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Glucose isomerase: an enzyme of industrial significance

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ABSTRACT: Microbial enzymes have shown useful in bio-industries such as food, animal feed, leather, and textiles, as well as in bioconversions and bioremediations.One of the microbial enzymes – glucose isomerase sought to be economically significant. Glucose isomerase has many biotechnological applications. Glucose isomerase is the key enzyme in the creation of high-fructose corn syrup (HFCS) where glucose isomerase catalyzes the isomerization of glucose to fructose. HFCS is widely used as a sweetener and preservative in the food and beverage industries.In the biofuel sector, glucose isomerase is also used to transform glucose into fructose, which can then be fermented into ethanol. Glucose isomerase is also used in the synthesis of uncommon sugars with potential medical and industrial uses, such as D-tagatose and D-xylose.Bacteria like *Escherichia coli*, yeast like *Saccharomyces cerevisiae*, and filamentous fungi like *Aspergillus niger* are reported to produce glucose isomerase. These organisms can be genetically modified to produce excess glucose isomerase, which will increase their productivity and effectiveness. Overall, the food, beverage, biofuel, and pharmaceutical industries benefit greatly from glucose isomerase and its applications, and future developments are possible.

KEYWORDS: Glucose Isomerase, Saccharification, D-Glucose, D-Fructose, Corn Syrup.

I. INTRODUCTION:

[1]Daily necessities including paper, textiles, feed, chemicals, and pharmaceuticals require a lot of energy and raw material and generate a lot of waste and severely influence our environment and quality of life. [2]Bacterial enzymes can operate as catalysts for different reactions in vitro and in vivo, including those that occur in the industry. At least 2000 years ago, microbeswere used in procedures like leavening bread and the saccharification of rice to generate koji to make items for human use. [3]The biological catalysts known as enzymes are present in all living systems. Proteinaceous in nature, enzymes catalyze a wide range of processes. For millennia, enzymes have been unintentionally utilized in the production of goods including leather and linen as well as wine, cheese, bread, beer, and vinegar in the form of bacterial or plant extracts. However, the widespread use of refined enzymes in manufacturing processes dates back only a few decades. [4]The expense involved in using enzymes in industrial processes is a significant problem. The synthesis of enzymes on a large scale requires a lot of capital, and the use of enzymes during various stages of manufacturing indirectly impacts product pricing. The equipment and installation of the plant account for a large portion of the annual operational cost of an enzyme production facility. However, raw materials account for 28% of the operating costs. [5]Xylose isomerase is another name for D-glucose isomerase. One of the most significant enzymes ever discovered. It is one of the three enzymes with the highest tonnage values. Protease and Amylase are the additional two. [6]The equilibrium reversible isomerization of D-glucose is catalyzed by glucose isomerase. The conversion of D-xylose into D-fructose and D-xylulose is also catalyzed by this enzyme. [7]Glucose Isomeraseis an intracellular enzyme made by a variety of genera, primarily *Streptomyces*, *Bacillus*, *Corynebacterium*, and *Arthobacter spp*. The organisms are grown in media that contain free sugars such as glucose and/or xylose. Additionally, *Pichia pastoris* and *Escherichia coli*have been used to create Glucose Isomerase. [8]It was discovered that bacteria and *Actinomycetes*generate Glucose Isomerase. The enzyme was released by *Lactobacillusbrevis*, *Streptomyces glaucescent*, and *S. flavogrisens*. Using traditional purification

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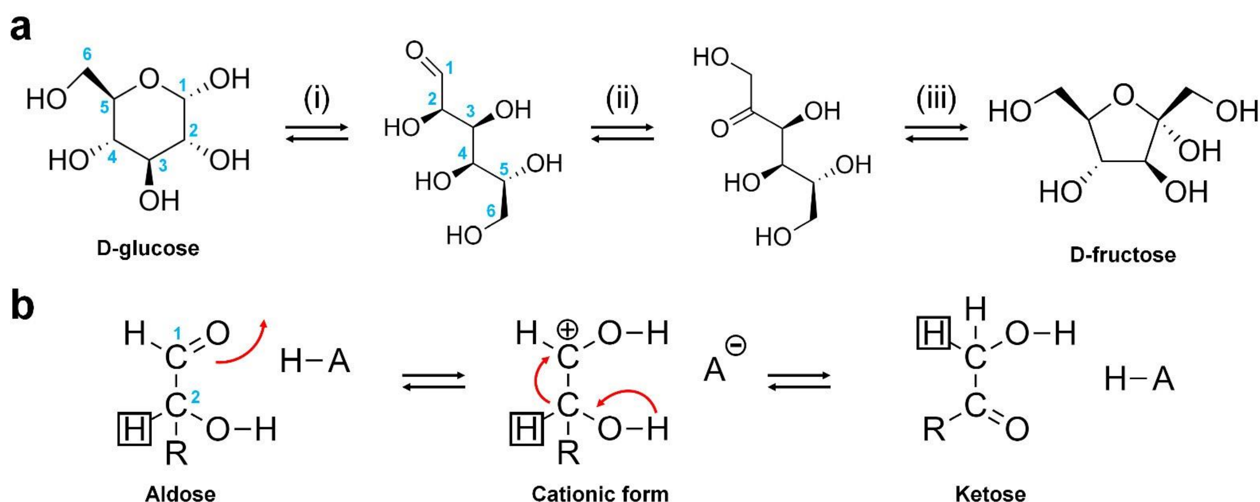
methods like gel filtration, ion exchange chromatography, and polyacrylamide gel electrophoresis, the extracellular GLUCOSE ISOMERASE from *Candida sp*. and alkali thermophilic *Bacillus sp*. has been homogeneously purified (PAGE). The fungus that has been linked to Glucose Isomeraseactivity is *Aspergillus oryzae*. [9, 10]Biocatalysts were immobilizedfor use either in fundamental research or in technical processes with economic value. The benefits immobilized enzymes have over their soluble counterparts have led to an increase in their use as catalysts in numerous industrial processes. The ability to recover and reuse the enzymes, increase enzymatic stability in harsh conditions of temperature, pH, and organic solvents, and in the case of proteases, the elimination or decrease of auto digestion are a few of these.

CATALYTIC MECHANISM OF GLUCOSE ISOMERASE:

D-glucose and D-xylose are reversibly isomerized into D-fructose and D-xylulose, respectively, by the enzyme glucose isomerase.



(Figure 1). [20, 21]The Glucose Isomerase's isomerization mechanism. (a) The configuration change from D-glucose to D-fructose is carried out in three main phases and is catalyzed by Glucose Isomerase. (b) The Glucose Isomerase's hydride shift mechanism.

[11]Saprophytic bacteria that live on decomposing plant matter require the interconversion of xylose to xylulose for nutrition, and it helps the bioconversion of hemicellulose to ethanol. In the process of making high-fructose corn syrup, the isomerization of glucose to fructose is important commercially. [16]Glucose Isomerasemay isomerize a variety of pentoses, hexoses, sugar alcohols, and sugar phosphates in addition to its native substrates. Although the substrate specificity of Glucose Isomerasescan vary depending on the source strain or organism, [12]It demonstrates activity against a wide range of sugar substrates, including D-ribose, [13]L-rhamnulose, [14]L-arabinose, and [15]D-allose. [17]There are two M1 and M2 metal binding sites in a typical Glucose Isomeraseactive site. The metals bound at the M1 and M2 sites are involved in the substrate binding and isomerization mechanisms, respectively, hence they are referred to as the structural metal and catalytic metal sites, respectively. [18, 19].In *S. rubiginous*, Glu181, Glu217, Asp245, and Asp287 coordinate the metal at the M1 site, and Glu217, His220, Asp255, and Asp257 bind the protein to the metal at the M2 site. The Glucose Isomerasefamily as a whole conserves these metal-bound amino acids. [20, 21].Based on findings from multiple studies using chemical modification, X-ray crystallography, and isotope exchange approaches, the action of Glucose Isomerasehas been linked to a hydride shift mechanism. Accordingly, it is hypothesized that the substrate at the Glucose Isomeraseactive site is in an open ring state and that, following isomerization, a closed ring form product is produced by a hydride shift from C2 to C1. The proton transfer from O1 to O5 is catalyzed by a conserved histidine residue during this procedure. According to crystallographic research, xylose initially binds to the enzyme in an open-chain conformation during its isomerization. As a result, it

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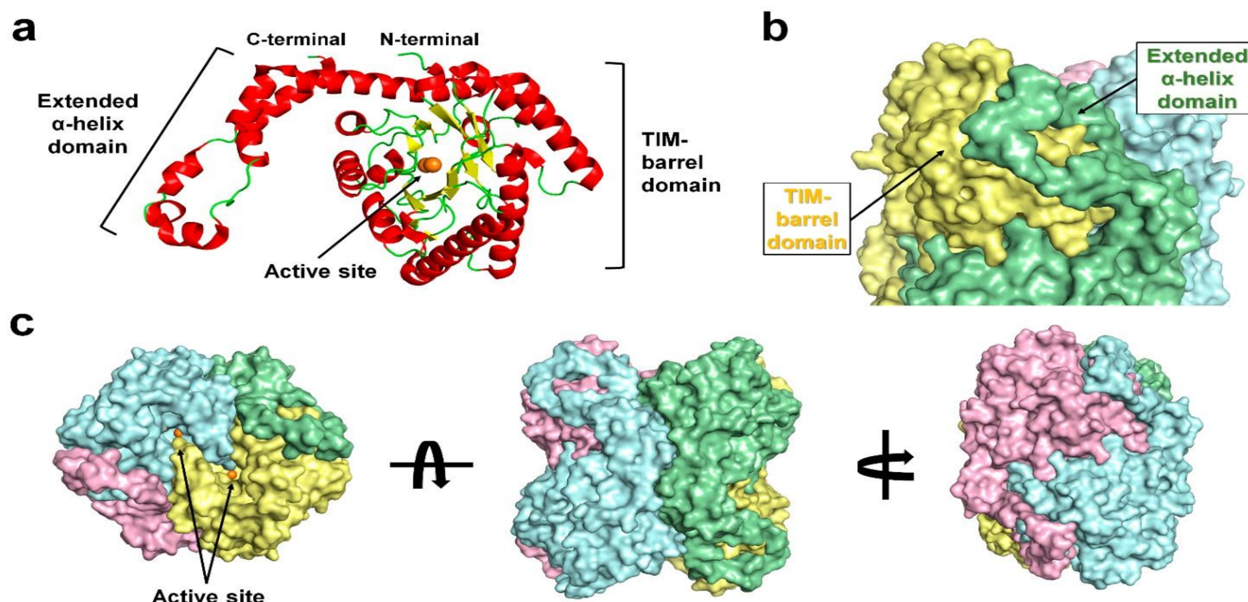
is suggested that the substrate at the Glucose Isomeraseactive site is in an open ring state and that isomerization results in a closed ring form product via a hydride shift from C2 to C1. The proton transfer from O1 to O5 is catalyzed by a conserved histidine residue during this procedure. According to crystallographic research, xylose initially binds to the enzyme in an open-chain conformation during its isomerization. Two locations (M1 and M2) in the Glucose Isomerasestructure can bind metals. The xylose molecule's O2 and O4 are bound by the metal ion at the M1 site, and after being bound, the metal ion at the M2 site binds to O1 and O2 in the transition state. These interactions aid in catalyzing the hydride shift required for isomerization, along with a conserved lysine residue. [22]*Bacillus thermoantarcticus* glucose isomerase was discovered to have the maximum activity at a reaction temperature and pH of 90°C and 7.0, respectively.

STRUCTURE OF GLUCOSE ISOMERASE:

[23-27]15 -helices and 8 -strands make up the SruGI monomer, which forms a TIM-barrel with an active site and an extended C-terminal -helix domain. The TIM-barrel domain contains the metal-binding site and the xylitol molecule. [28]The functional tetrameric structure shown in the crystal symmetry is further formed by the monomer subunit. The tetrameric assembly and subunit topology of SruGI were equivalent to those of SruGI's previously published crystal structures. Structure-wise, Glucose Isomeraseis made up of an extended-helix domain and a TIM barrel-like domain, which together form a functional tetramer. [29]The Glucose Isomerase active site is situated on the TIM-barrel fold, and two protomers form the substrate-binding pocket. The Glucose Isomerasehas four active sites for substrate isomerization and is a tetramer with 222 crystallographic symmetries.



(Figure 2). [28].*Streptomyces rubiginosus*Glucose Isomerasecrystal structure (SruGI). (A) The extended-helical domain and TIM-barrel domain make up the Glucose Isomeraseprotomer. (b) The extended-helical domain engages in interaction with the nearby GI molecule's TIM barrel domain. (C)Tetrameric Glucose Isomerase shows 222 symmetry.[30, 31]However, it was reportedthat other Glucose Isomerasecrystal structures have metal ions in their active sites that have reduced biological affinity. [32]Ca2+ is found in the M1 and M2 sites of the active site, for instance, in the crystal structures of Glucose Isomerases from *S. rubiginosus* (PDB code: 4W4Q), [30] *Paenibacillus sp*. R4 (PDB code: 6INT), and [31] *Piromyces*(6T8E), [33] although enzyme activity in the presence of Ca^{2+} has not been verified. Additionally, the metal ion was positioned somewhere other than the M1 and M2 locations in some crystal structures.

METAL-BINDING STATE AT THE GLUCOSE ISOMERASE ACTIVE SITE:

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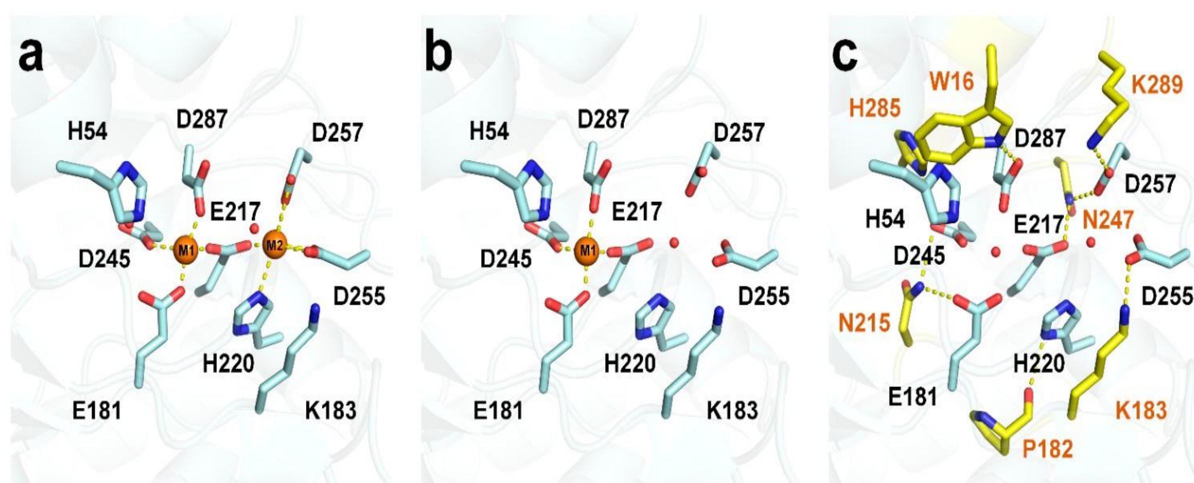
[19, 34, 35]The active site of Glucose Isomerasecan be in one of three states of metal binding: the metal-free state; the two metal-binding states; and the one metal-binding state. Although the active site of Glucose Isomerase crystal structures exhibits a variety of metal-binding states, the overall Glucose Isomerasefold is remarkably similar, suggesting that metal binding has little effect on how proteins fold. The M1 site, which is involved in substrate recognition, is the only location where the metal ion exists in the one-metal-binding mode of Glucose Isomerase, indicating that the M1 site has a higher affinity for metal binding than the M2 site.

[19]The sugar substrate or inhibitor may interact with the metal ion at the M1 site in the one-metal-binding mechanism. [35].To maintain their positions without experiencing significant conformational changes, the metal-binding residues are stabilized by the residues nearby the active sites (Glu181 *-Asn215, Glu217 *-Asn247, His220 *-Pro182, Asp245 *-Asn215, Asp245 *-His285, Asp255 *-Lys183, Asp257 *-Asn247, Asp257 * As a result, in the metal-free state, the configuration is at least open enough for the metal to detect the sites and bind to them.



(Figure 3). [19, 25, 34]*Streptomyces rubiginosus*GLUCOSE ISOMERASE active site has three distinct metal-binding states (SruGI). (a) Two-metal-binding mode (PDB code: 6IRK), (b) one-metal-binding mode (PDB code: 5Y4I), and (c) metal-free state of SruGI (PDB code: 7C).

XYLITOL BINDING TO THE ACTIVE SITE OF GLUCOSE ISOMERASE:

[33]By determining the settings of enzyme engineering to avoid product inhibition, FS and bioethanol production can be improved. [16]Certain divalent cations, such as Ca2+, Cu2+, Zn2+, Ni2+, Ag2+, and Hg2+, can decrease the isomerase activity of Glucose Isomerase. [36] Inhibitors of Glucose Isomeraseactivity include xylitol, arabitol, sorbitol, mannitol, lyxose, and tris. [33]By defining the parameters of enzyme engineering to avoid product inhibition, structure-based inhibitor studies of Glucose Isomeraseare beneficial for industrial applications like HGFS and the synthesis of bioethanol. [16]Certain divalent cations, such as Ca2+, Cu2+, Zn2+, Ni2+, Ag2+, and Hg2+, can decrease the isomerase activity of Glucose Isomerase. [36]Inhibitors of Glucose Isomeraseactivity include xylitol, arabitol, sorbitol, mannitol, lyxose, and tris. However, the exact mechanism by which these compounds inhibit the Glucose Isomerasesystem is still largely unknown.

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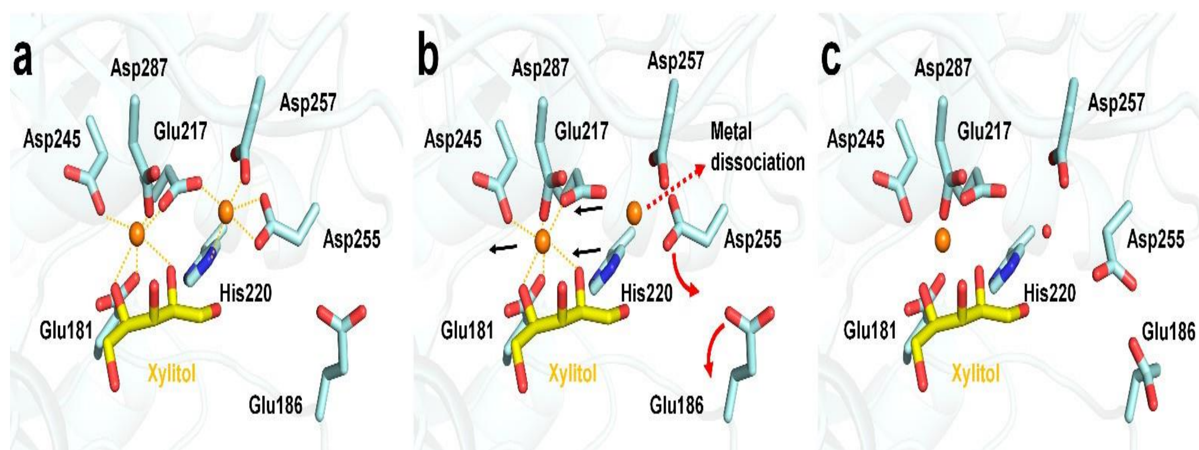

(Figure 4). [33]Proposed mechanism of xylitol-induced release of metal at the M2 site of Glucose Isomerase. (a) Xylitol-bound state of Glucose Isomerase. (b) Rearrangement of xylitol-binding residues. (c) Release of metal ion from M2 site of Glucose Isomerase.

[44,45]Well-researched structural analog of xylose. [17, 19, 33, 37, 38].Six xylitol-bound Glucose Isomerase crystal structures have been deposited in PDB as of this writing (PDB code 1XIG, 2XIS, 3GNX, 4DUO, 5Y4J, and 7DFK). All of these models demonstrate that the xylitol molecule binds to the Glucose Isomeraseactive site at the M1 site. The metal ion is maintained at the M1 site by three oxygen atoms from xylitol (O2, O3, and O4), octahedral coordination, the metal-interacting residues of the enzyme, and the xylitol molecules.

APPLICATIONS OF GLUCOSE ISOMERASE:

1. Advantages of High-Fructose Corn Syrup as a Sweetener:

The need for acceptable sucrose alternatives has arisen as a result of rising refined sugar demand, high production costs, and growing public awareness of the harmful consequences of sucrose and inverted sugar consumption on human health. Numerous artificial sweeteners that are noncalorific and carbohydrate-free, including saccharine, cyclamate, acesulfame-K, aspartame, and thaumatin, have been developed and disregarded because of potential health risks or other problems. Because aspartame hydrolyzes slowly at low pH, adding it to soft drinks makes them less sweet after extended storage. Glucose has a sweetening capability of 70–75%.Numerous studies have demonstrated that compared to glucose and sucrose, fructose has a higher sweetening index. HFCS is 1.3 times sweeter than sucrose and 1.7 times sweeter than glucose and contains almost equal proportions of glucose and fructose. Since glucose is the cells' preferred source of carbon and energy, it hasn't been widely recognised as a sweetening agent. The glucose syrup must be kept warm and properly protected from microbial attack in order to avoid crystallisation.Three processes are involved in turning starch into HFCS: (i) -amylase gelatinizes and liquefies the starch; (ii) amyloglucosidase and a debranching enzyme collaborate to saccharify the starch; and (iii) Glucose Isomeraseisomerizes the glucose. The end result is a maize syrup that contains a blend of glucose and fructose and has a greater potential to sweeten than sucrose. Studies have suggested that starch can be replaced with other ingredients including wheat, tapioca, rice, etc.

Gelatinization is the process of turning granulated starch into a gelatinous solution.Liquefaction is a starch hydrolysis process, while saccharification is the hydrolysis-based conversion of starch to glucose and maltose. Starch and water heated together can cause gelatinization. After being gelatinized, the starch is subsequently exposed to hydrolysis by acids or enzymes. Additionally, acidic or enzymatic hydrolysis is used to saccharify the hydrolyzed compounds. At a pH of 6.0–6.5, cold water (30–40%) is used to dissolve the granular starch into a slurry.20–80 ppm of Ca2+ are added to this slurry, stabilising and activating the enzyme. A dose of 0.5–0.6 kg tonne-1 (about 1500 U kg-1 dry matter) of starch is added together with the enzyme -amylase. Gelatinization happens quickly because of the combined action of mixing shear forces and enzyme hydrolysis, thus the contents are mingled. This partially gelatinized starch is transferred into many holding tubes that are

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kept at 100 to 105 degrees Celsius and retained for 5 minutes. The gelatinization process will be finished in this way. In holding tanks, the necessary DE (Dextrose Equivalent) is hydrolyzed for one to two hours at 90 to 100 degrees Celsius[39].

2. High-Fructose Corn Syrup (HFCS):

[40]Fructose is frequently employed in the production of ice cream and frozen desserts to affect taste and texture because of its advantageous features, which include a high solubility at low temperatures, a reduced inclination to crystallize than sucrose, and a high freezing point depression. [41, 42].Cake, biscuits, bread, and other confectionery products are also made with fructose[43].Additionally, fructose has a longer shelf life than sucrose syrup due to its high osmotic pressure in the solution, which makes it a superior preservative against microbial development[44].Additionally, fructose is employed in the pharmaceutical sector to create diabetic medications because it does not affect the levels of insulin and glucose in the blood[45].A-amylase liquefies starch, amyloglucosidase, and a debranching enzyme combine to saccharify starch, amyloglucosidase isomerizes glucose, and Glucose Isomeraseisomerizes glucose. These three processes are the main steps in the production of HFCS from starch. The result is corn syrup with a higher sweetening capacity than sucrose because it contains a mixture of glucose and fructose.

Debranching enzyme and glucoamylase are typically used to saccharify the liquid starch in order to produce glucose and maltose syrup.Finally, lowering the pH destroys the enzyme activity. The enzyme glucose isomerase converts the glucose that is generated into fructose. -D-glucopyranose is isomerized to -D-fructofuranose when cobalt ions are present. *Actinoplanesmissouriensis*, *Bacillus coagulans*, and several *Streptomyces* species are a few genera of bacteria that may make glucose isomerases. These enzymes have benefits because they can function at extremely high substrate concentrations, are resistant to thermal denaturation, and are stabilised at higher operating temperatures. The batch approach for immobilised enzyme had drawbacks since it was expensive, produced a lot of byproducts, and was challenging to remove additional ions and the catalyst from. The majority of isomerization today takes place in packed bed reactors (PBRs). The cofactors Mg2+ and Co2+ support the action of enzymes[39].

3. Production of Ethanol:

The Glucose Isomerase catalyzes both the isomerization of glucose and xylose. This enzyme's ability to isomerize xylose into xylulose, which normal yeasts can ultimately ferment into ethanol, is utilized in this process. Given the quick depletion of fossil fuels, it is crucial to convert renewable biomass into fermentable sugars and ethanol. Cellulose makes up 40% of the biomass, followed by hemicellulose at 30% and lignin at 30%. The hydrolysis of cellulose and hemicellulose to glucose and xylose, and their subsequent fermentation to ethanol by yeasts, determine the economic viability of biomass utilization.into usable carbon sources and further to ethanol is very important. Both the isomerization of glucose and xylose are catalysed by glucose isomerase. Glucose Isomeraseuses this capability to isomerase xylose to xylulose. This D-xylulose is then further fermented to ethanol using ordinary yeast. Biotransformation of feedstock Lignin, cellulose, and hemicellulose make up the feedstock. The breakdown of cellulose and hemicellulose to glucose and xylose (xylulose) and their subsequent fermentation to ethanol by yeasts are essential for the economic viability of feedstock utilisation[46]. The commercial use of yeasts is constrained by low ethanol tolerance and ethanol catabolism in the presence of oxygen [47].

By using conventional yeasts like *Saccharomyces cerevisiae*, *Schizosaccharomycespombe*, and *Candida tropicalis*, GI has been used to convert xylose into xylulose, which otherwise represents a significant metabolic roadblock in the process of fermentation of xylose to ethanol [48,49,50]. The oxidoreductive route is known to be utilised by a few yeasts, including *Pachysolentannophilus*, *Pichia stipitis*, *Candida utilis*, and *Candida shehatae*, however the rates of fermentation are quite low [47]. As the host, the flocculating industrial strain (YC-8) of *Saccharomyces cerevisiae* was used by Li and his colleagues (2015) to build the xylose isomerase (XI) pathway. When xylose was the only carbon source employed, both strains demonstrated improved growth and fermentation capacities.[51]

4. Paper and pulp industry:

Significant scientific efforts have been made in recent years todesigned to cut back on the amount of chlorine needed in bleachingafter the pulping procedures, of kraft pulp. Bleaching methods based on chlorine (chlorine, chlorine dioxide, and hypochlorite)can lead to the release of chloroorganics into the environment, including

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chlorinated phenols and dioxins. The pulp and paper industry has been forced by environmental regulatory demands to use new technologies in order to lessen or completely remove the presence of various pollutants in the effluents from bleaching plants.The three primary components of wood are lignin, cellulose, and hemicellulose. Enzymes are being used in pulp production research to break down or modify lignin and hemicellulose without harming the cellulose fibres.

Xylanase pretreatment of kraft pulp encourages a reduction in lignin concentration (kappa number) and an improvement in the brightness of the treated pulp. High-specificity lignin removal from lignin-carbohydrate complexes is facilitated by xylanase. Because of this enzymatic pretreatment, 20–30% less chlorine is required during chemical bleaching to achieve the desired brightness in the pulp. As a more affordable alternative to chlorine bleaches, the use of laccase (EC 1.10.3.2) to promote the degradation of lignin and bleaching of pulp has garnered a lot of interest. [52-64]

5. Dairy Industry:

Enzymes (proteases, lipases, esterases, lactase, and catalase) have a long history of use in dairy technology. In the initial stage of cheese making, rennets (rennin, a combination of chymosin and pepsin primarily derived from animal and microbial sources) are used to coagulate milk. Different types of proteases are employed to speed up the ripening of cheese, alter functional characteristics, and alter milk proteins to lessen the allergic effects of cow milk products on young children.For the development of lipolyticflavours, lipases are mostly used in the ripening of cheese. As a digestive aid and to increase the sweetness and solubility of various dairy products, lactase (-galactosidase, EC 3.2.1.23)produces glucose and galactose by hydrolyzing lactose. Many people lack enough lactase to break down milk sugar. These lactose-intolerant persons are able to consume milk and other dairy products thanks to lactose hydrolysis.Galactose inhibits the activity of the galactosidases from *K. fragilis*, *A. niger*, or *A. oryzae*. It is possible to get around the inhibition issue and reduce the expense of lactase use by using immobilised enzyme systems. Whey is a byproduct of the cheese manufacturing process, and lactose makes up 70–75 per cent of the whey solids. Whey is transformed into more beneficial food elements by the hydrolysis of lactose by lactase. Additionally, lactases have been utilised to process dairy wastes and as a tablet-based digestive assistance for people consuming dairy products.

As an efficient chemical sterilant, hydrogen peroxide can be used to treat raw milk instead of pasteurising it with heat. At the end of the procedure, the leftover peroxide is eliminated using catalase (EC 1.11.1.6), a substance that catalyses the breakdown of hydrogen peroxide. The synthesis of bitter-flavoured peptides from milk proteins accounts for the bitter off-flavours that appear in ripened cheese as it ages. Peptidases can break down the bitter peptides as they form, assisting in preserving the cheese's classic flavour. [52-64]

6. Detergent Industry:

About 30% of the industrial enzyme market is currently occupied by the detergent sector. Enzymes like cellulase, amylase, protease, and lipase can be found in more than half of laundry detergents. These enzymes must be very effective, operate at high temperatures and alkaline pH settings (pH 9–11), remain stable in the presence of chelating agents, perborates, and surfactants, and have long storage stability (>1 year) in order to function well in laundry detergent environments. Following a preliminary period of soaking, the use of enzymes permits lower temperatures and shorter periods of agitation.In the detergent sector, proteases are the most often used enzymes. In order to improve stability properties, DNA technology has been widely employed to change protein catalysts. They eliminate protein-based stains like those left behind by bodily secretions, blood, grass, eggs, and meat sauce. These detergent enzymes (serine proteases) are produced by the fermentation of *Bacillus sp*., *B. lichiniformis*, or *B. amyloliquefaciens*. Amylases eliminate the leftovers of starchy foods including porridge, spaghetti and mashed potatoes.

Lipases make it easier to get rid of resistant stains on collars and cuffs as well as fatty stains like lipstick, frying oil, butter, salad dressing, and sauces. At pH 12 and 60 C, *Humicolalanuginosa's* lipase is active. It was created using recombinant DNA technology.A fungal cellulase preparation that is alkali-stable has just been unveiled for use in washing cotton garments.Without appearing to harm the primary fibres, treatment with these cellulase enzymes removes the small fibres that extend from the fabric (known as pilling) and returns the fabric to "as new" condition by enhancing colour brightness, improving softness feel, and removing

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particle soiling.In the process of making textiles, cellulases are employed to partially remove the indigo colour from denim, giving it a stone-washed appearance. Amylase and protease, two enzymes that are resistant to bleach, are now available for use in automated dishwashing detergents.[52-64]

II. CONCLUSION:

Glucose Isomeraseis known to have a potential application in many industrial sector. However, their potency is due to the structural integrity in their catalytic site, thus it is necessary to understanding the structure as well as their isomerization mechanism to exploit the use of Glucose Isomerase. Extensive biochemical investigation and the engineering of novel Glucose Isomerase are required for the development of Glucose Isomerasebased industrial processes that are more efficient. There will be the scope of glucose isomerase, not only as whole cell or purified enzyme, but also in immobilized form as potent tool for industrial application.

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