Module-26: Fermentation Product Recovery and Purification-IV:

Liquid-Liquid& Two Phase Aqueous Extraction, Solvent recovery

Liquid-Liquid Extraction

- Liquid-liquid extraction is a method to separate compounds on the basis of their relative solubilities in two different immiscible liquids.
- The specific necessity is that a product must be obtained at higher proportion but concentrated, in a smaller volume of solvent.
- For liquid-liquid extraction it is important to find out on a small scale the solubility characteristics of the product using a wide range of solvents.
- A simple rule to remember is that 'like dissolves like'.
- The 'likeness' as far as solubility relations are concerned is in the polarities of molecules.
- Polar liquids mix with each other and dissolve salts and other polar solids.
- The solvents for nonpolar compounds are liquids of low or nil polarity.
- A measure of the degree of molar polarization of a compound is the dielectric constant*D*.
- The dielectric constant*D* of a substance can be measured by determiningthe electrostatic capacity C of a condenser containingthe substance between the plates.
- If Co is the valuefor the same condenser when completely evacuatedthen
  $D=C/Co$
- Experimentally, dielectric constants are obtained by comparing the capacity of the condenser when filled with a given liquid with the capacity of the same condenser containing a standard liquid whose dielectric constant is known very accurately.
- If $D_1$and $D_2$are the dielectric constants of the experimental and standard liquids and C_1 and C_2 are the electrostaticcapacities of a condenser when filled with each of the liquids then,$D_1/D_2 = C_1/C_2$
- The value of D_1can be calculated since C_1 and C_2 can be measured and $D_2$is known.
- If this value is known then it is possible to predict whether a compound will be polar or nonpolar.
- A high value indicates a highly polar compound.
- TABLE shows Dielectric constants of solvents at 25°C (arranged in order of increasing polarity)

Solvent	Dielectric constant
Hexane	1.90 (least polar)
Cyclohexane	2.02
Carbon tetrachloride	2.24
Benzene	2.28
Diethyl ether	4.34
Chloroform	4.87
Ethyl acetate	6.02
Butan-2-ol	15.8
Butan-1-ol	17.8
Propanol	20.1
Acetone	20.7
Ethanol	24.3
Methanol	32.6
Water	78.5

- Other than the dielectric constant partition coefficient also playsa deciding role in the choice of solvents.
- The final choice ofsolvent will be influenced by the distribution or partitioncoefficient *K* where,

  K = Concentration of solute in extract/Concentration of solute in raffinate
- The value of *K* defines the simplicity of extraction.

Significance of K Value in the Selection of Process

- High *K* value indicates good stability ofproduct as well asgood separation of the aqueous and solventphases.
- For example whenever a value of *K* is 50, the extraction should beeasy, possible bysingle-stage extraction system.

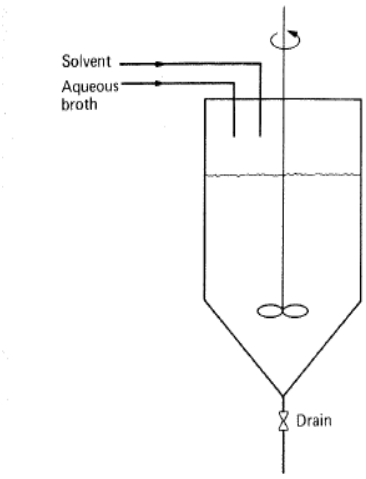

FIG. 10.24. Diagram of a single-stage extraction unit.

- If a value of *K*is 0.1, theextraction will be difficult and multistage process willbe necessary.
- In any system if the value of K is low, concurrent or countercurrent multistage systems have to beconsumed.
- In co-current system there are *n*mixer/separator vessels in line and the raffinate goes fromvessel 1 to vessel *n.*
- Fresh solvent is added to each stage, thefeed and extracting solvent pass through the cascade in the samedirection.
- Extract is recovered from each stage.
- Although arelatively large amount of solvent is used, a high degree ofextraction is achieved.

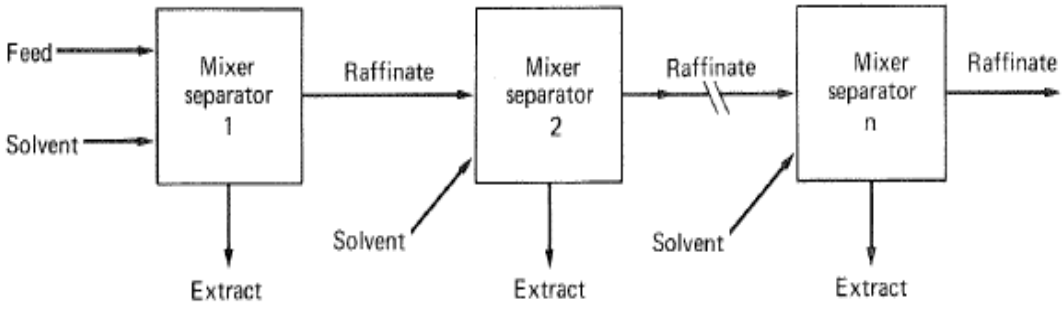

FIG. 10.25. Diagram of a co-current flow extraction system.

- In counter-current system,there are anumber of mixer/separators connected in series.
- The extractedraffinate passes from vessell to vessel *n* while the productenrichedsolvent is flowing from vessel *n* to vessel 1.
- So, in this the feedand extracting solvent pass through the cascade in *opposite*directions.

- The most efficient system for solvent utilization iscounter-current operation, showing a considerable advantageover batch and co-current systems.
- In practice, the series ofcounter-current extractions are conducted in a single continuousextractor using centrifugal forces to separate “the two liquidphases.

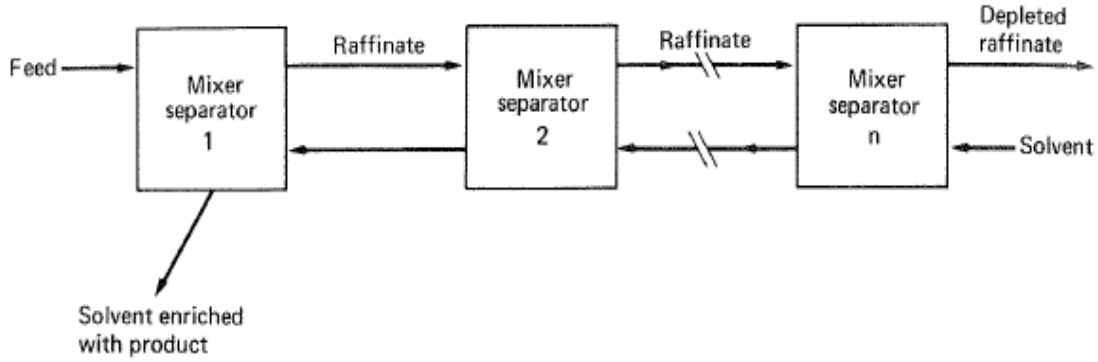

FIG. 10.26. Diagram of a counter-current extraction system.

- The Podbielniak centrifugal extractor consists of ahorizontal cylindrical drum revolving at up to 5000 rpm about ashaft passing through its axis.
- The liquids to be run countercurrentare introduced into the shaft, where the heavy liquidenters the drum at the shaft & the light liquid is run by aninternal route to the edge of the drum.

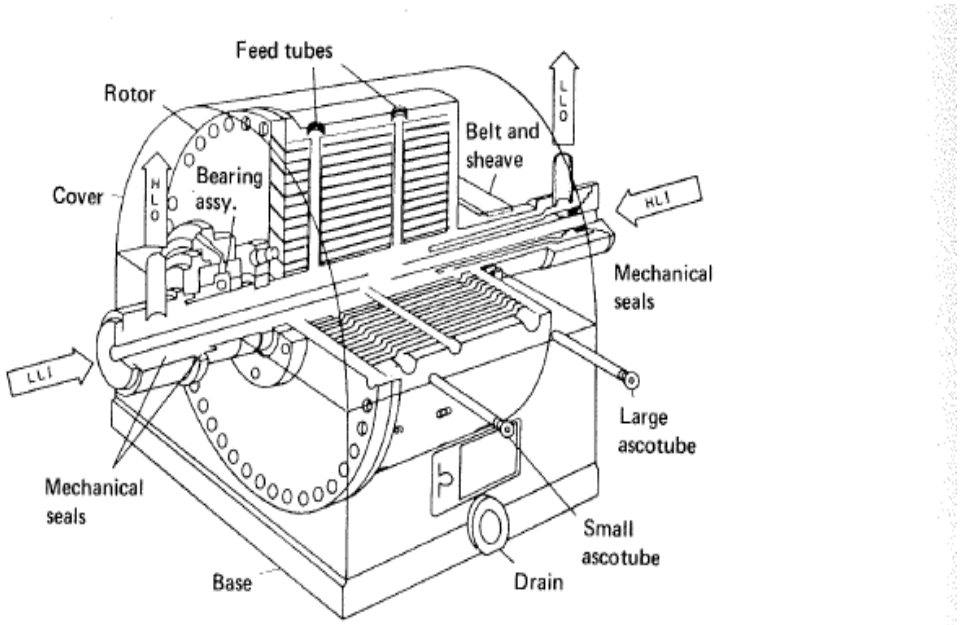

FIG. 10.27. Diagram of the Podbielniak extractor (Queener and Swartz, 1979). HLI, LLI, HLO and LLO indicate heavy and light liquid in and out.

- As the drum rotates, the heavy liquid is forced to the periphery of the drum by centrifugal action where it contacts the light liquid.

- The solute is transferred between the liquids and the light liquid is displaced back towards the axis of the drum.
- The heavy liquid isreturned to the drum's axis via internal channels.
- The two liquid streams are then discharged via the shaft.
- Penicillin G is an antibiotic which is recovered from fermentationbroths by centrifugal counter-current solvent extraction.
- The penicillin extraction process may involve the four followingstages:

1. Extraction of the penicillin G from the filtered broth into an organic solvent (amyl or butyl acetate or methyl isobutylketone)
2. Extraction from the organic solvent into an aqueous buffer
3. Extraction from aqueous buffer into organic solvent
4. Extraction of the solvent to obtain the penicillin salt

- At each extraction stage progressively smaller volumes ofextractant are used to achieve - concentration of the penicillin.
- Unfortunately, penicillin G has a half-life of 15 minutes at P^H2.0 &20°C.
- The harvested broth is therefore initially cooled to 0°to 3°.
- The cooled broth is then acidified to pH 2 to 3 withsulphuric or phosphoric acid immediately before extraction.
- This acidified broth is quickly passed through a Podbielniak centrifugal counter-current extractor using about 20% byvolume of the Solvent in the counter flow.
- Ideally, the hold-uptime should be about 60 to 90 seconds.
- The penicillin richsolvent then passes through a second Podbielniak extractorcounter-current to an aqueous NaOH or KOH solution (againabout 20% by volume) so that the penicillin is removed to theaqueous phase (pH 7.0 to 8.0) as the salt.

RCOOH(org) + NaOH(aq) →RCOO-Na++ H2O

- These two stages may be sufficient to concentrate the penicillinadequately from a broth with a high titer.
- At each stage the spent liquids should be checked for residualpenicillin and solvent usage carefully monitored.

- Since thesolvents are expensive and their disposal is environmentallysensitive they are re-covered for recirculation through theextraction process.
- The success of a process may depend on efficient solventrecovery and reuse.

Two-Phase Aqueous Extraction

- Liquid-liquid extraction is a well-established technologyin chemical laboratories for processing of chemicals.
- However, in the processing of sensitive biologicalsthe use of organicsolvents has limited application.
- Onthe other hand, two-phase aqueous systemsare regarded as beingbio friendlyas they require a high water content and lowsurface tension.
- These systems have been famous sincethe late nineteenth century.
- Nowadays a large variety ofhydrophilic polymers (natural and synthetic)are usedto create two (or more) aqueous phases.
- When hydrophilic polymers are addedto an aqueous solution, phaseseparation occurs.
- Two immiscible aqueous phasesare formed when the concentrationsgo beyond a certain value.
- Settling time for the two phases can beextended, depending on the components used andvessel geometry.
- Magnetic separators or centrifugal separators can be used to improve phase separation.
- Many systems are available:
  i) Non-ionic polymer/non-ionic polymer/water, e.g. polyethylene glycol/dextran.
  ii) Polyelectrolyte/non-ionic polymer/water, e.g. sodium carboxymethyl cellulose/polyethylene glycol.
  iii) Polyelectrolyte/polyelectrolyte/water, e.g. sodium dextran sulphates /sodium carboxymethyl cellulose

iv)Polymer/ Low molecular weight component/ water e.g. dextran/propyl alcohol.

- The distribution of a solute species between the phases is influenced by a number of factors such as temperature, polymer(type and molecular weight), salt concentration, ionicstrength, pH and properties (e.g. molecular weight ofthe solute.
- Affinity techniques such as those applied in chromatographic processes can be used to selectively recover and concentrate a solute.

- For purification of many solutes like enzymes, proteins, cells andsubcellular particles two phase aqueous systems have been used.
- Manysuch systems which utilized for large scale proteinseparation, uses PEG as the upper phase forming polymereither with concentrated salt solution,dextranor hydroxy propyl starch as the lower phase forming material.

Solvent Recovery

- Solvent recovery plant is a major component in any extraction process.
- It is usually a distillation unit.
- There are three stages of distillation:

1. The removal of solvent as a vapor from asolution i.e.Evaporation.
2. Separation of the lower boiling more volatile component from other less volatilecomponents by vapor-liquid separation in column.
3. Recovery of the more volatilesolvent fraction by condensation of the vapor.

- For evaporation wide range of evaporators are available which can operated either batch wiseor continuously.
- In batch distillation thevapor from the boiler passes up the column and is condensed.
- Part of the condensate will be returned as the reflux forcounter-current contact with the rising vapor in the column.
- The distillation is continued until a satisfactory recovery of thelower-boiling (more volatile) component(s) has been accomplished.
- Beginning of a continuous distillation is similar with a batch distillation, but condensate isnot withdrawn initially.
- There is total reflux of the condensate untilideal operating conditions are been established throughout thecolumn.
- At this stage the liquid feed is fed into the column atan intermediate level.
- The more volatile components moveupwards as vapor and are condensed, followed by partial refluxof the condensate.
- Meanwhile, the less volatile fractions movedown the column to the evaporator (reboiler).
- At this stage partof the bottoms fraction is continuously withdrawn and part isreboiled and returned to the column.
- Counter-current contacting of the vapor and liquid streams isachieved by causing:

a. vapor to be dispersed in the liquid phase (plate or traycolumn),

b. liquid to be dispersed in continuous vapor phase (packedcolumn).

- The plate or tray column consists of a number of distinctchambers separated by perforated plates or trays.
- The risingvapor bubbles through the liquid, which is flowing across eachplate, and is dispersed into the liquid from perforations (sieveplates) or bubble caps.
- The liquid flows across the plates andreaches the reboiler by a series of overflow wires and downpipes.
- A packed tower is filled with a randomly packed material such asrings, saddles, helices, spheres or beads.
- Their dimensions areapproximately one-tenth to one-fiftieth of the diameter of thecolumn and are designed to provide a large surface area forliquid-vapor contacting and high voidage to allow highthroughput of liquid and vapor.
- The heat input to a distillation column can be considerable.
- The simplest ways of conserving heat are to preheat the initialfeed by a heat exchanger using heat from:

a. hot vapors at the top of the column,

b. heat from the bottoms fraction when it is being removed ina continuous process

c. combination of both.

References

- **Principles of Fermentation Technology:** (2nd edition, by Peter F. Stanbury, Allan Whitaker and Stephen J. Hall, Butterworth-Heinemann, An imprint of Elsevier Science.)
- **Industrial Microbiology:** (By Casida L. E.New Age international (P) ltd publications)
- **A Text Book of Industrial Microbiology:** (2nd edition By Wulf Crueger & Anneliese Crueger)
- **Biotechnology:** Food Fermentation Microbiology, Biochemistry & Technology Vol. 1 & 2:(By V.K. Joshi & Ashok Pandey)
- **Manual of Industrial Microbiology and Biotechnology: (**2nd Edition by Arnold L. Demain and Julian E. Davies, Ronald M. Atlas, Gerald Cohen, Charles L. Hershberger, Wei-Shou Hu, David H. Sherman, Richard C. Willson and J. H. David Wu)
- **Industrial Microbiology-An introduction:** By Michael J. Waites, Neil L. Morgan, John S. Rockey and Gary Higton)
- **Comprehensive Biotechnology-The Principles, Applications and Rugulations of Biotechnology in Industry, Agriculture and Medicine:** (By Mrray Moo Young)
- **Fermentation Technology :** Up Stream Fermentation Technology- Vol-I: (By H. A. Modi-Pointer Publications)
- **Fermentation Technology :** Down Stream Fermentation Technology- Vol-II: (By H. A. Modi-Pointer Publications)
- **Industrial Microbiology by Prescott and Dunn's:** (4th edition, edited by Gerald Reed, CBR publications)
- **Fermentation Technology:** (By M.L. Srivastava, NAROSA publications)
- **Industrial Microbiology:** (By A.H. Patel)
- **International student edition: Microbiology- A laboratory Manual:** (4th edition. By James G. Chappuccino & Natalie Sherman)
- Bacteriological Techniques: (By F.J. Baker)
- Introduction to Microbial Techniques: (By Gunasekaran)
- **Mannual of Industrial Microbiology and Biotechnology:** (2nd Edition by Arnold L. Demain and Julian E. Davies, Ronald M. Atlas, Gerald Cohen, Charles L. Hershberger, Wei-Shou Hu, David H. Sherman, Richard C. Willson and J. H. David Wu)

Web references

- http://www.homebrew.net/ferment/
- http://www.soyinfocenter.com/HSS/fermentation.php
- http://www.ensymm.com/pdf/ensymm_fermentation_abstract.pdf
- http://scialert.net/fulltext/?doi=jm.2007.201.208
- http://aem.asm.org/content/7/1/57.full.pdf
- http://www.slideshare.net/yongkangbirdnest/lecture-4-sterilization
- http://www.ars.usda.gov/research/publications/publications.htm?seq_no_115=140721
- http://www.scribd.com/doc/30706834/Fermentation-Design
- http://www.wiley-vch.de/books/sample/3527318194_c01.pdf
- http://www.engineersirelandcork.ie/downloads/Biopharmaceuticals%2020Jan09%20-%202%20-%20Ian%20Marison%20DCU.pdf
- www.yobrew.co.uk/fermentation.php
- http://bioscipub.com/journals/bbb/pdf/19-24.pdf
- http://gertrude-old.case.edu/276/materials/web/immobilizedenzymereview.pdf
- http://download.bioon.com.cn/upload/month_0902/20090223_b809d1c59ba2a6e2abfdJtWiJOiFDm02.attach.pdf
- http://bioprocess-maulik.blogspot.in/2007/07/design-of-industrial-fermentation.html

- http://hsc.csu.edu.au/biology/options/biotechnology/3051/biotechnologyPart3.html
- http://www.rsc.org/ebooks/archive/free/BK9780854046065/BK9780854046065-00001.pdf
- http://www.biotech.upm.edu.my/academics/On%20Line%20Note/Bioprocess/BTK%205301/Lect6%28Inoculum%20Preparation%20and%20Development%29.pdf
- http://www.biotechresources.com/services-strain.shtml
- http://www.idosi.org/wjc/4%281%2909/14.pdf
- http://cheserver.ent.ohiou.edu/Paper-gu/DualFeed.pdf