ENZYME TECHNICAL ASSOCIATION

1776 K Street, N.W. | Washington, DC 20006 | Telephone: 202.719.4585 | Fax: 202.719.7049
www.enzymetechnicalassociation.org

Standard Manufacturing Process for Fermentation-based Enzymes Used in Food

The manufacturing process for fermentation-based food enzymes follows generally available and accepted standard industry practice.[1,2,3,4] Enzymes are produced in accordance with Good Manufacturing Practices (cGMP) in a 3-stage process that includes pure culture fermentation, recovery, and formulation (see Figure A).

Fermentation

A pure culture of the production microorganism is aseptically introduced to the fermentation media. The fermentation media includes raw materials (carbohydrates, proteins, vitamins, salts) that are safe and suitable for their intended use. Fermentation parameters such as temperature, pressure, oxygen feed, pH, and the concentration of nutrients are carefully managed to maximize enzyme protein production. The nutrients needed for enzyme production are consumed by the production microorganism during fermentation. After fermentation, the downstream process operations (recovery and formulation) are performed.

Recovery

Enzymes are recovered from the fermentation media by engineering operations widely used in enzyme production.[1,2,3,4] After fermentation, standard unit operations such as centrifugation and multiple types of filtration (i.e., microfiltration, diafiltration, rotary drum vacuum filtration, ultrafiltration, polish) are used to remove the production microorganism and other particulate matter including cell debris and fermentation raw materials, and to concentrate the enzyme resulting in a filtered enzyme concentrate. The objective is that the enzyme is separated from the production microorganism, and any remaining insignificant residues of the fermentation raw materials have no technical or functional effect in the final enzyme preparation.[5]

Formulation

The filtered concentrate is then finished with formulation ingredients acceptable for their intended use to stabilize and standardize the enzyme. Upon completion of the manufacturing process, the final formulated enzyme preparation is tested to verify compliance with quality specifications established for enzyme preparations, including those in the Food Chemicals Codex[6] and JECFA.[7]

---

1 **Aunstrup, K. (1979).** Production, Isolation, and Economics of Extracellular Enzymes. In: Wingard, LB, Katchalski-Katzir E, and Goldstein L (Eds), *Applied Biochemistry and Bioengineering Vol 2, Enzyme Technology, pp*. 28-68.

2 **Aunstrup, K, Andersen O, Falch EA, and Nielsen TK. (1979).** Production of Microbial Enzymes. Chapter 9 in: Peppler, HJ and Perlman D (Eds), *Microbial Technology* 2nd Ed. Vol 1., pp. 282-309.

3 **Kirk, O., Damhus, T., Borchert, T.V., Fuglsang, C.C., Hansen, T.T., Lund, H., Schiff, E.E., and Nielsen, L.K. (2004-2007).** Enzyme Applications, Industrial. In: Kroschwitz, J.L. (Ed.) Encyclopedia of Chemical Technology, 5th Edition. Volume 10. Wiley-Interscience, Hoboken, NJ, pp. 248-317.

4 **Sewalt, V., Shanahan, D., Gregg, L., La Marta, J., and Carrillo, R. (2016).** The Generally Recognized as Safe (GRAS) Process for Industrial Microbial Enzymes. Industrial Biotechnology 12(5): 295-302. http://doi.org/10.1089/ind.2016.0011.

5 **JECFA (2020).** Principles related to specific groups of substances, enzymes. In: *Environmental Health Criteria 240, Principles and Methods for the Risk Assessment of Chemicals in Food,* as updated in 2020.

6 **US Pharmacopeia (2020).** Monograph: Enzyme Preparations. In: *Food Chemicals Codex, 12th Ed.* The United States Pharmacopeial Convention, Rockville, Maryland. p. 404-410.

7 **Joint FAO/WHO Evaluation Committee for Food Additives (JECFA) (2006).** General specifications and considerations for enzyme preparations used in food processing. In: *Compendium of food additive specifications, volume 4. Analytical methods, test procedures and laboratory solutions used by and referenced in the food additive specifications.* FAO/JECFA Monographs 1. Rome, pp. xxi-xxv.

ENZYME TECHNICAL ASSOCIATION™

1776 K Street, N.W. | Washington, DC 20006 | Telephone: 202.719.4585 | Fax: 202.719.7049
www.enzymetechnicalassociation.org

Figure A: Standard Manufacturing Process for Fermentation-based Enzymes Used in Food