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ORGINAL PAPER

# Optimization of Arabitol Production by Karyoductant SP-K 7 of *S. cerevisiae* $V_{30}$ and *P. stipitis* CCY 39501 Using Response Surface Methodology

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Abstract

L-arabitol is used in the food and pharmaceutical industries. It can be secreted by genetically modified *Saccharomyces cerevisiae* carrying the genes responsible for pentose metabolism in yeast cells. The process of the biotransformation of L-arabinose to arabitol is highly dependent on culture conditions. The aim of this investigation was to use statistical response surface methodology (RSM) for optimization of biotransformation of L-arabinose to arabitol by a karyoductant of *S. cerevisiae* $V_{30}$ and *Pichia stipitis* CCY 39501, named SP-K7. Batch cultures of yeast were performed according to a Plackett-Burman design, and three factors, rotation speed, L-arabinose concentration, and temperature, were chosen for a central composite design (CCD) applied in order to optimize the production of the polyol by the karyoductant. On the basis of results obtained using 20 combinations of batch cultures of karyoductant SP-K7, the optimal levels of the factors were determined as: rotation speed 150 rpm, concentration of L-arabinose 32.5 g/l, and temperature 28°C. In such conditions, the predicted concentration of arabitol after two days of incubation of SP-K7 should be 18.367 g/l. The value of $R^2 = 0.93195$ suggested that this model was well-fitted to the experimental data. A verification of the model in experimental conditions confirmed its usefulness.

Key words: arabitol, karyoductants, L-arabinose, optimization, RSM

## Introduction

L-arabitol is five-carbon polyalcohol which, together with its enantiomer xylitol, has been identified as one of the top 12 biomass-derivable building block chemicals. Due to its health-promoting effects (low caloric – only 0.2 kcal/g, low-glycemic, low-insulinemic, anticariogenic, and prebiotic), arabitol can be used in many of the known applications of xylitol, as a natural sweetener, a dental caries reducer, and a sugar substitute for diabetic patients (Koganti *et al.*, 2011). Polyols are used in the food and pharmaceutical industries due to their technological properties; for instance, they can act as texturing agents, humectants, softeners, and color stabilizers. Industrial production of most sugar alcohols is performed by catalytic reduction of sugars with hydrogen gas and nickel at a high temperature and pressure, which is expensive and requires the use of chromatographic purification steps (Monedero *et al.*, 2010). Polyols are also produced by microorganisms from appropriate sugars, *e.g.*, those obtained from hydrolyzates of the hemicellulosic fraction of plant biomass (Saha and Bothast, 1996). Biotechnological production may represent an efficient and cost-effective alternative to chemical production. Arabitol is known to be produced from L-arabinose by yeast such as *Debaryomyces*, *Candida*, *Pichia*, *Wickerhamomyces*, and *Saccharomycopsis* (Koganti *et al.*, 2011). In a first screening of yeasts and fungi able to produce arabitol from L-arabinose under oxygen-limiting conditions Mc Millan and Boynton (1994) observed that the xylose-fermenting yeasts converted arabinose to arabitol, and not to ethanol, because of the relatively inefficient multistep redox assimilation pathways of the pentose sugar. Saha and Bothast (1996) observed that, among 49 yeast strains capable of growing on L-arabinose, *C. entomaea* NRRL Y-7785 and *P. guilliermondii* NRRL Y-2075 were superior secretors of L-arabitol (yield of about 0.7 g/g). Kordowska-Wiater *et al.* (2008) reported that *C. parapsilosis* DSM 70125 was an efficient producer of arabitol with a yield of 0.78 g/g.

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The yeast *Saccharomyces cerevisiae* is unable to assimilate and ferment pentose sugars such as D-xylose and L-arabinose. Some attempts have been made to obtain a modified *S. cerevisiae* capable of assimilating pentoses and secreting pentitols but only as byproducts of ethanol fermentation of ligninocellulosic hydrolyzates. The yeast has been modified by introducing genes of araBAD operon from *E. coli* (Sedlak and Ho, 2001), genes from fungal L-arabinose pathway (Bera *et al*., 2010), or genes from both bacteria and fungi (Karhumaa *et al*., 2006). The metabolism of the yeast has also been changed by fusion of its protoplast with that of another yeast strain *Torulaspora delbrueckii* (Lucca *et al*., 2002). In a previous study by Kordowska-Wiater and colleagues, a special kind of fusion between protoplasts of *S. cerevisiae* $V_{30}$ and nuclei of *P. stipitis* CCY 39501 was used to obtain karyoductants able to assimilate arabinose and secrete arabitol (Kordowska-Wiater and Targoński, 2001). One of them, named SP-K7, capable of producing large quantities of this polyol, is the subject of the present study.

The process of biotransformation of L-arabinose to arabitol is highly dependent on culture conditions, especially oxygen availability, temperature, pH, and kind and concentration of medium compounds. There are some reports concerning the influence of different conditions on the process studied (Fonseca *et al*., 2007; Kordowska-Wiater *et al*., 2008; Saha and Bothast, 1996), but there is no information about the application of statistical optimization methods to biotranformation of L-arabinose to arabitol. Response surface methodology (RSM) is a collection of mathematical and statistical techniques that are useful for modeling and analyzing situations in which a response of interest is influenced by several variables. The purpose of this method is to optimize a response by choosing the level of independent variables (factors) on the basis of a quadratic function describing the relationships between the response and the studied factors (Montgomery, 2001). This method is used in different biotechnological processes, *e.g.*, production of xylitol by yeasts (Sampaio *et al*., 2006; Sarrouh and da Silva, 2010; Vasquez *et al*., 2006), but there are no reports on the application of RSM in the process of arabitol production. The aim of this investigation was to use statistical methods of RSM to optimize biotransformation of L-arabinose to arabitol by karyoductant SP-K7 of *S. cerevisiae* $V_{30}$ and *P. stipitis* CCY 39501.

## Experimental

### Materials and Methods

**Microorganism.** Karyoductant SP-K7 of *S. cerevisiae* $V_{30}$ and *P. stipitis* CCY 39501 obtained by fusion of a *Saccharomyces* protoplast and a *Pichia* nucleus and able to assimilate L-arabinose (Kordowska-Wiater and Targoński, 2001) was used in experiments. This microorganism was maintained at 4°C on YPG agar slants. It was deposited in the Culture Collection of the Department of Biotechnology, Human Nutrition and Science of Food Commodities, University of Life Sciences in Lublin, Poland.

**Media composition.** Growth and cultivation media were composed of L-arabinose as the carbon source, yeast extract, malt extract, $(NH_4)_2SO_4$ and $KH_2PO_4$ in different concentrations according to a Plackett-Burman design (Table I). In a next stage, the medium was composed of yeast extract, malt extract, $(NH_4)_2SO_4$ and $KH_2PO_4$ at a concentration of 5 g/l and L-arabinose at concentrations from 3.1 to 61.9 g/l depending on the run number in CCD (Table II). The pH was adjusted to 5.5. Media for inoculum preparation were dispensed into tubes (5 ml per tube), and media for cultivation were dispensed into 100-ml Erlenmeyer flasks (20 ml per flask) and sterilized by autoclaving at 121°C for 15 min.

**Inoculum and culture conditions.** Inocula were prepared by transferring a loopful of cells from a slant into the tubes with media and incubation at 28°C for 24 hours. Then, cultivation media were inoculated with 2% (v/v) prepared culture and incubated in a rotary shaker (Infors HT Minitron, Infors AG, Switzerland)

Table I
A Plackett-Burman design for seven variables

| Run | Arabinose (g/l) | Yeast extract (g/l) | Malt extract (g/l) | $(NH_4)_2SO_4$ (g/l) | $KH_2PO_4$ (g/l) | Temp. (°C) | Rotation speed (rpm) | Max. arabitol conc. (g/l) | Arabitol yield (g/g) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 5 | 2 | 2 | 10 | 10 | 32 | 50 | 0.00 | 0.00 |
| 2 | 50 | 2 | 2 | 2 | 2 | 32 | 200 | 1.01 | 0.074 |
| 3 | 5 | 10 | 2 | 2 | 10 | 24 | 200 | 0.64 | 0.75 |
| 4 | 50 | 10 | 2 | 10 | 2 | 24 | 50 | 0.00 | 0.00 |
| 5 | 5 | 2 | 10 | 10 | 2 | 24 | 200 | 0.44 | 0.09 |
| 6 | 50 | 2 | 10 | 2 | 10 | 24 | 50 | 0.24 | 0.028 |
| 7 | 5 | 10 | 10 | 2 | 2 | 32 | 50 | 0.00 | 0.00 |
| 8 | 50 | 10 | 10 | 10 | 10 | 32 | 200 | 16.71 | 0.355 |

Table II
A central composite design matrix of the independent variables along with the experimental values of arabitol concentrations and yield

| Run | Rotation speed ($X_1$) (rpm) | Arabinose ($X_2$) (g/l) | Temperature ($X_3$) (°C) | Arabitol (g/l) | Arabitol yield (g/g) |
|---|---|---|---|---|---|
| 1 | 100 | 15 | 24 | 1.028 | 0.163 |
| 2 | 100 | 15 | 32 | 0.13 | 0.159 |
| 3 | 100 | 50 | 24 | 0.52 | 0.011 |
| 4 | 100 | 50 | 32 | 0.41 | 0.008 |
| 5 | 200 | 15 | 24 | 1.576 | 0.105 |
| 6 | 200 | 15 | 32 | 5.331 | 0.396 |
| 7 | 200 | 50 | 24 | 0.37 | 0.007 |
| 8 | 200 | 50 | 32 | 0.26 | 0.005 |
| 9 | 66 | 32.5 | 28 | 0.22 | 0.2 |
| 10 | 234 | 32.5 | 28 | 5.525 | 0.397 |
| 11 | 150 | 3.1 | 28 | 0.496 | 0.16 |
| 12 | 150 | 61.9 | 28 | 1.75 | 0.029 |
| 13 | 150 | 32.5 | 21.28 | 0.24 | 0.941 |
| 14 | 150 | 32.5 | 34.72 | 15.865 | 0.516 |
| 15 | 150 | 32.5 | 28 | 18.285 | 0.564 |
| 16 | 150 | 32.5 | 28 | 18.62 | 0.574 |
| 17 | 150 | 32.5 | 28 | 18.845 | 0.584 |
| 18 | 150 | 32.5 | 28 | 18.92 | 0.583 |
| 19 | 150 | 32.5 | 28 | 18.875 | 0.582 |
| 20 | 150 | 32.5 | 28 | 16.275 | 0.5 |

at 50 or 200 rpm and at 24°C or 32°C according to the Plackett-Burman design (Table I). In the second stage of the experiment, cultures were incubated at temperatures and rotation speeds according to CCD (Table II). After 48 and 72 h of incubation, the cells were collected by centrifugation at 6000 ×g for 15 min, and supernatants were used for further analysis.

**Verification of optimal model.** Inoculum of the investigated yeast strain was prepared as above. The medium was composed of yeast extract, malt extract, $(NH_4)_2SO_4$ and $KH_2PO_4$ at a concentration of 5 g/l and L-arabinose concentration of 32.5 g/l as estimated by RSM. The temperature of incubation and rotation speed were 28°C and 150 rpm, respectively. Cultures, in a volume of 100 ml, were incubated in 500-ml Erlenmeyer flasks on a rotary shaker (Infors HT Minitron, Infors AG, Switzerland). Samples for analysis were collected after 48 and 72 h and centrifuged at 6000 ×g for 15 min. Supernatants were used for L-arabinose and arabitol detection.

**Analytical methods**. L-arabinose and arabitol concentrations in supernatants were determined by HPLC (Gilson Inc., USA) equipped with a refractor index detector (Knauer GmbH, Germany) and Bio-Rad Aminex Carbohydrate HPX 42C (300 × 7.8 mm) column (Bio-Rad Laboratories Inc., USA). Deionized water was used as eluent at a flow rate of 0.5 ml/min, and the temperature of separation was 85°C. Integration and analysis of chromatograms were done using Chromax 2007 software version 1.0a.

**Experimental designs.** The Plackett-Burman design, which enables screening of *n* variables using only *n+1* experiments, was applied to limit the number of factors, selected from among media components and culture conditions, that were important for the process (Myers and Montgomery, 2002). In this experiment, a Plackett-Burman design matrix was constructed (Table I) in order to investigate the influence of the main effect of 7 selected variables on arabitol production. After the factors had been limited to three most strongly affecting the response (rotation speed, L-arabinose concentration, and temperature), a central composite design (CCD) based on three independent variables (Table II) was used to estimate response surfaces, following the general model equation:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j ,$$

where $Y$ is the response variable, $\beta_0$ is the interception, $\beta_i$ is the linear effect, $\beta_{ii}$ is the quadratic effect, and $\beta_{ij}$ are interaction effect coefficients. $X_i$ and $X_j$ are coded values of the factors selected as a result of the initial screening using the Plackett-Burman design. The significance of the obtained model was checked by an F-test, and

goodness of fit was tested by determining coefficient $R^2$. The relationships between experimental and predicted values were shown on the response surface plots. All design matrices were generated and analyzed using a Statistica software version 7 (2007).

## Results

**Plackett-Burman design.** Results of the experiments performed on the basis of the Plackett-Burman design are presented in Table I. The highest concentration of arabitol (16.71 g/l) and the highest arabitol yield (0.355 g/g) were obtained in experiment 8, in which all the variables were at high levels. Estimated values of the effect of independent factors are shown in Table III. The level of polyol concentration was mostly influenced by rotation speed (rpm) followed by concentration of L-arabinose and incubation temperature. The components of the medium such as extracts and mineral salts had less effect on biotransformation, but on the whole all the studied variables had a positive effect on arabitol production. Three factors with the highest effects were chosen for further investigation (Table III).

**CCD.** Full-factorial CCD consisted of five levels: the low and high levels, central points and star points with

Table III
Results of the Plackett-Burman design analysis

| Variable | Effect estimate | Coefficient | Ranking |
|---|---|---|---|
| Arabinose | 4.220 | 2.110 | 2 |
| Yeast extract | 3.915 | 1.957 | 6 |
| Malt extract | 3.935 | 1.967 | 5 |
| $(NH_4)_2SO_4$ | 3.815 | 1.907 | 7 |
| $KH_2PO_4$ | 4.035 | 2.017 | 4 |
| Temperature | 4.100 | 2.050 | 3 |
| Rotation speed | 4.640 | 2.320 | 1 |
| Mean/Interc. | 2.380 | 2.380 | – |

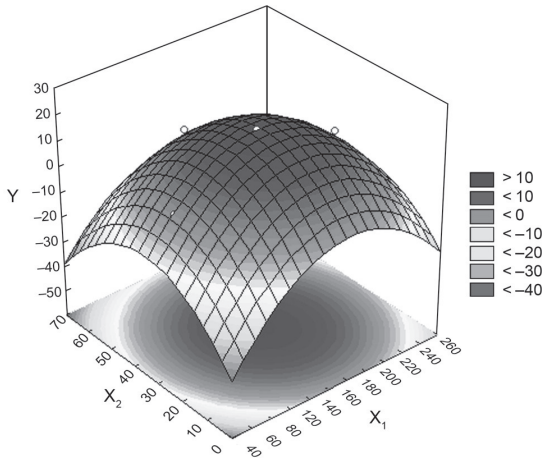


Fig. 1. Effects of rotation speed ($X_1$) and arabinose concentration ($X_2$) on arabitol concentration ($Y$) with temperature ($X_3$) at its center point level.

$\alpha = \pm 1.68$. Twenty combinations were run, as shown in Table II, and the concentration of arabitol in culture media was analyzed. Different concentrations and different yields of arabitol were obtained from 1 g of consumed sugar depending on the combination of variables. The regression coefficients obtained as a result of the CCD analysis are shown in Table IV. Since none of the factor interactions were significant, the model was simplified and the final quadratic model took the following form:

$$Y = -272 + 0.726X_1 - 0.002X_1^2 + 1.36X_2 - 0.021X_2^2 + 14.66X_3 - 0.252X_3^2$$

Table V presents the results of ANOVA which indicate that the model was statistically significant. The value of $R^2 = 0.93195$ suggests that this model was well fitted to the experimental data and only 6.8% of the total variations in the response were not explained by it. The response surface plots are shown in Figures 1–3. These

Table IV
Regression coefficients obtained on the basis of CCD

| | Regression | Std. Err. | t(10) | p |
|---|---|---|---|---|
| Mean/Interc. | –269.079 | 48.03999 | –5.60114 | 0.000227 |
| (1)X1-rotation speed (L) | 0.672 | 0.18003 | 3.73497 | 0.003878 |
| X1- rotation speed (Q) | –0.002 | 0.00031 | –7.47363 | 0.000021 |
| (2)X2-arabinose conc. (L) | 1.643 | 0.48981 | 3.35443 | 0.007311 |
| X2- arabinose conc. (Q) | –0.021 | 0.00257 | –8.26249 | 0.000009 |
| (3)X3-temp.(L) | 14.402 | 2.90978 | 4.94948 | 0.000579 |
| X3-temp.(Q) | –0.252 | 0.04911 | –5.13793 | 0.000439 |
| 1L by 2L | –0.001 | 0.00120 | –0.71810 | 0.489133 |
| 1L by 3L | 0.003 | 0.00526 | 0.55237 | 0.592822 |
| 2L by 3L | –0.005 | 0.01504 | –0.36528 | 0.722508 |

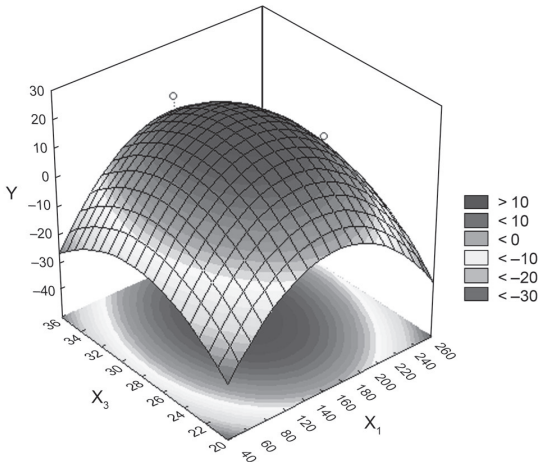


Fig. 2. Effects of rotation speed ($X_1$) and temperature ($X_3$) on arabitol concentration ($Y$) with arabinose concentration ($X_2$) at its center point level.

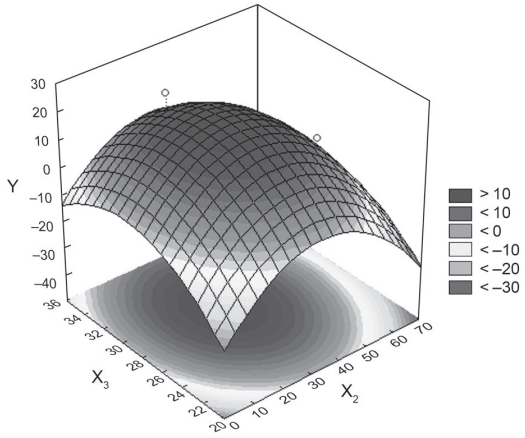


Fig. 3. Effects of arabinose concentration ($X_2$) and temperature ($X_3$) on arabitol concentration ($Y$) with rotation speed ($X_1$) at its center point level.

Table V
ANOVA for the simplified model of regression

| Source of variation | Sum of square | Degree of freedom | Mean square | F-value | p-value |
|---|---|---|---|---|---|
| Model | 1206.184 | 6.000 | 201.031 | 26.898 | 0.000 |
| Error | 97.160 | 13.000 | 7.474 | | |

figures clearly demonstrate that arabitol concentration was affected by all of the investigated factors and that the selected ranges of them were appropriate. On the basis RSM, the optimal levels of factors for arabitol production from L-arabinose by karyoductant SP-K7 were defined as follows: rotation speed 150 rpm, concentration of L-arabinose 32.5 g/l, and temperature 28°C. In such conditions, the predicted concentration of arabitol after two days of incubation should be 18.367 g/l.

**Verification of the model.** The medium containing arabinose at the optimal concentration and the remaining components at a concentration of 5 g/l was inoculated with the karyoductant strain and incubated at the optimal rotation speed and temperature in order to verify the usefulness of the statistical model. After 2 days of incubation 16.80 g/l of arabitol was obtained. The yield of this process was 0.52 g/g of consumed arabinose. The result of the experiment was about 9% lower than predicted, so it may be accepted as a confirmation of the usefulness of the model .

## Discussion

*Saccharomyces cerevisiae* has to be modified to obtain the ability to metabolize pentose sugars from hemicellulosic hydrolyzates and produce ethanol or pentitols as byproducts during ethanol fermentation. While a lot of investigations have focused on xylitol production from D-xylose (Hahn-Hagerdal *et al.*, 2007; Jeffries and Jin, 2004), there are few reports about the application of an engineered *S. cerevisiae* for L-arabinose utilization (Bera *et al.*, 2010; Bettiga *et al.*, 2009; Karhumaa *et al.*, 2006; Sanchez *et al.*, 2010; Sedlak and Ho, 2001). Karhumaa *et al.* (2006) constructed different recombinants of *S. cerevisiae* containing bacterial genes of the L-arabinose pathway and/or yeast genes of xylose metabolism, which were able to utilize xylose and/or arabinose. Strain BWY02.XA slowly consumed pentose sugars in anaerobic conditions and secreted arabitol with a yield of about 1 g/g of arabinose. Sanchez *et al.* (2010) continued the investigation of the engineered industrial strains of *S. cerevisiae* carrying genes responsible for xylose and arabinose metabolism, obtained by Karhumaa *et al.* (2006). Those authors used evolutionary engineering to improve strain TMB 3061 in continuous culture. Under anaerobic conditions, all of the modified strains almost stoichiometrically converted L-arabinose to arabitol (0.94–1.03 g/g), which suggested their inability to ferment arabinose to ethanol despite the presence of appropriate genes (Sanchez *et al.*, 2010). Bera *et al.* (2010) also obtained a recombinant *S. cerevisiae* 424A (LNH-ST) containing fungal genes of arabinose metabolism, which was able to ferment hemicellulosic sugars. While a control strain only produced arabitol from arabinose as sole carbon source with a metabolic yield of 82.5 ± 4.7%, the recombinant, bearing plasmid $pLXR_{NAD}$-LAD, produced both arabitol and ethanol from this pentose with the metabolic yields of 33.7 ± 0.3% and 42.6 ± 2.3%, respectively (Bera *et al.*, 2010). A recombinant obtained by Bettiga *et al.* (2009), TMB3664 carrying genes of the fungal

pathway, also produced arabitol from consumed arabinose with a yield of 0.48 g/g. Karyoductant SP-K7 of *S. cerevisiae* $V_{30}$ and *P. stipitis* CCY 39501 was shown to be able to assimilate arabinose and secrete arabitol with the yield of 0.45–0.95 g/g depending on culture conditions, but it could not produce ethanol from this pentose. On the other hand, the strain was demonstrated to be quite efficient producer of xylitol from D-xylose (Kordowska-Wiater and Targoński, 2001; unpublished data).

The results obtained by different scientists suggest that there are factors that might limit the L-arabinose metabolism. One such factor is an imbalance of redox cofactors which determine the kind of product that is secreted into the medium. Observations made by researchers indicate that it is necessary to check the influence of different environmental factors on L-arabinose assimilation and product secretion. On the basis of the literature and screening studies (unpublished data), 7 factors were chosen for the Plackett-Burman design (Table I) to find out which were the most important for the process. As it could be expected, the factors that influenced arabitol production most were rotation speed, which is responsible for oxygen availability in the medium, the initial concentration of L-arabinose, which is the substrate for the process, and temperature, which affects cell growth and enzymatic activity. These variables were selected for the second part of experiment – optimization on the basis of a CCD design. This statistical method is very useful in the optimization of biotechnological processes. There is no information about the use of the statistical methods of RSM for the optimization of arabitol production from L-arabinose. To the best of our knowledge, this is the first publication on this subject. The present results can, however, be compared with results for xylitol production from xylose because the substrates and the products are chemically and functionally similar and both sugars, being components of hemicelluloses, can be metabolized by similar yeast strains.

There are several publications reporting the application of RSM for xylitol production from D-xylose and other sugars of plant biomass origin by yeast *D. hansenii* (Sampaio *et al.*, 2006) and *C. guilliermondii* (Vasquez *et al.*, 2006; Sarrouh and da Silva, 2010). Sampaio *et al.*, (2006) ran a $3^3$ full factorial design on initial concentration of xylose, rotation speed and starting biomass concentration as independent variables, and maximum xylitol concentration, yield, productivity, and specific productivity as response variables. Four equations were obtained and $R^2$ of the model for maximum xylitol concentration was 0.9895, which meant that 98.95% of total variations in the response were explained by the model. The three remaining coefficients of determination were also above 0.9, which suggested a good fit of the model. It was shown that the increase in xylose concentration in the range 55–165 g/l resulted in a rise in xylitol concentration. A rotational speed was also shown to have positive influence on the response especially in the range 100–200 rpm (Sampaio *et al.*, 2006). In another study, Vasquez *et al.* (2006) used RSM in order to optimize xylitol production from D-xylose by *C. guilliermondii*. They investigated the influence of the oxygen transfer coefficient and initial cell mass on xylitol yield and productivity. The optimal point corresponded to a cell mass of 9.86 g and an oxygen transfer coefficient of 32.85/h, for which predicted productivity was $1.4 \pm 0.09$ g/h and predicted product yield was $0.7 \pm 0.02$ g/g with confidence level of 95% (Vasquez *et al.*, 2006). Sarrouh and da Silva (2010) used RSM ($2^3$ factorial design) to study the production of xylitol by *C. guilliermondii* from hemicellulosic hydrolysates rich in xylose in a fluidized bed reactor. They chose three independent variables, namely air flow, concentration of hydrolysate, and fluidization flux, and xylitol yield and productivity as response variables. The best values of the xylose to xylitol bioconversion parameters were observed in the central point of the factorial design (air flow, 600 ml/min; concentration of hydrolysate, 5x; and fluidization flux, 38) (Sarrouh and da Silva, 2010). Our results also showed the important role of culture aeration, which is determined by different factors (oxygen transfer, rotation speed, air flow) and can limit oxygenation processes in the cells and biomass growth. A second factor which is usually taken into consideration is the concentration of sugar as a sole carbon source and also mean values of it are the optimal for the process. It is interesting that different models are developed for different response variables of the same process, *e.g.*, product concentration (as in this report), product yield, productivity and so on. The variety of response variables, however, impedes comparison of results obtained by different scientists.

In summary, karyoductant SP-K7 of *S. cerevisiae* $V_{30}$ and *P. stipitis* CCY 39501 was able to produce arabitol from L-arabinose in batch cultures in different concentrations (0.13–18.92 g/l) and with different yields (0.005–0.94 g/g) depending on culture conditions and the amount of consumed pentose. RSM used in the optimization of this process showed that the most influential factors were rotation speed, concentration of L-arabinose and the temperature of incubation. The optimum values of these factors were 150 rpm, 32.5 g/l, and 28°C, respectively. The model predicted that the concentration of arabitol after two days of yeast incubation should be 18.367 g/l, which indicates that it is possible to achieve a yield of about 0.56 g/g during complete consumption of L-arabinose. The verification experiment confirmed the usefulness of the statistical model.

## Literature

**Bera A.K., M. Sedlak, A. Khan and N.W.Y. Ho.** 2010. Establishment of L-arabinose fermentation in glucose/xylose co-fermentation recombinant *Saccharomyces cerevisiae* 424 (LNH-ST) by genetic engineering. *Appl. Microbiol. Biotechnol*. 87: 1803–1811.

**Bettiga M., O. Bengtsson, B. Hahn-Hagerdal and M.F. Gorwa-Grauslund.** 2009. Arabinose and xylose fermentation by recombinant *Saccharomyces cerevisiae* expressing a fungal pentose utilization pathway. *Microbial Cell Factories* 8: 40.

**Fonseca C., I. Spencer-Martins and B. Hahn-Hagerdal.** 2007. L-arabinose metabolism in *Candida arabinofermentans* PYCC 5603$^T$ and *Pichia guilliermondii* PYCC 3012: influence of sugar and oxygen on product formation. *Appl. Microbiol. Biotechnol.* 75: 303–310.

**Hahn-Hagerdal B., K. Karhumaa, C. Fonseca, I. Spencer-Martins and M.F. Gorwa-Grauslund.** 2007. Towards industrial pentose-fermenting yeast strains. *Appl. Microbiol. Biotechnol*. 74: 937–953.

**Jeffries T.W. and Y.-S. Jin.** 2004. Metabolic engineering for improved fermentation of pentoses by yeasts. *Appl. Microbiol. Biotechnol*. 63: 495–509.

**Karhumaa K., B. Wiedemann, B. Hahn-Hagerdal, E. Boles and M.F. Gorwa-Grauslund.** 2006. Co-utilization of L-arabinose and D-xylose by laboratory and industrial *Saccharomyces cerevisiae* strains. *Microbial Cell Factories* 5: 18.

**Koganti S., T.M. Kuo, C.P. Kurtzman, N. Smith and L.-K. Ju.** 2011. Production of arabitol from glycerol: strain screening and study of factors affecting production yield. *Appl. Microbiol. Biotechnol.* 90: 257–267.

**Kordowska-Wiater M. and Z. Targoński.** 2001. Application of *Saccharomyces cerevisiae* and *Pichia stipitis* karyoductants to the production of ethanol from xylose. *Acta. Microbiol. Pol.* 3–4: 291–299.

**Kordowska-Wiater M., Targoński Z. and A. Jarosz.** 2008. Biotransformation of L-arabinose to arabitol by yeasts from genera *Pichia* and *Candida* (in Polish). *Biotechnologia* 1: 177–188.

**Lucca M.E., J.F.T. Spencer and L.I.C. de Figueroa.** 2002. Glycerol and arabitol production by an intergeneric hybrid, PB2, obtained by protoplast fusion between *Saccharomyces cerevisiae* and *Torulaspora delbrueckii*. *Appl. Microbiol. Biotechnol*. 59: 472–476.

**Mc Millan J.D. and B.L. Boynton.** 1994. Arabinose utilization by xylose-fermenting yeasts and fungi, *Appl. Biochem. Biotechnol*. 45/46: 569–584.

**Monedero V., G. Perez-Martines and M. Yebra.** 2010. Perspectives of engineering lactic acid bacteria for biotechnological polyol production. *Appl. Microbiol. Biotechnol*. 86: 1003–1015.

**Montgomery D.C.** 2001. *Design and analysis of experiments*, 5$^{th}$ ed. Willey, New York.

**Myers R.H. and D.C. Montgomery.** 2002. *Response Surface Methodology*. Willey, New York.

**Saha B.C. and R.J. Bothast.** 1996. Production of L-arabitol from L-arabinose by *Candida entomaea* and *Pichia guilliermondii*. *Appl. Microbiol. Biotechnol*. 45: 299–306.

**Sampaio F.C., D. de Faveri, H.C. Mantovani, F.M. Lopez Passos, P. Perego and A. Converti.** 2006. Use of response surface methodology for optimization of xylitol production by the new yeast strain *Debaryomyces hansenii* UFV-170. *J. Food Engin*. 76: 376–386.

**Sanchez R.G., K. Karhumaa, C. Fonseca, V. Nogue, J.R.M. Almeida, C.U. Larsson, O. Bengtsson, M. Bettiga, B. Hahn-Hagerdal and M. Gorwa-Grauslund.** 2010. Improved xylose and arabinose utilization by an industrial recombinant *Saccharomyces cerevisiae* strain using evolutionary engineering. *Biotechnol. Biofuels*. 3: 13.

**Sarrouh B.F. and S.S. da Silva.** 2010. Application of response surface methodology for optimization of xylitol production from lignocellulosic hydrolysate in a fluidized bed reactor. *Chem. Eng. Technol*. 33: 1481–1487.

**Sedlak M. and N. Ho.** 2001. Expression of *E. coli* ara BAD operon encoding enzymes for metabolizing L-arabinose in *Saccharomyces cerevisiae*. *Enzyme Microbial. Technol*. 28: 16–24.

StatSoft, Inc. 2007. STATISTICA version 8.0. www.statsoft.com

**Vasquez M.P., M.B. de Souza and N. Pereira.** 2006. RSM analysis of the effects of the oxygen transfer coefficient and inoculum size on the xylitol production by *Candida guilliermondii*. *Appl. Biochem. Biotechnol*. 129–132: 256–264.