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Statistical optimization of bioethanol production from waste bread hydrolysate

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Abstract: A recent trend in sustainable bioethanol production is the use of agricultural waste or food waste as an inexpensive and the most available feedstock. Bread waste is the major food waste that could be successfully used for the production of bioethanol. The aim of this study was to optimize ethanol production by the response surface methodology (RSM) using waste bread hydrolysate. Waste bread hydrolysate was obtained using crude hydrolytic enzymes that produce bacterial isolate Hymenobacter sp. CKS3. The influence of time of fermentation (24–72 h) and waste brewer's yeast inoculum (1–4 %) on ethanol production was studied. The optimal conditions, obtained by central composite design (CCD), were 48.6 h of fermentation and 2.85 % of inoculum. Under these conditions, a maximum of 2.06 % of ethanol concentration was reached. The obtained ethanol concentration was in good correlation, coefficient of 0.858, with yeast cell yield. The results obtained in this study imply that waste bread hydrolysate could be used as a biomass source for biofuel production with multiple benefits relating to environmental protection, reduction of production costs, and saving fossil fuels.

Keywords: waste bread; bioethanol; waste brewer's yeast; optimization; response surface methodology.

INTRODUCTION

During the last few decades, biofuels have gained more attention as an alternative to petroleum-based fuels.[1,2] Globalization and industrialization worldwide caused the depletion of fossil fuels that are major environmental pollutants. To reduce the negative environmental impact on climate changes caused by environ-

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mental pollutions, researchers over the world are searching for alternative and renewable energy resources that could replace the use of fossil fuels.[3]

Bioethanol is one of the dominant biofuel worldwide and especially in the transportation sector.[4] Mixing bioethanol and gasoline leads to reduction of greenhouse gas and carbon dioxide emission.[5] It was estimated that by 2030, fuel ethanol will displace 10–20 % of the gasoline demand.[6]

Bioethanol is produced by fermentation of simple sugars present in biomass or sugars obtained by prior chemical or enzymatic treatment of the biomass. As the biomass or feedstock for bioethanol production, different agricultural raw materials that contain appreciable amounts of sugar like molasses or starch (corn, wheat, rice, potatoes, *etc*.) and lignocellulosic raw materials (straw, grasses, *etc*.) could be converted into sugar and then fermented into ethanol.[7,8] In Europe, starch-based materials are currently most utilized for bioethanol production[9] but starchy raw materials are very costly.[10] To overcome this problem, utilization of food waste, rather than waste bread, for bioethanol production is very attractive. Bread is the most used food in many countries worldwide but, unfortunately, it becomes a major part of food waste. Apart from its good nutritional value, bread is one of the most wasted foods. Although a good part of the waste bread is used as animal fodder, a high amount is still wasted annually.[11] Therefore, the utilization of this organic waste for the production of fermentable sugars could be an option to reduce the costs of bioethanol production. In addition, by generating a value-added product from waste biomass (waste bread), the principles of a circular economy are respected while solid waste disposal is reduced.[12]

Bread contains a significant amount of starch that is hydrolyzed to simple sugars using enzymes amylases.[13] Additionally, proteins in bread after enzymatic hydrolysis to peptides and amino acids could be used for yeast growth and fermentation to obtain bioethanol.[13,14]

To develop an economically feasible process of enzymatic hydrolysis and thus bioethanol production, it is necessary to replace the commercial enzymes with in-house produced enzymes by adequate microorganisms. Today, there is a large number of microorganisms, natural isolates, that produce industrially important enzymes.[15] These microorganisms can use different agricultural by-products for their growth and to produce desired enzymes which are then used in the process of hydrolysis. From a large number of unexplored genera, special attention is drawn to the genus *Hymenobacter*, the enzymatic potential of which has not been revealed. It has previously been shown that the CKS3 strain, previously isolated from soil, possesses the ability to produce crude enzyme amylase as well as other industrially important enzymes.[16] In line with this, this study presents a continuation of previous research. Thus, enzymatically treated waste bread, by crude CKS3 amylase, was used in the optimization of bioethanol production using waste brewer's yeast. For this purpose, a statistical tool, response surface

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methodology (RSM), was used to describe the optimization of fermentation ethanol production. The parameters used in this optimization were the time of fermentation and yeast inoculum. Waste brewer's yeast, previously isolated from yeast biomass from the brewing industry, served as the inoculum for fermentation.

EXPERIMENTAL

Materials

Waste bread was kindly donated by the bakery "Skroz dobra pekara", Belgrade, Serbia. This waste bread (after shelf-life and without mold) was cut into cubes (2 cm×2 cm (±0.5 cm)).

Hydrolysis of waste bread

Enzymatic hydrolysis of waste bread was previously performed by crude enzyme amylase produced by *Hymenobacter* sp. CKS3, described in a previous work.[16] Hydrolysis was performed in an Erlenmeyer flask (300 mL) that contained 30 g of waste bread in 45 mL of 0.1 M acetate buffer pH 4.80. After sterilization (15 min, 120 °C), 75 mL of crude amylase was added at 50 °C. After 100 h of hydrolysis on an orbital shaker (200 rpm), the sample was centrifuged and the reducing sugars were quantified according to the method by Miller.[17]

Inoculum for ethanol fermentation

Waste brewer's yeast *Saccharomyces cerevisiae* was used for ethanol fermentation. The inoculum was prepared as described in a previous work by growing the yeast in malt extract broth (20 g L^{-1} malt extract) at 30 °C for 24 h.[18] A different amount of inoculum was used for ethanol fermentation according to the central composite design.

Optimization of ethanol production – central composite design

For ethanol production, waste bread hydrolysate was used. CCD was employed to evaluate the influence of two independent parameters: time of fermentation (A / h) and yeast inoculum concentration (B / %) on two dependent responses: ethanol production concentration ($Y1$ / %) and yeast cell yield ($Y2$, expressed as log (CFU / mL^{-1}). Each variable was studied at 3 different levels (Table I) and a set of 13 experiments were run (Table II).

TABLE I. Process parameters (ranges and levels) used in the CCD

Variable	–1	0	+1
A / h	24	48	72
B / %	1	2.5	4

The results showed that experimental data could be fitted with a quadratic polynomial model.

Analytical methods

The ethanol concentration was determined based on the density of alcohol distillate at 20 °C and expressed in wt. %.[19]

The number of viable yeast cells was determined by the indirect counting method – pour plate technique on malt extract agar plates. Serial dilutions of the samples were prepared, and after incubation at 30 °C for 48 h, the colonies were counted on malt extract agar plates. The changes in the viable cell number were calculated as:

$$\log (CFU / mL^{-1}) = \log ((CFU / mL^{-1})_1 - (CFU / mL^{-1})_0) \quad (1)$$

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where $(CFU / mL^{-1})_1$ is the number viable cell per milliliter after fermentation and $(CFU / mL^{-1})_0$ is the number viable cell per milliliter before fermentation.

TABLE II. CCD of RSM for ethanol production with actual (experimental) values

Run	Variable		Experimental values	
	A / h	*B* / %	*Y1* / %	*Y2*, as log (*CFU* / mL^{-1})
1	24	1	0.24	8.87
2	48	2.5	2.04	9.69
3	72	4	1.11	9.36
4	48	2.5	1.96	9.72
5	24	2.5	1.65	9.49
6	48	2.5	2.12	9.58
7	48	4	1.63	9.48
8	72	2.5	1.74	9.66
9	48	2.5	1.92	9.75
10	24	4	1.34	9.53
11	48	1	0.82	9.23
12	48	2.5	2.03	9.66
13	72	1	0.75	9.47

RESULTS AND DISCUSSION

Optimization of ethanol production – central composite design

Yeast fermentation using waste bread hydrolysate is a process in which bioethanol, as a value-added product, is obtained. In order to obtain maximum ethanol concentration, it is necessary to optimize the production process using simultaneously different parameters with a small number of experiments. In line with this, RSM was used as a very powerful tool for understanding the interactions between the independent parameters on one or several responses.

Ethanol production was optimized using a waste bread hydrolysate with a reducing sugar yield of 19.89 g L^{-1}, obtained in a previous study.[16] A set of 13 experiments were performed according to a designed matrix for CCD (Table II). The equations that relate ethanol concentration (*Y1* / %) and yeast cell yield (*Y2* expressed as log (*CFU* / mL^{-1})) as dependent variables to the independent variables time of fermentation (*A* / h) and inoculum concentration (*B* / %) could be expressed as follows:

$$Y1 = 2.02 + 0.062A + 0.38B - 0.18AB - 0.34A^2 - 0.81B^2 \quad (2)$$

$$Y2 = 9.67 + 0.10A + 0.13B - 0.19AB - 0.077A^2 - 0.30B^2 \quad (3)$$

An analysis of variance ANOVA was performed to determine the significance of the regression model for the two responses (Table III). The robustness of the two models was determined by calculating the determination coefficient R^2 and for the first response – ethanol concentration (*Y1*), it was 0.9933 and for the second response – yeast cell yield (*Y2*), R^2 was 0.9710. The high values of both R^2 (values close to 1) suggested that the models were reliable and could explain

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more than 99.33 and 97.10 %, respectively, of all variations. An adequate precision value higher than 4 (41.114 for *Y1* and 22.574 for *Y2*) indicate that the models could be used to predict the values for the responses.[20]

TABLE III. ANOVA table of the experimental results of the CCD (ethanol production); significant coefficient: $P < 0.05$

Source	*Y1*		*Y2*	
	F value	*P* value – *Prob* > *F*	*F* value	*P* value – *Prob* > *F*
Model	207.54	0.0001	46.95	0.0001
A / h	5.63	0.0493	20.83	0.0026
B / %	212.08	0.0001	37.03	0.0005
AB / h %	33.81	0.0007	51.46	0.0002
A^2 / h^2	79.41	0.0001	5.72	0.0480
B^2 / $\%^2$	448.83	0.0001	84.71	0.0001
Lack of fit	0.25	0.8602	0.25	0.8593
R^2	0.9933		0.9710	
Adjusted R^2	0.9885		0.9504	
Predicted R^2	0.9823		0.9291	
Coefficient of variation, %	4.28		0.56	
Adequate precision	41.114		22.574	

The relatively high adjusted determination coefficient adjusted R^2 (0.9885 for *Y1* and 0.9504 for *Y2*) refers to the significance of the tested models. Both response surface models for predicting ethanol production and yeast cell yield may be considered satisfactory.

The normal probability plot of the residuals *versus* externally studentized residuals for both models showed normally distributed points around a straight line, indicating that the models were suitable for use (Fig. 1a and b). The externally studentized residuals is a form of a Student's *t*-statistic, with the estimate of error varying between points, resulting from the division of a residual and its standard deviation.[20]

The regression analysis of the data (Table III) showed that ethanol production (*Y1*) was significantly affected by both tested parameters. The factors *A* and *B* were statistically significant ($P < 0.05$) as well as its quadratic coefficient and their interaction *AB* (time of fermentation-inoculum concentration). The influence of factors *A* and *B* on the response *Y1* (ethanol production) is presented on the perturbation plot (Fig. 2).

The quadratic coefficients A^2 and B^2 presented the maximum of the quadratic function indicating that ethanol production was highly influenced by these tested parameters. According to Eq. (2), parameter *B* (inoculum concentration) had the main influence on ethanol production, which describes a parabola with a pronounced maximum. With the increasing inoculum concentration up to 2.50 %, the ethanol production also increased. Higher inoculum concentration (2.5-2.85

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%) also showed increasing ethanol production but after reaching a maximum of 2.85 %, the ethanol concentration decreased.

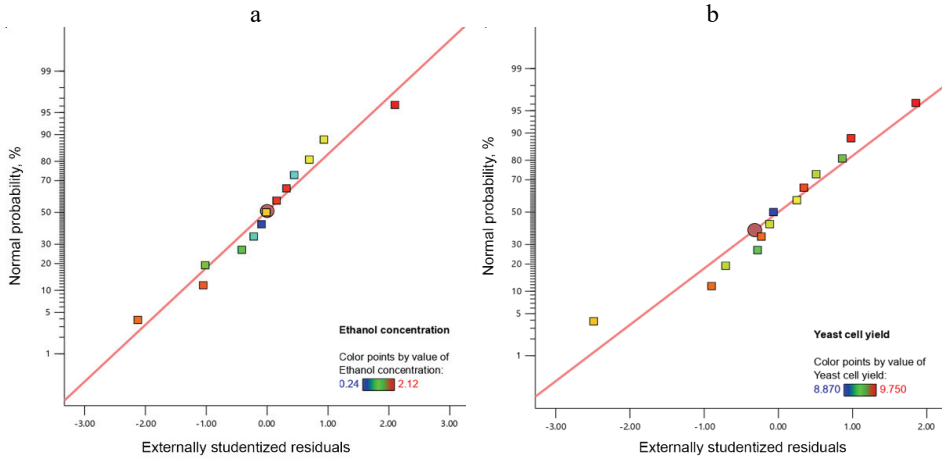

Fig.1. Normal probability plot of the residuals for: a) ethanol production (*Y1*) and b) yeast cell yield (*Y2*).

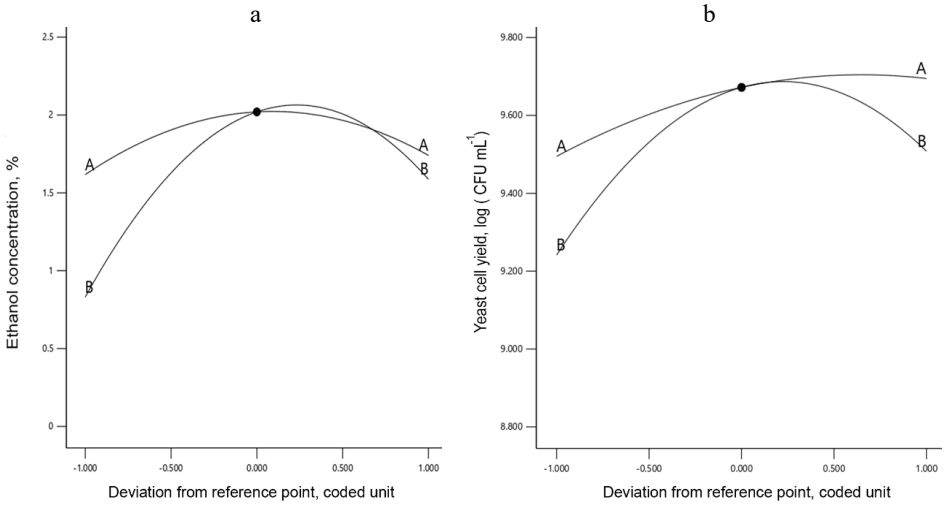

Fig. 2. Plot of the perturbation of time of fermentation (*A*) and inoculum concentration (*B*) on: a) ethanol production (*Y1*) and b) yeast cell yield (*Y2*).

The amount of inoculum for yeast fermentation is one of the crucial factors that influence ethanol production.[21] The increase in inoculum concentration leads to better utilization of reducing sugars by yeast, an increase in viable yeast biomass, and therefore the production of bioethanol.[21] However, further increase in

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inoculum concentration above the optimum results in reducing the number of viable yeast cells that are directly related to ethanol production.[22] Similar to this result, Izmirlioglu and Demirci[23] reported that 3 % of yeast inoculum was optimal for obtaining maximum ethanol production from waste potato mash. Furthermore, for maximum ethanol production from carob extract, a 3 % inoculum of *S. cerevisiae* was used. However, a higher inoculum concentration (up to 10 %) was also reported in the literature.[24,25]

The time of fermentation (*A*) also had a statistically positive influence on ethanol production (Table III). Compared with parameter *B*, the time of fermentation, *A* had a slightly curved line but a positive influence on ethanol production (Fig. 2a). Increasing the time of fermentation (*A*), the ethanol concentration increased, reaching a maximum at 48 h after which the ethanol concentration decreased. Observing the interaction *AB* (time of fermentation and inoculum fermentation, Fig. 3), increasing the time of fermentation (*A*) and inoculum concentration (*B*), ethanol concentration also increased, reaching a maximum and after further increasing of both variables, the ethanol concentration was decreased. The close relationship of these factors on the ethanol production could be confirmed. Namely, the time of fermentation affected the growth of the yeast cell. If the fermentation time is short and inoculum is low, there are not enough cells to multiply and fermentation is incomplete, while longer fermentation time and higher inoculum than optimal values, gave too many cells. Furthermore, the obtained ethanol under these fermentation conditions had a toxic effect on yeast cells and caused cell death.[24]

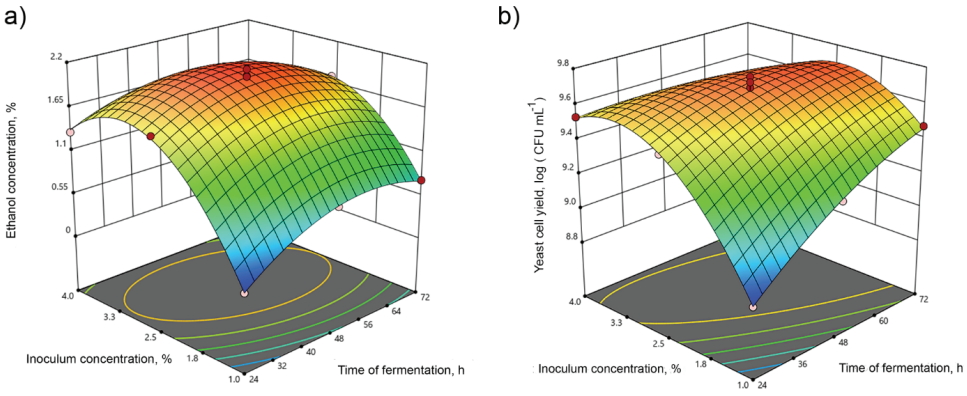

Fig. 3. The surface plot of time of fermentation and inoculum concentration on: a) ethanol production (*Y1*) and b) yeast cell yield (*Y2*).

During ethanol fermentation, the production of by-products (organic acids and higher alcohols) could decrease ethanol production by affecting some glycolytic intermediates in the corresponding metabolic pathways.[25] In addition, the

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effect of "environmental stresses" on yeast cells could cause yeast cell inhibition and further ethanol production.[25]

On the industrial scale, a prolonged fermentation time could increase ethanol production costs due to the higher energy consumption.[21] A fermentation time longer than 48 h obtained in this study was reported in the work of Awolu and coworkers.[26] They obtained 1.8 % of ethanol after 7 days of fermentation of waste bread using baker's yeast *S. caerevisiae.*[26] A shorter fermentation time with optimal ethanol yield is preferable from an economic point of view.

Datta and coworkers[27] obtained a higher concentration of ethanol 5.4 % than 2.12 % obtained in this study. They used an enzyme mixture that contained glucoamylase and protease, produced by the fungus *Aspergillus niger*, for enzymatic hydrolysis of waste bread and obtained 145 g L^{-1} glucose. This concentration of glucose was higher than 19.89 g L^{-1} of reducing sugars obtained in waste bread hydrolysate which was used for yeast fermentation and thus the ethanol concentration was lower. In most the published studies, enzymatic hydrolysis of waste bread was performed using commercial enzymes. In this study, waste bread hydrolysate was obtained by crude hydrolytic enzymes, mainly amylase, that produced a bacterial isolate *Hymenobacter* sp. CKS3. Our previous study showed that waste bread hydrolysis to fermentable sugars by CKS3 crude amylase was not complete and the end products of starch (waste bread) hydrolysis were mainly dextrins with the addition of maltotriose, maltose, and glucose.[16] Therefore, other published studies reported higher values of obtained ethanol concentration. In line with this, 10 % of the ethanol from bread residues was reported in the work of Ebrahimi and co-workers[28] while 8.31 % of ethanol was obtained using wheat-rye bread hydrolysate.[29] In both published studies,[28,29] commercial amylases were used for waste bread hydrolyze that released a higher amount of reducing sugar that is necessary for yeast fermentation and obtained higher ethanol concentration than in the present study (2.12 %). However, the use of raw enzymes in hydrolysis is a cheaper and cleaner process. Moreover, it is worth mentioning that this is one of the first studies that deals with the statistical optimization of bioethanol production using waste bread hydrolysate obtained by the hydrolytic enzymes produced by *Hymenobacter* sp. CKS3.

At lower ethanol concentrations, higher growth of yeast cells was detected, while with the achievement of maximum concentrations of ethanol of 2.12 % a decreased number of yeast cells was noted. Even earlier, Lind and coworkers[30] reported that ethanol altered the polarity of the cell membrane thus inhibiting the viability of yeast cells.

According to the regression analysis, the yeast cell yield was affected by the time of fermentation and inoculum concentration. Both parameters, their interaction, and their quadratic coefficients were statistically significant. The perturbation plot (Fig. 2b) shows the influence of factors *A* and *B* on yeast cell yield.

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The yeast cell yield gradually increased with increasing inoculum concentration but after reaching a maximum of 2.80 %, the yeast cell yield decreased (Fig. 2b). The time of fermentation had a positive influence on yeast cell yield (Fig. 2b). As could be noted from Table II (run 9), the maximum yeast cell yield (9.75log (*CFU* / mL^{-1})) was reached after 48 h of incubation and after that time, the yeast cell yield decreased. During fermentation, the yeast cells grow and multiply that lead to higher biomass (yeast cell yield).[31] The primary aim of a yeast cell is to reproduce and to produce cell biomass rather than produce ethanol.[32] Without significant yeast cell growth, ethanol cannot be produced.[32] On the other hand, yeast cells are sensitive to higher ethanol concentrations. As could be seen from Table II (run 6), after reaching a maximum concentration of produced ethanol (2.12 %), the yeast cell yield decreased. As mentioned before, a prolonged time of fermentation also had a negative effect on yeast cells, due to the accumulation of some toxic products.[24]

The high correlation coefficient of 0.890 between ethanol production (*Y1*) and yeast cell yield (*Y2*) showed that similar conditions affected both responses (Fig. 4). According to Bai[25] and Walker,[32] ethanol production is tightly linked with yeast cell growth.

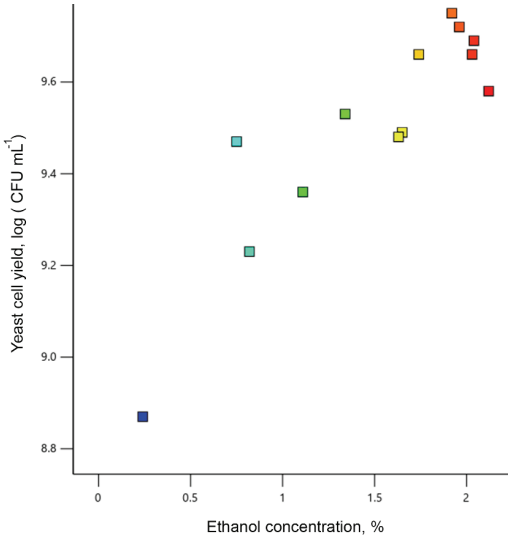

Fig. 4. Correlation between responses – ethanol production (*Y1*) and yeast cell yield (*Y2*).

Under the optimized conditions obtained from numerical optimization, the maximum ethanol production of 2.06 % was obtained after 48.68 h of fermentation with 2.85 % of inoculum. For this optimization, using software Design Expert 12, the criteria were to obtain maximum ethanol production with a selection of yeast cell yield "in range".

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Validation of the models

As mentioned before, the main goal of this study was to optimize the conditions that favor maximum ethanol production. Validation of the experiments was performed under the optimized conditions obtained from the desirability function: 48.86 h of fermentation and 2.85 % of inoculum. For these parameters, additional experiments were performed. The obtained ethanol concentration 2.06±0.10 % and yeast cell yield, log (*CFU* / mL^{-1}) = 9.72±0.1, fitted within the 95 % prediction intervals (PI) for ethanol production (1.90–2.23) and yeast cell yield (9.55–9.83). These results demonstrated that both models were reliable and acceptable for use.

CONCLUSIONS

An increasing interest worldwide in an alternative source of energy consequently leads to the use of various waste substrates in bioethanol production. Valorization of waste bread for bioethanol production is very promising due to its large availability, non-cost and protection from environmental pollution. For industrial demand, it is important to use low-cost waste substrates and "in house" produced enzymes that are economically very accepted. In this study, a statistical design – CCD was used to optimize the culture conditions for yeast fermentation, using for the first time waste bread hydrolysate previously obtained using hydrolytic enzymes produced by *Hymenobacter* sp. CKS3. Under optimal conditions, the maximum of produced ethanol was 2.06 %. This is the first study that deals with the optimization of ethanol production on waste bread hydrolysate, previously obtained using hydrolytic enzymes produced by *Hymenobacter* sp. CKS3, in the biotechnological process of obtaining bioethanol with the aim to reduce the number of wastes. In addition, the enzymatic potential of a novel strain of *Hymenobacter* may contribute to the development of the production of biofuels.

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И З В О Д

ОПТИМИЗАЦИЈА ПРОИЗВОДЊЕ БИОЕТАНОЛА ПОМОЋУ ХЛЕБНОГ ХИДРОЛИЗАТА

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Недавни тренд у одрживој производњи биоетанола је коришћење пољопривредног отпада или отпада од хране као јефтине и лако доступне сировине. Отпадни хлеб, као главни отпад од хране, се може веома успешно користити за производњу биоетанола. Циљ овог рада био је оптимизација услова производње биоетанола етанолном фермен-

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тацијом, односно ферментацијом квасца, на отпадном хлебном хидролизату, применом статистичке методе одзивне површине (RSM). Отпадни хидролизат хлеба је претходно добијен хидролизом отпадног хлеба помоћу сирових хидролитичких ензима, које производи бактеријски изолат *Hymenobacter* sp. CKS3. У овом раду, испитан је утицај времена ферментације отпадног пивског квасца (24-72 h) и концентрације инокулума отпадног пивског квасца (1–4 %) на производњу етанола користећи отпадни хлебни хидролизат. Оптимални услови, који су добијени применом централног композитног дизајна (CCD) у оквиру статистичке методе одзивне површине, били су 48,6 h ферментације и 2,85 % инокулума квасца. Под овим условима добијена је максимална концентрација етанола која износи 2,06 %. Ова концентрација етанола била је у добром коефицијенту корелације 0,858 са приносом ћелија квасца. Добијени резултати, у овој студији, указују на то да се хидролизат отпадног хлеба може веома успешно користити, као извор отпадне биомасе, за производњу биогорива. Оваква производња биогорива је еколошки и економски оправдана и утиче на смањење потрошње фосилних горива.

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REFERENCES

1. S. Tiwari, S. Jadhav, K. Tiwari, *Int. J. Environ. Sci. Technol.* **12** (2015) 3819 (https://doi.org/10.1007/s13762-014-0746-1)
2. S. Rezania, B. Oryani, J. Cho, A. Talaiekhozani, F. Sabbagh, B. Hashemi, P. F. Rupani, A. A. Mohammadi, *Energy* **199** (2020) 117457 (https://doi.org/10.1016/j.energy.2020.117457)
3. E. Demiray, S. E. Karatay, G. Dönmez, *Environ. Sci. Pollut. Res.* **26** (2019) 29366. (https://doi.org/10.1007/s11356-019-06020-1)
4. S. Nikolić, J. Pejin, L. Mojović, *Chem. Ind. Chem. Eng. Q.* **22** (2016) 1 (https://doi.org/10.2298/CICEQ151030001N)
5. H. S. Hafid, U. K. M. Shah, A. S. Baharuddin, A. B. Ariff, *Renew. Sustain. Energy Rev.* **74** (2017) 671 (https://doi.org/10.1016/j.rser.2017.02.071)
6. A. Walter, F. Rosillo-Calle, P. Dolzan, E. Piacente, K. B. da Cunha, *Biomass Bioenergy* **32** (2008) 730 (https://doi.org/10.1016/j.biombioe.2008.01.026)
7. P. Dinesha, S. Kumar, M. A. Rosen, *Environ. Sci. Pollut. Res.* **26** (2019) 8069 (https://doi.org/10.1007/s11356-019-04270-7)
8. J. Dodić, J. Grahovac, Z. Rončević, R. Pajović-Šćepanović, S. Dodić, B. Bajić, D. Vučurović, *J. Process. Energy Agric.* **22** (2018) 34 (https://doi.org/10.5937/JPEA1801034D)
9. C. A. Cardona, Ó. J. Sánchez, *Biores. Technol.* **98** (2007) 2415 (https://doi.opr/10.1016/j.biortech.2007.01.002)
10. J. R. Kwiatkowski, A. J. McAloon, F. Taylor, D. B. Johnston, *Ind. Crop Prod.* **23** (2006) 288 (https://doi.org/10.1016/j.indcrop.2005.08.004)
11. A. S. Demirci, I. Palabıyık, T. Gümüs, Ş. Özalp, *Waste Biomass Valor.* **8** (2017) 775 (https://doi.org/10.1016/j.indcrop.2005.08.004)
12. A. L. Young, *Environ. Sci. Pollut. Res*. **16** (2009) 117 (https://doi.org/10.1007/s11356-009-0106-8)
13. W. Pietrzak, J. Kawa-Rygielska, *Fuel* **134 (**2014) 250 (https://doi.org/10.1016/j.fuel.2014.05.081)
14. K. C. Thomas, W. Ingledew, *Appl. Environ. Microbiol.* **56** (1990) 2046 (https://doi.org/10.1007/BF02916454)

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15. R. Ravindran, S. Hassan, G. Williams, A. Jaiswal, *Bioeng.* **5** (2018) 93 (https://doi.org/10.3390/bioengineering5040093)
16. K. Mihajlovski, M. Rajilić-Stojanović, S. Dimitrijević-Branković, *Renew. Energy* **152** (2020) 627 (https://doi.org/10.1016/j.renene.2020.01.101)
17. G. L. Miller, *Anal. Chem.* **31** (1959) 426 (https://doi.org/10.1021/ac60147a030)
18. K. Mihajlovski, Ž. Radovanović, M. Carević, S. Dimitrijević-Branković, *Fuel* **224** (2018) 591 (https://doi:10.1016/j.fuel.2018.03.135)
19. W. Horwitz, P. Chichilo, H. Reynolds, *Official methods of analysis of the Association of Official Analytical Chemists*, 1970 (https://www.cabdirect.org/cabdirect/abstract/19720492404)
20. D. C. Montgomery, *Design and analysis of experiments*, John Wiley & Sons, New York, 2008 (ISBN: 9781119113478)
21. M. A. Saeed, H. Ma, S. Yue, Q. Wang, M. Tu, *Environ. Sci. Pollut. Res.* **25** (2018) 28851 (https://doi.org/10.1007/s11356-018-2972-4)
22. T. W. Nagodawithana, K. H. Steinkraus, *Appl. Environ. Microbiol.* **31** (1976) 158 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC169741/pdf/aem00002-0022.pdf)
23. G. Izmirlioglu, A. Demirci, *Appl. Sci.* **2** (2012) 738 (https://doi.org/10.3390/app2040738)
24. S. H. Mohd Azhar, R. Abdulla, S. A. Jambo, H. Marbawi, J. A. Gansau, A. A. Mohd Faik, K. F. Rodrigues, *B. B. Reports* **10 (**2017) 52 (https://doi.org/10.1016/j.bbrep.2017.03.003)
25. F. Bai, W. Anderson, M. Moo-Young, *Biotechnol. Adv.* **26** (2008) 89 (https://doi.org/10.1016/j.biotechadv.2007.09.002)
26. O. O. Awolu, I. O. Ibileke, *Afr. J. Food Sci.* **5** (2011)148 (https://academicjournals.org/journal/AJFS/article-full-text-pdf/CBEEF3D3037)
27. P. Datta, S. Tiwari, L. Pandey, in *Utilization and Management of Bioresources*, Springer, Singapore, 2018 (https://doi.org/10.1007/978-981-10-5349-8)
28. F. Ebrahimi, M. Khanahmadi, S. Roodpeyma, M. J. Taherzadeh, *Biomass Bioenergy* **32** (2008) 333 (https://doi.org/10.1016/j.biombioe.2007.10.007)
29. J. Kawa-Rygielska, W. Pietrzak, A. Czubaszek, *Biomass Bioenergy* **44** (2012) 17 (https://doi.org/10.1016/j.biombioe.2012.04.016)
30. L. R. Lynd, H.-J. Ahn, G. Anderson, P. Hill, D. S. Kersey, T. Klapatch, *Appl.Biochem. Biotechnol.* **28** (1991) 549 (https://doi.org/10.1007/BF02922633)
31. M. E. Ojewumi, O. E. Kolawole, D. Oyekunle, O. S. Taiwo, A. Adeyemi, *J. Ecol. Eng.* **20** (2019) 35 (https://doi.org/10.12911/22998993/102614)
32. G. M. Walker, G. G. Stewart, *Beverages* **2** (2016) 30 (https://doi.org/10.3390/beverages2040030).

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