



*a free-access multidisciplinary publication
of the Faculty of Engineering Hunedoara*

¹. Uroš MILJIĆ, ². Vesna VUČUROVIĆ, ³. Radojka RAZMOVSKI, ⁴. Vladimir PUŠKAŠ,
⁵. Marijana AČANSKI, ⁶. Kristian PASTOR

OPTIMISATION OF THERMO-CHEMICAL HYDROLYSIS OF KITCHEN WASTE FOR ETHANOL PRODUCTION USING RESPONSE SURFACE METHODOLOGY (RSM)

¹⁻⁴. Department of Biotechnology and Pharmaceutical Engineering, University of Novi Sad, 21000, Blvd. cara Lazara1, Novi Sad, SERBIA

⁵⁻⁶. Department of Applied and Engineering Chemistry, Faculty of Technology, University of Novi Sad, 21000, Blvd. cara Lazara1, Novi Sad, SERBIA

Abstract: Kitchen waste is highly biodegradable component of municipal solid waste, which mainly includes leftover food generated from the kitchen at various places of food preparation such as restaurants, hotels, canteens etc. This waste is a promising source for ethanol production due to its abundant availability, low cost and high carbohydrates content that can be converted to fermentable sugars. The aim of this study was to investigate and optimise the thermo-chemical pre-treatment of representative mixed sample of kitchen waste and to evaluate ethanol production from obtained fermentable sugars. Box-Behnken experimental design was employed to optimise the parameters of hydrolysis: pH, temperature and hydrolysis time. Second order polynomial models were developed to quantify the relationships between variables. Maximum yield of fermentable sugars of 130g and maximum ethanol concentration of 65 g per kilogram of kitchen waste containing 190 g of total sugar were obtained with the following optimum pre-treatment conditions: pH 1.0, temperature 120°C and hydrolysis time 60 min.

Keywords: ethanol; kitchen waste, optimization, hydrolysis

1. INTRODUCTION

Solid waste management is one of the biggest environmental challenges facing the world today due to the rapid increase of population. A sustainable approach to handle this will be to treat and reprocess organic waste on-site, to produce useful products. Kitchen waste is a highly biodegradable component of municipal solid waste. It mainly includes leftover food generated from the kitchen at various places of food preparation such as restaurants, hotels, canteens etc (Tembhurkar and Mhaisalkar, 2007). Most of kitchen waste is directly incinerated with other combustible waste and the residual ash is disposed of in landfills. However, the incineration of kitchen waste is problematic due to its high moisture and salt contents which makes this process expensive and energy consuming (Tang et al., 2008). This is the reason why the research in the recent decade was directed towards finding new alternative methods of treatment which would ensure pollution prevention and energy recovery. Different methods of kitchen waste management have been suggested. This includes biofuels production by anaerobic digestion process (biohydrogen and methane) (Tembhurkar and Mhaisalkar, 2007; Khalid et al., 2011), composting (Chang and Hsu, 2008), and ethanol fermentation (Tang et al., 2008; Wang et al., 2008; Vavouraki et al., 2013). Bioethanol production from solid waste conventionally includes sequential process consisted of a series of steps such as liquefaction, saccharification and fermentation. However, in order to reduce production time and investment costs, a process which implies simultaneous saccharification and fermentation has been proposed in the recent years (Kádár et al., 2004; Wang

et al., 2008). The available literature about the pre-treatment of kitchen waste prior to fermentation is very poor. Pre-treatment techniques proposed for saccharification of this food waste include enzymatic (Cekmecelioglu and Uncu, 2013) and thermo-chemical (Vavouraki et al., 2013) hydrolysis.

Due to abundant availability, low cost and high carbohydrates content that can be converted to fermentable sugars, kitchen waste is a promising source for ethanol production in Serbia. The aim of this study was to investigate and optimise the thermo-chemical pre-treatment of representative mixed sample of kitchen waste and to evaluate ethanol production from obtained fermentable sugars.

2. MATERIALS and METHODS

Materials

Kitchen waste

Starch based kitchen waste from local student's restaurant was grounded using the laboratory blender to obtain mash with diameter of particles smaller than 3 mm. Furthermore, obtained mash was analyzed in order to determine its composition (Table 1) as a substrate for ethanol production. Kitchen waste samples (44.0 g dry mass) were poured into an Erlenmeyer flask (0.5 l) and mixed with distilled water, keeping the dry matter to water ratio (hydromodule) at 1:5.

Yeast strain

Commercial pure-culture yeast *Saccharomyces cerevisiae* (Anchor WE372, Anchor Yeast, Johannesburg, South Africa) was used as a producing microorganism for ethanol fermentation. Yeast was activated by hydration in 0.1% sterile peptone pre-warmed to 35°C, and then inoculated into the fermentation medium (0.25g/kg).

Methods

Thermo-chemical pre-treatment

Thermo-chemical pre-treatment of the kitchen waste mash samples was conducted by the addition of 1 M HCl, and all process parameters (pH, temperature and reaction time) were set according to the experimental design. The samples were held in water bath for maintaining temperatures of 60°C and 90°C, while for 120°C samples were autoclaved. Afterwards, the pre-treated samples were cooled at room temperature and neutralized with 1 M NaOH up to pH 5, in order to obtain optimal pH of the fermentation medium.

Ethanol fermentation

After inoculation the flasks were fixed on a rotary shaker (GFL, Germany, Type 3015) at shaking frequency 120 rpm and shaking diameter 30 mm, and placed in a thermostat at 30°C. During the fermentation, the weight loss due to CO₂ release was measured at various time intervals from the beginning of each fermentation batch. At the end of each batch fermented liquid was distilled.

Analytical methods

Dry mass, starch, total nitrogen, protein content of the kitchen waste sample were estimated as per the standard AOAC methods. The ethanol concentration was determined based on the density of alcohol distillate at 20 °C by pycnometer method (AOAC methods, 2000).

Experimental design

Optimization of thermo-chemical pre-treatment of kitchen waste for ethanol production was carried out using RSM.

The experimental design and statistical analysis were performed using Stat-Ease software (Design-Expert 7.0.0 Trial, Minneapolis, MN, USA). Experiments with three independent variables, pH (1.0, 2.0, 3.0), reaction time (30, 45, 60 min) and temperature (60, 90, 120°C), were carried out by Box-Behnken experimental design. Response parameter was the amount of ethanol obtained per kilogram of kitchen waste. The design matrix of independent variables is shown in Table 2. The

Table 1. Composition of kitchen waste sample used for fermentation

Parameter	Content
Moisture (%)	75.6
Starch (%)	19.0
Starch (% dm*)	77.7
Total nitrogen (%)	0.5
Protein (%)	3.1

*dry mass

total of 15 experiments were conducted in the study toward the construction of a second-order polynomial model (1) which is used to calculate predicted responses:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3 \quad (1)$$

Where Y is the predicted response, X_1 (pH), X_2 (reaction time) and X_3 (temperature) are independent variables, b_0 is intercept, b_1 , b_2 and b_3 are linear effects, b_{11} , b_{22} and b_{33} are squared effects and b_{12} , b_{23} and b_{13} are interaction effects of the factors. The goodness of fitting and the significances of all terms in the polynomial equations were determined through appropriate statistical methods (coefficient of determination (R^2), F-value at a probability (P) of 0.05).

3. RESULTS and DISCUSSION

Multiple regression analysis was performed to fit the response function (Y), and second order polynomial equation (2) has been obtained. Regression coefficients (b_0 , b_1 , b_2 ... b_{13}) were used to generate response surface plots for investigated variable (Y_{1-3}). Response surface plots (Figure 1) are used to illustrate the effects of temperature, pH and reaction time on the saccharification of sugars present in kitchen waste through the values of obtained ethanol concentrations. The goodness of fitted model was evaluated by the coefficient of determination (R^2) while the adequacy and significance of the quadratic models was assessed by the analysis of variance (ANOVA) (Table 3).

$$Y = -4.0746 - 22.5266X_1 + 0.4133X_2 + 0.4207X_3 - 0.1402X_1X_2 - 0.4111X_1X_3 + 0.0011X_2X_3 + 12.9883X_1^2 - 0.0008X_2^2 + 0.0036X_3^2 \quad (2)$$

The obtained regression model was significant at 95% confidence level (F value = 6.34 and $P < 0.05$) with a satisfactory value of coefficient of determination ($R^2 = 0.9195$), implying that at least 91% of the variability in the response could be explained by the second-order model equation. The 3D plots were made using software tools by varying two variables within experimental range, while the third variable was kept constant (at middle level). Considering the amount of ethanol obtained after fermentation, it can be seen that thermo-chemical hydrolysis was more effective at higher values of applied temperature and reaction time and at lower pH. Model terms X_1 , X_3 , X_1^2 , and X_1X_3

Table 3. Analysis of variance (ANOVA) for response surface quadratic model

	F-value	P-value
Model	6.34	0.0279*
X_1	24.69	0.0042*
X_2	0.76	0.4222**
X_3	11.20	0.0204*
X_1X_2	0.28	0.6176**
X_1X_3	9.74	0.0262*
X_2X_3	0.018	0.8972**
X_1^2	9.96	0.0252*
X_2^2	0.0002	0.9642**
X_3^2	0.64	0.4592**

X_1 : pH; X_2 : reaction time (min); X_3 : Temperature ($^{\circ}\text{C}$);

*Significant at $P < 0.05$; ** not significant

Table 2. Box-Behnken design matrix

pH	Reaction time (min)	Temperature ($^{\circ}\text{C}$)	Ethanol (g/ kg waste)
X_1	X_2	X_3	Y_1
1.0	30	90	25.45
1.0	45	60	9.67
1.0	45	120	65
1.0	60	90	35.33
2.0	30	60	3.65
2.0	30	120	9.33
2.0	45	90	4.8
2.0	45	90	6.5
2.0	45	90	5
2.0	60	60	6.67
2.0	60	120	14.5
3.0	30	90	5.33
3.0	45	60	3.11
3.0	45	120	9.1
3.0	60	90	6.8

are significant at 0.05 level ($P < 0.05$) while terms X_2 , X_1X_3 , X_2X_3 , X_2^2 and X_3^2 do not have a significant effect on hydrolysis and ethanol production (Table 2). A positive sign of the coefficients indicates a synergistic effect, while a negative sign indicates an antagonistic effect on response. Ethanol yield was positively affected by the linear effects of reaction time and temperature, quadratic terms of pH and temperature and interaction between reaction time and temperature. On the other hand linear effect of pH, interactions between pH and reaction time, pH and temperature and quadratic term of reaction time have a negative effect on the response.

Optimization of investigated variables for thermo-chemical hydrolysis of kitchen waste for ethanol

production was carried out. The prediction of optimal reaction conditions was made by use of desirability function concept and Design Expert software (Popov et al., 2010). Maximal yield of fermentable sugars of 130g and maximal ethanol concentration of 65 g per kilogram of kitchen waste containing 190 g of total sugar were obtained with the following values of hydrolysis parameters: pH 1.0, temperature 120 °C and hydrolysis time 60 min. The predicted optimum was verified and the models were proven as adequate after a repeated experiment (triplicate set), with the optimal fermentation conditions (63.5 g of ethanol per kg of kitchen waste).

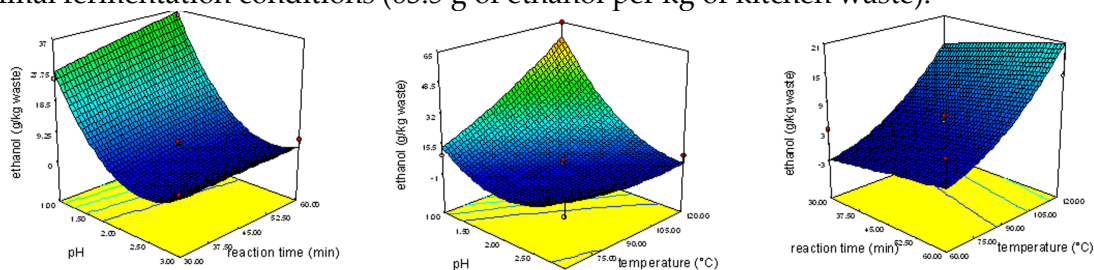


Figure 1. Response surface contour plots of the interaction of (a) pH vs. reaction time (temperature=90 °C), (b) temperature vs. pH (reaction time=45 min), and (c) temperature vs. reaction time (pH=2.0), and their influence on final ethanol content.

The obtained desirability function value was 0.950. Previous study (Vavouraki et al., 2013) reported the chemical pre-treatment using 1.17% HCl for 86 min at 100 °C as the most effective for hydrolysis of kitchen waste used. On the other hand, another study (Wang et al., 2008) reported that maximal ethanol concentration of 33.05 g/L was obtained under following optimal conditions of simultaneous saccharification and fermentation: time of 67.60 h, pH of 4.18 and temperature of 35 °C.

Acknowledgment

Financial support from the Provincial Secretariat For Science and Technological Development of the Republic of Serbia Autonomous Province of Vojvodina (Project 114-451-3830/2013-02) is greatly appreciated.

REFERENCES

- [1.] Association of Official Analytical Chemists International, 17th edition, Gaithersburg, AOAC, 2000. Official Methods: 966.20, 968.28, 970.57, 969.37, 977.08, 969.36, 942.06.
- [2.] Cekmecelioglu, D. & Uncu, O. N. (2013). Kinetic modeling of enzymatic hydrolysis of pretreated kitchen wastes for enhancing bioethanol production. *Waste Management*, 33, 735-739.
- [3.] Chang, J. I. & Hsu, T.-E. (2008). Effects of compositions on food waste composting. *Bioresource Technology*, 99, 8068–8074.
- [4.] Khalid, A., Arshad, M., Anjum, M., Mahmood, T., Dawson, L. (2011). The anaerobic digestion of solid organic waste. *Waste Management*, 31, 1737–1744.
- [5.] Popov, S., Ranković, J. Dodić, J. Dodić, S. Jokić, A. (2010). Bioethanol production from raw juice as intermediate of sugar beet processing: A response surface methodology approach. *Food Technology and Biotechnology*, 48(3), 376–383.
- [6.] Tang, Y.-Q., Koike, Y., Liu, K., An, M.-Z., Morimura, S., Wu, X.-L., Kida, K. (2008). Ethanol production from kitchen waste using the flocculating yeast *Saccharomyces cerevisiae* strain KF-7. *Biomass and Bioenergy*, 32, 1037-1045.
- [7.] Tembhurkar, A. R. & Mhaisalkar, V. A. (2007). Studies on hydrolysis and acidogenesis of kitchen waste in two phase anaerobic digestion. *Journal of the Institution of Public Health Engineers*, 2, 10-18.
- [8.] Vavouraki, A. I., Angelis, E. M., Kornaros, M. (2013). Optimization of thermo-chemical hydrolysis of kitchen waste. *Waste Management*, 33, 740-745.
- [9.] Kádár, Z., Szengyel, Z., Réczey, K. (2004). Simultaneous saccharification and fermentation (SSF) of industrial wastes for the production of ethanol. *Industrial Crops and Products*, 20, 103–110.
- [10.] Wang, Q., Ma, H., Xu, W., Gong, L., Zhang, W., Zou, D. (2008). Ethanol production from kitchen garbage using response surface methodology. *Biochemical Engineering Journal*, 39, 604-610.