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A Review Article on Zymase

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Abstract: Zymase is a complex enzyme which catalyze the fermentation of sugar especially fructose to form ethanol and carbon dioxide. The activity of zymase enzyme varies for different strains of yeast sp. They also have great economic importance and are also advantageous in industries as well. The aim of this review is to study the updated information on zymase, its optimum requirements on the extraction processes and advantages of using zymase over yeast as well as the industrial applications.
Keywords: Zymase, Fermentation, Extraction process, Yeast.

I. INTRODUCTION

Enzyme may be defined as part of living system which carries out the activities of a cell. Zymase enzyme is naturally found in yeast especially *Saccharomyces cerevisae* [G. I. Roshkov. *et.al,* 1996] secreted for alcoholic fermentation. It loses its strength outside the cell due to the presence of tryptic enzymes for example endotryptase which are destructive towards zymase and also due to oxidation. [Arindam Kuila. *et.al,* 2017].

$$C_6H_{12}O_6 \rightarrow 2\,C_2H_5OH + 2\,CO_2$$

Zymase has the ability to convert sugars such as maltose and sucrose into alcohol. Zymase is a complex of many enzymes like: Invertase, Hexogenase, Zymohexase, Isomerase, Phosphatase, Triosedehydrogenase, Mutase, Enolase, and Carboxylase. Zymase is composed of two elements namely a dialyzable and thermo stable element (is also called co-ferment which has the ability to ferment sugars) and the other is a non dialyzable and heat labile element (is also called inactive residue and do not possess sugar fermentation ability). This enzyme complex has a tertiary structure [Arindam Kuila. *et.al,* 2017].

II. EXTRACTION OF ZYMASE

With the passage of time many methods were put forward for the extraction of zymase enzyme from the yeast cells under different names like yeast zymin, yeast powdered form etc.

A. Ber. Dt. Chem. Method (1897)

Firstly one thousand grams of yeast had been carefully mixed with the same amount of quartz sand and 250 g Kieselguhr*. The above mixture was stirred until the mass has become moist and flexible. 100 ml of water was added to the paste, the solution was filtered using a filter cloth and gradually subjected to a pressure of 400–500 atmospheres using a hydraulic press, and almost about 350 cc press juice was obtained. Now, 100 ml of water was added to the residue obtained from the first filtration and was again subjected to the same pressure in the hydraulic press. Further 150 cc of press juice was obtained. Therefore one kg of yeast gave 500 cc press juice, which consists of about 300 cc cell substances. The trace of turbidity was removed by adding 4 g of Kieselguhr to the press juice and was filtered through a filter paper [A. Cornish-Bowden, 1997] The yeast juice or press juice is a brownish color fluid with strong yeast smell and consisted of large quantity of albumen. Due to which when yeast juice was boiled it got coagulated and almost became solid.

B. Maefadyen. et.al, Method(1900)

100 grams of yeast was mixed with equal parts of water. The suspension of yeast cells was centrifuged, where the cells settled down as a thick creamy mass at the bottom and the supernatant was decanted. Again water was added to this creamy mass and then centrifuged. This process was repeated until the last added water was clear and colorless. The final product of this process was a firm mass of yeast cells. The pasty mass of yeast was wrapped in a " hydraulic chain cloth'' and introduced into a series of shallow iron trays, such that the mass got strongly compressed in the hydraulic press to which a pressure of almost 70-100 atmospheres was applied and the expressed liquor ran off. The mass of yeast was removed from the cloth and a perfectly dry white powder, consisting of yeast-cells with approximately dry exteriors was obtained. Now100 grams silver sand was added to the dried yeast and was violently agitated, in such a manner that the cell wall got ruptured. Due to the rupture of cell wall, contents of cell wall expelled as a result the dry mass became moist. During the whole process i.e. between the rupture of the cell wall and the examination of the final product, the material was kept cool by means of a brine circulation.

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The brine was maintained at a temperature of - 5° C by means of expanding anhydrous ammonia. Then 80 grams of kieselguhr was added to the mixture. Kieselguhr acts as a filtering material and gives a perfectly clear opalescent product in which no suspended particles can be discovered. A pressure of from 200—300 atmospheres was applied to get about 30-35 cc of press juice [Maefadyen. *et.al*, 1900].

C. Mr. Arthur C. Tanqukkay Method (1904)

The pressed yeast was rubbed into a powder and then mixed with alcohol and ether for a few minutes, the solution was then rapidly filtered off and the process was repeated for a few times until a semi solid mass was obtained. The mass was washed with dry ether and finally dried in the air. Now here instead of alcohol acetone can also be used instead of alcohol and then ether. A fine white impalpable powder which contains about 5 percent of water was obtained. This material was termed as Dauerhefe (permanent yeast) or zymin i.e. yeast treated with ether and alcohol by Buchner. It can be preserved for a very long period without losing its fermenting power [Berichte, 1900].

D. Extraction from Rhizopus Species

100 g of the culture of *Rhizopus oryzae* (fungus) which was extracted from koji extract for about 10 days at 25^{o} C (The koji extract is cooked rice inoculated with fermentation culture. This is most prevalent in Japan.) was taken and washed several times using distilled water. The mass was filtered a filter paper to remove the excess water. The mass of fungus was chopped into small pieces with the help of sterile scissors. To these fine pieces of the fungus, 100g of fine silver sand and 20g of diatomaceous earth was added. The mixture was then stirred in a porcelain mortor until it changed into a doughy consistency. The doughy mass was wrapped in a silk cloth (Habutai), and exerted certain amount of pressure to press out the juice. Almost 30c.c. of juice was obtained in the first pressing. Some more water was added and the mass was pressed again. A further 20c.c. of juice was obtained in the second pressing. Therefore a total of 50c.c. juice was obtained from 100g of the culture [Teizo Takahashi. *et.al*, 1927].

E. Alcoholic Fermentation" 4TH Edition (1932).

To 500 g of yeast and 3 liters of acetone was mixed. The mixture was stirred together for 10 minutes and was filtered using a Buchner funnel. The filtrate obtained was then mixed with 1 liter of acetone for 2 minutes and filtered. The residue obtained was coarsely powdered. The powder was mixed with 250 cc. of ether for 3 minutes and filtered. The residue was spread out on paper as a thin layer and was set to dry at room temperature for an hour. The powder was dried at 40-45^{0}C for 24 hours. The average yield was about 25 per cent of the original weight of the yeast [Harden, 1932].

F. Method of Producing Fermenting Agent by Charles von Friedrich, (1938)

The yeast was placed in an abrading mill with sand or glass as abrasive. The mixture was subjected to the abrading operation to open the yeast cells and at the same time sufficient pressure was exerted on the cells to remove the enzymes like zymase and invertase. But be very careful as a very high pressure results in removal of enzymes like endotryptase which have a detrimental effect on zymase. After the opening the yeast cells, a suitable diluent such as 10% glycerine was added. The substance with the diluent was then pressed through a canvas bag exerting pressure of about 100 pounds per Square inch to remove the coarser abrasive. The solution bearing the enzymes invertase and zymase was then passed through a suitable filter to remove the finer abrasives. The strength of the solution was standardized by comparing the amount of carbon dioxide generated by given amount of yeast and the extract for a given period of time, and the ratio was compared. The solution can also be diluted with water or other inert diluents as well. For example, a 10% glycerine solution may be used to lower the fermentation activity. The solution can be made more active by the addition of an insoluble phosphate such as calcium phosphate [Charles von Friedrich. *et.al*, 1938].

G. Microencapsulation Of Zymase Complex

Microencapsules are regarded as a form of immobilized enzymes. One of the merits of these capsules is that the enzymes remain intact and also retain its properties as a result it can be used in multi enzyme systems as well. The encapsulated forms of zymase are easy to handle and can be used in continuous column reactions. They can also carry out multi stage reactions just like natural yeast cells and can also act as models for studying cellular reactions. More over these capsules can be filtered out easily and no precipitation is observed indicating the absence of free enzyme. The procedure for preparation of encapsulated zymase complex is as follows:

1) *Preparation of Zymase Complex:* Freshly fermented yeast solution was treated in a refrigerated cell fractional to destroy the cells. The solution was then centrifuged at 2000 rpm for 30 minutes and the supernatant was filtered using a filter paper. The filtrate was turbid.
2) *Muscle Enzyme Extract:* 100 g of leg muscle of rabbit was cut into small blocks of each 1 cm. it was homogenated by a homoblender for 15 minutes. 200 ml of 0.05 M phosphate buffer of pH 7 was added. The solution was then centrifuged at 9000 rpm for 20 minutes.

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3) *Microencapsulation:* 3.0 g of enzyme extract was emulsified in 15 ml of 5% benzene with ladder polymer of Sesqui phenylsiloxane using a homoblender for 3 minutes. The emulsion was added to 150 ml of 3% gelatin solution phosphate buffered at pH 7 at 20^{o} C. The temperature was then gradually increased to 37^{o}C for 1 hour such that the benzene gets evaporated. A hard solid capsule wall of silicone was formed. The microcapsules were recovered by centrifugation and washed using buffer for 3 times.
4) *Incubation:* The microcapsules were placed in 50 ml flask containing zymase complex and 30 ml of 400 mM of glucose and were set for air incubation at 30^{o}C.The concentration of alcohol was determined using gas chromatography method. The reaction rate was found to be 10-50 µmol of alcohol per gram of capsule per hour [Masao Kitajima. *et.al,* 1971].

III. CHARACTERISTICS OF ENZYME (YEAST EXTRACT)

A. *Optimum PH for CO_2 Production*

Take 6 g of zymin, 3 g of glucose, 1.0 cc. of toluene, and varying amounts of either acid or base(0.1 N HCl or 0.1 N NaOH) and H_2O such that the total volume of the solution becomes 25 cc. The pH was measured at different time intervals i.e. at 10, 20, and 30----up to 90 minutes. Optimum pH values for zymin fermentation are 5.6 to 6.1 for the initial rate of CO_2 production (0 to 30 minutes), and 5.8 to 6.3 for the steady rate (30 to 90 minutes). The optimum pH for CO_2 production by living yeast and dried yeast was observed at a range of 4.0 to 8.5 [Hagglund. *et.al,* 1926] .The evolution of CO_2 by living yeast was nearly the same at pH 5 to 7 [von Euler. *et.al,* 1919]. The reported values for sugar decomposition by yeast juice were at a range of 5.5 to 8.0 pH [Hiigglund. *et.al,* 1926]. It is to be noted that the above pH ranges are rather broad. Mahdihassan (1930) found the pH of the yeast cell interior to be 5.9 to 6.0.

B. *Effect of Salts Without Phosphate*

The reaction mixture consisted of 6 g of zymin, 3 g of glucose, 1.0 cc. of toluene, and 25 cc. of water. To this some amount of salt was added. Salts acted as activators for the zymase enzyme complex, when added in the proper concentration. The first 30 minutes showed a period of more rapid fermentation, and during this period most of the free phosphate in the zymin esterifies. The rate of fermentation is considered as a measure of the activity of the enzyme phosphatase after a steady state is reached. During this phase the rate of fermentation is conditioned by the amount of free phosphate available for esterification [Harden. *et.al,* 1921]. Based on this criterion, it was concluded that salts like NH_4Cl, $MgSO_4$, $CaCl_2$, and NaCl are activators which liberates organic phosphate, while the effect of KCl is little as compared to the other salts.

C. *Effect Of Iron*

In order to obtain enzymes of highest activity it is essential that the abrasives used be iron free. Iron is destructive to invertase and Zymase enzymes. The reason for the lack of practicability and decreased fermentive power of the enzyme solution obtained by many scientists was largely due to the use of sand and kieselguhr. These consisted of iron to a greater or less extent. Instead of the sand and glass abrasives other abrasives for example, emery, carborundum, calcite, granite, etc. can be used. Another alternative is that sand can be washed with warm hydrochloric acid and then wash it with pure water until the traces of acid have been removed. After this the sand can be ignited to expel organic matter.

IV. APPLICATIONS OF ZYMASE

A. *Alcohol Fermentation*

The enzyme zymase was used as biocatalyst to improve ethanol production. The operational parameters including the aeration rate, pH and agitation speed was observed. The maximum ethanol production of 82g/l was obtained at 0.2vvm/l, pH5.5, 300rpm and 5g/l of enzyme at a fixed temperature of 35^{o}C [M. Siddique. *et.al,* 2018].

B. *Urine Test*

Fermentation with yeast results in decomposition of glucose into alcohol and carbon dioxide. This method is to distinguish the kind of reducing substance present in the urine and also for the quantitative estimation of glucose. But this method has one disadvantage which is the necessity of using perfectly fresh yeast in its performance. Now take a few amount of the enzyme extract, mix it thoroughly with the urine to be tested and pour the mixture into a fermentation tube. Besides the fermentation test applied to the urine, two other control tests should be made, one with normal urine to which a little glucose is added to prove the activity of the yeast, and another of normal urine alone, to prove that there is no self fermentation of the yeast [Jacob Rosenbloom, 1914].

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V. ADVANTAGES OF ZYMASE

The fermented foods have its own benefits but some people have shown certain side effects as well. Due to the high probiotic content the most common side effect is gas formation and bloating. The recent studies have shown that bloating is good but for certain people bloating becomes severe and painful. Moreover high amounts of exogenous biogenic amines, especially Him and Tym, in the human diet contribute to a wide variety of toxic effects. These amines are categorized as psychoactive or vasoactive. Psychoactive amines act on the neural transmission of the central nervous system, while vasoactive amine acts on the vascular system. The severity of the intoxication is influenced by many factors, such as the presence of other amines (Put and Cad) in the diet and consumption of alcohol or use of drugs inhibiting amine oxidase activity as well as diseases of the gastrointestinal system. Histamine (Him) is one of the biogenic amine with the highest biological activity. Him poisoning symptoms include headache, nausea, vomiting, diarrhea, itching, oral burning sensation, red rash, and hypotension. Symptoms can be reduced by a Him-free diet or be eliminated by antihistamines. However, because of the multifaceted nature of the symptoms, the existence of Him intolerance has been underestimated or its symptoms are misinterpreted. Clinical symptoms and their provocation by certain foods and beverages appear similar in different diseases, such as food allergy and intolerance of sulfites, or other biogenic amines (eg, Tym) [Maintz. *et.al,* 2007].Generally fermented foods are safe but certain contaminated bacteria can also enter causing serious illness. A major outbreak was reported by *Salmonella typhi* in 2012 causing 89 outbreaks, followed by *Escherichia coli* in South Korean schools in 2013-14. But in case of zymase this cannot occur as pure zymase is extracted and there are no chances for contamination. The use of zymase enzyme can avoid all the above mentioned problems. The zymase enzyme will ferment the sugar content rapidly but in case of yeast the fermentation occurs continuously until sugar content is present and this result in growth of yeast cells as a result of which gas formation and acidity etc occurs. Moreover the fermentation period is also less as compared to that of live yeast cells.

VI. CONCLUSION

Zymase is a complex enzyme which is present in almost all yeast species. The enzyme converts the sugar and forms alcohol and carbon dioxide i.e. results in alcoholic fermentation. Zymase was isolated by a German scientist Edward Buchner in 1897 and was also awarded a Nobel Prize in 1907 for chemistry. Since the discovery of the fermentative activity of yeast zymase many attempts were made for the extraction of enzyme and to study its various properties .Therefore many extraction processes are discussed including preparation of press juice, powdered zymase, from rhizopus species and zymin. The most widely used preparations are yeast juice by Buchner and zymin. The activity of zymin is about one-eighth that of living yeast, whereas the ratio for yeast juice is about one-fortieth. This is because Buchner's press juice required the use of a hydraulic press due to which certain co-enzymes and active metal ions were removed resulting in losing the fermentive power.

They also differ in stability, the dry zymin retain its activity for months, while yeast juice autolysis rapidly. When the fermentive power of zymin was compared, it is much more vigorous than the yeast juice, and is capable of fermenting nearly six times as much sugar. Whereas 40 grams of yeast made into juice would only ferment about 2 grams of sugar, the same made into zymin by means of acetone or alcohol would ferment about 12 grams.

It was carefully studied that zymase loses its strength due to tryptic enzyme like endotryptase and also due to iron present in the abrasives for opening the cell wall of yeast. Moreover zymase acts in an alkaline medium with a pH range of 4-8.5. The fermentive power of zymase increases when salts like NH_4Cl, $MgSO_4$, $CaCl_2$, and NaCl are added.

The extraction of zymase is greatly advantageous in the emerging industrial fields as the fermenting period of zymase is also less as compared to that of live yeast cells. Moreover the encapsulated forms of zymase is also another better alternative as the microcapsules are easy to handle and can be used in continuous column reactions.

They can also carry out multi stage reactions just like natural yeast cells and can also act as models for studying cellular reactions. More over these capsules can be filtered out easily and no precipitation is observed indicating the absence of free enzyme. Recently it has been reported that fermented foods are causing certain diseases in consumers like gas formation, bloating, food poisoning to other serious disorders. The cultivation of yeast for fermentation results in growth of certain unwanted microorganisms resulting in contamination due to which cases of food poisoning has been reported whereas in the process of zymase extraction involvement of unwanted microorganisms does not occur. Also it has been discovered that bioactive amines are being used for fermentation which also results in headache, nausea, vomiting, diarrhea, itching, oral burning sensation, red rash, and hypotension which are the very basic symptoms of Him poisoning. Therefore to conclude use of zymase is a better alternative for yeast. Even though the process is a little laborious the results are satisfactory and safe for the human consumption.

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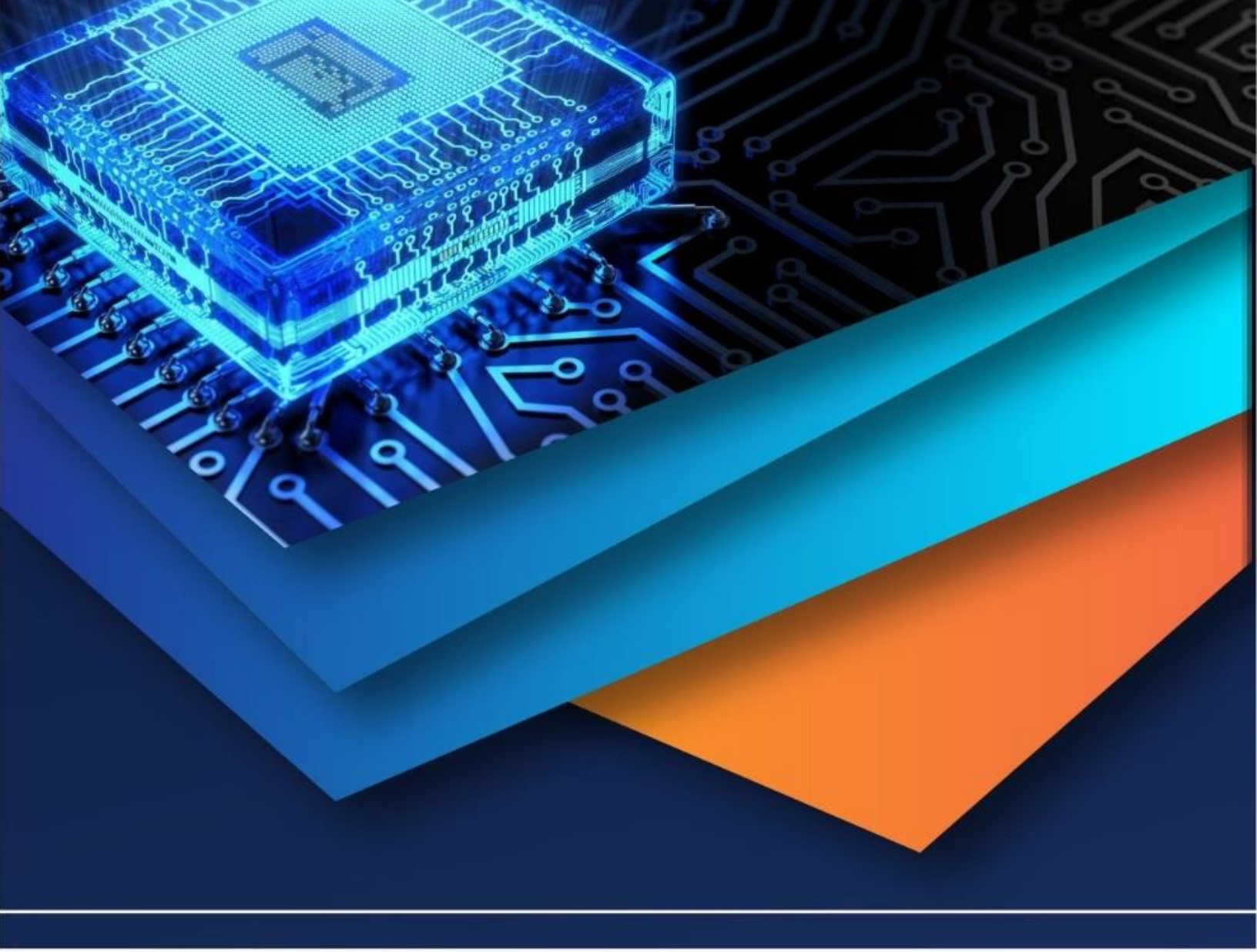

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