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# Optimization of various encapsulation systems for efficient immobilization of actinobacterial glucose isomerase

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ABSTRACT

Glucose isomerase (GI) converts glucose into fructose by a reversible reaction. This reaction is industrially valuable for the production of high fructose corn syrup (HFCS) which is used as a sweetener by food and beverage industries. GI being an expensive enzyme necessitates its usage in immobilized form for increasing its reusability and economic viability. Global HFCS production accounts to 10 million tons which is highest among products yielded by immobilized process. Current study evaluates different encapsulation systems for immobilization of GI produced by *Streptomyces* sp. T.S.A.KP which was found to be *Streptomyces enissocaesilis* (Accession number **MN911386**). This is the first report of extracellular GI production by a strain of *Streptomyces enissocaesilis.* GI was immobilized using chitosan, alginate and agar to enhance its stability and conversion efficiency. The concentration of polymeric gel with cross linking agent was optimized to get ideal pore size for maximum GI activity. Immobilization on chitosan significantly increased ($P < 0.05$) the GI activity by 47.18%. Agar increased the GI activity by 19.7% and alginate exhibited 18.5% higher activity than soluble enzyme. Thermal stability was increased by all three processes but maximally enhanced by chitosan immobilization. Chitosan immobilized GI exhibited enhanced activity at acidic pH whereas agar immobilization enhanced GI activity in alkaline range. The chitosan-TPP immobilization proved better than other encapsulation systems. HFCS was produced using immobilized GI. High performance liquid chromatography (HPLC) analysis of HFCS revealed that it comprised of an equilibrium mixture of glucose and fructose.

## 1. Introduction

Glucose isomerase (E.C. 5.3.1.5) isomerizes glucose into fructose and xylose into xylulose. It is also referred as xylose isomerase as it has higher affinity for xylose but the conversion reaction of glucose into fructose carries greater commercial importance. Glucose isomerase (GI) is widely utilized in production of HFCS, a chief sweetener used in food and beverages. Higher solubility and sweetening capacity of fructose than glucose makes it an attractive sweetener at industrial level. HFCS is cheaper than glucose and also has lower calorific value. For decades, the conversion of glucose to fructose has been of high interest and great commercial value (Fatima and Javed, 2020). HFCS finds wide application in food (confectionary, baking and softdrinks) and pharmaceutical industries to impart sweet taste in the products. Annual global production of GI is around 10 million tons GI is an expensive enzyme, therefore, to make the isomerization process economically feasible its immobilization is necessary. The immobilization of enzyme can be carried out by adsorption to insoluble carrier, entrapment within polymeric supports, cross linking and covalent binding with supports (Tumturk et al., 2008). Among these different methods of enzyme immobilization entrapment process causes least damage to the enzyme structure, therefore this method was opted for the current study. GI Immobilization has been reported by different methods using GAMM support (Yu et al., 2011), silica xerogel (Perminova et al., 2009), Eupergit C 250L (Katchalski-Katzir and Kraemer, 2000) and non porous glass surface (Chopda et al., 2014). Since GI is widely used in food industry, its immobilization carrier must be non-toxic and economical (Wang et al., 2009). In current investigation, chitosan, alginate and agar were used for immobilization of GI. These three carriers were chosen as they are easily available, economic, non-toxic and biodegradable.

Production of GI can be traced back to initial report by Marshall and Kooi in 1957 using *Pseudomonas hydrophila.* Since then *Actinoplanes* sp., *Streptomyces* sp., *Bifidobacterium* sp., *Bacillus* sp. and *Lactobacillus* sp. have been reported for GI production. Actinobacteria are well known

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producers of GI. Newly isolated strain of *Streptomyces* sp. T.S.A.KP (GenBank accession no. **MN911386**) identified as *Streptomyces enissocaesilis* by 16S rRNA sequencing has been utilized for the production of extracellular GI. This is the first report on production of GI by *Streptomyces enissocaesilis*. In majority the production of GI by actinobacteria has been reported to be intracellular which is recovered by the disintegration of cells. This step increases overall cost of downstream processing of GI before it can be utilized for industrial purposes (Chopda et al., 2014). In the present study we propose, immobilization method and its optimization for the extracellular GI. Industrially, the implementation of immobilized biocatalyst is profitable as it provides better advantage over its soluble counterpart because of reusability and increased stabilization (Illeova and Polakovic, 2018). The polymeric support involved in immobilization processes provides stability and better specificity to the enzyme (Demirel et al., 2006).

The major application of GI includes the production of high fructose corn syrups (HFCS) which is an equilibrium mixture of glucose and fructose with high sweetening capacity (Fatima and Javed, 2020). Its production is carried out using non-sweet and cheap sources such as starch or cellulose (Khalilpour and Roostaazad, 2008). HFCS is produced in two steps involving breakdown of starch into glucose and its isomerization into fructose. Fructose is sweeter than sucrose and unlike glucose it is absorbed without the involvement of insulin which makes it suitable for consumption in diabetes (Yu et al., 2011). The aim of immobilizing the GI biocatalyst is to efficiently carry out its applications at economic expenditures. The immobilization supports used here are biodegradable, non-toxic and prevent denaturation of the enzyme. The GI in our study has been utilized in the production of HFCS after optimizing the immobilization parameters to evaluate it efficiency in industrial processes.

This study is the first report on extracellular GI immobilization produced by *Streptomyces enissocaesilis*. The immobilization process was optimized to achieve greater enzyme encapsulation and enhanced activity. Activity range of GI and reusability for varied applications at broad range of pH and temperature was also worked out. We could derive the process for higher isomerization with immobilized GI as compared to free GI for its economical usage.

## 2. Materials and methods

All the chemicals were purchased from HiMedia and Merck.

### 2.1. Immobilization of glucose isomerase

GI was produced using an indigenously isolated *Streptomyces* sp. T.S.A.KP (GenBank accession no. MN911386). Submerged fermentation process was carried out in modified Bennett broth (xylose 1%, tryptone 0.1%, yeast extract 0.1% and beef extract 0.1%, pH 8.0) by inoculating 8 mm disc of 96hrs old culture. The process was conducted at 30 °C and 100 rpm for 5 days. Fermentation process was terminated and the extracellular GI was harvested from the broth by centrifugation at 5600*g* for 10min in cooling centrifuge (REMI CIS-24BL). The supernatant obtained was used as a source of extracellular crude enzyme (Bhasin and Modi, 2012).

### 2.2. Immobilization of GI using alginate

GI was immobilized in calcium alginate. The concentration of sodium alginate and calcium chloride was optimized to obtain suitable pore size of calcium alginate beads which can stabilize the enzyme as well as increase the efficiency of isomerization. Sodium alginate was prepared in different concentration such as 1% (w/v), 2% (w/v), 3% (w/v) and 4% (w/v). Extracellular GI was added to the sodium alginate solution to prepare the slurry and it was added dropwise to 0.2M $CaCl_2$ solution. Further to optimize the $CaCl_2$ concentration, GI was mixed with 1% (w/v) sodium alginate and added dropwise to four different solutions of $CaCl_2$ prepared as 0.1M, 0.2M, 0.3M and 0.4M concentration. Curing of beads was done for 1h. The calcium alginate beads formed with immobilized GI were washed thrice with distilled water before determining the enzyme activity. The enzyme activity was calculated at each optimization step for the determination of best combination of sodium alginate and $CaCl_2$.

### 2.3. Immobilization of GI using chitosan

The chitosan flakes were added to 4% glacial acetic acid solution to prepare slurry. Chitosan was prepared in varied concentrations of 1%w/v, 2%w/v, 3%w/v and 4%w/v. The crude enzyme (2 ml) was added to the all the above chitosan solutions (50 ml) and mixed well. The slurry was added dropwise to 15% (w/v) sodium tripolyphosphate (TPP) solution. Beads formed by all four concentrations were analyzed for GI activity and the concentration giving highest activity was selected to optimize TPP concentration.GI was now mixed with optimized chitosan concentration and added dropwise to four different concentrations of TPP i.e. 5%w/v, 10%w/v, 15%w/v and 20%w/v. The spherical beads formed during the process were allowed to cure for 1hr. For secondary curing the beads were washed and added to 1% w/v glutaraldehyde solution (Ganaie et al., 2014). The curing was carried out for 1hr in shaking condition at room temperature. The beads were washed thrice using distilled water before use.

### 2.4. Immobilization of GI using agar

Agar (1%w/v, 2%w/v, 3%w/v and 4%w/v and 5%w/v) was dissolved in water by boiling which thereafter was cooled and maintained at 50 °C. Extracellular crude GI (2 ml) was mixed with agar solution (50 ml). Later the warm slurry was added dropwise into the solvent mixture containing ethylene dichloride (ED) and ethyl acetate (EA) in a ratio of 3:1. GI activity of the beads formed by different concentrations of agar was analyzed and the optimum concentration was selected to further optimize ED:EA ratio. ED:EA ratio used for optimization were 1:1, 2:1, 3:1, 4:1 and 5:1. The agar beads formed were allowed to cure for 1hr. Later, the beads were washed thrice using distilled water before checking the activity.

### 2.5. Determination of immobilized GI activity

The activity of immobilized GI was assessed by using glucose as the substrate and estimating the amount of fructose yield. The reaction mixture comprised of 500 µl of 0.2M sodium phosphate buffer, 200 µl of 1M glucose, 100 µl of 0.1M $MgSO_4{\cdot}7H_2O$, 100 µl of 0.01M $CoCl_2$, 200 µl of crude enzyme and the volume was made up to 2 ml. The reaction mixture was incubated at 70 °C for 1hr. The reaction was stopped by adding 2 ml of 0.5M perchloric acid. The amount of fructose produced was estimated by taking 0.05 ml of above reaction mixture to which 0.95 ml distilled water was added. Furthermore, 200 µl of 1.5% (w/v) cysteine hydrochloride followed by 6 ml 70% $H_2SO_4$ and 200 µl of 0.12% (w/v) alcoholic carbazole was added. The generated purple color was measured spectrophotometrically at 560 nm (Hlima et al., 2012). One unit of GI activity was defined as the amount of enzyme required to produce 1 µmol of fructose per ml per min under assay conditions (Liu et al., 2015).

### 2.6. Loading efficiency

The amount of GI loaded in the different microspheres were determined using equation 1 as described by Wang et al. (2006).

$$L.E. = \frac{P_t - P_e}{P_t} \times 100$$

where $P_t$ is the total protein and $P_e$ represents the amount of protein

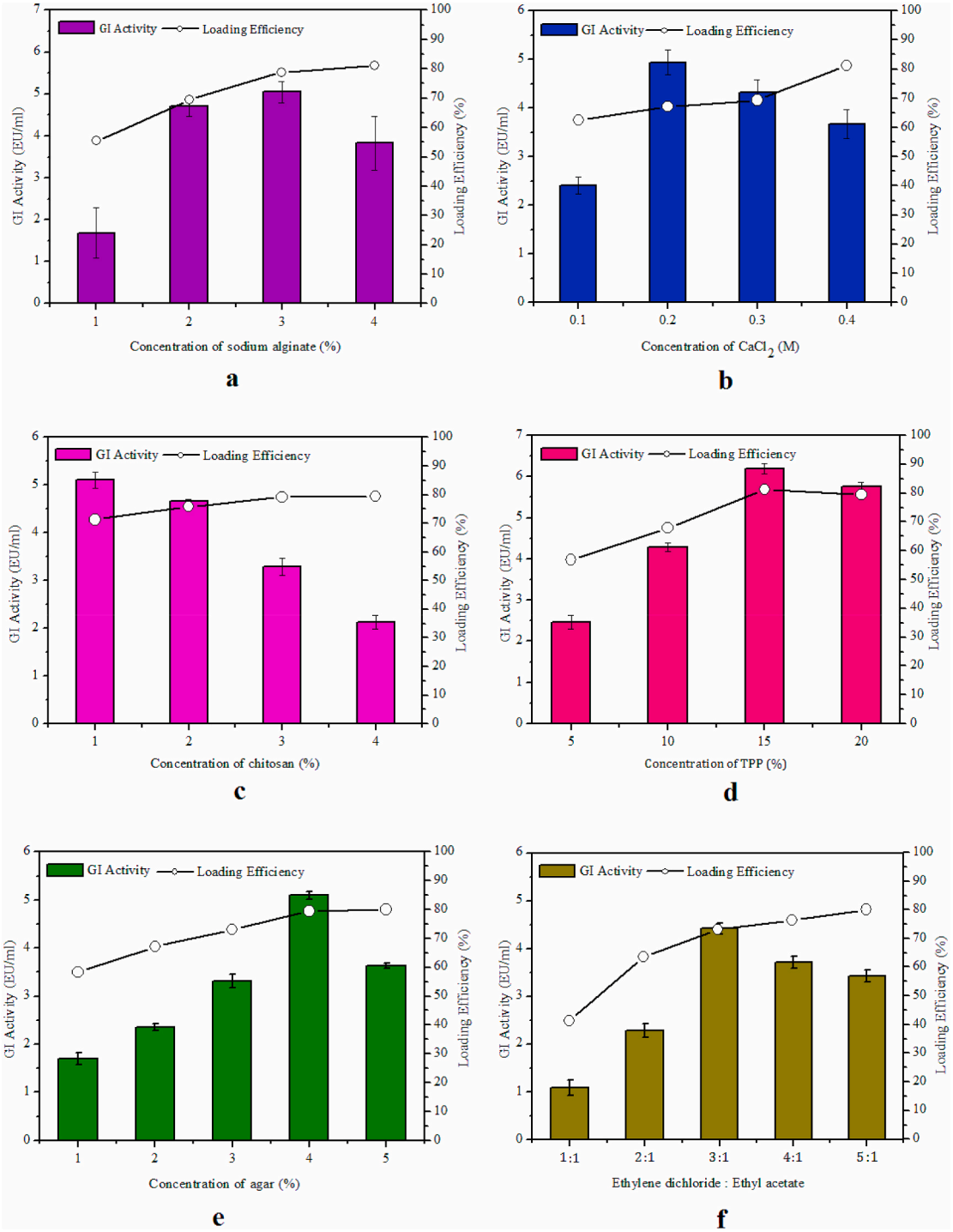


**Fig. 1.** **(a)** Effect of sodium alginate concentration on GI entrapment
**(b)** Effect of $CaCl_2$ concentration on GI entrapment
**(c)** Effect of chitosan concentration on GI entrapment
**(d)** Effect of TPP concentration on GI entrapment
**(e)** Effect of agar concentration on GI entrapment
**(f)** Effect of ethylene dichloride: ethyl acetate concentration on GI entrapment.

leached out in washings. The amount of protein in soluble enzyme and washings were determined using Lowry method. The absorbance was read at 680 nm.

### 2.7. *Effect of temperature and pH on immobilized enzyme*

Optimum temperature and pH for GI activity was determined. For determination of optimum temperature, isomerization was performed at a wide temperature range (50 °C, 60 °C, 70 °C, 80 °C, 90 °C and 100 °C) for free as well as immobilized GI. Favorable pH for GI activity was determined by carrying out the isomerization reaction in different buffers for a wide pH range. Acetate buffer was used for conducting the reaction at pH 4 and 5, phosphate buffer for pH 6 and 7, tris buffer for pH 8 and 9, glycine buffer for pH 10.

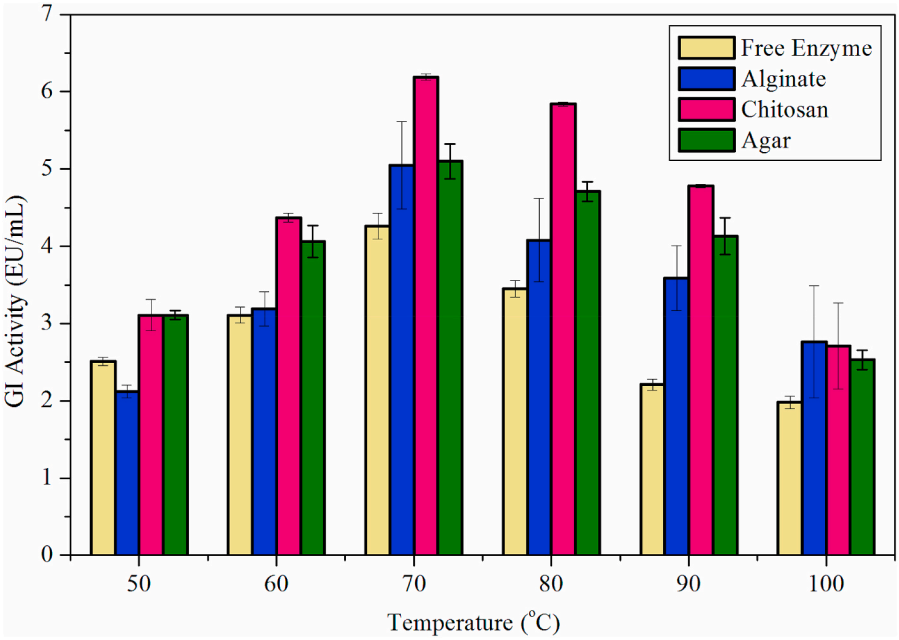


**Fig. 2.** Effect of temperature on GI activity.

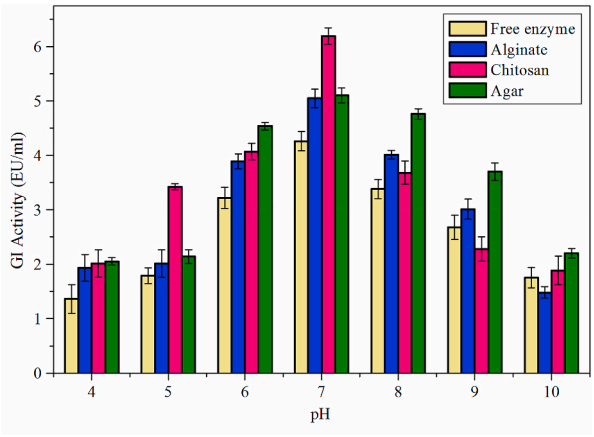


**Fig. 3.** Effect of pH on GI activity.

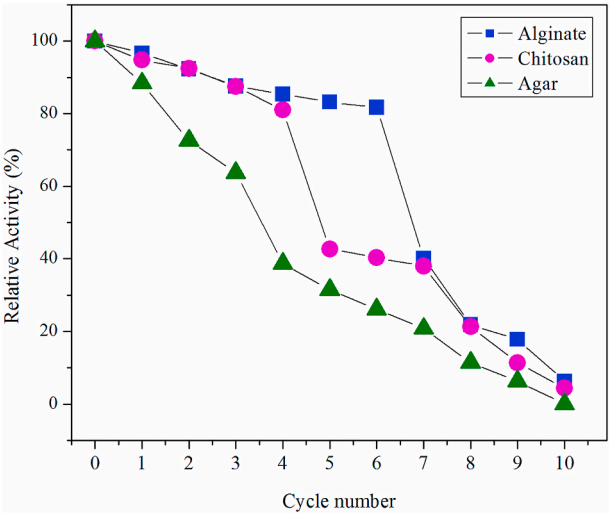


**Fig. 4.** Percentage change in GI activity after various cycles.

### 2.8. Reusability of immobilized GI

The enzyme immobilized using alginate, agar and chitosan was evaluated for its reusability. The immobilized GI was introduced to the assay mixture and incubated for 1h at 70 °C. The amount of fructose produced was analyzed using cysteine-carbazole method as described in section 2.5. The beads were recovered from mixture and washed thrice with distilled water for the next cycle. These washed beads were again added to fresh assay mixture and isomerization process was carried out for 1h at 70 °C. After second cycle washing with distilled water was repeated and third cycle of isomerization was performed. This process was carried out for 10 cycles. Production of fructose was estimated at the end of each cycle.

### 2.9. Production of high fructose corn syrup (HFCS) using immobilized GI

Production of HFCS was done in two steps.

### 2.10. Step I: Saccharification of corn starch

Saccharification of corn starch was performed by amylase produced by submerged fermentation process using *Streptomyces* sp. TSA.Dm1 (GenBank accession no. MN923073). Crude amylase extract was introduced into 1% (w/v) corn starch for saccharification. The hydrolysis of starch was carried out at 37 °C and the reduction in starch was estimated using starch-iodine complex method. The glucose units formed from starch hydrolysis was estimated through DNS method.

### 2.11. Step II: Isomerization of glucose into fructose

After complete hydrolysis; immobilized GI from *Streptomyces* sp. T.S. A.KP was added into the reaction mixture to isomerize glucose into fructose and amount of fructose units formed were estimated using cysteine-carbazole method.

### 2.12. End product analysis using HPLC

The HFCS produced by immobilizing GI using chitosan, alginate and agar was analyzed for the presence of glucose and fructose. The analysis of HFCS was done using HPLC, (Waters, USA) with 515 HPLC pump and

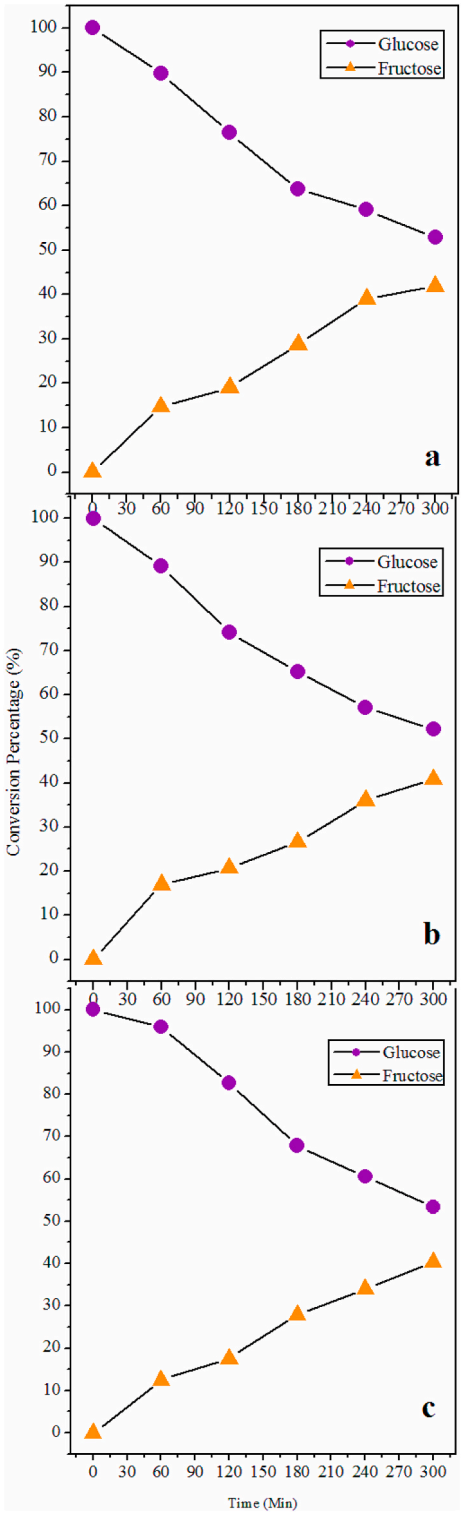


**Fig. 5.** (a) Percentage conversion of glucose into fructose using GI immobilized in chitosan
**(b)** Percentage conversion of glucose into fructose using GI immobilized in alginate
**(c)** Percentage conversion of glucose into fructose using GI immobilized in agar.

RI detector 2414. Sugar-Pak column of 6.5 × 300mm size was used for the identification of glucose and fructose. The injection volume was 20 µl and HPLC grade water was used as mobile phase with flow rate of 0.3 ml/min. The HFCS was filtered using 0.45µ PVDF syringe before introducing it into the HPLC system. The analysis was carried out using Empower 2 software. Most important precaution for this process was absence of suspended particles in the liquid i.e. HFCS. If the particles were observed it was filtered again using 0.45µ PVDF syringe to avoid blockage of flow system and damage of column.

### 2.13. Statistical analyses

All the experiments were carried out three times in triplicates and the results indicate the average of triplicate readings. The data in the tables and figures indicate mean ± standard deviation. Using one-way ANOVA, the difference was considered statistically significant if the P value was less than 0.05.

## 3. Results and discussion

The GI produced from *Streptomyces* sp. T.S.A.KP was immobilized using alginate, chitosan and agar supports. The concentration of polymeric support and cross-linking agent was standardized for best GI activity. A comparative analysis of all the three immobilization methods was done to find out the best one showing high GI activity. All the three methods involved in immobilization enhanced the activity of GI but to different extents.

### 3.1. Immobilization of GI in alginate

The immobilization parameters were optimized by varying the concentration of sodium alginate and $CaCl_2$ to get best activity. Alginate increased GI activity by 18.5% on immobilization. The free enzyme activity was 4.26 ± 0.165 EU/ml. immobilized GI activity obtained at 3% (w/v) sodium alginate and 0.2M $CaCl_2$ solution was 5.05 ± 0.154 EU/ml. Fig. 1a and Fig. 1b shows GI activity at various concentration combinations of sodium alginate and calcium chloride. The lowest GI activity of 1.69 ± 0.6 EU/ml was observed in case of 1% sodium alginate and 0.2M $CaCl_2$. Alginate is a widely used immobilization support in enzyme immobilization. Alginate forms a hydrogel in presence of $Ca^{2+}$ which acts as a crosslinker between the functional group of alginate chains. The GI activity increased with the increasing concentration of alginate. The GI activity was observed to be 1.69 ± 0.6, 4.7 ± 0.24 and 5.05 ± 0.154 EU/ml with the increasing concentration of 1%, 2% and 3% alginate respectively. The interaction of alginate with the cationic solution ($CaCl_2$) regulates the pore size and stability of formed beads which affects enzymatic interaction with substrate. This explains the increase in GI activity with increase in sodium alginate concentration. Further increase in sodium alginate concentration to 4% decreased the GI activity to 3.83 ± 0.64 indicating the steric hindrance between the substrate and active site of GI causing reduced isomerization activity. Furthermore the increase in concentration of sodium alginate reduces the pore size of the bead causing obstacle in the enzyme interaction with that of substrate (Jobanputra et al., 2011). The initial increase in GI activity with increase in loading efficiency in this study and then a sudden drop in the activity at 4% alginate concentration but increased enzyme content in beads further supports the basis of reduced accessibility of enzyme to the substrate. The concentration of cross-linking agent also regulates the pore size of the microsphere. Its higher concentration reduces the pore size causing difficulty in the diffusion of substrate and product in and out of the bead (Ganaie et al., 2014). This is evident from our results that increase in $CaCl_2$ concentration increases the loading efficiency, but decreases the enzyme activity.

### 3.2. Immobilization of GI in chitosan

Chitosan immobilized GI exhibited highest enhancement in isomerization efficiency. Chitosan is *N*-deacetylated derivative of polysaccharide chitin and is the second most abundant polysaccharide on earth. The chitosan beads were formed by the ionic crosslinking between cationic chitosan and anionic TPP (Ganaie et al., 2014). A significant

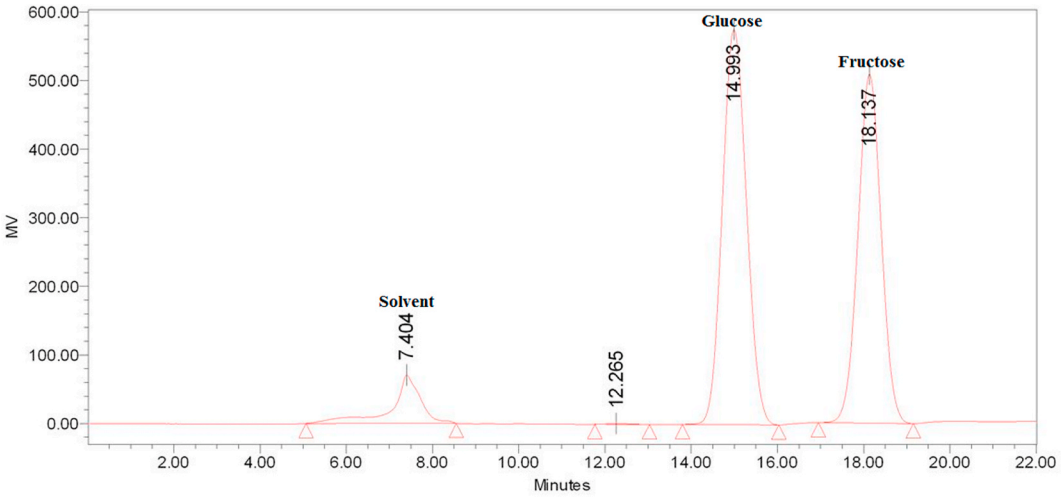


**Fig. 6.** HPLC chromatogram of HFCS produced using GI immobilized in chitosan.

increase of 47.18% ($P < 0.05$) in enzyme activity was observed when 1% (w/v) chitosan was added to 15% TPP, (Fig. 1c and 1d). The loading efficiency increased on raising the chitosan concentration but the GI activity reduced which might be due to the reduced accessibility of GI molecules to the substrate. The combination of 4% chitosan with 15% TPP yielded least activity of 2.13 ± 0.14EU/ml as retention of enzyme in the bead might be less. Optimum concentration of TPP was found to be 15%. Chitosan proves itself as the best immobilization support in the study. It provided better stability and significantly augmented the properties of the enzyme. Xu et al. (2014) also observed altered properties of GI and better stability at higher temperatures when immobilized using chitosan. These altered properties after immobilization can enhance or reduce the enzyme functionality which necessitates the optimization of immobilization parameters. The alteration in the property of biocatalyst depends upon its interaction with the support. In the current study a significant increase of 47.18% of enzyme activity was observed when immobilization was optimized using chitosan-TPP support. The optimum substrate-GI interaction was observed when 15% TPP was added to 1% chitosan. Lower TPP concentration creates large pores to the chitosan microsphere causing leakage of the biocatalyst. On the other hand tiny pores developed due to 20% (w/v) do not permit proper interaction between the substrate and the enzyme leading to reduced enzyme activity.

### 3.3. Immobilization of GI in agar

The enzyme entrapped using agar exhibited higher enzyme activity than free enzyme. The gelling ability, acid stability and non reactivity towards proteins make agar a suitable immobilization support. The immobilization parameters were standardized by varying concentrations of agar and cross-linking agent to get best activity. Fig. 1e and 1f shows that the highest enzyme activity of 5.10 ± 0.083 EU/ml was attained with combination of 4% (w/v) agar and 3:1 ED:EA solvent ratio. The agar forms a porous matrix on solidification with ethyl acetate and ethylene dichloride. This porosity depends on the concentration of agar which needs to be optimized for efficient immobilization of enzyme. In this study, it was observed that the maximum GI activity was at 4% agar concentration. Similar observations were reported by Rehman et al. (2014). The efficient immobilization not only depends on the concentration of agar but also on molecular weight of the enzyme (Rehman et al., 2014). Low concentration of agar i.e. 1%, 2% and 3% must have yielded larger pores leading to enzyme leakage thus enzyme activity was observed to be 1.7 ± 0.12EU/ml, 2.36 ± 0.07EU/ml and 3.31 ± 0.14EU/ml respectively. Effectivity of immobilized beads increased with increased agar concentration which is also supported by the increasing loading efficiency of the enzyme. On increasing the agar concentration to 5% the loading efficiency of the GI increased but the activity reduced which might be due to hindrance in interaction of active sites of enzyme with that of substrate. The agar gets solidified too quickly when its concentration was further raised to 6%. Minimum GI activity of 1.09 ± 0.162 EU/ml was observed when ED:EA ratio was 1:1. The beads formed with lower concentration of ED lacked hardness which suggests that the cross-linking lacked uniformity. On increasing EA in the solvent mixture, the beads lose their spherical conformation.

### 3.4. Effect of temperature on immobilized enzyme

As evident from Fig. 2 for the free and immobilized enzyme the optimum temperature was found to be 70 °C. The chitosan, alginate and agar bound enzyme exhibited higher GI activity than the free enzyme. A significant increase ($P < 0.05$) of 47.18% was observed in GI activity when it was immobilized with chitosan (Fig. 2). Isomerization efficiency of GI was increased by 18.5% and 19.7% when entrapped in alginate and agar respectively. Apart from this, the temperature range for isomerization was also increased which is an extremely significant outcome because isomerization processes need high temperature. This thermal stability was attained due to interaction of GI with the chitosan microsphere. The multi-interaction between the enzyme and solid carrier support can cause change in secondary structure of the biocatalyst (Sethuraman et al., 2004). These changes usually result in gaining β-sheet structure and losing α-helical conformation which is responsible for better stability and functioning of enzyme or even for its denaturation (Sethuraman et al., 2004; Kaneko et al., 2000). The enzyme immobilized using chitosan and agar exhibited better activity at higher temperature range of 80 °C and 90 °C. Drastic reduction of 23.4% in enzyme activity of free enzyme was observed when it was exposed to 80 °C but chitosan carrier act as a protection and immobilized GI exhibited only a marginal decrease of 5.3% activity.

The isomerization of glucose into fructose requires higher temperature and the polymeric supports provided better operational stability to the enzyme and enhance its range of catalysis even at high temperature. GI is available in the market since 1975 and has been worked upon for increase in enzyme activity but the thermal stability is not significantly enhanced (Illeova and Polakovik, 2018). In the present study we observed that thermal stability of GI was enhanced after immobilization using chitosan and alginate support. This is encouraging result as the GI working at high temperatures provides an additional advantage that there is no byproduct formation and also the viscosity of the substrate is reduced (Jia et al., 2018). The isomerization reaction at high temperature causes unfolding of enzyme polypeptide chains and thiol oxidation

of cysteine residues, also known as Maillard-like reaction, which lead to drastic reduction in enzyme activity. Source of the enzyme also contributes to the efficiency at a given temperature which is accounted by variability in their structure. Difference in the GI structure may leads to varying half lives of GI at operational temperatures. GI of varying molecular weights and structures from microbial sources has been reported by researchers (Mu et al., 2012). Increased stability of GI produced by *Streptomyces enissocaesilis* after immobilization proves its potential of high applicability. Thus, this strategy which has increased the GI activity by 47.18% and elaborated the working range of temperature till 90 °C shall prove promising at industrial level.

### 3.5. Effect of pH on immobilized GI

At pH 7.0 the free enzyme showed maximum enzyme activity of 4.26 ± 0.165 EU/ml which was significantly increased by 47.18% when GI was immobilized using chitosan. The agar and alginate bound GI also exhibited enhancement in enzyme activity by 19.7% and 18.5% respectively (Fig. 3). Immobilized GI displayed higher operational stability in a wide pH range.GI exhibited better stability and wider pH range for functioning after immobilization. In present study, it was observed that immobilization system improved the GI stability in acidic as well as alkaline pH range. The free GI activity at pH 5.0 was 1.72EU/ml, which increased 91.06%, when immobilized using chitosan. The glutaraldehyde treatment of chitosan beads must have imparted additional operational stability at the acidic pH (Cetinus and Oztop, 2006). There are very few reports on active GI working at acidic pH. Our study shows that chitosan immobilized GI can cater the Industrial applications requiring acidic pH optima. These findings are valuable as acidic pH prohibits the production of by-products like psycose and mannose (Neifar et al., 2019). Xu et al. (2014) also reported efficient working of GI at acidic pH after immobilizing it using chitosan-PAA support. Similarly, GI immobilized using agar was more stable at alkaline pH range of 8.0 and 9.0 when compared with free enzyme. This method can be suggested for industrial operations at alkaline pH. It is evident from this study that GI produced by *Streptomyces* sp. T.S.A.KP can be applied efficiently in alkaline as well as acidic pH range after immobilization. The present study indicates that the immobilized GI is more resistant towards the pH change. The chitosan bound GI showed stability at acidic pH of 5.0 and its activity was almost similar to that of free enzyme in neutral pH. Jin et al. (2017) also observed stability of GI at pH 5.0 when it was immobilized using glutaraldehyde, ethylenediamine and diatomaceous earth. GI immobilized using agar remained stable at alkaline pH of 8.0 and 9.0 and exhibited much higher activity than other supports. It retained more than 70% activity at pH 9.0. From this observation it is proposed that immobilization affects the catalytic properties of GI making it more stable and efficient at wide range of pH.

### 3.6. Reusability of entrapped GI

The immobilized system was evaluated for 10 continuous cycles of isomerization. The different immobilized systems exhibited variable reusability of the immobilized enzyme. In industrial application, the reusability of immobilized enzyme is a crucial aspect. An enhancement in stability after immobilization can make the enzyme more valuable than its free form. The isomerization of glucose into fructose is carried out at high temperature therefore, immobilization support should be able to resist the high temperature for multiple times to ensure better reusability. The chitosan beads retained 81% activity till 5th cycle after which a subsequent decrease in enzyme activity was observed. The loss of enzyme from the carrier might be a reason for this. Another reason for sudden decline in activity might be the loss in interaction of enzyme with the carrier support (Tumturk et al., 2008). The maximum reusability was observed in alginate bound enzyme which retained 81.7% of enzyme activity up to 6 cycles (Fig. 4). Rehman et al. (2014) reported retained enzyme activity of GI immobilized in alginate to be 70% after 8 cycles. In present study, the enzyme loss after each cycle was least in alginate support. The retained enzyme activity of 81.7% even after 6th cycle is appreciable as industrially it makes the isomerization process economic without compromising with the efficiency of the process. Demirel et al. (2006) reported 72% of retained enzyme activity when GI was immobilized with polyacrlamide/alginate.GI entrapped using agar retained 63.7% of the original activity till the third cycle after which steep decrease was observed. The reusability of immobilized enzyme ensures economic biocatalysis. If a biocatalyst can be recycled for 6 times for a given reaction, it certainly reduces the cost of catalysis by 6 times. The present study ensures economic usability of GI for carrying out isomerization process.

### 3.7. Production of HFCS using immobilized GI

The hydrolysis of 90.9% was attained using amylase from *Streptomyces* sp.TSA.Dm1. An isomerization of 41.08%, 42.9% and 40.3%was achieved using alginate, chitosan and agar immobilized GI respectively (Fig. 5a, Fig. 5b and 5c). HFCS commercially is available in two forms HFCS-42 and HFCS-55 which is characterized by the amount of fructose each contains, either 42% or 55%, respectively (Singh et al., 2014). The present study suggests that GI from *Streptomyces* sp. TSA.KP after immobilization is most suitable for production of HFCS 42. Chitosan bound GI exhibited highest isomerization of glucose into fructose but it was not significantly different from alginate and agar entrapped GI which also showed appreciable isomerization. HFCS production carried out using chitosan, alginate and agar exhibited appreciable increase in isomerization in the present study. Kaneko et al. (2000) has also reported 40% conversion of glucose into fructose using purified GI.

### 3.8. End product analysis using HPLC

The HFCS produced by the isomerization of glucose into fructose using GI immobilized through chitosan, alginate and agar was analyzed using high performance liquid chromatography (HPLC). This technique has been found to be very effective in the detection and separation of sugars. In the current study, the HPLC chromatogram revealed that the HFCS comprised of an equilibrium mixture of glucose and fructose (Fig. 6).The immobilized GI isomerized the glucose syrup into HFCS by developing an equilibrium mixture of glucose and fructose. The chitosan bound GI exhibited the maximum isomerization of 42.9% when compared with alginate and agar carriers. The retention time of glucose was observed to be ~15min and that of fructose to be ~18 min at the flow rate of 0.3 ml/min of solvent. For the production of HFCS at industrial scale, efficient immobilization process is obligatory. The HPLC chromatogram revealed the presence of glucose and fructose in the HFCS. The sharpness in the shape of the peak obtained in our result indicates the elution of single molecule at given retention time as also explained by Herpai et al. (2013). This suggests that the HFCS produced is free from other impurities. During our analysis, a clean separation and baseline resolution was attained for the detection of sugars i.e. glucose and fructose. The HPLC analysis confirmed that our steps for the production of HFCS are effective. The hydrolysis of starch carried out using amylase from *Streptomyces* sp. was maximally saccharified into glucose as no significant amount of starch was detected in the end product analysis.

## 4. Conclusion

In the current investigation GI from *Streptomyces* sp. T.S.A.KP was successfully immobilized using chitosan, alginate and agar support. As hypothesized, the immobilization altered the properties of the enzyme by providing it better stability and functionality. The immobilized biocatalyst was efficient in production of HFCS which is its major industrial purpose. Significant finding of the investigation is highly increased stability and catalytic efficiency of GI (47.18%) immobilized in chitosan

with TPP. The combination of chitosan and TPP for immobilization of GI using a *Streptomycete* strain identified as *Streptomyces enissocaesilis* is reported for the first time to best of our knowledge. The study also summarizes that immobilized GI has exhibited augmentation in characteristics which opens ways for its industrial applications. Further implications of the study are highly application oriented which suggest customized use of GI by *Streptomyces* sp. T.S.A.KP. Classified applications of this improvement study make chitosan immobilized GI highly suitable for isomerization process at acidic pH and high temperature. This immobilized GI has potential of carrying out operations at temperature range higher than that of optimum temperature of commonly available GI. Furthermore, to cater isomerization procedures at alkaline pH, we propose GI to be immobilized using agar for better stability and functioning. The alginate bound GI can be reused for catering isomerization process multiple times. Thus, the study proposes that immobilized GI is efficient in carrying out isomerization at different parameters based on the industrial requirements. The immobilization can enhance the efficiency of GI making it perfectly suitable for industrial applications.

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## CRediT authorship contribution statement

**Tanim Arpit Singh:** Writing - original draft, Methodology. **Anjana Jajoo:** Supervision, Validation, Writing - review & editing. **Sheetal Bhasin:** Supervision, Conceptualization, Writing - review & editing.

## Declaration of competing interest

The authors declare that they have no competing interests.

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