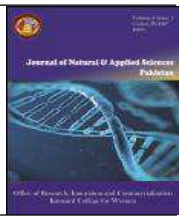
Contents lists available http://www.kinnaird.edu.pk/

Journal of Natural and Applied Sciences Pakistan

Journal homepage: http://jnasp.kinnaird.edu.pk/



OPTIMIZATION OF GROWTH MEDIUM AND CONDITIONS FOR THE MAXIMUM PRODUCTION OF GLUCOSE ISOMERASE BY CS1 BACTERIAL STRAIN

Rabia Iftikhar[1], Hooria Younas[2]*, Anum Waqar[3], Mishal Tariq[4]
[1] Department of Biochemistry, Kinnaird College for Women, Lahore, Pakistan.
[2] Department of Biochemistry, Kinnaird College for Women, Lahore, Pakistan..
[3] Department of Biochemistry, Kinnaird College for Women, Lahore, Pakistan.
[4] Department of Biochemistry, Kinnaird College for Women, Lahore, Pakistan.

Article Info

*Corresponding Author
Tel: +92 42 99203781– 84 (266)
Email Id: hooria.younas@kinnaird.edu.pk

Keywords
Glucose isomerase; Xylose isomerase; wheat straw; sugarcane bagasse, agriculture waste; Cysteine Carbazole assay.

Abstract

Glucose isomerase (GI) is an enzyme responsible for catalyzing the reversible interconversion of D-glucose and D-xylose to D-fructose and D-xylulose. The objective of this study was to optimize the growth conditions for maximum GI production of CS1 bacterial strain. Following three mediums (i) 2% xylose with peptone (soybean) and Mg2+, (ii) 1% wheat straw with peptone (casein) and Mn2+ and (iii) 1.5 % sugarcane bagasse with tryptone plus yeast extract and Fe2+ plus Co2+ gave optimized GI activity at pH 7 for 24 h incubation period. In this study, GI production was improved in xylose medium from 13.08U to 29.3U, in wheat straw medium from 7.65U to 14.1U and in sugarcane bagasse medium from 8.54U to 14.1U in 25mL mediums, respectively after optimizing different conditions. This study concludes that agricultural wastes, like sugarcane bagasse and wheat straw can be used for the cost-effective production of GI.

1. Introduction

Glucose isomerase (GI) is an enzyme that catalyzes the reversible isomerization of D-glucose and D-xylose to D-fructose and D-xylulose, respectively (1). This enzyme is involved in xylose catabolism and serves the purpose of providing nutrition to the saprophytic bacteria. The enzyme takes part in the isomerase pathway where it converts D-xylose into D-xylulose. D-xylulose is then phosphorylated by xylose kinase (XK) that utilizes an ATP, to result in D-xylulose-5-phosphate which enters the pentose phosphate pathway (2). The pentose phosphate pathway is an alternative to glycolysis and yields NADPH and pentoses. It is found prevalently in a number of microorganisms and is produced both intracellular and extracellular by different strains. The prevalence of GI activity in a few yeasts such as *Candida utilis* (3) *Candida albicans* (4) and *Candida boidinii* (5) has been reported. The only fungus found to produce the enzyme in a high amount (1126 U) is *Aspergillus oryzae*y (6).GI from different microorganisms is categorized according to their structure and sequence homology into two classes Class I and class II (7). The sedimentation constants and molecular weights of GI vary from 7.55 to 11.45 and from 52,000 to 191,000, respectively. Studies have exposed that GI is made up of a dimer or tetramer which may be similar or identical (1).

GI has commercial application in the production of ethanol and an artificial sweetener, high fructose corn syrup (HFCS), that is used in various food confectionary products and pharmaceutical industry. The most prevalent and abundant producers of GI are bacteria. Different bacterial strains require specific constituents in medium, presence of various metal ions and specific temperature and pH for their optimum growth and production of glucose isomerase. Hence optimization of fermentation medium for maximum production of GI is a crucial requirement for the industries today.

The divalent cations required by glucose isomerase are Mg^{2+}, Co^{2+}, Mn^{2+}, or a combination of these cations. Mg^{2+} and Co^{2+} are both crucial for GI activity. Mg^{2+} plays its role as an activator and Co^{2+} as the stabilizer of quaternary structure of the enzyme (8, 9). Each tetramer of GI in Strep*tomyces griseofuscus* has been reported to consist of four Co^{2+} ions by Kasumi *et al*. (10). Eliminating Co^{2+} from the medium is necessary due to the pollution caused by the spent media and due to the hazardous health effects of consuming Co^{2+} containing HFCS. The nitrogen and carbon sources are critical factors which need to be optimized for each source of enzyme. The GI-producing organisms that have been reported mostly require D-xylose as an inducer of the enzyme (1) but xylose is expensive and inconvenient for use on commercial scale. Hence, replacement for xylose is a necessity. Moreover, Pakistan is one of the most agricultural countries in the world. Growing bacteria on medium consisting of waste materials (wheat straw, rice straw and sugarcane bagasse *etc*.) from agricultural lands will prove to be cost-effective in the future. The possibility of utilizing such wastes for GI production and replace xylose in the production medium with cheaper inducers was evaluated in this study.

1. Methodology

The bacterial strain used in this study was isolated from a sugarcane field in Chenab, Punjab (11). AX medium (pH=7.0) was made with 10g of D-xylose, 5g of tryptone, 5g of yeast extract, 1g of K_2HPO_4, 0.2g of $MgSO_4.7H_2O$, 0.1g of $MnCl_2.4H_2O$, 0.05g of $CoSO_4.7H_2O$ and 11.68g of NaCl in 1 litre of distilled water.

2.1 Enzyme Assay. The enzymatic activity of GI was determined using colorimetric method i.e, Cysteine Carbazole Assay (12). 1 ml reaction mixture was made containing 100µl of 50mM $MgSO_4.7H_2O$, 100µl of 10mM $CoCl_2.6H_2O$, 100 µl of 1M D-glucose, 0.6 ml of 0.1 M Sodium phosphate buffer (pH 7.6) and 100µl of extracellular medium containing enzyme. The reaction mixture was placed in water bath at 70°C for 30 minutes. 0.1 ml of this enzyme reaction mixture was then immediately added to a test tube containing 0.4 ml of H_2O, 0.2 ml of 1.5 % cysteine hydrochloride, 3 ml of 70% sulfuric acid and 0.2 ml of 0.12 % alcoholic carbazole solution. The reaction mixture was heated at 60 ºC for 10 minutes. Intense violet colour was developed that showed the presence of fructose which was formed in the reaction mixture. Absorbance was measured at 560 nm using spectrophotometer. The unknown concentrations of samples were determined from the standard curve plotted using different (0.01 – 1.0 mg/ml) known fructose concentrations.

1 unit of enzyme activity was defined as the amount of enzyme that produced 1 μmole of D-fructose per min under the assay conditions.

2.2 Screening for Carbon Sources. The effect of medium components on growth and glucose isomerase activity of CS1 bacterial strain was evaluated. The experiments were performed on duplicates of each medium. The assay was performed after 24 hours of incubation in each case. The pure carbon sources, used at 1% concentration were xylose, glucose, fructose, and a combination of xylose and glucose. The crude carbon sources, used at a concentration of 1.5%, were rice straw, wheat straw, corn cob, sugarcane bagasse, hardwood stem, and newspaper.

1.2. Screening for Nitrogen Sources. The selected carbon sources were then used to screen for nitrogen sources. The nitrogen sources, used at 1% concentration, were yeast extract, tryptone, peptone (casein), peptone (soybean), meat extract, casein, urea, ammonium sulfate and a combination of tryptone and yeast extract (0.5% each).

1.3. Quantitative effect of Carbon Source. After optimization of carbon and nitrogen sources, the effect of increasing concentration (1%, 1.5%, 2%, 3%, and 5%) of carbon source on growth and GI activity of CS1 bacterial strain was determined.

1.4. Metal ions requirement. Metal co-factors required by CS1 bacterial strain for GI production were found out by adding them individually and in combinations (Table 1). The salts used to provide the respective metal ions were; $MgSO_4.7H_2O$, $MnCl_2.4H_2O$, $CoSO_4.7H_2O$ and $FeSO_4.7H_2O$.

1.5. $CoSO_4.7H_2O$ and $FeSO_4.7H_2O$.

1.6. Effect of pH. Three optimized mediums (Table 2) that were formulated were then subjected to different pH values (5, 6, 7, 8, 9 and10), in order to find the optimum pH for GI production by CS1 bacterial strain.

1.7. *Effect of incubation time.* The effect of incubation time on GI production and growth of CS1 bacterial strain was checked by growing it for different time periods, i.e., 6 h, 9 h, 24 h, 36 h, 48 h and 54 h.

3. Results

3.1 Screening for Carbon Sources. Glucose isomerase produced by CS1 bacterial strain exhibited maximum activity in the medium that contained both glucose and xylose as the carbon sources. The other carbon sources; xylose, fructose and glucose, were also able to produce a fairly good amount of enzyme as shown in Figure 1.

The results obtained with crude carbon sources were shown in Figure 2. The maximum enzymatic units were observed with medium containing sugarcane bagasse, followed by wheat straw and then by rice straw. The lowest enzyme units were produced with hardwood stem. Sugarcane and straw hemicellulose worked as inducers to increase the GI yield. Xylose, sugarcane and wheat straw were selected for further optimization.

3.2 Screening for Nitrogen Sources. Optimization results of nitrogen sources with xylose are shown in Figure 3. Maximum growth was observed with yeast extract whereas; the highest amount of GI was produced with peptone (soybean). Figure 4 shows the results obtained in wheat straw mediums. Peptone (casein), tryptone and a combination of tryptone + yeast extract were able to grow the strain maximally. GI activity was highest with peptone (casein). Sugarcane bagasse medium with yeast extract gave highest growth. Optimum enzyme activity was obtained in meat extract and yeast extract + tryptone mediums, followed by tryptone (Figure 5). A very low growth and GI activity was observed with urea and ammonium sulfate.

3.3 Quantitative effect of carbon sources. As the concentration of xylose was increased, GI activity of CS1 bacterial strain was also increased. Maximum growth was observed at 1.5% and highest GI units at 2% concentration of xylose (Figure 6C). With wheat straw, GI activity was highest at 1% concentration. It then reduced with concentration until a minimum activity was observed at 2%. Increasing concentration above 2% increased GI activity again as shown in Figure 6A. Varying sugarcane bagasse concentration did not have a profound effect on the GI activity. However, it was significantly lowered when the maximum concentration of 5% was used (figure 6C).

3.4 Metal ions requirement. Different metal co-factors used in xylose mediums gave the results as shown in Figure 7. The highest growth was observed with the combination of Fe2+ and Co2+, followed closely by Mn2+. Lowest growth was observed when all four metal ions were used together. The GI activity exhibited was optimum with Mg2+ and lowest with Mn2+. In the presence of Mn2+, it was able to produce the highest amount of GI in wheat straw mediums whereas, addition of Mg2+ along with Mn2+ to the medium lowered GI

production to a minimum value (Figure 8). In sugarcane bagasse mediums, there is no significant difference in GI activity among cultures grow with different metal ions but the combination of Fe2+ and Co2+ produced slightly better GI activity as compared to others (Figure 9).

3.5 Effect of pH. Three mediums were optimized with the composition shown in Table 2. The optimized wheat straw and sugarcane bagasse mediums were used to find the effect of pH on growth and GI activity of CS1 bacterial strain. The strain could grow and produce highest enzyme activities from pH 7.0 to 8.0.

3.6 Effect of incubation time. In all three optimized mediums, the strain showed a low growth and GI activity until 9h. GI activity increased with time and was highest at 24 h of incubation after which, it remained about similar and then slightly decrease after 54 hours (Figure 11).

4 Discussion

The present study was undertaken to optimize the fermentation medium composition and growth conditions for maximum production of Glucose isomerase by the CS1 bacterial strain. Glucose isomerase is capable of isomerizing glucose to fructose and xylose to xylulose. This makes it commercially important in production of HFCS from corn starch as well as ethanol from biomass. Glucose isomerase has been studied extensively ever since it was first discovered by Marshall and Kooi (13). It has been isolated from numerous source organisms and studied to optimize its fermentation medium for maximum production of the enzyme.

The bacterial strain used in this study was isolated from a sugarcane field in Chenab. It was found to produce high amounts of GI extracellularly using Cystein Carbazole Assay (12). GI has been reported to show high enzyme activities in mediums containing the pure carbon sources used in this study; fructose, glucose, xylose and a combination of xylose and glucose. Our strain was able to produce GI in highest amount when grown in both glucose plus xylose (figure 1) as was shown by Arthrobacter sp. grown by Lobanok (14). Addition of glucose with xylose increased the GI activity slightly in the strain used by Givry (15) as well. However, the increase caused by addition of glucose was not very significant compared to the use of xylose alone, the subsequent experiments were carried out with xylose being the natural inducer of GI as a control. The natural carbon sources selected for this study consist of high amounts of hemicelluloses, about 25-32%, that are majorly composed of xylose (16), sugarcane and wheat straw gave reasonably high GI activities (figure 2). Being rich in hemicelluloses, these two sources were utilized maximally by our strain to produce high enzyme units. For Streptomyces flavogriseus Sugarcane and wheat straw hemicellulose were able to work as inducers and to replace xylose and increase the GI yield (17). Another strain, Streptomyces sp. SB-P1, isolated by Bhasin and Modi (18) showed highest enzymatic activity in the medium with wheat husk and corn cob. Other investigators (19, 20) have reported high enzyme yield and growth of Streptomyces in presence of wheat bran. Hemicelluloses containing substances are thus useful as medium ingredients because of their lower cost than xylan and xylose. Takasaki and Tanabe (21) reported a Streptomyces strain YT-5 that was able to grow on xylan or xylan-containing material such as corn cob and wheat bran. Although we obtained a high activity with wheat straw, our results with corn cobs were contradictory and low GI activities were exhibited. Cheaper and easily available hemicellulosic materials rich in xylose sugars, such as sugarcane and wheat straw, can thus be used to replace the expensive xylose inducer for GI.

Various crude nitrogen sources (Peptone (casein), Peptone(soybean), urea, casein, yeast extract, tryptone, meat extract, and tryptone+yeast extract) along with a pure nitrogen source (ammonium sulfate) were selected. Our bacterial strain was unable to grow and produce GI sufficiently in urea and ammonium sulfate. This observation is in accordance with earlier reports that suggest that there is inability of inorganic salts to produce higher yields of GI (18). Givry (15) also reported that enzyme activity was not sufficiently affected when inorganic ammonium salts were used. Moreover, he reported that Lactobacillus bifermentas was able to produce GI only in the presence of organic sources; peptone, yeast extract and tryptone. These three sources along with meat extract proved to be the best nitrogen sources for maximum production of GI in our species as shown in Figures 3,4,5. Peptone that gave maximal GI production was also found to be the best nitrogen source by various scientists earlier. Bacillus coagulan (22) utilized peptone and yeast extract like our strain did but it also utilized ammoniums salts, which was not the case in our study. Moreover, unsuitability of urea

and nitrates in the medium was observed in both cases.

The catalytic activity of GI is dependent on bivalent cations like Mg2+, Mn2+, Co2+ and Fe2+ etc (23). They are a necessary component in the fermentation medium for maximum production of the enzyme. Results obtained after detailed investigation of the mechanism of action of GI in A. missouriensis have demonstrated that the two co-factors Mg2+ and Co2+ play an essential role in binding and stabilizing the open forms of the substrate and in catalyzing hydride transfer between the C-1 and C-2 positions. Co2+ is also necessary for protections against thermal inactivation of the GI (24). In this study, we provided the strain with the four metal co-factors (Mg2+, Mn2+, Co2+ and Fe2+) separately as well as in combinations. The two combinations made were Mg2++Mn2+ and Co2++Fe2+.The combination of Mg2++Mn2+ was chosen because it gave the highest acitivty in Strpetomyces flavogriseus studied by Chen (17). Depending on the source of enzyme, metal requirements of GI vary. In our study, the strain required different metals for GI productions when grown in different fermentation medium compositions. Growing it on xylose or wheat straw mediums with peptone as the nitrogen source in each, it required Mg2+ or Mn2+ for the maximum GI activity, respectively (Figures 7 and 8). Bacillus coagulans also required Mn2+ or Mg2+ for production of the enzyme (22). Deshmukh and Shankar (25) also produced GI in the absence of Co2+ and enhance its production with Mg2+ as seen in our xylose medium. Some organisms such as Arthrobacter spp. and Streptomyces olivaceus (25), as well as some mutants of Streptomyces olivochromogenes do not require cobalt for optimal production (23). When sugarcane mediums were used, the metal ions requirement varied. GI production was optimum in the fermentation medium with Co2+ and Fe2+ both. Streptomyces strain YT-5 has been reported to have an essential need for Co2+ (26). This difference in the metal requirement could be due to the fact that sugar cane was a rich source of metal ions like Mg2+ or Mn2+. In many areas, it has been reported that edible sugarcane tissues have metal ions due to metal ions wastes present in the soil. Sugarcane crops are able to grow in soil that has accumulation of metal ions. Hence, the necessary requirement of Mg2+ in the fermentation could have been fulfilled by sugarcane itself (27).

The Mn2+ ion has been reported as a stimulating factor in GI biosynthesis. Another observation made in our tests was that the glucose isomerase activity after growth in the presence of Mn2+ was always remarkably lower than the activity of the biomass grown without Mn2+. These results are similar to those obtained by Hasal (28).

An investigation into the effect of increasing concentration of the three selected carbon sources, i.e. xylose, sugarcane and wheat straw, was done in this study. Enzyme yield obtained with wheat straw was highest at 1% and 5% concentration. The concentrations used between these values gave lower GI activities. Optimum xylose concentration recorded was 2% however the maximum growth was observed at 1% concentration (Figure 6). Our results are in contrary with results obtained by Givry et al (15), where they observed highest enzyme activity in Lactobacillus bifermentas with 1% xylose and also by Sheetal Bhasin (18) who could produce maximum GI activity by Streptomyces sp. SB-P1 at 1% xylose. When grown at higher concentrations of xylose, our strain did not grow, this could be because the highest xylose concentration that the bacterial stain can bear was reached. Varying sugarcane concentration did not have a profound effect on the GI activity. However, it was significantly lowered when the maximum concentration of 5% was used.

pH is a very crucial factor in optimization of growth and enzymatic activities in bacteria. 7.0-8.0 is the range of pH of fermentation mediums in which most of the GI productions takes place (1, 23). In wheat straw mediums, pH 7 gave the highest GI activity as well as the growth of the bacterial strain. In sugarcane mediums, pH 7 produced maximum enzymatic activity but the growth was highest at a more alkaline condition; pH 9. These results are in accordance with Streptomyces thermonitrificans that could grow and produce similar enzyme activities from pH 7.0 to 8 (25). It is possible that the alkalinity of the mediums in our study caused hydrolysis of the sugarcane releasing various sugars in the medium and that these sugars improved the growth of the strain but did not improve GI synthesis. It is also possible that our strain did not have sufficient amount of hemicellulases to degrade hemicelluloses or that this enzyme was active at higher pH values and hence was unable to produce significantly high amounts of xylose for utilization by our bacterial strain.

Depending on the types of culture medium and source organism used, the period of fermentation of GI production varies from 6 to 48 h (23). Growth in xylose, wheat straw as well as sugarcane mediums was low till 9h of incubation. This could be due to the time taken by the strain to adapt to the growth conditions in the medium. It increased rapidly at 24h period, and was maximum at 36h (figure 11). After that, the growth reduced a little this could be because the nutrients in the medium got used up. The GI activity was lowest at 36 h when the growth was highest in xylose mediums and it was maximum when the growth was minimum (Figure 11). This observation was unexpected and occurred due to unidentified reasons. On wheat straw mediums, GI activity at 6h was low and it increased at 9h. It then started to reduce until it reached a minimum at 36h. After further incubation, GI activity increased and reached a maximum value at 54h period. A similar observation was made with sugarcane mediums (Figure 11). Deshmukh (25) observed maximal GI activity at 16 h of growth period, whereas, the strain used by Hasal (28) showed optimized activity after 20-24h of incubation.

5 Conclusion

The present study concludes that the CS1 bacterial strain could produce reasonable GI activities in mediums using agriculture waste such as sugarcane bagasse and wheat straw.

References

Bhosale, S.H., Rao, M.B. -& Deshpande, V.V. (1996). Molecular and Industrial Aspects of Glucose Isomerase. *Microbiological Reviews*, 60(2), 280-300

Ishizaki, H. & Hasumi, K. (2014). Ethanol Production from Biomass. Research Approaches to Sustainable Biomass Systems.Elsevier, USA. 243-258.

Wang, P. Y., B. F. Johnson, and H. Scneider. 1980. Fermentation of Dxylose by yeasts using glucose isomerase in the medium to convert D-xylose to D-xylulose. Biotechnol. Lett. 2:273–278.

Harcus, D., Dignard, D., Lépine, G., Askew, C., Raymond, M., Whiteway, M., Wu, C. (2013) Comparative xylose metabolism among the Ascomycetes *C. albicans, S. stipitis and S. cerevisiae.* PLoS One. 8(11):e80733.

Vongsuvanlert, V. & Tani, Y. (1988) Purification and characterization of xylose isomerase of a methanol yeast, *Candida boidinii*, which is involved in sorbitol production from glucose. *Agriculture and Biological Chemistry*, 52, 1817–1824.

Sayyed, R.Z. & Shimpi, G.B. (2010) Constitutive production of extracellular glucose isomerase by an osmophillic *Aspergillus* sp. under submerged conditions. *Journal of Food Science and Technology*, 47(5), 496-500.

Aehel, W. (ed.) (2007). Production and Applications, Industrial enzyme: Enzymes in Industry. John Wiley and Sons Ltd, England.

Callens, M.H., Vangrysperre, O. & Debruyne, C.K. (1988) D-Xylose isomerase from *Streptomyces violaceoruber*: structural and catalytic roles of bivalent metal ions. *Enzyme and Microbial Technology*, 10, 695–700.

Callens, M.H., Vangrysperre, O. & Debruyne, C.K. (1986) Catalytic properties of D-xylose isomerase from *Streptomyces violaceoruber*. *Enzyme and Microbial Technology*, 8, 696–700.

Kasumi, T.K., Hayashi, T. & Sumura, N. (1982) Role of cobalt in stabilizing the molecular structure of glucose isomerase from *Streptomyces griseofuscus* S-41. *Agriculture and Biological Chemistry*, 46, 21–30.

Waqar A. (2013) Production of glucose/xylose isomerase from different bacterial strains. Unpublished BSc (Honours) thesis. Kinnaird College for Women. Lahore, Pakistan.

Pandey, A., Webb, C., Soccol, C.R. & Larroche, C. (eds.) (2008). Enzyme technology. Asiatech publishers, New Delhi.

Marshall, R.O. & Kooi, E.R. (1957) Enzymatic conversion of D-glucose to D-fructose. *Science*, 125, 648–649.

Lobanok, A.G., Sapunova, L.I., Dikhtievski, Y.O. & Kazakevich, I.O. (1998) Screening of glucose isomerase-producing microorganisms. *World Journal of Microbiology and Biotechnology*, 14, 259-262.

Givry, S. & Duchiro, F. (2008) Optimization of culture medium and growth conditions for production of L-arabinose isomerase and D-xylose isomerase by *Lactobacillus bifermentas*. *Mikrobiologiia*, 77(3), 324-330

Shazadi, T., Mehmood, S., Irshad, M., Anwar, Z., Afroz, A., Zeeshan, N., Rashid, U. & Sughra, K. (2014) Advances in lignocellulosic biotechnology: A brief review on lignocellulosic biomass and cellulases. *Advances in Bioscience and Biotechnology*, 5(3), 246-251.

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

Bhasin, S. & Modi, H.A. (2012). Optimization of fermentation medium for production of glucose isomerase using *Streptomyces* sp. SP-B1. *Biotechnology Research International*, 2012, 874152.

Manhas, R.S. & Bala, S. (2004). Thermostable glucose isomerase production by some bacteria and *Streptomyces*. *Indian Journal of Microbiology*, 44(2), 129–132.

Pawar, H.S. & Deshmukh, D.R. (1994) Immobilization of D-xylose (D-glucose) from a Chainia species. *Preparative Biochemistry*, 24(2), 143-150.

Takasaki Y (1974) Formation of Glucose Isomerase by Streptomyces sp., Agricultural and Biological Chemistry, 38:3, 667-668.

Yoshimura, S., Danno, G. & Natake, M. (1966) Studies on D-glucose isomerizing activity of D-xylose grown cells from *Bacillus coagulans*, strain HN-68. I. Description of the strain and conditions for formation of the activity. *Agriculture Biological Chemistry*, 30, 1015-1023.

Thi Nguyen HY, Tran GB. (2018) Optimization of Fermentation Conditions and Media for Production of Glucose Isomerase from *Bacillus megaterium* Using Response Surface Methodology. *Scientifica*, 2018, 6842843.

Jenkins, J., Janin, J., Rey, F., Chiadmi, M., van Tilbeurgh, H., Lasters, I., De Maeyer, M., Van Belle, D., Wodak, S.J., Lauwereys, M., et al. (1992) Protein engineering of xylose (glucose) isomerase from *Actinoplanes missouriensis*. 1. Crystallography and site-directed mutagenesis of metal binding sites. Biochemistry, 31(24), 5449-58.

Deshmukh, S.S., Deshpande, M.V. & Shankar, V. (1994). Medium optimization for the production of glucose isomerase from thermophilic *Streptomyces thermonitrificans*. *World Journal of Microbiology & Biotechnology*, 10, 264-267

Takasaki, Y. (1966) Studies on sugar-isomerizing enzyme: production and utilization of glucose isomerase from *Streptomyces* sp. *Agricultural and Biological Chemistry*, 30, 1247-1253.

Sequro-munoz, S.I., da Silva Oveira, A., Nikaido, M., Trevilato, T.M., Bocio, A., Takayanaqui, A.M. & Domingo, J.L. (2006) Metal levels in sugar cane (*Saccharum* spp.) samples from an area under the influence of a municipal landfill and a medical waste treatment system in Brazil. *Environment International*, 32(1), 52-57.}

Hasal, P., Cejkova, A. & Vojtisek, V. (1992). Glucose Isomerase Production by *Streptomyces* sp. CCM, 4102. *Folia Microbiologia*, 37 (5), 365-371.

Tables.

Table 1: Metal-cofactor composition used in the mediums

Sr. No	**Metal ion**	**Concentration used**
1	Mg^{2+}	0.02%
2	Mn^{2+}	0.01%
3	Co^{2+}	0.005%
4	Fe^{2+}	0.005%
5	Co^{2+} and Fe^{2+}	0.005% each
6	Mg^{2+} and Mn^{2+}	0.02% and 0.01%, respectively
7	Mg^{2+}, Mn^{2+}, Co^{2+} and Fe^{2+}	0.02%, 0.01%, 0.005% and 0.005%, respectively

Table 2 Composition of the three optimized mediums

Component (%)	**Medium 1**	**Medium 2**	**Medium 3**
Xylose	-	-	2
Wheat straw	-	1	-
Sugarcane bagasse	1.5	-	-
Peptone (Soybean)	-	-	1
Peptone (Casein)	-	1	-
Tryptone	0.5	-	-
Yeast extract	0.5	-	-
NaCl	1.168	1.168	1.168
K_2HPO_4	0.1	0.1	0.1
$MgSO_4$	-	-	0.02
$MnCl_2$	-	0.01	-
$FeSo_4$	0.005	-	-
$CoCl_2$	0.005	-	-

Figures

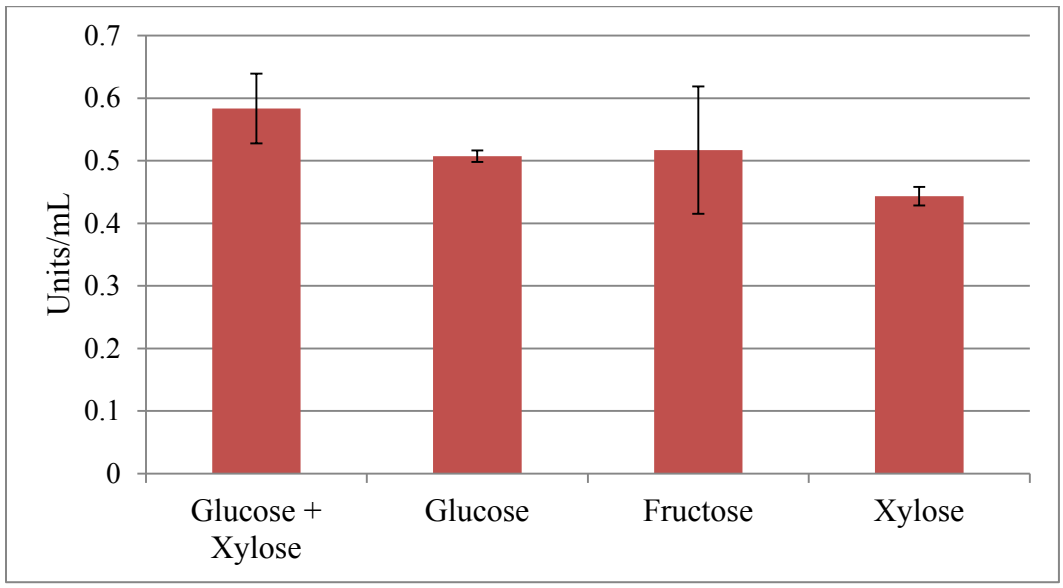


Fig. 1: Effect of pure carbon sources on GI activity of CS1 bacterial strain .Values are a mean of two-replicates ± SD.

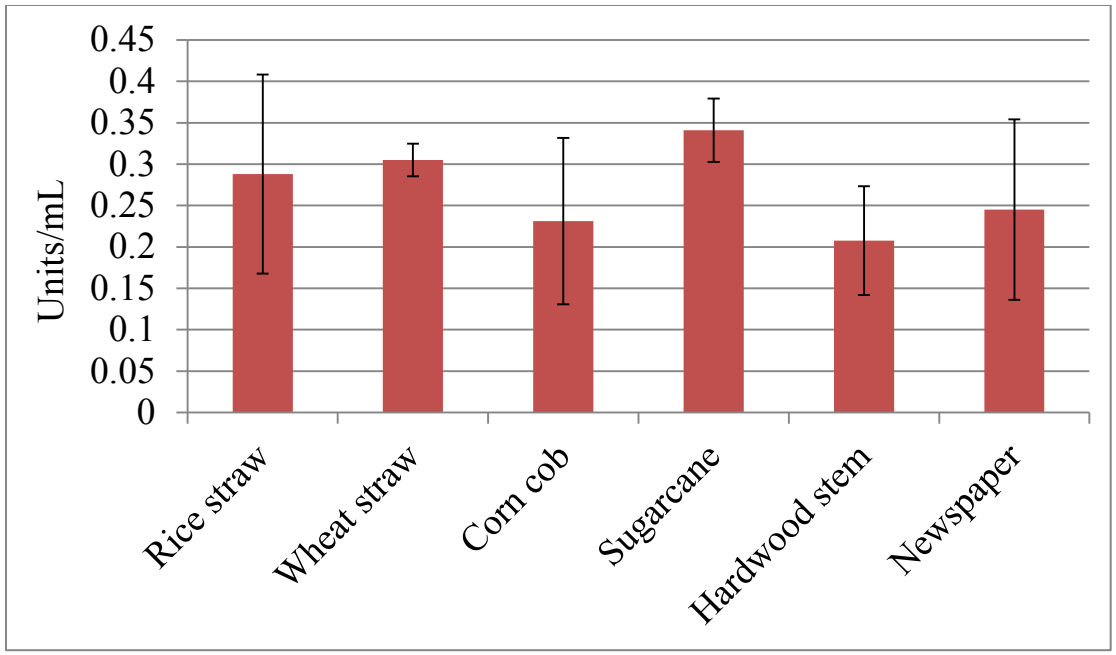


Fig. 2: Effect of natural carbon sources on GI activity of CS1 bacterial strain. Values are a mean of two-replicate ± SD.

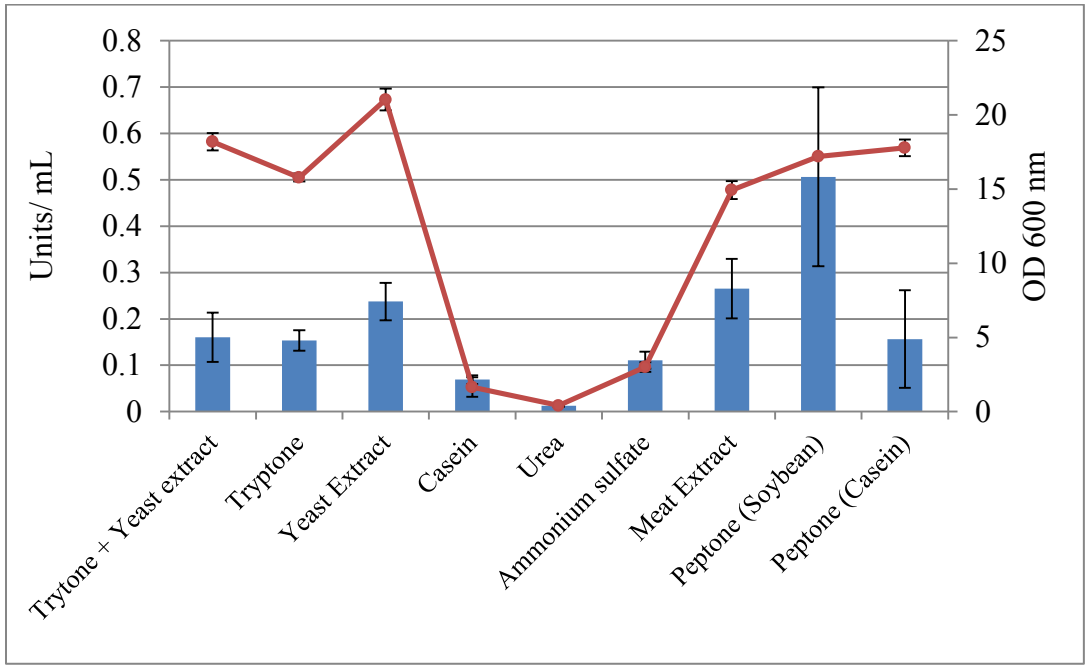


Fig. 3: Effect of nitrogen sources on growth and GI activity of CS1 bacterial strain grown on xylose. GI activity, Growth

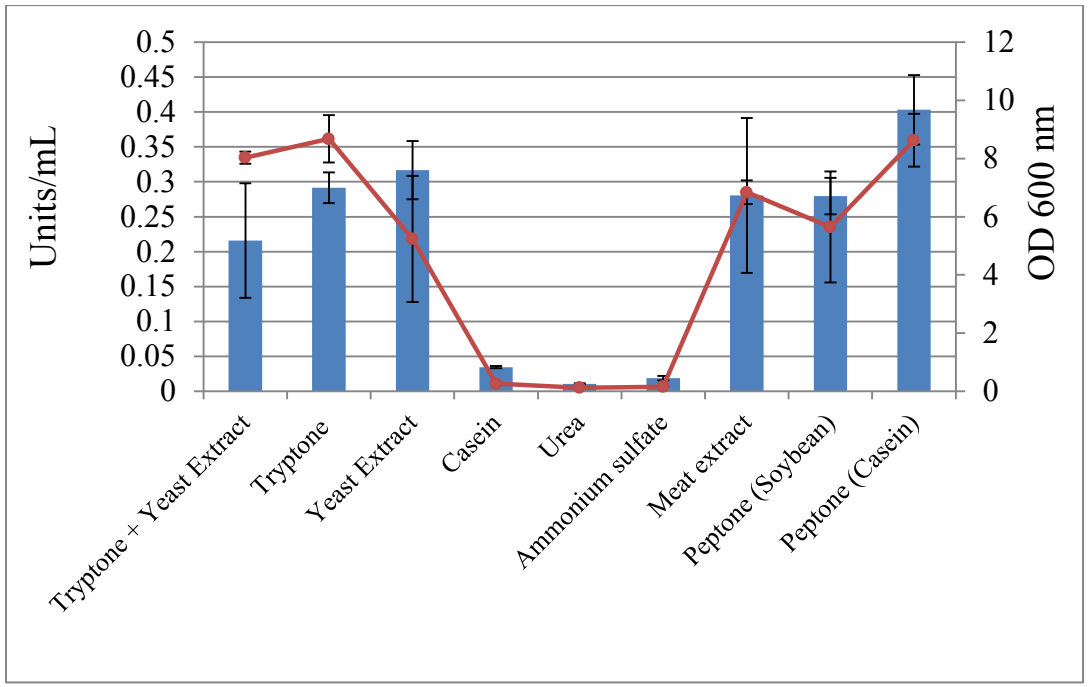


Fig. 4: Effect of nitrogen sources on growth and GI activity of CS1 bacterial strain grown on wheat straw. GI activity, Growth

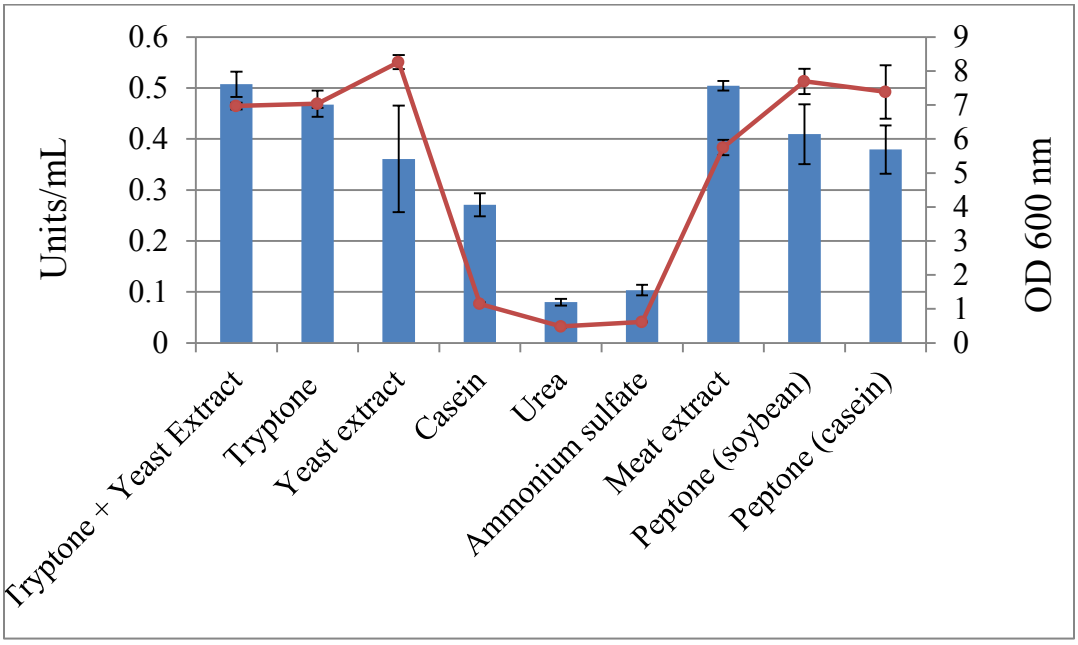
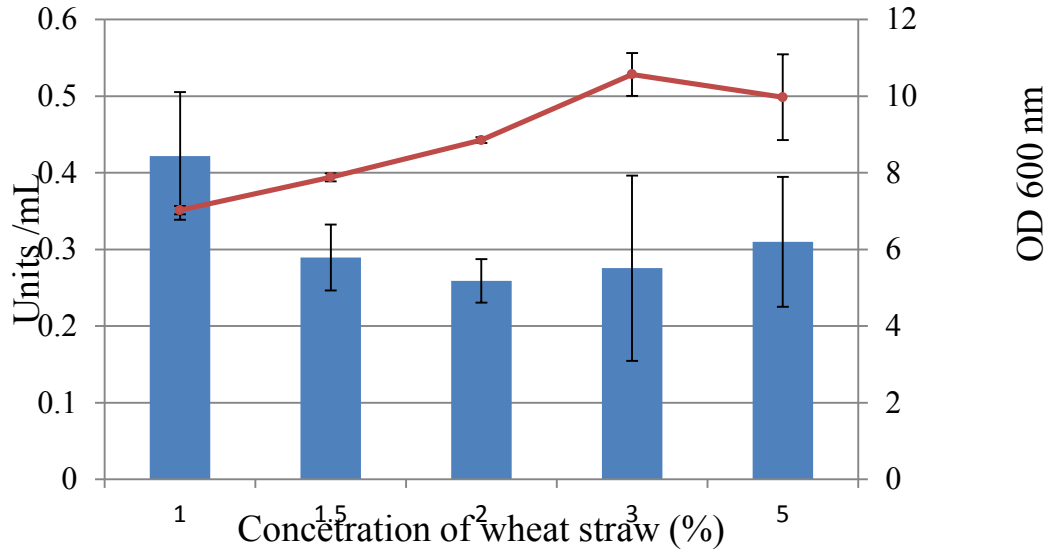
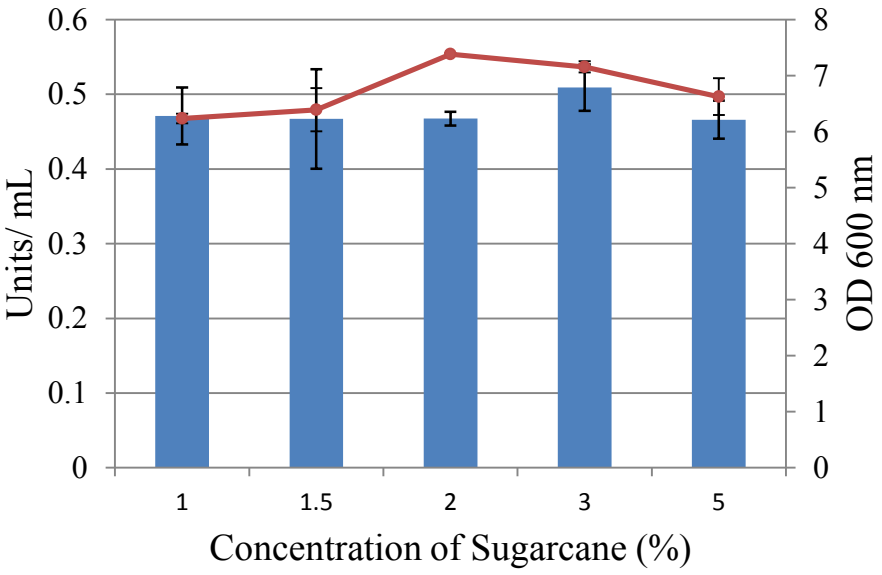


Fig. 5: Effect of nitrogen sources on growth and GI activity of CS1 bacterial strain grown on sugarcane bagasse. GI activity, Growth

A



B



C

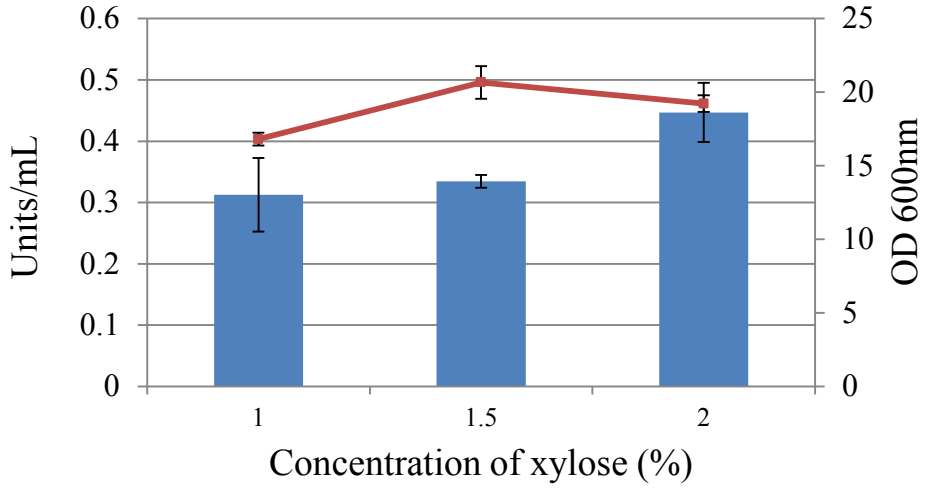


Fig. 6: Quantitative effect of different carbon sources on growth and GI activity of CS1 bacterial strain A) wheat straw B) sugarcane C) xylose. GI activity, Growth

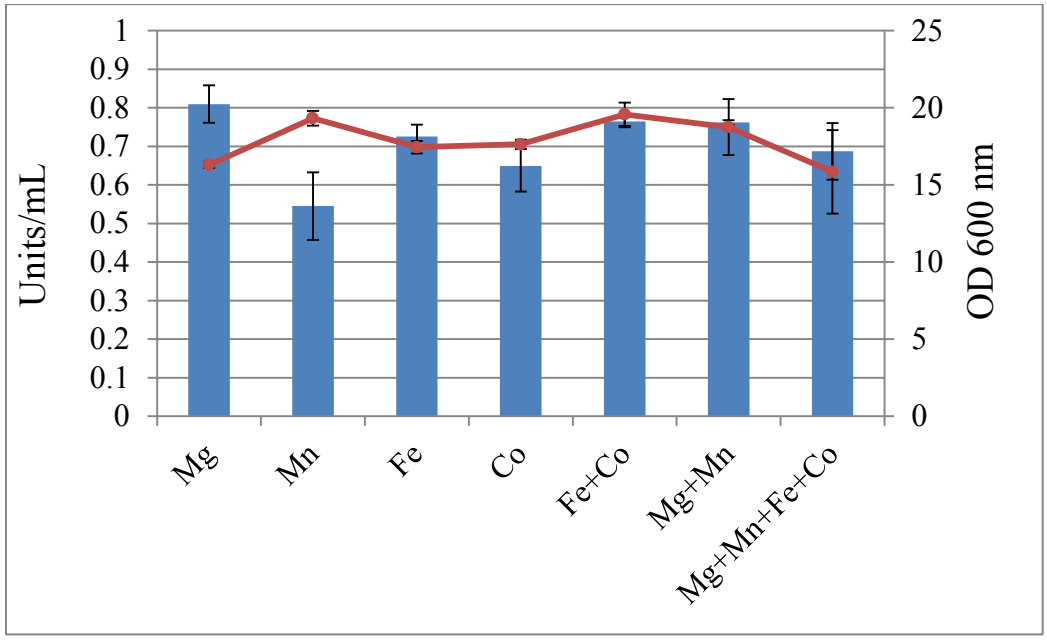


Figure 7: Effect of metal ions on glucose isomerase activity and growth of CS1 bacterial strain grown on xylose. GI activity, Growth

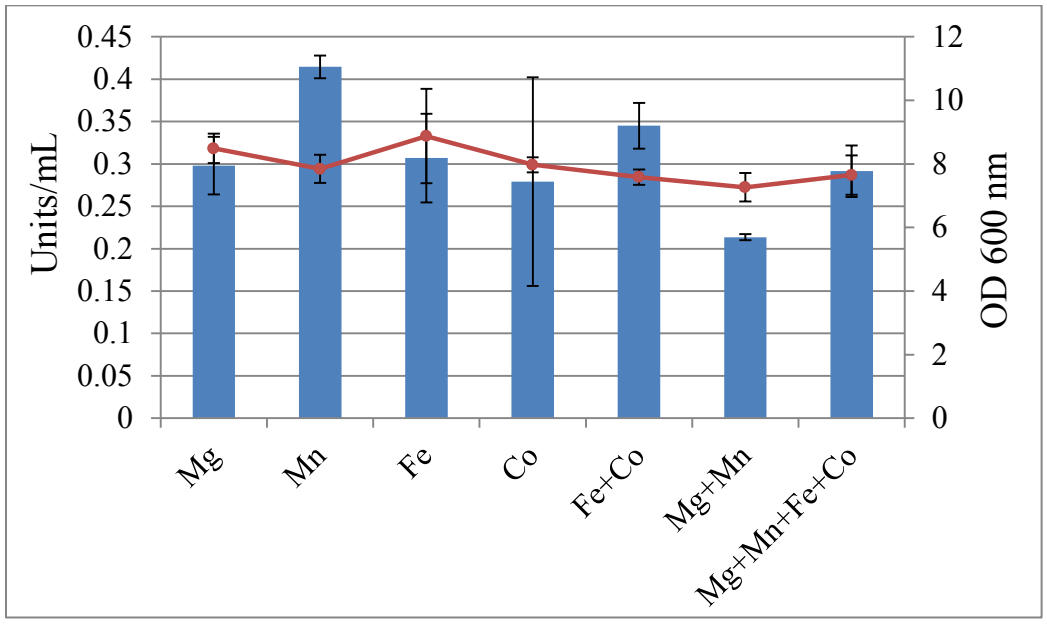


Fig. 8: Effect of metal ion combinations on glucose isomerase activity and growth of CS1 bacterial strain grown on wheat straw. GI activity, Growth

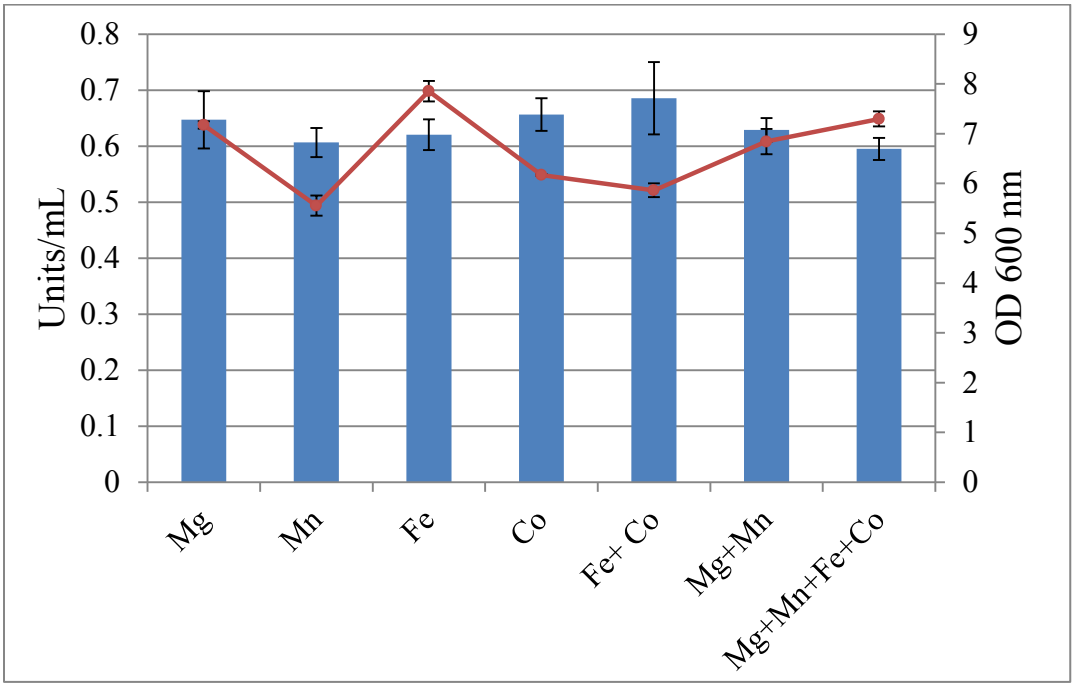


Fig. 9: Effect of metal ion combinations on GI activity and growth of CS1 bacterial strain grown on sugarcane bagasse. GI activity, Growth

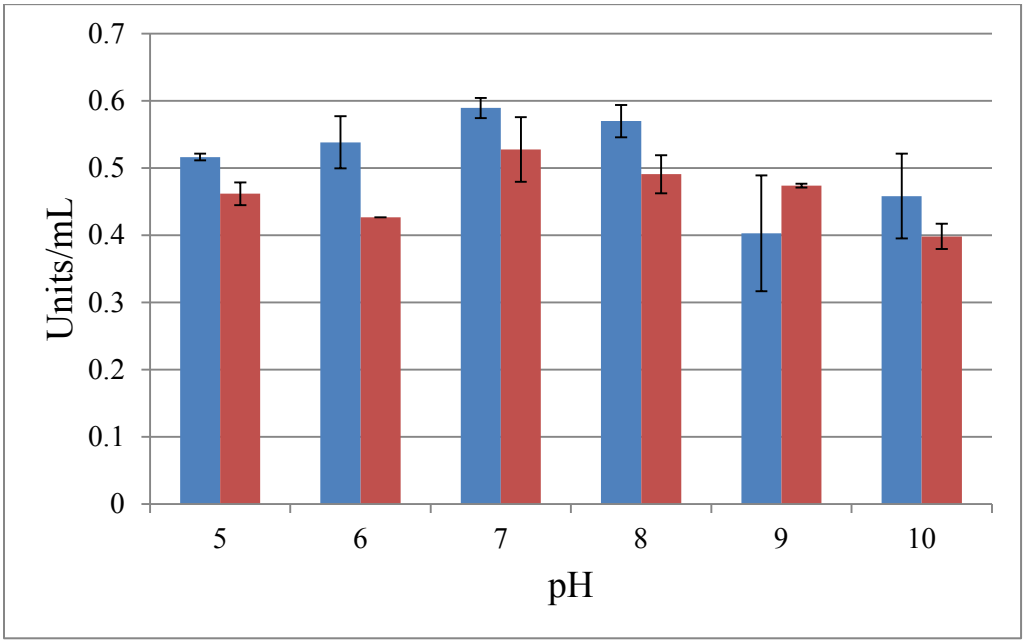


Fig. 10: pH dependence of GI activity of CS1 bacterial strain grown in optimized medium 1 and 2 .

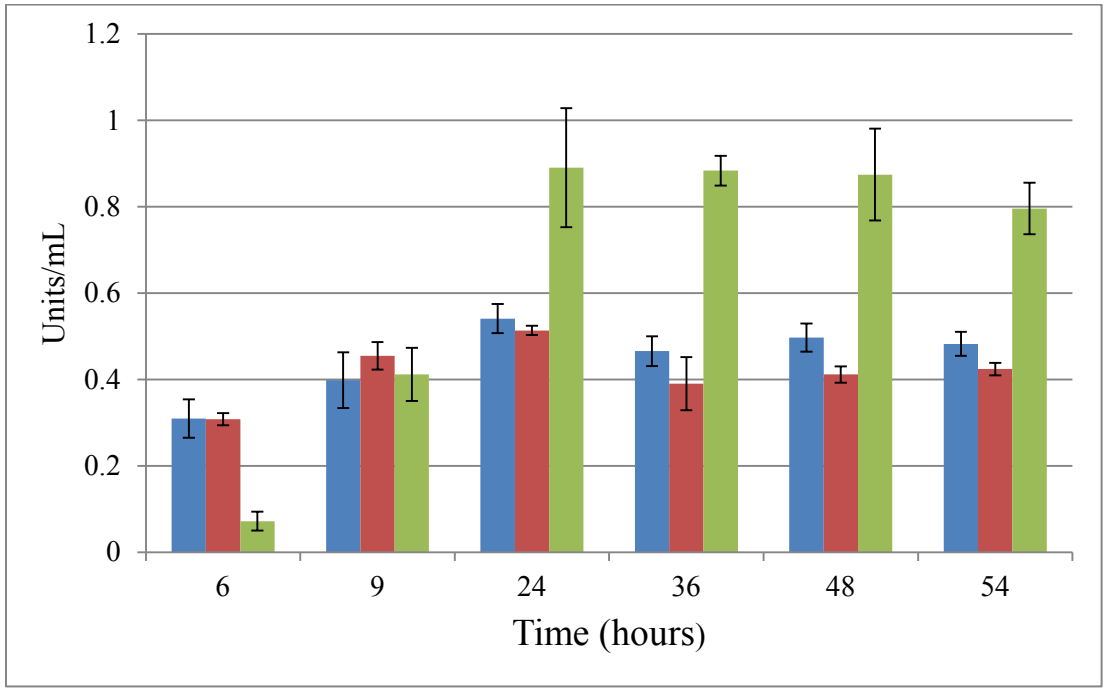


Fig. 11: Effect of incubation period on GI activity of CS1 bacterial strain grown on optimized medium 1 ■, medium 2 ■ and medium 3 ■.