

Full Length Research Paper

The effects of activation time on the production of fructose and bioethanol from date extract

Gaily, M. H.¹, Sulieman, A. K.¹, Zeinelabdeen, M. A.¹, Al-Zahrani, S. M.¹, Atiyeh, H. K.² and Abasaheed, A. E.^{1*}

¹Chemical Engineering Department, King Saud University, PO Box 800, Riyadh, 11241, Saudi Arabia.

²Biosystems and Agricultural Engineering, 227 Agricultural Hall, Oklahoma State University, Stillwater, OK 74078-6021, USA.

Accepted 30 March, 2012

In this study, fructose and bioethanol were produced from date's extract by fermentation using *Saccharomyces cerevisiae*, mutant strain ATCC 36858 and wild strain STAR brand, where the latter was activated at different periods: 30 min, 1, 2 and 3 days. Profiles of sugar consumption and bioethanol production using STAR were found to be almost having the same pattern for all activation periods, while fructose was consumed as well as glucose. Enhancement of fructose in sugar can only be obtained at the expense of ethanol. In order to obtain 75 and 90% fructose in sugar, the respective losses in fructose exceeded 39 and 63%. On the other hand, the results demonstrate that *S. cerevisiae* ATCC 36858 could selectively convert glucose to ethanol and biomass with minimal fructose conversion. A high fructose yield above 91% of the original fructose was obtained with ATCC 36858. In addition, the ethanol yield was found to be 63% of the theoretical.

Key words: *Saccharomyces cerevisiae*, fructose, glucose, bioethanol, fermentation.

INTRODUCTION

Sugars are carbohydrate materials produced each day from water and atmospheric carbon dioxide by photosynthesis (Hassoune et al., 2008). Dates are sugar-rich and low moisture fruits. On average, the sugar content is 0.8 g per gram dry matter (Guizani et al., 2010). Most of the carbohydrates in dates are in the form of reduced sugars, mainly fructose and glucose (Al-Farsi et al., 2007; Kulkarni et al., 2008). The global production of date fruits exceeds six million metric tons annually in the world (Boudries et al., 2007; Guizani et al., 2010). Dates are generally consumed as fresh or may be processed, especially low grade dates, into various products such as date paste, syrup or powder (Guizani et al., 2010).

Fructose is 60% sweeter than sucrose and 150% more than glucose. The major use of fructose syrup is in food and beverage industries at relatively high concentrations.

High fructose syrups (HFS) are produced from different raw materials including corn starch, sugar cane, and sugar beet, in addition to other starchy raw materials like rice and dates (Hanover and White, 1993; Vuilleumier, 1993). Enzymatic isomerization techniques are used to convert glucose into fructose, but the conversion is equilibrium-limited at around 42% fructose (Zhang et al., 2004). 90% HFS are produced through costly multistage chromatographic techniques (Atiyeh and Duvnjak, 2001; 2002).

Ethanol is also an important renewable and sustainable alternative clean fuel source (Balat et al., 2008; Sassner et al., 2008). Nowadays, the world fuel bioethanol production exceeds 20,000 millions of gallons per year (Renewable Fuels Association, 2010; Astudillo and Alzate, 2011). Selective fermentation is an efficient process for large scale production of fructose and bioethanol. In fermentation process, several microorganisms (*Saccharomyces cerevisiae* and *Zymomonas mobilis*) can be used to produce fructose and bioethanol. Generally, the use of mutants of these microorganisms

*Corresponding author. E-mail: abasaheed@ksu.edu.sa. Fax: +966-1-4678770.

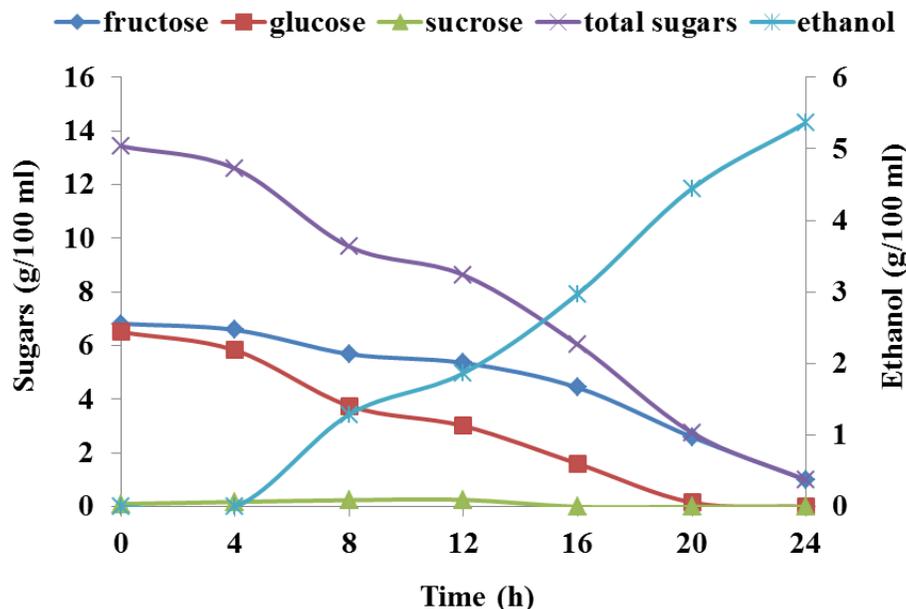


Figure 1. Profiles of sugars and ethanol for STAR yeast fermented at 33°C and zero activation days.

decreases the consumption of fructose (Atiyeh and Duvnjak, 2001, 2002, 2003).

The objective of this study was to evaluate the production of fructose and/or bioethanol from date's extract by fermentation at a fixed temperature using a mutant strain *S. cerevisiae* ATCC 36858 and a commercial *S. cerevisiae* (STAR brand) activated at different periods.

MATERIALS AND METHODS

The substrate was prepared from dates (Ruzaiz variety) using deionized water. The concentration of total sugars in the extracted date syrup was adjusted to 15% sugars (before dilution with growth medium), and was sterilized in an autoclave at 121°C for 15 min. Two strains of *S. cerevisiae* were used; mutant ATCC 36858 and commercial strain (STAR brand). Both strains were inoculated in Yeast Malt Broth (YM broth) prepared by adding 3 g bacto-yeast extract, 3 g bacto-malt extract, 5 g bacto-peptone, and 10 g bacto-dextrose to 1.0 L deionized water. The broth was sterilized at 121°C for 15 min. 2 g of the STAR yeast was added to 100 ml sterilized broth and then propagated for different activation periods. Activations of the yeast were conducted in a controlled temperature water bath shaker at 30°C and 120 rpm. The mutant *S. cerevisiae* ATCC 36858 obtained from American Type Culture Collection, USA in a pellet form was activated and transferred according to the standard procedure of the ATCC.

To study the effect of the activation time, four different sets of fermentation experiments were performed using the four different propagated broths (0, 1, 2 and 3 days) of STAR yeast at 33°C without controlling pH. 100 ml of the substrate and yeast medium was put in a sterile 500 ml conical flask for fermentation. Samples were taken every 4 h using a sterilized disposable pipette. A portion of the sample was centrifuged at 15000 rpm for 1 min to separate the cells from the solution and then the clear solution was

transferred to a small vial for sugars and ethanol analysis. The other portion was transferred to sterile test tubes for cell count determination. The sugars and ethanol were determined quantitatively using high performance liquid chromatography (HPLC; Agilent 1200 Infinitely series) equipped with an RID detector and Aminex® column. Cell count was determined by NucleoCounter® YC-100™ system cell counter.

RESULTS AND DISCUSSION

Fermentation with STAR yeast

The profiles of sugars and ethanol produced during fermentation at different activation periods of 0, 1, 2 and 3 days are illustrated in Figures 1 to 4, respectively. Along with fermentation, glucose and fructose were consumed; however, glucose consumption rate was higher than that of fructose. For the three activation periods 0, 1 and 2 days (Figures 1 to 3), there was a sharp drop in glucose from 4 to 8 h fermentation time, while for the 3 days activation, glucose consumption was almost linear (Figure 4). However, for all activation periods, glucose was consumed totally after 24 h. This proves that longer activation periods enhance sugar consumption at constant rates. This pattern of sugar consumption is reflected on ethanol production as shown in Figures 1 to 4.

For all activation periods under investigation, the ethanol yield was found to be in the range of 83.6 to 85.3% of the theoretical yield as shown in Table 1. The results show that over 93% of total sugars were consumed upon fermentation with *S. cerevisiae* STAR brand.

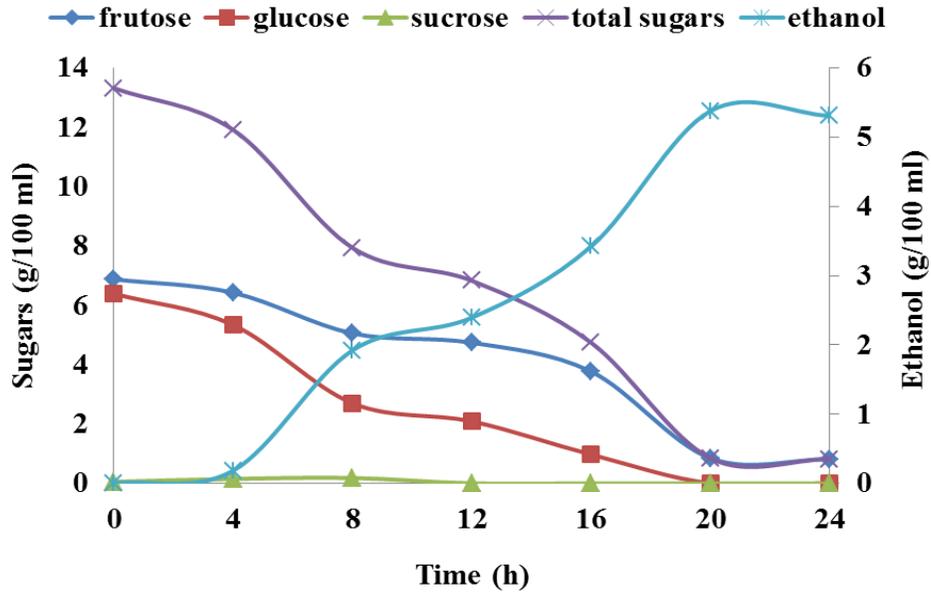


Figure 2. Profiles of sugars and ethanol for STAR yeast, fermented at 33°C and 1 activation day.

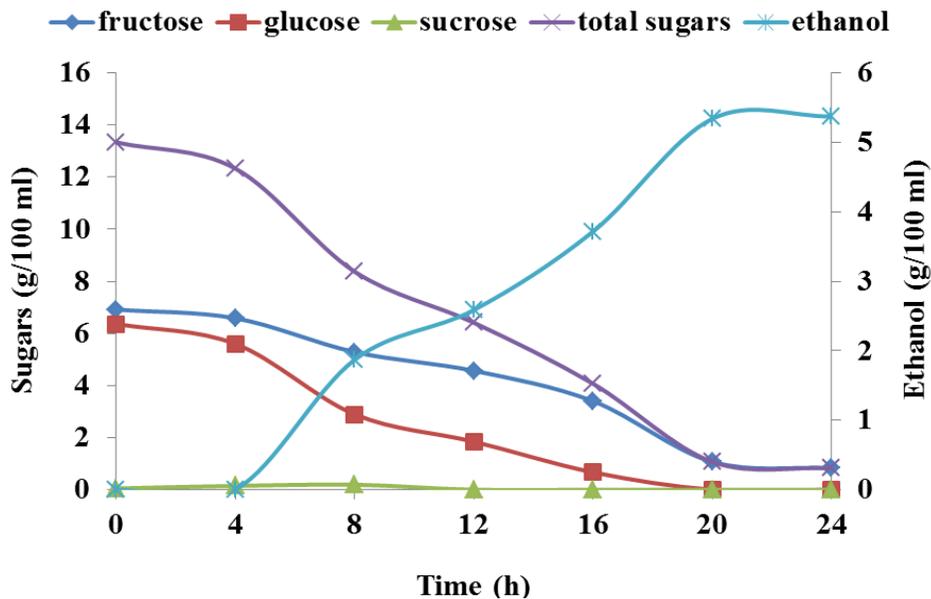


Figure 3. Profiles of sugars and ethanol for STAR yeast fermented at 33°C and 2 activation days.

In order to produce higher fructose concentrations, the fermentation should be stopped at intermediate times; stopping the fermentation after 12 h would produce 70% fructose in syrup for activation time of 3 days. Moreover, Figure 5 shows the percentage fructose losses for the four different activation time of fermentation with *S. cerevisiae* STAR brand at 33°C. Enhancement of fructose in sugar can only be obtained at the expense of

ethanol production. The average ethanol yield was found to be about 83% at higher fructose losses of 63%.

Fermentation pattern of ATCC 36858

Figure 6 shows the profiles of a typical fermentation process of sugars in dates syrup by *S. cerevisiae* ATCC

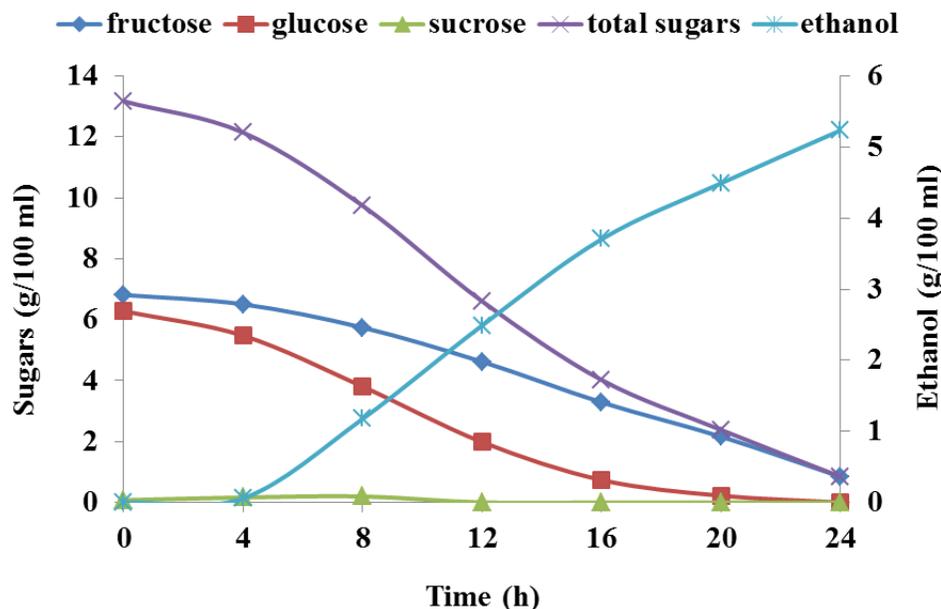


Figure 4. Profiles of sugars and ethanol for STAR yeast fermented at 33°C and 3 activation days.

Table 1. Summary of fermentation results with STAR yeast.

Activation time (day)	Initial total sugars (g/100 ml)	Average fructose consumption rate ($\text{g L}^{-1}\text{h}^{-1}$)	Average glucose consumption rate ($\text{g L}^{-1}\text{h}^{-1}$)	Ethanol yield (%)
0	13.43	2.1	2.3	85.3
1	13.32	2.2	2.3	83.6
2	13.33	2.2	2.3	84.6
3	13.17	2.1	2.2	83.8

36858. It is clear from the Figure that this strain can selectively ferment glucose with minimum effect on fructose, thus resulting in higher concentration of fructose in the remaining sugars after 48 h. All glucose was fermented and converted to ethanol and biomass, while fructose losses were less than 8.5% of the original fructose. On the other hand, production of ethanol from glucose was observed to increase as fermentation proceeded. The yield of ethanol produced on total sugars was found to be 63% of the theoretical ethanol yield. The biomass increased from 1.2 g/100 ml initially to 16.9 g/100 ml. In the beginning of the process, the yeast needed more than 4 h to start fermenting sugars, after that selective fermentation of glucose proceeded with an average glucose consumption rate of $1.4 \text{ g l}^{-1}\text{h}^{-1}$.

Although *S. cerevisiae* yeast was found to be a suitable microorganism among others for fermentation of sugars and for ethanol production, however, different strains of *S. cerevisiae* was found to show different fermentation pattern on sugars depending on the strain type. In this study, the results demonstrate the ability of *S.*

cerevisiae ATCC 36858 to selectively convert glucose to ethanol and biomass, while fructose accumulated. This strain is completely different from the STAR strain of *S. cerevisiae* that utilised both glucose and fructose to ethanol and biomass. Regarding fermentation temperature and shaking speed, both strains were found to be active at 33°C and 120 rpm, respectively, although the two strains fermented the sugars in different ways. This result agrees with the findings of Noor et al. (2003) who reported that for different *S. cerevisiae* strains, the best and suitable fermentation temperature was found to be in the range of 28 to 36°C with 120 rpm shaking speed. The pH was found to drop from 5.2 to around 4 at the end of the fermentation process for all fermentation runs under investigation.

Conclusions

Two *S. cerevisiae* yeast strains were used in this preliminary study; STAR and ATCC 36858. STAR yeast was

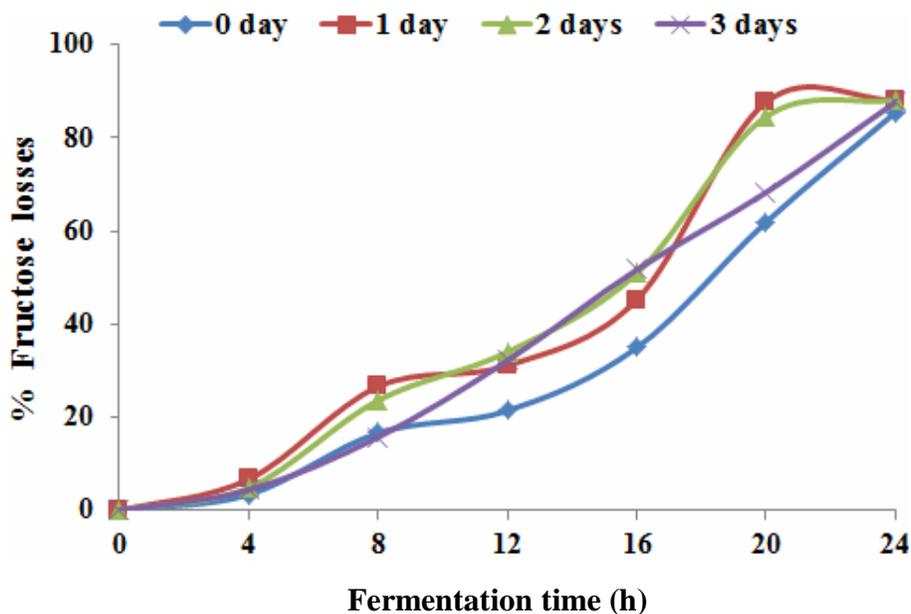


Figure 5. Percent (%) fructose losses during fermentation with STAR yeast.

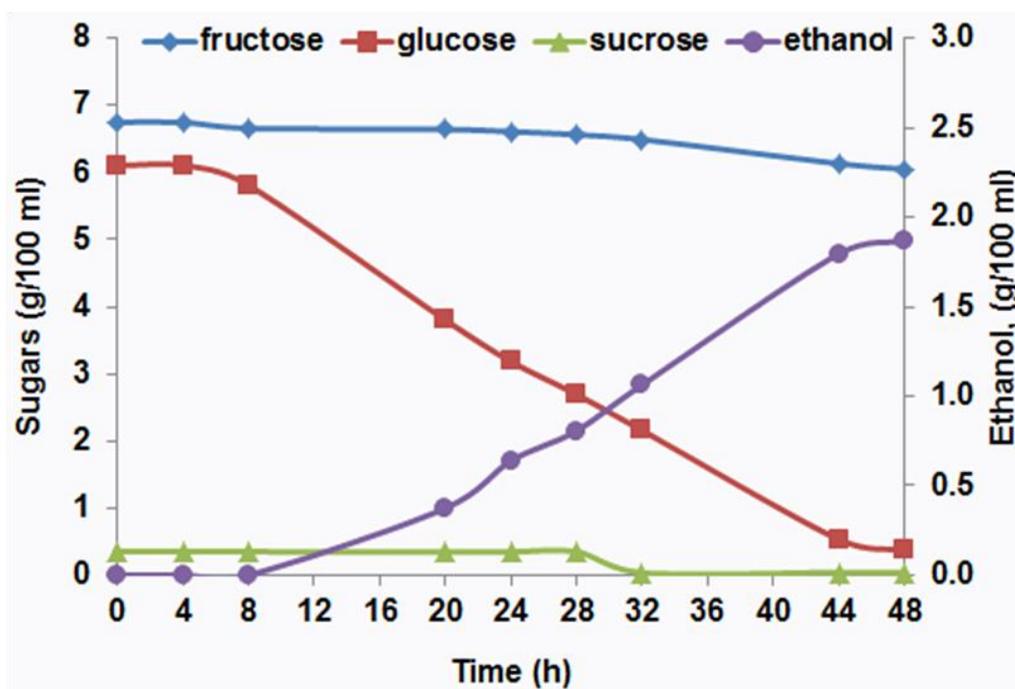


Figure 6. Profiles of sugars and ethanol for ATCC 36858 yeast fermented at 33°C.

found to ferment indiscriminately, glucose and fructose in the date's syrups to mainly ethanol. Production of high fructose concentration can only be achieved with this yeast by sacrificing ethanol. Attempts to enhance the selectivity of the fermentation towards glucose by changing activation times resulted in insignificant improvements

in performance. Hence, while STAR yeast is functional in fermenting dates syrups to ethanol, it is not a preferred candidate for producing higher fructose concentration. On the other hand, ATCC 36858 was able to selectively convert glucose to ethanol and biomass, and very high fructose yields were obtained with ATCC 36858.

ACKNOWLEDGEMENT

The authors extend their appreciation to the National Plan for Science and Technology (NPST) at King Saud University for their generous support and funding of this study as a part of project # 08-ADV391-02.

REFERENCES

- Al-Farsi M, Alasalvar C, Al-Abid M, Al-Shoaily K, Al-Amry M, Al-Rawahy F (2007). Compositional and functional characteristics of dates, syrups, and their by-products. *Food Chem.* 104: 943-947.
- Astudillo ICP, Alzate CAC (2011). Importance of stability study of continuous systems for ethanol production. *J. Biotechnol.* 151: 43-55.
- Atiyeh H, Duvnjak Z (2001). Production of fructose and ethanol from media with high sucrose concentrations by a mutant of *S. cerevisiae*. *J. Chem. Technol. Biotechnol.* 76: 1017-1022.
- Atiyeh H, Duvnjak Z (2002). Production of fructose and ethanol from sugar beet molasses using *S. cerevisiae* ATCC 36858. *Biotechnol. Prog.* 18: 234-239.
- Atiyeh H, Duvnjak Z (2003). Production of fructose and ethanol from cane molasses using *S. cerevisiae* ATCC 36858. *Acta Biotechnol.* 23(1): 37-48.
- Balat M, Balat H, Öz C (2008). Progress in bioethanol processing. *Prog. Energy Combust. Sci.* 34: 551-573.
- Boudries H, Kefalas P, Hornero-Méndez D (2007). Carotenoid composition of Algerian date varieties (*Phoenix Dactylifera*) at different edible maturation stages. *Food Chem.* 101: 1372-1377.
- Guizani N, Al-Saidi GS, Rahman MS, Bornaz S, Al-Alawi AA (2010). State diagram of dates: Glass transition, freezing curve and maximal-freeze-concentration condition. *J. Food Eng.* 99: 92-97.
- Hanover ML, White JS (1993). Manufacturing, composition, and applications of fructose. *Am. J. Clin. Nutr.* 58: 724S-732S.
- Hassoune H, Rhlalou T, Verchere JF (2008). Studies on sugars extraction across a supported liquid membrane: Complexation site of glucose and galactose with methyl cholate. *J. Membr. Sci.* 315: 180-186.
- Kulkarni SG, Vijayanand P, Aksha M, Reena P, Ramana KVR (2008). Effect of dehydration on the quality and storage stability of immature dates (*Phoenix dactylifera*). *LWT-Food Sci. Technol.* 41: 278-283.
- Noor AA, Hameed A, Bhatti KP, Tunio SA (2003). Bio-ethanol fermentation by the bioconversion of sugar from Dates by *S. cerevisiae* strains ASN-3 and HA-4. *Biotechnol.* 2(1): 8-17.
- Renewable Fuels Association (2010). Ethanol industry statistics: annual world ethanol production by country. Washington. Available from: <http://www.ethanolrfa.org/pages/statistics/#E> (11.10. 2010).
- Sassner P, Galbe M, Zacchi G (2008). Techno-economic evaluation of bioethanol production from three different lignocellulosic materials. *Biomass Bioenerg.* 32: 422-430.
- Vuilleumier S (1993). Worldwide production of high-fructose syrup and crystalline fructose. *Am. J. Clin. Nutr.* 58: 733S-736S.
- Zhang Y, Hidajat K, Ray AK (2004). Optimal design and operation of SMB bioreactor: production of high fructose syrup by isomerization of glucose. *Biochem. Eng. J.* 21: 111-121.