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ENZYME SYNTHESIS BY FERMENTATION METHOD : A REVIEW

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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 microorganisms, 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 microorganisms, which produce the enzymes either intracellular or extracellular. 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.

Key words: Enzyme, Fermentation, Solid state fermentation, Submerged fermentation.

INTRODUCTION

Enzymes commercially available now are at economically comparable to the chemical process. Hence, any substantial reduction in the cost of production of enzymes will be a positive stimulus for the commercialization of enzymatic depilation. Proteases are one of the most important groups of industrial enzymes and account for nearly 60% of the total enzyme sale. The major uses of free proteases occur in dry cleaning, detergents, meat processing, cheese making, and silver recovery from photographic film, production of digestive and certain medical treatments of inflammation and the virulent wounds[1]. A wide range of microorganisms including them are available commercially; and, they have almost completely replaced chemical hydrolysis of starch in starch processing industry. Although many microorganisms produce this enzyme, the most commonly used for their industrial application are *Bacillus licheniformis*, *Bacillus amyloliquifaciens* and *Aspergillus niger*. Amylases stand out as a class of enzymes, which are of useful applications in the food, brewing, textile, detergent and pharmaceutical industries[2]. *Rhizopus oligosporous* 1HS13, *Aspergillus niger*, *Rhizopus oryzae*, *Saccromyce cerevisiae* and *Conidiobolus spp* have ability to produce proteases. Their biomass can be easily determined after simple drying in oven as well as in dissector and weighing by digital balance. Fungal proteases are of particular importance in the

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food industry Aspergillus and Mucor have been studied intensively as protease producers although *Rhizopus oligosporus* also produces proteases, has a high proteolytic activity in the tempe fermentation and furthermore, does not produce toxins[3].

Amylases are enzymes that break down starch or glycogen. The amylases can be derived from several sources such as plants, animals and microbes. The major advantage of using microorganisms for production of amylases is in economical bulk production capacity and microbes are also easy to manipulate to obtain enzymes of desired characteristics. The microbial amylases meet industrial demands; a large number of them are available commercially and, they have almost completely replaced chemical hydrolysis of starch in starch processing industry.

Enzyme is protein which is synthesized as intra and extra cellular compounds. Enzymes energize and catalyze biochemical reaction with high specificity and enhance the reaction rate. Lipases are one of the highly commercialized enzymes; have an important role found and ranked after proteases and amylases. Lipases are widely used in industrial application such as, detergent industry, pharmaceutical industry, pulp and paper industry, production of biodiesel, dairy and bakery foods, fats and oils. Lipases are fond in animal, plant and microorganisms. Besides the stability, selectivity and broad substrate specificity microbial lipases are more promising in terms of availabilities and productivities. Among the various sources of lipases, fungi is recognized as the best enzyme producer and also used for industrial application[4].

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.

Methods of fermentation

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.

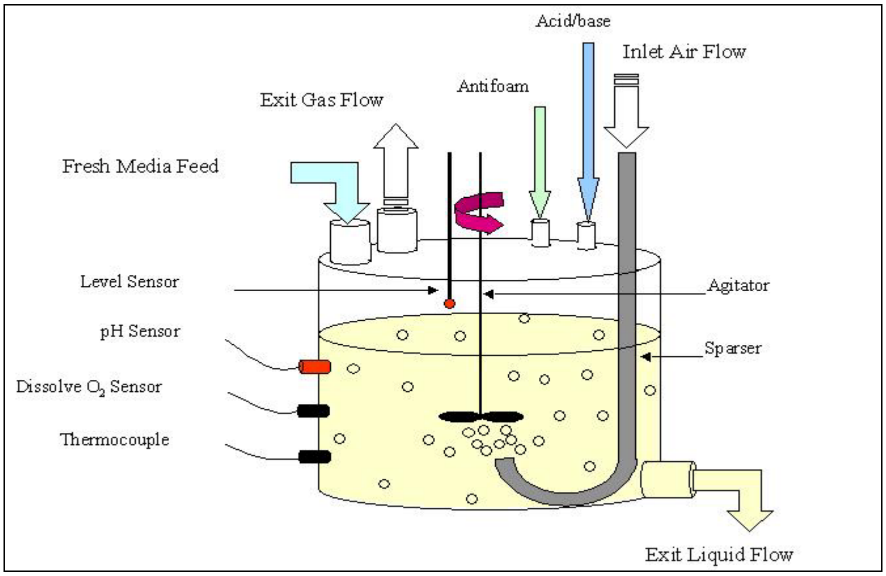

Fig. 1: Typical fermenter

Due to the development of large-scale fermentation technologies, the production of microbial enzymes accounts for a significant proportion of the biotechnology industries total output. Fermentation takes place in large vessels (fermenter) with volumes of upto 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.

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 physicochemical properties of the solid substrate. Enzymes of industrial importance have been produced by SSF. Some examples are proteases, pectinases, glucoamylases and cellulases[5].

Types of fermentation process

Fermentation in liquid media is of two types depending upon the mode of operation:

A. Batch fermentation

B. Continuous fermentation

Batch reactors are simplest type of mode of reactor operation. In this mode, the reactor is filled with medium and the fermentation is allowed to proceed. When the fermentation has finished the contents are emptied for downstream processing. The reactor is then cleaned, re-filled, re-inoculated and the fermentation process starts again.

Continuous reactors: Fresh media is continuously added and bioreactor fluid is continuously removed. As a result, cells continuously receive fresh medium and products and waste products and cells are continuously removed for processing. The reactor can thus be operated for long periods of time without having to be shut down. Continuous reactors can be many times more productive than batch reactors. This is partly due to the fact that the reactor does not have to be shut down as regularly and also due to the fact that the growth rate of the bacteria in the reactor can be more easily controlled and optimized.

In addition, cells can also be immobilized in continuous reactors, to prevent their removal and thus further increase the productivity of these reactors.

Continuous reactors are as yet not widely used in industry but do find major application in wastewater treatment. Fed batch reactor is the most common type of reactor used in industry. In this reactor, fresh media is continuous or sometimes periodically added to the bioreactor but unlike a continuous reactor, there is no continuous removal. The fermenter is emptied or partially emptied when reactor is full or fermentation is finished. As with the continuous reactor, it is possible to achieve high productivities due to the fact that the growth rate of the cells can be optimized by controlling the flow rate of the feed entering the reactor.

Factors influencing fermentation

A fermentation is influenced by numerous factors, including temperature, pH, nature and composition of the medium, dissolved O_2, dissolved CO_2, operational system (e.g. batch, fed-batch, continuous), feeding with precursors, mixing (cycling through varying environments), and shear rates in the fermenter. Variation in these factors may affect: the rate of fermentation; the product spectrum and yield; the organoleptic properties of the product (appearances, taste, smell, texture), The generation of toxins; nutritional quality; and other physic-chemical properties[6].

Effect of time

The effect of incubation period on enzyme production was investigated by checking the enzyme activity on 4th, 5th, 6th, 7th and 8th days of incubation in the different substrates at pH 7 and at room temperature.

Effect of temperature

The effect of temperature on enzyme production was investigated by fermentation in different substrates and incubated at 30^{o}C, 37^{o}C, 40^{o}C, 45^{o}C and 50^{o}C at pH 7 for 6 days.

Effect of pH

Fermentation investigated the effect of pH on enzyme production in different substrates by adjusting the pH of basal salt solutions to 4, 5.5, 6.5, 7, 7.5, 8 and 9. The substrates were then incubated for 6 days at room temperature.

Effect of carbon source

The effect of carbon sources on enzyme production was investigated by supplementing the basal salt solution, pH 7, with 2% of different carbon sources such as glucose, maltose, lactose, sucrose and starch. The substrates were then incubated for 6 days at room temperature.

Effect of nitrogen source

The effect of nitrogen source on enzyme production was studied by replacing the nitrogen source in basal salt solution, pH 7, with 2% of $NaNO_3$, $(NH_4)_2SO_4$, NH_4Cl, NH_4NO_3 and KNO_3, and incubated at room temperature for 6 days[7].

CONCLUSION

There are many products that are derived from the process of fermentation and the use of enzymes, 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. The micro organisms and enzymes cause the release of carbon dioxide and lactic acid. 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. Fermentation can yield acetic acid, lactate, ethanol and other simple products[5].

REFERENCES

1. Muhammad Nasir Iqbal, Optimization of Growth Conditions for Acidic Protease Production from Rhizopus Oligosporus Through Solid State Fermentation of Sunflower Meal, Int. J. Agri. Biolog. Sci., **1**, 1 (2010).
2. M. N. Hosseinpour, G. D. Najagpour, Submerged Culture Studies for Lipase Production by Abdul Rauf1, Muhammad Irfan1, Muhammad Nadeem1, Ishtiaq Ahmed* 1,2 and Hafiz Aspergillus Niger NCIM on Soya Flour (2011).
3. R. Paranthaman, K. Alagusundaram and J. Indhumathi, Production of Protease from Rice Mill Wastes by Aspergillus Niger in Solid State Fermentation, World J. Agri. Sci., **5(3)**, 308-312 (2009).
4. R. Vidyalakshmi, R. Paranthaman and J. Indhumathi, Amylase Production on Submerged Fermentation by Bacillus spp, World J. Chem., **4(1)**, 89-91 (2009).
5. R. Suganthi, J. F. Benazir, R. Santhi, V. Ramesh Kumar, Anjana Hari, Nitya Meenakshi, K. A. Nidhiya, G. Kavitha and R. Lakshmi, Amylase Production by Aspergillus Niger Under Solid State Fermentation Using Agroindustrial Waste, **3(2)** (2011).
6. Weir Emma, McSpadden Colette, Production of Industrial Enzymes in Fermentation.
7. Y. Chisti, Fermentation (Industrial): Basic Consideration, 663-674 (1999).