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Downstream processing of Fermented Foods/products

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1. INTRODUCTION

The extraction and purification of fermented products may be difficult and costly. Ideally, one is to obtain a high-quality product as quickly as possible at an efficient recovery rate using minimum investment operated at minimal costs. Unfortunately recovery costs of microbial fermented products may vary as low as 15% to as high as 70% of the total costs. Obviously, the chosen process, and therefore its relative cost, will depend on the specific product.

If a fermentation broth is analysed at the time of harvesting, it will be discovered that the specific product may be present at a low concentration in an aqueous solution that contains intact micro-organisms, cell fragments, soluble and insoluble medium components and other metabolic products. The product may also be intracellular, heat labile and easily broken down by contaminating microorganisms. All these factors tend to increase the difficulties of product recovery. To ensure good recovery or purification, speed of operation may be the over-riding factor because of the labile nature of a product. The processing equipment must therefore be of the correct type and also the correct size to ensure that the harvested broth can be processed within a satisfactory time limit.

The choice of recovery process is based on the following criteria:

1. The intracellular or extracellular location of the product.
2. The concentration of the product in the fermentation broth.
3. The physical and chemical properties of the desired product.
4. The intended use of the product.
5. The minimal acceptable standard of purity.
6. The magnitude of bio-hazard of the product or broth.
7. The impurities in the fermenter broth.
8. The marketable price for the product.

The various steps followed in the extraction of fermentation products together with the approximate level of purification obtained in each stage are given in Table 1. The procedure followed within each stage depends of course on the material being extracted. The product sought could be the cells themselves such as in yeast manufacture, or lodged in the cells (such as in streptomycin or some enzymes) or free in the medium as with penicillin.

Table 1 Conventional steps followed in the purification of products

Step Process	Hypothetical degree of purity (%)
1a. Removal of insolubles Filtration Centrifugation Decantation	0.1-1.0 (if product solubles) 90-99 if product is cell such as yeasts
1b. Disruption of cells	
2 Primary foam isolation of the product Sorption physical and/or ion exchange Solvent extraction Precipitation Ultracentrifugation	1-10
3 Purification Fractional precipitation Chromatography (adsorption, partition, ion exchange, affinity) Chemical derivatization Decolorization	50-80
4. Final product isolation Crystallization Drying Solvent removal	90-100

2. SOLIDS (INSOLUBLES) REMOVAL

In general the initial step separates solids from the liquid fraction thereby facilitating further extractive steps, such as sorption, solvent extraction which would be wasteful or near impossible if the cells were not separated. When the required product is solid or is lodged in the insoluble portion liquid removal helps concentrate the solids. In a few cases no separation takes place such as in the acetone butanol fermentation, where the entire beer is used. In most cases, however, the separation methods used are filtration, centrifugation, decantation, and foam fractionation. Where the required fraction is in the cells then much of the impurities are removed with the filtrate after the cells have been isolated. The various methods used in solids removal are discussed below.

Filtration

The rotary vacuum filter: One of the most commonly used filters in industry is the *rotary vacuum filter* which is available in several forms. Essentially the filter consists of a hollow rotating cylinder divided into four partitions and covered with a metal or cloth gauze. A vacuum is applied in the cylinder and as it rotates the vacuum sucks liquid materials from the shallow trough in which the rotating cylinder is immersed. For thickslurries which are difficult to filter (e.g. aminoglycoside broths) a thin layer of filter aid is first allowed to be absorbed on the cylinder. Later the filter cylinder with its thin coating of the filter aid is allowed to rotate in the trough in which the broth is now placed. The rotating cylinder, the vacuum still on, is washed with a sprinkle of water; a knife whose edge is positioned just short of the layer of filter aid scrapes off the solids picked up from the broth.

Ring and wire type filters: These filters consist of a coating of diatomaceous earth on a wire-mesh supported by a frame of metal rods. The liquid to be filtered is introduced under a

pressure of 75 p.s.i rather than under a vacuum as in the rotary vacuum filter. They are used when the load is light such as for polishing beer or fruit juices. They can be cleaned by back flushing with water.

Centrifugation

Centrifugation is not widely used for the primary separation of solids from broth in fermentation beer because of the thickness of these slurries and the fact that many industries have operated successfully with filters. Only in a few cases will a centrifuge de-water a broth to anywhere near the extend a filter would. In the enzyme isolation industry, however, centrifugation is preferred to filtration, probably because unwanted cell debris are quite efficiently removed by this method. A large number of centrifuges are available in the market and a new fermentation industry or a change in the production method of old processes may require the use of centrifuges for primary separation.

Coagulation and Flocculation

Coagulation is the cohesion of dispersed colloids into small flocs; in flocculation these flocs aggregate to form larger masses. The first is induced by electrolytes and the latter by polyelectrolytes, high molecular weight, water soluble compounds that can be obtained in ionic, anionic, forms. Bacteria and proteins being negatively charged colloids are easily flocculated by electrolytes or polyelectrolytes. Sometimes clay, oractivated charcoal may be used. The net effect of the flocculation is that colloid removal facilitates filtration. It may even be possible to merely decant the supernatant once large enough flocs remove the solid portion of the ‘beer’ of them which to use and low much to use among the various flocculants must be worked out by experimentation. Since flocculation depends on cell wall characteristics, the agents must meet the following requirements especially if the cells, and not the liquid, are the required products. The flocculants should have the following properties.

(i) They must react rapidly with the cells.
(ii) They must be non-toxic.
(iii) They should not alter the chemical constituents of the cell.
(iv) They should have a minimum cohesive power in order to allow for effective subsequent water removal by filtration.
(v) Neither high acidity nor high alkalinity should result from their addition.
(vi) They should be effective in small amounts and be low in cost.
(vii) They should preferably be washable for reuse.

Foam Fractionation

Foam formation has been described in Chapter 9. The principle of foam fractionation is that in a liquid foam system the chemical composition of a given substance in the bulk liquid is usually different from the chemical composition of some substance in the foam. Foam is formed by sparging the bulk liquid containing the substance to be fractionated with an inert gas. The gas is fed at the bottom (Fig. 10.2) of a tower and the foam created overflows at the top carrying with it the solutes to be fractionated. Surfactants or (surfaceactive substances that reduce surface tension e.g. teepol) may be added in liquids that do not foam. This method has been used to collect a wide range of microorganisms and although mainly experimental it may be used on a large scale in industry.

Whole-broth Treatment

As had been indicated earlier, in some fermentations such as the acetonebutanol fermentation, the whole unseparated broth is stripped of its content of the required product. This process saves the capital and recurrent expense of the initial separation of solids from the broth.

3. PRIMARY PRODUCT ISOLATION

After separation of the broth into soluble and insoluble fractions, the next process depends on the location of desired product as follows: the cells themselves as in yeasts may be desired product; they are dried or refrigerated and the liquid discarded. Further treatment such as drying is discussed later in the chapter.

The required product may be bound to the mycelia or to bacterial cells as in the case of bound enzymes or antibiotics. The cells then have to be disrupted with any of the several ways available – heat, mechanical disruption, etc. The cell debris are now removed by centrifugation, filtration or any of the other methods for removing solids, described above.

Where the material is extracellularly available or if it has been obtained by leaching with or without cell disruption then it is treated by one of the following methods: liquid extraction, dissociation extraction, sorption, or precipitation.

Cell Disruption

A lot of biological molecules are inside the cell, and they must be released from it. This is achieved by cell disruption (lysis). Cell disruption is a sensitive process because of the cell wall's resistance to the high osmotic pressure inside them. Furthermore, difficulties arise from a non-controlled cell disruption, that results from an unhindered release of all intracellular products (proteins nucleic acids, cell debris) as well as the requirements for cell disruption without the desired product's denaturation. There are mechanical and non-mechanical cell disruption methods.

Mechanical methods: When the target material is intracellular, the means microorganisms are disrupted mainly by mechanical disruption of the cells. Equipment for cell disruption includes: *Homogenizers, Ball Mills and Ultrasonic disruption.*
Non-mechanical methods: Cells can be caused to disrupt by permeabilization thorough a number of ways: *Chemical Permeabilization, Mechanical Permeabilization and Enzymatic Permeabilization*

Liquid Extraction

Also known as solvent extraction, or liquid-liquid extraction this procedure is widely used in industry. It is used to transfer a solute from one solvent into another in which it is more soluble. It also can be used to separate soluble solids from the mixture with insoluble material by treatment with a solvent.

In this method the broth to be extracted is shaken with a hydrophobic solvent (i.e., one that will not mix with water), allowed to settle and the solvent which should contain more of the material to be extracted is removed. This may be done in a small laboratory scale in separating funnels or in a stirred tank in industry.

The separation may be done in a stirred tank in one of several ways: (1) batch wise in a single tank and the solvent with its solute drained; or (2) continuous with a mixing and a setting tank. More efficient extractions are achieved with continuous addition of solvent in (3) a cross-current arrangement in which successive solvent extracts will be progressively more dilute or in a (4) counter-current fashion in which efficient extraction is achieved with less solvent usage.

Ion-exchange Adsorption

Ion exchange adsorption is one of several adoption methods which include chromatography, and charcoal adsorption. These will be discussed later. Ionic filtrates of fermentation broths can be purified and concentrated using ion exchange resins packed in columns. An ion exchange resin is a polymer (normally polystyrene) with electrically charged sites at which one ion may replace another.

Synthetic ion exchange resins are usually cast as porous beads with considerable external and pore surface where ions can attach. Whenever there is a great surface area, adsorption plays a role. If a substance is adsorbed to an ion exchange resin, no ion is liberated. Testing for ions in the effluent will distinguish between removal by adsorption and removal by ion exchange.

Precipitation

The insolubility of many salts is used in the selective isolation of some industrial products. It is particularly useful in the elimination of proteinaceous impurities or in the isolation of enzymes. Salts are precipitated by one of several methods: adding inorganic salts and (or) reducing the solubility with the addition of organic solvents such as alcohol in the case of enzymes. Lactate and oxalate salts of erythromycin have been so isolated and citric acid has been isolated with its calcium salt.

4. PURIFICATION

The methods described earlier isolate mixtures of materials similar in chemical and physical properties to the required product. The methods used in this section are finer and further eliminate the impurities thus leaving the desired product purer.

Chromatography

In chromatography, the components of a mixture of solutes migrate at different rates on a solid because of varying solubilities of the solutes in a particular solvent. The mixture of solutes is introduced (usually as a solution) at one end of the solid phase and the solvent (i.e., the solution which separates the mixture) flows through this initial point of the mixture application. Fermentation products are separated by any of the following chromatographic methods, where the separation of the solids occur for the reasons given in each of the following.
(i) Adsorption chromatography: (e.g., paper chromatography) variations in the weak (Van der Wall) forces binding solutes to the solid phase;
(ii) Partition chromatography: A mobile solvent is passed through a column containing an immobilized liquid phase; the solvent and immobilized liquid phase are immiscible.

Separation occurs by the different distribution or partition coefficients of the solutes between the mobile and immobilized liquid phases.
(iii) Ion exchange chromatography: The difference in the strength of the chemical bonding between the various solutes and the resin constitutes the basis for this method.
(iv) Gel Filtration: This depends on the ability of molecules of different sizes and shapes to permeate the matrix of a gel swollen in the desired solvent. The gel can be considered as containing two types of solvent; that within the gel particle and that outside it. Large particles which cannot penetrate the gel appear in the column effluent after a volume equivalent to the solvent outside the gel has emerged from the column. Small molecules which permeate the matrix appear in the effluent after a volume equivalent to the total liquid volume within the matrix has emerged.

Carbon Decolorization

Some solids are able to adsorb and concentrate certain substances on their surfaces when in contact with a liquid solution (or gaseous mixture). These include activated charcoal, oxides of silicon, aluminum, and titanium and various types of absorbent clays.

Absorbents have been used for the adsorption of antibiotics from broths, removal of colored impurities from a solution of an antibiotic, sugar or even from gasoline. In the fermentation industry activated charcoal has been most widely used because of its extensive pores which confer on it a large surface. Furthermore, the pores are large enough to allow the passage of the solvent.

Activated carbon, powdered or granular, is used to remove color. Thus penicillin solution is usually treated with activated carbon before the crystallization of the amino salt. A single-stage batch-wide system of mixing the solution with carbon followed by filtration may be used. Multi-stage counter-current decolorization is far more efficient per unit of carbon than batch. Before using an adsorbent it is important to determine experimentally the most efficient depth of the absorption zone which will thoroughly remove all color.

Crystallization

Crystallization is the final purification method for those materials which can stand heat. The solution is concentrated by heating and evaporation at atmospheric pressure to produce a super saturated solution. Many fermentation products will not however stand heat and the initial water removal is made by heating at reduced pressure or by lowering the temperature to form crystals which can be centrifuged off leaving a concentrated liquor. It yields compounds which are highly potent more stable and free from colored impurities. To obtain crystals, first a super saturated solution is produced; secondly, minute nuclei or seeds are formed and thirdly, the molecules of the solute build on the nuclei. Crystalline particles from a previous preparation may be deliberately introduced to produce the nuclei. In procaine penicillin production, fine crystals are used to induce crystallization whereas in dehydrostreptomycin sulfate, addition of methanol brings about crystallization.

5. PRODUCT ISOLATION

The final isolation of the product is done in one of the two following ways:

(i) Processing of crystalline products.

(ii) Drying of products direct from solution.

Crystalline Processing

Crystalline products are free-filtering and non-compressible and therefore may be filtered on thick beds under high pressure. This is usually done on a centrifugal machine capable of developing very high (about 1,000 fold) gravitational force. The crystals are washed to remove adhering mother liquor. After washing they are dried by spinning for further drying or solvent removal.

Drying

Drying consists of liquid removal (either organic solvent or water) from wet crystals such as was described above from a solution, or from solids or cells isolated from the very earliest operation. Several methods of drying exist and the one adopted will depend on such factors as the physical nature of the finished product, its heat sensitivity, the form acceptable to the consumer, and the competitiveness of the various methods in relation to the cost of the finished product. Drying can be considered under two heads: (i) liquid phase moisture removal, and (ii) solid-phase moisture removal.

Liquid-phase moisture removal

Liquid-phase moisture removal involves drying by heat. When drying is done by heating, the processes may be broken down to the supply of heat to the material and the removal of the resulting water vapor. The simplest method is by direct heating in which heated atmospheric air both heats the material and removes the water vapor. In others, the heating is done at reduced pressure to facilitate evaluation of the water vapor. Under such conditions, indirect heating from a heated surface, radiation (e.g., infra-red) or both is used to supplement the heat introduced by reduced vapor pressure.

The actual method of heating is done in a number of different mechanical contraptions such as *Tray Driers, Drum dryers and Spray drying*

Solid-phase moisture removal (freeze-drying)

The equipment used in freeze-drying is essentially the same as in the vacuum drier described earlier. The main difference is that the material is first frozen. In this frozen state, the water evaporates straight from the material. It is useful for heat-labile materials such as enzymes, bacteria, and antibiotics.

Conclusion: In some of the fermented factory, recovery equipment cost four times more than the fermentor. The necessity of having a well-planned and reliable recovery process and an efficient recovery plant is therefore of utmost importance.