IRJMETS
e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
Volume:02/Issue:07/July -2020 www.irjmets.com

STABILIZATION AND DELINEATION OF THE GLUCOAMYLASE BY THE ASPERGILLUS NIGER : REVIEW

Esha Dwivedi*[1], Lalit Kumar Singh*[2]

*[1,2]Department of Biochemical Engineering, School of Chemical Technology, Harcourt Butler Technical University, Kanpur, India

ABSTRACT

The glucoamylase production was carried out by the culture of *Aspergillus niger* and different substrates were taken in a solid-state fermentation. The wheat bran were estimated to test the competence and the productivity by using the sources of the carbon for the formation of the enzyme. The enzyme productivity was higher when wheat bran was used as a substrate. It was seen that when the wheat bran as a substrate was replaced by the other substrate such as rice bran, corn starch, Bengal gram, the production of the enzyme was extended and drifted.

Keywords: Glucoamylase, Starch, Glucohydrolase, Filamentous Fungi

I. INTRODUCTION

Glucoamylase

The one of the most important and the oldest used biocatalysts in the industry is the glucoamylase. A large number of filamentous fungi and a large number of the microorganisms have been produced by the glucoamylase. Glucoamylase, an exo-enzyme that plays a major role in starch breakdown into its glucose unit. Glucoamylase was loosely referred to as I-amylase, amyloglucosidase, or glucamylase (Solomon 1978). Chemically it is also known as α-1,4glucanglycohydrolase in which glucan refers to a sequence of connected glucose units and glycohydrolase made up of glycol and hydrolase which mines breakdown of glycosidic linkage present in between two glucose units. The enzyme identification code allocated to the Commission for Enzymes EC 3.2.1.3 by IUBM1992. Number 3 denotes the hydrolysis of large sugar molecule by the addition of water. While number 2 denote the glycosidase, which breaks the glycoside linkage present in between two glucose molecules.

Several researchers, like Fogarty Vihinenet, have studied microbial glucoamylase. Al. (back in 1989). This study looks closely at production of microbial glucoamylase, from *A. niger*.

II. ACTION OF GLUCOAMYLASE

Starch Structure

To know about the glucoamylase action, we have to observe starch's structure. Starch consists of amylase which is linear macromolecule and amylopectin which is highly branched macromolecule, in the ratio of one amylase part to three amylopectin parts. Both polysaccharides of elevated molecular weight are structured into semi crystalline granules (diameter 2-100 pm), where both macro-molecules are engaged in crystalline. Amylase is made up of α-1,4glycoside bound glucose (Mercier et al. 1988). One glucose unit's first carbon is connected to the other glucose unit's carbon no. 4. Therefore, the name is -l, 4 Bond (Solomon et. al. 1978; Colonna et. al. 1988). Many long linear monomeric chains are intertwined at α-l, 6 Glycosidic bonds to form a very strongly branched network. There is the same α-1,4Glycosidicrelation in Amylopectin between two units of glucose. Linear chains consisting of about 21-26, α-1,4linked chain of glucose interlinked by α-l,6 Glycosidic links to create multi-branched structures (Manners 1989). Therefore, strongly related system exists. The most abundant source and the widely available source of the energy in plants is the starch. The maltose, gulocose, organic acids, amino-acids etc. are produced by the starch.

Carbohydrases

Carbohydrases are enzymes capable of separating starch and various saccharides. Glycogen l,4-glycosidic bonds in carbohydrate can be hydrolyze by E.C. 3.2.1.1 (α- Amylase) in amylopectin and endo-fashion. All α-amylases break α -1,6glycosidic bonds, but it cannot brake. Upon amylase hydrolysis, followed by the following reaction;

amylopectin maltothriosis hydrolysis, maltose and maltotriosis are produced. α-amylase hydrolysis also produces a number of branched α-limit dextrin with a 4 or more-glucose residue and branched chains of α –1,6 glycoside chain connections. α-amylases are engineering innovation for ethanol and food sectors, including starch dilution, in the process of sugar fermentation. Digestion is a method that is accompanied by a thermal hydrolysis of amylase particulates (Mercier and Colonna 1988; Vihinen and Mantsala, 1989), disperses of insoluble starch granules in the aqueous solution. Glucoamylase an exo-active enzyme that develops D-glucose in successive α-l, 4 hydrolysis from non- reduction chain

(Fogarty et. al. 1983). The α-1, 6 Amylase and the rare α-l hydrolysis number 3 interfere greatly at much slower levels and if the enzyme is incubatorised for a longer period, it can hydrolyze starch fully. This is why, when incubated for long periods of time, that enzyme will hydrolyze starch entirely. It is also the scarifying enzyme. Wide connections of oligosaccharide to approx. 90% α-L are possible through glucoamylase, 6. Furthermore, the hydrolysis susceptibility plays a crucial role in the substrate width and orientation of the α-1, 6 contacts. Trans-glycosidase (EC 2.4.1.14) is also an impurity in untreated Glucoamylase processes. This enzyme catalyzes a glucose saccharine synthesis that is not fermentable by moving from α-l, 4 to α-l, 6 positions glucosyl residue.

The production of Panose and maltose affects the synthesis of final glucose. Different methods been developed for most successful muddy array of the low trans-glycosidase mutants to eliminate trans-glycosidase from consumer formulations of the glucoamylase (Fogarty 1983).

Glucoamylase Action

Glucoamylase is recommended that a particular chain structure be used concurrently in which enzyme is only operating on these all substrate molecules (Solmon et al. 1978). The planned ionizing process of the glycoside bond is shown. The amino group of hydrogen ions and Imidazole group of the active site is protonating D-glycosidic bond oxygen. AC-1 defective electron core receives electrons from a group of supporters like a hydroxyl system from the solvent and the serine component of the active site. Glucoamylase is proposed to function on all substrates molecules through a multi-chain random enzyme activity cycle (Solomon et al. 1978).For the glycosidic cleavage, the ionic mechanism suggested is shown. The D-Glycosidic bond oxygen being protonated by H^+ ions in Imidazole and amino group of active site enzyme. Electrons from donor group, such as a hydroxyl band from active fluid or a serine site receives an electron from the defect bond electron core at C-1. The resulting structure is divided on the side of the bond carbon (C-1) and leads to an intermediate carbon ion and positive

D-glycosyl component. The final step is to add a hydroxyl ion (or water molecule) to the 20 intermediate carbonium ion α-D-glucose hydrolysis (Solomon et al. 1978).Detailed information on the structure of amino acid at the active site of enzyme is presented in following section.

III. SOURCES AND FORMS OF GLUCOAMYLASE

Although the majority of glucoamylase are found in fungi, these are produced by various plants and animals and micro-organisms also. Both *Aspergillus niger* and *Rhizopus sp.* varieties are used to produce professionally working enzymes. Where glucose is used in maltose-oligosaccharide conversion (Fogarty et. al. 1983; Pandey et. al 1995). The Food & Drug Administration (FDA) generally finds these metabolites to be safe. Glucoamylase processes have been thoroughly explored elsewhere (Vihinen et. al. 1989).Several experiments on the heterogeneity of glucoamylase have been performed after two forms of black koji mold glucoamylase were described in the 1950s. Several mechanisms are believed to result from the different types of glucoamylase: variations in mRNA, tiny lyses protein, discrepancies in carbohydrate content and similar structural genes it being (Pretorius et. al. 1991).

Mold Glucoamylase

Glycoprotein's are 1 to 5 types of fungal glucoamylase. For a multitude of biotechnological sectors, the filamentous fungus sp. of *Aspergillus* is an organism of significant significance. In relation to glucoamylase use in starch processing for the manufacturing of glucose, maltose syrup, different species of mold were used in manufacturing of the secondary metabolites and of several hydrolytic enzymes, the most significant is glucoamylase. *Aspergillus niger* was widely used to manufacture extracellular glucoamylase e-commercially. Two glucoamylase forms have been separated from A. Niger, glucoamylase-I (GAI) the molecular weight of 99 kilo Dalton and glucoamylase-IIa (GA II) of 112 kilo Dalton. Both forms of glucoamylase carry covalently linked sugar like D-galactose, D-mannose, and D-glucose.

In glucoamylase both carbohydrate molecule and amino acid are percent and these carbohydrate and amino acid are linked together by glycosidic linkage. The OH group (hydroxyl group) of A.A. (amino acid) of L-threonine and L-threonine linked with carbohydrate by glycosidic linkage but glucosylamine bond links the carbohydrate and L-glutamine and asparagines. These enzymes are iso-glycozymes (Pazur et al. 1971).

A mutant of A. awamori has identified a less expensive, modern, stable and more efficient alternative to commercial glucoamylase preparations. (Shah et al. 1989). The media ingredients for the production of the glucoamylase ware optimized for greater production of the glucoamylase and ultra-filtration separation technique were used to concentrate the glucoamylase. These concentrated enzymes do the more efficient saccharification.

The variety of awamori in presence of zinc that seemed to boost the protease, kawachi glucoamylase was selectively

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
Volume:02/Issue:07/July -2020 www.irjmets.com

generated in various media. Pandey, 1995 found that the molecular weights of the enzymes were Glucoamylase I, 90,000; Glucoamylas II, 83,000; and Glucoamylase III, 57,000. The degraded form's properties were not similar, but being a great example, being responsible for numerous forms of the glucoamylase in proteolysis.

Suntornsuk et al. in 1994 and Yu et al. in 1991, recorded that L-lactic maize starch, and other cereals can be simulcast and fermented by *Rhizopusoryzae. Rhizopus sp.* is not commonly developed and has been frequently used for the transformation of the starch to the glucose with a maximum efficiency in Amyl (Yu and Hang 1991). Main strength of *Rhizopus spp.* Glucoamylasis. In other words, its higher activity with regard to crude starch material and its biochemical properties, the the saccharification of starch is performed at optimized pH (Ashikar et al. 1985).There was a clear pH for the raw starch digestion and all the raw starch enzyme's were consumed. Humicolalanuginosos (Taylor et. al. (1978), Hassam et. al. (1991)) and Myratheciumsp strains were tested for their thermo tolerating properties. They were examined. M1 (Husain and Malek, 1994). Glucose syrups are processed by thermo stable enzymes. The glucoamylase-producing plant-pathogenic fungus is the colletotrichumglioeosporoids (Krause et al. 1991).

Yeast Glucoamylase

Sasha et.al, Errant et.al, 1987.Glucoamylase was shown to be yeast-producing in 1989. S were diagnosed with glucoamylase. Cerevisiaevar, Moscow. Swanniomycesoccidentalis (Gellissenet.al.1991),Pichiaburlonii & Talaromyces spp. Diastaticus, Schwanniomycescastellii Cerevisiae are confined by only to sports stage of life cycle and not directly controlled by medium-sized development process (Pugh et al., 1989).

S. Cerevisiae is therefore not utilizing starch & other complicated oligosaccharide's during production and all sugars must be although supplied specifically during alcohol fermentation. Glucoamylase sporulation is developed intracellularly to break down glycogen which stores in center at time of the spore formation and encoded by SGA gene.

Bacterial Glucoamylase

Ther-mohydrosuffuricum. Clostridiumacetobutylicum, Clostridium thermosaccharolyticum and Lactobacillus amylovorus. With the exception of Lactobacillus amylovorus, most of Glucoamylase generated by these bacteria are thermo stable and are therefore potential enzymes for use in saccharification of starch for the development of glucose syrup where Temp. > 60 °C are used.

Aspergillus

Aspergillus is described as an asexual condition as a group of conidial fungi. Some of them are known as Ascomycota in telemorphic (sexual condition). There is a genus member capable of growing at elevated osmotic (elevated sugar, salt, etc.). *Aspergillus sp.* They are discovered in conditions that are extremely aerobic (wealthy in oxygen). On carbon-rich substrates such as monosaccharide (glucose) and polysaccharides (amylose), this fungus can develop.

Aspergillus niger

A. niger is categorized as the' conidiophores' that form hyphae or filaments of the organism. That name was taken from the Latin word ' aspergillum,' meaning' holy sprinkler of water.'

It was used in the pharmaceutical industry at industrial level to produce antibiotics. In cell factories, they are commonly used to produce various enzymes (amylases, pectinases, and proteases), antibiotics and food ingredients. It is used in citric acid manufacturing in the sector. In industrial fermentation, it generated many helpful enzymes.

A. niger glucoamylase enzyme is used in manufacturing of high fructose corn

syrup and pectinases used in cider and wine clarification. The *Aspergillus niger* fungus produces proteases enzyme. It can be used for wheat alteration in bread dough in the bakery and for cheese manufacturing in the dairies. It also plays an significant part in the tenderization of meat and the manufacturing of fermented food by cereal, soybean and rice molds.

Fungal amylase finds extensive use in the preparation of dried baby foods and cereal products. The sugars produced during enzyme treatment help impart a malt syrup flavor to the product and also help produce a smoother sheet on the drying rolls. This is a distinct advantage especially with the barley and rice type baby foods. Amylases enzyme are categorized into three types (alpha-amylase, beta-amylase and amyloglucosidase), these are used in syrup and dextrose manufacture from starch within recent years. Amylases are used in textile sizing and desizing.

Glucoamylase is an enzyme that A can produce. niger.-niger. Glucamylase (α-1,4-glucan glucose, amy-glucosidase) catalyses hydrolysis alpha-1,4 and α-1,6 glycoside compounds are frequently used in the food industry to produce high glucose syrup to extract beta-D glucose from non-decreasing starch ends, as well as associated Poly-and

oligosaccharides. It is also used in fermentation processes to manufacture beer and alcohol. It is also used in barley mash beer production. In the collection, development and production of glucose and high-fructose syrup, as feedstock for biological fermentations, the major industrial use is glucoamylase. Throughout the production of soy sauce and sake, glucoamylase is a vital ingredient.

IV. PRODUCTION

Glucose amylase are produced by numerous bacteria east and moulds species like *Aspergillus* bacillus sedona'setc have been employed for fermentative production of glucose mileage commercially glucoamylase are produced by solid state and submerged fermentation process both under control environmental condition.

Submerged fermentation process

Submerged fermentation process involves the growth of microorganism in liquid media submerged fermentation technology used for production of antibiotics is adopted by enzyme industry too. Most fermentation Industries today use the submerged fermentation process for production of microbial product (Sinha N.1999, Jan H 1995).

Submerged fermentation are generally preferably mixed reactions and thus offer many advantages over SSF.

- High water content leads to porosity.
- Low oxygen diffusion limitation.
- Easy to control environmental parameters.
- Low labour intensive.
- Less space requirement.
- Create uniformity.
- High conservation rate.
- Overcomes the problem of catabolic reparation.
- High productivity of prolonged period.
- Low handling cost

Solid state fermentation

The process also called "Koki fermentation" or "semi-solid fermentations", refers to the growth of microbes on solid substrates without Safe during fluid involvement. Your existence of humidity, however, is expected. A dense substratum serves as a source of carbon, nitrogen, minerals and other growth factors. Such materials are capable of absorbing moisture and this liquid satisfies micro-organisms' essential water requirements. Fermentation in solid state is a popular way to produce glucose fungal amylase. Filamentous fungi generally grow in nature on firm substrate as the seeds of symbiotic relationships originate from roots and plant leaves. In particular, the minimum water content for solid state fermentation is between 30 and 40 percent (Pandey A.1992).

SSF has an office server advantage compared to submerged fermentations such as• Increased productivity promotions.

- Improved and service focus.
- Further consistency of the material.
- Higher suppression for catabolises.
- Cultivation of water-insoluble soil and mixed cultivation of complex fungi microorganisms.
- Smaller sterility requirement because of high SSF water activity (Tonga R. et. al. 1998).

SSF limitations

- Diffusive challenges caused by the heavy design of the formatted volume typically faster.
- Moisture control challenge of the substrata.
- Challenge.
- Scale-up issue.
- Store energy metabolism.

- Modelling of the area.
- Really long period of fermentation.

For development of fungal enzymes the Solid State fermentation has been produced and identified with the advantages of easy acuity, lower production costs, high yields of enzymes and low output of waste water A. 1992, A. Pandey. D.A.mitchell 2000, C.R., Pandey 1999,2001 football. SSF has the benefits because it is a stationary system without physical expense all the complications that are concerned of, such as temperature control and pH balance.

Solid state fermentation

The process also called "Koki fermentation" or "semi-solid fermentations", refers to the growth of microbes on solid substrates without Safe during fluid involvement. Your existence of humidity, however, is expected. A dense substratum serves as a source of carbon, nitrogen, minerals and other growth factors. Such materials are capable of absorbing moisture and this liquid satisfies micro-organisms' essential water requirements. Fermentation in solid state is a popular way to produce glucose fungal amylase. Filamentous fungi generally grow in nature on firm substrate as the seeds of symbiotic relationships originate from roots and plant leaves. In particular, the minimum water content for solid state fermentation is between 30 and 40 percent (Pandey A.1992).

SSF has an office server advantage compared to submerged fermentations such as• Increased productivity promotions.

- Improved and service focus.
- Further consistency of the material.
- Higher suppression for catabolises.
- Cultivation of water-insoluble soil and mixed cultivation of complex fungi microorganisms.
- Smaller sterility requirement because of high SSF water activity (Tonga R. et. al. 1998).

SSF limitations

- Diffusive challenges caused by the heavy design of the formatted volume typically faster.
- Moisture control challenge of the substrata.
- Challenge.
- Scale-up issue.
- Store energy metabolism.
- Modelling of the area.
- Really long period of fermentation.

For development of fungal enzymes the Solid State fermentation has been produced and identified with the advantages of easy acuity, lower production costs, high yields of enzymes and low output of waste water A. 1992, A. Pandey. D.A.mitchell 2000, C.R., Pandey 1999,2001 football. SSF has the benefits because it is a stationary system without physical expense all the complications that are concerned of, such as temperature control and pH balance.

Solid state fermentation

The process also called "Koki fermentation" or "semi-solid fermentations", refers to the growth of microbes on solid substrates without Safe during fluid involvement. Your existence of humidity, however, is expected. A dense substratum serves as a source of carbon, nitrogen, minerals and other growth factors. Such materials are capable of absorbing moisture and this liquid satisfies micro-organisms' essential water requirements. Fermentation in solid state is a popular way to produce glucose fungal amylase. Filamentous fungi generally grow in nature on firm substrate as the seeds of symbiotic relationships originate from roots and plant leaves. In particular, the minimum water content for solid state fermentation is between 30 and 40 percent (Pandey A.1992).

SSF has an office server advantage compared to submerged fermentations such as• Increased productivity promotions.

- Improved and service focus.
- Further consistency of the material.
- Higher suppression for catabolises.

- Cultivation of water-insoluble soil and mixed cultivation of complex fungi microorganisms.
- Smaller sterility requirement because of high SSF water activity (Tonga R. et. al. 1998).

SSF limitations

- Diffusive challenges caused by the heavy design of the formatted volume typically faster.
- Moisture control challenge of the substrata.
- Challenge.
- Scale-up issue.
- Store energy metabolism.
- Modelling of the area.
- Really long period of fermentation.

For development of fungal enzymes the Solid State fermentation has been produced and identified with the advantages of easy acuity, lower production costs, high yields of enzymes and low output of waste water A. 1992, A. Pandey. D.A.mitchell 2000, C.R., Pandey 1999,2001 football. SSF has the benefits because it is a stationary system without physical expense all the complications that are concerned of, such as temperature control and pH balance.

V. REVIEW OF THE LITERATURE

Glucoamylase

Glucoamylase (EC 3.2.1.3) a part of amylase family hydrolyzes polysaccharide and oligosaccharides in to its monomeric units. Enzymetic breakdown of starch required collective action of exo and endo amylase action for this different amylase are used such as pullulanase, α-amylase, glucose isomerase, glucoamylase, and others (Antranikian, 1992)

Glucoamylase is also referred to as amyloglucosidase, glucamylase, I-amylase or 1-4 glucan glycohydrolase (Solomon 1978). In Glucan glycohydrolase glucan refers to the chain of glucose units connected to each other while glycohydrolase refers to the hydrolysis of glucose chain (breakdown of glycosidic bond present in-between glucose unit). Glucoamylase, an exo-acting enzyme produces α-D-glucose from non-reducing chain ends of amylase, amylopectin and glycogen through successive hydrolysis of α-l,4 connections (Fogarty 1983). It also hydrolyses α-1, 6 and the unusual α-l, but at very slower pace, 3 connections. Thus, if incubated for extended periods of time, this enzyme can completely hydrolyze starch. It's called the enzyme saccharifies. Glucoamylase are capable of degrading big oligosaccharides up to approximately 90% of α-l, 6 connections. However, in susceptibility to hydrolysis, the size of the substrate and the location of α-1,6 connections plays an important role (Fogarty 1983 &Vihinen et. al. 1989).

Glucoamylase are frequently used in different industries like textile, leather, paper, food, baking, liquor industries Joshi VK, PandeyAet. al.1999. It is also used in pharma industry for the preparation of glucose syrup, fructose syrup and alcohol (James JA and Lee Bah 1992).The natural source of glucoamylase are microorganisms. The glucoamylase are produced by a lot of microorganism such as bacteria, fungus etc. but filaments fungus can be a great source of glucoamylase while bacteria produce low amount of glucoamylase.

Microorganisms involve in production of glucoamylase

Glucoamylase is one of the catalyst which has very high demand in industries. There are different kinds of microorganisms are involve in production of glucoamylase such as bacteria, fungus, yeast etc. some of the micro organisms are listed bellowinvove in the production of glucoamylase-

Pichiasubpelliculosa	**Yeast**	Kumar et.al (2001)
Saccharomyces cerevisiae	**Yeast**	Tamaki (1978), Erratt and Stewart (1978)
Saccharomycopsiscapsularis	**Yeast**	Ebertova (1966)

Aspergillus niger	**Fungus**	Fogarty and Benson (1983)
Aspergillus awamori	**Fungus**	Yamasaki, Suzuki, and Ozawa (1977)
Picrophilustorridus	**Bacteria**	Dock, Hess, and Antranikian (2008)
Clostridium sp.	**Bacteria**	Ohnishi et. al. (1991)
B.stearothermophilus	**Bacteria**	Srivastava (1984)
Flavobacterium sp.	**Bacteria**	Bender et. al. (1981)
Bacillus spp.	**Bacteria**	Gill et. al (2004)
L. amylovorus	**Bacteria**	James et al. (1995, 1996)
C. acetobutylicum	**Bacteria**	Annous et al. (1990)

Purification and kinetics of microbial Glucoamylase

Conventional chromatographic approaches and modern techniques for the purification of microbial Glucoamylase were used. A high sufficient protein concentration is required to facilitate purification. A number of techniques have been used to concentrate raw glucoamylase preparations, as precipitation using organic solvents, ultra filtration sodium lyophilization, centrifuges and more. The most commonly used methods of cleansing Glucoamylase are Ion-exchange and Gel-filtration chromatography. Also chromatography methods for hydrophobic activity were also used.

In contrast to the above methods, polyethylene glycol (PEG) and potassium phosphate aqueous two facial systems(ATPS) have also been involved in the purification of Glucoamylase. (Minami,Cilicia, 1998). In the use of starch as bowlike (0.1% w/w), nine times, the glucoamylase partition variable was decreased, without altering the contaminant partition coefficient, thereby helping to distinguish the enzyme from pollutants quickly. (Gouveia and Cilicia 2000). 2000. The 3-phase method, developed by Mondale, Sharma and gupta (2003), in which 20 and 38 times of purification has been achieved, also purifies Glucoamylase.

Glucoamylase assays

Measuring the concentration of sugar produced by its operation against starch and related compounds such as amylopectin, dextrin, maltose, iso-maltose, pullulan, maltotriosis is a traditional method of glucoamylase analysis. This process takes place in ideal temperature and pH environments which ends in the production of glucose to decrease sugar, which is an example of the development of glucoamylasis. Some methods by Nelson-Somogyi (Nelson 1944 & Somogyi 1952) and DNS (Miller 1959) are being used more frequently for this purpose. Such approaches are constrained to contemplating total sugar removal instead of only fructose. Also used for glucoamylase activity assessment are the p-nitro phenyl alpha-D-glucopyranoside (pNPG) study. (Takahashi, Inokuchi and Irie, 1976; Okada, 1981); This reaction is used for calculating the ability of enzyme glucoamylase / alpha-glycosidase, and is used to catalyze the hydrolysis of p-nitro phenyl-alpha-D-glucopyranoside to glucose and p-nitro phenol. The yellow color of nitro phenol under alkaline pH is 400 nm and refers to activity of glucosidase. Alpha- glycosidase, in contrast to glucoamylase, can also catalyze the above-mentioned reaction with glucose and p-nitro phenol releases. The activity of glucoamylase and alpha-glycosidase, Beta glycoside, are however different, while alpha-D-glycoside, which is Beta-D-glycoside, is released through glucoamylase. (Anomers involve beta D-glucose and alpha-D-glucose).

Another method i.e. starch iodine assay is also being used to quantify glucoamylase is based on the color development because of binding of iodine to polymer of starch. (Xiao,Storms,Tsang,2006). Another method that is enzyme based glucose-oxidize, peroxidase assay being frequently used for assaying Glucoamylase, the advantage with this assay is that it is very specific and even can detect the glucose with even minute interference.

Inhibition and activation

Sugar analogues inhibit glucoamylase with basic nitrogen atom adjacent to C-1. This is the case with most of the glycosylases. The most potent glucoamylase inhibitor is a pseudo tetrasaccharide, Acarbose, which is having a kd of 10 to the power -12 M

(Svensson and Sierks,1992). Some other known glucoamylase inhibitors are castanospermine, 1-deoxynojirimycin, (+)-lentiginosine, 1,2,7-trihydroxyindolizidine (Brandi, et al., 1995; Hoti, Cardona, and Brandi, 1996; Cardina et al., 1997), iodoacetate, amino alcohols, guanidine-HCL, urea, EDTA, Tris, p- chloromercuribenzoic acid, trestatins, maltilol, N-bromoauccinimide and alpha-, Beta-, and gamma- cyclodextrins (Yamasaki, Suzuki, and Ozawa,1977a, 1977b; Yamasaki, Tsuboi and Suzuki, 1997; De Mot, Oudenduck, and Verachtert; De Not and Verachtert, 1987; Specka, Mayer, and Antranikian, 1991; Fagerstrom and Kalkkinen, 1995; James and Lee, 1996; Odibo and Ulbrich- Hofmann, 2001).

Calcium ion plays an important role in maintaining the activity and stability of alpha- amylase, however it's not the case with glucoamylase. Glucoamylase has shown stimulation and inhibition in presence of certain metal ions such as zinc, copper, lead, cadmium, nickel , iron, silver , gold, manganese (Yamasaki et. al. 1977a,. 1977b; Yamasaki, Tsuboi, and Suzuki, 1977; Takahashi, Tsuchida and Irie, 1978; Inokuchi, Takahashi, and Irie,1981; Fogarty and Benson, 1983; De Mot, Oudenduck, and Verachtert,1985; Abe et. al., 1988; Yasuda, Kuwae, and Matsushita, 1989; Ali and Hossain, 1991; Specka, Mayer, and Antranikian, 1991; Yamasaki and konno, 1991; Ali, Malek and Hossain, 1994; James and Lee, 1996: Spinelli et. al., 1996; da Silva and Peralta, 1998; Nguyen et. al., 2002; Kumar and Satyanarayan, 2003; Bhatti et. al., 2007b; Li et. al., 2007b) all these metal ion show inhibitory effects whereas barium, calcium, cobalt, copper, manganese, zinc ,sodium, magnesium, tin and iron are shown to increase glucoamylase activity (Spinelli et. al., 1996; Nguyen et. al., 2002; Bhatti et. al., Li et. al., 2007b).

Different forms of glucoamylase

Glucose mileage can be produced by different range of microorganism. Among them fungus produce almost 6 type of glucose mileage (Monma et al. 1987, Nagasaka et al. 1995) The commercially available A. awamori and A. Niger produce two different type of glucoamylase named as GAI and GAII. Both GAI and GAII are equally active for dissolve carbohydrates while GAII has sperate active site to above and digest starch. GAII glucoamylase is much larger than GAII (Takahashi et al. 1981). Both Two forms of glucoamylase (GAI and GAII) are produced by single gene (svensson et. al. 1983)

VI. CONCLUSIONS

It can be concluded that the Aspergillus niger gives good yield of Glucoamylase enzyme under optimized environmental conditions when wheat bran as the substrate for solid state fermentation and Aspergillus can utilize a broad range of the nitrogen sources, among them ammonium nitrate, amino acids such as peptone histidine or proline .Of particular interest is the pathway of the nitrate assimilation, a key process in the global nitrogen cycle with agricultural significance. Similarly effect of ionic strength need detailed investigation a part form effect of pH. However, study requires further detailed investigation for commercialization. The peptide bonds in a protein are cleaved by the protease enzyme.

VII. REFERENCES

[1] Ali-Hossain 1991, Characteristics of glucoamylase from Asp.terreus. J. Appl. Bacteriol 71 Page: 145–146.

[2] Abeet.al. 1988.Properties of raw starch digesting amylase of Asp. sp. K27: a synergistic action of glucoamylase&amylase. CarbohydrRes 175Page: 86–91.

[3] Antranikian, Specka, Mayer 1991. Purification, properties of thermo-active glucoamylase from the Clostridium thermo-saccharolyticum. Appl Environ. Microbiol 57 Page: 2318–2322.

[4] BhattiN, Rashid M, Nawaz, Asgher, Perveen, Jabbar 2007(b). Purification, characterization of novelglucoamylase from Fusariumsolani. Food Chem 103Page: 339–342.

[5] Bhatti HN, Rashid MH, Nawaz R, Asgher M, Perveen R, Jabbar A. 2007a. Optimization of media for enhanced glucoamylase prod.in SSF by Fusariumsolani. Food Technol Biotechnol 45Page: 51–56.

[6] Bender H. 1981.A bacterial glucoamylase degrading cyclodextrins, Partial purification, properties of enzyme

IRJMETS

e-ISSN: 2582-5208

International Research Journal of Modernization in Engineering Technology and Science

Volume:02/Issue:07/July -2020 www.irjmets.com

from Flavobacterium sp.EurJ.Biochem 115Page: 288–291.

[7] DeGouveia T., Kilikian 2000. Bio-affinity Extraction of Glucoamylase in Aq. 2 phase systems using starch as the free bioligand. J.Chromatogr B. 742Page: 242-246.

[8] da Silva BW, Peralta WM. 1998. Purification and characterization of a thermostableglucoamylase from Aspergillusfumigatus. Can J Microbiol 44Page: 494– 496.

[9] Erratt, Stewart 1978. Genetic and bio-chemical studies on yeast strains able to utilize dextrins. J Am Soc Brew Chem 36Page: 152–155.

[10] Ebertova H. 1966. Amylolytic enzymes of Endomycopsiscapsularis II: study of propertiesof isolated α -amylase, amyloglucosidase & maltosetransglucosidase. Folia Microbiol (Prague) 11Page: 422–438.

[11] Fagrstrom R, Kalkinen 1995. Characterization, subsitemaping and partial a.a. seq. of glucoamylase obtained from fil.fungi Trichodermaresei. BiotechAppln.Biochem.21 Page: 224–231.

[12] Fogarty WM, Benson CP. 1983. Purification and properties of thermophilicamylo- glucosidase from Aspergillussp.EurJ.Appl Microbiol Biotechnol 18Page: 272–275.

[13] Gill RK, Kaur J. 2004. A thermostableglucoamylase from a thermophilic Bacillus sp.: characterization and thermostability. J Ind Microbiol Biotechnol 11Page: 540–543.

[14] Inokuchi, Ire, Takahashi 1981. Purification and the characterization of minorglucoamylase from Aspergillus sp. J.Biochem 90Page: 1055–1067

[15] James, Lee 1995. Cultural conditions for prod.of glucoamylase from Lactobacillus amylovoms ATCC-33621. J ApplBacteriol 79Page: 499–505.

[16] James, Lee 1996. Characterization of glucoamylase from Lactobucillusamylovorus ATCC- 33621. BiotechLett18Page:1401–1406.

[17] James, Lee. 1996. Characterization of glucoamylase obtained from Lactobucillus amylovorus ATCC33621. Biotech Lett. 18Page: 1402–1405.

[18] Kumaret.al. 2003. Purification and kinetics of raw starchhydrolyzing, thermo-stable and neutral glucoamylase of a thermo-philic mouldThermomucorindicae- seudaticae. BiotechnolProg 19Page: 936–944.

[19] Li-H, Chi-Z, Wang, Duan, Gao 2007(b). Purification and characterization of extracellular amylase from the marine yeast Aureobasidiumpullulans N13d and its raw potato starch digestion. Enzyme MicrobTechnol 40Page: 1006–1012.

[20] Malek, Ali, Hossain 1994. Purification nd characterization of thermostableglucoamylase from amyrothecium isolate. J ApplBacteriol 76Page: 210–215.

[21] Miller1959. Use of DNS reagent for the determination of the reducing sugar. The Anal Chem 31 Page: 427–428Okada 1976. Enzymatic studies on cellulose system of the Trichodermaviride IV Purification and properties of less-random type cellulose. J Biochem (Tokyo) 80Page: 913–922

[22] Minami, Kilikian 1998. Separation and purification of glucoamylase in aq. 2 phase system by 2 step extraction. J Chromatogr (B) 771Page: 310–312.

[23] Monmaet.al. 1987.Purification ofglucoamylase components of Chalaraparadoxa by affinity chromatography and chromate-focusing. Carbohydr Res.159Page: 256–260.

[24] Nagasaka Y, Muraki N, Kimura A, Suto M, Yokota A, Tomita F. 1995. Cloning of Corticiumrolfsiiglucoamylasec DNA and its expression in Saccharomyces cerevisiae. Appl Microbiol Biotechnol 44Page: 452–457.

[25] Nelson 1944, A Photometric adaptation of Simonyi method for the determination of glucose. J.BiolChem 153 Page: 376–381.

[26] Odiboet.al.2001.Thermostable and amylase glucoamylase By Thermomyceslanuginosus F-1.ActaBiotechnol 21 Page: 142–153.

[27] Ohnishi, Sakai, Ohta 1991. Purification and properties ofglucoamylase from Clostridium sp. G0005.AgricBiolChem 55Page: 1901–2.

[28] Somogyi1952.Brief Notes regarding sugar determination. J Biol Chem 195Page: 20–22.

[29] Spinelliet. al. 1996.Biochemical characterization of glucoamylase from hyperproducerexomutant strain of Neurosporacrassa. FEMS Microbiol Lett 138Page: 174–176.

[30] Srivastava RAK. 1984. Studies on extracellular and intra-cellular purified amylases from thermophilic Bacillus stearothermophilus. Enzyme MicrobTechnol 6Page: 422–6.

[31] Svensonet. al. 1992.Roles of aromatic side chains in binding of substrates, inhibitors, cyclamate-oligosaccharides to glucoamylase by *Aspergillus sp. By* spectroscopy, chemical modification, mutagenesis. Carbhydr Resi. 227Page: 29–44.

[32] Svenson, Larsen, Svendsen, Boel 1983. The complete a.a. seq. of glycoprotein, glucoamylase G1 from Aspergillussp. Carlsberg Res Commun 48Page: 530–543.

[33] Speckaet.al. 1991. Purification, properties of thermo-active glucoamylase obtained from Clostridium thermo-saccharolyticm. Appl Environ Microbial 57Page: 2318–2322.

[34] Suzuki, Tsuboi,Yamasaki , 1977. 2 forms of Glucoamylase from Mucorrouxianus: Properties of 2-glucoamylases. AgricBiolChem 41Page: 2139–2148

[35] Takahashi et. al. 1981.The Purification and characterization of glucoamylase from *Aspergillus saitoi*. J Biochem (Tokyo) 89 Page: 125–134.

[36] Takahashiet.al.1981. Purification and characterization of glucoamylase from Aspergillussaitoi. J Biochem (Tokyo) 89Page: 126–133

[37] Takahashi et. al. 1978.Purification, properties of 3 forms of glucoamylase from a Rhizopus sp.J Biochem (Tokyo) 84Page: 1183–1194.

[38] Verachtert,,Oudenduck EV, De Mot R. 1985. Purification & characterization of extra cellular glucoamylase from yeast Candidatsukubaensis CBS 6389.Antonie van Leeuwenhoek 51 Page: 277–286.

[39] Xiao Z et.al. 2006. A quantitative Starch–Iodine method for measuring the α-amylase as well as glucoamylase activities. Anal Biochem 351 Page: 146–148.

[40] Yamasaki Y, Suzuki Y, Ozawa J. 1977b.Purification, Properties of 2 forms of glucoamylase from Pencillium-oxalicum.Agri. BiolChem. 41Page: 756–761.

[41] Yamasaki et. al. 1977(a). 3 forms of glycosidase and α-glucoamylase from Aspergillus awamori. Agric Biol-Chem 41Page: 2149–2161.

[42] Yamasaki, Suzuki, Tsuboi A. 1977. 2 forms of Glucoamylase by Mucorrouxianus: properties of two-Glucoamylase. AgricBiolChem 41Page: 2139–2148

[43] Yamasaki et. al. 1991. Purification, properties of glucoamylase form Mucorjavanicus. AgricBiolChem. 55Page: 2429.

[44] Yamasaki et. al.1977a. Three forms of glucosidase and aglucoamylase from Aspergillusawamori. AgricBiolChem 41Page: 2149–2161.