International Food Research Journal 23(2): 756-761 (2016)
Journal homepage: *http://www.ifrj.upm.edu.my*

Optimization of glucose isomerase production from *Streptomyces* sp. SH10 using the response surface methodology

[1*]Habeeb, S., [2]Yazaji, S. and [3]Al-Amir, L.

[1]*Research assistant, National Commission for Biotechnology, Damascus Syria P.O.Box.30621*
[2]*Department of Food Science Damascus University, Damascus Syria*
[3]*Assistant professor, National Commission for Biotechnology, Damascus Syria*

Article history

Received: 4 May 2015
Received in revised form: 7 July 2015
Accepted: 3 August 2015

Keywords

Glucose isomerase
Response Surface Methodology
Production
Optimization
Streptomyces sp.

Abstract

The effect of environmental factors on glucose isomerase production by *Streptomyces roseiscleroticus* was studied using the statistics test Response Surface Methodology (RSM). Results of RSM revealed that the highest production of glucose isomerase reached a maximum level of 13.6 U/ml. The optimum conditions for the production of the enzyme by submerged culture were achieved using broth medium containing 1.5% of xylose as a sole source of carbon and as an inducer for enzyme production, in addition to 0.05% magnesium sulfate and 0.05% cobalt chloride as metal ion sources. The initial pH was 6 during an incubation period of 48 hours at 25°C. Applying the optimum conditions obtained 13.6 U/ml enzyme activity, and indicated that the activity increased approximately 175% in comparison with 7.44 U/ml before applying the optimum conditions.

Introduction

Enzymes are stimulating factor proteins, they are responsible for a number of necessary chemical transformations, and play important roles in life. They are important in the industry of juices, pastries, pharmaceuticals, detergents and other applications (Novo, 2004). Glucose isomerase (GI) enzyme, follows the isomerases (Nelson and Cox, 2000), is considered one of the most important industrial enzymes (Sriprapundh *et al.*, 2003; Rao *et al*., 2008), it is the third important enzyme, after amylase and protease, in the industrial application, medicine and food (Prashant *et al.*, 2010) .

The main practical application of this enzyme is to isomerize D-glucose to D-fructose, and to produce a High-Fructose Corn Syrup (HFCS) (Schenck, 2000) which is used all over the world as an alternative to sucrose or invert sugar in the food and beverage industry (Fenn *et al*., 2004; Heo *et al*., 2008; Brat *et al*., 2009). Fructose is characterized by some physical and functional properties comparing with glucose, as its tendency to crystallize is small, and highly soluble in water, in addition to its high sweetness equals 1.73, 2.34 times more than sucrose and glucose respectively (Pritham, 1998). This enzyme has recently gained more interest due to its potential applications in the biofuel industry, as well as the GI enzyme has a potential application in the conversion of hemi cellulosic biomass to ethanol (Borgi *et al*., 2004; Joo *et al.*, 2005; Gromada *et al.*, 2008).

Currently, ethanol is the major form of biofuel, and numerous technologies have been employed to improve its production (Bangrak *et al*., 2011; Tao *et al*., 2011). Furthermore, ethanol fuel production from hemicellulosic hydrolysates by *Saccharomyces cerevisiae* became of a great economic interest as an alternative to fossil fuel (Mabee, 2007). Whereas wild-type *Saccharomyces cerevisiae* can ferment xylulose to ethanol via the pentose-phosphate pathway, it cannot ferment xylose (Van Maris *et al*., 2006). Glucose isomerase is one of the intracellular enzymes in majority of the cases (Manhas and Bala, 2004), so it requires a method of extraction that leads to the breakdown or crush the cell walls (Belfaquih and Penninckx, 2000). Although, the enzyme is widely distributed in various prokaryotes, most industrially GI enzymes are obtained from *Streptomyces* sp. (Martin and Aparicio, 2009).

GI is generally produced by submerged aerated fermentation. The optimization of the fermentation medium has been extensively studied to develop an economically viable fermentation technology for the production of GI (Bejar *et al*., 1994), but

*Corresponding author.
Email: *raedkhadoor@hotmail.com*
Tel: +963 988481145

there is no concrete composition of medium for the highest production of the enzyme from different microorganisms. Each organism or strain requires its own special conditions for maximum enzyme production (Bhosale *et al*., 1996).

Muhyaddin *et al* (2008) studied cultural requirements for the production of glucose isomerase by locally isolated *Streptomyces* sp. HM5, and the enzyme activity reached 9.59 U/ml when adding 1.5% xylose as a sole carbon source and an inducer for enzyme production, 1% peptone as a nitrogen source, 0.05% magnesium sulfate and 0.05% cobalt chloride as metal ion sources with an initial pH of 7.5 during an incubation period of 72 hours at 35°C.

The traditional statistical methods used in optimization of experimental conditions depending on fixing all the variables at one level and changing only one variable, may lead to erroneous conclusions (Pardeep and Satyanarayana, 2007). Statistical experimental designs have been used for many decades by several researchers in biotechnology for an optimization strategy (El-Naggar and. Abdelwahed, 2014) and can be adopted on several steps, the first step is to screen the important parameters and the second step is to optimize those parameters (Nawani and Kapadnis, 2005). These have several advantages that included less experiment numbers, suitability for multiple factor experiments, search for relativity between factors, and finding of the most suitable conditions and forecast response (Chang *et al*., 2006). Response surface methodology (RSM) is an efficient strategic experimental tool by which the optimal conditions of a multivariable system can be determined.

In this study, a high GI-producer, *Streptomyces roseiscleroticus*, was isolated from soil samples collected from Damascus (Habeeb and yazaji, 2009). The improvement of the enzyme-productivity of the selected isolate (SH10) was achieved through physiological optimization, including the formula and pH of the medium, the incubation period, and the temperature (Lowe, 2001). The aim of the present study was to investigate the optimum condition of GI enzyme production by *S. roseiscleroticus*.

Materials and Methods

Organisms

A strain of *Streptomyces roseiscleroticus*, a high producer of glucose isomerase (GI), was previously isolated from soil samples in Damascus/Syria (Habeeb and Yazaji, 2009). The purified isolate was maintained by starch nitrate agar (20 g soluble starch, 1 g KNO_3, 0.5 g K_2HPO_4, 0.5 g $MgSO_4$, 0.5 g NaCl, 15 mg $FeSO_4.7H_2O$, 20 g agar per liter) at 4°C and sub-cultured with the same medium every 4 weeks.

Preparation of inoculums

Sporulated cultures of *S. roseiscleroticus* 4-5 day old on starch-nitrate agar were harvested in sterile normal saline. The final count in the spore suspension was adjusted to about 10^5 CFU/ml, as determined by the standard viable count technique. The spore suspension was used as an inoculum to give a final count of 10^3 CFU/ml in the medium.

Enzyme production

Erlenmeyer flasks of 250 ml containing 50 ml of basal medium (1% peptone, 0.25% yeast extract, 1% D-xylose, 0.5% NaCl, 0.5% Beef extract, 0.05% MgSO4.7H2O w/v, pH 6.8) were inoculated with a spore suspension of the tested isolate (SH10) to give the required final count. Then, the cultures were incubated at 30°C for 72h in a cooling shaker incubator at 150 rpm (Chen *et al*., 1979).

Preparation of crude enzyme

The microbial cells were harvested from the 3 day old culture by centrifugation at 8,000 rpm for 15 min and washed with sterile distilled water twice. The cells were suspended in 0.05 M sodium phosphate buffer (pH 7.0) containing 0.1% cetyltrimethylammonium bromide (CTAB), and the suspension was incubated at 30°C for 24 h, and then was centrifuged and filtered. The filtrate was used as a crude enzyme.

Enzyme assay

We mixed 0.1 ml of enzyme solution with the reaction mixture composed of 0.5 ml of potassium phosphate buffer solution (0.1 M, pH 8), 0.2 ml $MgSO_4.7H_2O$ (0.05 M), and 0.2 ml of glucose substrate solution (1 M). After incubation in a water bath at 60°C for 30 min, 1 ml of Perchloric acid (0.5M) was added to stop the reaction. Fructose formed by enzyme was determined by the method of Dische and Borenfreund (1951) that a purple colour developed on adding 0.2 ml of 1.5% Cysteine hydrochloride, 6 ml of 70% H_2SO_4 and 0.2 ml of 0.12% Alcoholic Carbazole, then measured spectrophotometrically at 560 nm (Bhasin and Modi, 2012).

Optimization of glucose isomerase productivity of S. roseiscleroticus

The effect of different factors on GI production by *S. roseiscleroticus* was investigated. The basal medium used for this study was started by the following ingredients: 1% peptone, 0.25% yeast extract, 1.5 % D-xylose, 0.5% NaCl, 0.5% beef

extract and, 0.05% MgSO4 (w/v), and the pH adjusted to 7.0. All experiments were carried out using 250 ml Erlenmeyer flasks containing 50 ml of the fermentation medium inoculated with 1 ml of *S. roseiscleroticus* spore suspension. The flasks were incubated at specific temperatures and incubation periods according to the RSM test.

Effect of incubation period: A group of flasks containing basal medium were inoculated for 48, 72, 96, 120 and 146 h incubation periods.

Effect of incubation temperature: A set of flasks containing basal medium was incubated at different temperatures (25, 30, 35, 40 and 45°C).

Effect of carbohydrate: D-xylose was added in basal medium at different concentrations (5, 7.5, 10, 12.5 and 15g/l).

Effect of initial pH: Different flasks containing basal medium with initial pH values (6, 6.5, 7, 7.5 and 8) were inoculated with the tested organism and incubated.

Effect of Metal ions: The magnesium ion used in production medium was replaced by other different sources of metal ions with concentrations of 0.5%, these ions were Mn^{+2}, Co^{+2}, Ca^{+2}, Mg^{+2} and a mixture of Mg^{+2} and Co^{+2} in a primary experiment, the best source of metal ions was the mixture of Mg^{+2} and Co^{+2}, so it was added in the medium in different concentrations (0.1, 0.3, 0.5, 0.7 and 0.9%) for each of them.

Statistical analysis

Experimental design RSM included the effect of xylose concentration as a carbon source, the effect of addition of different ions, the effect of initial pH, the effect of fermentation temperature, and the effect of incubation periods. Boundaries of experimental domain and spacing of levels expressed in coded units were showed in Table (1).

This design contains 32 experimental plots. Experimental results of glucose isomerase production by a complete 5-factor, with 5 replications of the central point. The parameters of Eq. (1) were determined by multiple regression analysis, with the RSM method. The second-order polynomial regression equation, which showed the relationship between glucose isomerise activity (Y) and 5 tested variables in coded units, is represented by Eq (1):

$$Y=a+bX1+cX2+dX3+eX4+fX5+gX12+hX22+iX32+jX42+kX52+lX1X2+mX1X3+nX1X4+oX1X5+pX2X3+qX2X4+rX2X5+sX3X4+tX3X5+uX4X5$$

Y: response

a: constant

b, c, d, e, f: linear coefficients

Table 1. Boundaries of experimental domain and spacing of level expressed in coded units

Variable	Range and level of Variable				
	-2	-1	0	1	2
Xylose concentration g/L	5	7.5	10	12.5	15
Metal ion requirement %	0.01	0.03	0.05	0.07	0.09
initial pH	6	6.5	7	7.5	8
fermentation temperature°C	25	30	35	40	45
incubation period day	2	3	4	5	6

g, h, i, g, k: square coefficients

l, m, n, o, p, q, r, s, t, u: interaction coefficients

X1, X2, X3, X4 and X5: Variants (Xylose concentration, incubation period, pH, Temperature, Ions concentration)

Results and Discussion

Effect of ions on enzyme production

Results showed that the best treatment was by adding a mixture of magnesium and cobalt ions compared with the treatments using magnesium, manganese, cobalt, and calcium ions separately. The enzyme activity reached 9.12 U/ml with the mixture, while with the individual ions it was 7.39, 2.91, 6.65, 2.38 U/ml respectively (Figure 1).

Enzymatic activity of SH10 isolate (Streptomyces roseiscleroticus)

Table (2) shows the results of the enzymatic activity produced by SH10 isolate after optimizing medium conditions using the statistical test (RSM) as in the adopted statistical design (Table1) that contains xylose concentration, incubation period, pH, the temperature of fermentation medium and ions content.

The results in Table (2) clearly showed that there were differences in the enzymatic activity values in all experiments, and the experiment No (7) revealed the highest value with an enzymatic activity of 13.6 U/ml when the concentration of the substrate (xylose), incubation period, pH, temperature and the amount of magnesium and cobalt ions were 10 g/l, 2 days, 7, 35 °C, 0.05% respectively, while the minimal value was in the experiment No (18) that the enzymatic activity was 4.18U/ml when the concentration of the substrate, incubation period, pH, temperature and the amount of ions were 10g/l, 4 days , 6 , 35°C, 0.05% respectively.

The deterioration of the enzyme productivity of microorganisms after the ideal incubation period could be due to the entry of bacteria numerical stability

Table 2. Activity values for each treatment for the SH10 isolate

Blocks	Variants					Enzymatic activity U/ML
	A	B	C	D	E	
	Xylose con g/L	incubation period (Day)	pH	Temperature °C	Ions g/L	
1	10	4	7	25	0.05	9.13
2	10	4	7	35	0.05	8.91
3	10	4	7	35	0.09	10.48
4	10	4	7	35	0.05	7.98
5	7.5	3	7.5	40	0.07	4.52
6	10	4	7	35	0.05	9.32
7*	10	2	7	35	0.05	13.01
8	10	4	7	35	0.05	8.86
9	12.5	5	6.5	30	0.07	7.60
10	15	4	7	35	0.05	8.24
11	7.5	5	6.5	30	0.03	9.59
12	12.5	3	7.5	30	0.07	9.21
13	12.5	5	7.5	30	0.03	7.65
14	10	4	8	35	0.05	5.07
15	7.5	5	6.5	40	0.07	7.73
16	20	3	6.5	30	0.03	10.39
17	7.5	3	6.5	40	0.03	11.91
18	10	4	6	35	0.05	4.18
19	10	4	7	35	0.01	8.24
20	5	4	7	35	0.05	6.80
21	12.5	5	7.5	40	0.07	6.04
22	7.5	5	7.5	30	0.07	7.86
23	12.5	3	7.5	40	0.03	5.28
24	7.5	3	7.5	30	0.03	6.84
25	7.5	5	7.5	45	0.03	9.21
26	10	4	7	35	0.05	9.20

or the die stage, especially on submerged culture when the components of medium are consumed or a series of changes may arise and negatively affect the biomass and cause cell degradable, and emancipation of enzymes may affect the production of required enzyme.

Most studies showed that the optimal incubation period for glucose isomerase production ranges between 24-96 hours, so our results agreed with the findings of Joo and Rhee (1997) in that the optimal incubation period for the enzyme production from Streptomyces chibaensis J-59 was after 42 hours, and these results were close to the results of Muhyaddin *et al.* (2008) where the optimal enzyme production was during an incubation period of 72 hours. In addition, pH demonstrated a very important effect on the growth of microorganisms, as it effected the solubility of nutrients in the culture, and was reflected on the growth of bacteria and the enzyme production. These results were in conformity with the findings of Hong *et al* (1991) in a study of glucose isomerase production where the optimal pH for producing GI from *Streptomyces luteogrioseus* was 6.5.

Optimal condition for the enzyme production by the statistical test RSM

Optimal conditions for the production of glucose isomerise enzyme produced from *S. roseiscleroticus* after application of the statistical test RSM were indicated in (Figure 2). The maximum production of enzyme was 13.6 U/ml at a temperature of 25°C, pH 6, incubation time 48 hours, xylose concentration 15g/L and a mixture of 0.05% magnesium and cobalt ions. The statistical analysis showed that there was a significant interaction effect between pH and the incubation time at a confidence level of 5%.

Results of the RSM statistical test for glucose isomerase production

Table (3) showed the effect of the studied factors (the linear terms, the squared terms, and the interactions) on glucose isomerise production. The small P values for the incubation time and pH ($P < 0.05$) indicated that there were significant linear effects of each of these two variables on enzyme production, while temperature, xylose concentration and the amount of ions had no linear effects on

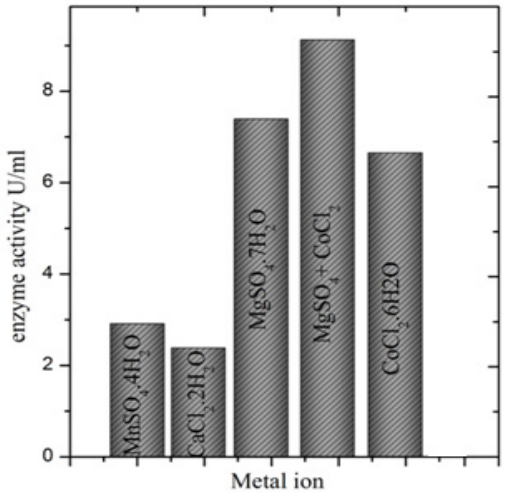

Figure 1. Effects of different metal ions on the glucose isomerase activity. Different types of metal ions were added to the reaction mixture at a concentration of 5%

enzyme production. On the other hand, the small P values for the effect of squared terms of incubation time and pH ($P < 0.05$) suggested that there was curvature in the response surface. Furthermore, the interactions between the incubation time and pH, and between temperature and pH were significant ($P < 0.05$). the high determination coefficient value ($R^2 = 86.3\%$) referred that the five studied factors were responsible for 86.3% changes in enzyme production. Consequently, the following equation could be deduced:

$$Y= - 206.023 -14.811\ X3 +60.815\ X4 +788\ X3X3 - 4.245\ X4X4+ 1.785\ X3X4 – 0.302\ X4X5$$

Conclusions

The isolate *Streptomyces* sp. SH10 was able to produce glucose isomerase and the optimum conditions for production of the enzyme were achieved on broth medium containing 1.5% of xylose as a sole carbon source and inducer for enzyme production, 0.05% magnesium sulfate and 0.05% cobalt chloride as metal ion source with an initial pH of 6 during an incubation period of 48 hours at 25°C. These optimum conditions for enzyme activity attained 13.6 U/ml. pH and the incubation time exhibited significant squared effects on enzyme production at a confidence level of 5%, and there were interaction effects between the incubation time and pH, and between the temperature and pH at a confidence level of 5%.

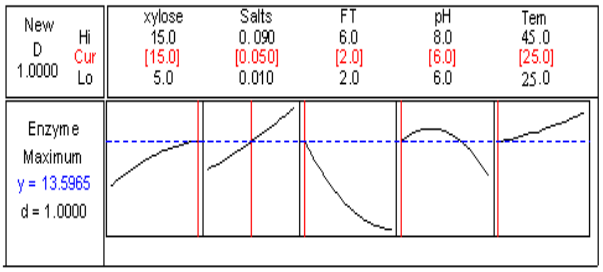

Figure 2. The optimal conditions for the glucose isomerase production from *S.roseiscleroticus* using statistical program RS

Acknowledgements

We are grateful to the National Commission for Biotechnology, and the Department of Food Science, and especial thanks to the technicians in this department.

References

Bangrak, P., Limtong, S. and Phisalaphong, M. 2011. Continuous ethanol production using immobilized yeast cells entrapped in loofa-reinforced alginate carriers. Brazilian Journal of Microbiology 42(2): 676-684.

Bejar, S., Belghith,K., Gargouri, R. and Ellouz. R. 1994. Construction of a new strain of *Streptomyces violaceoniger*, having strong, constitutive and stable glucose-isomerase activity. Biotechnology Letters 16(12): 1259–1264.

Belfaquih, N. and Penninckx, M.J. 2000. A bifunctional beta-xylosidase-xylose isomerase from *Sterptomyces* sp. EC10 . Enzyme and Microbial Technology 27(2): 114-121.

Bhasin, S. and Modi, H.A. 2012. Optimization of fermentation medium for the production of Glucose Isomerase using *Streptomyces* sp. SB-P1. Biotechnology Research International Article ID 874152: 1-10.

Bhosale, S.H., Rao, M.B. and Deshpande, V.V. 1996. Molecular and industrial aspects of glucose isomerase. Microbiological Reviews 60(2): 280-300.

Borgi, M.A., Srih-Belguith, K., Ben Ali, M., Mezghani, M., Tranier, S., Haser, R. and Bejar, S. 2004. Glucose isomerase of the Streptomyces sp. SK strain: purification, sequence analysis and implication of alanine 103 residue in the enzyme thermostability and acidotolerance. Biochimie 86(8): 561-568.

Brat, D., Boles, E. and Wiedemann, B. 2009. Functional expression of a bacterial xylose isomerase in *Saccharomyces cerevisiae*. Applied and Environmental Microbiology 75(8): 2304-2311.

Chang, C.Y., Lee, C.L. and Pan, T.M. 2006. Statistical optimization of medium components for the production of *Antrodia cinnamomea* AC0623 in submerged cultures. Applied Microbiology and Biotechnology 72(4): 654–661.

Chen, W.P., Anderson, A.W. and Han, Y.W. 1979. Production of glucose isomerase by *Streptomyces flavogriseus*. Applied and Environmental Microbiology 37(2): 324–331.

Dische.Z. and Borenfreund.A. 1951. A new spectrophotometric method for the detection and determination of keto sugar and trioses. Journal of Biological Chemistry 192(2): 583-587.

El-Naggar, N.E. and Abdelwahed, N.A.M. 2014. Application of statistical experimental design for optimization of silver nanoparticles biosynthesis by a nanofactory *Streptomyces viridochromogenes*. Journal of Microbiology 52(1): 53–63.

Fenn, T.D., Ringe, D. and Petsko, G.A. 2004. Xylose isomerase in substrate and inhibitor michaelis states: atomic resolution studies of a metal-mediated hydride shift. Biochemistry 43(21): 6464–6474.

Gromada, A., Fiedurek, J. and Szczodrak, J. 2008. Isoglucose production from raw starchy materials based on a two-stage enzymatic system. Polish Journal of Microbiology 57(2): 141-148.

Habeeb. S, and Yazaji. S. 2009. A study on bioactivity of local isolates of Streptomyces, MSc thesis, Damascus University, College of Agriculture. Syria.

Heo, G.Y., Kim, W.C., Joo, G.J., Kwak, Y.Y., Shin, J.H., Roh, D.H., Park, H.D. and Rhee. I.K. 2008. Deletion of xylR gene enhances expression of xylose isomerase in *Streptomyces lividans* TK24. Journal of Microbiology and Biotechnology 18(5): 837-844.

Hong, S.S., Baek, S.K., Lee, K.H., Kuk, S.U. and Park, K.H. 1991. Isolation of glucose isomerase-producing microorganisms, *Streptomyces luteogriseus* and determination of fermentation conditions. Korean Journal of Applied Microbiology and Biotechnology 19(1): 296-302.

Joo, G.J. and Rhee, I.K. 1997. Production of glucose isomerase from xy/b mutant *Streptomyces chibensis* J-59. Korean Journal of Applied Microbiology and Biotechnology 25: 75-81.

Joo, G.J., Shin, J.H., Heo, G.Y., Kim, Y.M., and Rhee, I.K. 2005. Molecular cloning and expression of a thermostable xylose (glucose) isomerase gene, xylA, from Streptomyces chibaensis J-59. Journal of Microbiology 43(1): 34-37.

Lowe, D.A. 2001. Production of enzymes, In C. Ratledge, and B. Kristiansen (eds.), Basic Biotechnology, 2nd ed. Cambridge University Press, Cambridge.2001: 392–408.

Mabee, W.E. 2007. Policy options to support biofuels production. Advances in Biochemical Engineering/ Biotechnology 108: 329-357.

Manhas, R.K. and Bala, S. 2004. Thermostable glucose isomerase production by some bacteria and Streptomyces. Indian Journal of Microbiology 44: 129–132.

Martin, J.F. and Aparicio, J.F. 2009. Enzymology of the polyenes pimaricin and candicidin biosynthesis. Methods in Enzymology 459(2009): 215-242.

Muhyaddin, M.O., Jabur, H.A. and Dalaly, B.K. 2008. Production of Glucose isomerase from local isolate of *Streptomyces* sp. HM5 2-Optimization of enzyme production conditions by submerged cultures. Journal of Research Diyala Humanity 31(2): 11-32.

Nawani, N.N. and Kapadnis, B.P. 2005. Optimization of chitinase production using statistics based experimental designs. Process Biochemistry 40(1): 651–660.

Nelson, d.L. and Cox, M.M. 2000. Lehninger Principles of Biochemistry. Worth Publishers: New York, USA.

Novo Nordisk, A/S. 2004. Enzymes at work . Bagsvaerd, Denmark.

Pardeep, K. and Satyanarayana, T. 2007. Optimization of culture variables for improving glucoamylase production by alginate-entrapped thermomucor indicae-seudaticae using statistical methods. Bioresource Technology 98: 1252-1259.

Prashant. S., Saurabh. S., Sanjay. K.C. and Gomase. V.S. 2010. Isolation, purification and characterization of Glucose Isomerase enzyme from *Streptomyces* species isolated from Parbhani Region. Journal of Enzyme Research 1(1): 1-10.

Pritham, G. H. 1998. Andersons Essentials in Biochemistry. P. 74, Mosby, New York, N. Y.

Rao, K., Chelikani, S., Relue, P. and Varanasi, S. 2008. A novel technique that enables efficient conduct of simultaneous isomerization and fermentation (SIF) of xylose. Applied Biochemistry and Biotechnology 146(1-3): 101-117.

Schenck, F.W. 2000. High Fructose syrups-review. International Sugar Journal 102(1218): 285-288.

Sriprapundh, D., Vieille, C. and Zeikus, J.G. 2003. Directed evolution of Thermotoga neapolitana xylose isomerase: high activity on glucose at low temperature and low pH. Protein Engineering Journal 16(9): 683–690.

Stephen, H. and Suraez, N. 2003. Sugar and sweetener: Situation and outlook. U. S. department of agriculture, 21(1): 1-74.

Tao, N.G., Gao, Y.M. and Liu, Y.J. 2011. Isolation and characterization of a *Pichia anomala* strain: a promising candidate for bioethanol production. Brazilian Journal of Microbiology 42(2): 668-675.

Van Maris, A.J.A., Abbott, D.A., Bellissimi, E., van den Brink, J.P., Kuyper, M., Luttik, M.A.H., Wisselink, H.W., Scheffers, W.A., van Dijken, J.P. and Pronk, J.T. 2006. Alcoholic fermentation of carbon sources in biomass hydro-lysates by *Saccharomyces cerevisiae*: current status. Antonie van Leeuwenhoek 90(4): 391-418.