DOWNSTREAM PROCESSING

Separation and recovery of fermentation products - Removal of microbial cells and solid matter, foam separation, precipitation, filtration, centrifugation, coagulation - cell disruption - physical and chemical methods, liquid-liquid extraction - solvent recovery, two phase aqueous extraction, supercritical fluid extraction.

REMOVAL OF INSOLUBLE

FOAM SEPARATION

Foam separation is a chemical process which falls into a category of separation techniques called "Adsorptive bubble separation methods". It is further divided into froth flotation and foam fractionation. A variety of materials can be concentrated as well as separated from one another using foam separation techniques that make use of the tendency of surface-active components in a solution to preferentially concentrate at the solution/gas interface. Nonsurface active agents that are capable of associating with surface-active agents can also be separated using these techniques. The various anions such as alkyl benzyl sulfonate; chromate; cyanide and phenolate; cations of, for example, dodecylamine. mercury, lead, and strontium; proteins; microorganisms; and minerals. The attractive feature of this group of techniques is its effectiveness in the concentration range that is too dilute for the successful use of most other techniques. Furthermore. these techniques are ideally suitable for also treating materials that are too sensitive to changes in temperature.

PRECIPITATION

Ammonium sulfate precipitation is a method of protein purification by altering the solubility of protein. Ammonium sulfate is commonly used due to its high solubility that allows salt solutions with high ionic strength. At sufficiently high ionic strength, the protein will be almost completely precipitated from the solution. Differential precipitation of proteins by ammonium sulfate is one of the most widely used preliminary purification procedures. It is based on proteins having differing solubility in ammonium sulfate solutions and can result in a two- to five-fold increase in specific activity. Provided that appropriately buffered ammonium sulfate solutions are used to protect the desired activity, recoveries approaching 100% can be expected. A typical protocol consists of adding ammonium sulfate to give specific percentage saturation, followed by a period of time for proteins to precipitate and a centrifugation step to collect the precipitate.

SEDIMENTATION:

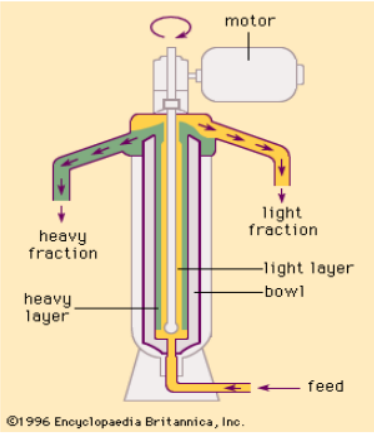
Sedimentation is the tendency for particles in suspension to settle out of the fluid in which they are entrained and come to rest against a barrier. This is due to their motion through the fluid in

response to the forces acting on them: these forces can be due to gravity, centrifugal acceleration, or electromagnetism. In geology, sedimentation is often used as the opposite of erosion, i.e., the terminal end of sediment transport. In that sense, it includes the termination of transport by saltationor true bedload transport. Settling is the falling of suspended particles through the liquid, whereas sedimentation is the termination of the settling process.Sedimentation may pertain to objects of various sizes, ranging from large rocks in flowing water to suspensions of dust and pollen particles to cellular suspensions to solutions of single molecules such as proteins and peptides. Even small molecules supply a sufficiently strong force to produce significant sedimentation.The term is typically used in geology to describe the deposition of sediment which results in the formation of sedimentary rock, but it is also used in various chemical and environmental fields to describe the motion of often-smaller particles and molecules. This process is also used in the biotech industry to separate cells from the culture media.

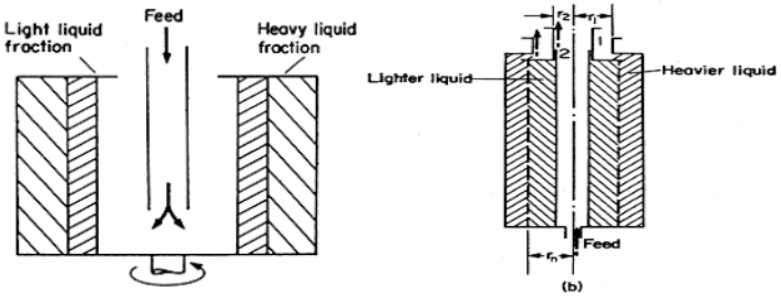
CENTRIFUGATION

Centrifugation is a process which involves the application of the centripetal force for the sedimentation of heterogeneous mixtures with a centrifuge, and is used in industrial and laboratory settings. This process is used to separate two immiscible substances. More-dense components of the mixture migrate away from the axis of the centrifuge, while less-dense components of the mixture migrate towards the axis. Chemists and biologists may increase the effective gravitational force on a test tube so as to more rapidly and completely cause the precipitate (pellet) to gather on the bottom of the tube.

- Feed added to spinning bowl
- Sedimentation of particles occurs in centrifugal field
- Flow is upwards at a particular rate which determines residence time in device
- Separation happens if sedimentation velocity is high enough for particle to reach side of bowl within residence time
- Large particles have higher settling velocities than small particles
- Both large and small are still particles, have small Reynolds no.s (<1) and obey Stokes' Law



- **Separation of milk into skimmed milk and cream is done with a centrifuge**



Centrifugal Motion

- Centrifugal acceleration = $r\omega^2$
- ω is the angular velocity in rad/s
- r is the radius of rotation
- Centrifugal force = $mr\omega^2$
- m is the mass of the particle

Sigma Factor

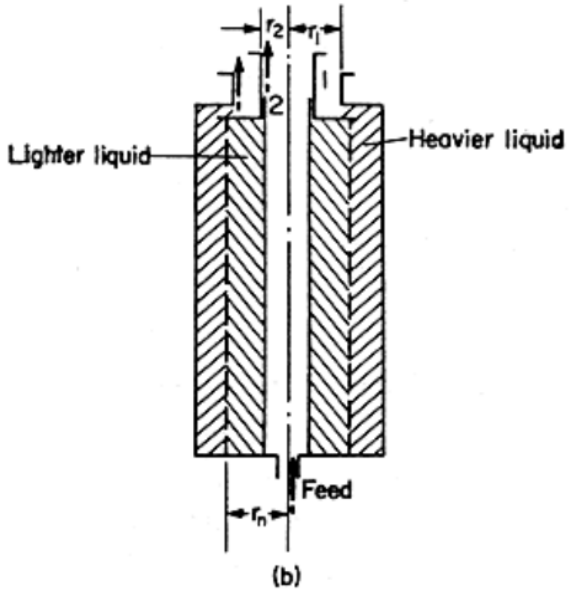
- The capacity of a centrifuge is defined by Σ
- Q is the throughput (m^3/s) at which all particles with a terminal velocity $\geq u_T$ (m/s) are retained
- Σ has units of m^2 and is equivalent to the cross sectional area of a thickener with the same capacity

Activity – Determine Σ

- **The contents of a fermenter are discharged to a centrifuge**
  - Volume of material is 100 m^3

- Centrifugation time is 5 hrs
- Particle size is 3 μm – all particles of this size are separated
- Density of solid phase 1090 kg/m^3
- Cell free liquid density 1025 kg/m^3
- Cell free liquid viscosity 0.005 Pa.s

- **Calculate the capacity factor, Σ**



Bowl Centrifuge

Sigma Factor – Bowl Centrifuge

$$\Sigma = \frac{\pi\omega^2\left(R^2 - r_c^{\,2}\right)H}{g\ln\left(R/r\right)}$$

ω is the angular velocity (rad/s)

R is the outer radius of the bowl (m)

r_c is the radius of the clarified discharge weir (m)

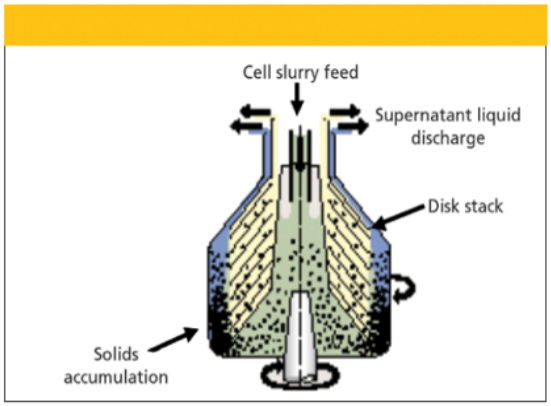
H is the height of the bowl (m)

r is the inner radius of the liquid in the bowl (m)

g is the acceleration due to gravity (m/s^2)

A long thin bowl gives good separation

The Disc Stack Centrifuge



Large particles have higher settling velocities than small particles

Cellular debris ends up at the outer edge of the bowl

Soluble intracellular material passes through with the clarified liquid

Discs give a higher sigma factor

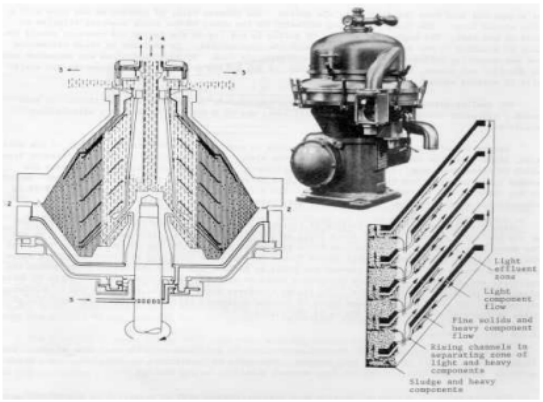
Benefit of Discs

The discs split the stream into a large number of very thin layers thereby improving separation

Solids flow downwards on bottom face of disc

Liquid flows upwards on top face of disc

Sigma factor $\propto$ no. of discs



Disc Stack Centrifuge Capacity

$$\Sigma = \frac{2}{3}\frac{\pi\omega^2 n\left(R^3 - r^3\right)}{g\tan\theta}$$

For the disc stack centrifuge:

ω is the angular velocity (rad/s)

n is the number of discs

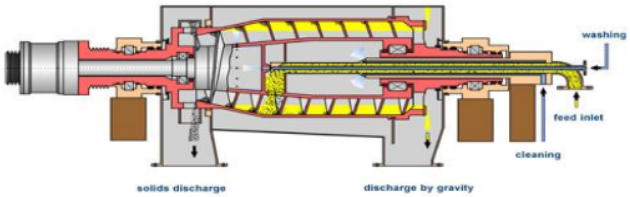
R is the outer radius of the discs (m)

r is the inner radius of the discs (m)

θ is the angle between disc and vertical (rad)

g is the acceleration due to gravity (m/s^2)

Decanter Centrifuge



Cross-section of a Flottweg DECANTER - in gas tight execution

FILTRATION

Filtration plays an important role in the natural treatment of groundwater as it percolates through the soil. It is also a major part of most water treatment. Groundwater that has been softened, or treated through iron and manganese oxidation, requires filtration to remove floc created by coagulation or oxidation processes. Since surface water is subject to run-off and does not undergo natural filtration, it must be filtered to remove particles and impurities.

Filtration can be compared to a sieve or micro-strainer that traps suspended material between the grains of filter media. However, since most suspended particles can easily pass through the spaces between grains of the filter media, straining is the least important process in filtration. Filtration primarily depends on a combination of complex physical and chemical mechanisms, the most important being adsorption. Adsorption is the process of particles sticking onto the surface of the individual filter grains or onto the previously deposited materials. Forces that attract and hold particles to the grains are the same as those that work in coagulation and flocculation. In fact, coagulation and flocculation may occur in the filter bed, especially if coagulation and flocculation before filtration was not properly controlled. Incomplete coagulation can cause serious problems in filter operation.

Theory of filtration

Depending on dispersing medium filtration is divided in two parts: 1) gas filtration and 2) liquid filtration. Gas filtration theory It mainly includes filtration of aerosols and lyosols. Membrane filters and nucleopore filters are based on these below mechanisms. Mechanism of gas filtration (Wilson & Cavanagh, 1969) Diffusion deposition: The trajectories of individual small particles do not coincide with the streamlines of the fluid because of Brownian motion. With decreasing particle size the intensity of Brownian motion increases and, as a consequence, so does the intensity of diffusion deposition. Direct interception: This mechanism involves the finite size of particles. A particle is intercepted as it approaches the collecting surface to a distance equal to its radius. A special case of this mechanism is the so-called sieve effect, or sieve mechanism. Inertial deposition: The presence of a body in the flowing fluid results in a curvature of the streamlines in the neighbourhood of the body. Because of their inertia, the individual particles do not follow the curved streamlines but are projected against the body and may deposit there. It is obvious that the intensity of this mechanism increases with increasing particle size and velocity of flow. Gravitational deposition: Individual particles have a certain sedimentation velocity due to gravity. As a consequence, the particles deviate from the streamlines of the fluid and, owing to this deviation; the particles may touch a fiber. Electrostatic deposition: Both the particles and the fibers in the filter may carry electric charges. Deposition of particles on the fibers may take place because of the forces acting between charges or induced forces. Liquid filtration theory (Melia & Weber, 1972). The term solid-liquid filtration covers all processes in which a liquid containing suspended solid is freed of some or the entire solid when the suspension is drawn through a porous medium.

Kozeny-Carman equation: $Idv/Adt = \Delta P / r \mu (l+L)$

Where, A = filter area; V = total volume of filtrate delivered; t = filtration time; ΔP = pressure drop across cake and medium; r = specific cake resistance; μ = filtrate viscosity; l = cake thickness; L = thickness of cake equivalent to medium resistance.

TYPES OF FILTERS

Several types of filters are used for water treatment. Early slow sand filters typically have filter rates of 0.05 gpm/ft2 of surface area and require large filter areas. The top several inches of the sand has to be removed regularly--usually by hand--due to the mass of growing material 'schmutzdecke' that collects in the filter. Sand removed is usually washed and returned to the filter. These filters are still in use in some small plants, especially in the western United States as well as in many developing countries. They may also be used as a final step in wastewater treatment. Modern filters are classified as: Gravity Filters (Rapid Sand or High Rate-Dualmedia-Multi- media) or Pressure Filters (Sand or Multi-media).

RAPID SAND FILTERS

Rapid sand filters have filter rates 40 times those of slow sand filters. The major parts of a rapid sand filter are:

•Filter tank or filter box

•Filter sand or mixed-media

•Gravel support bed

•Underdrain system

•Wash water troughs

•Filter bed agitators

The filter tank is generally constructed of concrete and is most often rectangular. Filters in large plants are usually constructed next to each other in a row, allowing piping from the sedimentation basins to feed the filters from the central pipe gallery. Some smaller plants are designed with filters forming a square of four filters with a central pipe gallery feeding the filters from a center wall.

Filter Sand

The filter sand used in rapid sand filters is manufactured specifically for the purpose of water filtration. Most rapid sand filters contain 24-30 inches of sand, but some newer filters are deeper. The sand used is generally 0.4 to 0.6 mm in diameter. This is larger than the sand used in slow rate filtration. The coarser sand in the rapid filters has larger voids that do not fill as easily.

PRESSURE FILTERS

Pressure filters fall into two categories: pressure sand and diatomaceous earth filters. Pressure filters are used extensively in iron and manganese removal plants.

A pressure sand filter is contained under pressure in a steel tank, which may be vertical or horizontal, depending on the space available. As with gravity filters, the media is usually sand or a combination of media, and filtration rates are similar to gravity filters.

Groundwater is first aerated to oxidize the iron or manganese, and then pumped through the filter to remove the suspended material.

FILTER AIDS

When water passes through a filter, the floc sometimes is torn apart into smaller particles that penetrate deeply into the filter media, causing premature turbidity breakthrough. This requires

more frequent filter backwashing of the filter and large volumes of backwash water to be able to remove the floc that has penetrated deeply into the filter bed.

A filter aid is a material that adds strength to the floc and prevents its breakup. Generally, a polymer is used as a filter aid because it creates strong bonds with the floc. Polymers are water soluble, organic compounds that can be purchased in either wet or dry form.

Polymers have very high molecular weight and cause the floc to coagulate and flocculate quickly. Polymers can have positive or negative charges, depending on the type needed to cause attraction to the specific floc filtered.

When used as a filter aid, the polymer strengthens the bonds and prevents the shearing forces in the filter from breaking the floc apart. For best results, the polymer should be added just ahead of the filter. A normal dose of polymer for filter aiding will be less than 0.1 ppm, but the exact dose will be decided by the result of a jar test and by experimentation in the treatment plant. Too much polymer will cause the bonds to become too strong, which may then cause the filter to plug, especially the top few inches of the filter media.

AQUEOUS TWO-PHASE EXTRACTION

Aqueous two-phase extraction which is a special case of liquid-liquid extraction involves transfer of solute from one aqueous phase to another. The two immiscible aqueous phases are generated in-situ by addition of substances such as polymers and salts to an aqueous solution. Historically, gelatin-agar and gelatin-soluble starch were used. Two types of aqueous two-phase systems are commonly used:

1.Polymer-polymer two-phase system

2.Polymer-salt two-phase system

Tab. 1. Two-Phase Aqueous Polymer Systems

Polymer/Salt Combination
Polyethylene glycol (PEG)-dextran
PEG-phosphate
PEG-citrate
PEG-hydroxypropyl starch
Ethylhydroxyethyl cellulose-hydroxypropyl starch
PEG-polyvinyl alcohol
PEG-pullulan
PEG-maltodextrins

A polymer-polymer two phase system can for instance be obtained by mixing dextran and PEG at a certain composition. By adding specific amounts of these polymers to an aqueous feed phase (which contains the solute), two aqueous phases, one rich in PEG and the other rich in dextran can be obtained. Aqueous two-phase systems can also be generated using a polymer (e.g. PEG or dextran) and a salt such as sodium or potassium phosphate. Aqueous two-phase separations take place at certain compositions only. The figure below shows a PEG-dextran phase diagram where a solubility curve separates the two-phase region (above the curve) from the single phase region (below the curve). Such "binary" phase diagrams which are based on the compositions of the two polymers (or polymer and salt) are used for determining the concentrations needed for an extraction process. These phase diagrams also predict the polymer/salt content of the raffinate and the extract phases. The composition of the individual phases generated can be obtained using tie-lines as shown in the figure.

Biomolecule	Recovery (%)
Xylanase	41
β-glucosidase	85 - 95
Polyphenol oxidase	50
Lipase	68
β-galactosidase	90
Glucoamylase	96
Horseradish peroxidase	75
Thaumatin	90 - 95
β-galactosidase	85 - 100
Fumarate hydratase (*Brevibacterium ammoniagenes*)	83
Fumarate hydratase (*E. coli*)	93
Aspartate-ammonia-lyase (*E. coli*)	96

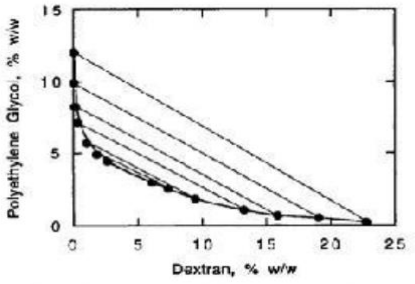


Fig. 2. Phase diagram for a PEG 6000-dextran D48 two-phase system at 20 °C (redrawn from Albertsson, 1986).

The partition of a solute between the two aqueous phases depends on its physicochemical properties as well as those of the two polymers (or polymer and salt).Factors Affecting Protein Partitioning in Two-Phase Aqueous Polymer Systems

1.Protein molecular weight

2.Protein charge, surface properties

3.Polymer molecular weight

4.Phase composition

5.Salt effects

6.Affinity ligands attached to polymers

7.pH

8.Temperature

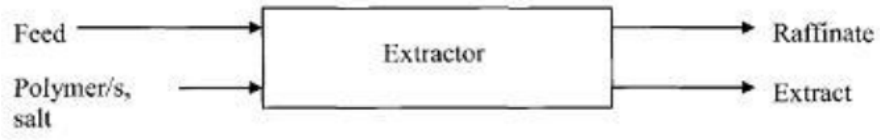


Fig. 7.4 Aqueous two-phase extraction

In PEG/dextran aqueous two-phase extraction of proteins, the partition behavior depends to a great extent on the relative polymer composition. It also depends on the solution pH and the molecular weight of the protein. Generally speaking, protein partitioning into the PEG rich phase is favored. When a polymer-salt combination is used to generate the aqueous two-phase system, a protein partitions favorably into the polymer rich phase. The general scheme for aqueous two-phase extraction is shown in Fig. 7.4.Extraction by an ATPS offers advantages for processing on a large scale, such as the possibility of obtaining a high yield, the possibility of continuous processing and a reduction in operational cost in relation to the costs of conventional processes.

PEG-DEXTRAN SYSTEMS

Effect of Polymer Molecular Mass (MM)

An increase in the molecular mass of dextran or of PEG will lower the concentration required for phase separation. The polymer molecular mass influences protein partitioning as a direct result of interactions between the two polymers. It has been found that the partitioned protein behaves as if it were more attracted by smaller polymer sizes and more repelled by larger polymers,

provided all other factors such as polymer concentrations, salt composition, temperature and pH are kept constant. It was observed that smaller protein molecules and amino acids were not affected as much as larger ones. For some proteins the partition coefficients increased as the MM of dextran increased if all other conditions were kept constant, but little effect was found for low MM proteins (Cytochrome C, 16,000). When the same proteins were partitioned in systems with different PEG MM, their partition coefficients decreased as the PEG MM increased, and for cytochrome C the effect was the smallest. This was attributed to the fact that when the PEG MM is increased, a weaker repulsion energy is required to cause phase separation. Repulsive interactions between the polymer and the protein become stronger as the polymer MM is increased, resulting in a distribution of the protein towards the phase containing the polymer with an unchanged MM. A weak net repulsion between the proteins and the polymer is sufficient to change the distribution when the polymer MM is changed.

Effect of Polymer Concentration

It was found that proteins with MM less than 20,000 showed a linear relationship between the ln K in PEG-dextran systems and a difference in PEG concentration between the phases, for any particular system. They found that it was possible to predict the partitioning of a protein at any concentration in that particular system if one partition coefficient in the system were known. However, others found that for some proteins the partition coefficient was inversely correlated to phase concentration in a PEG-dextran system, showing that better separation could be achieved at high polymer concentrations. This, however, may also affect the concentration of proteins that can be manipulated in the system as polymer concentration has a directly inverse effect on protein solubility.

Effect of Salts

Salts can affect protein partitioning in different ways in PEG-dextran systems: one is by altering the physical properties of the systems the hydrophobic difference between the phases and the other is by the partitioning of ions between the phases, which affects the partitioning of proteins according to their molecular charge.

Salts have been added to PEG-dextran systems to increase the selectivity of protein partitioning in the aqueous two-phase methodology application for biological separations.

It was observed that salt ions partition differently between the phases, causing an uneven distribution in the system that generates a difference in electrical potential between the phases. This difference in electrical potential would be independent of salt concentration, but linearly dependent on the partition behaviour of the ions.

It was also observed that polyvalent anions such as phosphate, sulphate and citrate partitioned preferentially into dextran-rich phases, while halides partitioned nearly equally. As an example, negatively charged materials have higher partition coefficients in phases containing sodium sulphate rather than sodium chloride, while the reverse holds for positively charged materials. Partition coefficients of negatively charged materials decrease when the cationic series is changed from lithium to sodium to potassium. The ratio between the phosphate ions, rather than the concentration, was decisive for the difference in electrical potential. This applies to multivalent ions, which show a series of pH-dependent dissociations and was clearly the reason for the potential difference found between the two phases (Kula et al., 1982).

PEG-SALT SYSTEMS

The formation of PEG-salt systems was first observed in the 1950s, but the theoretical fundamentals have not been well explained. It was found that for PEG solutions the addition of some inorganic salts (sulphates and carbonates) is more effective than the addition of others in reducing the critical concentration of cloud point curves. These inorganic salts dramatically reduced the PEG cloud point at high temperatures.

PEG-salts systems have been introduced for the practical application of large-scaleprotein separation because of the larger droplet size, greater difference in density between the phases, lower viscosity and lower costs, leading to a much faster separation than in PEG-dextran systems. Industrial application of PEG-salt systems

Biomolecule	Ligands attached to PEG	Recovery (%)
Lactate dehydrogenase	Tryazine dye-Cibacron Blue F3G-A	81.3
β-galactosidase	p-aminophenyl-β-D-thiogalactopiranoside – (APGP)	83
Protein A	IgG human	87
Lactate dehydrogenase	Eudrogit-Cibacron Blue	54
Penicillin acylase	Trimethylamina	97
Trypsin	Trypsin inhibitor	82

was improved by the availability of commercial separators, which allowed faster continuous protein.

Initially PEG-phosphate systems were widely used where scientists have studied ways of recycling the phosphate phase of the systems to minimize environmental pollution. The recycling of the phosphate phase was achieved by its separation from the solids by the use of alcohols. PEG from the top PEG-rich phase can also be successfully recycled.

More recently PEG-sulphate systems have begun to be used where separation of some biomaterial was achieved with PEG 4000 and (NH4)2SO4 at pH 7-7.5. The presence of 2%

NaCl (0.17 M) made the separation much worse. With 4% NaCl (0.34 M), a poor separation was obtained (a tenfold decrease in K for aspartase). Since a pH or phase ratio change was not observed, the dramatic change in K was considered to be due to a change in hydrophobicity between the phases.

AFFINITY PARTITIONING

In the last 30 years, several groups have studied methods to increase partitioning by the use of biospecific interactions in ATPSs.

The initial works on affinity partitioning in ATPSs were to purify trypsin by usingPEG-bound ligand p-aminobenzamidine and S-23 myeloma protein by using dinitrophenol as ligand.The degree of affinity partitioning, Kaff, can be described by the ratio between the partition coefficients of proteins with and without a ligand:

This equation describes the increase in the partition coefficient of a protein by the binding of a specific ligand to the PEG-rich phase.

Affinity partitioning results in specific extractions of proteins, nucleic acids, membranes, organelles and even cells, mainly when biospecific ligands are used.

Large Scale Extraction Schemes

Extraction processes can be divided into two general schemes:

•Batch extractions

•Continuous extractions,

Continuous extractions can also be further divided into the following schemes:

•Single stage continuous extraction

•Multi stage continuous extraction

In turn, multi stage continuous extraction can be divided into two general modes as:

•Crosscurrent continuous extraction

•Counter current continuous extraction These will be studied in the following sections.

Batch extraction

In a batch extraction process a batch of feed solution is mixed with a batch of extracting solvent in an appropriate vessel. The solute distributes between the two phases depending on its partition coefficient. The rate at which the transfer of solute takes place from the feed to the extracting solvent depends on the mixing rate. Once equilibrium is attained, the mixing is stopped and the extract and raffinate phases are allowed to separate. The separation funnel commonly seen in chemistry laboratories is the simplest small-scale batch extraction device. Mixer-settler units are usually used for large-scale batch extraction. The basic principle of batch extraction using a mixer settler unit is shown in Fig. 7.5. The mixer unit must be able to generate high interfacial area, must provide high solute mass transfer coefficient and cause low entrainment of air bubbles. The settler unit must have a low aspect ratio (L/D), i.e. be of flat geometry, must allow easy coalescence and phase separation, and must allow for easy collection of raffinate and extract as separate streams. The antibiotic penicillin partitions favorably in an organic solvent from an aqueous fermentation media at acidic conditions. However, at a neutral pH, the partitioning from organic phase to aqueous phase is favored. Thus the antibiotic could be purified by sequential reversed batch extraction, where the antibiotic is moved from aqueous to organic phase and back again (as shown in Fig. 7.6). This sequence is usually repeated a few times in order to obtain highly pure antibiotic.

If a batch of feed containing R volume of initial solvent and an initial solute concentration of C0 is mixed with S volume of pure extracting solvent, the concentration distribution in the extract and the raffinate at equilibrium is given by:

$CE = KCR$

Where

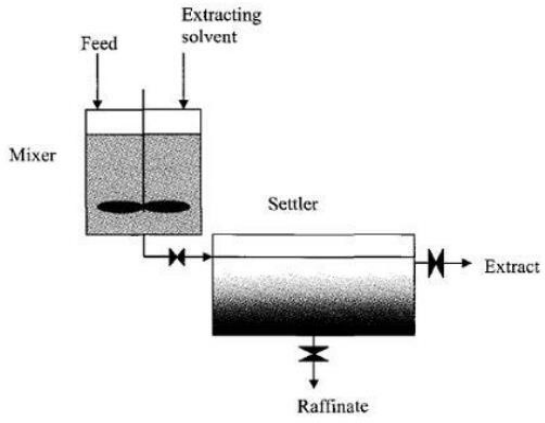


Fig. 7.5 Batch extraction using mixer settler unit

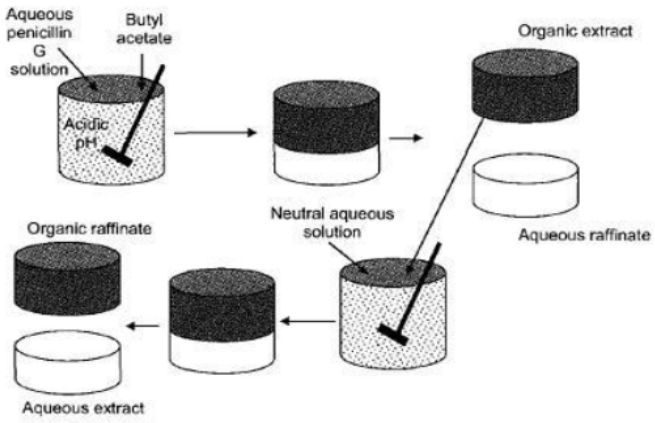


Fig. 7.6 Sequential reverse batch extraction

CE solute concentration in extract (kg/m3) CR solute concentration in raffinate (kg/m3)

Supercritical Fluid Extraction (SFE) is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent. Extraction is usually from a solid matrix, but can also be from liquids. SFE can be used as a sample preparation step for analytical purposes, or on a larger scale to either strip unwanted material from a product (e.g. decaffeination) or collect a desired product (e.g. essential oils). These essential oils can include limonene and other straight solvents. Carbon dioxide (CO_2) is the most used supercritical fluid, sometimes modified by co-solvents such as ethanol ormethanol. Extraction conditions for Supercritical carbon dioxide are above the critical temperature of 31°C and critical pressure of 74 bar. Addition of modifiers may slightly alter this. The discussion below will mainly refer to extraction with CO_2, except where specified. The properties of a supercritical fluid can be altered by varying the pressure and temperature, allowing selective extraction. For example, volatile oils can beextracted from a plant with low pressures (100 bar), whereas liquid extraction would also remove lipids. Lipids can be removed using pure CO_2 at higher pressures, and then phospholipids can be removed by adding ethanol to the solvent. The same principle can be used to extract polyphenold and unsaturated fatty acids separately from wine wastes.

The system must contain a pump for the CO_2, a pressure cell to contain the sample, a means of maintaining pressure in the system and a collecting vessel. The liquid is pumped to a heating zone, where it is heated to supercritical conditions. It then passes into the extraction vessel,

where it rapidly diffuses into the solid matrix and dissolves the material to be extracted. The dissolved material is swept from the extraction cell into a separator at lower pressure, and the extracted material settles out. The CO_2 can then be cooled, re-compressed and recycled, or discharged to atmosphere.

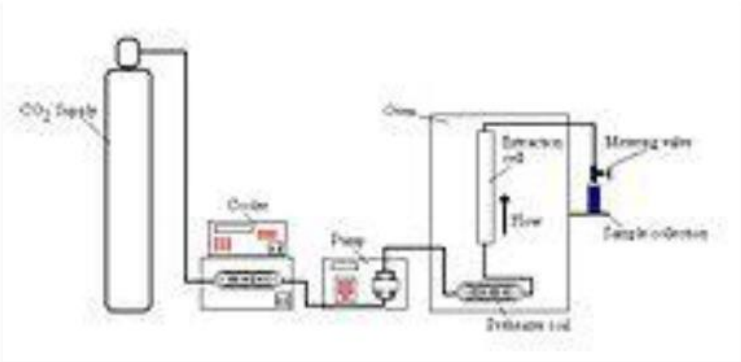


Figure 1. Schematic diagram of SFE apparatus