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PRODUCTION, KINETICS AND IMMOBILIZATION OF MICROBIAL INVERTASES FOR SOME COMMERCIAL APPLICATIONS - A REVIEW

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ABSTRACT

Invertase (EC. 3.2.1.26) is commercially important enzymes that hydrolyzes the sucrose and fructose, is a glycoprotein and has optimum pH of 4.5. Invertase is stable at a temperature of 50°C and has maximum activity at 55°C. This review explains and enlists different types and wide range of invertase sources from wide range of species. Especially different microbial sources that secrete either intracellular or extracellular are also discussed. Modification of invertase through genetic engineering is also discussed here. Different production techniques of invertase from different microorganisms either through submerged fermentation or solid state fermentation with their substrate requirements and optimum growth parameters of pH, temperature and incubation period are briefly discussed. This review also focused on the activity of enzyme obtained and isolated from microbial sources, its kinetics parameters, competitive inhibitors and heavy metal affect on enzymatic efficiency. Enzyme immobilization techniques of invertase on various materials such as chitosan, nanoparticles etc. and comparative study of catalytic activity of free and immobilized enzyme is part of this review. Wide range of commercial applications of enzyme in the fields of pharmaceutical industry, food industry, biosensors, confectionary industry and in the production of great range of biologically important compounds is comprehensively discussed in this review.

Keywords: Solid state fermentation, submerged fermentation, Invertase, Biosensors, Immobilization, Genetic engineering

INTRODUCTION

Enzymes are polymers made up of amino acid monomers and are also termed as globular proteins, due to possessing globular structure. The enzymes are Biological catalysts that speed up biochemical reactions in the body of living organisms (Gurung *et al.,* 2013). Enzymatic catalytic activity is important for their functioning. Enzyme can catalyze metabolic reaction in the body of living organisms, achieving a maximum rate of substrate conversion to product by lowering the need of the Gibb's free energy (DG) (Shinde *et al.,* 2015). Because of specific chemical nature and specific structure of enzymes, these catalysts can differentiate between their substrate and can carry out reactions over a wide range of pH and temperature conditions. Enzymes are essential bio-products that have a wide range of uses in the biotechnology and their demand and applications are increasing day by day (Anbu *et al.,* 2017).

Carbohydrates serve as primary source of energy for all type of living organisms. Even some non-reducing sugars that are disaccharides can serve as signaling molecules in the body like sucrose (Nadeem *et al.,* 2015). Different carbohydrates such as glucose or maltose and fructose have important roles in the metabolic pathways that are carried out by cell. Invertases (EC 3.2.1.26) are group of enzymes which are important in the industry of food and responsible for the sucrose inversion by producing D-fructose and D-glucose (Lincoln and More, 2017), as depicted in Fig. 1. It can be synthesized by yeasts and as well as by some filamentous fungi where it is inducible (Chen *et al.,* 1996; Romero *et al.,* 2000). In case of *A. japonicas* (Hayashi *et al.,* 1992), the glucose is the good inducer than sucrose but in case of *P. glabrum* the production increases, when the fructose is served as the main carbon source (Rubio *et al.,* 2003).

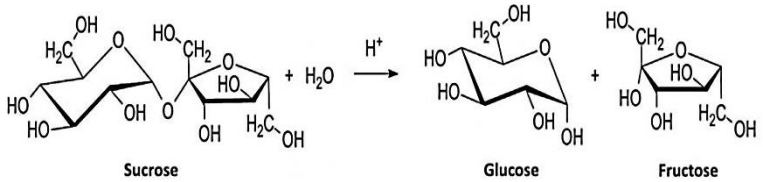


Fig. 1. Sucrose hydrolysis by invertase.

Therefore, invertase enzyme plays a key role because invertase can hydrolyze disaccharide sucrose. Invertase enzyme has been named due inversion that occurs in the optical rotation as sucrose is being the hydrolyzed (Kotwal

and Shankar, 2009).The mechanism of action of invertase and different amino acids involved in catalysis have been shown in the Fig. 2 depicts mechanism of action of invertases (Pang *et al.*, 2019).

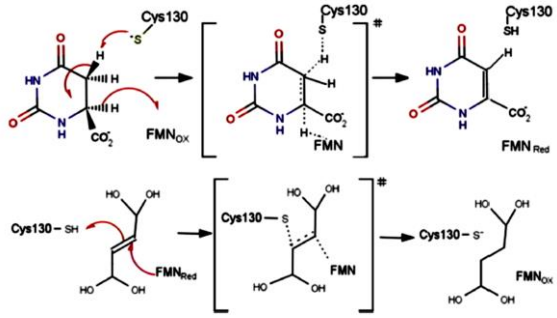


Fig. 2. Mechanism of action of invertases.

CLASSIFICATION OF INVERTASES

Sucrose is the one of the most abundant carbohydrate found in nature. Plant and other photosynthetic organisms are capable of production of their own food and it is derived from the process of photosynthesis and it plays a vital role in performing the biological functions as a soul sensor in the nutritional conditions of the plants. Therefore the invertase performs functions of plants and in providing the responses which are given in case of environmental stress (Vargas and Salerno, 2010). Sucrose and glucose have very key role in signaling pathways, especially in case of plants where the concentration of sucrose plays main role in the development and differentiation of cells. Although, humans and animals also indicates preferences for those diets which contains sucrose, but their genomes do not codes for the invertase. The genomes of microorganisms which are present in the human intestine i.e *Bifidobacterium longum* (Schell *et al.*, 2002) and *Bacteriodes thetiaotamicron* contain invertase genes which showed that these organisms get benefit due to intake of sucrose by humans. The sucrose which is used as carbon source and energy is dependent on the breakdown of glycosidic bond through the actions of the invertases that hydrolyze disaccharides present in sucrose and fructose, irreversibly. So, the three types of enzymes can hydrolyze the sucrose, the common invertases called as β-fructosidases, oligo α-1,6-glucosidases and α-1,4-glucosidases which have a broad range of specificity of substrates. Invertases can be divided into two types on the basis of its activity that are initially classified on the basis of difference between their optimal pH in vitro. The acidic invertases which have their optimum pH range between 4.5-5.0 and the neutral/alkaline invertases have their optimum range between pH of 6.5-8.0 (Tymowska-Lalanne and Kreis, 1998). Invertases are called as those enzymes that can hydrolyze fructose and sucrose. A few invertases are reported as very specific to sucrose i.e invertebrate alkaline carrot (Lee and Sturm, 1996).

On the other hand, most of the invertases have a wide range of specificity of substrates that can hydrolyze sucrose and B-fructosid bond in inulin, raffinose, levan and sucrose 6-phosphate (Lee and Strum, 1996). Due to some preferred substrates, B-Fructosidases can be named as fructanase, leavanase inulinase, sucrose and invertases. The invertases are present in the GH32 family bearing glycosyl hydrolases on the basis of sequence based classification. This family contains >370 members of bacterial, plant and fungal origin which consists of invertases. Besides invertases, this family also contains leavanases, inulinase, transfructosidases, fructans and exo-inulinases. Glycosyl hydrolases are a large group of enzymes which depicts broad varieties of substrate specificities and protein folding. They also share a same feature i.e presence of two critical sites for acid residue due to which the catalytic mechanism has become responsible for the denaturation of glycosidic bond. In case of yeast invertases, the above mentioned two invariant acid residues are considered as two different substrates i.e as aspartate which is located at N-terminal and there, it acts as a nucleophile and the second one is as glutamate that perform its function as general acid or base (Reddy and Maley, 1996).

PRODUCTION OF INVERTASES

There are different sources for the enzyme Invertase. It can be plant invertases, microbial invertases, and animal invertases. Here we give a look to the microbial (bacterial) invertases. Microbial invertases can be dimeric or multimeric enzymes, but the monomeric invertases are found in bacteria (Muramatsu *et al.*, 1993). Bacterial invertases can be present as extracellular or intracellular enzymes. Some gram positive bacteria present in *Actinomycetales* e.g *Arthrobacter* species (Win *et al.*, 2004) and *Brevibacterium* species (Yamamoto *et al.*,1986) produces invertases that are secreted from soil and also gram negative species e.g., *Zymonas mobilis* also investigated in term of producing invertases (Yanase *et al.*,1991) .

The external invertases are mostly glycoprotein in nature which comprises of mannose, carbohydrates and glucosamine. On the other hand, the intracellular invertases don't posses carbohydrates. The sequence of amino acids in both of the invertases is different (Workman and Day, 1983). For screening of invertase production, the ability of bacteria to hydrolyze the sucrose should be checked. After this, the strains which are isolated should be grown in the culture medium providing sucrose as the main source of carbon (Kaur and Sharma, 2005). For the metabolization of sucrose, microbes should perform invertase activity. This catabolic efficiency was measured by presence or absence or reducing sugar. Biochemical tests are performed to identify the colonies and by phylogenetic analysis and 16S rRNA sequencing of genes. Then the extracted strains were transferred to the agar slants set for incubation at 37°C for 24 to 48 h. After this process, the strains were placed in glycerol stocks for storage at -70°C or sometimes at -80°C for long time of preservation (Yoon *et al.,* 2007).

There are some of gram positive bacteria which are producing invertases that belonged to *Actinomycetales* such as *Anthrobacter* species, *Streptomyces* species and *Brevibacterium* species which were extracted from soil. Some strains of gram negative bacteria were also investigated. For the production of invertases, soil was considered as good source (Lincoln and More, 2017). Some scientists were also extracted extracellular invertases from the breast milk and that is a good source of important nutrients and enzymes which provides immunity, development and growth to the new born. Here in Table 1 Production of invertases from different bacterial invertases have been listed (Lincoln and More, 2017)

Table 1. Production of invertases from different bacteria.

Microorganism	**Incubation period (h)**	**pH**	**T (°C)**	**Agitation rate (rpm)**	**Inoculum size**	**C-source (%)**	**N-source (%)**
Arthrobacter globiformis	36	7.0	2	20	ND	ND	1% poly-peptone, 0.2 % yeast extract, 0.4% $(NH4)_2HPO_4$
Artrobacter sp.	20	ND	30	250 (further increased by 20%)	5%	4% sucrose	4% corn steep powder, 094% $(NH4)_2HPO_4$
Bacillus macerans	48	6.0	30	160	8	3% sucrose	0.5% peptone, 0.3% yeast extract
Bifidobacterium infanits	16	6.8	37	ND	5%	2% fructose	Semisynthetic medium
Lactobacillus reuter	16	ND	37	ND	ND	1% sucrose	MRS broth
Streptomyces sp.	24	5.0	37	ND	3 disc (9 mm)	1% sucrose	$NaNO_3$ + yeast extract

*ND (not determined)

There are many useful bacteria present in milk that are *Staphylococci*, *Micrococci, Bifidobacterium, Streptococci, Lactobacilli* and *Enterococci* have various applications in biotechnology. Milk is considered as the natural source of probiotics. In a study conducted by Award and colloquies, approximately thirty different species of Lactobacillus were extracted from breast milk and the strain which is producing high amount of invertase was identified (Awad *et al.,* 2013).

Invertases have been produced from microbial sources due to their high production rates and ease of extraction and purification. The invertases from animal and plant sources are not preferred because of release of harmful chemicals during their production (Zouaoui *et al.,* 2016). In recent research and industry production of invertases is achieved through submerged fermentation (SmF) and after that solid state fermentation (SSF). This is the best strategy used for traditionally production of invertases on commercial scale by microbes. This process is opted due to its low cost, high production rate and ease of control and harvesting the product (Lincoln and More, 2017). In SmF fermentation microbes are cultivated in fermenters with controlled conditions and are provided with oxygen and essential nutrient for fermentation process. Microbes produce invertases and these invertases are released extracellular medium. Genetically altered microorganisms can be easily grown in SmF. In solid state fermentation microbes are grown on the solid medium and this method is suitable for the growth of microbes that require low

moisture content for growth. Growth environment in SSF is similar to natural habitat of microbes. This method is not suitable for large scale production of invertases (Renge *et al.,* 2012).

Another organism *Schizosaccharomyces pombe* which have extreme similarities with multicellular organisms have been used for the recombinant invertase production. Invertase produced by *S. pombe* was stable at a temperature less than 50^{o}c and a Km value of 0.026 mol/L. In recent research *Pichia pastoris* have been employed for production of heterologous invertase from baker's yeast, plant and *Elsholtzia haichowensis*. *Pichia pastoris* produces enzyme at a high rate and have efficient glycosylation capability. Table 2 and 3 shows over view of invertase production through submerged and solid state fermentation and required optimum growth parameters (Pang *et al.,* 2019).

Table 2. Invertase production through submerged fermentation

Source	**Substrate**	**Optimum production condition**			**Yield**
		pH	**Temperature (°C)**	**Incubation time**	
Fusarium solani	Molasses	5.0	30	4 days	9.90 U/mL
Clasdosporium clasdosporioides	Pomegranate peel	4.0	30	4 days	~45.0 IU/mL
A. niger	Fruits peel	5.0	30	4 days	16.25 ± 0.60 µM
A. flavus	Agriculture-based-by-products	6.5	40	2 days	7.41 U/mL
A. niger	Agro-industrial waste	6.5	25	6 days	15.9 ± 2.44µ/g
A. nidulans and *Emericela nidulans*	Rye flour	4.8-5.6	54-62		927.0 ± 35.3 U

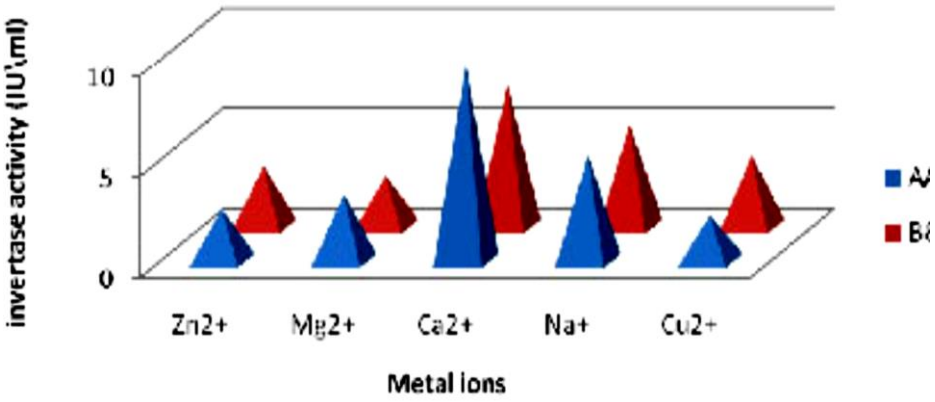
Table 3. Invertase production through solid state fermentation

Source	**Substrate**	**Production condition**			**Yield/Enzyme activity**
		pH	**Temperature (°C)**	**Incubation time**	
S. cerevisiae	Red carrot residue	---	30	72 hours	272.5 U/g
S. cerevisiae	Sugarcane press mud	5.0	40	3 days	430 U/mg
A. niger	Wheat bran	5.5	30	3 days	194.71 U/g
Aspergillus sp.	Carrot peel	7.0	at room temperature	5 days	6.2 U/mL/min
A. niger	Agro-industrial wastes		30 (At 50% moisture)	3 days	154.27 ± 9.38 μg^{-1}
A. niger	Fruits peels	5.0	30	4 days	51 U/Ml

Recombinant DNA technology or the heterologous gene expression is technique that refers to series of event that are carried out for isolation of genes ,modification of genes and their transfer to different host for cloning or expression of specific genes and products of genes (Yesilirmak and Sayers, 2009). It is great technique and strategy for the commercial production of different biological products that have very low production cost (Palomares *et al.,* 2004). The different achievements and advancements in the technology of gene manipulation have enabled us for the commercial production of enzymes in different microorganisms. The heterologous expression of invertase is preamble done in the eukaryotic hosts as eukaryotic hosts able to carry out different post translational changes that prokaryotic organism cannot do (Desai *et al.,* 2010).Recombinant DNA technology (RDT) or genetic engineering is employed for the production of invertases by yeast/fungal sources. *S. cerevisiae* is used for production of invertase and preferred due to extracellular release of enzyme and ease of purification (Yesilirmak and Sayers, 2009).

KINETICS OF INVERTASES

As compared with the kinetics statics of many enzymes, invertases have a high catalytic efficiency of its reactions over a wide range of pH. The pH for invertase optimal activity is between 3.5 to 4.5 pH. The optimum temperature for enzyme is 55^{o}C at this temperature invertase have maximum activity. Free invertase has Km (Michaelis-Menten value) of 30Mm (Workman and Day, 1983). Invertase is glycoprotein in nature and still stable at a temperature of 50^{o}C. The invertase is inhibited by Hg^{+2}, Cu^{+2}, Ca^{+2} and Ag^{+}. Invertase is competitively inhibited by 2, 5-anhydro-D-manitol because of resemblance to furanose type of ring (Kulshrestha *et al.*, 2013). Fig. 3 shows the affect of different metals on the activity of the invertase (Uma and Kumar, 2010). The amount activation energy and substrate binding affinity for sucrose fermentation were found to be very important in yeast culture (Sikander, 2007).



A - *A. fumigatus* B - *P. brevicompactum*

Fig. 3. Effect of metals on enzyme activity.

The mechanism of enzyme action is dependent upon its purity, source and stability. Hence as five step method having ten parameters can be made and all of the reactions are reversible. According to Michaelis–Menten kinetics, in first step sucrose as substrate binds with the enzyme and forms an enzyme substrate complex, i.e substrate sucrose (Suc). In the next step the hydrolysis of the sucrose to fructose and glucose is shown. In the equations trans-fructosylation is also shown. This model also explains the competitive inhibition of enzyme by glucose; in that case glucose binds to active site of enzyme and competitively inhibits the enzyme. In the Fig. 4 and Fig. 5 different equations has been shown to elaborate mechanism of action of invertase (Khandekar *et al.*, 2014).

$$E + Suc \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} ESuc$$

$$ESuc \underset{k_{-2}}{\overset{k_2}{\longleftrightarrow}} EFru + Glu$$

$$EFru \underset{k_{-3}}{\overset{k_3}{\longleftrightarrow}} E + Fru$$

$$EFru + Suc \underset{k_{-4}}{\overset{k_4}{\longleftrightarrow}} E + FOS$$

$$E + Glu \underset{k_{-5}}{\overset{k_5}{\longleftrightarrow}} EGlu$$

Fig. 4. Mechanism of invertase action (Khandekar *et al.*, 2014).

$$\frac{d[E]}{dt} = -k_1[E][Suc] + k_{-1}[ESuc] + k_3[EFru] - k_{-3}[E][Fru] + k_4[EFru][Suc] - k_{-4}[E][FOS] - k_5[E][Glu] + k_{-5}[EGlu]$$

$$\frac{d[ESuc]}{dt} = k_1[E][Suc] - k_{-1}[ESuc] - k_2[ESuc] + k_{-2}[EFru][Glu]$$

$$\frac{d[EFru]}{dt} = k_2[ESuc] - k_{-2}[EFru][Glu] - k_3[EFru] + k_{-3}[E][Fru] - k_4[EFru][Suc] + k_{-4}[E][FOS]$$

$$\frac{d[EGlu]}{dt} = k_5[E][Glu] - k_{-5}[EGlu]$$

$$\frac{d[Suc]}{dt} = -k_1[E][Suc] + k_{-1}[ESuc] - k_4[EFru][Suc] + k_{-4}[E][FOS]$$

$$\frac{d[Glu]}{dt} = k_2[ESuc] - k_{-2}[EFru][Glu] - k_5[E][Glu] + k_{-5}[EGlu]$$

$$\frac{d[Fru]}{dt} = k_3[EFru] - k_{-3}[E][Fru]$$

$$\frac{d[FOS]}{dt} = k_4[EFru][Suc] - k_{-4}[E][FOS]$$

Fig. 5. Mechanism of enzyme action parameters (Khandekar *et al.*, 2014).

IMMOBILIZATION OF INVERTASES

Immobilized enzymes are extensively used in different industries ties due to their high conversion rate. Invertase (β-D-fructofuranosidase, E.C. 3.2.1.26) has been used in the industry for the production of invert sugar syrup. The successful biocatalysts and reaction rate depends upon the support and the reagents that form suitable groups for the linking of enzymes to the support (Rosa *et al.*, 2000). Due to high commercial demand the chemically stable and active immobilization of invertase is important. In immobilization of different enzymes the properties of the matrix and the method of attachment of enzyme are important for the properties of bounded enzyme (Torres *et al.*, 2002). Here Fig. 6 shows a comparative study of kinetics of free and immobilized enzyme on various surfaces (Malhotra and Basir, 2020).

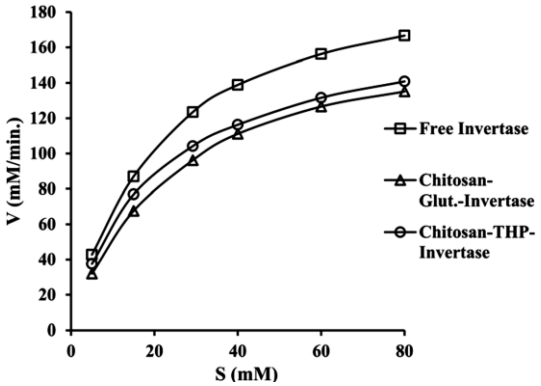


Fig. 6. Michaelis-Menten kinetics of free and immobilized invertase (Malhotra and Basir, 2020).

Mostly, hydrophilic matrices like cellulose, Sephadex, Sepharose and the derivatives of these compounds are used for the immobilization of enzymes because these hydrophilic matrices increase the stability of bounded enzyme. Invertase is immobilized on many hydrophilic as well as on the inorganic matrices like polystyrene resins. Comparatively, polystyrene derived matrices are more advantageous because they are resistive to microbial invasion and mechanical compression. Immobilization of invertase enzyme was achieved on charcoal and aluminium hydroxide and scientists demonstrated that immobilized invertase still retained its activity. Immobilization of invertase on different material like microcrystalline cellulose (46% activity), DEAE- (41% activity), and CM-Sephadex (70% activity), and Con A-agarose (73% activity). Con A-agarose immobilized invertase is more active as compared to other matrices (Kotwal and Shankar, 2009).

Gelatin (G) is compound that is obtained by partially hydrolyzing collagen protein and is widely used for immobilizing enzymes. Gelatin possesses different very reactive functional groups like amino, hydroxyl and carboxyl functional groups. Gelatin is hardened by alum and chromium salts by the formation of cross links with carboxyl groups (Emregul *et al.*, 2006). In recent research studies invertase enzyme was immobilized on chitosan by cross linking agent glutaraldehyde or tris (hydroxymethyl) phosphine. Optimum conditions of pH for immobilized enzyme was 5.5 and for free enzyme it was also 5.5 (Kotwal and Shankar, 2009). The invertase enzyme optimum temperature was the same for both the immobilized and free enzyme and it was 55 °C. The invertase immobilized by using THP cross linker was much stable both thermally and from pH aspect. Fig. 7 depicts the schematic diagram of immobilization of enzyme (Malhotra and Basir, 2020).

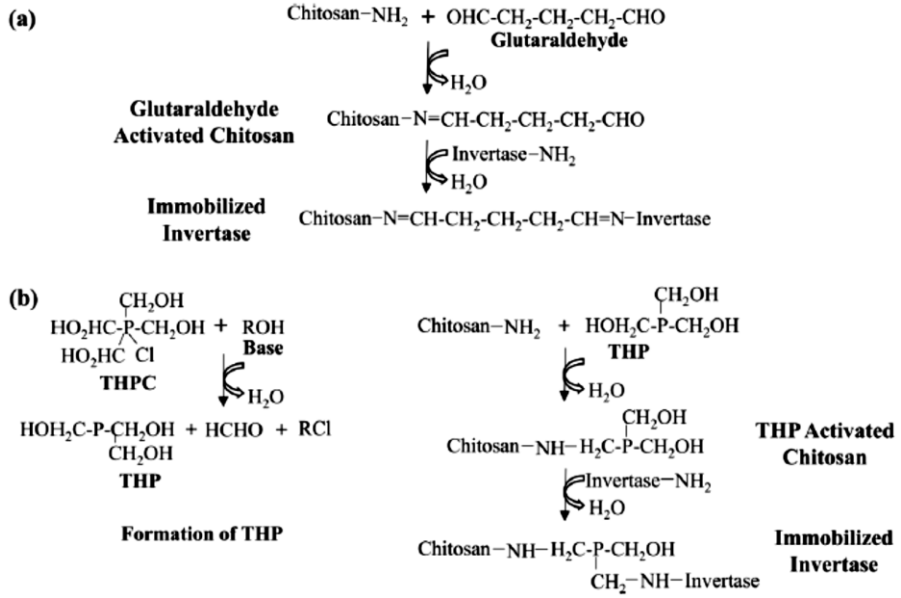


Fig. 7. A schematic representation of immobilization of invertase (Malhotra and Basir, 2020).

In other research magnetic polyvinyl alcohol (PVAL) microspheres were made by cross linking the glutaraldehyde. 1, 10-Car bonyldiimidazole (CDI), was adopted for the activation of hydroxyl groups of polyvinyl alcohol. Invertase was immobilized by covalent bonds through amino groups with magnetic PVAL microspheres. Here the catalytic efficiency of the enzyme was very high and it retained 74% catalytic activity. Here comparative study of free and immobilized enzyme was also conducted and Kinetic parameters were studied and the Km values for immobilized invertase (55 mM sucrose). These Km values were higher as compared from free invertase (24 mM sucrose). But Vmax values that were obtained from experiments were greater for free enzyme as compared to the immobilized invertase. The optimum temperature for high efficiency was 5^{o}C higher for enzyme immobilized on PVAL as compared to free invertase. Here Fig. 8 shows immobilization of invertase on PVAL (Akgol *et al.*, 2001).

COMMERCIAL APPLICATIONS OF INVERTASES

Invertases have a wide range of applications in different industries. These enzymes are an important source of heavy metal pollutant detections because heavy metals can inhibit these enzymes and therefore are used in biosensors. In pharmaceutical industries invertases are used for the production of high fructose syrup and invert syrup production. In food industry it is important for confectionary industry.

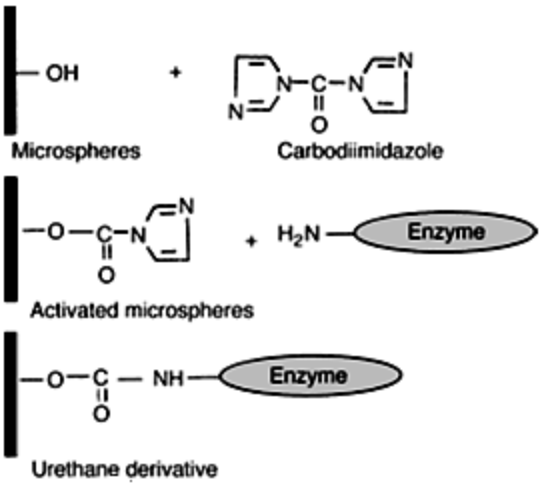


Fig. 8. Activation of PVAL (Akgol *et al.*, 2001).

Invertase biosensors

Enzyme based biosensors have gained enormous importance in research due to their selectivity and sensitivity .Enzyme based biosensors are used in various fields of life and have very a high commercial value (Karube and Nomura, 2000). Different enzymes blocking or inhibition by inhibitor molecules is a unique characteristic that is used by different industries for analytical aims such as to detect the presence of different polluting chemicals like heavy metals (e.g. lead, mercury) , or other polluting agents (Hosseinpour *et al.*, 2003). These heavy metals have very an adverse effect on enzymes as they retard enzyme activity by their irreversible binding to thiol groups in enzymes. Enzyme based biosensors are also made that are based on the mechanism of enzyme inhibition by different inhibitory molecules. Due to their high specificity and sensitivity to heavy metal pollutants invertase based biosensors have been used frequently (Verma and Singh, 2006). Invertase based biosensors have been preferred due to their effective cost and stability. The immobilized invertase along with glucose oxidase has been used for the estimation of sucrose in the solution. The working principle of this biosensor is dependent on the formation of glucose by invertase hydrolytic activity and subsequent glucose oxidation of glucose molecule into glucose hydrogen per oxide that is detected and measured by Ag/AgCl reference electrode (Bagal-Kestwal *et al.*, 2008).

Prostate cancer is a cancer which adversely affects the reproductive system of males and is becoming most common cancers in men. There is no effective treatment for prostate cancer to prolong life of patients. However, an early diagnosis of prostate cancer is necessary to save life of patients. Prostate specific antigen (PSA) is considered to be most affective biomarker in the detection and early diagnosis of prostate cancer in males. Identification of this biomarker is important subject in research. A highly sensitive biosensor was developed for the prostate-specific antigen (PSA). A Schematic diagram has been shown below in Fig. 9 (Hun *et al.*, 2015).

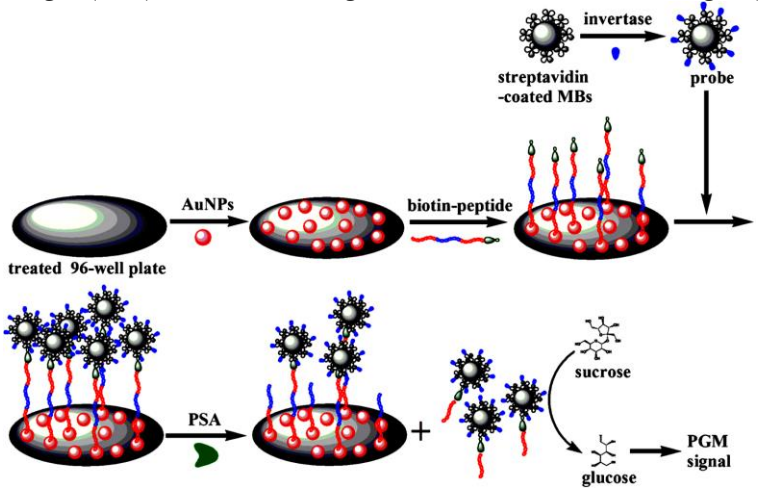


Fig. 9. Schematic diagram of invertase based biosensor of prostate-specific antigen determination (Hun *et al.*, 2015).

Biosensors are basically used for the monitoring of polluting agents mostly in aqueous environment mostly like river or household water. Many biosensors which use enzymes are made for measuring various environmental pollutants. With the immobilization techniques of enzymes their specificity and reactivity is increased (Karube and Nomura, 2000). Electrochemical detection of the inhibitory effect of heavy metal on invertase as a biosensor has been shown in Table 4 (Bagal-Kestwal *et al.,* 2008).

Table 4. Electrochemical detection of the inhibitory effect of heavy metal on invertase as a biosensor

Metal ions	Dynamic concentration range (M)	Lower detection limit (M)
Hg^{2+}	5×10^{-10} to 12.5×10^{-10}	5×10^{-10}
Pb^{2+}	5×10^{-8} to 2.5×10^{-7}	3×10^{-8}
Ag^{+}	5×10^{-8} to 5×10^{-7}	5×10^{-8}
Cd^{2+}	2.5×10^{-8} to 12.5×10^{-8}	2.5×10^{-8}

(Bagal-Kestwal *et al.,* 2008)

Invert syrup production

Invertase is an important enzyme for the generation of equimolar concentration of fructose and glucose by hydrolysis of sucrose and it result in the production of invert syrup that is sweeter than sucrose due to the presence fructose which has high degree of sweetness and subsequently the sugar contents of syrup can be increased without crystallization of sucrose as hydrolysis of sucrose prevents its crystallization. Therefore, the production of a sugar syrup that is non-crystalizable is one the major applications of invertase enzyme. Due to its hygroscopic nature invert syrup is used in the production of candies and fondants as humectant (Kotwal and Shankar, 2009).

Pharmaceutical applications of invertase

Moreover, invertases have key importance in the field of pharmaceutical industry for the formation of drugs, like cough syrup, digestive tablets, nutraceuticals, and infant foods (Lincoln and More, 2017). Besides, this commercial importance of invertases in the pharmaceutical filed is further interested to find invertases having unique transfructosylating, which form FOS. FOS is carbohydrate oligosaccharides that are composed of short chains of fructose which are less sweet as compared to sucrose, and resulting oligosaccharide has low calories and is perfect diet for diabetic patients (Dominguez *et al.* 2013). . Invertases are also used for the production of FOS (Fructo oligosaccharides) which is used as prebiotics, which are used for the stimulation of growth of prebiotics that reside in our body and confers health benefits to us (Pang *et al.,* 2019).

Invertases are very efficient anti-oxidant and anti-microbial enzyme and helpful in prevention of gut fermentations and infestations due to oxidation reactions. In Indian subcontinent raw honey was used affectively against bacterial infections and was also given to the patients having weak hearts. Invertase and some other enzymes are also affective against cold, flu and other respiratory system diseases (Kulshrestha *et al.,* 2013). Invertase enzyme has shown as significant affect as an antioxidant and antibacterial agent. Therefore it finds potential use as antibacterial agent to prevent different infections caused by bacteria. It has been employed to treat different diseases of alimentary canal. Honey has special properties like hygrospcic, moisture retaining, this property inhibits growth of bacteria in body and these characteristics conferred by invertase to honey. Invertase produces low molecular weight sugars that can be easily digested and will not persist long in the stomach, hence toxic effects will not be shown. In this way these sugars can be easily absorbed in the body and ulcer can be prevented. Invertase present in the honey has capability to convert glucose into hydrogen peroxide and formation of hydrogen peroxide kills bacteria (Manoochehri *et al.,* 2020).

Some studies have indicated that invertase possesses chemotherapeutic properties and can be used in the treatment of cancer. Researchers have found that invertase present in the honey can be used to treat stomach and bone tumors and it is efficient agent to cause regression of tumors. Enzyme based therapy can reduce adverse effects of chemotherapy and radiotherapy. Employment of enzyme in the treatment of cancer leads to prolong life (Benkeblia *et al.,* 2008).

Production important bio-products

Ethanol, lactic acid and glycerol have been produced by fermentation that uses invertases. Invertases also used for sucrose hydrolysis of sucrose in canned molasses (Pang *et al.,* 2019). Ethanol was from the cane molasses by culturing yeast in the presence of immobilized invertase on THP-activated chitosan. A comparative study was also conducted to compare the activity of free and immobilized enzyme for production of ethanol. The large scale demand for fuel having high efficiency, eco-friendly, and high energy security has drawn attention of the scientists to produce liquid biofuels from fermentation techniques such as bioethanol and biodiesel. However, efficiency of

ethanol is almost 34% less energy per unit volume as compared to fossil fuels such as gasoline. But ethanol is an environment friendly compound and its combustion releases less amount of green house gases. Therefore it is good alternative to fossil fuels. In India, bioethanols is majorly produced from sugarcane molasses (Malhotra and Basir, 2020). Invertases have been employed in paper and cosmetic industry as well (Mckenzie *et al.,* 2013).

Invertase in confectionery foods

Invertases or β-d-fructofuranosidase have key used to hydrolyze disaccharide sucrose and polysaccharides, which possess the bond called β-d-fructofuranosyl bond, to form fructose and glucose as end products of reaction. These invertases have capability to carry out this catalytic reaction in the backward direction as well (Ojwach, 2018). The solution or mixture that is formed as a result of invertase catalytic reaction is has fructose and glucose is also called as "inverted sugar" due to the inversion of optical properties of the mixture. Invertases are frequently employed in confectionery food industry for the formation of artificial sweetener in catalytic reactions (Veana *et al.,* 2018).

Therefore, invertase is needed to prepare formulas that have ability to prevent crystal formations in the certain sweets, like in the chocolate industry. In some other syrup, β-d-fructofuranosidase is used to enhance its sweetening characteristics such as formation of the soft caramel fillings (Lincoln and More, 2017). The most commonly used type of this inverted sugar is honey, which is actually a supersaturated mixture containing glucose and fructose. Moreover, invertases are also capable to form fructooligosaccharides from fructotransferase which have high amount sucrose. The fructooligosaccharides have key role in the improvement of human health (Veana *et al.,* 2018). Fig. 10 shows area of application of invertase (Yushkova *et al.,* 2019). Invertase is extensively employed for the production of non-crystallizing creams and to make jams (Emregul *et al.,* 2006).

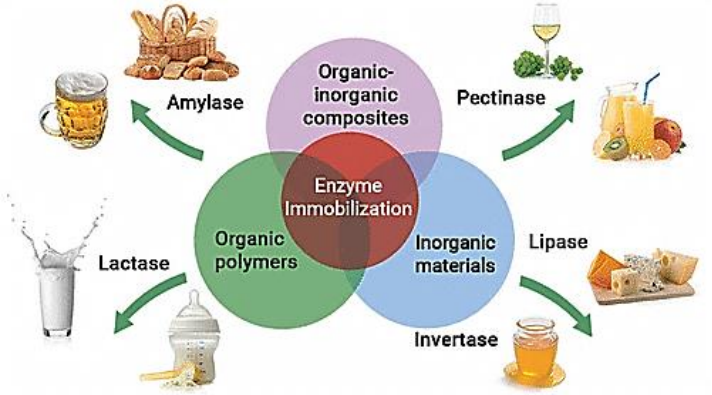


Fig. 10. Application of various immobilized enzymes (Yushkova *et al.,* 2019).

CONCLUSION

Invertases are very important industrial enzyme with wide range of applications and are frequently used in the processing of sucrose. Invertase has gained commercial importance because of its immobilization on various materials and subsequent stability, recovery, retention and catalytic efficiency. Many attempts in research are made upon the immobilization methodologies of invertase for production of economically beneficial and cheap bio-products. But commercially economical immobilization methods are yet more to be discovered because of high cost issues. The activity, efficiency and stability towards industrial process can be elevated to higher levels by searching new sources of invertase, with unique characteristics. Invertases produced from extremophiles sources can be used in wide range of industries under very extreme conditions of pH and temperature .Moreover, genetic engineering can be used in efficient way to improve the thermal stability and tolerance level of invertase in various industries by production of novel invertases can be increased through their heterologous expression in different sources.

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