Bioprocess technology Strategies, Production and Purification of Amylases: An overview

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Citation

Abstract

The Present paper deals with bioprocess strategies involved in the production of Amylases from different microbial sources. Amylases purification and different sources sub categories of amylase production discussed in detailed .The paper also deals with Glucoamylase, Molecular Biology of Amylases , and application of commercially available enzymes.

Amylases are among the most important enzymes and are of great significance in present-day biotechnology. Although they can be derived from several sources, such as plants, animals and microorganisms, the enzymes from microbial sources generally meet industrial demands. Microbial amylases could be potentially useful in the pharmaceutical and fine-chemical industries if enzymes with suitable properties could be prepared. Interestingly, the first enzymes produced industrially were an amylase from a fungal source in 1894, which was used as a pharmaceutical aid for the treatment of digestive disorders [1]. With the advent of new frontiers in biotechnology, the spectrum of amylase application has widened in many other fields, such as clinical, medicinal and analytical chemistries, as well as their widespread application in starch analytical chemistries, as well as their widespread application in starch saccharification and in the textile, food ,brewing and distilling industries. Some of the applications of commercial available enzymes available enzymes are summarized in Table1.

Amylases have most widely been reported to occur in microorganisms, although they are also found in plants and animals. Two major classes of amylases have been identified in microorganisms, namely ? -Amylase and Glucoamylase. In addition, ? -amylase and, which is generally of plant origin, has also been reported from a few microbial sources. ? -Amylases (endo-1,4-?--D-glucan glucohydrolase,EC 3.2.1.1) are extra cellular enzymes that randomly cleave the 1,4- ? -D-glucosidic linkages between adjacent glucose units in the linear amylase chain. These are endozymes that split the substrate in the interior of the molecules and are classified according to their action and properties. For examples, amylases that produce free sugars are termed 'saccharogenic' and those that liquefy starch without producing free sugars are known as 'starch-liquefying' . ? Amylases (?-1,4-glucan maltohydrolase,EC 3.2.1.2) is usually of plant origin, but a few microbial strains are also known to produce it. It is an exoacting enzymes that cleaves non-reducing chain ends of amylase, amylopectin and glycogen molecules. It hydrolyses alternate glycosidic linkages, yielding glycosidc linkages maltose (? meric form).Since ? amylase is unable to by pass -1,6-glycosidic ? linkages in amylopectin, it results in incomplete degradation of the molecule, yielding 50-60% maltose and limit dextrin. Glucoamylase (synonym amyloglucosidasse, 'glucanogenic enzymes','starch glucogenase' and 'Y-amylase'; exo-1,4- ? -D-glucan glucano-hydrolase,EC 3.2.1.3) hydrolyses single glucose units from the non-reducing ends of amylase and amylopectin in a stepwise manner. Unlike ? amylase, most glucoamylopectin, although at a lower rate than 1,4- ? -linkages. Thus glucose, maltose and limit dextrins are the end products of glucoamylase action. Some of the properties of amylases are summarized in Table2.

▯-AMYLASE

▯ -Amylase may be derived from several bacteria, yeasts and fungi. Bacterial amylase, however, is generally reefed over fungal amylase due to several characteristic advantages that it offers. Strain of Aspergillus sp [2,3,4] and Bacillus sp[5,6,7], mainly Bacillus amyloliquefaciens and B.licheniformis, are employed for commercial applications. Themostable ▯-amylases are generally preferred as their application minimizes contamination risk and reduces reaction time,

thus providing considerable energy saving. Hydrolysis carried out at higher temperature also minimizes polymerization of D-glucose to Isomaltose.

⬚ AMYLASE

Unlike other members of the amylase family, only a few attempts have been made to study ⬚ amylases of microbial origin a few attempts have been made to study ⬚ amylase have generally been obtained from plant sources. Bacterial strains belonging to Bacillus [8],Pseudomoas (aerobic) [8] and Clostridium (anaerobic) sp. [9,10,11] actinomycete strains belonging to Streptomyces sp. [8] and fungal strains belonging to Rhizopus sp.[8] have been reported to synthesize ⬚ amylase.

GLUCOAMYLASE

They can be derived from a number of sources, such as plants , animals and microorganisms. Commercial need, however, is met by glucoamylase obtained from microbial sources. Filamentous fungi apparently constitute the major source of glucoamylase among all microbes[12].

PRODUCTION OF AMYLASES

Although several microorganisms can produce amylases, it remains a challenging task to obtain a strain is the most significant factor in the amylase production process. Sometimes a single strain can produce more than one enzyme, i.e. alpha-amylase as well as glucoamylase. For example, the strains of A.niger can produce as many as 19 enzymes, whereas alpha-amylase can be produced in reasonably good titres by as many as 28 strains [13]. Commercial production of amylases is carried out in various steps, essentially because the environmental factors required for the optimum growth for microorganism being employed factors required for the production of enzymes. These parameters include nutrient supplementation, pH of the medium, osmotic relationship, and degree of aeration, temperature and the control of contamination during fermentation. Maintaining the purity of the medium is also a very important factor, especially when the fermentation is carried out under aerobic conditions. Although the details of the specific fermentation processes adopted by different manufacturers vary, there remain two main methods for amylase production, submerged fermentation and solid–state fermentation. Solid-state fermentation has gained renewed interest from researchers for the production of these enzymes in view of its several economic and engineering advantages and has been often employed to produce amylases [14,15].Since thermo stability is a feature of most of the

enzymes sold in bulk for industrial applications, thermophilic microorganisms are of special interest for the production of especially ⬚, ⬚ –amylase, has concentrate on the enzymes of thermophlies and extreme thermophlies. Between the thermophiles and mesophilic groups lie the much less common and largely unexplored facultative thermophiles. Whereas their growth may be optimum at 450C, they are capable of growing well at higher temperature, thus covering both the mesophilic and thermophilic ranges .However, not much is known about the process for enzymes production involving such organisms. Comparative analysis of the structure and physico-chemical properties of these two groups (mesophilic and thermophilic)may provide some information about the molecular basis of the high-temperature tolerance ability of thermostable amylases.

PRODUCTION OF ⬚-AMYLASE

Bacillus species are considered to be the important sources of ⬚-amylase and have been used for enzymes production using solid substrate fermentation (SSF) [16,17,18] or submerged fermentation (SmF) [19,20]. The SSF process is a potential tool for achieving economy in enzyme production and starch hydrolysis. Omidiji et al [20] and Bajpai et. al., [21] developed simple and cheap media based on cheese whey, corn steep liquor and soya bean meal for ⬚-amylase production. It was claimed that the medium could be exploited for the industrial production of ⬚-amylase. Salva and Moraes [22] studied the effects of different carbon sources on ⬚-amylase production. Where as lactose, dextran and soluble starch were found suitable for enzyme production, the highest enzymes yield was obtained when glucose was used. EI Helow and EI Gazaerly [23] compared ⬚-amylase production in three different nutritional media. Different patterns of enzymes induction were obtained when beet pulp, corn cob, rice husk, wheat bran and wheat straw were used separately to partially replace the nutrient content of the selected medium. ⬚-Amylase was maximally expressed in the effects of different carbon sources (glucose,maltose,xylose and starch) on ⬚-amylase production. Higher cell density and higher specific growth rate were obtained from glucose but higher enzymes activity and higher specific enzyme activity were obtained from starch. Using a defined synthetic medium, Hiller et al., [25] demonstrated the effects of lactose nitrogen on cell physiology and ⬚-amylase production. Results showed cell-growth and ⬚-amylase production patterns to be similar regardless of the limiting nutrient and suggested stationary

phase gene control of ⍺-amylase production as opposed to a direct response to nutrient limitation. Key et al.[19] described the production of ⍺-amylase in a bi-phasic process in which high ⍺-amylase activities were achieved. Oxygen transfer condition, and especially the dissolved oxygen tension, was reported as vital factors for ⍺-amylase production [26]. High aeration rates were found to be essential for good yields of enzymes. Control of dissolved oxygen tension, however, was not found to be advantageous. Babu and Satyanarayna [16] also achieved maximum ⍺-amylase yield in aerated reactors. In view of the apparent advantages offered by cell recycling in bioprocesses, Gron et al.[28] investigated ⍺-amylase production in a cell recycling in bioreactor incorporating a membrane filtration module for cell separation. The reactor gave increased enzymes yield and volumetric productivity compared with conventional continuous fermentation. Coupling of fermentation and microfiltration for ⍺-amylase production was also studied by Morcel and Beidermann [29]. Compared with a batch process, continuous fermentation with cell recycling led to a reduction in ⍺-amylase concentration but to doubling of volumetric productivities. Tari et al.[30] also demonstrated operation strategies of a two-stage bio-reactor for ⍺-amylase production. They claimed that this would improve enzymes production. During laboratory studies on enzyme production, as a practice, single-stage inoculum is used for fermentation processes. Generally, it is carried out in a routine way without being given critical attention. Keeping this mind, miner et al.[27] studies one-stage and two stage inoculum. Since filamentous fungi are generally consider to be the most prolific producers of extra cellular enzymes, attempts have been made to study ⍺-amylase production of the using them. The thermophilic fungus Thermomyceslanuginosa was reported to be an excellent producer of ⍺-amylase [31,32]. Increased production of the enzyme could be obtained by manipulating the growth conditions and medium composition. Sudo et al.[33] compared acid –stable ⍺-amylase production in SmF and SSF and examined the the reason why A. kawachil IFO 4308 produced larger amounts of acid-stable ⍺-amylase in SSF than in SmF .Some of the SSF characteristics were given as the major reasons for higher enzyme production in SSF . Krishna and Chandrasekaran [17] cultivated Aeromonas caviae (CBTK 185) on banana waste. The results indicated the excellent scope for utilizing this strain and banana wastes for commercial production of ⍺-amylase in SSF.

The cell- immobilization technique has also been employed for ⍺-amylase production. Lvanova et al. [34] compared various immobilization techniques including entrapment in gels such as calcium alginate, ⍺-carrageenan, agar and their combinations with such as polyethylene oxide and fixation on formaldehyde-activated acrylonitrile\acrylamide membranes for ⍺-amylase production. Among these, agar, ⍺-carrageenan , agar/polyethylene oxide gels and the membranes were found to be suitable.

PRODUCTION OF ⍺-AMYLASE

⍺-amylase are usually of plant origin, and not much work has been done on the production of ⍺-amylase using microorganisms. Some of the microorganisms reported to produce ⍺-amylase include Bacillus polymyxa, B.cereus,B.megatarium,streptomyces sp., pseudomonas sp. and Rhizopus japonicus [1,8]. Ray et al. [35] compared the production of ⍺-amylase from starch waste by a hyper-amylolytic strain of B.megaterium B6 mutant UN12 in SmF and SSF . The starchy wastes used as substrates were from arrowroot, arum , maize, potato, pulse, rice , rice husk, tamarind , kernel, cassava, water chestnut wheat and wheat bran. Arum and wheat bran gave the highest yields.

PRODUCTION OF GLUCOAMYLASE

Extensive work has been carried out on the production of glucoamylases in solid cultures using A.niger [12,36,37]. The study included screening of a number of agro-industrial residues such as wheat bran, rice bran, rice husk, gram flour, wheat flour , corn flour, tea waste ,copra waste etc.[36,37,38,39]. Apart from the substrate particle size , which showed profound impact on fugal growth and activity, substrate particle size, which showed profound impact on fugal growth and activity, substrate moisture and water activity also influenced the enzymes yield significantly [12,40,41,42]. Design and configuration of fermentor, its mode of operation and fermentation influence the enzymes production . These included flaks, trays, rotary reactors and columns (vertical and horizontal)[42,43,44, 45]. Fermentation techniques included SmF, SSF, Semi-solid and in aqueous two- phase system [46, 47]. Enzyme production in trays occurred in maximum quantities in 36 h compared with 96 h typically required in flaks [42].

PURIFICATION OF AMYLASES

Enzyme application in pharmaceutical and clinical sectors requires high purity amylases. Thus, it is significant to developed economic process for their purification to obtain chemically pure enzymes with maximum specific activity. Traditionally the purification of amylases from fermentation media has been done in several steps, which include centrifugation of the culture (a step of extraction may be

required for solid media), selective concentration of the supernatant usually by ultra filtration, and selective precipitation of the enzyme by ammonium sulphate or organic solvents such as ethanol in the cold. Then the crude enzyme is subjected to chromatography (usually affinity or ion-exchange chromatography) and gel filtration.

MOLECULAR BIOLOGY OF AMYLASES

Genetic engineering has been used extensively for cloning of amylase producing strains, mainly ⍺-amylase and GA, in order to achieve desirable characteristics in the cloned host. The purpose of gene cloning can be, amongst other, the expression of thermostable enzymes, higher enzyme productivity and co-expression of two enzyme by the same organism. A great deal of work has been done on the cloning of ⍺-amylase genes in different microbes, mostly in Escherichia coli or saccharomyces cerevisiae. Apparently, no attention has been paid to the cloning of genes for ⍺-amylase of microbial origin, although a few reports are available on its expression on its expression in E.coli, mostly from Bacillus sp. and at least one from Thermoanaerobacterium. Like ⍺-amylase, the number of glucoamylase-encoding genes also varies between strains.

{image:1}

{image:2}

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