VERSITA

Cent. Eur. J. Chem. • 12(8) • 2014 • 868-876
DOI: 10.2478/s11532-014-0542-2

Central European Journal of **Chemistry**

Cultivating conditions optimization of the anaerobic digestion of corn ethanol distillery residuals using response surface methodology+

RICCCE 18

László Gyenge[1], Botond Ráduly[2*], Silvia Crognale[3], Szabolcs Lányi[2], Beáta Ábrahám[2]

[1]*Department of Inorganic Substances Technology and Environmental Protection, University of "Politehnica", Bucharest 060042, Romania*

[2]*Department of Bioengineering, Sapientia Hungarian University of Transylvania, 530104 Miercurea Ciuc, Romania*

[3]*Department for Innovation in Biological, Agro-food and Forest systems (DIBAF), Tuscia University, 01100 Viterbo, Italy*

Received 7 September 2013; Accepted 18 December 2013

Abstract: This study investigated the individual and interactive effects of three factors – temperature, inoculum/substrate ratio (ISR) and inoculum typology – on the anaerobic digestion of corn ethanol distillery wastewater. Biochemical methane potential assays planned with factorial design with two independent quantitative variables on three levels (ISR: 1:1, 2:1 and 3:1; temperature: 30°C, 33.5°C, 37°C) and one independent qualitative variable (inoculum type: suspended, granular, mixed) have been performed. Response Surface Methodology has been used to study the effect of the factors with the aim of maximizing the specific methane yields (Y_{CH4}) obtainable with this substrate. The results show that all three investigated factors influence in a significant matter the Y_{CH4}, the ISR having the strongest effect on it. The temperature has significant influence on the Y_{CH4} only in combination with high ISR values. The optimal conditions for the maximum Y_{CH4} (551 mL CH_4 g^{-1} VS_{added}) have been found at 37°C operating temperature, ISR=3:1 and using granular inoculum. These conditions gave rise to a 4-fold increase of Y_{CH4} with respect to the worst combination of factors (Y_{CH4}=129 mL g^{-1} VS_{added} for the suspended inoculum type, at 30°C and ISR=1:1). The results improve the knowledge on the digestion of this substrate, providing information for successful process up-scaling.

Keywords: *Anaerobic digestion optimization • Response surface methodology • Corn DDGS • Process conditions • Inoculum type*

1. Introduction

The anaerobic digestion (AD) of the corn ethanol distillery residuals became a process of interest in the last years, as a consequence of the fast expansion of the corn-bioethanol industry [1,2]. Despite the tenfold growth of the corn-ethanol production in the last decade [3], the corn-based bioethanol production still fights two major problems, namely the large amount of byproducts and the related energy intensive processing operations currently practiced. Besides every liter of corn ethanol also 10-20 L of distillery wastewater (a.k.a. whole stillage) is produced [4,5]. Due to its nutrient content the dried form of whole stillage (dried distiller's grains with solubles, DDGS) is normally valorized as cattle feedstock. However, the processing of whole stillage into DDGS requires large amounts of energy, accounting for almost half of the of total energy consumption of a dry-grind corn bioethanol plant [5]. This additional energy consumption increases the cost and worsens the net energy balance ratio of the corn bioethanol, making it less competitive on the fuel market [6]. AD is seen as a promising alternative stillage processing method, which on one hand could reduce the byproduct volumes, and on the other hand would enable

* *E-mail: radulybotond@sapientia.siculorum.ro*

+ *The article has been presented at the 18th Romanian International Conference on Chemistry and Chemical Engineering - RICCCE18 - held in New Montana, Sinaia, Romania on 4-7 September, 2013.*

energy recovery by converting the organic fraction of the residuals into biogas [1,5,6]. Besides the energetic valorization of the residual organic matter, the AD could also eliminate other problems related to the traditional stillage handling, such as the solids build-up, toxicity to yeasts and chemical oxygen demand (COD) raise of the distillery wastewater caused by recycling the soluble part of the stillage [7,8].

AD has been successfully utilized to produce methane from a series of food processing wastes but has not yet been used much in the ethanol industry [2]. The biomethanation process is based on the anaerobic bacterial degradation of organic substrate in 4 consecutive stages carried out by unique functional groups of microbes, which use as substrate the products of the previous stage [9]: (i) complex organic molecules are broken down through hydrolysis to amino acids, long-chain fatty acids, and sugars; (ii) these products are then fermented during acidogenesis to form volatile fatty acids (VFAs); (iii) in the acetogenesis, syntrophic acetogenic bacteria consume VFAs and generate acetic acid, carbon dioxide, and hydrogen; and (iv) methanogenic organisms consume the acetate, hydrogen, and some of the carbon dioxide to produce methane. Due to the synthropic associations, the competition between microorganisms, the different sensitivity to environmental conditions and the different nutritional requirements of the involved microorganisms the AD is a very complex process, influenced by a large number of environmental factors [10]. Temperature, pH, inoculum type or concentration of trace-elements are just a few of the factors influencing the specific methane yield (Y_{CH4}) obtainable from this substrate, and thus the practical applicability of the AD to the processing of bioethanol by-products [11]. Given the large number of potentially influential factors involved in the process, it is not obvious to determine which are the most important. Previous studies highlighted the importance of inoculum choice, as well as the effect of inoculum/substrate ratio (ISR) on the digestion under mesophilic conditions of raw and dried corn whole stillage [11,12]. No studies have been performed, however, on the effect of temperature within the mesophilic temperature range on the digestion of this substrate, and the combined effect of these factors has not been investigated either. Hence there is no knowledge regarding which factor is most important for the efficiency of biomethanation, and the eventual synergies between the different factors are unknown. Temperature is known to have an important influence on the methane production efficiency; especially for the net energy generation it is very important to obtain accurate information concerning the operating temperatures versus gas yield correlation [13]. Hence temperature-related studies have been carried out on several other substrates [14-16].

In this paper a Response Surface Methodology (RSM) has been applied to define the individual and interactive effect of the ISR, temperature and inoculum typology on the Y_{CH4}. The RSM is a statistical technique for designing experiments, building models, evaluating the effects of several factors, searching the optimum condition for desirable responses and reducing the number of experiments. Unlike the conventional, laborious and time-consuming 'change-one-factor-at-a-time' method, the RSM makes possible to determine the influence of various factors with only a small number of trials. Moreover, the factorial design makes it possible to take advantage of practical knowledge about the process during the final response surface analysis. Because of these advantages, RSM is often used for the optimization of cultivating conditions, of metabolite production and of biochemical processes in general [17,18]. It is worth noting that, for methane production, literature reports that RSM has been used mainly for experiments of co-digestion [19-21]. To the best of our knowledge this is the first experimental work using RSM for the optimization of the biomethanation of corn-ethanol byproducts.

2. Experimental procedures

2.1. Substrate and inocula

In the digestion experiments rehydrated corn-DDGS has been used as substrate. The DDGS was acquired from SC Bio Fuel Energy SRL a dry-grind corn ethanol plant located in Zimnicea (Romania), and it was kept in a cool and dry place until use. For the methane potential assays the DDGS was hydrated with distilled water in such a way to match the average water content of its precursor, the whole stillage (7% of dry matter).

Two types of inoculum seeds and the mix of them (in 1:1 ratio expressed in Total Solids) have been used in this experiment. The first inoculum (suspended type) was collected from the effluent line of the suspended-growth anaerobic digester of the municipal wastewater treatment plant of Sfantu Gheorghe (Romania). The second inoculum (granular type) was collected from the up-flow anaerobic sludge blanket reactor (UASBR) of the anaerobic wastewater treatment facility treating the brewery wastewater of Heineken S.A. Miercurea Ciuc (Romania). Before their use in the experiments, the inocula were stored in 37°C incubators for 14 days. This "starving period" was necessary to minimize the residual methane production of the inocula.

Table 1. Physical-chemical characteristics of the substrate and inocula used in the experiments. The values presented are the mean of at least two measurements.

Parameter	Substrate	Inoculum type		
		Granular	Suspended	Mixed
pH	3.53	7.29	7.24	7.27
TS [%, w/w]	7.04	8.01	10.44	9.06
VS [%, w/w]	6.74	3.85	4.87	4.27
VS/TS [%]	95.7	48.1	46.2	47.2
COD [mg L^{-1}]	117 609	70834	57698	63847
SCOD [mg L^{-1}]	28 232	879.0	3450	1691
TKN [mg NL^{-1}]	3460	-	-	-

2.2. Chemical characterization of substrate and inocula

The determinations of Total Solids (TS) and Volatile Solids (VS) content of the inoculum seeds and of the substrate were performed according to Standard Methods [22]. The Total Kjeldahl Nitrogen (TKN) content of the substrate has been determined chemically using an UDK 159 VELP Automatic Kjeldahl Distillation & Titration System apparatus (Velp Scientifica, Italy). Digestion of samples (5 g) was made with concentrated H_2SO_4 (96%) and cupric catalyst in DK6 Heating Digester Unit (Velp Scientifica, Italy). Chemical Oxygen Demand (COD) determinations were accomplished using the Open Reflux Method [23]. The soluble COD (SCOD) determinations were performed on the filtrate passing the 0.45 μm glass-fiber filter. All reagents used for the chemical analyses were of analytical grade. The physical-chemical characteristics of the inocula and substrate are provided in Table 1.

2.3. Biogas determination

The volume of the produced biogas has been measured by displacement of acidulated water (pH=2 in concordance with [23]) in an upside-down graduated cylinder. At each volume measurement the biogas has been let out of the serum bottles through a syringe needle inserted in the septum (and conducted into the upside-down cylinder) until atmospheric pressure inside the bottles was reached. The observed biogas volumes were in each case corrected for temperature, hence methane volumes reported in this paper are referred to normal conditions (101325 Pa, 0°C).

Biogas composition was determined using a gas chromatograph (Hewlett Packard 5890A) equipped with thermal conductivity detector (TCD). The mounted column type was Porapack Q 3 mts × 1/8" × 2.1 mm D.I., (Teknokrama). Helium has been used as carrier gas in splitless mode with a back pressure of 338 kPa. The oven was maintained at a constant temperature of 70°C for 3 min. The injector and detector temperatures were at 150°C and 180°C, respectively. The system was calibrated with analytical methane and carbon-dioxide.

2.4. Factorial design by RSM of the experiments

The combined effect of the temperature, inoculum type and ISR on the specific methane production (response variable) has been optimized by D-optimal design. Two independent quantitative variables, namely ISR (X_1) and temperature (X_2), as well as an independent qualitative variable – inoculum type (Z) – have been investigated in this study. The experimental design included a set of 17 variable combinations and one center point replicated 4 times (hence a total of 21 runs). The two quantitative dimensional variables (X_i) were coded as dimensionless terms (x_i) using the equation

$$x_i = (X_i - X_0^*)/\Delta X \quad (1)$$

where x_i is the coded value and X_i the actual value of the variable, X_0^* is the actual value of the same variable at the center point and ΔX is the step change in the variable. The qualitative factor (Z_i) was coded in a logical dimensionless term (z_i, taking the value 1 if present and 0 if missing). The tested parameter range was 30-37°C for the temperature and 1:1-3:1 for the ISR (see also Table 2 for details). The selected temperature range is known to be optimal for mesophilic digestion [10]. The ISR range used in this work is also typical to AD optimization studies [24,25]. The qualitative parameter (inoculum type) took three values: "granular", "suspended" and "mixed", this latter being the 1:1 mixture of the other two. Interrelationship between process variables and Y_{CH4} was established by the following second-degree polynomial equation

$$Y = b_0 + \sum b_i x_i + \sum b_{ii} x_i^2 + \\ + \sum b_{ij} x_i x_j + \sum \beta_i z_i + \sum b_i \beta_i x_i z_i \quad (2)$$

where Y is the predicted response variable, b_0 is the intercept, b_i and b_{ii} are linear and quadratic coefficients of the quantitative input variables, β_i is the linear coefficient of the qualitative input, b_{ij} and $b_i\beta_i$ are the interaction coefficients and x_i, x_j and z_i are the coded forms of the input variables.

Statistical examination of results and response surface study were carried out by the MODDE 9.1 software (Umetrics AB, Sweden).

2.5. Biochemical methane potential (BMP) assays

The anaerobic degradation assays were conducted in 50 mL serum bottles. The inoculum quantity has been determined so that ISR expressed in TS was 1:1, 2:1 and 3:1 (as required by the factorial design). Alkalinity was added to the mixtures in order to adjust the pH to 7. The serum bottles were than adjusted to 25 mL with distilled water, so that the remaining headspace in each bottle was equal. For each of the 21 theses blank probes (containing only inoculum), and for each inoculum control probes (with reference substrate) have been performed. Starch has been used as reference substrate, having a known biogas yield.

All bottles were gassed with nitrogen gas for 2 min and sealed immediately using rubber septa and aluminum crimp caps, to ensure anaerobic conditions. All bottles were placed in incubator at the mesophilic temperatures as required by the factorial design; mixing was assured by inverting the bottles three times, once a day. The volume and the methane concentration of the produced biogas were measured each day at the beginning of the experiment, and less frequently later on, as the intensity of biogas production decreased. The results of the blank probes (the biogas production of the inocula due to endogenous respiration, were subtracted from the observed biogas volumes). Degradation tests were ended after 31 days, when all cumulative biogas curves reached the plateau phase.

3. Results and discussion

The specific methane yields observed at the final time of the BMP assays are presented in Table 2 ("Observed" column). The methane yields vary widely within the studied parameter range: the lowest value Y_{CH4}=129 mL g^{-1} VS_{added} has been observed for the suspended inoculum type, at 30°C and ISR=1:1, while the granular inoculum gave the highest yield (Y_{CH4}=551 mL g^{-1} VS_{added}) at 37°C and with an ISR=3:1. This means that the best parameter combination gave 4.3 times higher specific methane yield than the worst combination of the parameters. The results are comparable to values reported in the literature for the digestion of corn bioethanol residuals. The specific methane yields reported for the mesophilic and termophilic digestion of rehydrated corn-DDGS are in the range 0.3-0.5 L g^{-1} VS_{added} [11,26]. The best Y_{CH4} obtained in this study exceeds this range, and it is actually higher than values reported for the mesophilic digestion of corn whole stillage (0.45-0.5 L g^{-1} VS_{added} [12]). This is an excellent result, considering that DDGS may lack part of the soluble organic fraction of whole stillage (if solubles recycling is applied in the ethanol production facility [2]), so it might have a somewhat lower methane potential than the raw whole stillage. It is to be mentioned, that the conditions giving the highest methane yield also gave a high methane concentration of the produced biogas (63%). The final values of methane percentage in the BMP bottles were in the range of 45-64%.

Experimental data were best fitted by a polynomial quadratic equation, with coefficients presented in Table 3. The equation describes well the experimental data on the investigated parameter domain, as it is also shown by the good agreement of observed data with those estimated by the model (Table 2). The correlation coefficient (R^2) adjusted for degrees of freedom was 0.97, indicating that the statistical model can explain more than 95% of the variability in the response. The Q^2 value close to 1 indicates a very good model with excellent predictive power. The model F-value of 99.63 indicates that model terms were highly significant, while the F_{ME} value of 1.55, calculated as the ratio between mean squares of model error and replicate error, indicates that the probability for lack of fit of the model is not statistically significant. As can be seen in Table 3 the intercept and first-order coefficients of independent variables were all highly significant, while among the second-order coefficients only that of the ISR was significant for a 95% confidence level. Among the interaction terms the ISR·temperature and ISR·Inoculum_type have statistically significant coefficients. The reproducibility of the model (the variation of the response for replications, compared to the total variation of the response) is also very good.

When comparing the coefficient values of Table 3 one can see that the most significant influence on the response variable has X_1 (both its first-order and second-order term coefficients have high values). This means that the ISR has by far the highest influence on the specific methane yield, while for example the temperature has a less strong effect on it. The same

Table 2. D-optimal design showing coded and actual values of the independent variables ISR (X_1), incubation temperature (X_2) and inoculum type (Z_i) and observed and predicted values of specific methane yields (volume of methane produced from a unit mass of substrate volatile solids) of the 21 runs.

Experiment	Independent variables			Specific methane yield (mL g^{-1} VS_{added})	
	X_1	X_2	Z_i*	Observed	Predicted
1	-1 (1)	-1 (30.0)	granular	174	172.8
2	1 (3)	-1 (30.0)	granular	465	478.4
3	-1 (1)	1 (37.0)	granular	170	185.4
4	1 (3)	1 (37.0)	granular	551	551.8
5	0 (2)	0 (33.5)	granular	335	306.6
6	-1 (1)	-1 (30.0)	suspended	129	128.0
7	1 (3)	-1 (30.0)	suspended	308	300.7
8	-1 (1)	1 (37.0)	suspended	133	140.7
9	1 (3)	1 (37.0)	suspended	369	374.0
10	-1 (1)	0 (33.5)	suspended	134	134.4
11	1 (3)	0 (33.5)	suspended	328	337.4
12	0 (2)	-1 (30.0)	suspended	152	173.9
13	0 (2)	1 (37.0)	suspended	253	216.9
14	-1 (1)	-1 (30.0)	mixed	164	151.5
15	1 (3)	-1 (30.0)	mixed	504	478.7
16	-1 (1)	1 (37.0)	mixed	173	164.2
17	1 (3)	1 (37.0)	mixed	548	552.0
18	0 (2)	0 (33.5)	mixed	278	296.1
19	0 (2)	0 (33.5)	mixed	292	296.1
20	0 (2)	0 (33.5)	mixed	265	296.1
21	0 (2)	0 (33.5)	mixed	307	296.1

** Z_i takes the value 1 if the respective inoculum is present in a thesis and 0 if not present*

Table 3. Coefficients of the input parameters (estimated coefficient ± standard error). Statistical parameters measuring the correlation and significance of the model are shown in the last two columns.

Coefficient	Value	Parameter	Value
b_0	266.1 ± 8.6***	R^2	0.98
b_1	149.4 ± 5.9***	R^2_{adj}	0.97
b_2	21.5 ± 5.8**	Q^2	0.95
$\beta_{ganular}$	40.6 ± 7.5***	F	99.63
$\beta_{suspened}$	-70.7 ± 6.6***	F_{ME}	1.55
β_{mixed}	30.1 ± 6.8***	Reproducibility	0.98
b_{11}	40.5 ± 10.4**	Confidence level	0.95
b_1 b_2	15.2 ± 6.2*		
b_1 $\beta_{granular}$	18.6 ± 8.5*		
b_1 $\beta_{suspended}$	-47.9 ± 7.7***		
b_1 β_{mixed}	29.3 ± 8.5**		

** $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$*

is confirmed by the main effect plot (Fig. 1). The plot shows the predicted values of Y_{CH4}, when the ISR varies from its low to its high level, all other factors in the design held constant at their averages. It can be clearly seen that the ISR alone causes a threefold change in the Y_{CH4} over to the factor levels defined by the model (1:1 to 3:1). This is in contrast with the observations of Eskicioglu and Ghorbani on corn whole stillage digestion using a suspended type inoculum, suggesting that the ISR has no influence on the specific methane yield [12]. Note that in our experiment the effect of ISR was far stronger in the case of granular inoculum, while it was less evident when using suspended inoculum, showing that the effect of ISR on the methane yield is inoculum-dependent.

Fig. 2 shows the response surfaces predicted by the model for the three inocula types, together with the contour diagrams of the specific methane production in function of ISR and temperature. The main evidence the

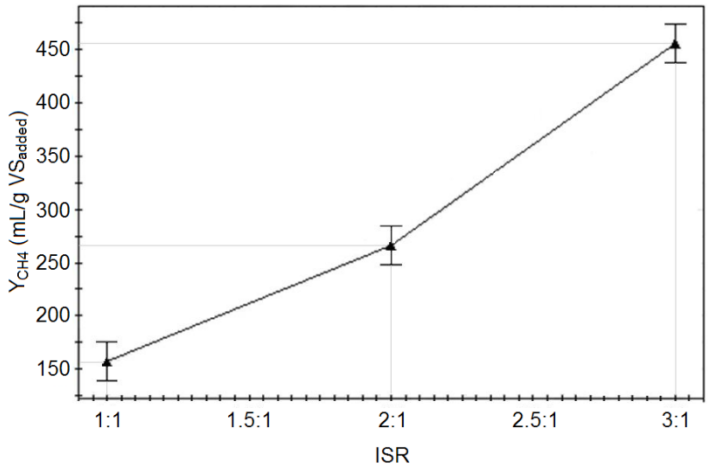

Figure 1. Main effect plot: predicted influence of ISR on the specific methane yield when all other factors are held constant at their averages.

figure shows is that the granular inoculum outperforms by approx. 50% the suspended one in terms of specific methane production. Indeed, the highest values of the methane yields are around 550 mL g^{-1} VS_{added} and 370 mL g^{-1} VS_{added} for the granular and suspended inocula, respectively. The better performance observed with granular inoculum on methanogenic activity are in agreement with the findings of Pereira *et al.* on a number of different substrates [27].

The mixed inoculum performs very closely to the granular one in terms of methane production. There are no significant differences between their response surfaces (Figs. 2a and 2b, respectively) except in the regions where both ISR and temperature values are low. The results show that the inoculum type has a strong influence on the methane production. Considering the absolute performance of the two inoculum types, and their relative proportions in the mixed inoculum an easy synergetic effect can be observed as well. However this synergy does not manifest in a higher absolute biomethanation performance, because the mixed inoculum did not give better results than the granular one in terms of specific methane yield. Also note that the model does not give information on the synergetic effect between inocula. To obtain a model which considers interactions between inoculum types the factorial design should be reformulated and qualitative factors should be treated as quantitative ones.

On all three response surface plots a steep increase in the methane yield can be observed with increasing ISR values, especially at the higher end of the tested temperature range. Also the effect of temperature can be clearly seen at high ISR values: at ISR 3:1 moving from 30°C to 37°C causes a 23% increase in the specific methane yield in the case of the suspended inoculum, and an increase of 18% in the case of the granular one. At low ISR values the effect of temperature was not significant for the granular inoculum, and affected only easily the methane yields obtained with the suspended inoculum. The fact that the performance of the suspended inoculum is affected more by the increase of temperature may suggest that the temperature optimum of the microbial consortia of this inoculum is shifted towards high temperatures, possibly exceeding the investigated temperature range. This hypothesis is supported by the above mentioned synergetic effect between the two inocula, detectable only at high temperatures. The higher the temperature the greater the contribution of the suspended inoculum on the biomethanation efficiency when co-inoculated.

Summarizing, in order to optimize the specific methane yields from the anaerobic digestion of the corn ethanol residuals, high ISR has to be applied and the temperature should be kept at the higher end of the mesophilic range, possibly at 37°C. The correlation found between operating temperature and methane yield is very important considering the common practice of operating the anaerobic digesters at temperatures below 35°C [13]. In many cases it is advantageous for the net energy production to operate the digester at such low temperatures, but it is not the case of corn stillage digestion, where no auxiliary heating is necessary for keeping the digester at 37°C, due to the residual heat of the hot stillage. Operating the digester at 37°C instead of 33°C would bring a 10% higher specific methane production, as can be read from the contour plot of Fig. 2a. The obtained model predicts even higher methane yields at higher ISR values. However, extrapolation in biochemical processes is very uncertain, so this prediction has to be verified experimentally.

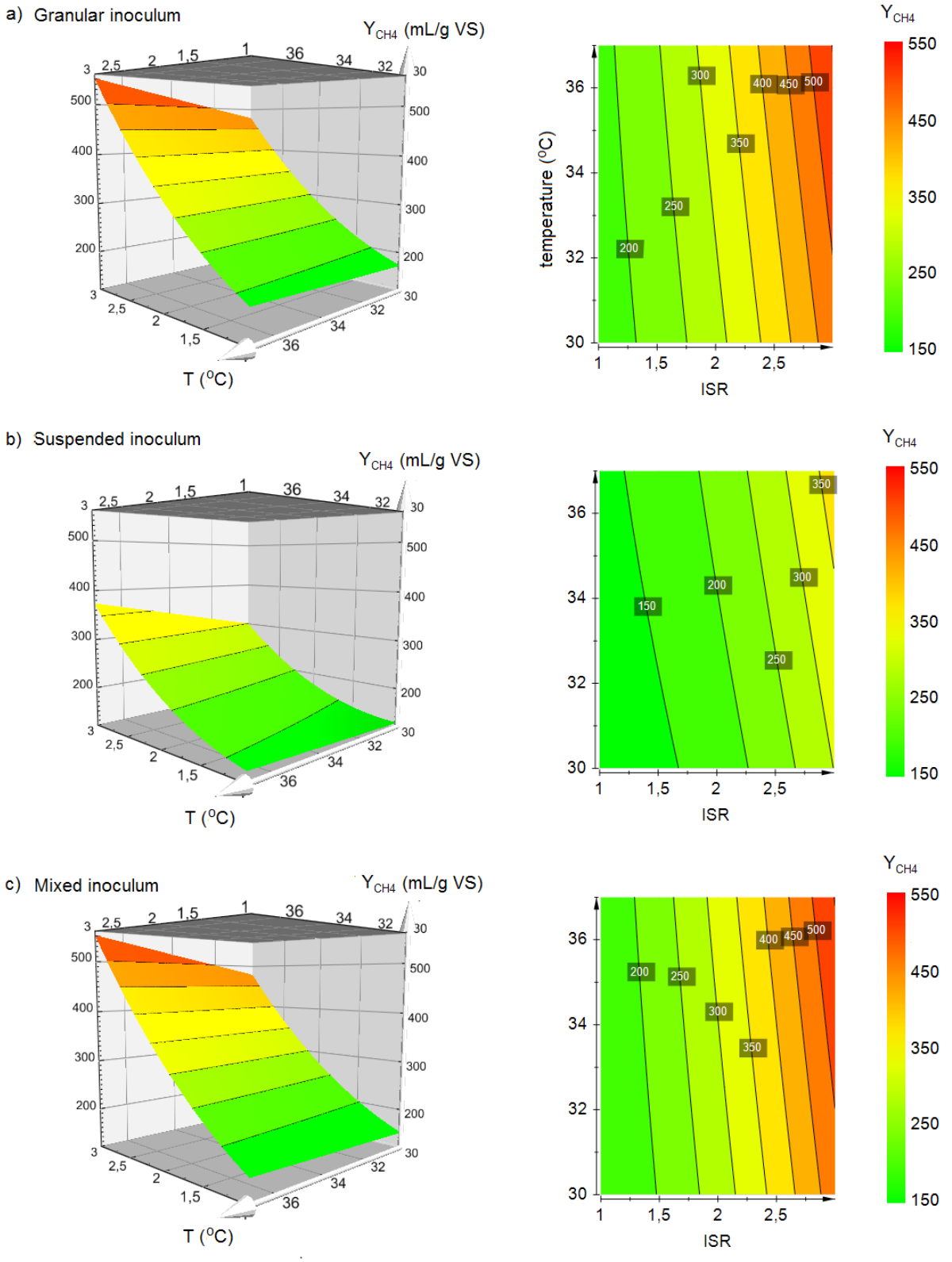

Figure 2. Response surfaces and the corresponding contour diagrams of the specific methane production in function of ISR and temperature for the three inoculum types tested: a) granular inoculum b) suspended inoculum and c) mixed inoculum.

4. Conclusions

The RSM statistical method enabled the assessment of the effect of the three selected parameters on the methane yield over the full parameter domain, using a reduced number of experiments and resulted in a model with great predictive power (R^2=0.985). All three studied parameters were found to significantly influence the methane yield. The optimal parameter set for maximal specific methane yield was found to be 37°C, ISR 3:1 and granular inoculum type. This condition gave 4.3 times higher Y_{CH4} than the worst parameter combination (suspended inoculum, 30°C, ISR=1:1). The response surface study shows that the parameter with the highest influence on the Y_{CH4} of corn-DDGS substrate is the ISR. In the same time our model shows that the effect of ISR is strongly influenced by the inoculum typology. Temperature has a significant influence only in combination with high ISR values. The obtained model predicts further possible increase of the specific methane yield at higher ISR values, conditions to be tested in the future.

The results obtained from the BMP assays allowed for the definition of optimal temperature, ISR and type of the inoculum for the studied process. However, a further step of optimization has to be considered in view of possible scaling up of the process, where other parameters such as organic loading rate and stirring of the reactor have to be evaluated.

Acknowledgements

This work has been funded by the Sectorial Operational Programme Human Resources Development 2007-2013 of the Romanian Ministry of Labour, Family and Social Protection through the Financial Agreement POSDRU/107/1.5/S/76903. Experimental work has been carried out in the Biochemical and Biotechnological Research Center, Sapientia Hungarian University of Transylvania. We would like to thank the Sapientia Foundation – Institute for Scientific Research for the support provided in this research.

References

[1] S.H. Schaefer, S. Sung, Water Environ. Res. 80, 101 (2008)
[2] K. Liu, K.A. Rosentrater, Distillers Grains. Production, Properties and Utilization (CRC Press, Boca Raton, 2012)
[3] B.A. Babcock, J.F. Fabiosa, CARD Policy Brief 11, 1 (2011)
[4] M. Krzywonos, E. Cibis, T. Miskiewicz, A. Ryznar-Luty, Electron. J. Biotechn. 12 (2009)
[5] C. Eskicioglu, K. Kennedy, J. Marin, B. Strehler, Bioresource Technol. 102, 1079 (2011)
[6] M. Agler, M. Garcia, E. Lee, M. Schliche, L. Angenent, Environ. Sci. Technol. 42, 6723 (2008)
[7] S.A. Shojaosadati, H.R. Sanaei, S.M. Fatemi, J. Chem. Technol. Biot. 67, 362 (1996)
[8] A.C. Wilkie, K.J. Riedesel, J.M. Owens, Biomass. Bioenerg. 19, 63 (2000)
[9] T. Abbasi, S.M. Tauseef, S.A. Abbasi, Biogas Energy (Springer, New York, 2012)
[10] G.K. Anderson, P.J. Sallis, S. Ujanik, In: D. Mara, N.J. Horan (Eds.), Handbook of water and wastewater microbiology (Academic press, San Diego, 2003)
[11] L. Gyenge, B. Raduly, R. Barrena, X. Font, Sz. Lanyi, B. Abraham, Energy (IYCE) 1 (2013)
[12] C. Eskicioglu, M. Ghorbani, Process Biochem. 46, 1682 (2011)
[13] D. Hawkes, R. Horton, In: G. Milazzo (Ed.), Energetics and Technology of Biological Elimination of Wastes (Elsevier, New York, Amsterdam, 1981) 131
[14] R. Alvarez, G. Liden, Bioresource Technol. 99, 7278 (2008)
[15] W. Choorit, P. Wisarnwan, Electron. J. Biotechn. 10, 376 (2007)
[16] K.J. Chae, Am Jang, S.K. Yim, In S. Kim, Bioresource Technol. 99, 1 (2008)
[17] S.J. Kalil, F. Maugeri, M.I. Rodrigues, Process Biochem. 35, 539 (2000)
[18] P. Barghini, D. Moscatelli, A.M.V. Garzillo, S. Crognale, M. Fenice, Enzyme Microb. Tech. 53, 331 (2013)
[19] X. Wang, G. Yang, Y. Feng, G. Ren, X. Han, Bioresource Technol. 120, 78 (2012)
[20] M.J. Han, S.K. Behera, H.S. Park, J. Chem. Technol. Biot. 87, 1541 (2012)
[21] C. Gonzalez-Fernandez, B. Molinuevo-Salces, M.C. Garcia-Gonzalez, Appl. Energ. 88, 3448 (2011)
[22] L.S. Clesceri, A.E. Greenberg, A.D. Eaton (Eds.), Standard Methods for the Examination of Water and Wastewater, 20th edition (APHA, Washington D.C., 1999)
[23] M. Walker, Y. Zhang, S. Heaven, C. Banks, Bioresource Technol. 100, 6339 (2009)
[24] K.S.B. Kameswari, C. Kalyanaraman, S. Porselvam,

K.Thanasekaran, Clean. Technol. Envir. 14, 241 (2012)
[25] F. Raposo, R. Borja, M.A. Martin, A. Martin, M.A. De la Rubia, B. Rincon, Chem. Eng. J. 149, 70 (2009)
[26] W. Wu-haan, MSc thesis (Iowa State University, Ames, USA, 2008)
[27] M.A. Pereira, O.C. Pires, M. Mota, M.M. Alves, Water Sci. Technol. 45, 139 (2005)