-fouling problem is considerably minimized. It will be surprising if a more competitive process technology exists at present time. It is therefore our adamant belief that this new process technology can not only positively impact on this sector of food industry, but also establish itself as a model approach for panoply of other protein products in industry.

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References

1. Sorgentini DA, Wagner JR (1999) Comparative study of structural characteristics and thermal behavior of whey and isolate soybean proteins. J Food Biochem 23: 489-507.
2. Zhu GF, Li JZ, Wu P, Jin HZ, Wang Z (2008) The performance and phase separated characteristics of an anaerobic baffled reactor treating soybean protein processing wastewater. Bioresour Technol 99: 8027-8033.
3. Yamasaki Y (2003) Beta-amylase in germinating millet seeds. Phytochemistry 64: 935-939.
4. Qi JC, Zhang GP, Zhou MX (2006) Protein and hordein content in barley seeds as affected by nitrogen level and their relationship to beta-amylase activity. J Cereal Sci 43: 102-107.
5. Li Z, Youravong W, H-Kittikun A (2006) Separation of proteases from yellowfin tuna spleen by ultrafiltration. Bioresour Technol 97: 2364-2370.
6. Enevoldsen AD, Hansen EB, Jonsson G (2007) Electro-ultrafiltration of amylase enzymes: Process design and economy. Chem Eng Sci 62: 6716-6725.
7. Mireles D, Morrissey MT (2002) Parameters for the recovery of proteases from surimi wash water. Bioresour Technol 81: 241-247.
8. Powell MJ, Timperman AT (2005) Quantitative analysis of protein recovery from dilute, large volume samples by tangential flow ultrafiltration. J Memb Sci 252: 227-236.
9. Dicko MH, Searle-van Leeuwen MJF, Hilhorst R, Traore AS (2000) Extraction, partial purification and characterization of β-amylase from the bulbs of *G. klattianus*. Bioresour Technol 73: 183-185.
10. Bernfeld P (1955) Amylases α and β. In: Colowwick SP, Kaplan N (eds). Methods in Enzymology. Academic Press, New York, USA, pp. 149-158.
11. Bourseau P, Vandanjon L, Jaouen P, Chaplain-Derouiniot M, Massé A, et

al. (2009) Fractionation of fish protein hydrolysates by ultrafiltration and nanofiltration: impact on peptidic populations. Desalination 244: 303-320.

12. Ziegler P (1999) Cereal Beta-Amylase. J Cereal Sci 29: 195-204.
13. Almécija MC, Ibánez R, Guadix A, Guadix EM (2007) Effect of pH on the fractionation of whey proteins with a ceramic ultrafiltration membrane. J Membr Sci 288: 28-35.
14. Kumar RSS, Vishwanath KS, Singh SA, Rao AGA (2006) Entrapment of a-amylase in alginate beads: Single step protocol for purification and thermal stabilization. Process Biochem 41: 2282-2288.
15. Li, J, Lin YS, Kuo ST, Jiang CM, Wu MC (2009) Purification of soybean amylase by superparamagnetic particles. Food Chem 117: 94-98.
16. Kolawole AO, Ajele JO, Sirdeshmukh R (2011) Purification and characterization of alkaline-stable-β-amylase in malted African finger millet (*Eleusine coracana*) seed. Process Biochem 46: 2178-2186.
17. Swamy MV, Sai Ram M, Seenayya G (1994) β-Amylase from *Clostridium thermocellum* SS 8-a thermophillic, anaerobic, cellulolytic bacterium. Letters in Applied Microbiology 18: 301-304.