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DEVELOPMENT OF A STATISTICAL MODEL TO PREDICT METHANE PRODUCTION FROM WASTE ACTIVATED SLUDGE CO-DIGESTED WITH OLIVE MILL WASTEWATER AND CATTLE DUNG BY RESPONSE SURFACE METHODOLOGY

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Abstract. Nowadays, population growth is likely to lead to a wide variety of biomass wastes generation from the diversified human, industrial, and agricultural activities. Anaerobic digestion is mostly applied to manage biomass wastes and mitigate a huge spectrum of environmental damages. This paper aims to enhance the anaerobic digestion efficiency of multicomponent substrates, using a mixture of waste activated sludge (WAS), olive mill wastewater (OMW), and cattle manure (CM). A Response Surface Methodology is employed in experimental design to determine individual and interactive effects on methane yield and chemical oxygen demand reduction. After numerical optimization using Design Expert®, the optimum values of the test factors in actual were as follows: initial pH = 8, COD/N ratio = 47, 42, CM/WAS-OMW ratio = 0.352, TS = 42.94 g/L. The obtained results indicate that anaerobic co-digestion performance could be achieved by optimising substrate composition to assure a larger microbial synergistic effect.

Keywords: waste activated sludge; olive mill wastewater; cattle manure; response surface methodology; anaerobic co-digestion.

1. Introduction

The escalation of wastewater treatment plants worldwide caused a gigantic production of waste activated sludge (WAS), which constituted a severe environmental problem.[1] WAS contain high organics, and it necessitates to be stabilized sufficiently to decrease organic contents, odor problems, and pathogen contaminations before ultimate disposal.[2]

Anaerobic digestion (AD) has been recognized as an efficient bioprocess for the management of WAS,[3] by offering several environmental and economic benefits.[4] However, the AD process efficiency utilizing WAS as the sole substrate is limited[5] and present low methane potentials,[6] mainly for some reasons. Firstly, this WAS is originating from prolonged aeration processes.[7] Consequently, it has relatively low degradability. Secondly, the hydrolysis step's deficient performance is caused by rigid cell walls and substantially secreted extracellular biopolymers.[3] Then slow microorganisms growth rates in methanogenesis.[8-10] Moreover, possible volatile fatty acids (VFAs) and ammonia inhibition.[11]

To solve the above-cited problems and enhance the bioenergy recovery, the WAS co-digestion with other sorts of wastes has been considered extensively.[12] This approach is a simultaneous anaerobic treatment of two or more raw materials sorts.[13] It is the best possible manner in this critical situation,[14] as it assures a high energy improvement with little or no drawbacks,[15] and that increases the AD efficiency of both biomass waste rather than their mono-digestion.[16,17] This strategy usually compromises its economic advantages resulted from the equipment sharing, easier managing of mixed wastes, and synergistic effect.[18] The interests of the co-digestion process covering: dilution of the potentially toxic compounds eventually existing in any treated materials; an augmented load of biodegradable organic matter, then better biogas yield due to synergistic effects; tuning of the moisture content and pH; strength the essential buffer capacity to the mixture; enlarging the range of bacterial strains taking part in the process.[15,17-19]

Therefore, significant issues have been performed by digesting simultaneously WAS with other biological wastes.[20-28]

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Olive Mill Wastewater (OMW) and Cattle Manure (CM) are attractive co-substrates for anaerobic co-digestion because the co-digestion of ammonia-rich with carbon-rich feedstocks is an exciting option.[29] Livestock manure is one of the most typically used co-substrate for its good buffering capacity, richness in micronutrients, and high microbial activity.[30]

The methane yield optimization from the co-digestion of WAS with OMW and CM by thermophilic culture was examined in the present work. The experiments were carried out systematically for proper investigation of synergistic and/or antagonistic interactions of these wastes. The Response Surface Methodology (RSM) was used to consider the effects of the initial pH, the COD/N ratio, the CM/WAS-OMW mass mixing ratios, and the total solid contents (TS) in the process.

2. Materials and Methods

2.1. Wastes sources

Waste activated sludge was taken away from the decanter of Boumerdes urban wastewater treatment plant. The average sludge retention time in the extended aeration process (sludge age) was 18 days. Fresh CM was collected from the Cow Farm located near the city of Boumerdes in Eastern Algeria. The OMW used in this study was taken from a three-phase olive mill processing plant located at the Issers city in Boumerdes during the harvesting period. The characteristics of wastes are presented in Table 1.

2.2. Analytical Methods

Soluble and total chemical oxygen demand (COD) total nitrogen (TN) and total phosphorus (TP), Total solids (TS) and Volatile Solids (VS) were quantified according to Standard Methods.[31] The concentration of total phenolic compounds TP_c was determined spectrophotometrically according to the Folin–Ciocalteu method. According to the Method cited by Liu *et al.*, heavy metals were determined by the atomic absorption spectrophotometer (Perkin Elmer, Optima 8000). The biogas composition (CH_4 + CO_2) was measured using a gas chromatograph (GC-HP 5890) coupled with a thermal conductivity detector (TCD) and stainless steel column that was 2 m long with a 5 mm OD and 2 mm ID and contained Porapak Q 100 that had a mesh range from 80 to100. The carrier gas was N_2, and the analysis was carried out at a carrier gas flow rate of 30 $mL{\cdot}min^{-1}$ with the injector, column, and detector temperatures at 393, 363, and 393 K, respectively.

Table 1. Chemical characterization of substrates used in the co-digestion

Parameters	Waste activated sludge	Olive Mill Wastewater	Cattle Manure
pH	7.8 ± 0.15	4.8 ± 0.1	7.58 ± 0.2
CODt (g L^{-1})	38.6 ± 1.3	128.1 ± 5.4	149.05 ± 7
CODs (g L^{-1})	3.70 ± 0,6	64.7 ± 1.4	65.15 ± 0.4
TS (g L^{-1})	61.2 ± 0.4	69.5 ± 3.1	154.80 ± 0.8
VS (g L^{-1})	48.1 ± 0.5	57.4 ± 4.5	145.24 ± 0.3
TN (g L^{-1})	1.72 ± 0.02	1.26 ± 2.2	1.88 ± 0.4
TP (g L^{-1})	0.303 ± 0.07	0,48 ± 0.09	0.93 ± 0.5
TPc (eqgallic acid, g L^{-1})	/	4.11 ± 0.3	1.5 ± 0.05
Oil and grease (g L^{-1})	/	17.4 ±1.7	/
Cd (mg L^{-1})	201	$<1\times10^{-3}$	0.24
Cr (mg L^{-1})	508.9	0.655	0.106
Pb (mg L^{-1})	335.5	0.186	0.022
Mn (mg L^{-1})	922.5	$<1\times10^{-3}$	0.915
Ni (mg L^{-1})	$<1\times10^{-3}$	3.96×10^{-2}	0.051
Fe (mg L^{-1})	4520	1.504	0.145
Zn (mg L^{-1})	30.63	0.24	0.235
Cu (mg L^{-1})	1116	0.33	0.036

COD_t: total chemical oxygen demand
COD_s: Soluble chemical oxygen demand
TN: total nitrogen
TP: total phosphorus
TS: Total solids
VS: Volatile solids
TPc: total phenolic compounds

2.3. Operating Procedure

Jacketed batch fermenters of 5 L are used for the thermophilic anaerobic digestion at 328 K (Fig. 1). The working volume of each bioreactor was maintained at 4.5 L. The substrate was prepared according to the initial conditions fixed in Tables 2 and 3. However, the WAS-OMW is held at a constant ratio of 3 : 1. The bioreactors were purged with helium gas to eliminate air from the reactor before fermentation. The generated biogas volume was measured by liquid displacement (water, pH 2, NaCl 10 %).

Fig. 1. Photo of the anaerobic digestion system

2.4. Experimental Design and Statistical Analyses

To investigate the influence of parameters like initial pH, COD/N ratio, CM/ WAS-OMW ratio, and TS content on the specific methane production, response surface methodology (RSM) was chosen to optimize the studied parameters. This statistical technique is a practical tool when the response may be influenced by various variables.[32]

We have used Central Composite Design (CCD) generated by the Design Expert® 10 software. There are four independent quantitative variables, each at five levels (Table 2). According to this CCD, 21 experiments included 17 variable combinations and one center point replicated four times (Table 3). The value range of the quantitative variables used was based on preliminary experiments and the literature data.

A second-order polynomial equation was utilized to analyze methane yield potential (P: mL CH_4/g $_{VSlod}$) and the COD reduction (COD_R: %). The resulted data were simulated to the equation by multiple regression measures. The usual form of the predictive polynomial quadratic equation is:

$$Y=\beta_0+\sum\beta_n X_n+\sum\beta_{nn} X_n^2+\sum\beta_{nm} X_n X_m \qquad (1)$$

where Y is the predicted response, β_o offset term, β_n linear coefficient, β_{nn} squared coefficient, β_{nm} interaction coefficient, X_n nth independent variable, X_n^2 squared effect, and X_nX_m interaction effects.

The statistical analysis of the regression coefficient was implemented using an ANOVA (analysis of variance). The polynomial model fit quality was expressed by the determination coefficient, R^2, and Adj R^2, and its statistical significance was verified by the Fisher's F-test in the same program. Model terms were selected or rejected based on the P-value (probability) with a 95 % confidence level ($p < 0.05$).

Each acting parameter was examined at five different levels assigned as -2, -1, 0, +1, and +2. The P and COD_R were used as the output variables. The factorial design matrix and the results of P and COD_R measured for each experiment (the average of tree replication) are established in Table 3.

Three-dimensional (3D) plots with their respective contour plots were acquired based on the two factors' effects at five levels. Moreover, the perturbation plot would help compare the impact of all the factors at a particular point in the design space. The regression equation adequacy was checked by comparing the experimental data with predicted values founded by the models.

Table 2. Factors and levels used in the factorial design

Symbols	Independent variables	Factor levels				
		-α	Low	Mid	High	+α
		(-2)	(-1)	0	-1	(+2)
A	pH	5	6	7	8	9
B	COD / N	20	30	40	50	60
C	CM / WAS-OMW Ratio	0.1	0.2	0.3	0.4	0.5
D	TS (g · L^{-1})	20	30	40	50	60

CM / WAS-OMW: the ratio of the mixture of Cattle Manure/waste activated sludge- Olive Mill Wastewater
COD /N: *the ratio of total chemical oxygen demand / total nitrogen*
TS: Total solids

Table 3. CCD matrix of studied factors in coded and real values

Standard Order	Coded levels				Real values				P (mL · g^{-1} VS_{lod})		COD_R (%)	
	A	B	C	D	pH	COD/N	Ratio CM/ WAS-OMW	TS (g/Kg)	Observed values	Predicted values	Observed values	Predicted values
1	1	1	1	-1	8	50	0.4	30	625.12	622.12	41.22	41.19
2	1	1	-1	-1	8	50	0.2	30	279.48	283.34	44.1	43.49
3	1	-1	1	1	8	30	0.4	50	379.34	357	41.46	40.70
4	-1	1	-1	1	6	50	0.2	50	271.25	286.68	40.86	40.25
5	1	-1	-1	1	8	30	0.2	50	410.55	395.06	45.9	46.01
6	-1	-1	1	-1	6	30	0.4	30	280.66	269.89	46.98	46.22
7	-1	1	1	1	6	50	0.4	50	180.66	189.23	42.6	42.56
8	-1	-1	-1	-1	6	30	0.2	30	363.33	359.41	40.8	40.91
9	-2	0	0	0	5	40	0.3	40	380.33	372.21	39	39.33
10	2	0	0	0	9	40	0.3	40	633.33	648.36	45	45.33
11	0	-2	0	0	7	20	0.3	40	264.12	260.24	35.76	36.09
12	0	2	0	0	7	60	0.3	40	249.45	260.24	48.24	48.57
13	0	0	-2	0	7	40	0.1	40	228.5	225.1	43.2	43.68
14	0	0	2	0	7	40	0.5	40	271.66	281.97	43.5	43.68
15	0	0	0	-2	7	40	0.3	20	234.33	237.78	45	45.33
16	0	0	0	2	7	40	0.3	60	473.37	476.83	39	39.33
17	0	0	0	0	7	40	0.3	40	614.56	605.52	61.14	59.704
18	0	0	0	0	7	40	0.3	40	609.45	605.52	59.26	59.704
19	0	0	0	0	7	40	0.3	40	593.12	605.52	58.84	59.704
20	0	0	0	0	7	40	0.3	40	606.12	605.52	59.08	59.704
21	0	0	0	0	7	40	0.3	40	604.33	605.52	60.2	59.704

CM / WAS-OMW: the ratio of the mixture of Cattle Manure/waste activated sludge- Olive Mill Wastewater
COD /N: *the ratio of total chemical oxygen demand / total nitrogen*
TS: Total solids
P: methane yield potential
COD_R: chemical oxygen demand reduction

3. Results

3.1. Fitting the response surface models to significant independent variables

CCD was selected for finding out the relationship between the response function (Y) and variables (X). The values of the independent variables as well as their variation limits, plus the experimental and predicted responses (Y_P–Y_{CODR}), are presented in Table 3.

The Fitting of the data to different models (linear, 2FI, quadratic, and cubic) and their ensuing analysis of variance indicated that a quadratic model more fittingly described P and CODR. The model was improved based on the insignificance of some model terms. The final reduced model to predict responses is presented as follows:

$$Y(P\ mL\ CH_4 / g_{\ VSlod}) = +605.52 + 69.04 \cdot A + 14.22 \cdot C + 59.76 \cdot D + 98.11 \cdot AB + 60.96 \cdot AC + 46.12 \cdot BC - 48.10 \cdot CD - 23.81 \cdot A^2 - 86.32 \cdot B^2 - 87.99 \cdot C^2 - 62.05 \cdot D^2 . \quad (2)$$

$$Y_{(CODR\%)} = +59.70 + 1.50 \cdot A + 3.12 \cdot B - 1.50 \cdot D - 1.21 \cdot AB - 1.91 \cdot AC + 3.91 \cdot AD + 1.32 \cdot BD - 0.75 \cdot CD - 4.3 \cdot A^2 - 4.34 \cdot B^2 - 4.01 \cdot C^2 - 4.34 \cdot D^2 . \quad (3)$$

From equations 2 and 3, the P and COD_R have linear and quadratic effects of the four-process variable. The positive sign in front of the coefficients estimates a synergistic effect. In contrast, the negative sign indicates an antagonistic impact on the response.[33] It was detected that all the linear coefficients of the two equations presented a positive effect except the TS (D) coefficient in the COD_R model.

These regression equations were assessed statistically through analysis of variance (ANOVA). The results are summarized in Table 4. In the first response, ANOVA of the regression model for methane yield demonstrated that the F-value of 213.89 implies the model's significance. There is alone a 0.01 % chance that F-Value is due to noise. Then the Values of Prob > F less than 0.05 indicate that the model terms are significant.[34,35] In this response, A, C, D, AB, AC, BC, CD, A^2, B^2, C^2, D^2 are significant. The Lack of Fit F-value "5.61" implies the existence of a 5.99 % chance that a Lack of Fit

F-value due to noise. Lack of Fit is not significant but good, according to Qian *et al.*[36] The adjusted correlation coefficient (Adj R^2) is 0.9915, so the model is stronger and predicts a greater response when the R^2 value is closer to 1.[37] The predicted determination coefficient (Pred R^2) is 0.9558. This value indicates the model's tremendous significance and gives a significant agreement between the observed and P's predicted values (Fig. 2a).

Table 4. Analysis of variance (ANOVA) for Response Surface Quadratic model

	Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Methane Yield Potential (mL/ g VS_{fed})	Model	5.290E+005	11	48089.53	213.89	< 0.0001	significant
	A-pH	76257.44	1	76257.44	339.17	< 0.0001	
	C-Ratio CM/WAS-OMW	3234.48	1	3234.48	14.39	0.0043	
	D-TS	28570.06	1	28570.06	127.07	< 0.0001	
	AB	38501.31	1	38501.31	171.24	< 0.0001	
	AC	29730.19	1	29730.19	132.23	< 0.0001	
	BC	17013.67	1	17013.67	75.67	< 0.0001	
	CD	18505.99	1	18505.99	82.31	< 0.0001	
	A^2	14225.42	1	14225.42	63.27	< 0.0001	
	B^2	1.870E+005	1	1.870E+005	831.73	< 0.0001	
	C^2	1.943E+005	1	1.943E+005	864.34	< 0.0001	
	D^2	96640.13	1	96640.13	429.82	< 0.0001	
	Residual	2023.54	9	224.84			
	Lack of Fit	1770.84	5	354.17	5.61	0.0599	not significant
	Pure Error	252.70	4	63.18			
	Cor Total	5.310E+005	20				
	Adj R^2=0.9915Pred R^2 = 0.9558; Adequate precision (AP)= 40.506; Coefficient of variation (CV) = 3.68%						
COD_R (%)	**Source**	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
	Model	1262.28	12	105.19	130.05	< 0.0001	significant
	A-pH	18.00	1	18.00	22.25	0.0015	
	B-COD/N	77.88	1	77.88	96.28	< 0.0001	
	D-TS	18.00	1	18.00	22.25	0.0015	
	AB	5.90	1	5.90	7.30	0.0270	
	AC	29.03	1	29.03	35.89	0.0003	
	AD	61.31	1	61.31	75.80	< 0.0001	
	BD	6.97	1	6.97	8.62	0.0188	
	CD	4.50	1	4.50	5.56	0.0461	
	A^2	473.70	1	473.70	585.64	< 0.0001	
	B^2	473.70	1	473.70	585.64	< 0.0001	
	C^2	402.96	1	402.96	498.18	< 0.0001	
	D^2	473.70	1	473.70	585.64	< 0.0001	
	Residual	6.47	8	0.81			
	Lack of Fit	2.83	4	0.71	0.78	0.5935	not significant
	Pure Error	3.64	4	0.91			
	Cor Total	1268.75	20				
Adj R^2=0.9872; Pred R^2= 0.8504; Adequate precision (AP)= 33.376; Coefficient of variation (CV) = 1.92%							

CM / WAS-OMW: ratio of mixture of Cattle Manure/waste activated sludge- Olive Mill Wastewater
COD / N: *ratio of total chemical oxygen demand / total nitrogen*
TS: Total solids

In the second response, ANOVA of the regression model for COD_R confirmed that the F-value "130.05" indicates the model's significance. In this case, there is a 0.01 % chance that F-Value" is due to noise. Then, the values of Prob > F less than 0.05 suggest a significant model term.[34,35] Subsequently, A, B, D, AB, AC, AD, BD, CD, A^2, B^2, C^2, D^2 make significant model terms. When the Lack of Fit F-value is 0.78, it implies the Lack of Fit insignificance with a chance percentage of 59.35 % for Lack of Fit F-value could occur at random. Based on the adjusted correlation coefficient (*Adj* R^2 =0.9872) and the predicted determination coefficient (Pred R^2= 0.8504), the observed and the predicted values of COD_R (Fig. 2b) approve an excellent agreement and advocates greater significance of the model. According to Niladevi *et al.*, to reassures the model best fit, the R^2 must be in the range of 0.75–1.0.[38]

Adequate precision (AP) compared the predicted values' series at the design points to the average prediction error. The two model's ratios of (40.50 and 33.376) were superior to 4, which advocated adequate signals.[17] Simultaneously, a small coefficient of variation (CV) (3.68 % and 1.92 %) is a good precision and experiments reliability indicator.[34]

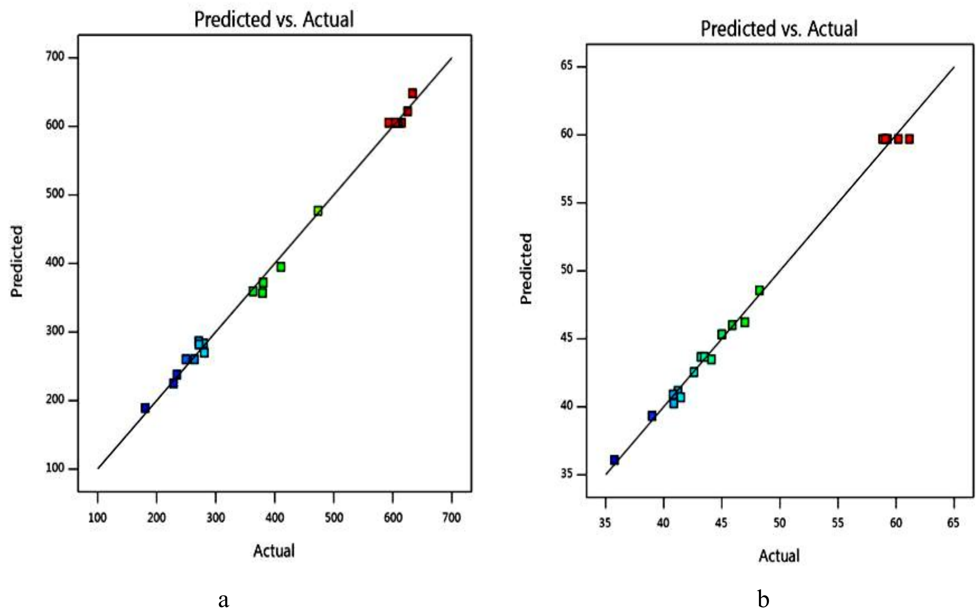


Fig. 2. Scatter plot of predicted value *vs.* observed value, (a) methane yield potential ($PmLCH_4.g^{-1}VS_{lod}$), (b) COD reduction (COD_R%)

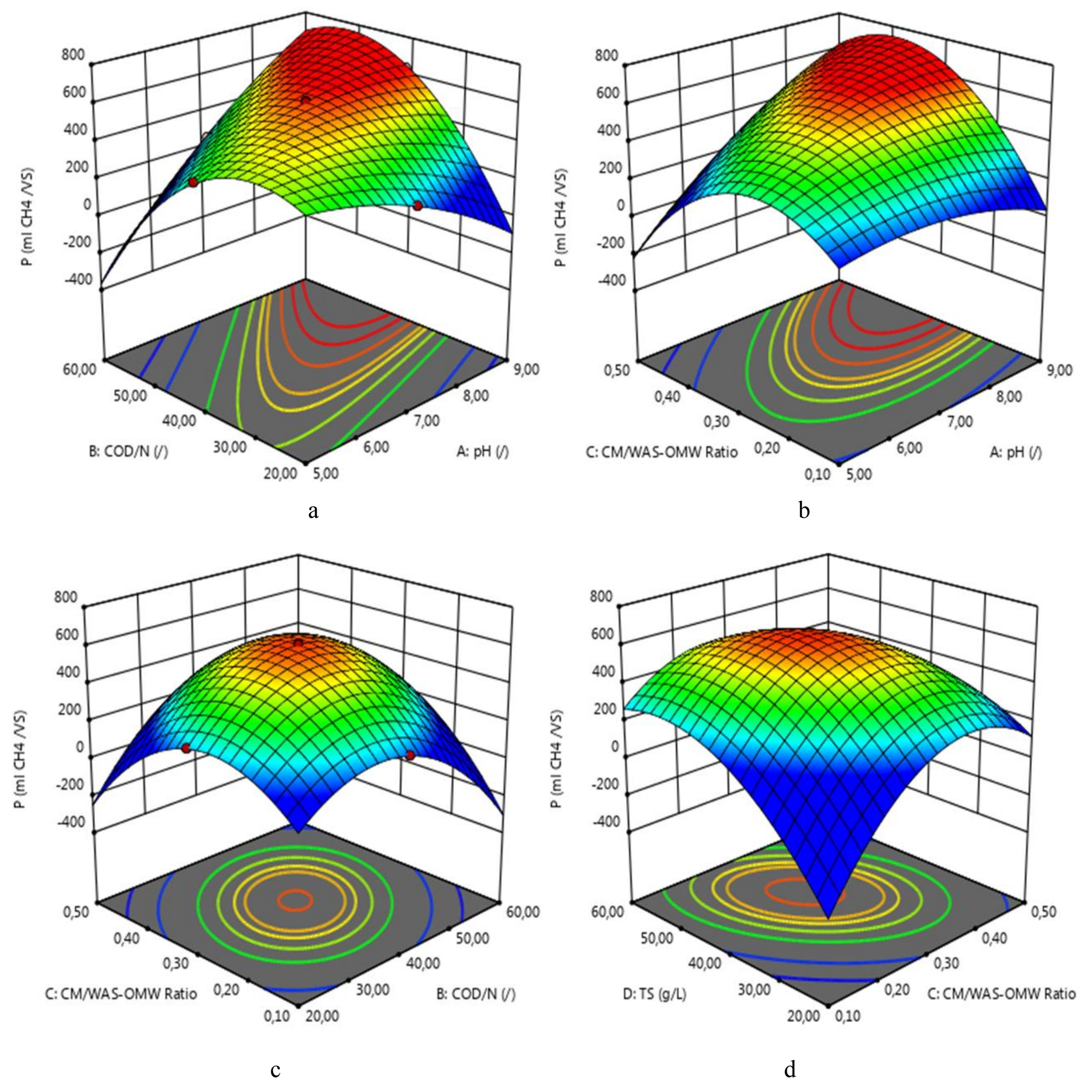
3.2. Effect of factors on the response analysis

The 3D surface plots were used to comprehend the process factor interaction effects that are compulsory for maximum P and COD_R. Response surface curves for variation in P and COD_R were created. In every set, two factors varied within their tested range, while the other two parameters are set at their middle (0) levels.

3.2.1. Methane yield (P)

The terms in equation (2) display that interactions between factors significantly impact the P. Therefore, instead of examining a single variable, the interaction will be investigated in a complete optimisation study. Fig. 3a shows the effects of initial pH and the COD/N ratio on the P (mL CH_4 / g $_{VSlod}$). An initial increased pH and COD/N ratio gives an increase in P. But, the increase in one factor only provides a lower result. The Fig. 3b data displays a rounded contour range running obliquely on the plot, indicating that initial pH and CM/ WAS-OMW Ratio were slightly interdependent or had a considerable interactive effect. Equally, it shows that P augmented by increase CM/ WAS-OMW ratio and initial pH from 0.1 to 0.35 and 5 to 8, respectively, and after these ranges, P decreased. Response surface plot for interaction between CM/ WAS-OMW and COD/N ratios on the P (mL CH_4/ g VS_{lod}) was presented in Fig. 3c. The P augmented with an increase in the CM/ WAS-OMW ratios and COD/N from 0.1 to 0.35 and 20 to 48, respectively. However, P decreased after these ratios ranges. The results presented in Fig. 3d display an elliptical nature and a clear contour range elongated successively and diagonally on the plot. It indicates a significant interactive effect on P between the two independent variables, CM/ WAS-OMW ratio and TS.

Equally, as seen in Fig. 3e and (Eq 2), it shows that initial pH affects it in a steeper slope direction until pH=8; after this value, the opposite effect is next; this is due to the greater positive linear effect and the smaller negative quadratic effect. Contrary it was apparent that CM/ WAS-OMW, COD/N ratios, and TS had a positive linear impact less than their negative quadratic effect on overall methane yield potential (Y_P) (Eq 2). Here positive effect reveals that the corresponding response (Y_P) increases as the effect factor level increases. However, the negative effect means that the matching response (Y_P) diminishes as the level increases.



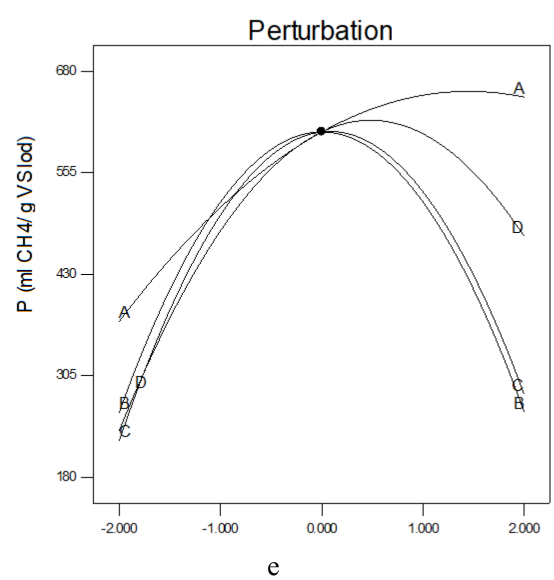


Fig. 3. Design-expert response surface plot for methane yield potential: (a) Interaction between initial pH and COD/N ratio, on the P; (b) Interaction between initial pH and CM/ WAS-OMW ratio on the P; (c) Interaction between CM/ WAS-OMW ratio and COD/N ratio on the P; (d) Interaction between CM/ WAS-OMW Ratio and TS on the P; (e) Perturbation graph

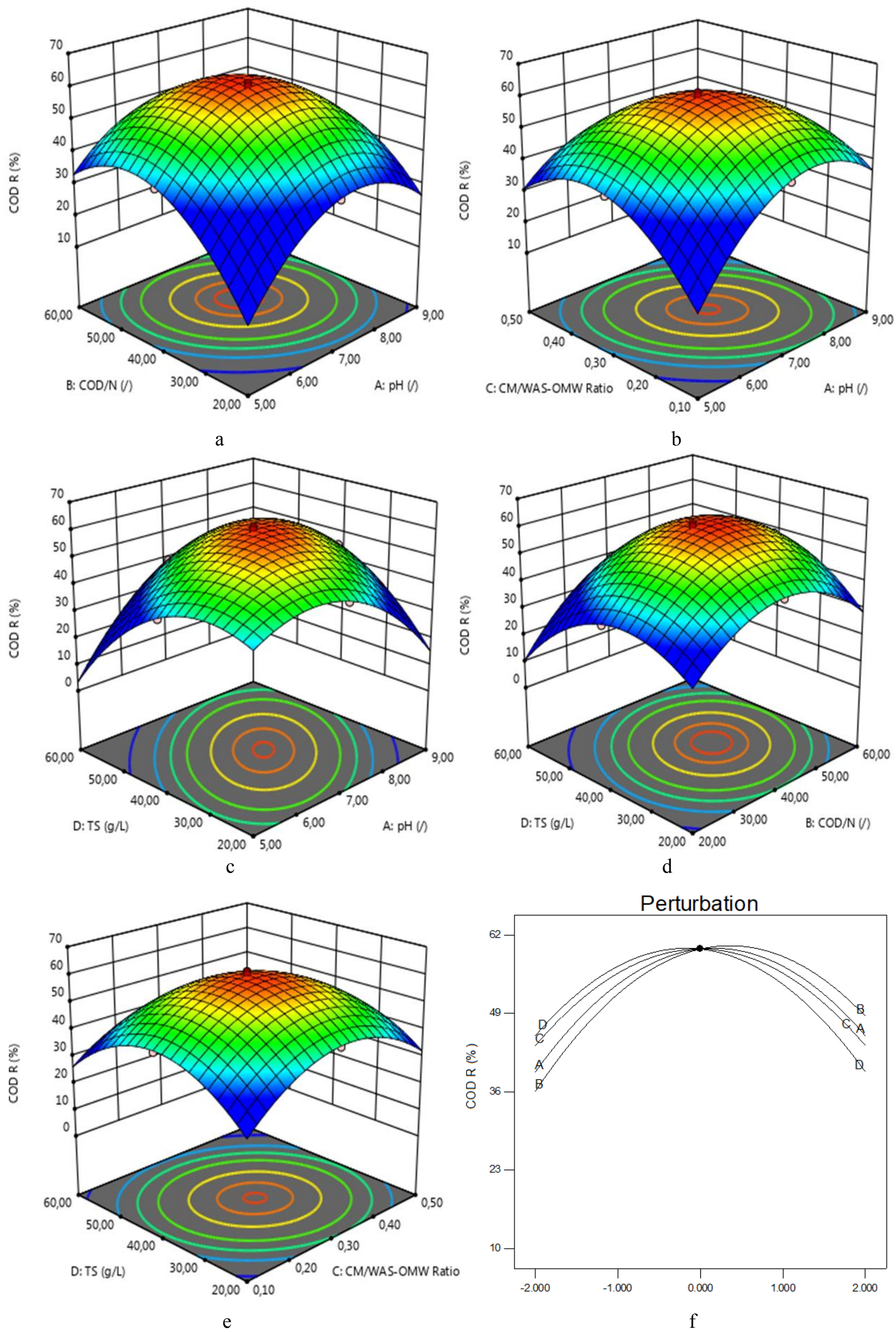


Fig. 4. Design-expert plot; response surface plot for COD_R: (a) Interaction between initial pH and COD/N ratio on the COD_R; (b) Interaction between initial pH and CM/WAS-OMW ratio on the COD_R; (c) Interaction between initial pH and TS on the COD_R; (d) Interaction between initial TS and COD/N ratio on the COD_R; (e) Interaction between CM/WAS-OMW ratio and TS on the COD_R (%); (f) Perturbation graph

3.2.2. Chemical oxygen demand reduction (COD_R)

It can be noted that there are opposite effects of D, AB, AC, CD, A^2, B^2, C^2, and D^2 on Y_{CODR} (Eq 3). Then the initial pH gives the bigger positive linear effect. The interactions were showed in Fig. 4a, b, c, d and e. The three-dimensional surface plots in all figures are nearly symmetrical in profile with circular contours. The COD_R (%) responses plot shows a clear peak, which describes that the optimum settings for the maximum response value are delimited by all design factors inside the design margin. The decline in these response efficiencies is observed when moving away from this point, this indicating that neither increase nor decrease in any tested variables is desired. The perturbation plot (Fig. 4f) illustrates the effect of all the factors at the center point in the design space. The results enabled identifying the COD_R maximum point as a function of these four factors (A, B, C, and D) involved.

3.3. Validation of the experimental model under optimized settings

A Numerical optimization (Fig. 5) was generated by encapsulating the possible response values range in the factor space and the limits that fit the optimization parameters. The optimum values of the tested factors in actual were as follows: the initial pH = 8, the COD/N ratio = = 47, 42, the CM/WAS-OMW ratio = 0.352, the TS = 42.94 $g \cdot L^{-1}$. While the responses predicted were P = 713.96 mL CH_4/gVS_{lod}, and COD_R 54.31 %. Verification of the results in these conditions was accomplished by executing the experiments in triplicate. The P average value obtained through the experiment was 713.327 mL CH_4/gVS_{lod} at COD_R average percentage of 54.0667 %. These experimental data were in agreement with the model prediction Table 5.

4. Discussion

Referring to equations 2 and 3, the P and COD_R have linear and quadratic effects of initial pH, COD/N ratio, CM / WAS-OMW ratios, and TS. According to Lay *et al.* the pH is known to influence enzymatic activity. Each enzyme is active, particularly inside a restricted pH area, and gives the maximum activity at an optimal pH.[39] Further, lower P values at pH below 6.5 could be observed, which could be explained by methanogenic bacteria sensitivity and their metabolic destruction.[40,41] The methanogenic bacteria are most efficient at pH 6.5–8;[42] this pH range is comparable to our obtained one. When adding an alkali agent (NaOH) to WAS, COD solubilization intensifies.[43] Some reactions such as saponification of uronic acids and acetyl esters, reactions happening with free carboxylic groups and neutralization of various acids formed from particular substrates' metabolism.[44] Then, alkali pretreatment is benefiting for OMW digestion because it presents a variable quantity of lipids compounds.[45,46]

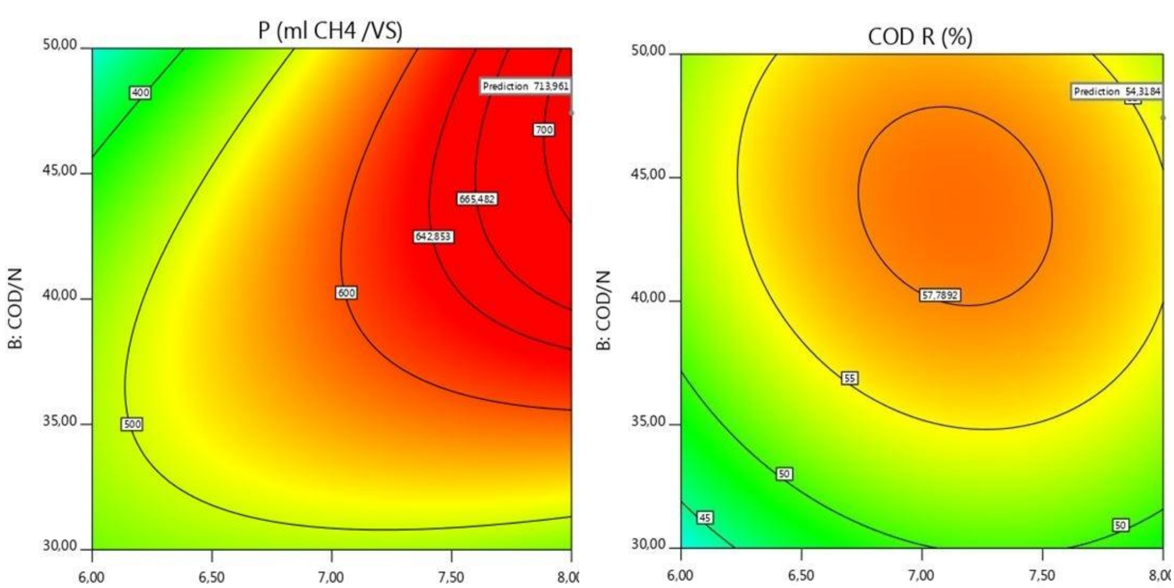


Fig. 5. Numerical optimization of process

Table 5. Confirmation (Lower Bound Confidence = 95 %)

Solution 25 of 55 Response	Predicted Mean	Predicted Median	Std Dev	n	SE Pred	95% PI low	Data Mean
P*	713.961	713.961	14.9946	3	13.027	690.081	713.327
COD R*	54.3184	54.3184	0.899371	3	0.896425	52.6515	54.0667

Further, the saponification consists of the reaction between lipid and an alkali, giving a glycerol release and Long-Chain Fatty Acid salts (soluble soap), improving the contact between the substrate and microorganisms.[47] CM has founded an important substrate for methane production. Besides, it enhanced the buffer capacity of the medium.[48] According to Mao *et al*., alkali treatment of the organic polymer compound of CM destroys the bonds between lignin and polysaccharides, making the ligno-celluloses ideal maters for hydrolysis and saponification reactions. Therefore, the specific surface area of substrate compounds augments, and it became merely accessible to anaerobic microbes involved in biogas production.[49] Zhang *et al*. reported that higher Volatile Fatty Acid concentrations were obtained at 328 K, pH 8 in waste activated sludge alkaline fermentation.[50]

The peak profile in the Perturbation graph in Fig. 4(f) shows that the CODR (%) responses describe that the optimum settings for the maximum response value are delimited at the center point. It illustrates the decline in these response efficiencies is observed when moving away from this point. The carbon degraded is converted to CH_4, and CO_2, then the Methane yield (P) responses give a positive correlation with COD reduction; the difference is in the impact of pH factor in the tow response CODR (%) and Methane yield (P). The Methane yield curve with pH impact is not in the form of a clear peak in the center of design Fig. 3(e); the peak is at the value pH=8. There is a decrease at pH>8. This alkali medium affects the acetogenic bacteria and acetoclastic methanogens.[51] So, it proves that the CH_4 yielded is resulted from hydrogenotrophic basophilic methanogens action. According to Wormald *et al.*[52] and Xu *et al.*,[53] hyd-rogenotrophic methanogenesis is dominant under alkaline conditions. Then Jin and Kirk,[54] suggest that the production of methane through the acetoclastic pathway, although energetically possible with respect to bicarbonate, does not proceed under alkaline conditions (>pH 9.0). This is explained by the dissociation of acetic acid to its anion (CH_3COO^-) under high pH conditions and then pre-venting transmembrane diffusion.[51] But in acid conditions, all groups of the methanogenic have metabolic dest-ruction.[40,41]

Our result of biomethane yields (P), in optimal conditions, is comparable to that reported by Carrère *et al.*[55] whose studied the co-digestion of waste activated sludge and fatty residues with alkaline pretreatments by determining the methane potential (P) of WAS increase from 190 mL $CH_4.g^{-1}$ to 700 mL $CH_4.g^{-1}$ at a thermophilic condition (353 K) and the pH=8.

The AD is extremely sensitive to the COD/N ratio.[49] The obtained results show clearly that our res-ponses (P, COD_R) moderately varied with the COD/N ratio (Figs. 3a, c; 4a, d), which can be mainly due to the selective effect of bacterial communities. Li *et al.*,[56] in their study of the AD system of cattle and/or swine manure by metagenomics assays, noted that the substrate type, the ratio of co-substrate, play major roles in -N ratio of substrate and free ammonia, which play a central factor in the development and structuring of the bacterial communities in AD systems. According to the particular waste's physicochemical characteristics (Table 1), it is easily verified that OMW and WAS alone lack nitrogen deficiencies. The mixing of WAS and OMW with CM ensures a sufficient amount of nitrogen and important ruminal microorganisms adapted to destroy polyphenolic compounds.[57,58] This polyphenolic compound is consi-dered toxic in the AD system of OMW.[46,59] The resulted optimal COD/N ratio is in agreement with that obtained by different authors, but in some cases, our result is lower than the values listed in the other research papers. For example, Zhang *et al.*[60] added supplementary nutrients nitrogen (NH_4Cl) to generate a COD/N ratio of 250 : 5 (~50) in the digestion of palm oil mill effluent. This range is comparable to our best COD/N ratio (47, 42). As well in keeping with Khoufi *et al.*,[61] a COD/N ratio of 50.7 is required for a balanced carbon to nitrogen feed in the co-digestion of olive mill wastewater and liquid poultry manure.

However, in the strategies of Gonçalves *et al.*,[62] to convert OMW efficiently to methane, the lipids and phe-nolics degradation required an additional nitrogen source (NH_4Cl) to obtain a COD/N ratio of 100/1 and to increase methane yield from 18 % to 76 % and COD removal effi-ciencies from 81 to 87 %. This COD/N ratio is higher than our obtained result. Eventually, this difference in COD/N range was influenced by the operating conditions and waste nature. A combined anaerobic co-digestion of OMW effluent with swine manure showed that a COD : N ratio in the range of 65 : 1 to 126 : 1 was necessary for the optimal degradation process.[45]

Concerning the influence of TS on P and COD_R appears clearly with a peak in the interactions' results (Figs. 3c; 4c, d, e) and an antagonistic effect on the COD_R (Eq. 3). Abbassi-Guendouz *et al.*[63] have highlighted in their research that high TS amount affects AD performance considerably via decrease of the microbial hydrolysis capacity through physical limitation associated with the liquid/gas mass transfer.

5. Conclusions

The co-digestion process of WAS with OMW and CM was studied by a Central composite Design of experiments. Factors such as initial pH, COD/N ratio, CM/WAS-OMW ratio, and TS have strongly affected the methane yield and chemical oxygen demand reduction. OMW and CM's addition to the WAS digestion induced an increase in COD_R and methane yield. The obtained results, in the optimum conditions, showed a good agreement between experimental and model predictions. If the limit of factors such as pH, COD/N ratio, and TS was not adequate with the substrate composition (CM/WAS-OMW), the synergistic effect of microbial community could not be avoided inhibitory effects associated with the accumulation of some product from the enzymatic pathways.

Ethical approval

This article does not contain any investigations with human participants or animals performed by any of the authors.

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РОЗРОБЛЕННЯ СТАТИСТИЧНОЇ МОДЕЛІ ДЛЯ ПРОГНОЗУВАННЯ ВИРОБНИЦТВА МЕТАНУ З ВІДХОДІВ АКТИВНОГО МУЛУ ЧЕРЕЗ СПІЛЬНЕ БРОДІННЯ ЗІ СТІЧНИМИ ВОДАМИ ВИРОБНИЦТВА ОЛИВКОВОЇ ОЛІЇ ТА ГНОЄМ ВЕЛИКОЇ РОГАТОЇ ХУДОБИ ЗА ДОПОМОГОЮ МЕТОДОЛОГІЇ ПОВЕРХНІ ВІДГУКУ

Анотація. *У наш час зростання кількості населення, призводить до утворення великої кількості відходів біомаси внаслідок різноманітної людської, промислової та сільськогосподарської діяльності. Для регулювання відходів біомаси та пом'якшення величезного спектра шкоди навколишньому середовищу застосовують переважно анаеробне бродіння. Метою цієї статті є підвищення ефективності анаеробного бродіння багатокомпонентних субстратів з використанням суміші відходів активного мулу (ВАМ), стічних вод виробництва оливкової олії (СВВОО) і гною великої рогатої худоби (ГВРХ). У плануванні експерименту використано методологію поверхні відгуку для визначення індивідуального впливу й інтерактивного ефекту на вихід метану та хімічне зниження потреби в кисні. Після числової оптимізації за допомогою Design Expert® оптимальні фактичні значення тестових факторів були такими: початкове pH = 8, співвідношення загальна хімічна потреба в кисні : загальний азот = 47, 42, співвідношення ГВРХ/ВАМ-СВВОО = 0,352, загальний сухий залишок ЗСЗ = 42,94 г/л. Отримані результати вказують, що ефективності анаеробного спільного бродіння можна досягти через оптимізацію складу субстрату для забезпечення більшого мікробного синергічного ефекту.*

Ключові слова: *відходи активного мулу; стічні води виробництва оливкової олії; гній великої рогатої худоби; методологія поверхні відгуку; анаеробне спільне бродіння.*