Biblid: 1821-4487 (2019) 23; 3; p 132-137
UDK: 582.661

Original Scientific Paper
Originalni naučni rad

KINETIC ANALYSIS OF ALCOHOLIC FERMENTATION USING INTERMEDIATE AND BY-PRODUCTS OF SUGAR BEET PROCESSING IN LABORATORY BIOREACTOR

KINETIČKA ANALIZA ALKOHOLNE FERMENTACIJE MEĐU I NUSPROIZVODA PRERADE ŠEĆERNE REPE U LABORATORIJSKOM BIOREAKTORU

Jovana GRAHOVAC, Vanja VLAJKOV, Ivana PAJČIN, Zorana RONČEVIĆ, Aleksandar JOKIĆ, Siniša DODIĆ, Jelena DODIĆ
University of Novi Sad, Faculty of Technology Novi Sad, 21000 Novi Sad, Bulevar cara Lazara 1, Serbia
e-mail: vanja.vlajkov@uns.ac.rs

ABSTRACT

Bioethanol production in batch culture by free Saccharomyces cerevisiae cells from intermediate and by-products of sugar beet processing was investigated. This research relays on the previouslyconducted experiments in the bioreactor of 1.5 L working volumewhere key fermentation parameters, including initial sugar concentration and fermentation time, were optimized. This study was performed to validate the applicability of obtained results in the enlarged scale i.e. in 10 L laboratory bioreactor. The main aim was to analyze the bioprocess time course by monitoring the yeast cell number and concentrations of sugar, amino nitrogen, total soluble salts,and ethanol. Also, the kinetic analysis of ethanol and yeast biomass formation as well as sugar consumption during fermentation of media based on raw juice, thin juice, thick juice,and molasses was performed.

Key words: *bioethanol, sugar beet, kinetics, laboratory bioreactor, Saccharomyces cerevisiae.*

REZIME

U ovom radu ispitana je šaržna proizvodnja bioetanola između i nusproizvoda prerade šećerne repe submerznom kultivacijom Saccharomyces cerevisiae. Ključni parametri fermentacije, uključujući početnu koncentraciju šećera i dužinu trajanja fermentacije, optimizovani su u eksperimentima izvedenim u bioreaktoru radne zapremine 1,5 L. Ovo istraživanje izvedeno je sa ciljem validacije primenljivosti ostavrenih rezultata u uvećanim zapreminama odnosno u 10 L laboratorijskom bioreaktoru. Osnovni cilj ovog istraživanja je bila analiza toka bioprocesa praćenjem broja ćelija kvasca i koncentracija šećera, amino azota, ukupnih rastvorljivih soli kao i etanola. Takođe, izvedena je i kinetička analiza nastanka etanola i biomase kvasca kao i potrošnje šećera tokom fermentacije hranljivih podloga na bazi ekstrakcionog, retkog, gustog soka i melase.

Rezultati ove studije otvaraju nove perspektive za među i nusproizvode prilikom prerade šećerne repe u proizvodnji bioetanola, koja bi se u poslednje vreme mogla koristiti kao biogorivo ili za primenu u različitim industrijama, uključujući prehrambenu, farmaceutsku i hemijsku industriju. Istraživanje predstavlja neophodnu osnovu za razvoj proizvodnje bioetanola u srpskim pogonima šećera.

Ključne reči: *bioetanol, šećerna repa, kinetika, laboratorijski bioreaktor, Saccharomyces cerevisiae.*

INTRODUCTION

The enthusiasm of scientist working on the development of new approaches for renewable energy production is matched with the global problem of rapid depletion of fossil fuels, which has been occurring much faster than it had been previously predicted (*Grahovac et al., 2012a*). Biofuels are recognized as a possible alternative solution to the growing world energy demand and a substitution for fossil fuels.The promotion of biofuels sector is instigated by the fact that they present an alternative without negative influences on the environment, with a tendency of contributing to public savings (*Bhatia et al., 2017*).

The productivity and cost-effectiveness of bioethanol production, as one of the most important biofuels,is strongly dependent on low-cost substrates utilization (*Gnansounou et al., 2005*). Bioethanol co-production in sugar plants is considered as a promising possibility for the utilization of sugar beet outside the food industry. The optimization of bioethanol production from intermediates and byproducts of sugar beet processing means a step closer to the mass production of this biofuel (*Grahovac et al., 2016; Pajčin et al., 2017*).

The most commonly used feedstock for bioethanol production is molasses. It is a well-known by-product obtained at the end of sugar beet processing and the cost of its production is considerably higher comparing to the cost of raw juice produced at the beginning of the process by water extraction of sliced sugar beet. Raw juice contains about 15–20 % of dry solids and its purity ranges between 85 and 90 %, which means that it consists of about 85–90 % of sugars and 10–15 % of non-sugars in dry matter. The obtained raw juice can be used either directly for ethanol and sugar production during the sugar beet harvest season, or it can be concentrated and stored for several months. Thick juice is the relatively pure intermediate product of sugar beet processing due to a largeamount of fermentable sugars (55–65 %) accompanied by a profuse mineral composition which makes it suitable for bioethanol production(*Grahovac et al., 2012b*). On the other hand, thin juice contains significantly lower sugar content (12-14 %), but it is also successfully used as a substrate for bioethanol production. Which substrate will be used is influenced by the time of the year and whether there is an ongoing annual campaign of sugar beet processing, when all fresh intermediate products are available, or there are only stored raw materials (*Dodić et al., 2018*).

In order to provide producers in sugar factories with reliable information whether it is more economically efficient to produce sugar or ethanol, what is the optimal ratio between them and which is the most suitable substrate for bioethanol production (raw juice, thin juice, thick juice or molasses) it is crucial to determine the yield, cost-effectiveness, technical requirements and optimal conditions of bioethanol production by using each of the side-products mentioned above. The aim of this study was to describe changes of yeast cell number, total sugar content, total nitrogen content, total dissolved salts (TDS) content, pH value and ethanol fraction volume and perform kinetic analysis of biomass growth, product formation and sugar consumption during the batch fermentation of each previously described substrate by using *Saccharomyces cerevisiae* in a laboratory scale bioreactor (10 L).

MATERIAL AND METHOD

Microorganism and inoculum preparation

The producing microorganism used in the experiments was *Saccharomyces cerevisiae* - fresh baker's yeast – FB (Alltech, Serbia), which is at the same time the most commonly used yeast type in distilleries in our region. Prior to inoculation the yeast cells were suspended in a small quantity of the same fermentation mediumthat was used for ethanol production (see next section).

Raw materialand fermentation medium preparation

Substrates used for the preparation of fermentation medium were raw juice, thin juice, thick juice and molasses obtained from the local sugar beet processing factories including "Crvenka" JSC (Crvenka, Serbia), "TE-TO" JSC (Senta, Serbia), and "Šajkaška-Hellenic Sugar Industry" JSC (Žabalj, Serbia). All substrates mentioned above were diluted using distilled water to achieve optimal values of initial total sugar content, as determined in the previous study (*Popov et al., 2010; Grahovac et al., 2012b; Grahovac et al., 2016*). The initial pH value of fermentation medium was adjusted to 5.0 with 10% (v/v) sulphuric acid.

Fermentation conditions

The fermentation process was carried out in a laboratory scale bioreactor (CH-8708, Chemap AG, Switzerland) with a total volume of 14 L (working volume 10 L) under anaerobic conditions at 28 °C (internal temperature regulation) with mixing using two parallel Rushton turbines (agitation rate 150 rpm) and 4 baffles during 48 h. The fermentation time was measured from the moment of inoculation. The samples were collected immediately after the inoculation and every 2 h from the moment of inoculation.

Analytical methods

Biomass

Biomass concentration was determined by the spectrophotometric method based on predefined dependence of absorbance as a function of yeast cell concentration. The absorbance was measured by a spectrophotometer (UV Spectrophotometer, UV-1800, Shimadzu, Japan) at 660 nm. Before the fermentation starts fresh commercial baker's yeast suspensions, which will be later used for bioethanol production, were made by using saline to achieve different concentrations (5, 10, 20, 40 and 50 g/L) in order to perform calibration. Biomass concentration in these suspensions was determined by measuring the mass of dry cells for each concentration, by centrifuging a certain volume of each suspension (10 mL) for 10 minutes at 3000 rpm (LC-321, TehtnicaŽelezniki, Slovenia), pouring off the supernatant, placing the flasks together with the sludge on drying at 105 ºC and measuring them till a constant mass was reached.

Fermentable sugars

Fermentation broth samples were centrifuged for 10 minutes at 3000 rpm (technical centrifuge LC-321, TehtnicaŽelezniki, Slovenia), and then the content of fermentable sugars (sucrose, glucose, fructose) was analyzed using the obtained supernatants by HPLC (PU-980 pump, detector RI-930, autosampler A-950 (Jasco Inc., USA)), using the column Shodex Sugar KS-801 (Showa Denko K.K., Japan) and water as the mobile phase, under the following conditions: 20 μL injection volume, flow rate 0.6 mL/min and elution time 30 min. The results of these measurements are presented hereafter as a concentration offermentable sugars.

Bioethanol

The bioethanol concentration was determined in the distillates of fermentation broth samples by gas chromatography, using a system of Hewlett Packard HP 5890 Series II GC (Agilent Technologies Inc., USA) equipped with a flame ionization detector, and Carbowax 20 M column (Agilent Technologies Inc., USA) at a temperature of 85 ºC, while the gas carrier was helium. Injector and detector temperature were maintained at 150 ºC. For the construction of the calibration curve, standard aqueous solutions of ethanol with concentrations of 0, 3, 5, 8, 10, 15 and 20 % (v/v) were made.

Statistical analysis

All experiments in this study were carried out in triplicate andthe results were averaged. The reproducibility of these measurementswere good, the deviation between parallel experimentswere in the range of ±4.6%.

Kinetic analysis

Microbial growth was described by using Monod kinetic model, based on the following assumptions: stirring in the bioreactor provides homogenous conditions in the entire volume and stirring rate of 150 rpm provides more than sufficient mass transfer and uniform substrate availability.

The rate of biomass production (R_x), substrate consumption (R_s) and ethanol production (R_p) were calculated by dividing the "moving average" (Data Analysis ToolPak, Microsoft Office Excel 2007, Microsoft, USA) of biomass content, total sugar content and ethanol content, respectively (from the first to the third sample of three consecutive sampling) with a time interval of sampling.

The coefficient of ethanol yield $Y_{P/S}$ (%) was calculated using the following equation:

$$Y_{P/S} = \frac{P_t - P_0}{S_0 - S} \cdot 100 \quad (1)$$

where$P_t$is ethanol content at the sampling point (g/L), P_0 is ethanol content at the moment of inoculation (g/L), S_0 is total sugar content at the moment of inoculation (g/L), while S is total sugar content at the sampling point (g/L).

The coefficient of yeast biomass yield $Y_{X/S}$ (g/g) was calculated using the following equation:

$$Y_{X/S} = \frac{X_t - X_0}{S_0 - S} \quad (2)$$

whereX_t is biomass content at the sampling point (g/L), while X_0 is biomass content at the moment of inoculation (g/L).

The degree of substrate conversion$K$(%) was calculated using the following equation:

$$K = \frac{S_0 - S_t}{S_0} \cdot 100 \quad (3)$$

RESULTS AND DISCUSSION

The optimal initial substrate concentration and optimal fermentation time were determined in the previous study by performing fermentation in the laboratory bioreactor of smaller volume (1.5 L). The fermentation media were based on raw juice, thin juice, thick juice and molasses with optimized initial sugar mass content of 12.71, 12.75, 21.12 and 11.59 % (m/v), respectively. The fermentation time values, also optimized in the previous research step and employed in this study, were 38, 42, 47 and 34 h, for the media based on raw juice, thin juice, thick juice,and molasses, respectively (*Popov et al., 2010; Grahovac et al., 2011; Grahovac et al., 2012b; Grahovac et al., 2016)*. In order to verify the applicability of the previously obtained mathematical model for fermentation of each aforementioned substrate, fermentation experiments were performed under the optimal conditions in a larger scale laboratory bioreactor (10 L).

Before the fermentation starts, it is necessary to adjust the pH value of the fermentation medium based on raw juice to 5.0 and perform sterilization. Raw juice is recognized as one of the most promising substrates for the economically efficient bioethanol production, compared to other byproducts, due to its affordable price (*Hinková and Bubník, 2001*).

The course of raw juice fermentation is given in Fig. 1A. After 38 hours of fermentation, the mean value of ethanol content was 7.65±0.22 % (v/v). The mean values of yeast cells number and total sugar content were 2.25±0.14×10^8 CFU/mL and 0.09±0.03 % (w/v), respectively. Values of the selected variables predicted by the previously obtained model were: number of yeast cells 2.31×10^8 CFU/mL, ethanol content 7.99 % (v/v) and total sugar content approximately equal to zero. It could be concluded that good agreement of the experimental results with the variables' values predicted by the model can be confirmed (the maximum deviation is about 4%), suggesting the validity of the obtained model, which could also be applied in a larger volume bioreactor.

Thin juice is recognized as a very suitable substrate for bioethanol production. However, its utilization for this purpose is limited due to lack of possibility to store this raw material over a long time period, since it contains sugar in concentration range optimal for growth of different microorganisms*(Hinková and Bubník, 2001)*.

The course of thin juice fermentation is given in Fig. 1B. After 42 hours of fermentation, the mean value of ethanol content was 7.78±0.09 % (v/v). The mean values of yeast cells number and total sugar content at the end of fermentation were 2.33±0.21×10^8 CFU/mL and 0.26±0.07 % (w/v), respectively.

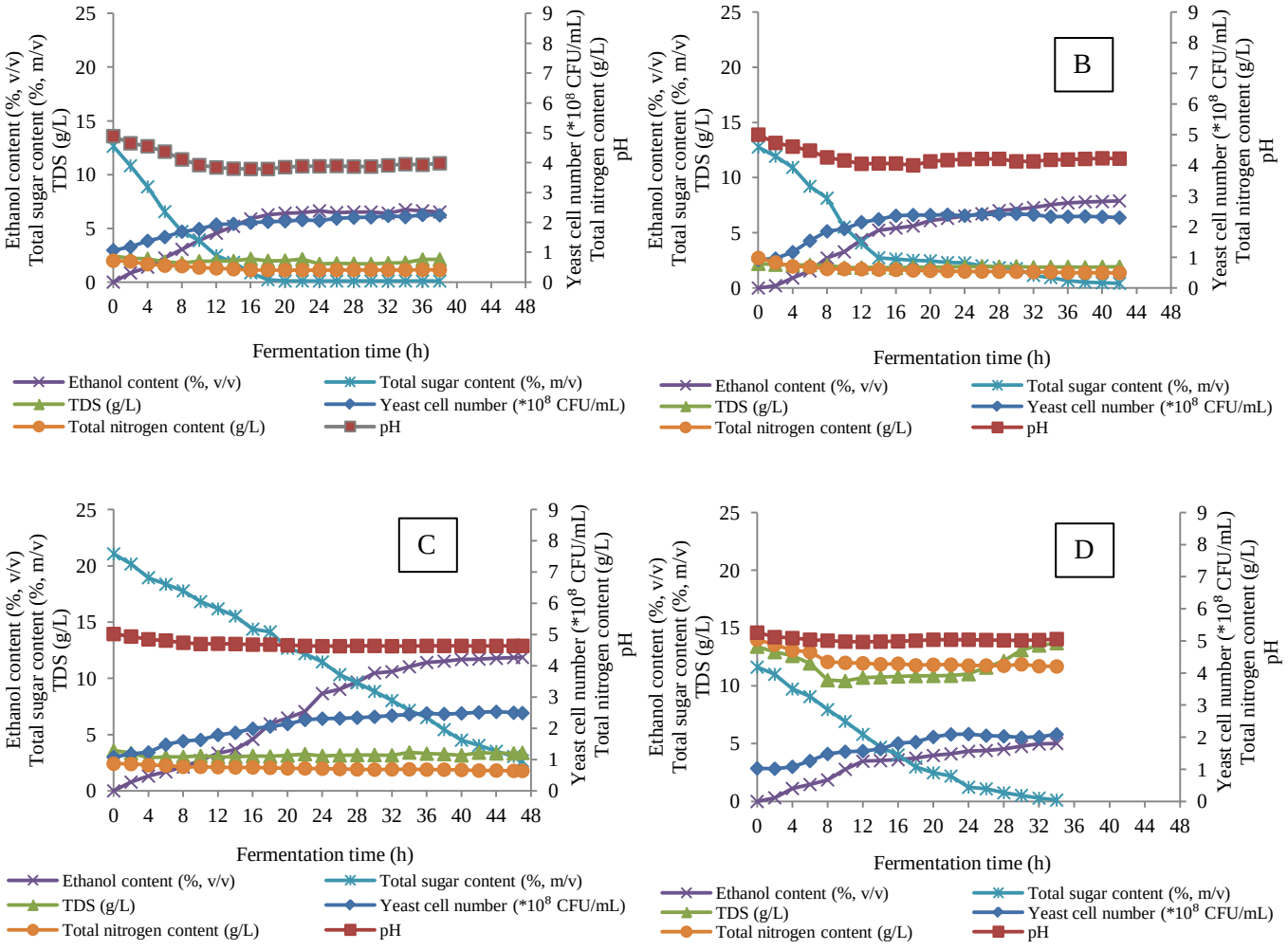

Fig.1. Cultivation course of bioethanol production in a laboratory scale bioreactor (10 L) using: A - raw juice, B – thin juice, C – thick juice, D – molasses, as substrates

Model-predicted values were: the number of yeast cells 2.27×10^8 CFU/mL, ethanol content 8.01 % (v/v) and total sugar content 0.03 % (w/v). Based on the presented data, good agreement of the experimental results with the values predicted by the model can be confirmed(the maximum deviation is about 3%).

Due to high sugar content, thick juice could be stored over a long period of time, almost as long as molasses. On the other hand, thick juice production is a demanding and expensiveprocedure,which affects bioethanol final price significantly*(Krajnc and Glavič, 2009).*

The course of thick juice fermentation is given in Fig. 1C. After 47 hours of fermentation, the mean value of ethanol content was 11.09±0.20 % (v/v). The mean values of yeast cells number and total sugar content were $2.35\pm0.19\times10^8$ CFU/mL and 3.08±0.29 % (w/v), respectively. The previously obtained model has predicted the following values: a number of yeast cells 2.37×10^8 CFU/mL, ethanol content 11.38 % (v/v) and total sugar content 3.02 % (w/v). Once again, good agreement of the experimental results with the model predicted values can be observed.

The course of molasses fermentation is given in Fig. 1D. After 34 hours of fermentation, the mean value of ethanol content was 4.92±0.18 % (v/v). The mean values of yeast cells number and total sugar content were $2.80\pm0.17\times10^8$ CFU/mL and 0.08±0.01 % (w/v), respectively. The predicted values from the previously obtained model were: number of yeast cells 1.84×10^8 CFU/mL, ethanol content 4.54 % (v/v) and total sugar content close to zero (w/v). It could be concluded there is a good agreement of the experimental results with the model predicted values.

Fig. 2A shows the dependence of ethanol production rate, sugar consumption rate and yeast biomass production rate on the fermentation time during the raw juice fermentation. Since the moment of inoculation, yeast biomass production rate was increasing intensively and after 6 hours it attained a maximal value of 0.31 g/L·h, after which it was decreasing until 26 h when it reached a value close to zero, which is in accordance with the start of the stationary growth phase. The sugar consumption rate was increasing intensively in the first 6 hours of fermentation when it reached its maximal value (11.55 g/L·h), and after that, it was continuously decreasing, while after 22 hours of fermentation its value became close to zero. The ethanol production rate reached a maximum value of 3.49 g/L·h after 4 hours of fermentation and after that, it has slowly decreased, while after 30 h, ithas reached a value almost equal to zero.

Fig. 2B presents the ethanol production rate, sugar consumption rate,and yeast biomass production rate dependence on the fermentation time for thin juice fermentation. At the beginning of the fermentation yeast biomass production rate was increasing continuously and after 10 hours attained a maximal value of 0.72 g/L·h. Further changes in biomass production rate showed a decrease until the end of the bioprocess when it reached a value close to zero. The sugar consumption rate was intensively increasing until 10 hours of cultivation when it attained maximal value (13.10 g/L·h), and after that, it was

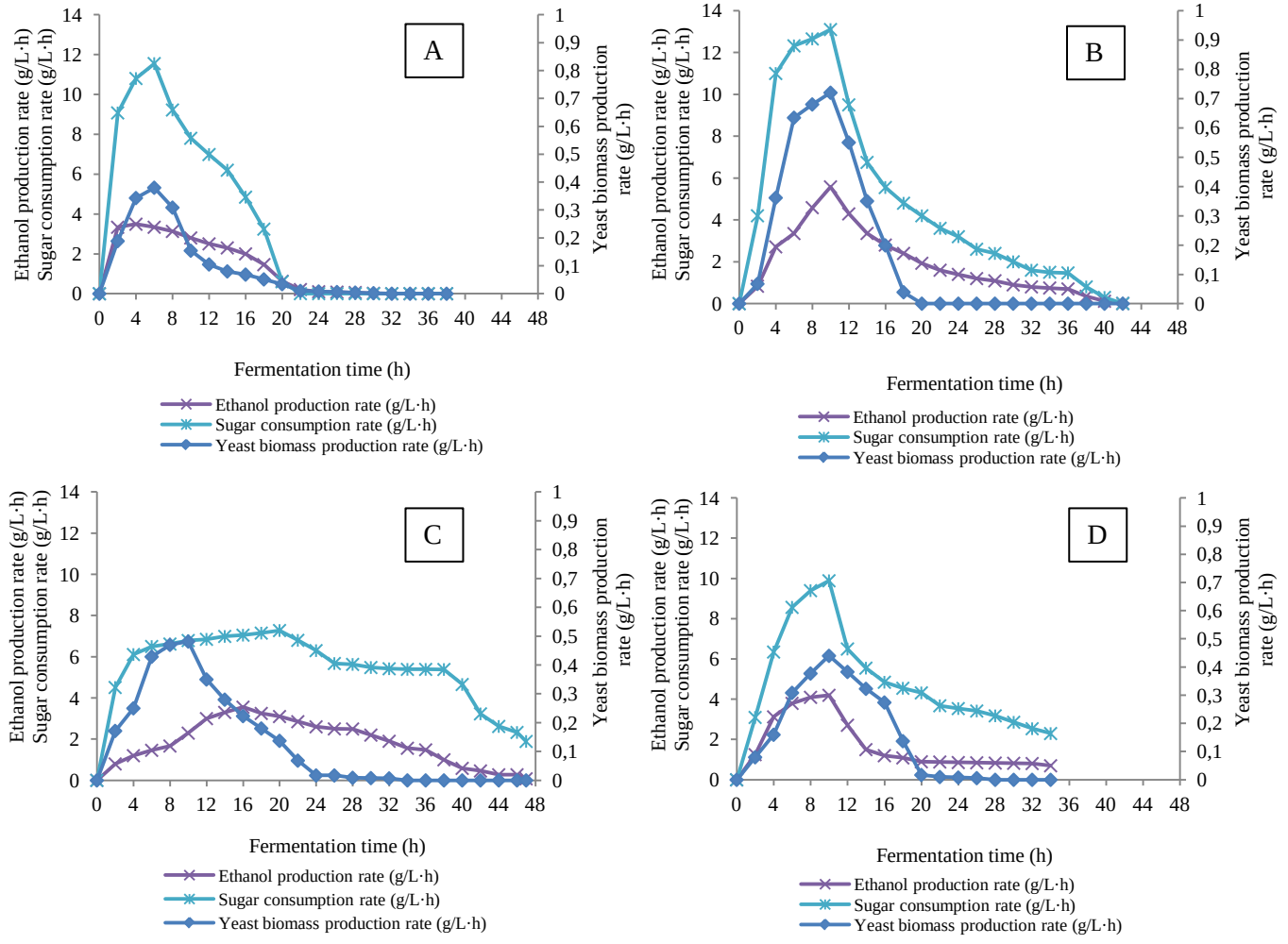

Fig. 2. Ethanol production rate, sugar consumption rate,and yeast biomass production rate during fermentation of A - raw juice, B – thin juice, C – thick juice, D – molasses, in a laboratory scale bioreactor (10 L)

continuously decreasing to the minimal value (close to zero) achieved at the end of the fermentation process. The trend of ethanol production rate was quite similar since it has also reached a maximum value of 5.58 g/L·h after 10 hours of fermentation and then it was slowly decreasing to the end of the bioprocess when it has reached a value almost equal to zero.

Fig. 2C presents the dependence of ethanol production rate, sugar consumption rate and yeast biomass production rate onfermentation time during fermentation of thick juice. Since the moment of inoculation, the yeast biomass production rate was increasing and it reached a maximal value of 0.48 g/L·h after 10 hours of fermentation. The minimal value of biomass production rate, close to zero, was achieved after 32 hours of fermentation. Relatively high level of sugar consumption rate during the entire fermentation process was in accordance with yeast biomass and ethanol production. Since biomass growth was more intensive during the first 10 hours of fermentation, it was clear that sugar consumption has been highly dependent on ethanol production. The maximal value of sugar consumption rate, achieved after 20 hours of fermentation, was 7.28 g/L·h. The ethanol production rate was increasing until 16 hours of the bioprocess when it achieved maximal value (3.55 g/L·h), and after that, it was gradually decreasing to the minimal value (close to zero) at the end of the fermentation.

Fig. 2D presents the dependence of ethanol production rate, sugar consumption rate and yeast biomass production rate onfermentation time during the fermentation of molasses. Yeast biomass production rate was increasing gradually and it reached a maximal value of 0.44 g/L·h after 10 hours of fermentation. The maximal value of sugar consumption rate was 9.89 g/L·h after 10 hours of fermentation, while the minimal value of this parameter (2.3 g/L·h) was achieved simultaneously with biomass growth decrease after 34 hours of the bioprocess. The same trend could be noticed for ethanol production rate, since it was increasing until 10 hours of fermentation, attaining its maximal value of 4.21 g/L·h at this sampling point. Further changes have resulted in intensive decrease until 14 hours of fermentation, after which decrease was gradually continued until the end of the fermentation process.

The theoretical coefficient of ethanol yield, when sucrose is used as a carbon source in fermentation by *Saccharomyces cerevisiae*, amounts up to 53.8 %. Due to expected consumption of sugar for biomass growth and for production of undesired metabolites, as well as due to the losses caused by non-quantitative ethanol recovery, the real ethanol yield coefficient is lower than the theoretical one. The coefficient of ethanol yield obtained in the performed experiment, when raw juice was used as a substrate for bioethanol production, was 41.88 % (Fig. 3A), or 77.84 % of the theoretical value. The degree of substrate conversion for raw juice has a maximum value of 99.24% after 38 h of fermentation, but its change is significant only during the first 22 h of fermentation.

The coefficient of ethanol yield obtained in the experiment where thin juice was utilized as substrate was higher compared

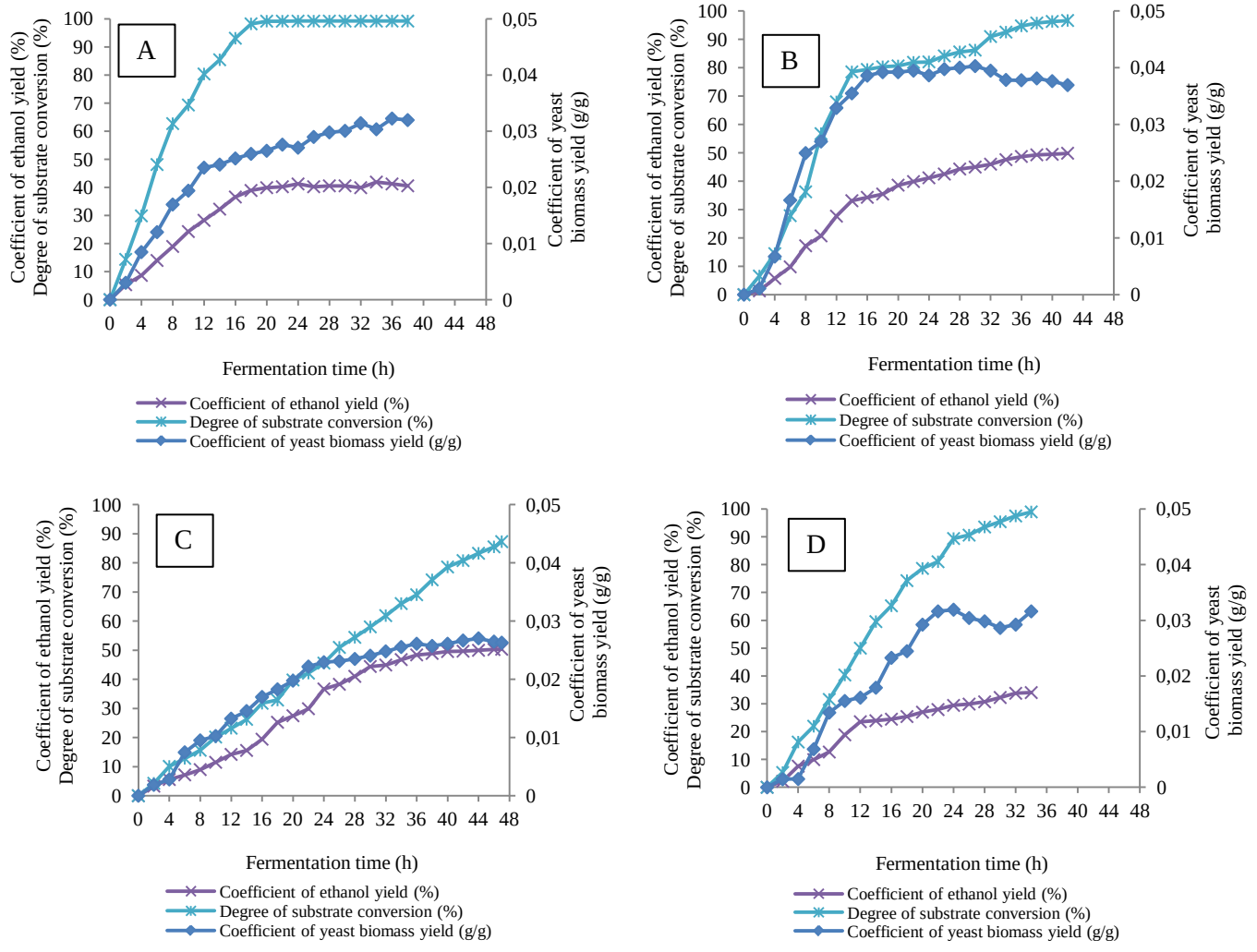

Fig. 3. Coefficients of ethanol and biomass yield and degree of sugar conversion during fermentation of A - raw juice, B – thin juice, C – thick juice, D – molasses, in a laboratory scale bioreactor (10 L)

to the previous one, where raw juice was used for medium preparation (Fig. 3B). The obtained value was 49.86 % or 92.68 % of the theoretical value. The degree of substrate conversion for thin juice is significantly increased during the first 14 h of fermentation and reaches a maximum value of 96.65% after 42 h.The coefficient of ethanol yield of 50.34%, achieved in the experiment where thick juice was used as a substrate for bioethanol production (Fig. 3C), presents 93.57 % of the theoretical value. Close values for ethanol yield obtained in the experiments where thick and thin juices were used as substrates are expected due to the similarity in their content, which varies only in water quantity. The degree of substrate conversion for thick juice is almost linearly increased during the first 47 h of fermentation. On the other hand, its maximal value is 87.29%. Lower value of this kinetic parameter achieved for thick juice is in accordance with the fact that, in this case, at the end of fermentation about 3% of sugar remained in fermentation mash.

The value of the coefficient of ethanol yield during fermentation of molasses was 34.05 % (Fig. 3D), or 63.29 % of the theoretical value. Since the molasses composition is complex and it contains different non-sugar components, lower ethanol yield could be a consequence of metabolic inhibition caused by higher osmotic pressure, even after the raw material dilution (*Ergun and FerdaMutlu, 2000*).The degree of substrate conversion for molasses is increased during whole fermentation time and values 98.97% at the end of process.

CONCLUSION

Investigation of possible valorization routes of side products generated in the sugar industry and fulfilling the maximal potential of their usage in bioethanol production is an important step towards the mass production of this biofuel. The results of this study contribute to the development of the higher capacity of bioethanol production and clearly indicate the great potential of using by-products of sugar beet processing in bioethanol production in a laboratory scale bioreactors of different volumes. Determination of kinetic parameters of the fermentation process, based on intermediates and byproducts of sugar beet processing in domestic plants, provides a good starting point for further optimization concerning techno-economic parameters of this bioprocess.The model predicted values of the initial sugar content and fermentation time from the experiments performed previously were verified in this research in a laboratory scale bioreactor with a larger volume, as a next scale-up step, with a good perspective to be applied successfully in bioethanol production at the industrial level. The results of this study open up new perspectives forintermediates and byproductsof sugar beet processing utilization in bioethanol production, which could be lately used whether as a biofuel or for application in different industries, including food, pharmaceutical,and chemical industry. The performed research presents a necessary basis for bioethanol co-production development in Serbian sugar plants.

ACKNOWLEDGMENT: The authors gratefully acknowledge the support of the Ministry of Education, Science and Technological Development of the Republic of Serbia through the research fundingin the framework of the project TR31002 entitled "Improving the production of bioethanol from sugar beet processing products".

REFERENCES

Bhatia, S.K., Kim, S.H., Yoon, J.J., Yang, Y.H. (2017). Current status and strategies for second generation biofuel production using microbial systems. Energy Conversion and Management, 148, 1142-1156.

Dodić, J., Grahovac, J., Rončević, Z., Pajović-Šćepanović, R., Dodić, S., Bajić, B., Vučurović D. (2018).Challenges in bioethanol production from intermediate and by-products of sugar beet processing in the Republic of Serbia. Journal on Processing and Energy in Agriculture, 22(1), 34-39.

Ergun, M., Ferda Mutlu S. (2000). Application of a statistical technique to the production of ethanol from sugar beet molasses by *Saccharomyces cerevisiae*.Bioresource Technology, 73(3), 251-255.

Gnansounou, E., Dauriat, A., Wyman, C.E. (2005). Refining sweet sorghum to ethanol and sugar: economic trade-offs in the context of North China. Bioresource Technology, 96(9), 985-1002.

Grahovac, J. (2011). Optimizacija dobijanja etanola fermentacijom međuproizvoda tehnologije prerade šećerne repe. Doktorska disertacija. Tehnološki fakultet Novi Sad, Srbija.

Grahovac, J., Dodić, J., Dodić, S., Popov, S., Jokić, A., Zavargo, Z. (2011). Optimisation of bioethanol production from intermediates of sugar beet processing by response surface methodology. Biomass and Bioenergy, 35(10), 4290-4296.

Grahovac, J., Dodić, J., Dodić, S., Popov, S., Vučurović, D., Jokić, A. (2012a). Future trends of bioethanol co-production in Serbian sugar plants. Renewable and Sustainable Energy Reviews, 16(1), 3270-3274.

Grahovac, J., Dodić, J., Jokić, A., Dodić, S., Popov, S. (2012b). Optimization of ethanol production from thick juice: A response surface methodology approach. Fuel, 93, 221-228.

Grahovac J., Jokić A., Dodić J., Vučurović D., Dodić S. (2016). Modelling and prediction of bioethanol production from intermediates and byproduct of sugar beet processing using neural networks. Renewable Energy, 85, 953-958.

Hinková, A.,Bubník, Z. (2001). Sugar beet as a raw material for bioethanol production. Czech Journal of Food Science, 19(6), 224-234.

Krajnc, D., Glavič, P. (2009). Assessment of different strategies for the co-production of bioethanol and beet sugar.Chemical Engineering Research and Design,87(9), 1217-1231.

Microsoft Office Excel 2007 (Data Analysis ToolPak), Microsoft, USA.

Pajčin, I., Grahovac, J., Dodić, J., Jokić, A., Dodić, S., Vučurović D., Milović N. (2017). Application of artificial neural networks in modeling and optimization of biofuels production. Journal on Processing and Energy in Agriculture, 21(2), 66-70.

Popov, S., Ranković, J., Dodić, J., Dodić, S., Jokić, A. (2010).Bioethanol from sugar beet juice. Food Technology and Biotechnology, 48(3), 376-383.

Received: 20. 02. 2019. Accepted: 03. 07. 2019.