ISSN NO. 2320–7418 DOI: 10.22270/jmpas.V10I4.1387

International open access journal

Journal of Medical Pharmaceutical and Allied Sciences

Journal homepage: www.jmpas.com

Research article

Kinetic study comparison of immobilized glucose isomerase (gensweet and sweetzyme it) in stirred tank reactor and packed bed reactor

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ABSTRACT

D- Glucose/xylose isomerase catalysis the reversible isomerization of aldoses to ketoses such as D-glucose and D-xylose to D-fructose and D-xylose respectively. High fructose corn syrup (HFCS), a low calorie sugar substitute for cane sugar, utilizes Glucose isomerase enzyme for conversion of glucose to fructose. The conversion of glucose to fructose favors more at high temperature, providing an incentive to utilize thermostable and thermoactive glucose isomerase in High fructose corn syrup (HFCS) production. Present studies emphasize on enzymatic conversion and optimization using Sweetzyme IT extra & Gensweet, commercially available glucose isomerases. The experiments were carried out for enzymatic conversion of glucose to fructose using Gensweet and Sweetzyme in Packed bed reactor (PBR) and Stirred tank reactor (STR). Maximum conversion was seen in Stirred tank reactor (STR) using both of these enzymes, approx 10 % more Fructose conversion comparing it to packed bed reactor (PBR). Also, Stirred tank reactor (STR) reaction conditions such as pH, buffers, cofactor (MgSO4) requirement were optimized to achieve optimum enzyme activity. Analysis of enzymatic conversion samples was done using HPLC-RID (using Zorbax Column). The importance of the divalent cation MgCl2 for optimal enzyme activity was investigated. The enzyme performed best at pH 7.5 and 60°C, using 10mM MgSO4 as a cofactor. Utilizing Gensweet in Stirred tank reactor (STR), the maximum fructose transformation was 44 %. The most activity was detected with Sodium phosphate buffers, and EPPS buffers at pH 7 and 8, accordingly, whereas the least activity was reported with TRIS HCl buffer.

Keywords: High fructose corn syrup, Stirred tank reactor, packed bed reactor, Optimization, Sodium phosphate, EPPS buffers and TRIS HCl buffer.

Received - 16/06/2021, Reviewed - 10/07/2021, Revised/ Accepted- 28/07/2021

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INTRODUCTION

Glucose isomerase has been manufactured worldwide for enzymatic conversion of glucose to fructose.[1,2] It catalysis the isomerization of substrates having hydroxyl groups at carbons 3 and 4 in equatorial position and hence this enzyme can utilize substrate like D-Ribose, L-arabinose, and L- Xylose.[3] For its activity and stability it requires divalent cations. It exists as a tetramer or dimer of identical 45-50KDa subunits. GI is widely distributed in prokaryotes, after its discovery in Pseudomonas hydrophila, a large number of bacteria and actinomycetes were found to produce GI Novo's new Glucose isomerase, Sweetzyme is produced from a specially selected strain of Bacillus coagulans and immobilized according to Novo's patented method.[4,5] Method of immobilization is cross linking of cell material with glutaraldehyde. Another commercially available glucose isomerase is Gensweet, which is manufactured by Genencor International; the enzyme source is Streptomyces rubigonosis and the enzyme is cross linked with or without cellular debris using (PEI) poly ethylene imine & glutaraldehyde. GI require Mg2+, Mn2+ or CO2+ for activity and stability [6, 7]. Its mechanism of action is to bind, α-d-Pyranose form of substrate and isomerizes by 1, 2- hydride shift mechanism [8].

MATERIALS AND METHODS

Materials: Glucose, Glucose Isomerase (Gensweet & Sweetzyme IT extra), GE- XK 16/40 Column, Filtration unit, Whatmann paper 1.2µ

filter membrane, magnesium sulfate, sodium carbonate, Tris HCl, EPPS, Sodium phosphate etc.

Enzymatic conversion using Immobilized-Glucose isomerase in Packed Bed Reactor (PBR)

For biocatalysts, packed bed reactors, also known as fixed bed reactors, are frequently utilized. They are easy to design; more products are generated as a result of greater reactant/catalyst interaction; they are inexpensive to build, operate, and maintain; and they are also efficient at high temperatures and pressures.

Repeated batch conversion using Sweetzyme in PBR

45% Glucose (w/v) (substrate) was weighed, dissolved and its pH was adjusted to 7.5 using sodium carbonate. Later, it was filtered using What Mann filter paper followed by 1.2µ filter membrane. Temperature was maintained at 60°C using water bath throughout the process. Im-GI (Sweetzyme) was washed with several volumes of deionized water until fine particles were removed from it and packed into the XK-16/40 Column. To avoid air gaps, packing were done in access deionized water. Before starting the conversion the substrate and the enzyme were equilibrated at 60°C temperature. The repeated batch cycle's reaction was carried out at 10ml/min flow rate, with bottom to top flow pattern. Before proceeding with next cycle, three column volumes of collected fructose-glucose mixture was passed through the Sweetzyme column at same flow rate to equilibrate. After completion of every cycle 1ml samples were collected in sterile Eppendorf's tubes & given for HPLC-RID analysis.

Time dependent conversion kinetics of Sweetzyme IT extra in Stirred Tank Reactor (STR)

Time dependent conversion kinetics was done to determine the reaction velocity of immobilized Sweetzyme in STR. 50 ml of substrate was prepared using 15% & 45% of Glucose. Later 10mM. MgSO4 was added and pH was adjusted to 7.5 using Sodium carbonate. The reaction mixture was filtered using Whatt Mann filter paper followed by 1.2µ filter membrane and was kept for equilibration at 60°C water bath for 10 mins at 400 rpm. After equilibrating, 1g of Sweetzyme was added in both substrates and the reaction was commenced at 400 rpm. The reaction was continued for 90 and 180 mins for 15% and 45% glucose (w/v) respectively. After every 10 minutes 200µl of sample was collected and given for HPLC-RID analysis.

METHODS

Effect of superficial velocity on fructose isomerization using Gensweet in PBR

Flow rate determination

Different flow rates were checked to determine the effect of flow rate on isomerization of glucose to fructose in packed bed reactor using Gensweet. 45%. Glucose (w/v) (substrate) was weighed, dissolved and its pH was adjusted to 7.5 using sodium carbonate. Later, it was filtered using What Mann filter paper followed by 1.2µ filter membrane. Gensweet was washed with several volumes of deionized water until fine particles were removed from it and packed into the XK-16/40 Column. The column temperature was maintained at 60°C using submergible pump. The flow rates checked were 25, 20. 15, 10, 7.5, 5, 2.5, 1.5, 0.6 ml/min from bottom to top flow pattern. Before proceeding to another flow rate, the column was equilibrated with three column volumes of substrate at desired flow rate. The samples of the above mentioned flow rate were collected and analysis was done using HPLC-RID.

Repeated batch conversion using Gensweet in PBR

7.5 ml/min flow rate was selected on the basis of fructose conversion and reaction was continued for 11 cycles. After completion of each cycles, 1 ml samples was collected and given for HPLC- RID analysis.

Time dependent conversion kinetics of Gensweet in STR

The reaction velocity of Gensweet in stirred tank reactor was done using the following reaction conditions: 50 ml of reaction mixture was prepared using 15% Glucose (w/v). Later 10mM MgSO4 was added and pH was adjusted to 7.5 using Sodium carbonate. The reaction mixture was filtered using Whatt Mann filter paper followed by 1.2µ filter membrane and was kept for equilibration at 60°C water bath for 10 mins at 400 rpm. After equilibration, 2g of Gensweet enzyme was added in substrates and the reaction was commenced at 400 rpm. The reaction was continued for 180 mins. After every 15 minutes 200µl of sample was collected and given for HPLC-RID analysis.

PH optimization of Gensweet enzyme

To determine the pH and buffer at which optimum enzyme activity will be seen, various buffer such as sodium phosphate and Tris HCl, EPPS at pH 6, 6.5,7 and 7, 7.5 and 8.0 respectively was screened. 20% Glucose was used as substrate, 10mM MgSO4 as a cofactor and 400 rpm at 60°C temperature. The reaction mixture was filtered with Whatt Mann filter followed by 1.2µ filter membrane and was equilibrated at 60°C for 10 mins, before adding enzyme. 1g of Gensweet was used for each reaction, followed by equilibration with respective buffers of 50mM strength until the desired pH was achieved. After equilibration, reaction was continued for 30 mins. Enzyme deactivation was done at 95°C for 10 mins. Samples were analyzed using HPLC-RID.

RESULTS AND DISCUSSION

Repeated batch conversion using Sweetzyme in packed bed reactor (PBR):

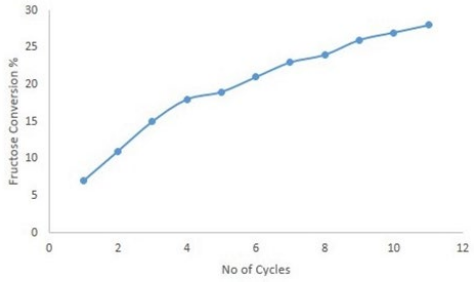
Enzymatic conversion of Glucose to fructose was done using Sweetzyme in PBR:

Bioconversion of Glucose to Fructose using Sweetzyme IT extra in PBR was done at 10 ml/min flow rate using 45% Glucose, 10mM MgSO4; Sweetzyme (bed volume 20 ml) packed in XK 16/40 Column, pH: 7.5 adjusted using Sodium carbonate salt at 60°C. The result was shown in table 1 and figure 1

Table: 1: Fructose production in different cycle

Cycle	Fructose %
1	7
2	11
3	15
4	18
5	19
6	21
7	23
8	24
9	26
10	27
11	28

Figure 1: Fructose production % in different cycle



Observation

From the above data it shows that the doubling of conversion is seen only first two cycles after that the conversion of glucose to fructose has reduced to 1% or 2%. It was expected to increase ~4% with every cycles, but this has not happened. It may be due to decline in enzyme activity or substrate limitation. It may be due to decline in enzyme activity or substrate limitation.

Time dependent conversion kinetics of Sweetzyme IT extra in Stirred Tank Reactor (STR)

To determine the reaction velocity of immobilized Sweetzyme in stirred tank reactor, following reaction was done in 50 ml reaction volume using 1g of enzyme (dry weight), 15% and 45 % Glucose, 10 mM MgSO4, pH adjusted to 7.5 using sodium carbonate at 60 °C at 400 rpm and was continue for 90 min and 180 min respectively.

Observation

Maximum 40% fructose conversion was obtained using different substrate amounts. Using 15%, after 70 mins reaction rates declines. This may be due to substrate limitation. Thus, reaction was carried using 45% glucose keeping rest of condition same for 180 mins. No change in fructose conversion was seen viz., maximum ~40% fructose conversion. This may be due to non-accessibility of substrate to enzyme due to higher viscosity of substrate and lower agitation rate.

The same set of reaction should have been done with increase agitation rate to achieve higher fructose conversion. Increased fructose conversion was obtained in STR compare to PBR. Thus reaction conditions such as buffer pH, Substrate concentration, temperature etc. need to optimized to obtained higher conversion.

Effect of superficial velocity on fructose isomerization using Gensweet in PBR

Enzymatic conversion of Glucose to fructose was done using Gensweet in packed bed reactor.

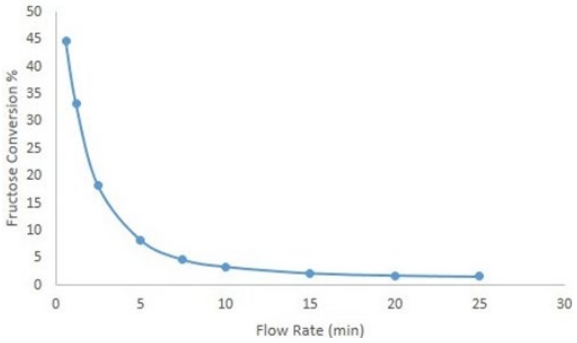
Flow rate determination

Different flow rates were set to determine the effect of flow rate on isomerization of glucose to fructose in packed bed reactor using Gensweet .The following were the reaction condition for the same. The result was shown in table 2 and figure 2.

Table 2: Effect of flow rate on Fructose production

Flow rate (ml/min)	Fructose Conversion (%)	Productivity (g/min)	Pressure (Mpa)
25	1.6	0.174	0.52
20	1.8	0.158	0.44
15	2.2	0.149	0.39
10	3.4	0.151	0.24
7.5	4.7	0.159	**0.2**
5	8.3	0.186	0.16
2.5	18.2	0.205	0.06
1.2	33.2	0.179	0.04
0.6	44.6	0.12	0.02

Figure 2: Effect of flow rate on fructose production

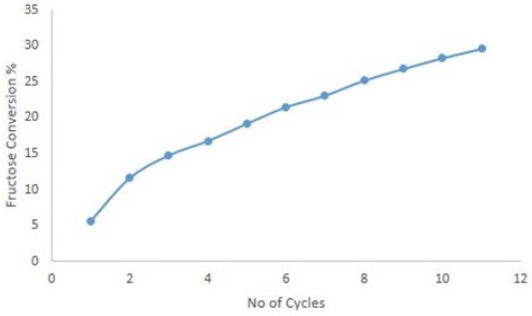


With the increase in flow rate, conversion decreases. This difference in conversion is because of the residence time; at higher flow rate enzyme was getting less time to interact with substrate hence the conversion was less. 7.5 ml/min flow rate was selected on the basis of fructose conversion, productivity and pressure drop. Stability studies of Gensweet were done using 7.5 ml/min flow rate. The result was shown in table 3 and figure 3.

Table 3 Repeated batch conversion using Gensweet in PBR (2nd batch)

Cycle	Fructose %
1	5.6
2	11.6
3	14.8
4	16.8
5	19.2
6	21.5
7	23.1
8	25.2
9	26.8
10	28.3
11	29.6

Figure 3: Fructose production in different cycle (2nd batch)



Maximum 30% of fructose was obtained after completion of 11 cycles. The doubling of conversion is seen only in first two cycles, after that the conversion of glucose to fructose has reduced to 1% or 2%. It indicates reaction progresses very slowly after initial two cycles. Decrease in conversion rate is may be due to decrease enzyme activity with increase fructose conversion. STR reaction will be performed to determine maximum conversion of fructose.

Table 4: Fructose production in different times

Time (min)	Fructose (%)	IU/g
0	0	0
15	2	33.1
30	7	50.7
45	13	59.9
60	18	64.1
75	24	66
90	29	66.4
105	32	63.9
120	36	62
135	39	60
150	41	56.4
165	43	54.4
180	44	51.3

Figure 4: Fructose production in different time

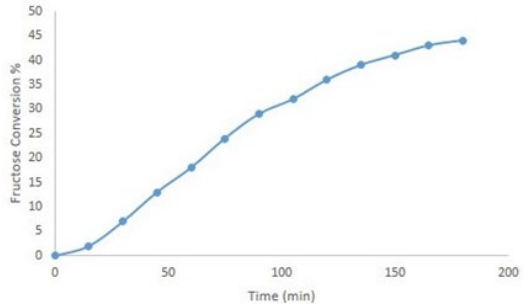
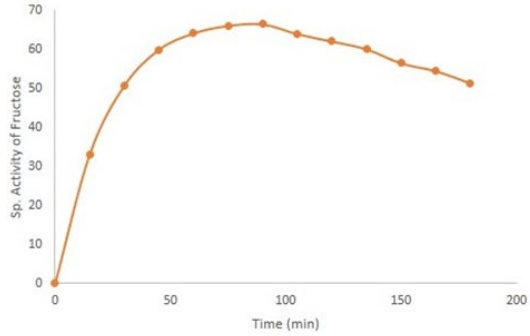


Figure 5: Effect of fructose production on specific activity of enzyme



Maximum 44% fructose conversion was obtained. It was observed that after 90 minutes reaction was progressing slowly. From HPLC analysis it was observed that evaporation occurred during the reaction. The reaction is still incomplete, as it has not reached its saturation point. After 90 minutes of reaction the specific activity reduced. The reaction time has to be increase or the amount of enzyme has to be decrease to attain the saturation point. The result was shown in table 4 and figures 4 and 5.

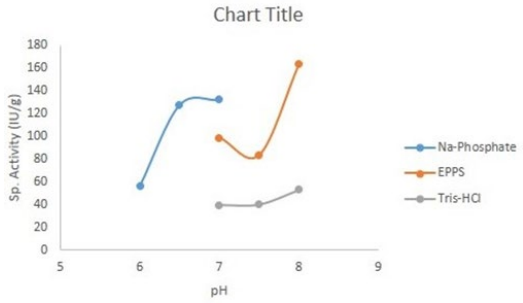
PH optimization of Gensweet enzyme

To determine the optimum pH for Glucose isomerase (Gensweet) at 60°C using 50mM Sodium phosphate, TRIS, EPPS buffers. The result was shown in table 1.5 and figure 1.6.

Table 5: Effect of pH on fructose production

BUFFER	pH	Time (min)	IU/g	Time (min)	IU/g
Na-Phosphate	6	15 min	56.4	30 min	77.9
	6.5		127.7		140.2
	7		132.3		148.5
EPPS	7		98.9		107.9
	7.5		83.5		126.7
	8		164.2		188.4
Tris-HCl	7		39.6		65.9
	7.5		40.1		65.5
	8		53.4		96.8

Figure 6: Effect of different buffer on fructose production



Maximum activity was obtained using 50 mM EPPS buffer & least activity was obtained using TRIS buffer. Frothing was not seen in any reaction. No buffer interference was observed in HPLC analysis.

CONCLUSION

During these studies, requirement of divalent cation, MgCl2, for optimum enzyme activity was examined. Enzyme showed optimum activity at pH 7.5 at 60˚C temperature, along with 10mM MgSO4 as a co-factor. Maximum fructose conversion obtained was 44% using Gensweet in STR. pH optimization using various buffers showed maximum activity with Sodium phosphate and EPPS buffer at pH 7 and 8 respectively, least activity was seen with TRIS HCl buffer. The ideal GI would have a neutral pH optimum, a higher temperature optimum, be resistant to Ca2+ inhibition, and have a higher affinity for glucose than currently available enzymes. The effort of incorporating all of these features into a single protein is Herculean, which has hampered the development of a commercially viable enzymatic isomerization of glucose to fructose method. Advances in recombinant protein and DNA technology engineering have brought up new and exciting opportunities for combining desired traits in a single organism to create a custom protein. The pairing of starch saccharification and isomerization will mean a reduction in reaction time and a significant reduction in equipment costs. The large difference in optimum catalytic activity for the two enzymes tends to impair the effectiveness of a simultaneous system, which is a fundamental issue in the development of the uni-pH method.

ACKNOWLEDGMENT

The authors want to acknowledge Jadavpur University,

Kolkata-700032, India, for providing samples and the necessary instrumental facilities to complete the work.

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How to cite this article

Pinaki Saha, Dibya Das, Madhumita Saha, Sudipta Saha, Pratyusa Sar, Debabrata Bera, 2021. "Kinetic study comparison of immobilized glucose isomerase (gensweet and sweetzyme it) in stirred tank reactor and packed bed reactor". Jour. of Med. P'ceutical & Alli. Sci. V 10 - I 4, 1387 P-3115-3119. doi: 10.22270/jmpas.V10I4.1387.