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RECOVERY OF PROTEIN & MICROBIAL CELLS USING ADSORPTIVE BUBBLE SEPARATION TECHNIQUE

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Abstract:

Separation of various chemical components from each other is often the most difficult step in analytical procedures. The problems attached to separation become further magnifiedwhen the species concentrations are extremely low. A group of techniques that has proven useful especially in dilute solutions for separating and concentrating metallic as well as non-metallic ions and complexes, protein microorganisms, particulates, etc. is the adsorptive bubble separation techniques.The success or these processes is primarily dependent upon differences in the natural surface activity of various species or particulates in the system or in their tendency to associate with surfactants. The efficiency of the process is determined by such variables as solution pH,ionicstrength, concentration or various activating and depressing agents, and temperature.A proper control or variables offers an opportunity to recovery of proteins and microbial cells..In this paper the principles governing recovery of proteins and microorganisms from cultivation by foam flotation along with the calculation of mass balance in the continuous foam separation to achieve complete removal of proteins from microbial cells.

Keywords:

Protein,Foam flotation, Absorptive Bubble Surfactant.

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1. Introduction:

Adsorptive bubble separation technique, the generic name was first proposed by Lemlich(1966). This technique is based on differences in surface activity. Material, which may be molecular colloidal, or macro particulate in size is selectively adsorbed or attached at the surface of bubbles rising through the liquidand is thereby concentrated or separated. A substance, which is not surface active itself, can often be madeeffectively surface active through union with or adherence to a surface-active collector. The substance is so name is termed ascolligend.This extends Adsorptive Bubble Separation method applicable to a quite wide range of biological products;furthermore, high separation efficiency can be obtained by this process in the early stages of downstream purification regime.

1.1 Mode of operation:

a) Simple Batch
b) Simple Continuous Flow
c) Continuous Flow Enriched by Reflux
d) Continuous Flow Stripping
e) Combined Enriching and Stripping
f)Staged Operation

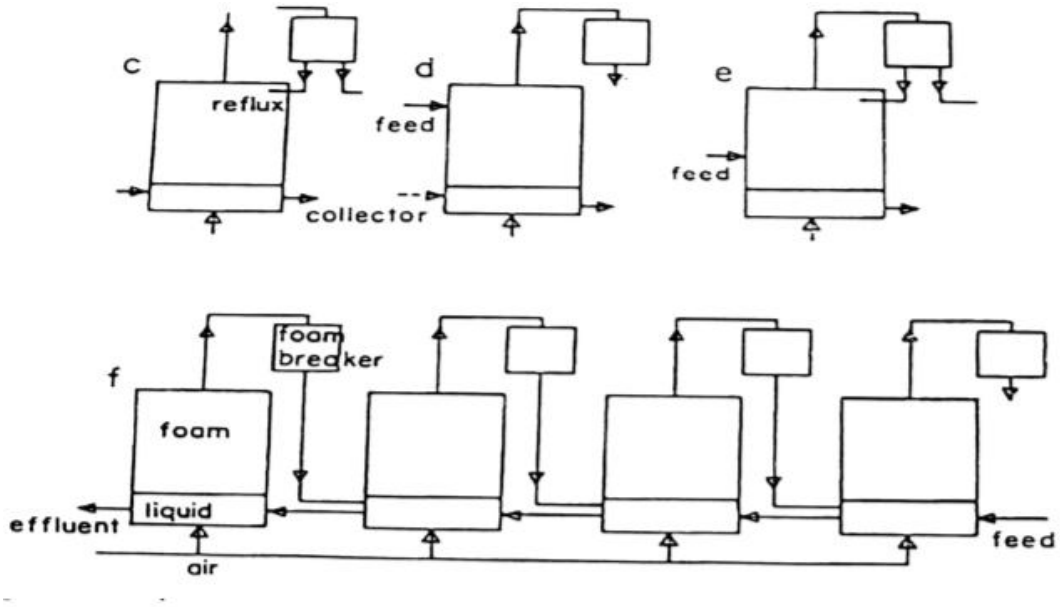


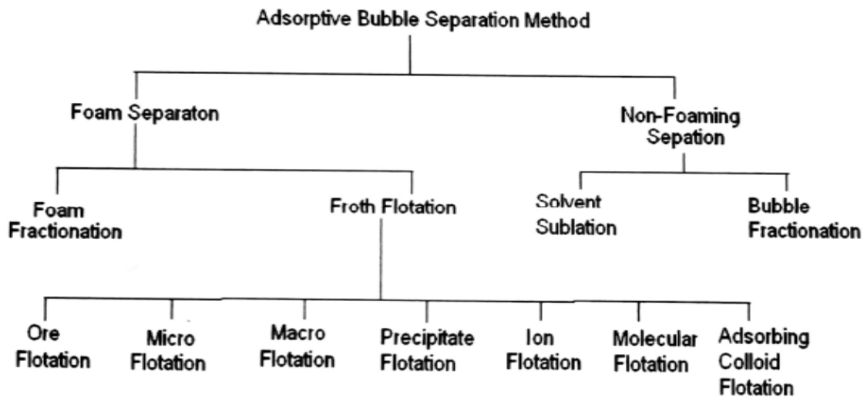
FIG. 1, Different types of mode of operation in ABSM

1.2 Potential area of application:

- Foam fractionation for separation of enzymes, proteins of biological materials.
- Recovery of protein and microorganism from cultivation medium.
- Separation of fatty acids.
- Separation of organic dyes.
- Foam fractionation in protein skimming.
- Separation of drug components or purification of drugs from a mixture of components.
- Separation of enantiomeric drugs mixture.
- Separation of chemical constituents from plant source.

2. Literature survey:

In the year of 1972 Lemlich M [1]edited a book on adsorptive bubble separation technique, in which he discussed the principle of column operation and different types of technique like ion flotation , precipitation, mineral flotation, macro flotation, bubble fraction and solvent sublation etc.



In the year 1995,Anand.K[2] et al.,studied the Kinetic adsorption of Lysozymes and BovineSerum Albumin (BSA) at the air water interface from binary mixture.They showed that the increase in BSA concentration in bulk phase caused a decrease in the extent of adsorption of lysozymes and an exponential decrease in its apparent diffusion coefficient,the lag time for its adsorption,however decreased progressively with the increasing bulk concentration of BSA.The ratio of BSA to Lysozyme in the mixed monolayer increased with increasing in the ratio of BSA to Lysozyme in the bulk phase.However the unit cell dimensions occupied byBSA and Lysozyme in the mixed monolayer were the same as those in single component monolayer, suggesting that both BSAand Lysozyme adsorbed independently and the thermodynamic state of one protein was not modified by other protein in the interface.

In the year 1998,Brown, A.K[3],investigated the foam separation of different protein mixture like β-casein:Lysozyme,BSA:Lysozyme and β-casein:BSA.The foaming device usedfor the investigation

consists of glass,having height 0.51m,with a flat plate sparger of perforated ceramic plate 2cm in diameter with 20 evenly distributed 0.1cm diameter holes.Humidified N2 gas was passed through the base of column via sparger at the rate of 600 cc/min,the foam height was 0.34m.The result revealed that the enrichment ratio in the foam was found to decreased when the concentrations of proteins in the feed solution was increased.

The other process variables were gas flow rate, pH, feed flow rate, column height,foam height,shape and size of flat sparger.

In the year 2000, Schugerl[4] had studied the recovery of proteins and microorganisms from cultivation media by foam floatation method. He also presented a clear description of foaminess of proteins and microbial cell cultivation system in his paper. He investigated the recovery of Bovine serum Albumin (BSA), a globular protein from aqueous solution by foam flotation. The foaming device used consists of a thermostated column with 23mm internal diameter, 49cm bubbling liquid height and 30 cm from layer.

In the year 2001, S Bhattacharya [5] and co-worker studied of the separation of proteins from multicomponent mixtures by a semi-batch foaming process. Their experiences was conducted to obtained the values of the average bubble size, gas holdup, interfacial area, the bulk phase concentration and the heat desorption (which determines the concentration of absorbed components) in the liquid solution using a 75mm diameter, 530mm long glass column fitted with stainless steel sparger for bubble generation. Here the mass transfer coefficient was determined from the analysis of concentration of foam and feed at times using the above

mentioned parameters.

In the year 2003, Darton [6]and his co-worker worked on the development of a multistage foam fractionation column. Here, surface-active materials stabilize foam by adsorption at the gas/liquid interface. In foam fractionation, the foam is condensed to give a 'foamate', liquid rich surfactant, which is extracted and concentrated on organic solute. The measured liquid composition were in good agreement with a model, which described the equilibrium using an adsorption isotherm, which makes a mass balance for each stage in the column.

Ekici.P., Backleh-sohrt.M., Parlar. H., High effiency enrichment of total and single whey proteins by pH controlled foam fractionation, International Journal of Food Science and Nutrition, 2005; [7] worked on high effiency enrichment of total and single whey proteins by pH controlled foam fractionation. The feasibility of foam fractionation, as an alternative to other more commonly used methods, to effectively separate whey protein concentrate and single fraction has been studied. The investigation focused on the effects of different process parameters such as the pH value, the initial protein concentration as a surfactant. Albuminbovine, b-lactoglobulin and a- lactalbumin from whey solutions in the presence of sodium dodecyl sulfate could be transferred into the foam fraction .The results demonstrate that enriching whey proteins using foam fractionation can be quantitative and effective according to process parameters.

Zaid.S. Stanley. R., and Nigam. M., Extraction of Polyphenolics from Apple Juice by Foam Fractionation, International Journal of FoodEngineering, 2006; [8] investigated the extraction of polyphenolics from Apple Juice by Foam Fractionation for use as functional food ingredients. The separation performance like enrichment ratio, selectivity & percentage recovery, was determinedas a function of operating variables, namely N2-flow rate, initial feed concentration, bubble size, solution pH and the presence of alcohol to modify the surface tension. The bulk phase concentrations of the polyphenolics in the feed & foam fractions were analysed for total phenolic content by Folin assay and phenolic composition of the Polyphenolics in the feed & foam fractions were analysed for total phenolic content by Folin assay and phenolic composition by reverse phase HPLC. Recoveries were low at around 30% of total phenolics and selectivity was poor.

Ko. S., Cherry.J., and Prokop. A., Effect of Natural Contaminant on Foam Fractionation of Bromelin, Applied Biochemistry and Biotechnology, 2007 [9] studies the effect of a natural contaminant on foam fractionation of bromelin. They found a dilute bromelin solution with a pH (2.0 – 7.0)foams very well when bubbles were introduced into a foam fractionation column. It was observed that the dilute enzyme solution only foamed between pH – 2.0-3.0 when inner wall of the fractionation column was coated with a natural contaminant. They studied the separation ratio and the protein mass recovery to explore the effect of anaturalbifoaming agent on the foam fractionation of a dilute bromelin solution. The control variables used in this process were initial bulk solution pH, which rangedfrom 2.0 – 7.0 and superficial air velocity (1.7 – 6.2) cm/sec.).

Qu. Y.H., Zeng. G.M., Huang J.H., Xu. Ke, Fang. Y.Y., Li Xue,LiuL.H., Recovery of Surfactant SDS and Cd2+ From permeate In MEUFusing continuous Foam Fractionator, Journal of Hazardous materials, 2009[10] investigated on solvent sublation of l- lysineand found out that it can be used to separate the low concentration l- lysine from theaqueous solution by using dodecylbenzenesulfuric acid (DBSA) as the surfactant, di-(2-ethylhexyl) phosphoric acid (D2EHPA) as the extractant, n-heptane as the extraction solvent.The results showed that the optimum conditions of initial pH, D2EHPA concentration in the organic phase, DBSA and NaCl.

B Burghoff - Journal of Biotechnology, 2012 [11]Biotechnological downstream processing faces several challenges, such as dilute product streamslike the recovery of phytonutrients, metabolites and proteins. However, no large scale applications of foam fractionation in biotechnological downstream processing exist yet. This is due to the complexity of various biotechnological media.

Technology of streptomycin sulfate separation by two□stage foam separation
J Li, Z Wu, R Li - Biotechnology progress, 2012 [12] Industrial discharges from manufacturing streptomycin sulfate (SS) are inhibitory to biological wastewater treatment and need to be stripped of residual SS. For effective SS recovery from the wastewater, a two□stage foam separation technology was investigated using a column with a vertical ellipsoid□shaped channel (VEC) and a conventional one, and sodium dodecyl sulfate (SDS) served as the collector. The mechanism of enhancing foam drainage by VEC was theoretically analyzed.

E Karaoğul, P Parlar, H Parlar, MH Alma - Journal of analytical methods in chemistry ,2016[13] The main aim of this study was to enrich glycyrrhizic acid ammonium salt known as one of the main compounds of licorice roots (Glycyrrhizaglabra L.) by isoelectric focused adsorptive bubble separation technique with different foaming agents. . The results showed that highest enrichment values were obtained from β-lactoglobulin as much as 368.3 times. The lowest enrichment values (5.9 times) were determined for the application.

3. DISCUSSION:

3.1 Protein& it's structure:.

Proteins are essential nutrients for the human body.The name 'protein' is derived from the Greek word protos. They are one of the building blocks of body tissue and can also serve as a fuel source. As a fuel, proteins provide as much energy density as carbohydrates: 4 kcal (17 kJ) per gram; in contrast, lipids provide 9 kcal (37 kJ) per gram. The most important aspect and defining characteristic of protein from a nutritional standpoint is its amino composition. Proteins are polymer chains made of amino acids linked together by peptide bonds. During human digestion, proteins are broken down in the stomach to smaller polypeptide chains via hydrochloric acid and protease actions.

Protein ⟶ Protease ⟶ Peptone ⟶ Polypeptide ⟶ Amino acid, Purine, Pyrimidine

This is crucial for the absorption of the essential amino acids that cannot be biosynthesized by the body. There are nine essential amino acids which humans must obtain from their diet in order to prevent protein-energy malnutrition and resulting death. They are phenylalanine, valine, threonine, tryptophan, methionine, leucine, isoleucine, lysine, and histidine. There has been debate as to whether there are 8 or 9 essential amino acids. The consensus seems to lean towards 9 since histidineis not synthesized in adults. There are five amino acids which humans are able to synthesize in the body. These five are alanine, aspartic acid, asparagine, glutamic acid and serine. There are six conditionally essential amino acids whose synthesis can be limited under special pathophysiological conditions, such as prematurity in the infant or individuals in severe catabolic distress. These six are arginine, cysteine, glycine, glutamine, proline and tyrosine.

3.2Fundamental Consideration:

a. Formation of a surface phase.
b. Driving force for protein movement from bulk surface.
c. Relationship between heat of desorption and surface tension.
d. Measurement of protein surface tension.
e. Surface tension concentration diagram.

3.3Adsorption stages:

1. Diffusion of native protein molecules to the interface and their adsorption.

2. Uncoiling of polypeptide chains at the interface.

3.Aggregation of the surface denatured protein into coagulum largely devoid of the surface activity.

3.4Biological foams:

The foam capacity of a surfactant protein solution is characterized by the foaminess (Σ).

$\Sigma = Vs/VtG$

Vs = the equilibrium volume of the foam above the liquid layer

VtG = the volumetric gas flow rate

3.5 Protein recovery by continuous flotation:

The separation of proteins from a cultivation medium is usually carried out by ultrafiltration, adsorption,

flocculation or precipitation. It is well-known thatprotein is enriched in foam, however, no systematic investigations of the influence of equipment andoperating parameters on protein enrichment and separation factors (Clarke and Wilson 1983)[14] havebeen carried out. Therefore, the objective of these investigations was to assess the feasibility of proteinremoval from aqueous solutions or their enrichment by foam flotation.

Three flotation columns were used:

A. a three-stage column (Fig. 1),
B. the same column without stage-separating plates and with greater length
C. a single-stage column used by Viehweg and Schfigerl (1983).

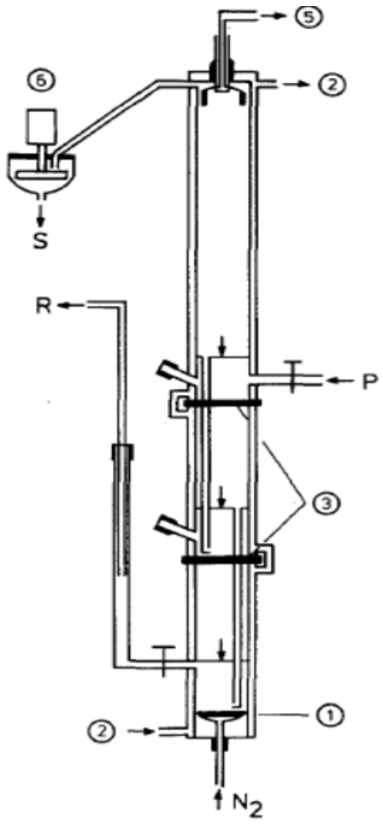


Fig. 1. Three-stage thermostated flotation column A, 25 mm i diameter. *P* feed; *R* liquid outlet with protein residue; *S* foa liquid outlet; N_2 gas inlet; *1)* glass frit *2)* thermostat; *3)* perforate plates with overflow and downcomer; *4)* liquid level in the stages *5)* electrical conductivity probe; *6)* foam breaker (3.000 rpm)

4. CONCLUSION:

1.. Separation of drug components or purification of drugs from a mixture of components

2. Separation of enantiomeric drugs mixture

3. Separation of chemical constituents from plant source, e.g. salts of alkaloid mixture, separation from soap, and enrichment of active components by foam fractionation method

4. Enrichment of plant proteins with adsorptive foam separation method

5. Foam fractionation of fruit juice enzymes for example, brumelin from pineapple

6. Removal of drug components from wastewater

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