EVALUATION OF THE BIOMETHANE POTENTIAL OF ENZYMES-ENRICHED SUNFLOWER SEED CAKE

Andreea D. DIMA[1], Carmen MATEESCU[2]

This paper aims at evaluating the biomethane potential (BMP) of sunflower seed cake (SSC) and the influence of enzymatic slight treatment on the anaerobic digestion of SSC. For a total fermentation period of 74 days, the enzymatic 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 a noticeable slowdown of the process corresponding to a lag phase was observed during the first days of anaerobic digestion. The kinetics of anaerobic degradation was determined using the Cone model in order to comparatively analyze the degradation performance and biogas production.

Keywords: anaerobic digestion, biomethane potential, enzymatic pretreatment, sunflower

1. Introduction

Developing the renewable energy technologies is considered among the most important policies for greenhouse gas emission reduction and sustainable energy supply [1,2]. Anaerobic digestion (AD) is a complex biochemical process in which organic materials are decomposed to biogas and fermented sludge and it can naturally occur in landfill sites causing environmental pollution and human health concerns [3].

Although usually employed for municipal wastewater treatment, anaerobic digestion has more recently been directed towards the controlled degradation of industrial and agricultural wastes which is considered an advantageous strategy for diminishing the environmental impact of waste along with fuel gas generation [4,5]. AD could also be used to improve the energy balance of other processes such as the ethanol production by on-site cogeneration of heat and electricity or supply energy for isolated consumers [6,7]. However, when dealing with waste organic materials, difficulties may arise from the unsteady chemical and

[1] Ph.D. Student, Dept. of Chemical and Biochemical Engineering, University POLITEHNICA of Bucharest, Romania; Research Assistant, Dept. of Environment/Energy and Climate Change, National Institute for Research and Development in Electrical Engineering ICPE-CA, Bucharest, Romania

[2] PhD, Researcher, Dept. of Environment/Energy and Climate Change, National Institute for Research and Development in Electrical Engineering ICPE-CA, Bucharest, Romania, e-mail: carmen.mateescu@icpe-ca.ro

microbiological composition, high quantity of hardly biodegradable structures and possible material interactions [8,9]. Hence, prior to industrial use, more in-depth knowledge of the substrate behaviour when submitted to anaerobic digestion is widely advised, so that time spending and financial losses be considered and avoided.

Biomethane potential (BMP) tests are productive tools for gaining information about the bio-methanation of specific substrates which could significantly contribute to the biogas production process efficiency. BMP tests are easy to perform and relatively inexpensive [10]. They could be used to study various strategies for obtaining increased biomethane production, such as pretreatment methods to enhance substrate biodegradability, addition of fermentation catalyzers to fasten biochemical reactions, adjustment of operating parameters for process optimization etc. [11,12].

Sunflower seed cake (SSC), which is a highly proteic by-product resulted from oil extraction processes, has the third largest production in Europe after soy-bean and rapeseed cake [13]. They are generally utilized for feed purposes, but the dietary use of SSC in ruminants, pigs and poultry is limited due to some concerns associated to adverse effects on digestion [14,15]. Additionally, in some cases, SSC have been reported to contain high amounts of toxins caused by poor storage and post-harvesting handling which make it non-compliant for usage as feed due to potential threat to animal health [16]. Therefore, sunflower oil industry produces large quantities of residuals requiring storage, but further treatment to minimize the environmental impact of such waste is highly advised. Although pelleting and subsequent burning for heating might be applied for SSC, these are regarded as non-environmentally friendly. Thus, directing the over plus of SSC to clean fuel production in on-site biogas plants is an eco-friendly practice that could also bring several benefits to the industrial producers, such as costs reduction for waste processing, freeing warehouses, savings from coverage of heat consumption, or important financial gains from injecting biomethane into the grid.

The energy value of biogas is given by methane, which generally accounts for 50-70% of the total biogas volume; carbon dioxide is the second major product of anaerobic digestion, being the non-combustible share of biogas. Other components are present in biogas, such as water vapours, nitrogen, hydrogen sulphide, etc. [4]. It is therefore essential that in anaerobic digestion processes, quantitatively reasonable biogas volumes with high calorific value and efficient organic matter reduction should be obtained to support feasibility of choosing this technology over other waste management technologies.

Anaerobic digestion comprises four basic phases (hydrolysis, acidogenesis, acetogenesis, and methanogenesis), where hydrolysis has been reported as the rate determining stage of the bioconversion [17]. For hardly degradable substrates, several physical, chemical and biological treatments have

been previously employed to enhance biomass disintegration and consequently to enhance the accessibility of anaerobic bacteria to organic contents. When compared to some other methods, the substrate biological pretreatment is arguably a more environment-friendly method to improve anaerobic digestion and requires less capital investments [11,18].

Sunflower seed cake composition varies with the mechanical configuration of oil extraction system, but it generally shows a significant protein and fiber content [19,20]. Enzymatic substrate pretreatment could enhance the biomethane production of SSC by improving the hydrolysis of proteins and fiber to smaller molecules which become readily available to fermentation microorganisms. Amylases and peptidases have been previously used to increase the biomethane yields in anaerobic digestion of several waste materials. Enzyme α-amylase is known to hydrolyze alpha bonds in large polysaccharides into fermentable sugars, while proteinase K is an enzyme that catalyzes the breakdown of proteins into smaller molecules such as polypeptides or amino acids [21-23].

In this paper, the biomethane potential of sunflower seed cake 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.

2. Experimental

2.1. Materials

The substrate used for this study was sunflower seed cake that was provided by a farmer in Prahova country (Romania). SSC resulted after the oil-extraction of un-decorticated sunflower seeds in a semi-automated process, using a four head screw oil press machine designed for semi-industrial applications. SSC samples were delivered to the laboratory as 30-100 cm long pellets of 8-10 mm thickness and were stored in plastic bags at room temperature. SSC pellets had a dry-greasy feel on touch and specific strong odor following heating exposure during the mechanical pressing. Representative samples were grounded to pass a 3 mm mesh before subjected to physico-chemical analysis and use in the BMP tests, as shown in Fig. 1.

The SSC composition was analyzed for total solids (TS) and volatile solids (VS) according to the APHA standard methods [24]. Total carbon (TC) was determined by combustion with the TC analyzer vario TOC cube (Elementar, De), while total nitrogen was analyzed using a Photolab S12 equipment (WTW, De).

Fig. 1. Feedstock materials: sunflower seeds (SS), sunflower seed cake (SSC) and grinded SSC

The oil content was determined using hexane as extraction solvent in a Soxtherm fat extraction system (Gerhardt, De).

Fermented sludge supplied from an industrial biogas plant treating various agro-industrial organic waste and wastewaters and operating in mesophilic conditions was used to inoculate the fermentation mass with fermentation microbiota. The inoculum source is important for providing an appropriate microbial community to the fermentation medium which ensures a proper start for the anaerobic digestion process [25,26]. Inoculum was tested for TS, VS and pH and preserved at room temperature, protected from light and kept in strict anaerobic conditions until used in the anaerobic digestion experiments. The biogas produced during storage was carefully released to prevent oxygen penetration, until no notable production was observed due to nutrient depletion.

Pure analytical grade enzymes α-amylase (Fluka, CH) and proteinase K (Merck, De) were used in the study. α-Amylase was obtained from bacteria and had 25 IU/mg, while proteinase K was fungus derived and had 10-15 U/mg.

Chemical composition analyzes were performed in duplicate and the mean value was used for calculations.

2.2. Equipment and procedures

The BMP tests were conducted in batch, at mesophilic temperature (37±0.5°C) according to the VDI 4630 standard method [27]. The total fermentation time was 74 days (d). For the laboratory BMP testing, a simple, reliable and convenient BMP experimental set-up has been developed, as shown in Fig. 2.

Digesters were brown glass fermentation bottles (Supelco, US) with a volume of 240 mL. Substrates consisting of grinded SSC (S1), respectively grinded SSC with 1% α-amylase and 1% proteinase K (w/w) (S2) were weighted, introduced in the bottles and supplemented with the inoculum. The inoculum to substrate ratio was 0.5 (VS basis), while the TS mass of SSC substrate in the total fermentation sludge volume was 8% (w/v). Distilled water was added to fill up the working volume of 120 mL.

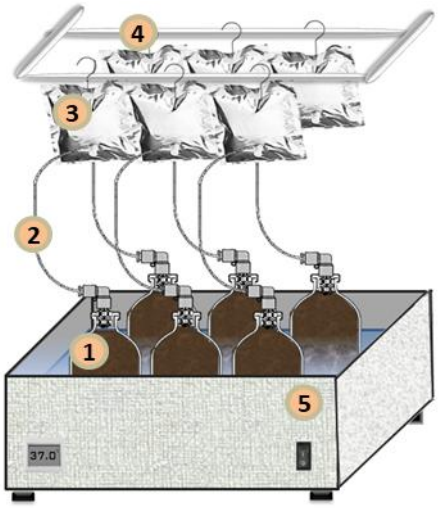

Fig. 2. BMP experimental set-up: (1) amber glass fermentation bottle; (2) connection tube; (3) gas sampling bag (4) hanging system; (5) thermostatic water bath.

A blank test consisting of inoculum and distilled water was also performed in order to determine the gas production of inoculum alone. This production was subtracted from the results obtained by S1 and S2 so that they would reflect exclusively the production of the organic substrate [28].

Fermentation mass was homogenized by stirring and the headspace of each bottle was flushed with nitrogen, then immediately sealed in order to ensure anaerobic conditions in the digesters during the experiment. A PTFE washer and a straight, fast joint copper connector (AirTech Solutions, Ro) coupled to a PTFE tube was used to ensure the connection from the fermentation bottle to the 5L multi-layered gas bags (Supelco, US). The gas bags were fitted with valves, thus allowing simple decoupling of the gas bag from the system for biogas analysis.

Fermentation bottles were placed in the 22.5 L thermostatic water bath WBO1 Series (lbx Instruments, ES), at 37± 1°C. Substrates were manually stirred twice a day by slow circular motion of glass bottles, in order to ensure substrate homogeneity during the experiment, but avoiding disturbances on the bacterial population. Biogas analysis was performed periodically, starting with the 7th day of the experiment. The digestion experiments were carried out until the volume of accumulated gas remained significantly low, that is the average daily production was lower than 2% of the accumulated biogas. The assessment of the quantity and quality of the biogas was performed separately for each fermentation mixture, by periodic analysis of biomethane quantity and biogas volume accumulated in the collecting bags. After each analysis, the collection bags were emptied and replaced in position. The mass percentage of methane in biogas at analysis day, $P_{ex}(t)$ (%), was measured for each sample by gas chromatography using a Varian 450-GC chromatograph (Agilent Technologies), coupled to a flame ionization

detector (FID) as described in a previous publication [29]. The experimental biogas volume was measured using the water displacement method [30] and used to compute the experimental biogas yield $Y_{ex}(t)$ (mL/g VS).

In order that the gas formation path to be completely recognizable, the frequency of biogas analyzes was set to be higher for the first 38 days of experiment during biogas production growth, compared to the last days of experiment, when biogas production decreased. All tests were performed in duplicate. Results of biogas/biomethane production are displayed for 1g VS substrate after subtracting the specific production of inoculum.

3. Results and discussions

3.1. Physico-chemical substrate characterization

Results of chemical analysis for sunflower seed cake and inoculum are displayed in Table 1.

Table 1

Characteristics of SSC and inoculum

Parameter	SSC	Inoculum
TS (%)	93.3	8.54
VS (% of TS)	93.6	53.75
C (% of TS)	49.8	-
N (% of TS)	2.76	-
C/N	18.04	-
pH	-	8.3

Sunflower fat content is about 50% [31]. The oil quantity of SSC was found to be 10.88%, which means that the extraction process using the screw oil press machine is moderate-high. The average yield of mechanical extraction of sunflower seeds is generally around 75%, but factors such as the pressing temperature and fresh seed moisture content are affecting the oil production [32].

3.2. Experimental and predicted kinetics of AD process

The experimental and predicted biogas/methane yields for both substrates, $Y(t)$ (mL/g VS), are plotted in Fig. 3, where bullet points indicate the experimental data and lines indicate the predicted data using Cone model.

Depicted data reveal that the substrates are starting to produce important biomethane volumes after 7 d of AD, which is noticeable by a sharp increase in the slope of biomethane production. Following d 7 of AD, the methanogenic activity intensifies; the methanogens take over the non-methanogenic microorganisms, leading to important methane volume formation up to d 38, when a significant decrease in production is observed, most probably linked to substrate nutrient depletion. The methane production of S2 is on the other hand constantly below the methane production of S1 until the 50th day of anaerobic

digestion. The behaviour of S2 could be correlated to a lag phase due to a slight inhibition of anaerobic bacteria in the presence of proteinase K and α-amylase in the fermentation medium. Enzymes are known to be proteins capable of converting specific compounds in the substrate into other products at high reaction rates [33]. It is therefore possible that the hydrolysis step had been accelerated, leading to high production of both volatile fatty acids and ammonia, which could have resulted in a slight imbalance in the biochemical processes during anaerobic digestion rather than a stimulation of biogas production. This phenomenon is known as "inhibited steady state", being characterized by process apparent stability, but rather lower methane yields [34]. Interestingly, the equilibria between non-methanogenic and methanogenic microorganisms re-establishes after about 50 d of AD, when Y_{S2} exceeds Y_{S1} in terms of biomethane production. For a total fermentation period of 74 days, the enzymatic treatment of the fermentation mass increased the overall BMP with 8.5%. However, this increase is not remarkable considering the long fermentation period. The substrate external enzymatic treatment followed by pH adjustment before the start of anaerobic digestion process might have been a better approach for obtaining significant process improvement.

Experimental BMP of S1 and S2 were found to be about 351 mL/ g VS and 381 mL/ g VS, respectively. These results suggest that SSC obtained after semi-industrial extraction of sunflower seeds are potential substrates for AD, proving to be comparable to various other productive waste substrates used in AD [35]. Moreover, these are slightly higher than that obtained by other researchers using similar feedstock, being most probably associated to a larger quantity of residual oil in the treated waste. For instance, Monlau et al. found a maximum biomethane potential of 195 mL/ g VS after anaerobic digestion of SSC containing 0.7% oil, while Raposo et al. found a BMP of 227 mL/g VS for SSC with 1.7 % fat content [13,19].

Experimental data regarding the kinetics of anaerobic degradation were processed using several mathematical models (data not shown) aiming at analyzing and comparing the degradation performance and biogas/biomethane production of samples. Experimental data processing by using mathematical models is an effective strategy for better understanding of (bio)chemical systems, which facilitates the assessment of various process variables for process optimization and technological scale-up [29,36,37]. In Fig. 3, the kinetics of anaerobic degradation was determined using the Cone model which showed a better agreement between experimental and predicted data.

The Cone model [29,38] is expressed by Eq. (1), where $Y(t)$ (mL/g VS) is the cumulative biogas/methane production at time t (d), Y_∞ (mL/g VS) the maximum cumulative biogas/methane production predicted by the kinetic model at $t \rightarrow \infty$, k (d^{-1}) the rate constant, and n the shape factor.

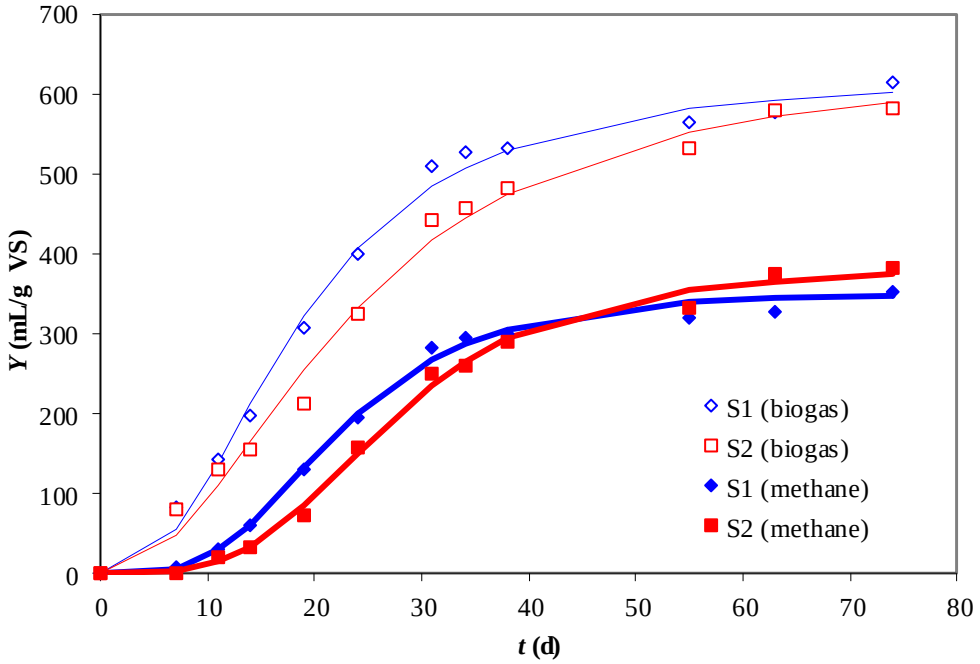

Fig. 3. Time variation of biogas and methane yields (bullets: experimental data, lines: data predicted by Cone model (Eq. (1)).

Adjustable parameters of Cone model, Y_∞, k, and n, which were estimated from experimental data using Solver add-in program (Microsoft Excel), are summarized in Tables 2 and 3. The values of experimental ultimate biogas/methane yield, Y_{ex}, as well as those of root mean square error (*RMSE*) and coefficient of variation (*CV*) defined by Eqs. (2) and (3), where $Y_{ex,mn}$ represents the mean value of $Y_{ex}(t)$, are also specified in Tables 2 and 3. Tabulated results reveal an acceptable agreement between experimental and predicted data (*CV*=4.204-6.046%). Moreover, the values of k are higher for S1 substrate, *i.e.*, k_{S1}/k_{S2}=1.26 for biogas production and k_{S1}/k_{S2}=1.23 for methane production, indicating a decrease in mean process rate in the presence of enzymes.

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

$$RMSE = \sqrt{\frac{\sum_{i=1}^{12}\left(Y_i(t) - Y_{i,ex}(t)\right)^2}{12}} \tag{2}$$

$$CV = \frac{RMSE}{Y_{ex,mn}} \times 100 \quad (3)$$

Table 2

Experimental ultimate biogas yield and adjustable parameters of Cone model for biogas production

Parameter \ Substrate	S1 (SSC)	S2 (SSC+enzymes)
$Y_{B,mn,ex}$ (mL/g VS)	614.016	582.314
$Y_{B,\infty}$ (mL/g VS)	623.022	641.359
k (d^{-1})	0.054	0.043
n	2.416	2.101
RMSE (mL/g VS)	15.597	20.068
CV (%)	4.204	6.046

Table 3

Experimental ultimate methane yield and adjustable parameters of Cone model for methane production

Parameter \ Substrate	S1 (SSC)	S2 (SSC+enzymes)
$Y_{M,m,ex}$ (mL/g VS)	351.432	381.247
$Y_{M,\infty}$ (mL/g VS)	354.462	385.176
k (d^{-1})	0.045	0.037
n	3.393	3.501
RMSE (mL/g VS)	8.805	10.003
CV (%)	4.594	5.527

3.3. Mass percentage of methane in biogas

For a better understanding of the biomethane production of S1 and S2, the mass percentage of methane in biogas resulted in the anaerobic fermentation tests, $P_{ex}(t)$, is displayed in Fig. 4. This is an extremely important parameter to characterize the AD of substrates, as the concentration of biomethane in biogas is proportional to the energy potential or the caloric value of biogas [39].

Initially, for both samples, P_{ex} is low, but progressively increases to exceed 50%, after 14 days of AD. The maximum value of 77.97% is reached after 31 days of fermentation for S1, and of 87.01% after 38 days of fermentation for S2, respectively. Starting with day 34, there is a remarkably different behavior between the outcome of S1 and S2 tests, in terms of biomethane content in biogas.

While for S1, the methane content sharply decreases, for S2, the methane percentage in biogas remains relatively high until the end of the test period. If Figs. 3 and 4 are to be compared, the significant fall in biomethane yield showed by S1 after 38 d of AD could be linked to decrease of methanogenic activity due to substrate depletion.

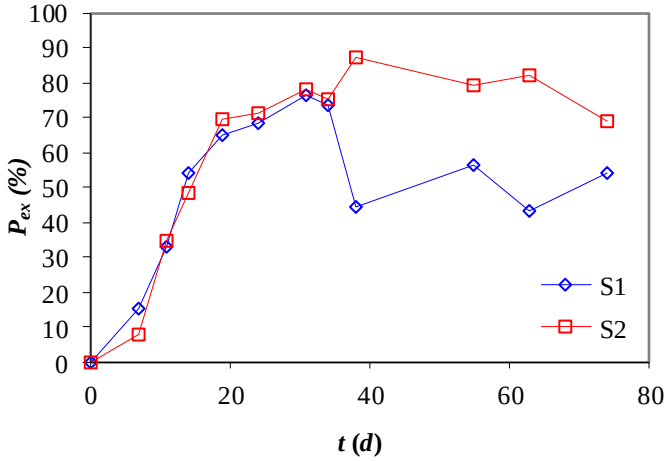

Fig. 4. Time variation of mass percentage of methane in biogas

On the other hand, 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 S2, respectively.

4. Conclusions

Results of biomethane potential tests suggest that sunflower seed cake 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 S2, 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 S2, the AD of S2 provides a more concentrated and thus a more calorific gas which may require less expenditure for purification.

Acknowledgements: This work was supported by a grant of the Romanian Ministry of Research and Innovation, CCCDI - UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0541/ 32PCCD/2018, within PNCDI III.

R E F E R E N C E S

[1]. *D. Gielen, F. Boshell, D. Saygin, M. D. Bazilian, N. Wagner, R. Gorini,* The role of renewable energy in the global energy transformation, Energy Strategy Rev., **vol. 24**, 2019, pp. 38-50. https://doi.org/10.1016/j.esr.2019.01.006

[2]. *Y. S. Park, J. Szmerekovsky, A. Dybing,* Optimal location of biogas plants in supply chains under carbon effects: Insight from a case study on animal manure in North Dakota, J. Adv. Transp., **vol. 21**, no. 13, 2019, article ID 5978753. https://doi.org/10.1155/2019/5978753

[3]. *I. Kushkevych, M. Vítězová, T. Vítěz, J. Kováč, P. Kaucká, W. Jesionek, M. Bartoš, L. Barton,* A new combination of substrates: Biogas production and diversity of the methanogenic microorganisms, Open Life Sci., **vol. 13**, no. 1, 2018. https://doi.org/10.1515/biol-2018-0017

[4]. *I. Angelidaki, L. Treu, P. Tsapekos, G. Luo, S. Campanaro, H. Wenzel, P.G. Kougias,* Biogas upgrading and utilization: Current status and perspectives, Biotechnol. Adv., **vol. 36**, no. 2, 2018, pp. 452–466. https://doi.org/10.1016/j.biotechadv.2018.01.011

[5]. *C. Mateescu, A.D. Dima,* Innovation and technology transfer in energy recovery systems from agro-zootechnical waste, J. Sustain. Energ., **vol. 10**, no. 2, 2019, pp.78-82.

[6]. *L. Gyenge, S. Crognale, S. Lányi, B. Ábrahám, B. Ráduly,* Anaerobic digestion of corn-ddgs: effect of pH control, agitation and batch repetition, U.P.B. Sci. Bull., Series B, **vol.** 76, no. 4, 2014, pp. 163-172.

[7]. *C. Mateescu, A. D. Dima, O. C. Pârvulescu,* Improving the biogas performance of selected waste materials by substrate ratio optimization and microalgae addition, J. Eng. Sci. Innovation, vol. 4, no. 3, 2019, pp. 263-272.

[8]. *M. C. Gould,* Bioenergy and anaerobic digestion, In book: Bioenergy, 2015, pp. 297-317. https://doi.org/10.1016/B978-0-12-407909-0.00018-3

[9]. *C. Mateescu, I. Constantinescu,* Comparative analysis of inoculum biomass for biogas potential in the anaerobic digestion, U.P.B. Sci. Bull., Series B, **vol. 73**, no. 3, 2011, pp. 99-104.

[10]. *G. Esposito, L. Frunzo, A. Giordano, F. Liotta, A. Panico, F. Pirozzi,* Anaerobic co-digestion of organic wastes, Rev. Environ. Sci. Bio., **vol. 11**, no. 4, 2012, pp. 325–341. https://doi.org/10.1007/s11157-012-9277-8

[11]. *A. Salihu, M. Zahangir Alam,* Pretreatment methods of organic wastes for biogas production, J. Appl. Sci., **vol**. **16**, no. 3, 2016, pp. 124-137. https://doi.org/10.3923/jas.2016.124.137

[12]. *A.D. Dima, O.C. Pârvulescu, C. Mateescu, T. Dobre,* Optimization of substrate composition in anaerobic co-digestion of agricultural waste using central composite design, Biomass Bioenerg., **vol. 138**, 2020, article ID 105602. https://doi.org/10.1016/j.biombioe.2020.105602

[13]. *F. Monlau, E. Latrille, A. Carvalho Da Costa, J. P. Steyer, H. Carrère,* Enhancement of methane production from sunflower oil cakes by dilute acid pretreatment, Appl. Energy, **vol. 102**, 2013, pp. 1105-111. https://doi.org/10.1016/j.apenergy.2012.06.042

[14]. *A. Kargopoulos, E. Bonos, Z. Basdagianni, I. Nikolakakis,* Use of sunflower cake in the diet of ISA brown pullets, J. Agr. Sci. Technol. A, **vol. 7**, no. 2, 2017, pp. 129-134. https://doi.org/10.17265/2161-6256/2017.02.008

[15]. *R. Kalmendal, K. Elwinger, L. Holm, R. Tauson,* High-fibre sunflower cake affects small intestinal digestion and health in broiler chickens, Br. Poult. Sci., **vol. 52**, no.1, 2011, pp. 86-96. https://doi.org/10.1080/00071668.2010.547843

[16]. *J. A. Mmongoyo, F. Wu, J. E. Linz, M. G. Nair, J. K. Mugula, R. J. Tempelman, G. M. Strasburg,* Aflatoxin levels in sunflower seeds and cakes collected from micro- and small-scale sunflower oil processors in Tanzania, Plos One, **vol. 12**, no. 4, 2017, article ID 0175801. https://doi.org/10.1371/journal.pone.0175801

[17]. *M. Dincă, G. Voicu, M. Ferdeş, L. Toma, I. Voicea,* Anaerobic digestion of animal manure and maize silage in pilot plant for biogas production, U.P.B. Sci. Bull., Series B, **vol. 78**, no. 2, 2016, pp. 71-80.

[18]. *C. Mateescu, N. O. Nicula, A.D. Dima, E. M. Lungulescu,* Enzymatic pretreatment of algal biomass for enhanced conversion to biogas, J. Eng. Sci. Innovation, vol. 4, no. 4, 2019, pp. 361-370.

[19]. *F. Raposo, R. Borja, B. Rincon, A. M. Jimenez,* Assessment of process control parameters in the biochemical methane potential of sunflower oil cake, Biomass and Bioenergy, **vol. 32**, 2008, pp. 1235–1244. https://doi.org/10.1016/j.biombioe.2008.02.019

[20]. National sunflower Association, https://www.sunflowernsa.com/wholeseed/sunflower-as-a-feed/#top, accessed 19 February 2020.

[21]. *K. Oszust, A. Pawlik, G. Janusz, K. Ziemiński, M. Cyran, A. Siczek, A. Gryta, N. Bilińska-Wielgus, M. Frąc,* Characterization and influence of a multi-enzymatic biopreparation for biogas yield enhancement, Bioresources, **vol. 12**, no. 3, 2017, pp. 6187-6206. https://doi.org/10.15376/biores.12.3.6187-6206

[22]. *J. Li, K. Sun, J. He and Q. Chen,* Using an Amylase Pretreatment of Pig Manure to Enhance Biogas Production, 2011 Asia-Pacific Power and Energy Engineering Conference, Wuhan, 2011, pp. 1-5. https://doi.org/10.1109/APPEEC.2011.5748924

[23]. *S. Bonilla, Z. Choolaei, T. Meyer, E. A. Edwards, A. F. Yakunin, D. G. Allen,* Evaluating the effect of enzymatic pretreatment on the anaerobic digestibility of pulp and paper biosludge, Biotechnol. Rep., **vol. 17,** 2018, pp. 77-85. https://doi.org/10.1016/j.btre.2017.12.009

[24]. APHA-AWWA-WPCF Standards 20th edition, American Public Health Association, American Water Works Association and Water Environment Federation, Washington DC, 1998.

[25] *Liu T., Sun L., Müller B., Schnürer A.,* Importance of inoculum source and initial community structure for biogas production from agricultural substrates, Bioresour. Technol., **vol. 245,** part A, 2017, pp. 768-777. https://doi.org/10.1016/j.biortech.2017.08.213

[26] *Wojcieszak M., Pyzik A., Poszytek K., Krawczyk P.S., Sobczak A., Lipinski L., Roubinek O., Palige J., Sklodowska A., Drewniak L.,* Adaptation of methanogenic inocula to anaerobic digestion of maize silage, Front. Microbiol., 2017. https://doi.org/10.3389/fmicb.2017.01881

[27]. VDI 4630, Verein Deutscher Ingenieure e.V., Düsseldorf 2016.

[28]. *I. Angelidaki, M. Alves, D. Bolzonella, L. Borzacconi, J. L. Campos, A. J. Guwy, S. Kalyuzhnyi, P. Jenicek and J. B. van Lier,* Defining the biomethane potential (BMP) of solid organic wastes and energy crops: A proposed protocol for batch assays, Water Sci. Technol., **vol. 59**, no. 5, 2009, pp. 927-34. https://doi.org/10.2166/wst.2009.040

[29]. *A. D. Dima, C. Mateescu, O. C. Parvulescu, E. M. Lungulescu, N. O. Nicula,* Theoretical and Experimental Results on the Recovery of Potato Processing Residuals by Anaerobic Digestion, Rev. Chim. (Bucharest), **vol. 70,** no. 7, 2019, pp. 2524-2529.

[30]. *R. Moletta, G. Albagnac,* A gas meter for low rates of gas flow: Application to the methane preparatation, Biotechnol. Lett., **vol. 4**, no. 5, 1982, pp. 319–322. https://doi.org/10.1007/BF00132833

[31]. *A. Popescu,* Romania's sunflower seeds production, export and import- analysis of the 2007-2017 period and forecast for 2018-2022 Horizon, Sci. Papers S. Manage., Econ. Eng. Agr. Rural Dev., **vol. 18**, no. 4, 2018, pp. 261-270.

[32]. *C. Lacaze-Dufaure, J. Leyris, L. Rigal, Z. Mouloungui,* A twin-screw extruder for oil extraction: I. Direct expression of oleic sunflower seeds, J. Am. Oil Chem. Soc., **vol. 76**, no. 9, 1999, pp. 1073-1079. https://doi.org/10.1007/s11746-999-0206-0

[33]. *M. Goyal, U. G. Phutela,* Protease production from biodigested slurry of biogas plants: A review, Curr. Microbiol., **vol. 7**, no. 1, 2018, pp. 369-379. https://doi.org/10.20546/ijcmas.2018.701.042

[34]. *Y. Chen, J.J. Cheng, K.S. Creamer,* Inhibition of anaerobic digestion process: A review, Bioresour. Technol., **vol. 99**, 2008, pp. 4044–4064. https://doi.org/10.1016/j.biortech.2007.01.057

[35]. *D. Barik,* Energy extraction from toxic waste originating from food processing industries, in Energy from toxic organic waste for heat and power generation, Woodhead Publishing Series in Energy, 2018, pp. 17-42. https://doi.org/10.1016/B978-0-08-102528-4.00003-1

[36]. *Dobre T., Pârvulescu O. C., Stoica-Guzun A., Stroescu M., Jipa I., Al Janabi A.A.A.,* Heat and mass transfer in fixed bed drying of non-deformable porous particles, Int. J. Heat Mass Tran., **vol. 103**, 2016, pp. 478-485. https://doi.org/10.1016/j.ijheatmasstransfer.2016.07.079

[37]. *Stoica A., Dobre T., Stroescu M., Sturzoiu A., Parvulescu O.,* From laboratory to scale-up by modelling in two cases of β-carotene extraction from vegetable products, Food Bioprod. Process., **vol. 94**, 2015, pp. 218–228. https://doi.org/10.1016/j.fbp.2014.02.005

[38]. *S.Achinas, J. Krooneman, G. J. W. Euverink,* Enhanced Biogas Production from the Anaerobic Batch Treatment of Banana Peels, Eng. J., **vol. 5**, 2019, pp. 970–978. https://doi.org/10.1016/j.eng.2018.11.036

[39]. *M. A. Abdel-Hadi,* Determination of methane content by measurements of flame temperature and voltage from biogas burner, J. Agric. Eng., **vol. 26**, no. 1, 2009, pp. 498- 513. https://doi.org/10.21608/mjae.2020.110168