Production of Industrial Enzymes in Fermentation

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Abstract

Enzymes are proteins, which act as catalysts. Enzymes lower the energy required for a reaction to occur, without being used up in the reaction. Many types of industries, to aid in the generation of their products, utilize enzymes. Examples of these products are; cheese, alcohol and bread.

Fermentation is a method of generating enzymes for industrial purposes. Fermentation involves the use of micro organisms, like bacteria and yeast to produce the enzymes. There are two methods of fermentation used to produce enzymes. These are submerged fermentation and solid-state fermentation. Submerged fermentation involves the production of enzymes by microorganisms in a liquid nutrient media. Solid-state fermentation is the cultivation of microorganisms, and hence enzymes on a solid substrate. Carbon containing compounds in or on the substrate are broken down by the micro organisms, which produce the enzymes either intracellularly or extracellularly. The enzymes are recovered by methods such as centrifugation, for extracellularly produced enzymes and lysing of cells for intracellular enzymes.

Many industries are dependent on enzymes for the production of their goods. Industries that use enzymes generated by fermentation are the brewing, wine making, baking and cheese making.

Introduction

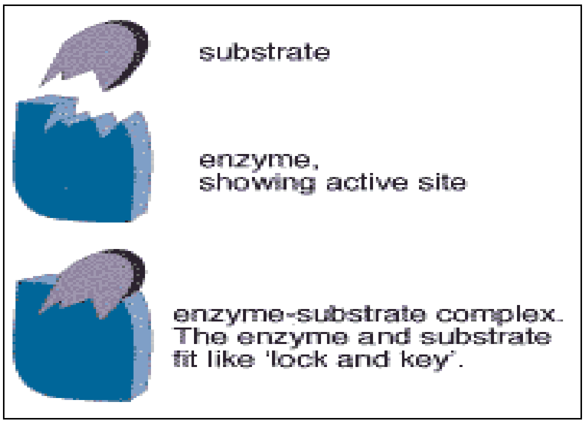
Enzymes are among the most important products obtained for human needs through microbial sources. They are catalysts. They speed up a reaction without being used up in the reaction. This was first observed in 1783 by Spallonzani (Italy)

In 1857, Pasteur showed that fermentation is closely linked to live yeast. He distinguished between the actions of organised ferments (cellular) and unorganised ferments (soluble). The soluble ferments are not bound to a living cell and were labelled enzymes by Kuhne (1878).

An essential property of a catalyst is that it must be able to regenerate the initial substrate from its products.

A large number of industrial processes in the areas of industrial, environmental and food use enzymes at some stage of the process.

Enzymes are large, three-dimensional protein molecules with an active site at a defined location on the surface. This site can be seen as a pocket that will only allow entry to specific substrates for a reaction to occur. This is described as the lock and key rule.



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Bonds between enzyme and substrate loosen bonds that hold the substrate together. The energy barrier is lowered and the reaction can proceed to reach equilibrium at room temperature.

Fermentation is defined as the energy yielding anaerobic metabolic breakdown of carbon containing compounds, for example glucose, without net oxidation. Fermentation can yield acetic acid, lactate, ethanol and other simple products.

In the fermentation of alcohol, which occurs with brewers yeast and some bacteria, the production of lactic acid is bypassed. The glucose molecules are degraded into two molecules of the two-carbon alcohol, ethanol, and also, two molecules of carbon dioxide. Some of the enzymes that are involved in the production of lactic acid and alcohol fermentation are identical to the enzymes utilized in the metabolic conversion of glycolysis.

When referring to fermentation regarding food, there are no distinctions between anaerobic and aerobic metabolism. Fermentation changes the characteristics of the food by the action of the enzymes produced by bacteria, mould and yeasts, which can occur in aerobic or anaerobic conditions.

There are many products that are derived from the process of fermentation and the use of enzymes. As mention above, alcohol is one product produced by enzymes and fermentation. The process of brewing and wine making produces alcohol. Other products include, cheese, yoghurt and bread. Bread making utilizes yeast and lactobacilli (in sourdough) to leaven the bread. The micro organisms and enzymes cause the release of carbon dioxide and lactic acid. Carbon dioxide enables the bread to rise.

The process of fermentation requires a food source (e.g. glucose); enzymes form bacteria or yeast and (depending on the product) anaerobic or aerobic conditions.

In this essay, we will concentrate on the production of industrial enzymes in fermentation processes for alcohol production and food.

Fermentation

Enzymes have been used for thousands of years to produce food and beverages, such as cheese, yoghurt, beer and wine.

Yeast is a fungus whose enzymes aid the breakdown of glucose into ethanol and carbon dioxide anaerobically.

The enzymes in yeast break down sugar (glucose) into alcohol (ethanol) and carbon dioxide gas:

$$\text{glucose} \xrightarrow{\text{yeast}} \text{ethanol} + \text{carbon dioxide}$$

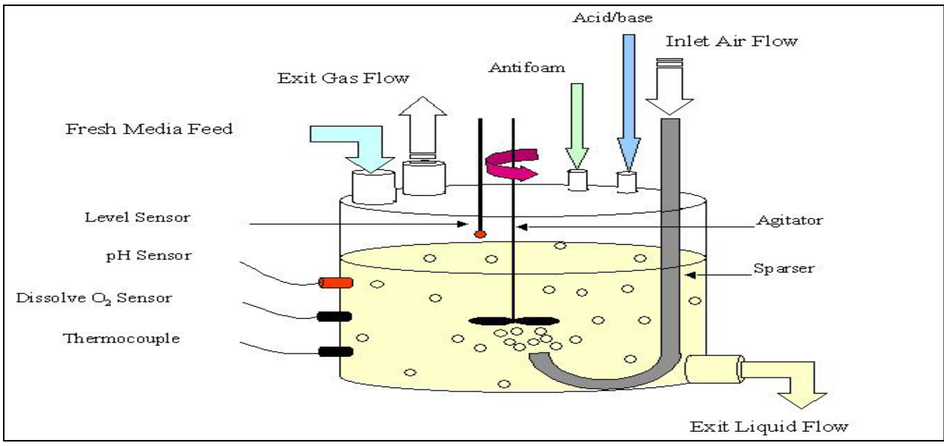
$$C_6H_{12}O_6\ (aq) \xrightarrow{\text{yeast}} 2\ C_2H_5OH\ (aq) + 2\ CO_2\ (g)$$

This reaction, which takes place in the absence of oxygen, is called fermentation.

Fermentation works best when the yeast and glucose solution is kept warm. Enzymes will also become ineffective if the temperature becomes too high.

Fermentation is used in all production of alcoholic drinks. For stronger alcohol, such as whiskey and vodka, these need to be distilled after fermentation to increase the concentration of ethanol in the fermented mixture. This is due to the fact that ethanol poisons the yeast and stops it working when the concentration builds up about 18 % by volume.

Fermentation is also used in the baking industry to make bread rise. After the dough has been prepared, it is left to rest in a warm place before going into the oven. This gives the enzymes in the yeast a chance to break down the sugar and make carbon dioxide.



Typical fermenter

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Submerged Fermentation

Submerged fermentation is the cultivation of microorganisms in liquid nutrient broth. Industrial enzymes can be produced using this process. This involves growing carefully selected micro organisms (bacteria and fungi) in closed vessels containing a rich broth of nutrients (the fermentation medium) and a high concentration of oxygen. As the microorganisms break down the nutrients, they release the desired enzymes into solution.

Due to the development of large-scale fermentation technologies, the production of microbial enzymes accounts for a significant proportion of the biotechnology industry's total output. Fermentation takes place in large vessels (fermenter) with volumes of up to 1,000 cubic metres.

The fermentation media sterilises nutrients based on renewable raw materials like maize, sugars and soya. Most industrial enzymes are secreted by microorganisms into the fermentation medium in order to break down the carbon and nitrogen sources.

Batch-fed and continuous fermentation processes are common. In the batch-fed process, sterilised nutrients are added to the fermenter during the growth of the biomass. In the continuous process, sterilised liquid nutrients are fed into the fermenter at the same flow rate as the fermentation broth leaving the system.

This will achieve a steady-state production. Parameters like temperature, pH, oxygen consumption and carbon dioxide formation are measured and controlled to optimise the fermentation process.

Firstly, in harvesting enzymes from the fermentation medium one must remove insoluble products, e.g. microbial cells. This is normally done by centrifugation. As most industrial enzymes are extracellular (secreted by cells into the external environment), they remain in the fermented broth after the biomass has been removed. The biomass can be recycled as a fertiliser, but first it must be treated with lime to inactivate the microorganisms and stabilise it during storage.

The enzymes in the remaining broth are then concentrated by evaporation, membrane filtration or crystallization depending on their intended application. If pure enzyme preparations are required, they are usually isolated by gel or ion exchange chromatography.

Certain applications require solid enzyme products, so the crude powder enzymes are made into granules to make them more convenient to use. Sometimes liquid formulations are preferred because they are easier to handle and dose along with other liquid ingredients. Enzymes used in starch conversion to convert glucose into fructose are immobilised, typically on the surfaces of inert granules held in reaction columns or towers. This is carried out to prolong their working life as these enzymes normally go on working for over a year.

Advantages:

Measure of process parameters is easier than with solid-state fermentation.

Bacterial and yeast cells are evenly distributed throughout the medium.

There is a high water content which is ideal for bacteria.

Disadvantages:

High costs due to the expensive media

Large reactors are needed and the behaviour of the organism cannot be predicted at times.

There is also a risk of contamination.

Solid State Fermentation

Solid-state fermentation (SSF) is another method used for the production of enzymes. Solid-state fermentation involves the cultivation of microorganisms on a solid substrate, such as grains, rice and wheat bran. This method is an alternative to the production of enzymes in liquid by submerged fermentation. SSF has many advantages over submerged fermentation. These include, high volumetric productivity, relatively high concentration of product, less effluent generated and simple fermentation equipment.

There are many substrates that can be utilized for the production of enzymes by SSF. These include wheat bran, rice bran, sugar beet pulp and wheat and corn flour. The selection of substrate depends on many factors, which is mainly related to the cost and the availability of the substrate. Other factors include particle size and the level of moisture. Smaller substrate particles have a larger surface area for the proliferation of the microorganisms, but if too small the efficiency of respiration will be impeded and poor growth and hence poor production of enzymes will result. Larger particles provide more efficient aeration and respiration, but there is a reduction in the surface area. A compromise must be reached, regarding the particle size of the substrate for a particular process. SSF requires moisture to be present on the substrate, for the microorganisms to produce enzymes. As a consequence the water content of the substrate must also be optimized, as a higher or lower presence of water may adversely affect the microbial activity. Water also has implications for the physico-chemical properties of the solid substrate. Enzymes of industrial importance have been produced by SSF. Some examples are, proteases, pectinases, glucoamylases and cellulases.

Industrial Enzymes and their uses:

Enzyme	Application	Sector
Beta-glucanase	Avoid filtration problems	Brewing
Amylase	Process control	Baking
Urease, pectinase	Removal of urea, increased yields	Wine
Chymosin	Clotting in cheese manufacturing	Dairy

Fermentation/Enzymes in the brewing industry

Fermentation is where the most important step takes place. This is where the production of the ethanol occurs. Usually it takes approximately 8-10 hours for fermentation to start. Once the desired ethanol is reached, the beer is chilled by means of a cooling plate within the fermenter. The beer is then sent to the ageing tank where it matures. The fermenter is then cleaned for thirty minutes using an 180^{o}C caustic solution.

Enzymes are extremely useful in the brewing industry for the production of beer. They can work as filtration improvers to get rid of any polysaccharides such as glucans and xylans. They can also aid in faster maturation of beer after fermentation. The main reason why enzymes are used in the beer industry is due to the fact that they are mush less expensive than the malting process.

β-amylases, from a strain of Bacillus and β-glucanases are examples of industrial enzymes used. α-Acetolactate decarboxylase is produced by submerged fermentation of Bacillus subtilis carrying the gene (AldB) coding for alpha-acetolactate decarboxylase from Bacillus brevis and is used as a processing aid in the brewing and alcohol industry.

Cheese Production

The first step in this process is curd formation. This occurs with the enzymatic cleavage of κ-casein, which destroys the stability of the milk system causing casein to aggregate. This is the introduction of a coagulating enzyme, adding salts and adjusting the temperature. Most cheeses use rennet. Rennet coagulates milk by precipitating casein. The technical term for rennin is chymosin.

The majority of milk protein is casein and there are four major types of casein molecules: alpha-s1, alpha-s2, beta and kappa. The alpha and beta caseins are hydrophobic proteins that are readily precipitated by calcium. The normal calcium concentration in milk is far in excess of that required to precipitate these proteins. However, kappa casein is a distinctly different molecule - it is not calcium-perceptible. As the caseins are secreted, they self-associate into aggregates called micelles in which the alpha and beta caseins are kept from precipitating by their interactions with kappa casein. In essence, kappa casein normally keeps the majority of milk protein soluble and prevents it from spontaneously coagulating. Chymosin cuts and inactivates kappa casein, converting it into para-kappa-casein and a smaller protein called macro peptide. Para-kappa-casein does not have the ability to stabilize the micellar structure and the calcium-insoluble caseins precipitate, forming a curd.

Chymosin is also a very important industrial enzyme because it is widely used in cheese making. Chymosin was extracted from dried calf stomachs for this purpose, but the cheese making industry has expanded beyond the supply of available calf stomachs. Many proteases are able to coagulate milk by converting casein to paracasein and alternatives to chymosin are readily available. Rennet is the name given to any enzymatic preparation that clots milk, specificity Chymosin splits κ-casein at a specific bond, namely Phe-150—Met-106.

Related Industry: **MEGAZYME**

Megazyme is the main producer of enzymes in Ireland. It was set up in Australia in 1989 and relocated to Bray, Ireland in 1996. It is a technology based, research Biotechnology Company. It manufactures analytical test kits for cereal, food, feed and fermentation industries. It focuses especially on the measurement of enzymes and carbohydrates.

Megazyme introduces methodology into industries that are traditionally conservative, for example, the baking and brewing industries. For any methods adopted, they must first be validated. This is carried out through inter-laboratory evaluation under the guidelines of relevant international associations such as the Association of Official Analytical Chemists International.

Some of the industries that Megazyme have been involved with are;

1. Flour milling and baking industry
2. Brewing and malting industry.

In the natural environment, starch occurs in granules. Portions of these granules are damaged physically while milling wheat. Unlike native starch granules, damaged granules absorb large quantities of water and affect the formation of the dough. The hydrated starch in damaged granules is quickly hydrolysed by amylase enzymes in the dough, producing fermentable sugars, which are used by yeast. These in turn produce carbon dioxide gas, required for dough development. An excessive level of α-amylase in the flour leads to excessive starch breakdown and to a sticky crumb texture in the final loaf.

In the malting process, barley is germinated under controlled conditions of temperature, moisture and oxygen. A major objective is to maximize the production of α-amylase enzyme and minimize the respiration of sugars. α-amylase plays an important role in degrading starch to fermentable sugars. Effective malting also leads to breakdown of cell-wall carbohydrates called beta-glucans. A high level of beta-glucan in the wort leads to problems in filtration and the formation of hazes and precipitates in the beer. Megazyme developed a range of methods for monitoring changes in the levels of key carbohydrates and enzymes.

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