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NEW POSSIBILITIES FOR THE VALORIZATION OF SUGAR INDUSTRY BY-PRODUCTS

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This paper presents researches in developing new ways to exploit the fermentable sugars from beet-pulp that could be used as substrate (mainly as carbon source) in obtaining the fodder yeast, at industrial level.
The research aimed at developing a fabrication recipe for manufacturing the fodder yeast using as a main substrate the beet-pulp hydrolyzate obtained after a concentrated sulphuric acid treatment. The recipe was optimized according to the influence of the nutrient amounts and sugar content in the substrate over accumulation of biomass, using two strains of yeast *Candida utilis* and *Candida arboreea.*
The results lead to the conclusions that the beet-pulp hydrolyzate can be used in the manufacture of fodder yeast, constituting an effective source of sugar, with a yield of 89%. Offering a better response under experimental conditions, *Candida utilis* may be considered an appropriate candidate for the manufacture of fodder yeast from beet-pulp hydrolyzate, but it does not exclude the possibility of using both strains at the industrial level, because the differences in biomass gain were minimal.

INTRODUCTION

The food industry, through its large range of by-products and waste from manufacturing streams, has a significant impact on the environment. The major by-product of sugar processing plants (225.4 kg pressed beet pulp with 24.3% dry matter/1000 kg of processed beet pulp), the beet-pulp, is generally used as fodder in cattle-raising industry[1] and, more recently, it can serve as a substrate for bio-hydrogen production,[2,3] in obtaining pectin extract[4,5] and biodegradable plastics[6], antioxidants[7-9] or sugar beet cellulose nanofibril-reinforced composites.[10,11]

Sugar beet pulp is a material susceptible to unwanted aerobic and/or anaerobic biodegradation reactions, especially when the product is stored in unsuitable conditions, due to its complex chemical composition, with a negative impact over the environment.[12] Beet pulp average composition is: 94.4% water, 4.7% insoluble substances in water (cellulose 1.2%, hemicelluloses 1.1%, pectin substances 2.4%), 0.7% minerals and soluble organic substances, and 0.2% soluble sugar.[13,14]

The conversion of hemicelluloses and cellulose from the sugar beet pulp in fermentable sugar can be achieved by enzymatic[15-18] and acid hydrolysis[19] (with diluted or concentrated mineral acids).

The most used method is the enzymatic hydrolysis, but it requires a strict control of working parameters, with considerable energy consumption and reduced working capacity.

The treatment of sugar beet with sulphuric acid was conducted in two stages, to avoid the destructions of valuable compounds sensitive to high acid concentrations. In the first stage, the hydrolysis of hemicelluloses using sulphuric acid at the concentration of 38% w/w and then, in the second stage, the hydrolysis of cellulose using a concentration of 63% w/w.[19]

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After using Amberlite IR-118 for a chromatographic separation of the residual sulphuric acid[20] (>70%), the product was neutralized with CaO, filtered and concentrated from an initial content of 8.8 ± 0.5 g/L sugar at 18 ± 0.5 g/L.[19]

To test the viability of the outcome and given the high concentrations of sugar that is obtained after hydrolysis, the product was used as the main source of carbon for the development of fodder yeasts.

RESULTS AND DISCUSSION

There were a total of 27 runs for optimizing individual parameters in the current t-test, which was applied to the accumulation of biomass and total nitrogen for the two species of fodder yeast. The response values at different experimental combinations for coded variables are listed in Table 1.

Table 1

T-test and the response for biomass and total nitrogen accumulation for the two strains of yeasts

Run	x_1	x_2	x_3	Response g biomass CU*/L	Response g biomass CA*/L	Response % total nitrogen CU*	Response % total nitrogen CA*
1	-1	-1	-1	3.95	3.87	30.1	28.4
2	-1	-1	0	4.23	4.12	31.6	29.6
3	-1	-1	1	4.24	4.17	32.3	30.6
4	-1	0	-1	4.65	4.57	35.8	33.9
5	-1	0	0	4.95	4.87	38.5	36.4
6	-1	0	1	5.14	5.02	39.9	37.6
7	-1	1	-1	4.95	4.87	38.5	36.4
8	-1	1	0	5.65	5.56	42.7	40.4
9	-1	1	1	5.80	5.73	44.2	42.0
10	0	-1	0	6.65	6.44	50.7	47.3
11	0	-1	0	5.70	5.62	44.5	42.2
12	0	-1	0	6.75	6.67	52.4	49.9
13	0	0	0	6.50	6.44	50.5	50.0
14	0	0	0	6.60	6.53	50.3	47.9
15	0	0	0	6.90	6.77	53.1	47.7
16	0	1	0	6.57	6.35	50.1	46.6
17	0	1	0	6.60	6.52	50.3	47.8
18	0	1	0	6.40	6.42	48.8	47.1
19	1	-1	1	6.55	6.46	49.9	47.4
20	1	-1	0	6.95	6.71	52.7	48.9
21	1	-1	-1	5.40	5.32	41.2	39.0
22	1	0	1	5.73	5.45	43.7	40.0
23	1	0	0	5.73	5.65	43.4	41.2
24	1	0	-1	5.43	5.40	41.4	39.6
25	1	1	1	5.53	5.40	42.6	40.0
26	1	1	0	5.33	4.88	40.6	35.8
27	1	1	-1	5.10	4.56	38.9	33.5

* CU – *Candida utilis*; CA – *Candida arboreea*

Accumulation of biomass recorded significant values when using a concentration of 16 g/L reducing sugar. Comparable but slightly lower values were obtained using 18 g/L reducing sugar.

The experimental program aimed at full utilization of the hydrolyzate from sugar beet pulp. That is why the final product consists in a mixture of reducing sugars: 40% obtained from hemicelluloses hydrolysis and the remaining 60% from cellulose hydrolysis. It was observed that increasing the amount of hydrolyzed beet pulp in the recipe leads to inhibition of metabolic activity for both strains of yeast, within 48 hours of incubation. This can be explained by the low affinity of yeasts for using the reducing sugars from hydrolysis of hemicelluloses as a main source of carbon.

Similar behaviour is observed in the accumulation of biomass for the two strains, the maximum values are 6.95 ± 0.02 g/L for *Candida utilis* and 6.77 ± 0.02 g/L for *Candida arboreea*. The average value of the differences obtained in the same experimental conditions in biomass accumulation for *Candida utilis* as compared to *Candida arboreea* is 0.13 ± 0.02 g/L.

By applying the multiple regression analysis on the experimental data for biomass accumulation at *Candida utilis*, the response variable and the test variable were related by the following second-order polynomial equation:

$$y = 6.597 + 0.45 \cdot x_1 + 0.085 \cdot x_2 + 0.0072 \cdot x_3 - 0.579 \cdot x_1 \cdot x_2 - 0.2925 \cdot x_1 \cdot x_3 + \\ + 0.08 \cdot x_2 \cdot x_3 + 0.02 \cdot x_1 \cdot x_2 \cdot x_3 - 1.225 \cdot x_1^2 - 0.0505 \cdot x_2^2 - 0.07 \cdot x_3^2 \quad (1)$$

Some insignificant terms were neglected and the predicted model can be described by the following equation in terms of coded values:

$$y = 6.597 + 0.45 \cdot x_1 + 0.085 \cdot x_2 - 0.579 \cdot x_1 \cdot x_2 - 0.2925 \cdot x_1 \cdot x_3 + 0.08 \cdot x_2 \cdot x_3 - 1.225 \cdot x_1^2 \quad (2)$$

The optimum response function was determined through the derivatives method:

$$\begin{cases} \dfrac{\partial y}{\partial x_1} = 0.45 - 0.579 x_2 - 0.2925 x_3 - 2.45 x_1 \\ \dfrac{\partial y}{\partial x_2} = 0.085 - 0.579 x_1 + 0.08 x_3 \\ \dfrac{\partial y}{\partial x_3} = -0.2925 x_1 + 0.08 x_2 \end{cases} \quad (3)$$

In Figs. 1, 2 and 3, the response functions are plotted in 3D (response surface plot) and 2D (contour plot) using PTC - Mathcad v.14, allowing us to visualize the maximum value determined.

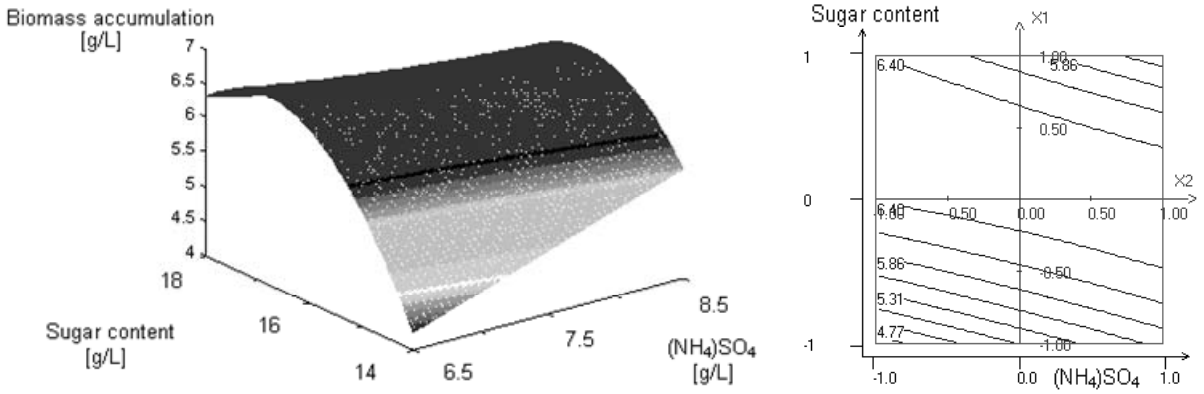

Fig. 1 – The biomass accumulation based on variation of sugar and ammonium sulphate content, the quantity of magnesium sulphate being in the centre field for *Candida utilis* (response surface and contour plot).

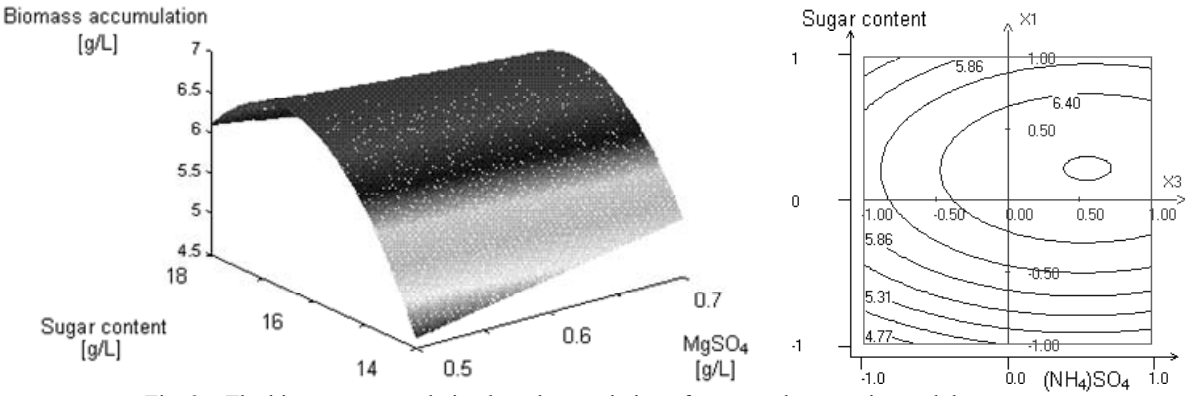

Fig. 2 – The biomass accumulation based on variation of sugar and magnesium sulphate content, the quantity of ammonium sulphate being in the centre field for *Candida utilis* (response surface and contour plot).

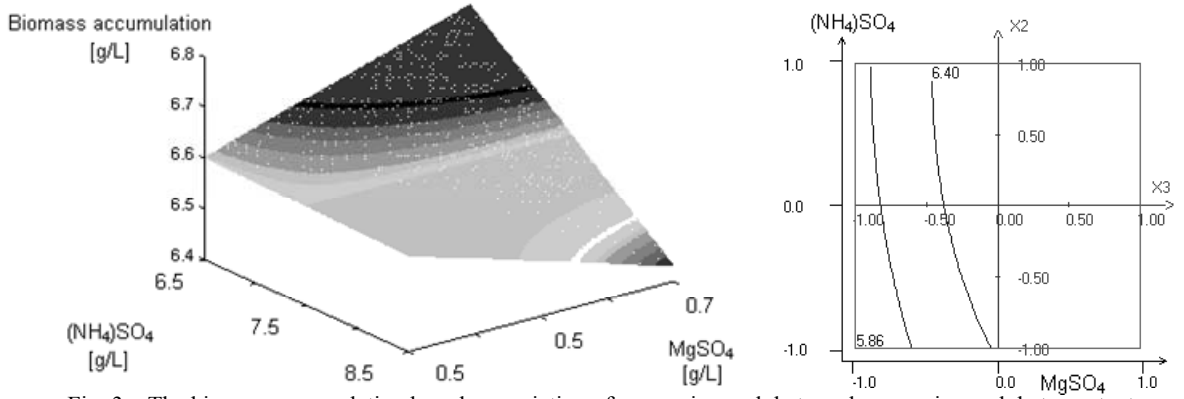

Fig. 3 – The biomass accumulation based on variation of ammonium sulphate and magnesium sulphate content, the quantity of sugar being in the centre field for *Candida utilis* (response surface and contour plot).

The real values of independent variables for the optimum value were calculated or determined by over-imposing the graphic representations from Figs. 1, 2 and 3, and they are: X_1 = 16.228 g/L sugar content, X_2 = 7.925 g/L $(NH_4)_2SO_4$, X_3 = 0.574 g/L $MgSO_4$.

By applying the multiple regression analysis on the experimental data on biomass accumulation at *Candida arboreea*, the response variable and the test variable were related through the following second-order polynomial equation:

$$y = 7.8 + 2.179 \cdot x_1 + 0.05 \cdot x_2 + 0.011 \cdot x_3 - 0.644 \cdot x_1 \cdot x_2 - 0.303 \cdot x_1 \cdot x_3 + \\ + 0.058 \cdot x_2 \cdot x_3 - 0.032 \cdot x_1 \cdot x_2 \cdot x_3 - 1.271 \cdot x_1^2 - 0.262 \cdot x_2^2 - 0.05 \cdot x_3^2 \quad (4)$$

Some insignificant terms were neglected and the predicted model can be described by the following equation in terms of coded values:

$$y = 7.8 + 2.179 \cdot x_1 + 0.05 \cdot x_2 - 0.644 \cdot x_1 \cdot x_2 - 0.303 \cdot x_1 \cdot x_3 + 0.058 \cdot x_2 \cdot x_3 - 1.271 \cdot x_1^2 \\ - 0.262 \cdot x_2^2 - 0.05 \cdot x_3^2 \quad (5)$$

The optimum response function was determined through the derivatives method:

$$\begin{cases} \dfrac{\partial y}{\partial x_1} = 2.179 - 0.644 x_2 - 0.303 x_3 - 2.542 x_1 \\ \dfrac{\partial y}{\partial x_2} = 0.05 - 0.644 x_1 + 0.058 x_3 - 0.524 x_2 \\ \dfrac{\partial y}{\partial x_3} = -0.303 x_1 + 0.508 x_2 - 0.1 x_3 \end{cases} \quad (6)$$

In Figs. 4, 5 and 6 the response functions are plotted in 3D (response surface plot) and 2D (contour plot) using PTC - Mathcad v.14, allowing us to visualize the maximum value determined.

The real values of the independent variables for the optimum result were calculated or determined by over-imposing the graphic representations from Figs. 4, 5 and 6, and they are: X_1 = 17.124 g/L sugar content, X_2 = 7.239 g/L $(NH_4)_2SO_4$, X_3 = 0.637 g/L $MgSO_4$.

After weighing the biomass was used to determine the total nitrogen, in all 27 runs (Table 1). Accumulation of total nitrogen shows a similar behaviour as biomass accumulation because it is directly influenced by proteic nitrogen found in biomass.

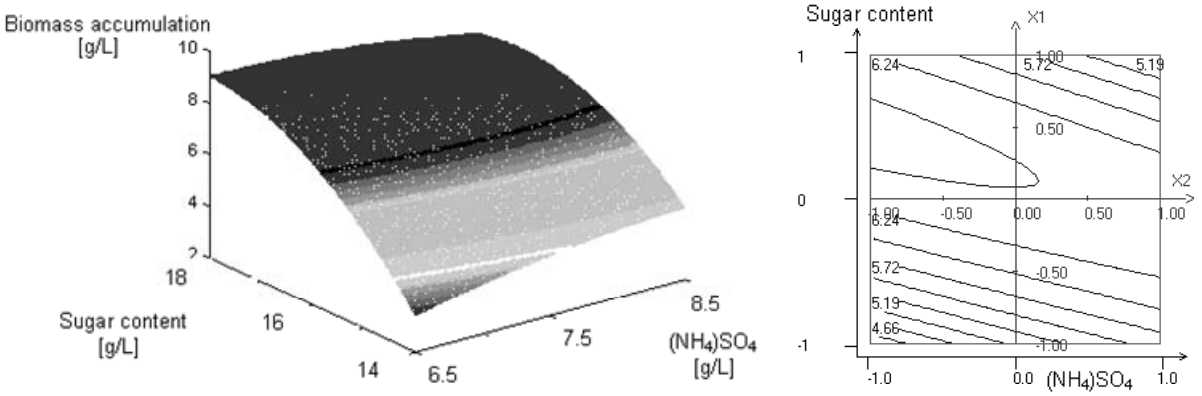

Fig. 4 – The biomass accumulation based on variation of sugar and ammonium sulphate content, the quantity of magnesium sulphate being in the cantered field for *Candida arboreea* (response surface and contour plot).

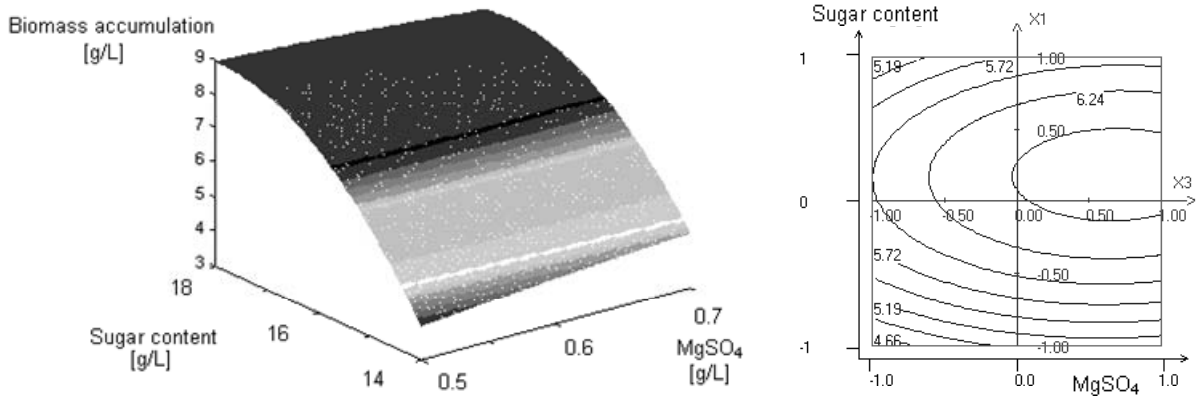

Fig. 5 – The biomass accumulation based on variation of sugar and magnesium sulphate content, the quantity of ammonium sulphate being in the centre field for *Candida arboreea* (response surface and contour plot).

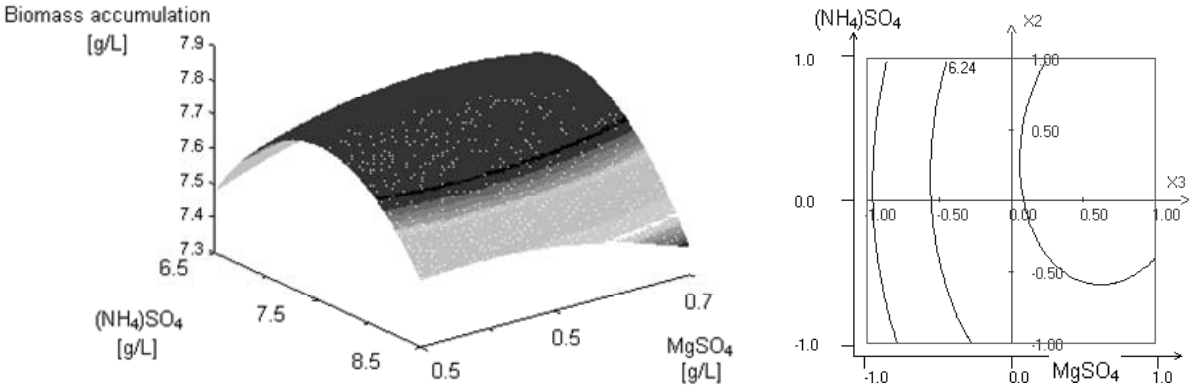

Fig. 6 – The biomass accumulation based on variation of ammonium sulphate and magnesium sulphate content, the quantity of sugar being in the centre field for *Candida arboreea* (response surface and contour plot).

The total nitrogen accumulation for the two strains reaches the maximum value at 52% for *Candida utilis* and 50% for *Candida arboreea*. The average value of the differences obtained in the same experimental conditions in biomass accumulation for *Candida utilis* as compared to *Candida arboreea* is 2.64%.

By applying multiple regression analysis on the experimental data on the total nitrogen accumulation for *Candida utilis*, the response variable and the test variable were related by the following second-order polynomial equation:

$$y = 52.07 + 3.37 \cdot x_1 + 0.62 \cdot x_2 + 0.016 \cdot x_3 - 4.425 \cdot x_1 \cdot x_2 - 2.225 \cdot x_1 \cdot x_3 + \\ + 0.458 \cdot x_2 \cdot x_3 + 0.187 \cdot x_1 \cdot x_2 \cdot x_3 - 9.63 \cdot x_1^2 - 0.5 \cdot x_2^2 - 2.5 \cdot x_3^2 \quad (7)$$

Some insignificant terms were neglected and the predicted model can be described by the following equation in terms of coded values:

$$y = 52.07 + 3.37 \cdot x_1 + 0.62 \cdot x_2 - 4.425 \cdot x_1 \cdot x_2 - 2.225 \cdot x_1 \cdot x_3 - 9.63 \cdot x_1^2 - 2.5 \cdot x_3^2 \quad (8)$$

The optimum response function was determined through the derivatives method:

$$\begin{cases} \dfrac{\partial y}{\partial x_1} = 3.37 - 4.425 x_2 - 2.225 x_3 - 19.26 x_1 \\ \dfrac{\partial y}{\partial x_2} = 0.62 - 4.425 x_1 \\ \dfrac{\partial y}{\partial x_3} = -0.225 x_1 - 5 x_3 \end{cases} \quad (9)$$

In Figs. 7, 8 and 9 the response functions are plotted in 3D (response surface plot) and 2D (contour plot) using PTC - Mathcad v.14, allowing us to visualize the maximum value determined.

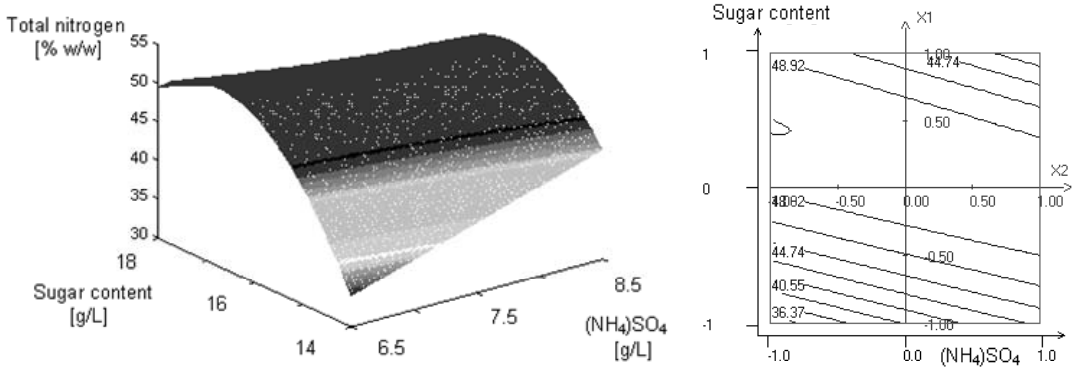

Fig. 7 – The total nitrogen percentage based on variation of sugar and ammonium sulphate content, the quantity of magnesium sulphate being in the centred field for *Candida utilis* (response surface and contour plot).

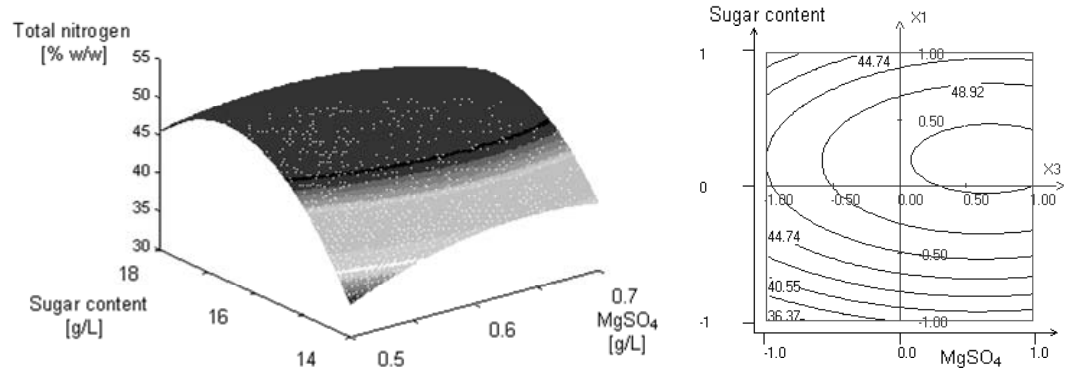

Fig. 8 – The total nitrogen percentage based on variation of sugar and magnesium sulphate content, the quantity of ammonium sulphate being in the centred field for *Candida utilis* (response surface and contour plot).

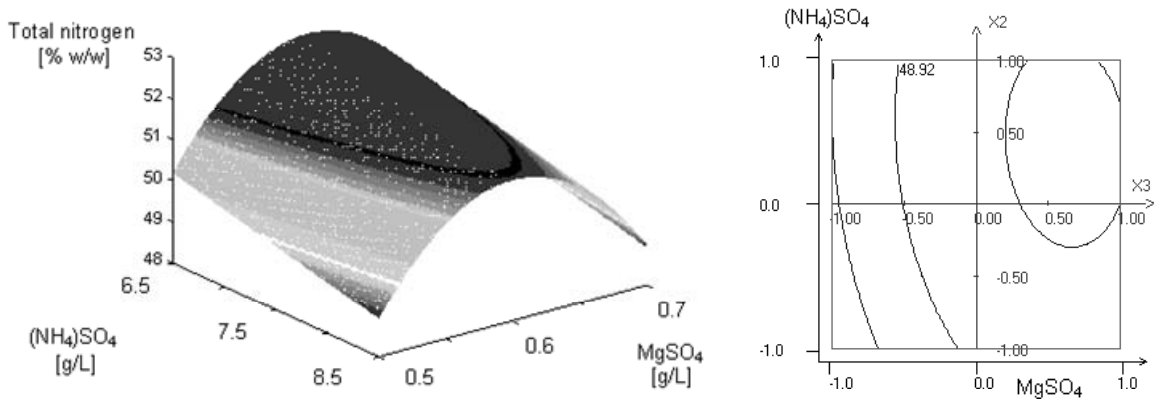

Fig. 9 – The total nitrogen percentage based on variation of ammonium sulphate and magnesium sulphate content, the quantity of sugar being in the cantered field for *Candida utilis* (response surface and contour plot).

The real values of the independent variables for the optimum result were calculated or determined by over-imposing the graphic representations from Figs. 7, 8 and 9, and they are: X_1 = 16.28 g/L sugar content, X_2 = 7.493 g/L $(NH_4)_2SO_4$, X_3 = 0.668 g/L $MgSO_4$.

By applying the multiple regression analysis on the experimental data on total nitrogen accumulation for *Candida arboreea*, the response variable and the test variable were related by the following second-order polynomial equation:

$$y = 48.084 + 2.78 \cdot x_1 + 1.794 \cdot x_2 - 0.16 \cdot x_3 - 4.683 \cdot x_1 \cdot x_2 - 2.233 \cdot x_1 \cdot x_3 + \\ + 0.266 \cdot x_2 \cdot x_3 - 1.425 \cdot x_1 \cdot x_2 \cdot x_3 - 9.56 \cdot x_1^2 - 0.871 \cdot x_2^2 - 0.195 \cdot x_3^2 \quad (10)$$

Some insignificant terms were neglected and the predicted model can be described by the following equation in terms of coded values:

$$y = 48.084 + 2.78 \cdot x_1 + 1.794 \cdot x_2 - 4.683 \cdot x_1 \cdot x_2 - 2.233 \cdot x_1 \cdot x_3 - 9.56 \cdot x_1^2 - 0.871 x_2^2 \quad (11)$$

The optimum response function was determined through the derivatives method:

$$\begin{cases} \dfrac{\partial y}{\partial x_1} = 2.78 - 4.683 x_2 - 2.233 x_3 - 19.12 x_1 \\ \dfrac{\partial y}{\partial x_2} = 1.794 - 4.683 x_1 - 1.742 x_2 \\ \dfrac{\partial y}{\partial x_3} = -2.233 x_1 \end{cases} \quad (12)$$

In Figs. 10, 11 and 12 the response functions are plotted in 3D (response surface plot) and 2D (contour plot) using PTC - Mathcad v.14, allowing us to visualize the maximum value determined.

The real values of the independent variables for optimum results were calculated or determined by over-imposing the graphic representations from Figs. 10, 11 and 12, and they are: X_1 = 16.00 g/L sugar content, X_2 = 8.54 g/L $(NH_4)_2SO_4$, X_3 = 0.507 g/L $MgSO_4$.

In order to verify the optimized values obtained for biomass and the total nitrogen accumulations for the two strains of yeast from the statistical model, a total of three experiments were conducted for each one. The lack of major differences between the experimental results led to the formulation of a common recipe that was later used to determine the level of consumption of sugar in 24 hours.

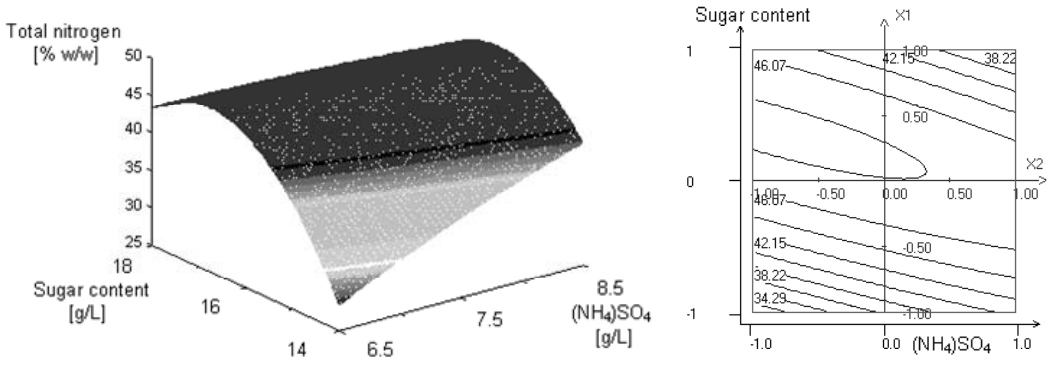

Fig. 10 – The total nitrogen percentage based on variation of sugar and ammonium sulphate content, the quantity of magnesium sulphate being in the centred field for *Candida arboreea* (response surface and contour plot).

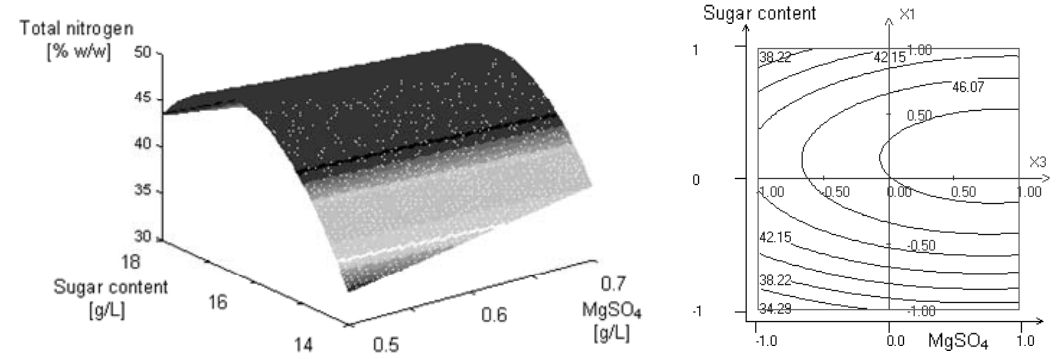

Fig. 11 – The total nitrogen percentage based on variation of sugar and magnesium sulphate content, the quantity of ammonium sulphate being in the centred field for *Candida arboreea* (response surface and contour plot).

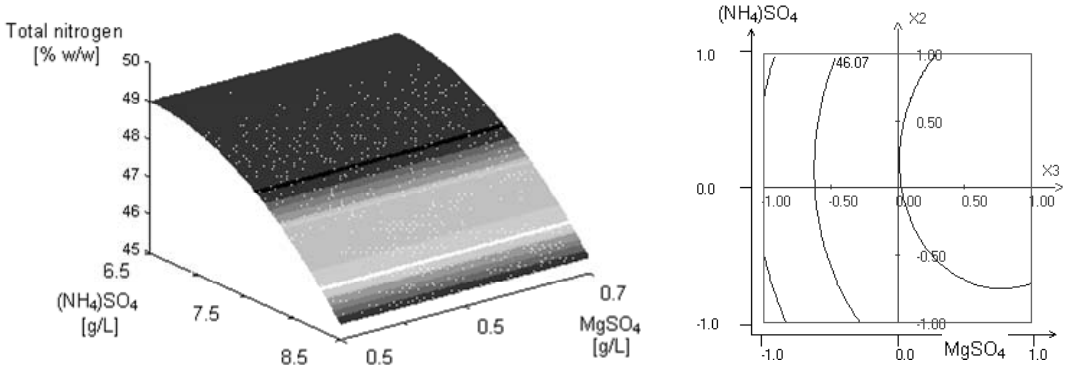

Fig. 12 – The total nitrogen percentage based on variation of ammonium sulphate and magnesium sulphate content, the quantity of sugar being in the centred field for *Candida arboreea* (response surface and contour plot).

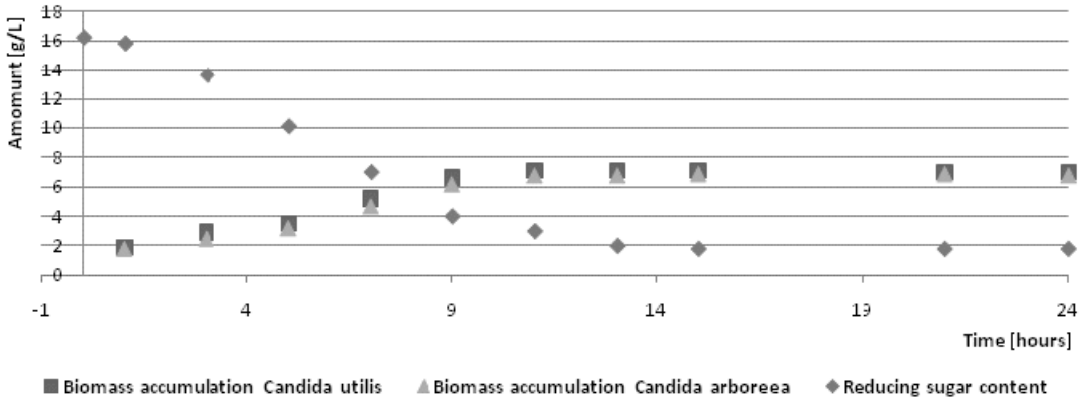

Fig. 13 – Accumulation of biomass in 24 h for the two types of yeast and sugar consumption.

The recipe that was used for these experiments was: beet pulp hydrolyzed with sugar content of 16.2 g/L, ammonium sulphate between 7.5 g/L, magnesium sulphate 420 mg/L, potassium chloride 1 g/L, ammonium phosphate 420 mg/L and 10^6 cells/cm^3 fodder yeast inoculums. The work parameters were pH 5 and 34 °C.

The results obtained had a low degree of difference in terms of biomass accumulation for both *Candida utilis* and *Candida arboreea*. The evolution of the sugar consumption was almost identical for the two types of yeast, the conversion degree being 89%, Fig. 13.

EXPERIMENTAL

For a better understanding and reproducibility of the experiment, two strains of yeasts (*Candida arboreea* and *Candida utilis*) have been chosen and, in the experimental conditions imposed, their behaviour in the accumulation of biomass and percentage of total nitrogen have been studied.

Yeast multiplication. A concentration of 10^6 cells/cm^3 in each culture medium was immersed, with the growth medium composition imposed by the experimental algorithm, incubated for 48 hours at 34 °C and then the cell biomass concentration was measured gravimetrically after centrifuging 7.5 cm^3 of sample, washing the cells with distilled water and then drying them at 105 °C until a constant weight was achieved. For determining the total nitrogen in the cell biomass, the samples were mineralized.

Experimental apparatus used in this stage were: trinocular microscope NOVEX, K Series, Model 85 340, 10x, 40x, 100x, with a vertical photo tube, hood with sterile air (laminar flow), "SPACE" PBI, 120/180, LEEC Classic Incubators C157 for *in vitro* culture, Hettich Table Centrifuge EBA III, 4x15 g load, adjustable speed 800-6000 rpm and Kern MLB 50-3 Moisture Balance.

The recipe which has been chosen for culture medium was: beet pulp hydrolyzed with sugar content between 14 and 18 g/L (variation imposed by the experimental algorithm); ammonium sulphate between 6.5 and 8.5 g/L (variation imposed by the experimental algorithm); magnesium sulphate between 500 mg and 700 mg/L (variation imposed by the experimental algorithm); potassium chloride 1 g/L; ammonium phosphate 420 mg/L; 10^6 cells/cm^3 fodder yeast inoculums.

To ensure the proper development conditions for the two strains of yeast, the pH was maintained at 5 and the incubation temperature at 34 °C.

Sugar content and total nitrogen determination. Hydrolyzate obtained by sugar beet pulp treatment with concentrated sulphuric acid with content in reducing sugar of 18 ± 0.5 g/L has been used as a main carbon source for *in vitro* growth of yeasts.

An FT-IR Bruker Tensor 27 spectrophotometer, at 540 nm and 3,5-Dinitrosalicylic acid (DNS) reagent were used for the determination of reducing sugar in the hydrolyzed product.

The content of total nitrogen from biomass (and the estimation of protein percentage in biomass) was determined through the Kjeldahl method using a Hach – Digesdahl Digestion Apparatus and an Auto Analyzer, model 1030, Tecantor, Hoganas.

Mathematical model. The t-test design was used to statistically optimize the formulation parameters and to evaluate the main effects, interaction effects and quadratic effects of the formulation ingredients on biomass accumulation and the total nitrogen gain for the two strains of fodder yeasts. According to the principle of the t-test, the content of reducing sugars in beet pulp hydrolyzate, ammonium sulphate and magnesium sulphate concentrations, which have a strong effect on the growth medium, respectively on yeast development, were taken as variables tested in a 27-run experiment. As shown in Table 2, the three factors chosen for the study were designated as X_1, X_2 and X_3, and were prescribed into three levels, coded +1, 0, -1 for high, intermediate and low value, respectively.

The test variables were coded according to the following equation:

$$x_i = \left(X_i - X_0\right)/\Delta X \tag{13}$$

where x_i is the coded value of an independent variable; X_i is the actual value of an independent variable at the centre point and ΔX is the step change value of an independent variable. For predicting the optimal point, a second-order polynomial model was fitted to correlate the relationship between independent variables and response (biomass and total nitrogen accumulation). For the three factors, the equation was:

$$Y = A_0 + \sum_{i=1}^{3} A_i X_i + \sum_{i=1}^{3} A_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} A_{ij} X_i X_j \tag{14}$$

where Y is the response (biomass and total nitrogen accumulation). A_0, A_i, A_j, and A_{ij} are the regression coefficients of variables for the intercept, linear, quadratic and interaction terms, respectively. X_i and X_j are the independent variables ($i \neq j$).[21-23]

Analyses of the experimental design and data were carried out using PTC - Mathcad v.14 and Microsoft Office Excel 2007.

Table 2

Code and level of independent variable chosen the for t-test, for both strains of *Candida utilis* and *Candida arboreea*

Variables	Symbol		Levels			ΔX
	Coded	Uncoded	-1	0	1	
			Actual values			
Reducing sugars in beet pulp hydrolyzate concentration, g/L	x_1	X_1	14	16	18	2
Ammonium sulphate concentration, g/L	x_2	X_2	6.5	7.5	8.5	1
Magnesium sulphate concentration, g/L	x_3	X_3	0.5	0.6	0.7	0.1

CONCLUSIONS

The interpretation of the results has led to the conclusion that the optimal values of the culture medium composition in terms of sugar content, the amount of ammonium sulphate and magnesium for the biomass accumulation and total nitrogen percentage for *Candida utilis* yeast are: beet pulp hydrolyzed with sugar content of 16.228 g/L, ammonium sulphate 7.925 g/L, magnesium sulphate 574 mg/L for a biomass accumulation of 6.9 g/L and beet pulp hydrolyzed with sugar content of 16.28 g/L, ammonium sulphate 7.493 g/L, magnesium sulphate 668 mg/L for the total nitrogen percentage of 52%.

The optimal values for the culture medium composition in terms of sugar content, the amount of ammonium sulphate and magnesium for the biomass accumulation and the total nitrogen percentage for *Candida arboreea* yeast are: beet pulp hydrolyzed with sugar content of 17.124 g/L, ammonium sulphate 7.239 g/L, magnesium sulphate 637 mg/L for a biomass accumulation of 6.7 g/L and beet pulp hydrolyzed with sugar content of 16.00 g/L, ammonium sulphate 8.5 g/L, magnesium sulphate 507 mg/L for a total nitrogen percentage of 50%.

The recipes contain a constant amount of potassium chloride 1 g/L and ammonium phosphate 420 mg/L. The fodder yeast inoculums contain 106 cells/cm3 and the experiments were run at pH 5 and 34 °C.

It is noted that for the two types of yeast the optimal values are close (16.5 to 17 g/L sugar content, 7.5 to 7.9 g/L $(NH_4)_2SO_4$ and 0.57 to 0.63 g/L $MgSO_4$; certain differences appear only in the biomass accumulation when *Candida utilis* has a slight advantage. The amount of total nitrogen is proportional to the increase in biomass.

The beet pulp hydrolyzate can be used in the manufacture of fodder yeast, constituting an effective source of sugar, with a conversion of 89%.

Better adapted, the *Candida utilis* strain may be considered an appropriate candidate for the manufacture of fodder yeast from beet pulp hydrolyzate. A mixture of the two yeasts can also be used in the manufacturing process because of the minor differences in accumulation of biomass and total nitrogen.

REFERENCES

1. J. C. Pereira and J. González, *Anim. Res*., **2004**, *53*, 99-110.
2. M. Hutnan, M. Drtil, J. Derco, L. Mrafkova, M. Hornak and S. Mico, *Pl. J. Env. Stud*., **2001**, *10*, 237-243.
3. L. Ozkan, H. Erguder and T. Demirer, *Int. J. Hydrogen. Energ.*, **2010**, *36*, 382-389.
4. E. Favela-Torres, T. Volke-Sepúlveda and G. Viniegra-González, *Food Technol. Biotechnol.*, **2006**, *44*, 221-227.
5. C.K. Siew and P.A. Williams, *J. Agric. Food Chem.*, **2008**, *56*, 4164-4171.
6. L. Liu, M. L. Fishman, K. B. Hicks and C. Liu, *J. Agric. Food Chem.*, **2005**, *53*, 9017-9022.
7. M. Sakac, J. Gyura, A. Mišan, Z. Šereš and B. Pajin., *Sugar Ind.*, **2011**, *136*, 151-157.
8. M. Sakac, J. Gyura, A. Mišan, Z. Šereš and B. Pajin., *Sugar Ind.*, **2009**, *134*, 418-425.
9. A. A. Mohdaly, M. A. Sarhan, I. Smetanska and A. Mahmoud, *J. Sci. Food Agric.*, **2010**, *90*, 218-226.
10. J. Leitner, B. Hinterstoisser, M. Wastyn. J. Keckes and W. Gindl, *Cellulose*, **2007**, *14*, 419-425.
11. A. Bendahou, Y. Habibi, H. Kaddami, A. Dufresne and *Rev. Roum. Chim.*, **2009**, *54*, 557-563.
12. Y. Akcay and M. Uzunoz, *J. Appl. Sci*., **2006**, *6 (5)*, 1123-1127.
13. M. Hutnan, M. Drtil, J. Derco and L. Mrafkova, *Biodegradation*, **2000**, *11*, 203-211.
14. A. R. Kirby, A. J. MacDougall and V. J. Morris, *Food Biophy.*, **2006**, *1*, 51-56.
15. Y. Kato and M. Kobayashi and *J. Appl. Glycosci*, **2000**, *1*, 45-48.
16. M. Spagnuolo, C. Crecchio, M. D. R. Pizzigallo and P. Ruggiero, *Biotec. Bioeng*., **1999**, *64*, 685-691.
17. M. F. Gailing, A. Guibert and D. Combes, *Bioprocess Eng*., **2000**, *22*, 69-74.
18. L. Sidi Ali, N. Cochet, T.K. Ghose and J.M. Lebeault, *Biotech. Lett.*, **1984**, *11*, 723-728.
19. A. Simion, "*Research regarding methods and technologies for valorization of by-products from sugar beet industry-technical and environmental impact"*, **2010**, "Gh. Asachi" Technical University of Iaşi, PhD Thesis.
20. J. Heinonen, and T. Sainio, *Ind. Eng. Chem. Res.*, **2010**, *49*, 2907-2915.
21. X. L. Bai, T. L. Yue, Y.H. Yuan and H. W. Zhang, *J. Sep. Sci.*, **2010**, *33*, 1-8.
22. S. Wang, F. Chen, J. Wu, Z. Wang, X. Liao and X.Hu, *J. of Food Eng.*, **2007**, *78*, 693-700.
23. M. Tulbure, D. Ciobanu and G. Feraru, *Env. and Mgmt. J.*, **2009**, *8*, 425-428.