Topic: GENERAL ASPECTS OF ENZYME PRODUCTION

Subject: Botany

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Most enzyme production is carried out in deep submerged fermentation; however, a few are best produced in semi-solid media.

Related information can also be found at following link.

http://www.biologydiscussion.com/enzymes/enzyme-technology/enzyme-technology-application-and-commercial-production-of-enzymes/10185

https://royalsocietypublishing.org/doi/pdf/10.1098/rstb.1983.0004

Semi solid medium

"This system, also known as the 'Koji' or 'moldy bran' method of 'solid state' fermentation is still widely used in Japan. The medium consists of moist sterile wheat or rice bran acidified with HCl; mineral salts including trace minerals are added. An inducer is also usually added; 10% starch is used for amylase, and gelatin and pectin for protein and pectinase production respectively. The organisms used are fungi, which appear amenable to high enzyme production because of the low moisture condition and high degree of aeration of the semi-soluble medium. The moist bran, inoculated with spores of the appropriate fungi, is distributed either in flat trays or placed in a revolving drum. Moisture (about 8%) is maintained by occasionally spraying water on the trays and by circulating moist air over the preparation. The temperature of the bran is kept at about 30°C by the circulating cool air. The production period is usually 30-40 hours, but could be as long as seven days. The optimum production is determined by withdrawing the growth from time to time and assaying for enzyme. The material is dried with hot air at about 37°C–40°C and ground. The enzyme is usually preserved in this manner. If it is desired, the enzyme can be extracted. Growth in a semi-solid medium seems sometimes to encourage an enzyme range different from that produced in submerged growth. Thus, *Aspergillus oryzae* on semi-solid medium will produce a large number of enzymes, primarily amylase, glucoamylose, and protease. In submerged culture amylase production rises at the expense of the other enzymes. Similarly, if *Aspergillus oryzae* producing takadiastase (a commercial powder containing amylase and some protease) is grown in submerged culture four protease components are formed whereas on semi-solid medium not only are two proteases formed, but these are less heat resistant than those produced in submerged fermentation."[1]

[1] Okafor, Nduka (2007). Modern industrial microbiology and biotechnology. Science Publishers, USA.

Submerged medium

“Most enzyme production is in fact by submerged cultivation in a deep fermentor. Submerged production has replaced semi-solid production wherever possible because the latter is labor intensive and therefore expensive where labor is scarce, and because of the risk of infection and the generally greater ease of controlling temperature, pH and other environmental factors in a fermentor. The medium must contain all the requirements for growth, including adequate sources of carbon, nitrogen, various metals, trace elements, growth substances, etc. However, a medium adequate for growth may not be satisfactory for enzyme production. For the production of inducible enzymes, the inducers must be present. Thus, pectic substances need to be in the medium when pectinolytic enzymes are being sought. Similarly, in the production of microbial rennets soy bean proteins are added into the medium to induce protease production by most fungi. The inducer may not always be the substrate but sometimes a breakdown or end-product may serve. For example, cellobiose may stimulate cellulose production. Sometimes some easily metabolizable components of the medium may repress enzyme production by catabolite repression. Strong repression is often seen in media containing glucose. Thus, α-amylase synthesis is repressed by glucose in *Bacillus licheniformis* and *B. subtilis.* Fructose on the other hand represses the synthesis of the enzyme in *B. stearothermophilus.* In many organisms protease synthesis is repressed by amino acids as well as by glucose. It is therefore usual to replace glucose by more slowly metabolized carbohydrates such as partly hydrolyzed starch. High enzyme yield may also be obtained by adding constantly, low amounts of the inducer. End-product inhibition has also been widely observed. Some specific amino acids inhibit protease production in some organisms. Thus, isoleucine and proline are involved in the case of *B. megaterium* while sulphur amino acids inhibit protease formation in *Aspergillus niger.* Temperature and pH requirements have to be worked out for each organism and each desired product. The temperature and pH requirements for optimum growth, enzyme production, and stability of the enzyme once it is produced are not necessarily the same for all enzymes. The temperature adopted for the fermentation is usually a compromise taking all three requirements into account. The oxygen requirement is usually high as most of the organisms employed in enzyme production are aerobic. Vigorous aeration and agitation are therefore done in the submerged fermentations for enzyme production. Batch fermentation is usually employed in commercial enzyme fermentation and lasts from one to seven days. Continuous fermentation, while successful experimentally, does not appear to have been used in industry. In a few cases the enzyme production is highest during the exponential phase of growth. In most others, however, it occurs post-exponentially. Furthermore, different enzymes are produced at different stages of the growth cycle. Thus *A. niger* produces mostly α–amylases in the first 72 hours but mainly maltase thereafter.

Enzyme Extraction

The procedures for the extraction of fermentation products are applicable to enzyme extraction also. In order to limit contamination and degradation of the enzyme the broth is cooled to about 20°C as soon as the fermentation is over. Stabilizers such as calcium salts, proteins, sugar, and starch hydrolysates may be added and destabilizing metals may be removed with EDTA. Antimicrobials if used at all are those that are normally allowed in food such as benzoates and sorbate. Most industrial enzymes are extra-cellular in nature. In the case of cell bound enzymes, the cells are disrupted before centrifugation and/or vacuum filtration. The extent of the purification after the clarification depends on the purpose for which the enzyme is to be used. Sometimes enzymes may be precipitated using a variety of chemicals such as methanol, acetone, ethyl alcohol or ammonium sulfate. The precipitate may be further purified by dialysis, chromatography, etc., before being dried in a drum drier or a low temperature vacuum drier depending on the stability of the enzymes to high temperature. Ultra-filtration separation technique based on molecular size may be used."

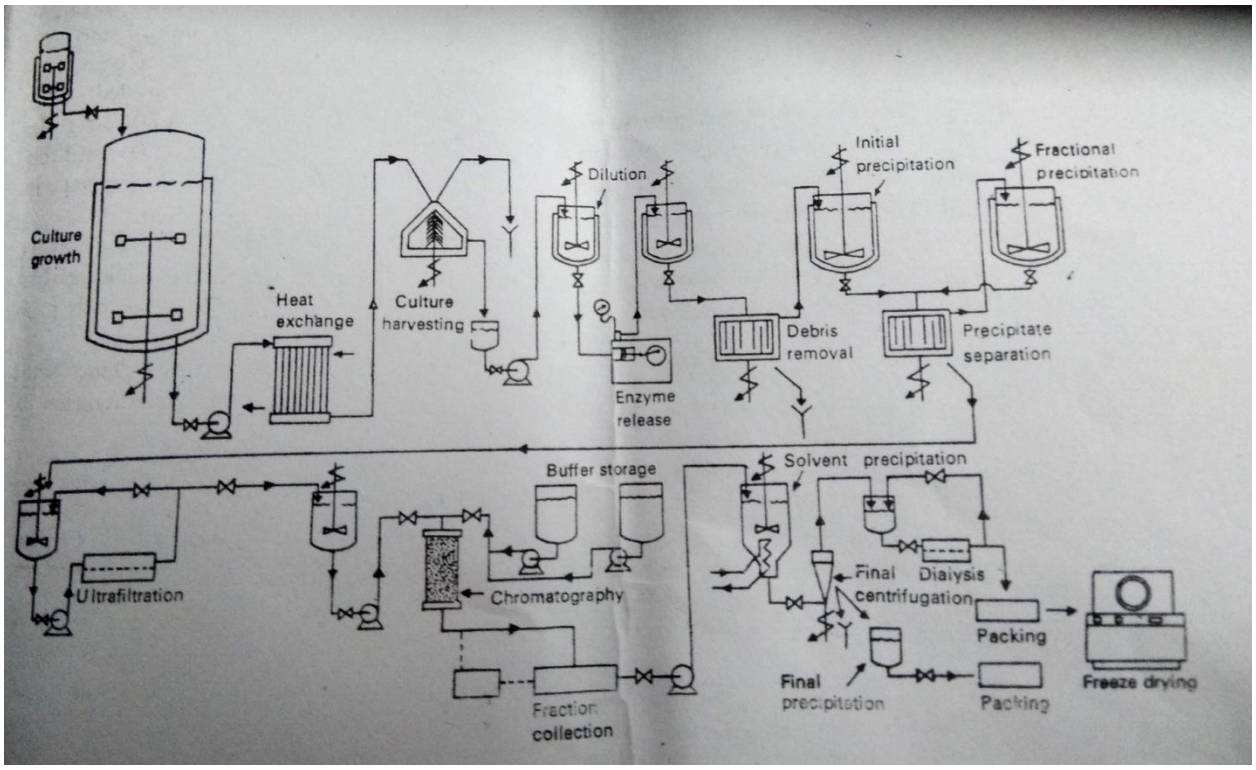

Fig. 1 Flowchart of production of enzymes

Packaging and Finishing

"The packing of enzymes has become extremely important since the experience of the allergic effect of enzyme dust inhalation by detergent works. Nowadays, enzymes are packaged preferably in liquid form but where solids are used, the enzyme is mixed with filler and it is now common practice to coat the particles with wax so that enzyme dusts are not formed.

Toxicity Testing and Standardization

The enzyme preparation should be tested by animal feeding to show that it is not toxic. This test not only assays the enzyme itself but any toxic side-product released by the microorganisms. For a new product extensive testing should be undertaken, but only spot checks need to done for a proven non-toxic enzyme in production. The potency of the enzyme preparation, based on tests carried out with the substrate should be determined. The shelf life and conditions of storage for optimal activity should also be determined."[2]

[2] Okafor, Nduka (2007). Modern industrial microbiology and biotechnology. Science Publishers, USA.

Suggested readings

Industrial microbiology- A. H. Patel

Enzymes in Industry (Production and Applications)- Wolfgang Aehle