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ABSTRACT
DOCTORAL THESIS

ASSESING AND OPTIMIZATION OF BIOGAS PRODUCTION FROM AGRO-INDUSTRIAL WASTE

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KEY WORDS:

anaerobic digestion, biogas, biorefining, pretratment, renewable energy, waste recovery

CHAPTER 1. CRITICAL STUDY OF LITERATURE

1.1. INTRODUCTORY CONCEPTS AND RESEARCH PREMISES

Finding solutions for the transition from fossil fuels-based energy to energy produced from renewable fuels, which are known for their minor carbon footprint, has become one of the emerging problems of the century. This is mainly due to depletable reserves of conventional fuels along with increasing emissions of greenhouse gases that lead to extreme climatic phenomena (Kapoor et al., 2019).

There is a growing worldwide interest in using biomass residues as raw material for green energy production and for obtaining value-added products (Casoni et al., 2018). Many of the waste materials have no utility and must be disposed in such a way that they do not induce environmental pollution or endanger the public health (Katsuyama, 1979). At the same time, the uncontrolled discharge of organic waste exerts increasing pressure on the environment, being an important source of pollution that accentuates global warming (Li et al., 2018). Organic waste are responsible for countless health and environmental risks, as many dangerous contaminants are spread in the air, soil and water, while nutrients such as nitrates and phosphates cause water eutrophication (Mateescu and Constantinescu, 2010). Consequently, sustainable waste management strategies are effective approaches for facing current environmental challenges. Recovery of waste to electricity offers new possibilities to the energy market, although it may raise major problems of competitiveness between producers and add pressure on increasing the efficiency of existing power plants.

Among the currently used valorization strategies for waste, anaerobic digestion (AD) is widely applied for the conversion of organic waste into biogas, yielding economic value in large-scale applications (Achinas and Euverink, 2019). The present paper addresses various aspects of AD technology which represents an advantageous alternative for the controlled disposal of organic waste and also a solution for the production of renewable energy which may be used for power supply, heating and fuel gas, while providing important economic and environmental benefits (Angelidaki et al., 2018; Scarlat et al., 2018).

1.2. STATE OF THE ART IN ANAEROBIC DIGESTION PROCESS

1.2.1. Significance of anaerobic digestion

Anaerobic digestion is an effective biochemical conversion method for biodegradable organic materials. The degradation process takes place in the absence of air and with the presence of specific microorganisms and leads to obtaining biogas, liquid digestate and solid digestate as fermentation products. Due to its multiple advantages, AD is widely used to treat and recover energy from several types of biomass-based raw materials, such as energy crops, wood residues, grass and other plants or weeds, agricultural and forestry residues, the organic part of municipal or industrial waste etc. (Cioabla et al., 2013).

The bio-conversion of organic matter into energy-rich methane gas is considered both a good practice for reducing environmental pollution and a long-term strategy for finding alternative sources of energy and renewable fuels in pursuit of economic development and energy security (Mateescu and Constantinescu, 2010; Cogan and Antizar-Ladislao, 2016; Chen et al., 2008; Esposito et al., 2012; Franke-Whittle et al., 2014; Pagés Díaz et al., 2011; Siddique and Wahid, 2018).

There are multiple benefits of biogas production, including reduction of conventional fuel imports, provision of heating, gas and electricity to both regular consumers and those from isolated areas and sustaining ecological agriculture, by the fact that the fermented residue generated as a by-product of AD is a valuable natural fertilizer (Tambone et al., 2010). Thus, anaerobic digestion can deliver plenty of economic and ecological benefits, especially in the case of application in industrial energy generation processes, combining production of renewable energy with sustainable treatment of a huge variety of biodegradable waste, coming from municipal waste water treatment facilities, from agriculture or various domestic or industrial processes, etc. (Cioabla et al., 2017; Galvão, 2014; Jørgensen, 2009; Molino et al., 2013). Moreover, AD has proven to be a more suitable method for the disposal of wet organic waste than many other waste treatment techniques that are more energy-intensive, such as incineration or pyrolysis (Dumitrel et al. et al., 2017; Yan et al., 2017).

1.2.2. Substrates for anaerobic digestion

The main resources used for bioenergy production are agricultural crops, agro-industrial wastes and by-products, lignocellulosic products, animal manure, algae and other aquatic plants or organically loaded waste water, such as municipal sewage sludge or waste water from farming, etc. (Sayara and Sánchez, 2019). Agro-industrial residues represent one of the most advantageous raw materials for AD, showing multiple benefits (Bharathiraja et al., 2016).

1.2.3. Biochemistry of anaerobic digestion

AD is a complex biochemical process in which biochemical degradation reactions of organic matter and product formation take place successively and simultaneously. Conventionally, there are four key biochemical stages of AD process, as shown in Figure 1.1.

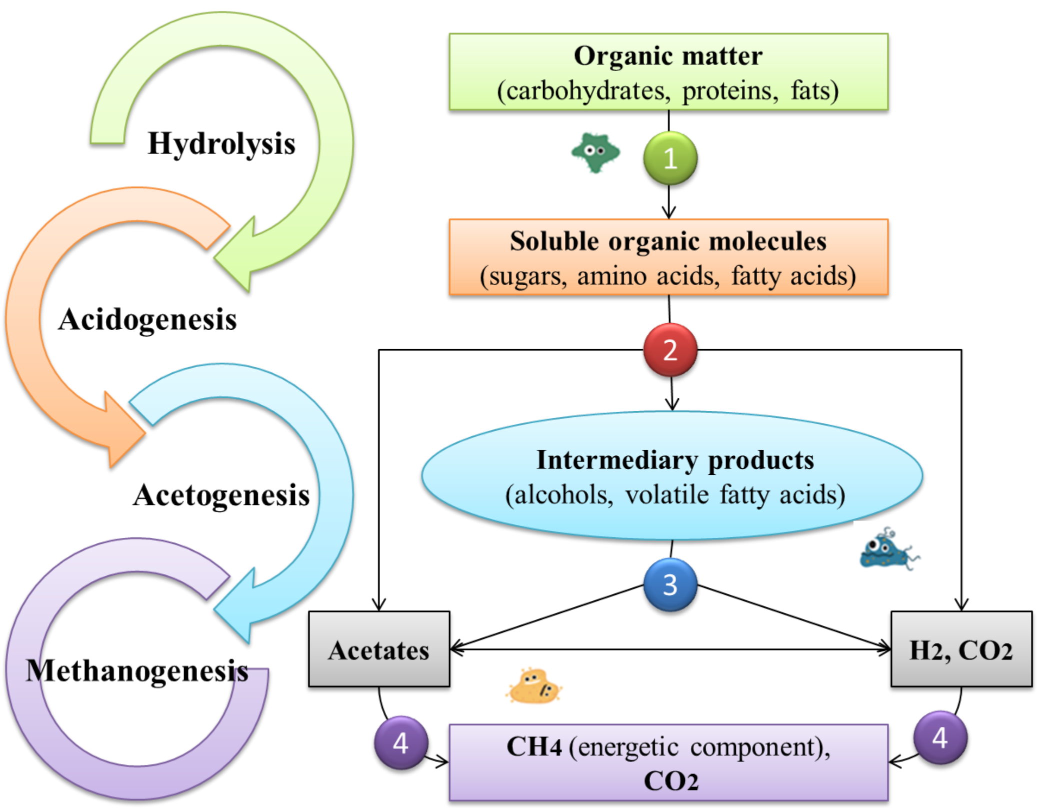

Figure 1.1. Simplified scheme of biochemical steps in anaerobic digestion process (adapted from Dussadee et al., 2016; Angelidaki et al., 2002)

1.2.4. Anaerobic digestion products - biogas, liquid digestate and solid digestate

Following the AD process, biogas and digestate are the main degradation final products. The graphical representation of AD products and their uses is shown in Figure 1.2.

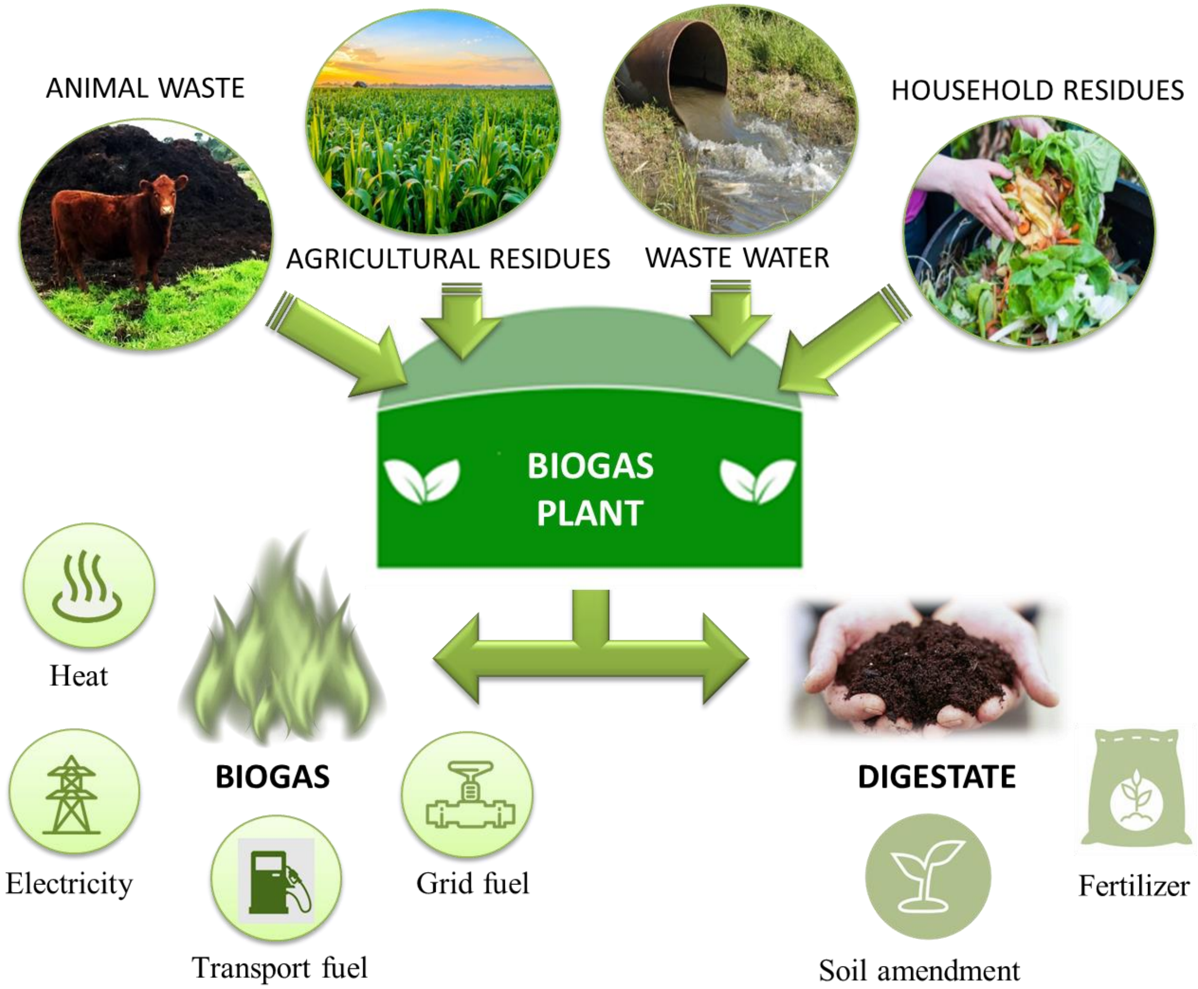

Figure 1.2. Main substrates used in AD processes, digestion products and their uses (adaptated from EESI, 2017)

1.2.5. Factors affecting anaerobic digestion

The stability of the AD process depends mostly on the equilibria established between microbial consortia in the digester. More specifically, for a better outcome of the process, it is essential to ensure optimal life conditions for all fermentative microorganisms, of which methanogens have the strictest environmental requirements.

The progress of AD processes is higly influenced by the environmental factors, substrate characteristics and operational parameters which all affect biogas production and its quality.

1.2.5.1. Factors that are characteristic to the digestion substrate/mixture

1.2.5.1.1. pH level
1.2.5.1.2. Alcalinity
1.2.5.1.3. Redox potential
1.2.5.1.4. Volatile fatty acids
1.2.5.1.5. Ammonia and ammonium
1.2.5.1.6. C/N ratio and nutrients
1.2.5.1.7. Other inhibition thresholds

1.2.5.2. Independent parameters (factors) of the process

Both technical and economic aspects should be considered when developing and operating the biogas plant. The choice of system design (size and type of digester) or operational parameters are always based on a compromise between obtaining the highest biogas yield and a justified economy. The influence of the most important process factors on AD is further discussed.

1.2.5.2.1. Temperature
1.2.5.2.2. Organic loading and hydraulic retention time
1.2.5.2.3. Total solids content in fermenter
1.2.5.2.4. Mixing
1.2.5.2.5. Particle size

1.2.6. Strategies for increasing biomethane production

Although biogas technology is already a mature technology, the efficiency of anaerobic conversion processes of organic materials for biogas production requires several improvements to increase the profitability of the investment.

1.2.6.1. Anaerobic co-digestion

Anaerobic co-digestion (AcoD) of materials with complementary characteristics can be a very effective option to improve the performance of the process. AcoD usually aims at balancing the C/N ratio, pH, solids, micro- and macronutrient content, as well as improving buffering capacity, diluting inhibitory/toxic compounds in the bioreactor and expanding the range of microorganisms involved in the process. Co-digestion can ensure the optimization of the AD process, improving the biotransformation efficiency of the complex organic substrate into biomethane (Maile et al., 2016; Wang et al., 2012).

1.2.6.2. Substrate pretreatment

Increasing the competitiveness of biogas installations in the biofuels sector by using substrate pretreatment has gained increasing interest in the scientific literature. Research in this field is mainly oriented towards the development of efficient methods for breaking down the recalcitrant biopolymers chains inside the structure of raw materials.

1.2.6.3. Mathematical modelling of (co-)digestion process

Mathematical modeling is an effective tool for designing, controlling, optimizing and predicting the performance of unit operations and chemical/biochemical processes (Batstone et al., 2002; Dima et al., 2019; Dobre et al., 2016; Nguyen et al., 2019; Stoica et al., 2015; Zahan et al., 2018).

1.3. CLASSIFICATION OF INSTALLATIONS USED FOR BIOGAS PRODUCTION

1.3.1. Household, agricultural and industrial installations

In developed contries, large and medium-sized biogas plants can be generally seen, which use advanced fermentation technologies to obtain biomethane and other value-added products, many of them being coupled with purification units. In the less developed regions, however, small and medium installations are mainly used for the production of biogas (EU, 2017; Martinov et al., 2020).

1.3.2. Laboratory installations for the determination of methane potential

Laboratory AD installations are used to determine the biomethane potential (BMP), aiming for the study of anaerobic digestion processes, determination of the energy value of different substrates or process optimization.

CHAPTER 2. CASE STUDY - THE BIOGAS SECTOR IN ROMANIA

2.1. OVERVIEW OF THE BIOGAS SECTOR IN ROMANIA

Romania is one of the European countries balanced in terms of availability of primary and renewable energy resources. The purpose of the case study was to identify the major factors that have hindered the development of the biogas sector and which have slowed down the implementation of biogas projects by economic agents and/or local authorities in Romania. Several possible approaches for overcoming the identified obstacles have been discussed in order that the environment and the national economy gain from the energy recovery of bioresources.

2.2. CHALLENGES OF THE BIOGAS SECTOR IN ROMANIA

The most important factors that have influenced the development of the biogas industry in Romania:

2.2.1. Legislative obstacles

2.2.2. Financial obstacles

2.2.3. Research

2.2.4. Collaboration

2.2.5. Support offered by the administration

2.2.6. Knowledge in the biogas field

2.3. SOME RECOMMENDATION FOR THE DEVELOPMENT OF THE BIOGAS SECTOR IN ROMANIA

In Romania, development of an adequate and stable regulatory framework to support investments in biogas projects is a solution that must swiftly be adopted at a government level (EC, 2017).

Figure 2.2. presents the some recommendations for the decision-makers in Romania that could lead to the growth of the biogas sector.

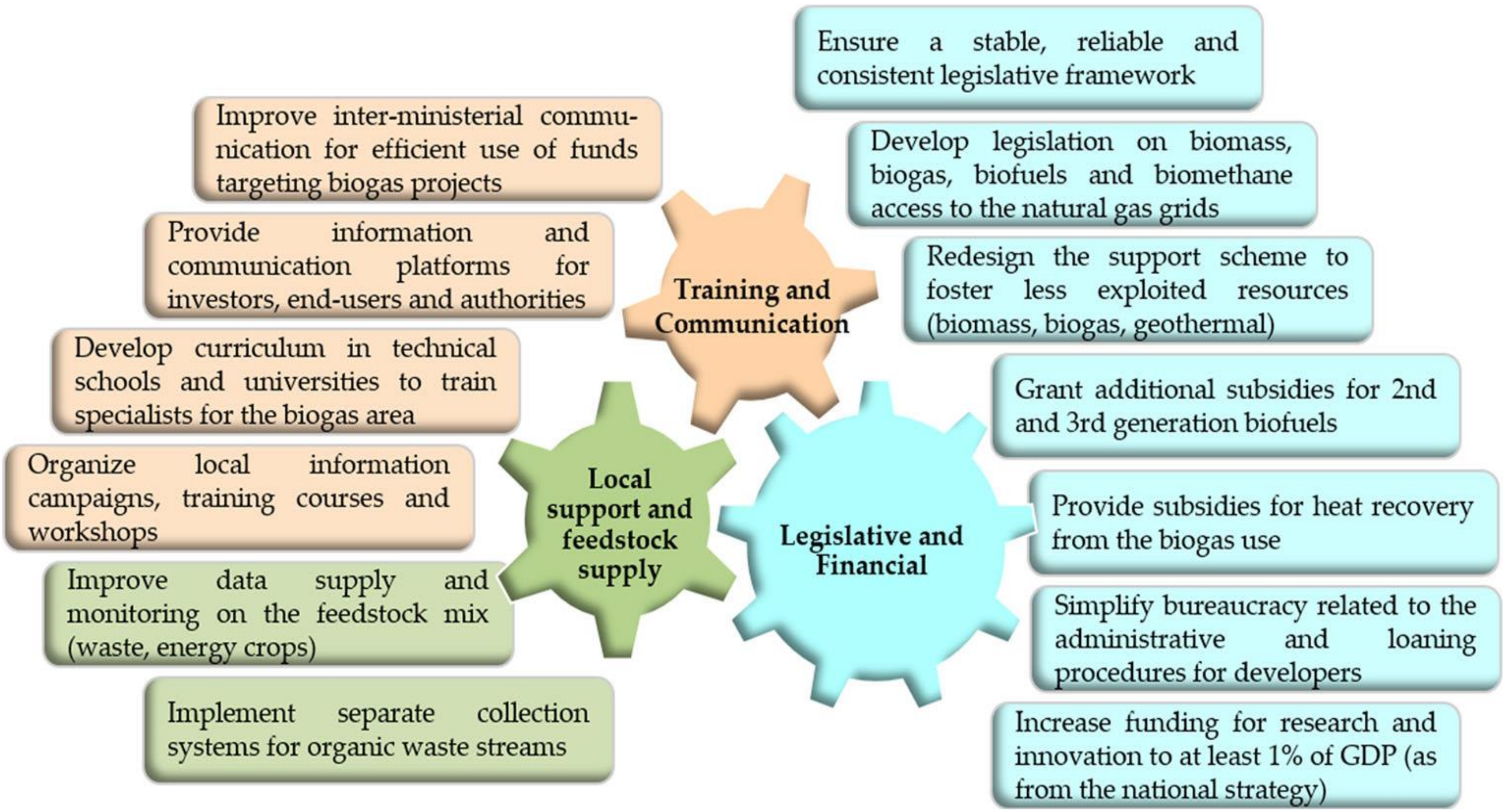

Figure 2.2. The most important measures that could be implemented for the development of the biogas sector in Romania (Mateescu and Dima, 2020)

CHAPTER 3. ORIGINAL CONTRIBUTIONS

3.1. SOME REMARKS REGARDING THE COURSE OF THE EXPERIMENTAL RESEARCH

The experimental activity was focused on the valorization of local residual matter for biogas production, consisting mostly of agricultural and farm residues. Thus, the biogas production of different biomass mixtures, composed of potato waste (PW), sugar beet root waste (BW), cow dung (CD), poultry manure (PM), corn silage (CS) and sunflower seed cake (SSC) was studied. Moreover, possible strategies to increase fuel gas production were investigated, *e.g.*, innovative techniques for substrate pretreatment or biostimulators addition, and the use of mathematical modeling for the optimization of fermentation mixtures.

3.2. SUBSTRATES FOR ANAEROBIC DIGESTION. GENERAL DESCRIPTION

Several types of raw materials and materials were used in the experimental study:

3.2.1. Potato waste (PW)

3.2.2. Sugar beet root waste (BW)

3.2.3. Cow dung (CD) and poultry manure (PM)

3.2.4. Corn silage (CS)

3.2.5. Sunflower seed cake (SSC)

3.2.6. Microalgae

3.2.7. Inoculum

3.3. ANALYSIS METHODS

3.3.1. Determination of total solids (*TS*) and volatile solids content (*VS*)

3.3.2. Determination of carbon content (% *C*)

3.3.3. Determination of nitrogen content (% *N*)

3.3.4. Determination of pH

3.3.5. Simultaneous determination of C, N, S, O, N content

3.3.6. Determination of the biogas volume

3.3.7. Determination of methane concentration in biogas

3.3.8. Laboratory installations for BMP determination and working procedure

3.3.8.1. Laboratory installation for BMP tests using a climatic chamber (I-EXP-1)

Anaerobic digestion tests for the first experimental study were carried out with the I-EXP-1 installation using a climatic chamber.

3.3.8.2. Laboratory installation for BMP tests using a water bath (I-EXP-2)

The laboratory facility for BMP tests using a water bath (I-EXP-2) was used in the experimental activities no. 2-5. The experimental assembly is shown in Figure 3.7.

Figure 3.7. Experimental installation for conducting BMP tests: (1) thermostatic water bath; (2) dark glass fermentation bottle; (3) Teflon connector tube; (4) multi-layer gas bag; (5) hanging system (Dima et al., 2020)

3.4. Recovery of Potato Processing Residuals by Anaerobic Digestion and the influence of adding microalgal extracts of *Chlorella* sp. and *Spirulina* sp. to the substrate (EXP-1)

3.4.1. Objectives

Assessing the experimental biomethane production of potato processing waste, by using batch anaerobic digestion tests was the main objective of the experiment. Also, the determination of the theoretical biomethane potential and biodegradability of the substrate were investigated. At the same time, co-digestion of potato waste and microalgal extracts of *Chlorella* sp. and *Spirulina* sp. and their effect on the biogas production were evaluated.

3.4.2. Procedures

3.4.2.1. Preparation of the fermentation mixture and the BMP tests

Potato residuals *(PW)*, consisting of 70% peel and 30% pulp, were used as digestion substrates in this experiment. The total duration of the fermentation tests was 21 days, which was observed to be sufficient for adequate anaerobic digestion of the substrate.

3.4.2.2. Theoretical biochemical methane potential (TBMP)

Theoretical biochemical methane potential of the material, *TBMP* (mL/g VS), can be estimated using Equations (3.7) and (3.8) (Achinas și Everink, 2016; Boyle, 1976; Buswell și Muller, 1952; Deublein și Steinhauser, 2008; Feng et al., 2013; Herout et al., 2011; Nguyen et al., 2019).

$$C_aH_bO_cN_dS_e + \left(a - \frac{b}{4} - \frac{c}{2} + \frac{3d}{4} + \frac{e}{2}\right)H_2O \rightarrow \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}\right)CH_4 + \\ + \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4} + \frac{3d}{8} + \frac{e}{4}\right)CO_2 + dNH_3 + eH_2S \tag{3.7}$$

$$TBMP = \frac{1000V_m\left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}\right)}{M_{C_aH_bO_cN_dS_e}} \tag{3.8}$$

3.4.2.3. Kinetic modelling

Cone and modified Gompertz models have been widely used to predict the dynamics of biomethane production (Feng et al., 2013; Nguyen et al., 2019; Hassan et al., 2017; Yu et al., 2019). They are described by Equations (3.11) and (3.12).

$$Y_M(t) = \frac{Y_{M,m}}{1+(kt)^{-n}} \tag{3.11}$$

$$Y_M(t) = Y_{M,m}\exp\left\{-\exp\left[\frac{r_m \exp(1)}{Y_{M,m}}(\lambda - t) + 1\right]\right\} \tag{3.12}$$

3.4.3. Results and discussions

3.4.3.1. Characteristics of potato waste

Physicochemical analysis results of potato waste are summarized in Table 3.1.

3.4.3.2. Theoretical biomethane potential

Subscripts *a*, *b*, *c* și *d* in the chemical formula $C_aH_bO_cN_d$ can be determined based on the elemental analysis – data in Table 3.2.

Table 3.2. Determination of subscripts in the chemical formula $C_aH_bO_cN_d$ based on the elemental analysis

Element *i*	**c_i (%)**	**x_i (g/g)**	**M_i (g/mol)**	**$R_i = x_i/M_i$ (mol/g)**	**R_i/R_N**	
C	42.27	0.423	12.0107	0.03519	*a*	36.51
H	6.22	0.062	1.00784	0.06172	*b*	64.03
O	50.16	0.502	15.999	0.03135	*c*	32.53
N	1.35	0.014	14.0067	0.00096	*d*	1

3.4.3.3. Experimental biogas and biomethane productions

Values of $Y_{M,exp}(t)$ are presented in Figure 3.9. Depicted data reveal that the potato waste sample without the addition of microalgae generated lower cumulative biogas volumes, but significantly higher cumulative methane productions compared to potato samples with the addition of microalgal extracts. Results revealed an inhibitory effect of microalgae for the methanogenesis of potato waste.

3.4.3.4. Methane-based biodegradability

Biodegradability of potato waste, which was estimated using Equation (3.10), depending on $Y_{M,m,exp}$ = *EBMP* = 148.24 mL/g VS and *TBMP* = 383 mL/g VS, was 38.7%, which indicates that the biochemical processes have faced some inhibitory influences.

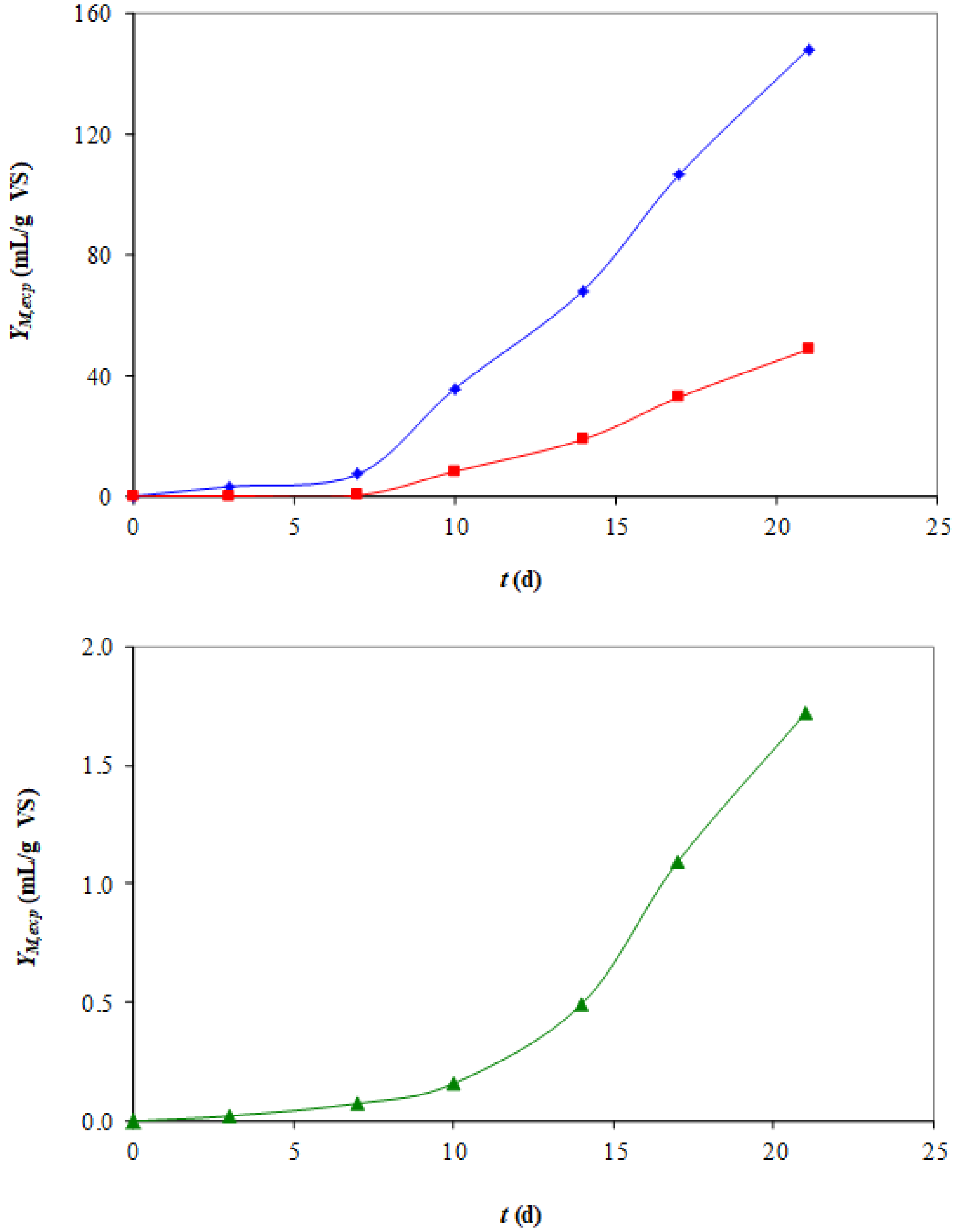

Figura 3.9. Time variation of cumulative biomethane production for potato waste (♦) and PW with extracts of *Chlorella* sp. (■) and *Spirulina* sp. (▲)

3.4.3.5. *Kinetic modeling*

Characteristic parameters of Cone model (Equation (3.11)) and and modified Gompertz model (Equation (3.12)), which were estimated based on experimental data using Solver add-in program (Microsoft Excel) and the values of root mean square error (RMSE) for the substrates subjected to anaerobic digestion are summarized in Table 3.4.

Table 3.4. Characteristic parameters of kinetic models

Model	**Substrate / Parameter**	**PW**	**PW and extract of *Chlorella* sp.**	**PW and extract of *Spirulina* sp.**
Cone	$Y_{M,m}$ (mL/g VS)	277.15	80.03	2.63
	k (d^{-1})	0.050	0.053	0.054
	n	2.98	3.83	4.93
	RMSE (mL/g VS)	2.76	0.90	0.03
Modified Gompertz	$Y_{M,m}$ (mL/g VS)	245.83	83.75	3.28
	r_m (mL/g VS/d)	11.64	4.31	0.18
	λ (d)	7.89	9.48	11.09
	RMSE (mL/g VS)	2.78	0.90	0.04

3.4.4. Conclusions

The present study aimed at assessing the theoretical and experimental biomethane potential of potato processing residuals as well as at studying the effects of Chlorella sp. and Spirulina sp. microalgae extracts on the anaerobic digestion process of potato waste. After 21 days, a cumulative methane production of 148.24 mL/g VS was obtained from potato waste inoculated with manure without microalgal extract, whereas the substrate with *Chlorella* sp. had a cumulative methane yield of 48.65 mL/g VS, and that with *Spirulina* sp. had a very low cumulative methane yield of 1.72 mL/g VS. The inhibitory effect of microalgal extracts on the methane production is probably due to stabilizers in the extract composition as well as to the toxic effect of ammonia generated in excess by the extract rich in nitrogen. The biodegradability of potato waste was 38.7%. Dynamics of cumulative methane production were accurately predicted by Cone and modified Gompertz models. Both kinetic models could be used to design, control, and optimize the anaerobic digestion process.

3.5. Improving the biogas performance of selected waste materials by substrate ratio optimization and microalgae addition (EXP-2)

3.5.1. Objectives

The main objective of the co-degestion experimental study was to determine the optimal composition of a complex mixture, consisting of several types of residual biomass for enhanced biogas production. Also, the influence that addition of 5% microalgal biomass had on the fermentability of the substrate was evaluated.

3.5.2. Materials characterisation and procedures

For this experimental work, several types of agro-industrial residuals were used to prepare the substrates for anaerobic digestion. They were provided by INCDCSZ Brasov and consisted of: beet root waste (BW), potato waste (PW), corn silage (CS), cow dung (CD) and chicken manure (CM). Dry powder of microalgal biomass was supplied by INCDCP-ICECHIM Bucharest.

Chemical substrate composition for each test is given in Table 3.5.

Table 3.5. Chemical substrate composition for the BMP tests

Test no.	**Substrate composition**					**Substrate characteristics**			**Additive**
	BW (%)	PW (%)	CS (%)	CD (%)	CM (%)	*TS* (g/L)	*VS* (g/L)	C/N	Microalgal biomass (%)
1	10	10	30	20	20	99.99	83.72	24.90	-
1A	10	10	30	20	20	99.99	83.72	24.90	5
2	10	20	10	40	20	100.08	81.49	24.31	-
2A	10	20	10	40	20	100.08	81.49	24.31	5
3	10	10	20	30	30	100.13	79.05	22.92	-
3A	10	10	20	30	30	100.13	79.05	22.92	5
4	5	10	20	40	25	99.86	78.99	21.57	-
4A	5	10	20	40	25	99.86	78.99	21.57	5

3.5.3. Results and discussions

Time variation, t (d), of the cumulative experimental biogas volume, $V_{B,exp}$ (mL/g VS), and that of the cumulative experimental biomethane production , $Y_{M,exp}$ (mL/g VS) for the 4 mixtures of substrates prepared for anaerobic co-digestion, representing a total of 8 samples with and without the addition of microalgal biomass are shown in Figures 3.12. and 3.13.

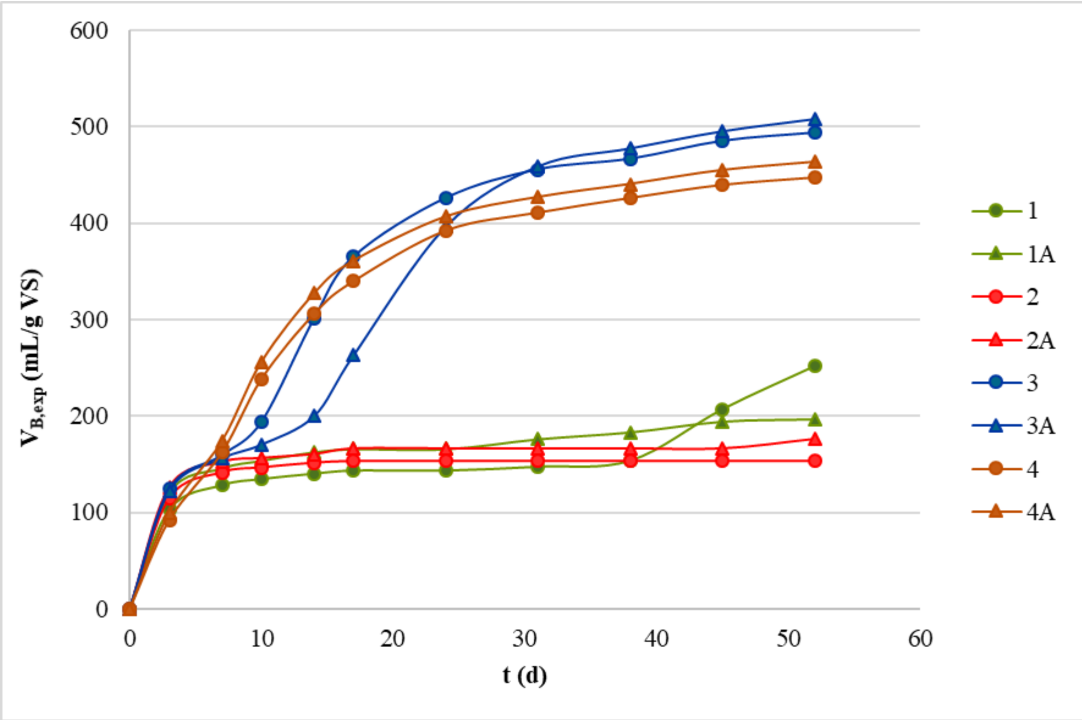

Figure 3.12. The cumulative experimental biogas volume (● mere sample; ▲ additivated sample)

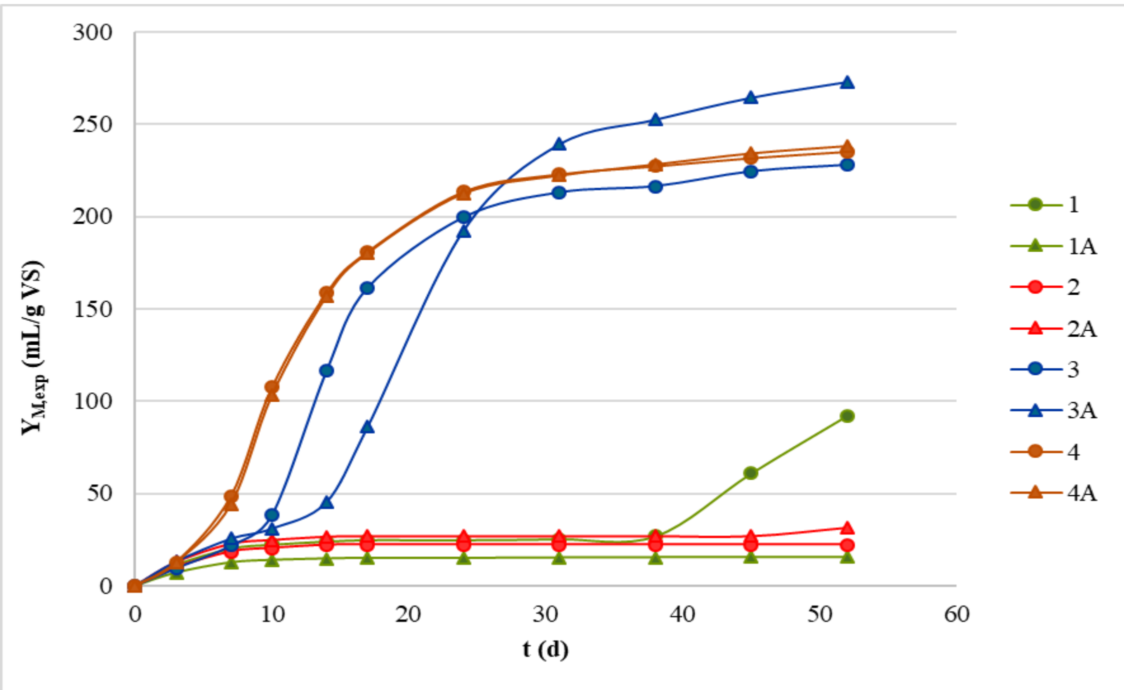

Figure 3.13. The cumulative experimental biomethane production (● mere sample; ▲ additivated sample)

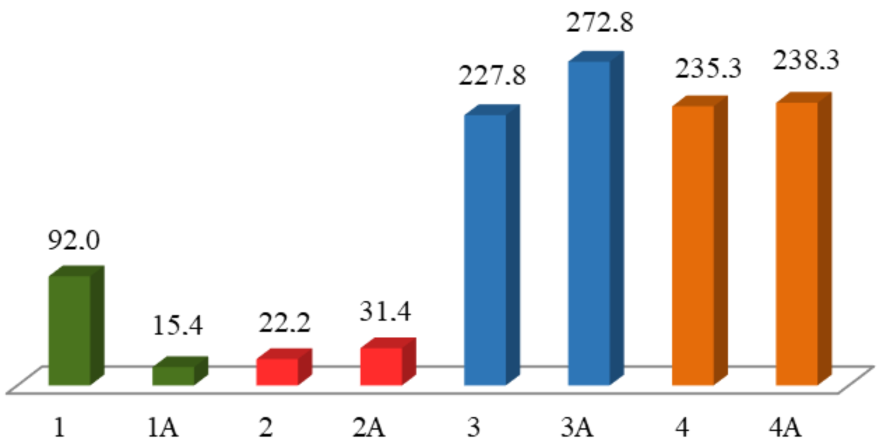

Figure 3.14. Biomethane potential (EBMP) for the test samples (mL CH_4/g VS)

Figure 3.14. shows the biomethane potential (EBMP) of the 8 samples, expressed in mL CH4/gVS, equivalent to the maximum (final) experimental methane production ($Y_{M,m,exp}$).

The highest EBMP value of 272.8 mL CH4/g VS was recorded for sample 3A consisting of BW, PW, CS, CD, CM in the ratios of 1:1:2:3:3, additivated with 5% microalgal biomass, while the lowest BMP of 15.4 mL CH4/gVS was obtained for sample 1A consisting of BW, PW, CS, CD, CM in the ratios of 1:1:3:2:2, additivated with 5% microalgal biomass. It is obvious that the mixture of components and the contribution of micronutrients are essential factors in attaining a favorable fermentation environment, implicitly affecting the degree of biomass to biomethane conversion.

The significant differences noticed between the values of biomethane concentration in biogas for the selected mixtures that were tested in this research indicate that the chemical and microbial interactions play a significant role in the bioreactor and they are highly influenced by the specific composition of the substrate.

3.5.4. Conclusions

The experimental results proved that the ratio between different materials in the digestor is an important factor in creating an optimal nutrient balance to facilitate the activity of methanogens; the biomethane concentration and the biogas volume were strongly influenced by the quality of the digestion substrate, specifically by the organic wastes mixing ratio. Also, the experiments showed that microalgal biomass slightly enhanced the biogas production of the selected substrate mixtures.

3.6. Optimization of substrate composition in anaerobic co-digestion of agricultural waste using central composite design (EXP-3)

3.6.1. Obiectives

The aim of the experiment was to study the AcoD process using several ternary mixtures of agricultural wastes, consisting of sugar beet root waste (BW), manure (CD) and poultry manure (PM). The possibility of optimizing the process using the desirability function combined with the response surface methodology (RSM) was also investigated, using as process factors the mass fraction of CD in the animal waste mixture and the C/N ratio of the ternary waste mixture, according to a Central Composite factorial design.

3.6.2. Materials characterisation and procedures

Fresh organic wastes (BW, CD, and PM) were provided by INCDCSZ Brasov (Romania). All experimental runs were performed in triplicate for 30 d until the biogas production substantially decreased or ceased.

3.6.2.1. Experimental design, statistical analysis, and optimization

According to a CCD involving 2 process factors and 4 centre point runs, 12 experimental runs were performed simultaneously. Uncoded (natural) and coded factor levels for each experimental run are summarized in Tabele 3.7.

3.6.2.2. Theoretical biochemical methane potential and biodegradability of each substrate

Theoretical BMP of substrate *j* (BW, CD, and PM), $TBMP_j$ (mL/g VS), was determined based on Equations (3.16) and (3.17). Biodegrability of substrate *j*, BD_j (%),was calculated using Equation (3.18), where $EBMP_j$ (mL/g VS) is the experimental BMP of substrate *j*.

$$C_{a_j}H_{b_j}O_{c_j}N_{d_j}S_{e_j} + \left(a_j - \frac{b_j}{4} - \frac{c_j}{2} + \frac{3d_j}{4} + \frac{e_j}{2}\right)H_2O \rightarrow \left(\frac{a_j}{2} + \frac{b_j}{8} - \frac{c_j}{4} - \frac{3d_j}{8} - \frac{e_j}{4}\right)CH_4 + \\ + \left(\frac{a_j}{2} - \frac{b_j}{8} + \frac{c_j}{4} + \frac{3d_j}{8} + \frac{e_j}{4}\right)CO_2 + d_j NH_3 + e_j H_2S \quad (3.16)$$

$$TBMP_j = \frac{1000V_M\left(\frac{a_j}{2}+\frac{b_j}{8}-\frac{c_j}{4}-\frac{3d_j}{8}-\frac{e_j}{4}\right)}{a_jM_C+b_jM_H+c_jM_O+d_jM_N+e_jM_S} \quad (3.17)$$

$$BD_j = 100\frac{EBMP_j}{TBMP_j} \quad (3.18)$$

3.6.3. Results and discussions

3.6.3.1. Substrate and inoculum characteristics

Physico-chemical analysis results for agricultural wastes used as substrates and for inoculum are specified in Tabele 3.8.

Table 3.8. Characteristics of sugar beet root waste (BW), cow dung (CD), poultry manure (PM), and inoculum

Parameter	BW	CD	PM	Inoculum
TS (%)	54.20	16.0	36.6	8.54
VS (% of *TS*)	96.30	82.9	54.0	53.75
C (% of *TS*)	41.90	41.6	24.4	-
H (% of *TS*)	6.44	5.07	3.01	-
O (% of *TS*)	50.90	51.2	69.2	-
N (% of *TS*)	0.73	2.14	2.87	-
S (% of *TS*)	0	0	0.53	-
C/N ratio	57.5	19.4	8.49	-

3.6.3.2. Experimental and predicted kinetics of AcoD process

Time variations of experimental methane yield at different levels of process factors are shown in Figure 3.15.

$$Y_M(t) = Y_{M,\infty}\left[1-\exp(-kt)\right] \quad (3.19)$$

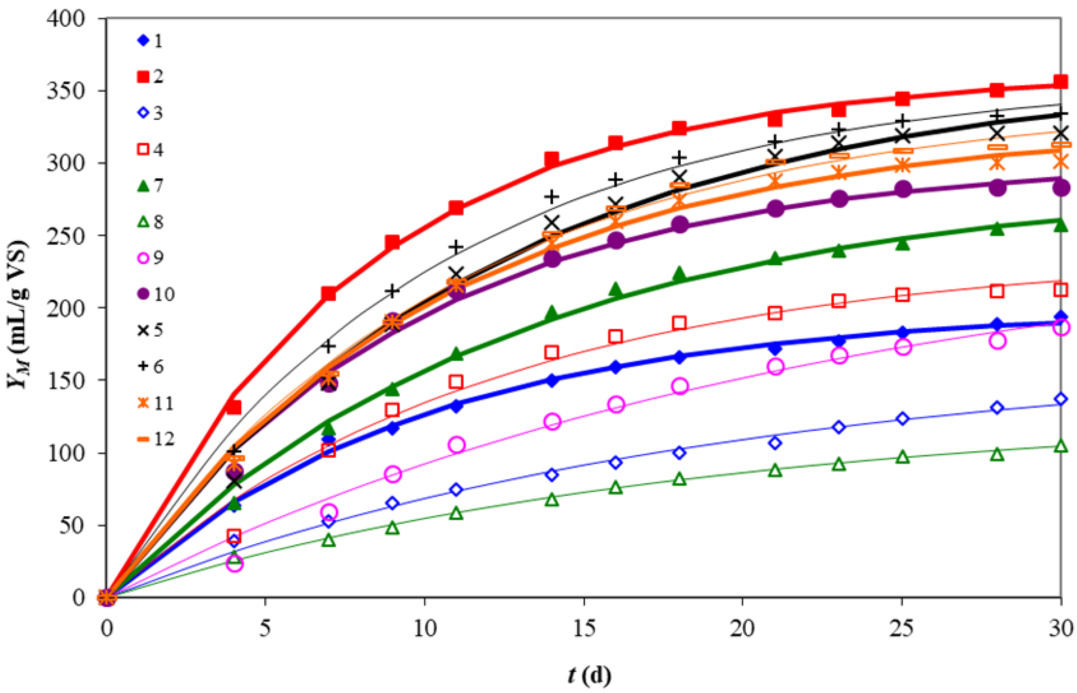

Figure 3.15. Time variation of methane yield (bullets: experimental data, lines: data predicted by first-order rate model [Equation (3.19)]

3.6.3.3. Statistical models

The statistical model expressed by Equation (3.20), where regression coefficients were estimated based on experimental values of maximum methane yield ($Y_{M,m,exp}$) obtained after 30 d of AcoD, was obtained using STATISTICA 10 software.

$$Y_{M,m} = 317{,}374 - 51{,}872X_1 + 46{,}696X_2 - 63{,}938X_1^2 - 36{,}934X_2^2 - 21{,}698X_1X_2 \quad (3.20)$$

Effects of process coded factors (X_1 şi X_2) on maximum methane yield ($Y_{M,m}$), predicted by Equation (3.20), are shown in 3D response surface plot and its corresponding 2D contour plot represented in Figure 3.16. Contour plot indicates the highest levels of $Y_{M,m}$ for X_1 between –0,9 and –0,1 and X_2 between 0,3 and 1,3, corresponding to values of ω = 0.275 – 0.475 and R = 23.80 – 29.80.

The optimum values of coded factors for maximizing the response of $Y_{M,m}$ are X_1 = –0.707 (ω = 0.323) and X_2 = 0.707 (R = 26.24). Under these optimum conditions, the process response is $Y_{M,m,opt}$ = 347.48 mL/g VS and desirability function is $d(Y_{M,m,opt})$ = 0.966.

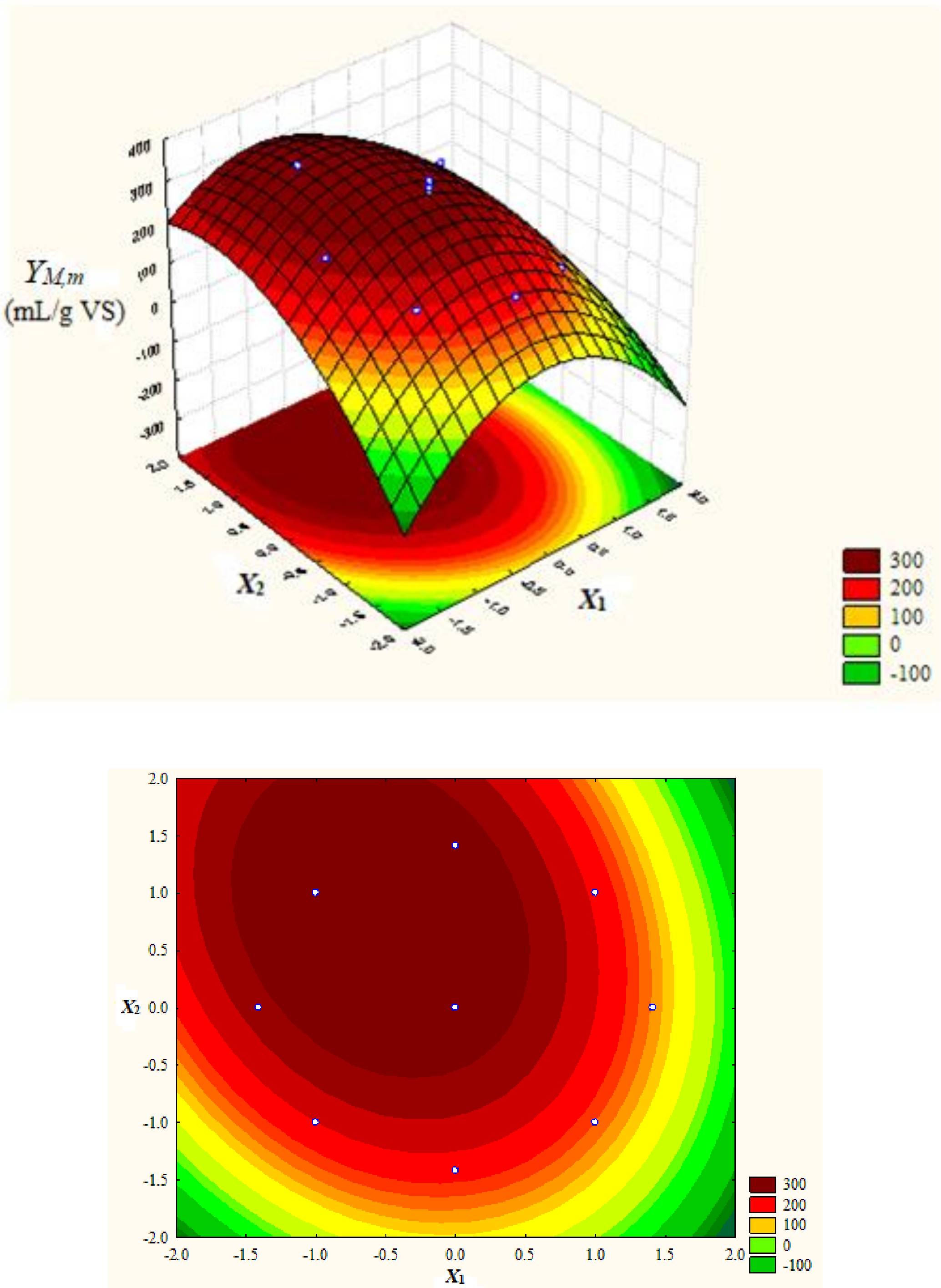

Figure 3.16. 3D surface response plot and 2D contour plot of maximum methane yield depending on coded factors ([Equation (3.20)]

3.6.3.4. Theoretical biochemical methane potential and substrates biodegradability

Values of constants a_j, b_j, c_j, d_j și e_j in Equation (3.17) estimated based on the procedure described by Dima et al. (2019) are summarized in Table 3.12.

Table 3.12. Constantele din Ecuaţia (3.17), potenţialul teoretic și experimental de biometan şi biodegradabilitatea substratului *j*

Substrate *j*	a_j	b_j	c_j	d_j	e_j	$TBMP_j$ (mL/g VS)	$EBMP_j$ (mL/g VS)	BD_j (%)
BW	67.00	122.60	61.03	1.00	0	387.50	306.26	79.03
CD	22.66	32.93	20.95	1.00	0	336.64	252.27	74.94
PM	122.76	180.69	261.75	12.40	1.00	50.45	31.84	63.11

3.6.3.5. Synergistic and antagonistic effects of AcoD

Weighed experimental biochemical methane potential of a mixture of substrates *j*, *WEBMP* (mL/g VS), was calculated using Equation (3.25) [24, 26], where m_j (g VS) represents the VS mass of substrate *j* in the mixture and $EBMP_j = Y_{M,m,exp,j}$ (mL/g VS).

$$WEBMP = \frac{\sum_{j=1}^{3} m_j EBMP_j}{\sum_{j=1}^{3} m_j} \tag{3.25}$$

In all experiments where synergistic effects were observed, *i.e.*, runs 1, 2, 5–7, 9–13, CD mass fraction in the fresh ternary mixture was relatively low (0.087–0.348).

3.6.4. Conclusions

Experimental results revealed values of experimental ultimate methane yield ($Y_{M,m,exp}$) of 105.32–356.10 mL/g VS. Process kinetics was simulated using a first-order rate model. Higher values of process rate, evaluated as rate constant (k = 0,044 - 0,123 d^{-1}), were obtained for lower levels of ω and higher levels of R. RSM was used to establish the effects of process factors on its performance, evaluated as ultimate methane yield ($Y_{M,m}$), and to optimize the process. The maximum performance under conditions considered in CCD, *i.e.*, $Y_{M,m,opt}$ = 347.48 mL/g VS, was predicted for $\omega = 0.323$ și $R = 26.24$. Under these optimum conditions, $Y_{M,m,exp,opt}$ = 358.45 ± 33.40 mL/g VS and a strong synergistic effect (an improvement in methane yield by 41.2%) related to the weighed experimental BMP of ternary waste mixture was observed. The results obtained in this study reveal that AcoD of sugar beet root waste with cow dung and poultry manure at $\omega = 0.275 - 0.475$ și $R = 23.80 - 29.80$ is a suitable option for obtaining increased methane production by AcoD.

3.7. Influence of gamma-ray irradiation on the biomethane production of sunflower seed cake (EXP-4)

3.7.1. Objectives

The objective of this study was to investigate the convenience of using sunflower seed cake (SSC), which is an abundant agro-industrial waste in Romania, for biogas production by mesophilic anaerobic digestion. Moreover, the influence of γ-ray irradiation (at doses of 50-150 kGy) on digestibility of SSC has been evaluated, knowing that irradiation can improve the digestability of hadly degradable lignocellulosic structures in the shell of sunflower seeds.

Dynamics of biogas and biomethane yields were compared for γ-ray pretreated and untreated substrates. Cone and modified Gompertz models were applied to simulate the performances of anaerobic digestion process.

3.7.2. Materials characterisation and procedures

The sunflower seed cake used in this experiment was collected from a local farmer in Prahova country. SSC substrate exposure to γ-rays was carried out in a laboratory irradiator ObServo Sanguis (Institute of Isotopes, Budapest) equipped with 60Co source and rotary rack for homogenous irradiation.

A lab-scale experimental set-up was used for BMP tests. All BMP tests lasted 74 days (d), after this period the daily biogas production dropping to less than 2% from thecumulative gas volume.

3.7.3. Results and discussions

3.7.3.1. Experimental biogas and biomethane production

Dynamics of experimental cumulative biogas (B) and biomethane (M) productions, $Y_{B,exp}(t)$ and $Y_{M,exp}(t)$, where t (d) is the digestion time, are shown in Figures 3.20 and 3.21.

3.7.3.2. Kinetic modelling

Cone and modified Gompertz models described by Equations (3.26) and (3.27), were applied to predict the dynamics of biogas, $Y_B(t)$, and biomethane, $Y_M(t)$, productions. Analogously, the equations applied to simulate the dynamics of biomethane production can be obtained.

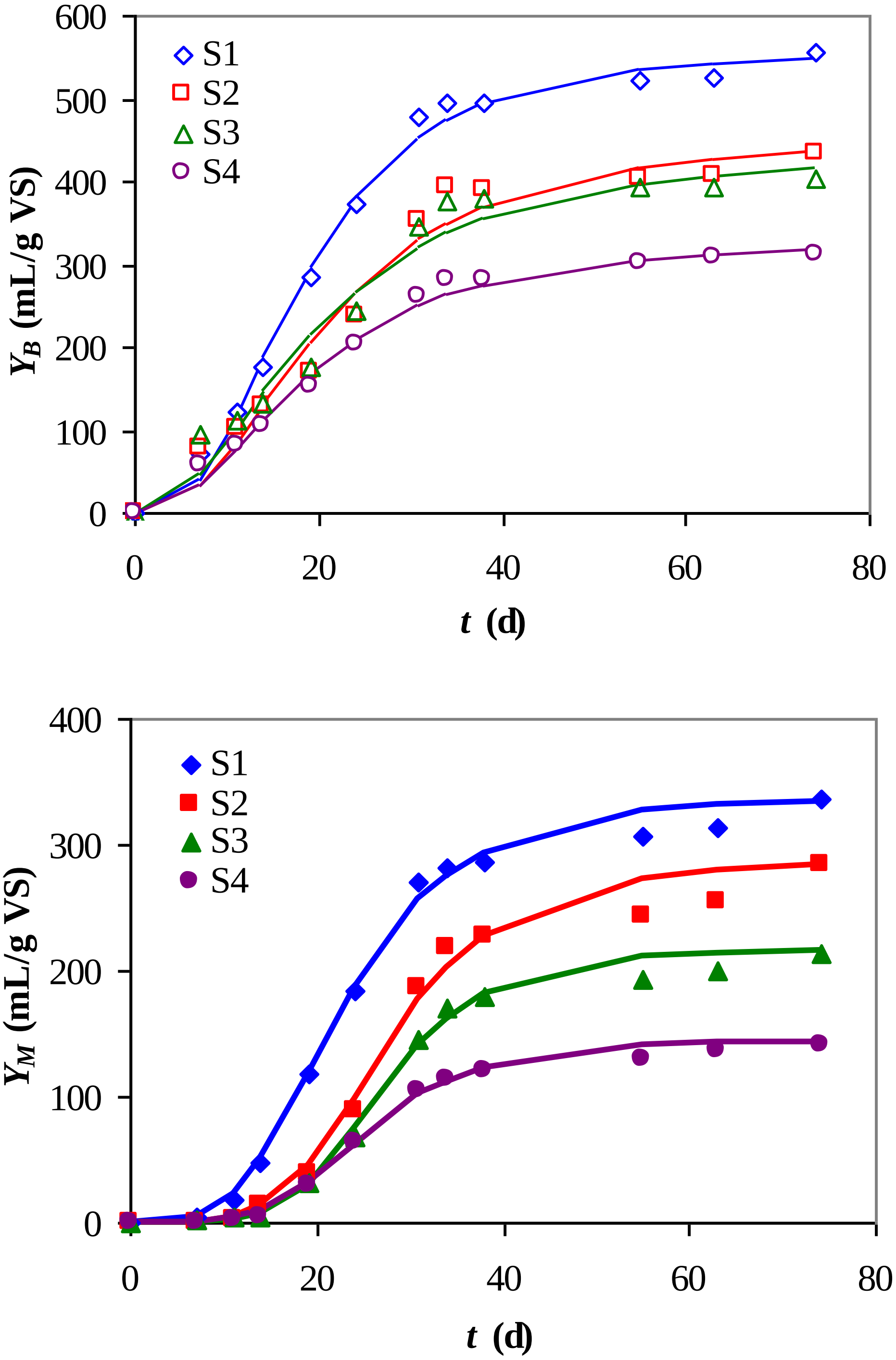

Figure 3.25. Time variation of biogas and biomethane production for untreated (S1) and pretreated (S2-S4) substrates (bullets: experimental data, lines: data predicted by Cone model)

$$Y_B(t) = \frac{Y_{B,\infty}}{1+(k_B t)^{-n_B}} \tag{3.26}$$

$$Y_B(t) = Y_{B,m} \exp\left\{-\exp\left[\frac{r_B \exp(1)}{Y_{B,m}}(\lambda_B - t) + 1\right]\right\} \tag{3.27}$$

Experimental and predicted dynamics of biogas and biomethane production are shown in Figures 3.25 and 3.26. The γ-ray irradiation had a significant negative effect on the biomethanation of SuSC, increasing the lag-phase period of methane production.

Although the purpose of the irradiation pretreatment was to decrease the intermolecular hydrogen interactions in the feedstock material and to provide an easier access to microbial attack, some unanticipated interactions impeded the anaerobic digestion of the pretreated substrate (Kassim et al., 2016).

3.7.4. Conclusions

Results indicate that besides its current recovery options, SSC may also be a promising substrate for biomethane production by anaerobic digestion. The values of experimental maximum (ultimate) biogas and biomethane yields, $Y_{B,m,exp}$ and $Y_{M,m,exp}$,obtained after 74 d in batch mesophilic anaerobic digestion tests using untreated SSC as a vegetal substrate, were 557.2 mL/g VS and 336.5 mL/g VS, respectively, proving a relatively high gas production compared to other agricultural waste substrates. The pretreatment of SSC using γ-ray irradiation doses (D) of 50, 100, and 150 kGy disturbed the methanogenic activity in the fermentation broth and decreased the process rate compared to the case of untreated sample. The biogas and biomethane productions decreased linearly with an increase in γ-ray irradiation dose. Over-acidification of the fermentation suspension and occurrence of some competing cross-linking reactions in the fermentation environment could be responsible for poor performance of γ-ray irradiation pretreatment. Cone and modified Gompertz models were used to predict the dynamics of biogas and biomethane yields, revealing highly similar results.

3.8. Evaluation of the biomethane potential of enzymes-enriched sunflower seed cake (EXP-5)

3.8.1. Objectives

In this experimental study, the biomethane potential of sunflower seed cake (SSC) was evaluated by batch anaerobic digestion in mesophilic conditions in order to assess the opportunity of using SSC as substrate for biogas production. In this regard, a reliable and convenient BMP lab-scale experimental set-up has been developed and tested using SSC as substrate. Moreover, the influence of enzymatic pretreatment on the biomethane production has been experimented using pure α-amylase and proteinase K enzymes to act as biological catalyzers for the anaerobic digestion processes.

3.8.2. Materials characterisation and procedures

The substrate used for this study was sunflower seed cake that was provided by a farmer in Prahova country (Romania). Pure analytical grade enzymes α-amylase (Fluka, CH) and proteinase K (Merck, De) were used in the study.

The BMP tests were conducted in batch, at mesophilic temperature (37±0.5°C) according to the VDI 4630 standard method (VDI 4630, 2016). The total fermentation time was 74 days.

3.8.3. Results and discussions

3.8.3.1. Physico-chemical substrate characterization

Results of chemical analysis for sunflower seed cake and inoculum are displayed in Tabele 3.13.

3.8.3.2. Experimental and predicted kinetics of AD process

The experimental and predicted biogas/methane yields for both substrates, $Y_B(t)$ and $Y_M(t)$ (mL/g VS), are plotted in Figure 3.27, where bullet points indicate the experimental data and lines indicate the predicted data using the Cone model.

Experimental BMP of S1 and S5 were found to be about 351 mL/ g VS and 381 mL/ g VS, respectively.

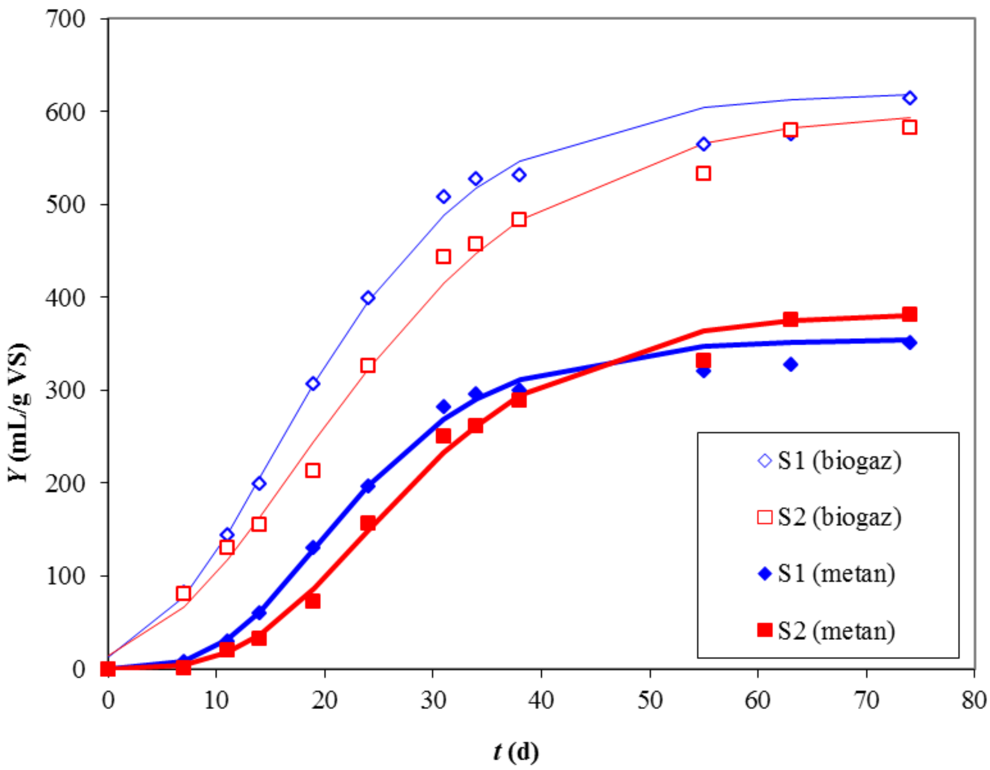

Figure 3.27. Time variation of biogas and methane yields [bullets: experimental data, lines: data predicted by Cone model (Equation 3.30)]

The kinetics of anaerobic degradation, determined by using the Cone model is shown in Figure 3.27.

The Cone model (Achinas et al., 2019; Dima et al., 2019) is expressed by Equation (3.30).

$$Y(t) = \frac{Y_{\infty}}{1+(kt)^{-n}} \tag{3.30}$$

$$RMSE = \sqrt{\frac{\sum_{j=1}^{N_{\exp}} \left[Y(t_j) - Y_{\exp}(t_j)\right]^2}{N_{\exp}}} \tag{3.31}$$

$$CV = \frac{RMSE}{Y_{\exp,mn}} \times 100 \tag{3.32}$$

3.8.3.3. Mass percentage of methane in biogas

The time variation of mass percentage of methane in biogas resulted in the anaerobic fermentation tests, $c_{M,t}(t)$, is displayed in Figure 3.28.

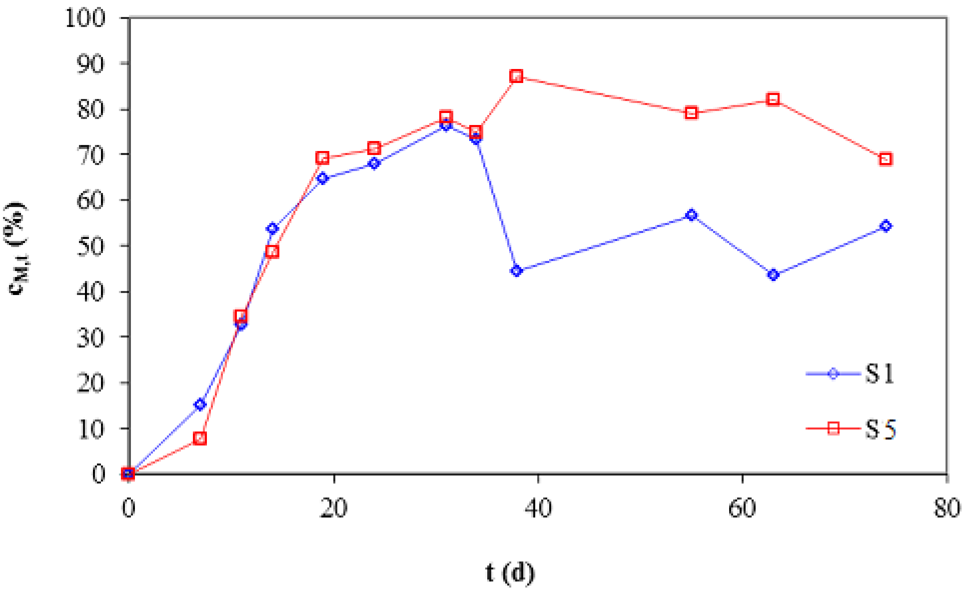

Figure 3.28. Time variation of mass percentage of methane in biogas, $c_{M,t}$

The slight increase in the methane production of S2 which leads to a higher BMP for the enzyme enriched SSC sample at the end of AD, could be associated to a better digestibility of the substrate following enzymatic exposure. The average methane concentration in biogas for the entire fermentation duration was 57.2% for S1 and 65.5% for S5, respectively.

3.8.4. Conclusions

Results of biomethane potential tests suggest that sunflower seed cake (SSC) is an appropriate waste substrate for AD, showing a methane production of 351 mL/g VS for a total 74 days of anaerobic digestion. The addition of 1% (w/w) α-amylase and 1% proteinase K to the SSC substrate increased the overall BMP with about 8.5% compared to the control, despite the fact that a slight inhibition was observed during the first days of anaerobic digestion. The kinetics of anaerobic degradation was evaluated using the Cone model, where the values of the rate constant for S1 substrate were higher compared to S5, indicating a decrease in mean process rate in the presence of enzymes. On the other hand, findings suggest that although the methane yield of S1 is higher compared to S5, the AD of S5 provides a more concentrated and thus a more calorific gas which may require less expenditure for purification.

GENERAL CONCLUSIONS

Several strategies to increase the production of fuel gas in biogas plants were proposed in this study. In order to determine the degree of their effectiveness, comparative determinations of biomethane potential were carried out, using different substrates or mixtures of raw materials, both in their unaltered form and the modified form. Thus, five experimental studies were performed (EXP-1, EXP-2...EXP-5), using different substrates and substrate preparation technologies.

The main conclusions of the conducted studies are the following:

- within EXP-1, the influence of adding microalgal extracts of *Chlorella* sp. and *Spirulina* sp. to the potato processing residuals substrate was evaluated. Although the high proportion of nitrogen in the microalgae composition suggested a possible increase in biogas production due to a more balanced composition of the mixture, results indicated a decrease in the amount of biogas compared to the control sample using unadded potato residues as substrate;
- within EXP-2, improving the biogas production of selected waste materials by substrate ratio optimization and microalgae addition was considered; the experimental results showed that the volume of biogas and the biomethane concentration in biogas were strongly influenced by the quality of the fermentation substrate;
- within EXP-3, the anaerobic co-fermentation of agro-industrial beet root residues with animal manure was evaluated. Also, the biogas production of the substrate was optimized by using the central composite design (CCD). Acoording to the experimental design, the mass fraction of cow dung in the manure mixture (ω = 0,146 - 0,854) and the C/N ratio of the ternary waste mixture (R = 13.515 - 30.485) were used as process factors, resulting in values of the maximum experimental methane production of 105.32 - 356.10 mL/g VS. A first-order rate model was employed for simulating the kinetics of the process. Higher values of process rate were obtained for lower levels of ω and higher levels of R. For process optimization, the response surface methodology was used. The maximum performance of the process was predicted for ω = 0.323 and R = 26.24; under these optimal conditions, a strong synergistic effect was observed, namely an improvement of the methane yield by 41.2%. The obtained results highlight the importance of determining the optimal proportion of substrates involved in anaerobic co-digestion, while mathematical modeling proves to be a suitable strategy for process optimization.

- within EXP-4, the influence of irradiation pretreatment on anaerobic digestion of sunflower seed cake was investigated. Results indicate that sunflower seed cake is a promising substrate for biomethane production showing a biomethane production of 336.5 mL/g VS, which is relatively high compared to that of other agricultural substrates. Irradiation pretreatment using γ-ray irradiation doses of 50, 100 and 150 kGy disrupted the methanogenic activity in the fermentation mixture and decreased the rate of the process compared to the untreated sample.
- within EXP-5, supplementation of the substrate with enzymes led to optimistic results; the addition of 1% α-amylase and 1% proteinase K to the sunflower seed cake substrate increased the methane production by 8.5% compared to the control, despite the fact that a slight inhibition was observed in the first days of anaerobic digestion. Results also suggest that the anaerobic digestion of the additivated substrate can lead to a highly concentrated methane gas.
- comparing results of all 5 conducted experimental studies, it can be concluded that, under the described experimental conditions, optimization of biogas production by using the Central Composite experimental plan and by adjusting the proportion between substrates led to the best results. The use of enzymes to increase the degradability of the fermentation mixture also showed a high potential for improving the anaerobic digestion process, while the addition of the substrate with microalgae appeared ineffective. The gamma irradiation pretreatment of the substrate is not recommended, drastically affecting the biochemical balance in the biofermenter and lowering the biomethane yield;
- the experimental study revealed a number of advantages when using the lab-scale experimental set-up I-EXP-2 for the determination of the biochemical methane potential, compared to I-EXP-2 which showed certain inconveniences;

Given that the experimental results open some discussions regarding the phenomena that might explain the obtained data, several possible directions for further research were revealed. Moreover, following this research, complementary experimental activities that might lead to increasing the biogas production were identified. Therefore, the perspectives of further research are separately discussed.

PROSPECTS FOR FUTURE RESEARCH

The experimental research presented in this paper reveal some optimization strategies for increasing the biogas production of agro-industrial waste. The overall aim of the study was to promote the economic profitability of biogas plants by supplying effective methods to enhance the biomethane yield of substrates subjected to the anaerobic digestion process. Results provide important indications regarding the impact that the compositions of the substrate and its pretreatment methods have on the stability of the process or the biodegradability of the digestion mixture. Moreover, the mathematical modelling showed to be an effective tool for process optimization.

With respect to the way of planning and development of the experimental part, this work opens new prospects for future research, by offering the possibility of carrying out related experimental activities, such as:

- Evaluation of same strategies to increase the biogas production starting from other types of substrates, analyzed under similar experimental conditions;
- Carrying out experimental studies involving different concentrations of microalgal biomass/ enzymes as fermentation additives;
- Confirmation of the general effect of gamma ray irradiation on fermentation processes, by using other types of substrates;
- Conducting research on other innovative pretreatment strategies;
- Extending physico-chemical/biological analysis means for complex description of the fermentation environment, as well as for the intermediary phases of the process;
- Conducting anaerobic digestion experiments in continuous flow digestion installations and process optimizing by mathematical modelling.

The field of biogas is wide, offering a multitude of original approaches. Further research in the field of anaerobic digestion in Romania can lead to a larger number of specialists in this field, higher interest for renewable energy production and awareness of the legislative structures towards the needs of this industry. Consequently, a stronger biogas sector in our country would be possible, driven by larger investments in biogas technologies. By this means, reliable technologies for the production of renewable energy and sustainable management of organic waste could be implemented al local or regional levels, with important social, energetic and environmental benefits.

PERSONAL CONTRIBUTIONS

Dissemination of results

The results obtained during the conducted research were presented to the scientific community through publications in specialized journals (articles and book chapters), patents, oral and poster presentations.

Scientific articles

1. **Andreea D. Dima**, Oana C. Pârvulescu, Carmen Mateescu, Tănase Dobre. Optimization of substrate composition in anaerobic co-digestion of agricultural waste using central composite design, Biomass and Bioenergy, Vol. 138, 2020, 105602. https://doi.org/10.1016/j.biombioe.2020.105602. WOS:000540918400012. Impact factor 2021: 5,774 (Q1).

2. Carmen Mateescu, **Andreea D. Dima**[*]. Critical analysis of key barriers and challenges to the growth of the biogas sector: a case study for Romania, Biomass Conversion and Biorefinery, 2020. https://doi.org/10.1007/s13399-020-01054-9. WOS:000578234300001. Impact factor 2021: 4,987 (Q1).

3. **Andreea D. Dima**, Carmen Mateescu, Oana C. Pârvulescu, Eduard M. Lungulescu, Nicoleta O. Nicula. Theoretical and experimental results on the recovery of potato processing residuals by anaerobic digestion. Revista de Chimie, Vol. 70(7), 2019, pp. 2524-2529. https://doi.org/10.37358/RC.19.7.7373. WOS:000485843500046. Impact factor 2019: 1,755.

4. **Andreea D. Dima**, Oana C. Pârvulescu, Carmen Mateescu, Eduard M. Lungulescu. Influence of gamma-ray irradiation on the biomethane production of sunflower seed cake, U.P.B. Scientific Bulletin, Series B, Vol. 83(1), 2021, pp. 59-72. WOS:000627764100006.

5. **Andreea D. Dima**, Carmen Mateescu, Evaluation of the biomethane potential of enzymes-enriched sunflower seed cake, U.P.B. Scientific Bulletin, Series B, Vol. 82(4), 2020, pp. 39-50. WOS:000610101300004.

6. Carmen Mateescu, **Andreea D. Dima**, Oana C. Pârvulescu. Improving the biogas performance of selected waste materials by substrate ratio optimization and microalgae addition, Journal of Engineering Sciences and Innovation, Vol. 4(3), 2019, pp. 263-272.

Oral presentations at national/international conferences

1. **Andreea D. Dima**, *Monitorizarea factorilor care influențează producția de biogaz în vederea creșterii biodegradabilității masei de fermentare*, Simpozionul național „Orizonturi noi în valorificarea biomasei algale şi remedierea habitatelor acvatice” (25 octombrie 2018, INCDIE ICPE-CA, Bucureşti).
2. Carmen Mateescu, Eduard M. Lungulescu, **Andreea D. Dima**, Nicoleta O. Nicula, *Biomethane from potato waste for energy recovery in biogas plants*, Conferința „Zilele ASTR” (October 17-19 2018, UPG, Ploiesti).
3. **Andreea D. Dima**, Carmen Mateescu, *Cercetări privind optimizarea fermentării anaerobe a reziduurilor organice și a microalgelor*. Definirea amestecurilor optime de fermentare și elaborarea tehnologiei de laborator, în cadrul Workshopului: Energetic efficiency biogas plants improvement by integrated system: biogas-microalgae-biofuels in frame of biorefinery concept (AlgalBiogasConceptEnergy) (October 30, 2019, ICECHIM, Bucharest).
4. **Andreea D. Dima**, Carmen Mateescu, *Biotehnologie inovativă de valorificare energetică a deșeurilor agricole pentru producerea de biogaz și îngrășământ organic*, Conferința Națională cu Participare Internatională, Cercetare Științifică și Inovare în Contextual Extinderii Agriculturii de Precizie și Modernizării Sistemelor Tehnologice (November 27-28, 2019, INCDCSZ, Brasov).
5. Carmen Mateescu, **Andreea D. Dima**, *Challenges in implementing biogas tehnologies in Romania: A comprehensive approach of obstacles and solutions,* International Conference on Renewable Energy - ICREN 2019 (April 24-26, 2019, UNESCO, Paris, France).

Poster presentations

1. **Andreea D. Dima**, Carmen Mateescu, *Biomethane recovery from agro-industrial waste for energy supply and climatic change mitigation*, International Conference on Renewable Energy - ICREN 2019, April 24-26, 2019, UNESCO, Paris, France.
2. **Andreea D. Dima**, Oana C. Pârvulescu, Carmen Mateescu, *Optimization of biomethane recovery from agro-industrial waste for energy supply and environement protection*, 21st Romanian International Conference on Chemistry and Chemical Engineering (RICCCE), September 4-7, 2019, Mamaia, Romania.
3. **Andreea D. Dima**, *Influence of microalgae on the organic matter conversion to renewable fuel gas*, ASMES 2019, September 20-22, 2019, Predeal, Romania.

4. Carmen Mateescu, **Andreea D. Dima**, *Biotehnologii în sprijinul reducerii riscului patogen al deșeurilor biodegradabile*, Conferința INGIMED XX - „Ingineria biomedicală la răscruce de concepte în lume și de generații în România”, November 7, 2019, Bucharest, Romania.
5. **Andreea D. Dima**, Carmen Mateescu, Oana C. Pârvulescu, *Assesing an advanced pretreatment method on the biodegradability of sunflower seed cake to biogas*, International Symposium of Chemistry and Chemical Engineering (SICHEM), September 17-18, 2020, Bucharest, Romania.

Book chapters

1. Carmen Mateescu, **Andreea D. Dima**, *Biochemical Conversion of Residual Biomass: An Approach to Fuel Gas and Green Fertilizers*, In book: Catalysis for Clean Energy and Environmental Sustainability, Springer Nature Switzerland AG 2021, K. K. Pant et al. (eds.), pp. 49-88, 2021, https://www.researchgate.net/deref/https%3A%2F%2Fdoi.org%2F10.1007%2F978-3-030-65017-9_2%23DOI

Patents

1. Carmen Mateescu, Andreea D. Dima, Eduard M. Lungulescu, A. Militaru, *Procedeu de stimulare a producției de biogaz și biometan în procese de codigestie,* Patent application no. A/00773/20.11.2019 - contribution: 40%.

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