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Partition of tannery wastewater proteins in aqueous two-phase poly (ethylene glycol)-magnesium sulfate systems: Effects of molecular weights and pH

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Abstract

The partitioning behavior of soluble proteins from tannery wastewater using aqueous two-phase system (ATPS) was investigated. An ATPS polyethylene glycol (PEG)/$MgSO_4$ was examined with regard to the effects of PEG molecular weight (MW) and concentration, $MgSO_4$ concentration, pH and NaCl concentration on protein partition and extraction. The partition coefficients measured for soluble proteins were proportional to the difference in PEG concentration between the phases. The MW and concentration of PEG were found to have significant effects on protein partition and extraction with low MW PEG4000 showing the best conditions for the partitioning of protein in PEG + $MgSO_4$ + water system. Sulfate salt was chosen as the phase-forming salt because of its ability to promote hydrophobic difference between the phases. This system was operated at room temperature (30 °C). Increase in pH of the system increases the partition coefficient of proteins from tannery wastewater. The addition of sodium chloride showed significant influence on the partition coefficient. ATPS comprising PEG4000-magnesium sulfate provided a means for the recovery of proteins from tannery wastewater. The maximum percentage yield of protein extracted is 82.68%.

Keywords: Aqueous two-phase systems; Globular proteins; Polyethylene glycol; Bovine serum albumin; Partition coefficient; Magnesium sulfate

1. Introduction

Conventional leather processing involves chemical treatment of the biological matrix. This leads to the removal of non-fibrous proteins from the skin matrix (Heidemann, 1993). The amount of non-fibrous proteins namely albumins and globulins present in the skin/hide matrix is 1.5–2.0% of the weight of the skins/hides (Kemp and Stainsby, 1981). These globular proteins are discharged as liquid wastes. This liquid waste also contains debris like degraded products of proteoglycans and fibrous proteins. Globally, nine million metric tons of skins/hides are being processed annually (Thanikaivelan et al., 2005). It is estimated that about 100 metric tons of globular proteins are removed during leather processing, globally. The estimated value of this protein is around 52 billion US $. The presence of these proteins significantly increases the biochemical oxygen demand (BOD) and chemical oxygen demand (COD) level in the liquid wastes (Saravanabhavan et al., 2005; Aloy et al., 1976). These globular proteins have potential application in food and biopharmaceutical industries (Muller, 1973; Rito-Palomares and Middelberg, 2002; Rito-Palomares, 2004). Hence, there is scope for the recovery of valuable material from the tannery wastewater.

Biotechnology and recombinant DNA technology have made an advance in the production of a large number of new proteins important to the food, pharmaceutical, medical, and chemical industries (Rito-Palomares and Middelberg, 2002; Rito-Palomares, 2004; Deepa et al., 2003). In most cases the proteins of interest are present in a highly complex mixture of contaminated proteins, cell wall material, and nucleic acids. Separation from these complex mixtures is further complicated by the labile nature of the desired products and stringent specifications of the final product purity. Usually, downstream process accounts for 50–80% of the total production costs of proteins (Albertsson, 1986; Walter et al., 1985; Albertsson et al., 1990). Thus, separation and purification of proteins is

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a critical element of modern process biotechnology because it provides a vital link between laboratory discoveries and large-scale productions. Conventional methods used for protein purification are usually expensive and difficult to scale up. In addition, liquid two-phase systems consisting of immiscible aqueous and organic solvents are not usually suitable for extraction of proteins where maintenance of biological activity is required (Zaslavsky, 1995; Hustedt et al., 1985). Hence, in recent years there has been an ongoing interest in the biotechnology for the development of innovative separation and purification methods that are both economically viable and gentle enough to preserve biological activity of proteins. Aqueous two-phase system (ATPS) is one of the novel techniques to provide secured separation and purification of bio-molecules (Veide et al., 1983; Huddleston et al., 1996).

ATPS is composed of two different polymers or one polymer and one salt are mixed at certain concentrations in an aqueous solution. The solution separates into two immiscible phases, with each dissolved component predominating in one or the other phase with water as a solvent in both phases (Albertsson, 1986). Due to higher water content, the ATPSs have several advantages as compared to the commonly used separation and purification techniques (Zaslavsky, 1995). ATPS have low interfacial tension, are safe, non-toxic, non-flammable and biocompatible. ATPS also has the advantage that bio-molecular degradation is low in it. They also show high resolution, have relatively high capacity and are easy to scale-up. Therefore, this system can provide an innocuous environment for the separation and purification of biomaterials (Diamond and Hsu, 1992; Huddleston et al., 1991).

A wide variety of biological systems, such as proteins, nucleic acids, microorganisms, animal and plant cells, have been successfully separated using ATPS (Silva and Franco, 2000). The partitioning of biological materials is a result of van der walls, hydrophobic, hydrogen bond, and ionic interactions of the biomolecules with the surrounding phase. Therefore, the partition coefficient is influenced by many factors, including the concentrations and molecular masses of polymer, type and concentration of added salts, temperature and pH (Lebreton and Lyddiatt, 2000; Su and Chiang, 2006). The polymer–salt systems have certain advantages than the polymer–polymer systems. The most commonly studied aqueous two-phase polymer–polymer system, which is composed of PEG and dextran, is very expensive because of the high cost of dextran. The PEG–salt system is relatively inexpensive and easy to attain the phase equilibrium. Hence PEG–magnesium sulfate salt system has been selected for the separation and purification of proteins from the tannery wastewaters. The sulfate salt system was chosen as the phase-forming salt because of its ability to stabilize the hydrophobic interactions as seen from the Hofmeister series (Cacace et al., 1997). The PEG–magnesium sulfate ATPS is a better choice for protein partitioning due to dissimilarities in both the phase-forming components, resulting in high ΔG^{CH_2}. The magnesium sulfate salt is mildly buffering and therefore produces solutions of different concentrations varying slightly in pH (Eiteman and Gainer, 1990).

In this work, an attempt has been made to recover the globular proteins from tannery wastewater employing a PEG–magnesium sulfate salt based ATPS. The effect of polymer molecular weights, polymer and salt concentrations, addition of sodium chloride and pH on the partitioning behavior of protein have been investigated.

2. Experimental section

2.1. Materials

Polyethylene glycol with molecular weights of 4000, 6000 and 10 000 was obtained from Merck-Schuchardt (Munich, Germany) and bovine serum albumin (BSA) was purchased from Sigma (St. Louis, MO, USA). Magnesium sulfate ($MgSO_4.7H_2O$) was obtained from Merck-Schuchardt (Munich, Germany). All chemicals were of analytical grade. The polymer and salts were used without further purification. Millipore water was used throughout the experiments. Spent lime processed wastewater was collected from commercial tanning unit in India.

2.2. Preparation of sample

A known weight of green raw skin/hides was taken for alkali operation. In this stage most of the non-fibrous proteins are removed as a waste. This sectional stream wastewater was collected, filtered and neutralized before taking for extraction. This wastewater was used for preliminary investigations on partitioning of the soluble proteins present in the tannery wastewater.

2.3. Preparation of aqueous two-phase systems

ATPSs were prepared from stock solutions of poly (ethylene glycol) of molecular weight 4000, 6000 and 10 000 of about 40%w/w and magnesium sulfate of about 30%w/w were prepared. A known amount of 40%w/w PEG (desired molecular weight) solution was taken into a glass jacketed vessel of volume 50 cm^3 for the experimental determination of liquid–liquid equilibrium (Gonzalez-Tello et al., 1996). The glass vessel was provided with an external jacket in which water at constant temperature (30 °C) was circulated using a thermostat. The temperature was controlled to within ±0.05 °C. The binodal curves were determined using the turbidity method (Eiteman and Gainer, 1990; Alves et al., 2000). The determination of the tie-lines involve preparation of the feed samples (about 20 cm^3) by mixing appropriate amounts of polymer, salt and water in the vessel. The thermostat was set at constant temperature and the sample was stirred for 1 h. Then the mixture of PEG and salt was allowed to settle for 24 h. After separation of the two-phases (PEG rich top phase and salt rich bottom phase), the concentration of PEG with desired molecular weight in top and bottom phases was determined using refractive index measurements (Salabat, 2001). The concentration of magnesium sulfate in the top and bottom phase of the PEG + magnesium sulfate + water system were determined by