STATISTICAL OPTIMIZATION OF FERMENTATION PARAMETERS USING PLACKET-BURMAN FOR ENHANCED XYLITOL PRODUCTION BY *PICHIA STIPITIS* NCIM 3498.

Jyosthna K. Goli[1], Smita H. Panda[2], Venkateswar R. Linga[1] and Hameeda Bee[1*]

[1*]Department of Microbiology, Osmania University, Hyderabad-500007 (Telangana) India.
[2]Department of Zoology, North Orissa University, Baripada-757003 (Odisha) India.

Abstract

Xylitol a polyol, gaining its importance in food and pharmacological industry due to its low calorific value, anti-cariogenic nature and effective in treating diabetes, anaemia, acute otitis media and osteoporosis. Physico-chemical factors such as temperature, pH, rpm, inoculum size, xylose concentration, co-substrate and nitrogen source play a crucial role on xylitol production. Influence of above said variables on production of xylitol by one factor at a time (OFAT) strategy using *Pichia stipitis* NCIM 3498 was performed and 30°C, pH5, 200rpm, 48h, 15% inoculum, 7% xylose with glucose as co-substrate and NH_4NO_3 as nitrogen source was found to be optimum for xylitol production. Further, when Plackett-Burman statistical model was used to screen effective medium components, variables like xylose, peptone, magnesium sulphate, ammonium nitrate and di-sodium hydrogen phosphate were found to be highly significant for xylitol production and represented a confidence level of 97%. Validation of xylitol production under optimum conditions has shown maximum xylitol yield concentration and productivity of 52g/l, 0.74g/g and 1.08g/l/h respectively. Xylitol production has increased nearly threefold from 19 g/l to 52g/l using Plackett Burman design.

Key words: xylitol, Anaemia, Osteoporosis, (OFAT), Plackett-Burman.

Introduction

As the consumers are health and weight consciousness, they are inclining towards sugar-free and low-calorie food products due to which the demand for xylitol is increasing (Rao *et al.,* 2016). Xylitol is a low-calorie five-carbon pentinol, became an attractive alternative sweetener for the treatment of diabetics as it is insulin independent Xylitol has numerous applications in the preparation of food, pharmaceutical and cosmetic products (Zhang *et al.,* 2014; Tizazu *et al.,* 2018). It has high heat of dissolution and endothermic solution of heat (34.8 cal g/l1), that gives pleasant cool and fresh mint sensation, hence used in the manufacture of oral products like chewing gum, toothpaste, mouthwash (Branco *et al.,* 2011).

Xylitol is anti-cariogenic, it inhibits *Streptococcus mutans* and avoid acid formation that attack the tooth enamel (Ramesh *et al.,* 2013; Mohamad *et al.,* 2015). Xylitol considerably improves the biomechanical properties of the bones and prevents the reduction in density, calcium and phosphorus mineral content, hence used for the treatment of osteoporosis (Jeevan *et al.,* 2011). Erythrocytic glucose-6-phosphate dehydrogenase deficiency can also be treated with xylitol (J.K. Goli *et al.,* 2012).

Industrial xylitol production is carried out by chemical synthesis which involves toxic catalyst and operated at increased temperature and pressure with expensive steps of purification. Hence microbial production of xylitol is gaining the attention of researchers (Kogie and Ghosalkar 2016; Guirimand *et al.,* 2019). Production of xylitol by microorganisms is an environmentally friendly process as it does not need the use of toxic catalysts nor produces any racemic mixtures and is environmentally safe (Deng *et al.,* 2014). Bacteria, fungi and yeasts like microbes are usually studied for production of xylitol. Especially those belonging to *Candida guilliermondii*, *Debaromyces hansenii*, *Candida tropicalis*, *Candida boidinii*, *Pachysolen tannophilus* and *Pichia stipitis* are known to be the best xylitol producers (Prakasham *et al.,* 2009; López-Linares *et al.,* 2018). Xylose-assimilating yeasts have xylose reductase (XR) which

Author for correspondence : E-mail: drhami2009@gmail.com

reduces D-xylose to xylitol by oxidation of NADPH. Whereas xylitol dehydrogenase (XDH) which cause the oxidation of xylitol to D-xylulose which in presence of xylulose kinase gets converted into D-xylulose 5-phosphate that finally enters the pentose phosphate pathway (Rafiqul *et al.,* 2015).

The current study is to evaluate the ability of *Pichia stipitis* NCIM 3498 for enhanced xylitol production from pure D-xylose using Plackett Burman statistical design.

Materials and Methods

Microorganism and maintenance

Pichia stipitis NCIM 3498 was procured from NCIM, Pune and was maintained on YEPX (yeast extract-peptone-xylose) agar containing (g/l) yeast extract, 10; peptone, 20; Xylose, 30 and agar, 25; pH, 5.0 and stored at 4°C.

Inoculum preparation

A loopful of culture from agar slant was transferred in 250 ml Erlenmeyer flask that contained 50 ml of medium (g/l): D-xylose 30; yeast extract 3; peptone 5 and were then incubated in an shaker incubator at 150 rpm, 30°C for 24 h. The broth was centrifuged at 10,000 rpm for 10 min and the cell pellet obtained was washed, resuspended in sterile distilled water and further used for fermentation.

Effect of agitation

To find out the agitation effect on growth of organism and xylitol production, fermentation was carried out by incubation of inoculated media at 30°C for 96h by agitating at various rpm such as 100, 150, 200 and 250. Sampling was done after every 24h to estimate the concentration of xylose utilized and xylitol produced.

Effect of temperature

To determine the temperature effect on xylitol production, fermentation was conducted at temperature range of 30°, 35°, 40° and 45°C respectively at 200rpm for 96 h. The fermentative broth was collected after every 24h of incubation, centrifuged and the supernatant was estimated for leftover xylose and xylitol concentrations.

Effect of pH

To find out optimum pH on production of xylitol by *P. stipitis*, the fermentation media is maintained at different pH such as 4, 5, 6 and 7 and left for incubation at 30°C, 200 rpm for 96 h. For every 24h of incubation, samples were collected and estimated for xylose utilized and xylitol produced.

Effect of size of inoculum

To determine the effect of inoculum size on xylitol production, fermentation has been carried out at 30°C, 200 rpm for 96h by varying the inoculum percentage from 5, 10, 15 to 20.

Effect of nitrogen source

The effect of various nitrogen sources on xylitol fermentation was determined by incorporating 1.5% of various nitrogen sources like peptone, urea, ammonium sulphate, ammonium nitrate to the basal media and incubated at 30°C, 200 rpm for 96h.

Effect of co-substrate concentration

The influence of co-substrate on xylitol production was determined by adding 1.5% of different sugars like arabinose, glucose, maltose and galactose to the basal media and incubated at 30°C, 200 rpm for 96 h. The amount of xylose and xylitol in the fermentative broth was analyzed after every 24 h.

Effect of initial xylose on xylitol yield

To evaluate the optimum xylose concentration on xylitol production, fermentation is carried by varying the concentration of xylose from 2% to 8% and incubated at 30°C for 96h at 200 rpm. Samples were estimated for concentration of xylose and xylitol after every 24 h for.

Fermentation conditions

One-factor-at-a-time strategy was followed for initial optimization of physicochemical parameters by using basic media containing g/l xylose 50; yeast extract 1.5; peptone 1.5; $MgSO_4$. $7H_2O$, 1; $CaCl_2.7H_2O$ 0.5 and KH_2PO_4 0.5. However, the composition of medium for Plackett Burman studies was varied according to the design. The media is inoculated with 15% (v/v) inoculum of *P. stipitis* and incubated at 30°C with 200 rpm of agitation. Samples were collected after every 24h and centrifuged at 10,000g for 10min. The supernatant was filtered with a 0.02 -micron cellulose filter and analyzed for xylitol and residual sugar concentration using High-Performance Liquid Chromatography (HPLC) (Schimadzu). The growth was estimated using UV-VIS spectrophotometer by determining the optical density of the culture at 600 nm (OD600).

Analytical procedure

The concentrations of xylitol and leftover sugars were assessed using HPLC fitted with Repromer Ca column (USP-L19) (9 μm. 300 × 8 mm) (Dr Maisch GmbH, Germany). Water is used as the mobile phase with a flow rate of 0.6 ml/min and at column temperature 75°C using the RI detector.

Experimental design

Plackett-Burman experimental design for the screening different medium components Plackett Burman model is a statistical design used for identification of critical

parameters during medium optimization. This design evaluates N-1 variables by n number of experiments. The factors designated X1-X12 represents medium constituents. A total of 12 factors were selected for this study which includes two levels of concentrations for each factor. Every variable is represented at higher concentration (+) and lower concentration (-) *i.e.* two levels in 20 trials or runs. Each row represents a trial and each column an independent variable. (Table 1). The total number of positive and negative signs per trial is (K+1)/2 and (K-1)/2, respectively (Table 1). Every column should possess positive and negative signs in equal number. The following equation is used to find out effect of each variable.

$$E_{xi} = 2 \, (\Sigma \, M_{i+} - \Sigma \, M_{i-})/N.$$

Where E_{xi} is the effect of concentration of variables tested and M_{i+} and M_{i-} represent the quantity of xylitol produced from the trials. The variable (X_i) measured is available at high and low concentration with number of trials represented by N. The concentration of xylitol produced in g/L. was calculated as the response. All experiments were carried in duplicates and by using t-test the coefficient, the effect and significant level of each variable was calculated.

Results and Discussion

One factor at a time approach (OFAT) is a simple experimental strategy where the effects of individual factors on production of xylitol can be observed on graphs. Influence of factors like agitation speed, temperature, pH, inoculum percentage, nitrogen source, initial xylose concentration and co-substrate on xylitol production from synthetic media was determined by OFAT as follows.

Effect of agitation speed on xylitol production

Oxygen plays a major role in fermentation of xylitol. As agitation increases oxygen transfer also increases and thereby yield.

In the present study, formation of xylitol by *P. stipitis* NCIM 3498 was found to be too negligible at static condition, however xylitol production rose when agitated

Table 1: Variables used for screening using PB design.

Variables	Parameter	Low level (-) (g/l)	High level (+) (g/l)
X_1	Xylose	50	70
X_2	Yeast	1.5	2.5
X_3	Peptone	1.5	2.5
X_4	Urea	1.5	2.5
X_5	$(NH_4)_2SO_4$	1.5	2.5
X_6	NH_4NO_3	1.5	2.5
X_7	Glucose	2	4
X_8	$ZnSO_4$	0.5	1
X_9	$CaCl_2$	0.5	1
X_{10}	$MgSO_4$	0.5	1
X_{11}	KH_2PO_4	0.5	1
X_{12}	Na_2HPO_4	0.5	1

Table 2: Affect of variables on xylitol production by *P. stipitis* NCIM 3498 using Plackett Burman experimental design.

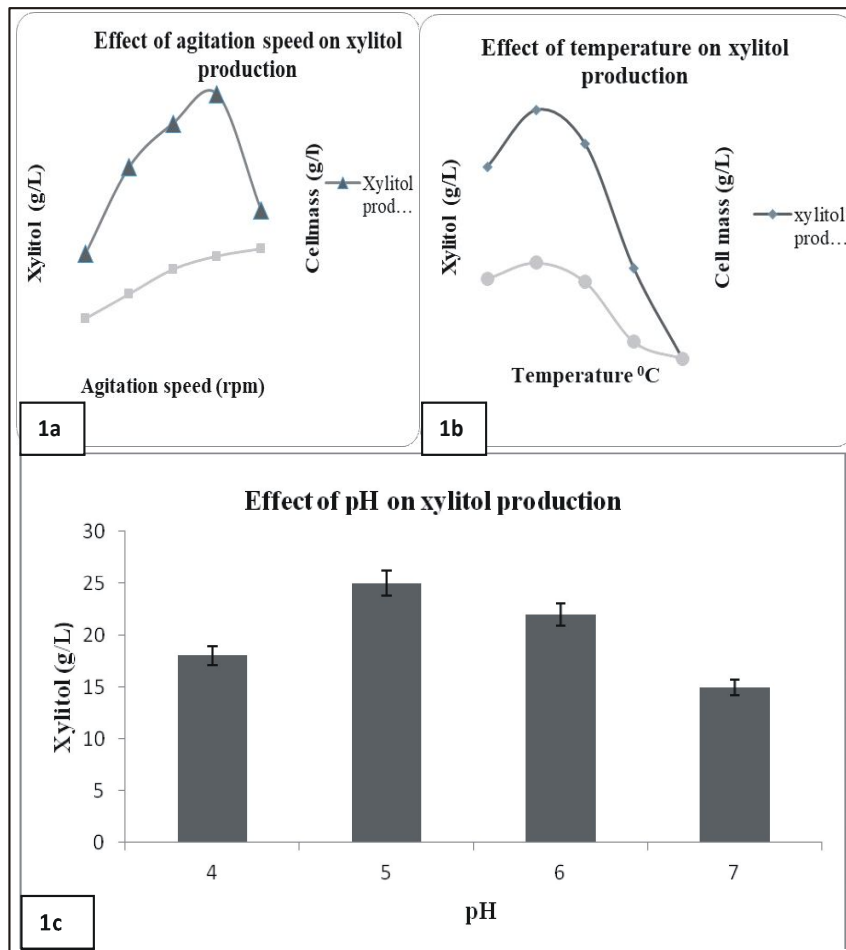| Run	Xylose	Yeast	Peptone	Urea	NH_4SO_4	NH_4NO_3	Glucose	$ZnSO_4$	$CaCl_2$	$MgSO_4$	KH_2PO_4	Na_2HPO_4	Xylitol (g/l)
1	+	-	+	-	-	-	+	+	+	+	-	+	31.41
2	-	+	-	-	-	-	+	-	+	-	-	+	26.09
3	-	-	+	+	+	+	+	-	-	-	-	+	30.33
4	+	+	-	+	+	-	+	+	+	-	+	+	31.62
5	-	+	+	+	+	-	-	+	-	-	-	+	30.12
6	+	+	+	-	+	-	-	-	-	+	+	+	32.62
7	-	-	+	+	-	+	-	-	+	+	+	+	25.04
8	+	-	+	+	-	-	+	+	-	+	-	-	35.04
9	-	+	-	+	-	-	-	+	-	+	+	-	21.91
10	-	-	-	-	+	+	+	+	-	+	+	+	26.94
11	+	-	-	-	-	+	-	+	-	-	+	+	37.43
12	-	-	-	+	+	-	+	-	+	+	+	-	27.29
13	-	+	+	-	-	+	+	+	+	-	+	-	25.66
14	+	+	+	-	-	+	+	-	-	-	+	-	41.36
15	+	+	-	+	-	+	-	-	+	+	-	+	46.00
16	+	-	-	+	+	+	-	+	+	-	-	-	28.23
17	-	+	+	-	+	+	-	+	+	+	-	-	23.93
18	+	-	+	-	+	-	-	-	+	-	+	-	32.46
19	-	-	-	-	-	-	-	-	-	-	-	-	25.67
20	+	+	-	-	+	+	+	-	-	+	-	-	30.09

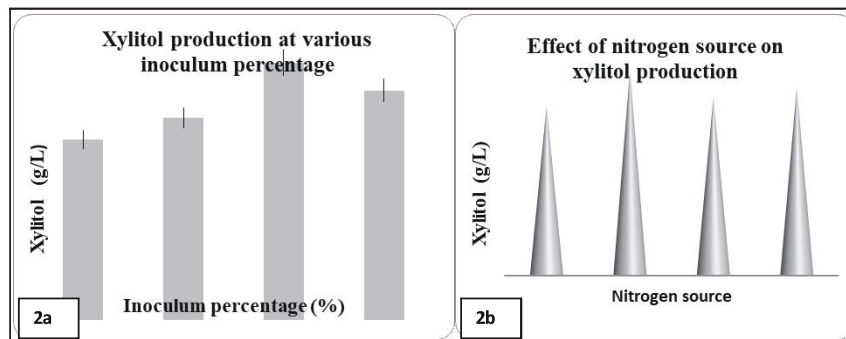**Fig. 1:** Effect of, agitation speed, temperature and pH on xylitol production.

Fig. 2: Effect of inoculum percentage and nitrogen source on xylitol production.
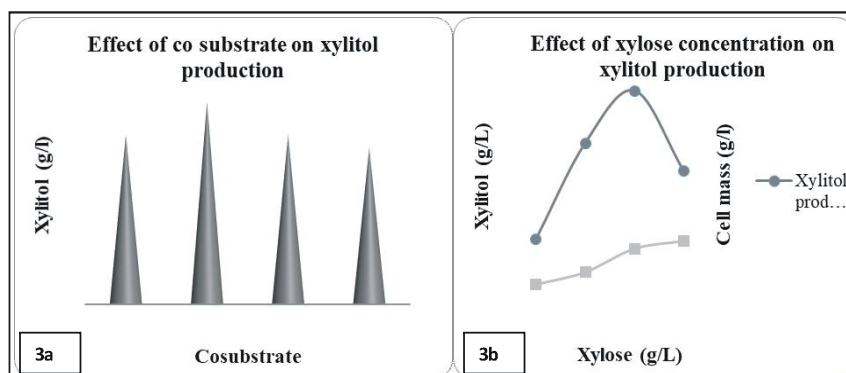
Fig. 3: Effect of co-substrate and xylose concentration on xylitol production.

at different rpm and attained high concentration (19g/l) at 200 rpm (Fig. 1a). In contrast to our study Bhattacharya *et al.*, (2016) reported maximum xylitol yield of 0.61g/g by *Pichia stipitis* (NCIM 3500) at 150 rpm.

Our results are in agreement with Jin *et al.*, (2005) who investigated fermentation by *P. stipitis* FPL-YS30, under aerobic and oxygen-limited culture conditions and reported xylitol yields of 0.30g/g and 0.27 g/g respectively at aeration rates of 100 and 200rpm.

Our results are similar to the studies of Sampoi *et al.*, (2008) who reported maximum xylitol production with *D. hansenii* at an agitation speed of 200 rpm.

Effect of temperature on xylitol production

When optimization of xylitol production is carried out at a temperature range of 25°C-45°C, 30°C was found to be optimum for maximum xylitol production of 22g/l (Fig. 1b). Jeevan *et al.,* (2011) reported 28°C as the optimal temperature for xylitol production by *Pichia* sp from corncob hydrolyzates, which is lesser than 30°C reported in our study.

Similar to our study, Misra *et al.*, (2013) obtained maximum xylitol production of 11.89 g/l at 30°C from corncob hydrolyzate using *C. tropicalis*.

Effect of media pH on xylitol production

Optimization of pH range on xylitol fermentation was observed in a range of 4-7. Maximum xylitol production of 25g/l was attained at pH 5 (Fig. 1c). Our reports are in accordance with Mareczky *et al.,* (2015) who reported pH 4.5 as optimum for maximum xylitol production of 21.7 g/L by *H. anomala.*

In contrast to our result, Srivani and Setty, (2012) reported pH 3.5 as optimum for maximum xylitol productivity by *Candida parapsilosis*

Table 3: Xylitol production as obtained and calculated effect, Coefficients, *t* values and probability as per Plackett Burman design.

Variable	Effect	Coefficient	t -value	P- value
Xylose	**3.72**	**2.03**	**1.32**	**0.000**
Yeast	2.32	0.35	0.56	0.085
Peptone	**7.11**	**3.54**	**-2.12**	**0.000**
Urea	3.53	1.87	-1.82	0.076
$(NH_4)_2SO_4$	2.58	1.56	-0.75	0.076
NH_4NO_3	**3.78**	**1.25**	**-1.12**	**0.000**
Glucose	1.84	0.98	0.45	0.155
$ZnSO_4$	1.22	0.45	0.35	0.285
$CaCl_2$	1.54	0.87	-0.56	0.198
$MgSO_4$	**4.86**	**3.87**	**-1.67**	**0.000**
KH_2PO_4	2.78	1.34	-1.02	0.421
Na_2HPO_4	**4.25**	**2.06**	**-1.86**	**0.000**

NCIM-3323 above this, there was a decrease in the production of xylitol.

Effect of inoculum percentage on xylitol production

When inoculum percentage was used in the range of 5- 20% v/v for xylitol production, 15% was found to be most favorable for highest xylitol production of 27g/l (Fig. 2a). Our results are supported by observations of Kresnowati *et al*., (2012) that greater initial cell concentration led to higher xylitol production. However, indifference to our study Bhattacharya *et al*., (2018) reported 10% (v/v) inoculum of *Pichia stipitis* (NCIM 3500) as optimum, for formation of maximum xylitol of 32.3 g/L with a yield of 0.63 g /g from concentrated, detoxified water hyacinth hemicellulosic hydrolysate.

Effect of the nitrogen source on xylitol synthesis

Evaluation of the nitrogen source on xylitol production is illustrated in fig. 2b. Ammonium nitrate is shown as best nitrogen source among others for high xylitol accumulation of 29g/l. In contrast to our study Rodrigues *et al*., (2011) employed *Pichia stipitis* YS-30 for production of xylitol by providing urea and ammonium

sulphate as nitrogen sources and found that xylitol production increased by the addition of urea to the medium.

The xylitol yield obtained in our work is less compared to Mukherji *et al*., (2013) who reported maximum xylitol yield of 0.852 gm/gm, using cotton seed flour as the organic nitrogen source by novel yeast *Pichia Caribbica* (HQ222812).

Bhattacharya *et al*., (2016) reported maximum xylitol production using ammonium sulphate as a nitrogen source. They observed an increase in xylitol concentration and yield from 32.45 g/L to 40.5 g/L and 0.49 g/g to 0.61 g/g respectively with the enhancement of concentration of ammonium sulphate from 1 g/L to 2.5 g/L by *P. stipitis* (NCIM, 3500).

Effect of co-substrate on xylitol production

The biggest challenge for xylitol production by yeasts is xylose uptake, to regenerate NADPH for xylose reductase activity. Co-substrate utilization has become one of the strategies to overcome this challenge by the continuous provision of NADPH to meet the energy and carbon intermediates required for cell growth and maintenance and thereby improving xylitol production. Glucose is used as the preferred co-substrate over other sugars, due to its low cost and abundant availability (Tamburini *et al*., 2010; Uppada *et al*., 2014).

Among the four sugars, arabinose, glucose, maltose and galactose optimized in our study (Fig. 3a) glucose is found as better co-substrate for increased production of xylitol of 31g/L from xylose synthetic media. In contrast to our report, Perez *et al.,* (2016) proposed sucrose to be an ideal co-substrate for maximum xylitol production of 36.11 g/l.

Our result is in accordance with observations of Silva and Felipe, (2006) who obtained an increased yield of xylitol (0.59 g/g) using *Candida guilliermondii* FTI 20037 when glucose and xylose are used in the ratio of 1:5 which is similar to our study.

Effect of xylose concentration on xylitol production

The influence of xylose on xylitol formation is determined in range of 30g/L to 60 g/L and is depicted in fig. 3b. The xylitol formation has been increased steadily and a maximum xylitol production of 36g/L was obtained at xylose concentration of 60g/L and thereafter gradually decreased. Our results are similar to Ping *et al*., (2013)
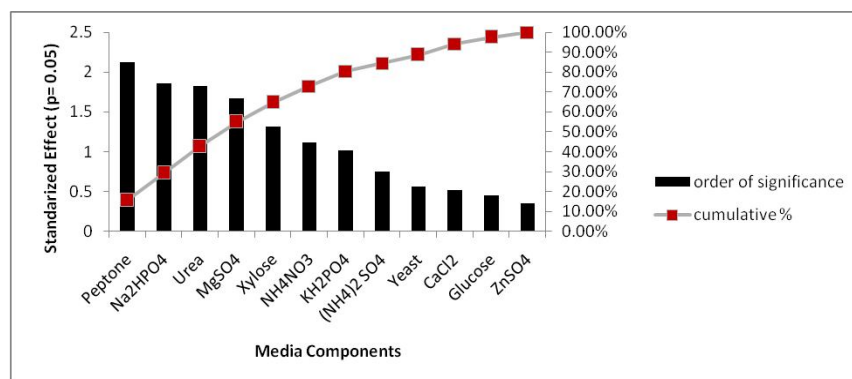
Fig. 4: Pareto chart for Plackett–Burman design of experiments for xylitol production by *P.stipitis* NCIM 3498.
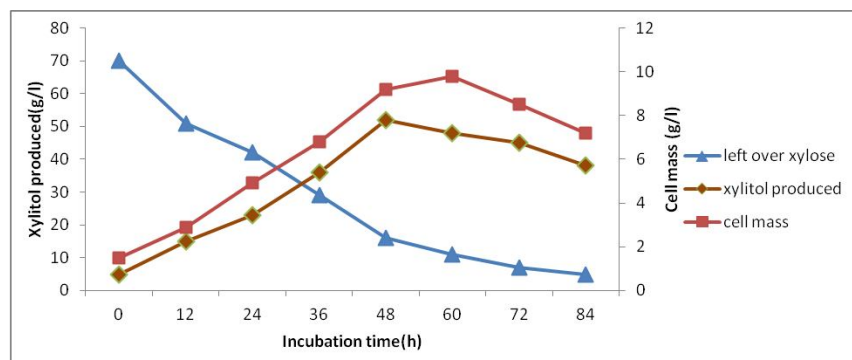
Fig. 5: Validation model for xylitol production using Plackett Burman design.

who observed utmost xylitol yield of 0.71 g/ g at initial xylose concentration of 60 g/L.

The concentration of xylose 60g/L for maximum xylitol production in our study is less compared to the reports of Kim *et al.*, (2019) who observed highest xylitol production using xylose concentration of 300g/L.

Our results are supported by the findings of Tamburini *et al.*, (2015), who demonstrated that *Candida tropicalis* DSM 7524 has shown maximum xylitol yield of 71-83%, by consuming xylose between 60 g/L and 80 g/L and further increase of xylose beyond 80 g/L, led to drastic decrease in xylitol yield.

Plackett Burman design

Plackett Burman is orthogonal, fractional two-level factorial design used for identification of important parameters that influence the process of fermentation. This design gives the effect of every variable purely, without confounded interaction with other variables (Naveena *et al.*, 2005).

In the current study, 12 chemical components are screened for their influence on xylitol production by *P. stipitis* NCIM 3498 using statistical design, Plackett Burman. The experimental design and their responses (xylitol production) are shown in table 2. In the table, each row represents experiments that involve 12 independent variables and each variable with two levels of concentration. The standard T-test was performed using the MINITAB (Release 17, PA, USA). The effect of parameter E, standard error (SE), t-value and probability (P) for the design are represented in table 3. It is observed that the t-test values for xylose, peptone, ammonium nitrate, magnesium sulphate and di-sodium hydrogen phosphate have highly significant values with $\geq$ 97% confidence level (Table 3). The analysis also confirms that the above 5 variables played a key role in xylitol production. From a statistical point of view, all other remaining factors do not have any significant effect on xylitol production. It is evident from the t-test that 4 variables have the sign of the effect as positive hence

these variables has greater influence on the xylitol production at a high level whereas the variables with negative effect are taken in low levels for xylitol production.

The results of our study are supported by reports of Kumdam *et al.*, (2012) that di-sodium hydrogen phosphate along with K_2HPO_4 creates a suitable buffer system in the production medium and hence increase in its concentration lead to increase in xylitol production.

Our study is in accordance to report of Ramesh *et al.*, (2013) who observed peptone, xylose, $MgSO_4.7H_2O$ and yeast extract to be significant variables for xylitol production from corncob hydrolysate using *Pachysolen tannophilus* (MTTC, 1077) by Plackett Burman model.

Using Plackett Burman design, Hongzhi *et al.*, (2011) reported $(NH_4)_2SO_4$, KH_2PO_4, yeast extract and $MgSO_4.7H_2O$ as significant variables affecting xylitol production.

Pareto Chart

Pareto chart is a graphical representation of Student's t-test. It illustrates different variables affecting xylitol production in significant order (Fig. 4).

Validation

Validation of the model for xylitol production was carried out based on the optimized conditions predicted by Plackett-Burman design. Xylitol production and yield under these optimum conditions were found to be 52g/L and 0.74g/g. respectively (Fig. 5). Xylitol yield in current study 0.74g/g is close to xylitol yield 0.73g/g obtained by Neeru *et al.*, (2013) under optimized fermentative conditions of 32°C, pH 5.7 for 72 h using *Pichia stiptis CBS 5773*.

Conclusion

Optimization of xylitol production using a statistical model, Plackett Burman was proved to be efficient and can successfully be used for the optimization of components of media to increase xylitol production.

Acknowledgements

References

Bhattacharya, Anamica, Amit Ganguly, Anup Kumar Sadhukhan and Pradip Kumar Chatterjee (2018). Investigations on the

effect of driving parameters for xylitol production from water hyacinth biomass. *Indian Journal of Biotechnology*., **17:** 272-283.

Bhattacharya, A., A.K. Sadhukhan, A. Ganguly and P.K. Chatterjeea (2016). Investigations on Microbial Fermentation of Hemicellulose Hydrolysate for Xylitol Production. *Banat's Journal of Biotechnology*., **VII(14):** DOI: 10.7904/2068–4738– VII(14): 13.

Branco, R.D.F., J.C.D. Santos and S.S.D. Silva (2011). A novel use for sugarcane bagasse hemicellulosic fraction: Xylitol enzymatic production. *Biomass Bioenergy*., **35:** 3241-3246.

Deng, L.H., Y. Tang and Y. Liu (2014). Detoxification of corncob acid hydrolysate with SAA pretreatment and xylitol production by immobilized *Candida tropicalis*. *Sci. World J.*, 214632 doi: 10.1155/2014/214632.

Goli, J.K., S.H. Panda and V.R. Linga **(**2012). Molecular Mechanism of d-Xylitol Production in Yeasts: Focus on Molecular Transportation, Catabolic Sensing and Stress Response Published in book entitled 'D-Xylitol: Fermentative Production, Application and Commercialization'. *Springer Berlin Heidelberg.,* 85-107.

Guirimand K. Inokuma, T. Bamba, M. Matsuda, K. Morita, K. Sasaki, C. Ogino, J. Berrin, T. Hasunuma and A. Kondo*,* (2019). Cell-surface display technology and metabolic engineering of *Saccharomyces cerevisiae* for enhancing xylitol production from woody biomass. *Green Chem*., DOI: 10.1039/C8GC03864C.

Hernández-Pérez, A.F., I.A.L. Costa, D.D.V. Silva, K.J. Dussán, T.R. Villela, E.V. Canettieri, J.A. Carvalho Jr, T.G. Soares Neto and M.G.A. Felipe (2016). Biochemical conversion of sugarcane straw hemicellulosic hydrolyzate supplemented with co-substrates for xylitol production. *Bioresource Technol*., **200:** 1085-1088. doi.org/10.1016/j.biortech 2015.11.036.

Jeevan, P., R. Nelson and A. Edith Rena (2011). Microbial production of xylitol from corn cob hydrolysate using *Pichia sp. Adv Environ Biol*., **5:** 3613-3619.

Jin, Y.S., J. Cruz and T. Jeffries (2005). Xylitol production by a *Pichia stipitis* D-xylulokinase mutant. *Appl Microbiol Biotechnol.,* **68(1):** 42-45.

Kogje, Anushree and Anand Ghosalkar (2016). Xylitol production by *Saccharomyces cerevisiae* overexpressing different xylose reductases using non-detoxified hemicellulosic hydrolysate of corncob. *3 Biotech*., **6(2):** 127.

Kim, S., J. Lee and B.H. Sung (2019). Isolation and Characterization of the Stress-Tolerant Candida tropicalis YHJ1 and Evaluation of Its Xylose Reductase for Xylitol Production from Acid Pre-treatment Wastewater. *Front. Bioeng. Biotechnol*., **7:** 138. doi: 10.3389/fbioe.2019.00138.

Kresnowati, M., A.B. Ardina and V.P. Oetomo (2012). From palm oil waste to valuable products: microbial production of xylitol. 19th Regional Symposium of Chemical Engineering, Bali, Indonesia.

Kumdam, H.B., S.N. Murthy and S.N. Gummadi (2012). A Statistical Approach to Optimize Xylitol Production by *Debaryomyces nepalensis* NCYC 3413 *in Vitro. Food Nutr. Sci.*, **3:** 1027-1036.

Ling, H., K. Cheng, J. Ge and W. Ping (2011). Statistical optimization of xylitol production from corncob hemicellulose hydrolysate by Candida tropicalis HDY-02. *New Biotechnol.,* **28(6):** 673-678.

López-Linares, J.C., I. Romero, C. Cara, E. Castro and S.I. Mussatto (2018). Xylitol production by Debaryomyces hansenii and Candida guilliermondii from rapeseed straw hemicellulosic hydrolysate. *Bioresour. Technol.*, **247:** 736-743.

Mareczky, M., A. Fehér, C. Fehér, Z. Barta and K. Réczey (2015). Effects of pH and aeration conditions on xylitol production by *Candida and Hansenula* Yeasts. *Periodica Polytechnica Chemical Engineering.*, DOI: 10.3311/PPch.8116.

Misra, S., S. Raghuwanshi and R.K. Saxena (2013). Evaluation of corncob hemicellulosic hydrolysate for xylitol production by adapted strain of *Candida tropicalis. Carbohyd Polym.*, **92:** 1596-1601.

Mohamad, N.L., N.L. Kamal, S.M. Mustapa and M.N. Mokhtar (2015). Xylitol biological production: a review of recent studies. *Food Ver. Int.*, **31:** 74-89.

Mukherji, R., K. Joshi-Navare and A. Prabhune (2013). Crystalline xylitol production by a novel yeast, Pichia caribbica (HQ222812) and its application for quorum sensing inhibition in gram-negative marker strain Chromobacterium violaceum CV026. *Appl Biochem Biotechnol. 2013 Mar.*, **169(6):** 1753-63.

Naveena, B.J., M. Altaf, K. Bhadriah and G. Reddy (2005). Selection of Medium Components by Plackett-Burman Design for Production of l (+) Lactic Acid by *Lactobacillus amylophilus* GV6 in SSF Using Wheat Bran. *Bioresour. Technol.*, **96(4):** 485-490.

Neeru, C., C. Balomajumder and V. Jagati (2013). Biological production of xylitol from corn husk and switch grass by *Pichia stipitis. Research Journal of Chemical Sciences.*, **3(3):** 58-64.

Ping, Y., H. Ling, G. Song and J. Ge (2013). Xylitol production from non-detoxified corncob hemicellulose acid hydrolysate by *Candida tropicalis. Biochem. Eng. J.*, **75:** 86-91.

Prakasham, R.S. and R.S. Rao (2009). Hobbs PJ. Current trends in biotechnological production of xylitol. *Curr Trends Biotechnol Pharm Future Prospects.*, **3(1):** 8-36.

Ramesh, R.S., R. Muthuvelayudham, R. Kannan and T. Viruthagiri (2013). Enhanced production of xylitol from corncob by *Pachysolen tannophilus* using response surface methodology. *International Journal of Food Science.*, dx.doi.org/10.1155/2013/514676.

Rafiqula, I.S.M., A.M.M. Sakinaha and A.W. Zularisamba (2015). Evaluation of sawdust hemicellulosic hydrolysate for

bioproduction of xylitol by enzyme xylose reductase. *Food and bioproducts processing.*, **9(4):** 82-89.

Rodrigues, R.C.L.B., W.R. Kenealy and T.W. Jeffries (2011). Xylitol production from DEO hydrolysate of corn stover by *Pichia stipitis* YS-30 *J Ind Microbiol Biotechnol.*, **38:** 1649-55.

Silva, D.D.V. and M.G.A. Felipe (2006). Effect of glucose: xylose ratio on xylose reductase and xylitol dehydrogenase activities from *Candida guilliermondii* in sugarcane bagasse hydrolysate. *J Chem Technol Biotechnol.*, **81:** 1294-1300.

Sampaio, F.C., V.M. Chaves-Alves, A. Converti, F.M.L. Passos and J.L.C. Coelho (2008). Influence of cultivation conditions on xylose-to-xylitol bioconversion by a new isolate of Debaryomyces hansenii. *Bioresource Technol.*, **99:** 502-508.

Srivani, K. and Y. PydiSetty (2012). parametric optimization of xylitol production from xylose by fermentation. *Asia Pac. J. Chem. Eng.*, **7(3):** 280-284.

Tamburini, E., S. Costa, M.G. Marchetti and P. Pedrini (2015). Optimized production of xylitol from xylose using a hyper-acidophilic *candida tropicalis. Biomolecules.*, **5:** 1979-1989.

Tamburini, E., E. Bianchinib, A. Brunia and G. Forlani (2010). Cosubstrate effect on xylose reductase and xylitol dehydrogenase activity levels and its consequence on xylitol production by *Candida tropicalis. Enzyme Microb. Technol.*, **46:** 352-359.

Uppada, V., S. Bhaduri and S.B. Noronha (2014). Cofactor generation- an important aspect of biocatalysis. *Curr. Sci.*, **106:** 946-957.

Venkateswar Rao, L., J.K. Goli, J. Gentela and S. Koti (2016). Bioconversion of Lignocellulosic biomass to xylitol: An overview. *Bioresource Technology.*, **213:** 299-310.

Zegale Tizazu, B., K. Roy and V.S. Moholkar (2018). Ultrasonic Enhancement of Xylitol Production from Sugarcane Bagasse using Immobilized Candida tropicalis MTCC 184, *Bioresource Technology.,* doi: https://doi.org/10.1016/j.biortech.2018.07.1.

Zhang, J., B. Zhang, D. Wang, X. Gao and J. Hong (2014). Improving xylitol production at elevated temperature with engineered *Kluyveromyces marxianus* through over-expressing transporters. *Bioresour Technol.*, **175:** 642-645.