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Proteins from common bean (*Phaseolus vulgaris*) seed as a natural coagulant for potential application in water turbidity removal

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ABSTRACT

The ability of coagulation active proteins from common bean (*Phaseolus vulgaris*) seed for the removal of water turbidity was studied. Partial purification of protein coagulant was performed by precipitation with ammonium sulphate, dialysis and anion exchange chromatography. Adsorption parameters for ion-exchange process were established using dialysate extract. Results revealed that the highest values of the adsorbed protein were achieved in 50 mmol/L phosphate buffer at pH 7.5 and the maximum adsorption capacity was calculated to be 0.51 mg protein/mL matrix. Partially purified coagulant at initial turbidity 35 NTU expressed the highest value of coagulation activity, 72.3%, which was almost 22 times higher than those obtained by crude extract considering applied dosages. At the same time, the increase in organic matter that remained in water after coagulation with purified protein coagulant was more than 16 times lower than those with crude extract, relatively to its content in blank.

1. Introduction

Coagulation/flocculation as a step in water treatment processes is applying for removal of turbidity in raw water that comes from suspended particles and colloidal material. Materials that are used in this stage of water treatment can be inorganic coagulants, synthetic organic polymers or coagulants from natural sources. Aluminium sulfate (alum) is a common coagulant globally used in water treatment. In spite of its undoubtfull effusiveness in turbidity removal, alum increases concerns towards ecotoxicological impact when introduced into the environment as post-treatment sludge having large volumes. Regarding the application of synthetic polymers, the presence of residual monomers is undesirable because of their neurotoxicity and strong carcinogenic properties (Mallevialle et al., 1984).

A part of possible solution of these problems might be development of new coagulants, preferably from natural and renewable sources, which have to be safe for human health as well as biodegradable. Because their production relies on local materials, renewable resources and food grade plant material, and is relatively inexpensive, it can contribute to achieving sustainable water treatment technologies. By using natural coagulants considerable savings in chemicals and sludge handling cost may be achieved along with production of readily biodegradable and less voluminous sludge that amounts only 20–30% that of alum treated counterpart (Narasiah et al., 2002).

In recent numerous studies variety of plant materials as a source of natural coagulants has been reported (Raghuwanshi et al., 2002; Diaz et al., 1999; Miller et al., 2008) but the most studied is *Moringa oleifera* whose efficiency has been reported for turbidity removal (Ndabigengensere and Narasiah, 1998; Okuda et al., 2001a; Ghebremichael et al., 2006) as well as antimicrobial properties (Ghebremichael et al., 2005). Apart of all previously mentioned preferences of using natural coagulants instead of synthetic ones, major disadvantage of their application as crude extracts in water treatment is an increase of organic matter in water. This complicates further processing and adversely affects water quality but could be overcome by purification of the coagulant (Ghebremichael et al., 2006).

During the course of plants' screening program in our laboratory (Šćiban et al., 2005, 2009), crude extract from common bean (*Phaseolus vulgaris*) seed showed the ability to act as a natural coagulant. Seed from common bean as potential source of coagulant for water treatment would be promising considering its food grade nature. Moreover, it offers a few advantages over *M. oleifera* seed – because of no oil present in it there is no need for extraction by organic solvents thus avoiding delipidation step which is beneficial for both economic and environmental reasons.

The objective of the study was partial purification of the coagulation active components extracted from common bean seed. Optimal conditions for ion-exchange chromatographic purification of coagulant protein regarding the process of adsorption were established. In addition, application of the partially purified common bean coagulant was evaluated as well as its suitability in comparison to crude extract regarding organic load.

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2. Methods

2.1. Extraction of active component from common bean seed

The locally obtained common bean (*P. vulgaris*) dry seed was ground to a fine powder by using a laboratory mill and sieved through 0.4 mm sieve. The fraction with particle size less than 0.4 mm was used in experiments. Fifty grams of seed powder was suspended in 1 L of 0.5 mol NaCl/L water. The suspensions were stirred using a magnetic stirrer for 10 min to accomplish extraction and then filtered through a rugged filter paper (Macherey-Nagel, MN 651/120) to obtain filtrates – crude extracts of active component.

2.2. Precipitation of active component

The coagulation active component from common bean was further processed by precipitation and dialysis. Crude extracts were saturated to 80% by addition of $(NH_4)_2SO_4$ and centrifuged at 4000*g* (5804-R, Eppendorf) for 10 min. Precipitate was redissolved in 10 mmol/L appropriate buffer (i.e. some of the buffers listed in the following text) and dialysed overnight at 4 °C against Millipore water in dialysis bag with molecular cut-off 12–14 kDa.

2.3. Adsorption studies

Adsorption studies were conducted using dialysate extracts obtained above in series of buffers in batch ion-exchange (IEX) experiments with Amberlite™ IRA 900 Cl (Rohm and Haas) as matrix. Amberlite™ IRA 900 Cl is a macroreticular polystyrene type 1 strong base anion exchange resin containing quarternary ammonium groups whose shipping weight is 700 g/L and total exchange capacity $\geqslant$ 1.00 eq/L (Cl^- form). In order to find the optimum pH of the buffer for the adsorption, dialysate extract was diluted in universal Britton and Robinson (I) buffer having pH from 7 to 9 with an increment of increase of pH 0.5. The choice of the buffering substance was made by measuring the amount of bound protein in phosphate, Tris–HCl or ammonium acetate buffer at pH 7.5. The effect of ionic strength of the buffer on the adsorption of active compounds to anion exchange resin was evaluated by varying concentration of phosphate buffer (10, 25, 50, and 100 mmol/L) at pH 7.5.

In order to estimate optimum volume of IEX matrix, adsorption experiments were carried out by adding 0.33 mg dialysate extract in phosphate buffer (50 mmol/L, pH 7.5) to different volumes of the matrix ranging from 0.33 mL to 1.0 mL.

2.4. Kinetic studies and adsorption isotherm

Kinetics of adsorption was studied using 3.3 mg dialysate extract in 10 mL phosphate buffer (50 mmol/L, pH 7.5). Protein solution was added to 10 mL IEX matrix and mixed in magnetic stirrer at 100 rpm. Samples were collected in certain time intervals, centrifuged immediately and supernatants were analysed for protein content. Blanks were carried out without matrix to check out if any measurable loss of protein came out from reasons other than its adsorption to matrix.

Adsorption capacity of the matrix was estimated using 1.78–6.44 mg dialysate extract in 10 mL phosphate buffer (50 mmol/L, pH 7.5). Protein solution were added to 10 mL of IEX matrix and mixed at 100 rpm for 90 min at room temperature. After that unadsorbed protein concentration was measured and amount of adsorbed protein was calculated from a mass balance.

Maximum adsorption capacity of the matrix and the dissociation constant of the adsorption were determined form the Langmuir adsorption model (Faust and Aly, 1987):

$$\frac{C_e}{q_e} = \frac{1}{b \cdot X_m} + \frac{C_e}{X_m}, \quad (1)$$

where C_e is the concentration of protein in solution in equilibrium (mg/mL); q_e is the amount of protein adsorbed per volume of adsorbent (mg/mL); b is a constant that is related to the enthalpy of adsorption (mL/mg) and X_m is the maximum adsorption capacity (mg/mL).

2.5. Purification of active component

Dialysate extract was loaded onto column (10 mm × 150 mm glass column) packed with 10 mL of Amberlite™ IRA-900 Cl previously equilibrated with 50 mol/L phosphate buffer, pH 7.5. Active components were eluted from resin by linear gradient of ionic strength of NaCl solution from 0 to 1 mol/L at a flow rate 1 mL/min. Protein content and coagulation activity of fractions (2 mL) were determined.

2.6. Preparation of turbid water

Turbid water for coagulation tests was prepared by adding 1 g kaolin to 1 L tap water. The suspension was stirred for 1 h to achieve uniform dispersion of kaolin particles, and then it was allowed to remain for 24 h for completing hydration of the particles. This suspension was used as the stock suspension. Turbid water with 50 mg/L kaolin (about 35 nephelometric turbidity units – NTU) was prepared by diluting 50 mL of stock suspension to 1000 mL tap water just before the coagulation test. The initial pH of the synthetic water was adjusted to 9.0 with 1 mol/L NaOH solution, in accordance with previous investigations (Okuda et al., 2001a; Šćiban et al., 2005).

2.7. Coagulation test

Coagulation activity of the fraction eluted from column as well as crude extract was evaluated in jar tester VELP, model FC6S. Samples were added to the beakers at different dosages (0.5, 1.0 or 2.0 mL/L turbid water) and the content was stirred at 200 rpm for 2 min. The mixing speed was then reduced to 80 rpm and was kept for 30 min. Then, the suspensions were left to allow sedimentation. After 1 h of sedimentation, an aliquot of 100 mL of clarified sample was collected from the top of the beaker and residual turbidity was measured. The residual turbidity of sample was RT_S. The same coagulation test was performed with no coagulant as the blank. The residual turbidity in the blank was RT_B. Coagulation activity was calculated as:

$$\text{Coagulation activity } (\%) = \frac{RT_B - RT_S}{RT_B} \times 100. \quad (2)$$

2.8. Analytical methods

Protein concentration was measured according to Bradford (1976) with bovine serum albumin as standard. Turbidity was measured using a turbidimeter (TURB 550 IR) and it was expressed in nephelometric turbidity units (NTU). The amount of organic matter released from common bean seed crude extract and partially purified protein were determined as chemical oxygen demand (COD) according to Standard Methods (APHA, 1998).

All experiment were run in duplicate (the accuracy is considered to be ±5%) and the mean value is presented herein.