INFLUENCE OF GAMMA-RAY IRRADIATION ON THE BIOMETHANE PRODUCTION OF SUNFLOWER SEED CAKE

Andreea D. DIMA[1], Oana C. PÂRVULESCU[2*], Carmen MATEESCU[3*], Eduard M. LUNGULESCU[4]

Batch mesophilic anaerobic digestion tests were performed to investigate the biogas and biomethane potential of sunflower seed cake (SuSC) after irradiation pretreatment applying various doses of γ-radiation (0-150 kGy). Sample exposure to γ-rays was carried out using a ^{60}Co source at a dose rate of 1.0 kGy/h. The experimental maximum biogas and biomethane yields were 312.4-557.2 mL/g VS and 141.2-336.5 mL/g VS, respectively, the maximum levels being achieved for untreated SuSC substrate. Compared to the untreated substrate, the biogas production of irradiated samples decreased by 1.3-1.8 times and that of biomethane by 1.2-2.4 times with an increase in the irradiation dose. Some hypotheses were discussed to explain the negative effect of γ-radiation on the process performances. Cone and modified Gompertz models were applied to predict the dynamics of biogas and biomethane production.

Keywords: anaerobic digestion, biomethane, gamma irradiation, sunflower seed cake, kinetic models

1. Introduction

The large amount of waste generated around the world is a matter of high environmental, social, and economic concern, particularly in the view of a strong demographic growth, rise in urbanization, and continuous agro-industrial development [1]. Compared to other waste disposal techniques (*e.g.*, landfilling, open dumping or thermal treatment by pyrolysis, gasification, incineration), anaerobic digestion can better reduce the waste volume and provide superior energy recovery, limiting greenhouse gases emission and harmful pollutants release into the environment [2]. Anaerobic digestion has thus been credited not only for its remarkable waste management efficiency, but also for the considerable energy production in the form of biogas (composed of 55-75% methane) and the good nutrient recycling for soil amendment in the form of

[1] PhD 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*] Associate Prof., Dept. of Chemical and Biochemical Engineering, University POLITEHNICA of Bucharest, Romania,
[3*] PhD, Researcher, Dept. of Environment/Energy and Climate Change, National Institute for Research and Development in Electrical Engineering ICPE-CA, Bucharest, Romania
[4] PhD, Researcher, Dept. of Metallic, Composite and Polymeric Materials, National Institute for Research and Development in Electrical Engineering ICPE-CA, Bucharest, Romania

digestate. However, in spite of its benefits, anaerobic digestion faces several problems associated to operating parameters and substrate quality [2-4]. For instance, the characteristics of the digested substrate could cause process instability, significantly lowering the methane yield of biogas production unit.

When dealing with lignocellulosic materials, the low biodegradability of hemicelluloses and lignin structures in waste substrates hampers the activity of fermentative bacteria which causes higher time requirements for the hydrolysis stage of anaerobic digestion [5,6]. The biogas production is known to develop in four main stages, *i.e.*, hydrolysis, acidogenesis, acetogenesis, and methanogenesis, from which hydrolysis is the rate determining step. Consequently, a longer duration of hydrolysis demands for a longer substrate hydraulic retention time in the bioreactor, which implies lowering efficiency and increasing the overall cost of biogas production [7,8]. In this respect, several substrate pretreatment methods have been developed, willing to achieve a proper cleavage of macromolecules into smaller, solvable compounds prior to anaerobic digestion.

Mechanical pretreatment techniques, such as grinding, chipping or milling, have been widely employed to intensify the enzymatic hydrolysis by increasing the surface area of biomass. However, they generally need coupling to other methods due to low effectiveness and they are seldom used in industrial applications due to excessive energy consumption [9]. Chemical (acid/basic hydrolysis) and enzymatic pretreatments are often more energy intensive, but the use of chemicals comes with the disadvantage of a possible contamination of the end products or could influence the biochemical balance in the fermentation reactors [6]. More recently, other advanced technologies (*e.g.*, *γ*-ray irradiation, microwave, ultrasound, electron beam, pulsed electric field) have drawn attention in the light of encouraging results in treating lignocellulosic biomass, both in terms of efficiency and time economy, resulting in increased return on biogas plant investment [10-14].

High energy radiations in the form of *γ*-rays could induce changes in the chemical structure of exposed materials by radiolysis reactions. In the case of lignocellulosic biomass, studies indicated that *γ*-ray irradiation enhanced the specific surface area of the matter, reduced the polymerization degree of cellulose, improved the hydrolysis of hemicellulose, and caused partial depolymerisation of lignin [15,16]. The efficiency of *γ*-ray irradiation pretreatment depends on substrate composition, irradiation atmosphere, dose rate, and exposure time [10,17]. Macromolecular scission results mainly by chain radical reactions generating fragments with a lower degree of polymerization. Pretreated biomass is thus more readily available for enzymatic attack and easily convertible to energy-carriers such as gaseous and liquid fuels [17]. The effect of *γ*-ray irradiation on the hydrolysis of lignocellulosic materials, including poplar sawdust, bamboo, oil palm empty fruit bunches, wheat straw, soft and hardwood was reported in the related literature [16-18]. For example, the structure of wheat straw was strongly affected by irradiation, a maximum level of glucose yield being reached after enzymatic hydrolysis at a 500 kGy irradiation dose. Moreover, crushing treatment applied to γ-ray pretreated straw resulted in an increase in the rate of enzymatic hydrolysis [18].

Sunflower (*Helianthus annuus*) is one of the most important crops used for the production of vegetable oil. According to the European Institute of Statistics, Romania ranks among the first countries in the European Union in cereal production. In 2018, Romania recorded the highest production in the EU for maize and sunflower and the fourth largest production for wheat. Regarding sunflower seeds harvest, production increased with about 15% compared to 2017, accounting for 3.35 Mt in 2018, at a yield of 2.9 t/ha [19]. Sunflower seeds contain about 50% oil, higher than rape or soybean, which makes them very attractive for oil production. By-products from sunflower oil production include sunflower seeds hulls, cake or meal, depending on whether seed decortication is performed or not prior to oil pressing or extraction. Generally, for processes commonly lacking in a decortication step, the obtained sunflower seed cake (SuSC) is rich in proteins (28-40%) and fibres (15-35%), but the exact composition varies with the characteristics of the used technology, *i.e.*, solvent extraction or pressing [20,21]. It is generally capitalized by using as livestock feed, by burning for heat, or by composting for production of mushroom substrate. Although an important share of SuSC is used for feed purposes, excess or contaminated SuSC biomass which is not proper for animal feeding could be successfully directed to bioenergy production, offering a higher recovery than heat generation by direct combustion. However, this material contains about 15% lignin, impeding processing and specifically causing delay in the hydrolysis step of biogas production [20].

The objective of this study was to investigate the influence of γ-ray irradiation dose (50-150 kGy) on digestibility of SuSC in batch mesophilic anaerobic digestion. 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.

2. Materials and methods

2.1. Materials and physico-chemical analysis

SuSC collected from a local farmer in Prahova country was used as substrate. It was a by-product obtained in the process of oil extraction from undecorticated seeds using a semi-industrial oil pressing machine. Substrate samples were dark brown pellets of maximum 100 mm length and about 10 mm diameter. Prior to being used in the experiments of anaerobic digestion, SuSC pellets were ground and sieved to get a fraction with particle size less than 3 mm.

Fermented sludge type inoculum used for biomethane potential tests was taken from an industrial biogas plant treating agro-biomass and farming residuals. The fermented sludge was kept at room temperature under anaerobic conditions until used to provide fermentation microbiota into the digester. The physico-chemical characterization of SuSC and inoculum was done in compliance to specific standard methods according to a previous paper [22]. All analyses were performed in duplicate.

2.2. Substrate pretreatment

SuSC sample exposure to *γ*-rays was carried out in a laboratory irradiator ObServo Sanguis (Institute of Isotopes, Budapest) equipped with ^{60}Co source and rotary rack for homogenous irradiation. SuSC samples were transferred to zip lock bags, wrapped in aluminium foils, and then irradiated at normal pressure and temperature using an irradiation dose rate of 1.0 kGy/h. Irradiation doses (D) applied to SuSC were 50, 100, and 150 kGy.

2.3. Biochemical methane potential (BMP) tests

A lab-scale experimental set-up (Fig. 1) was used for BMP tests. Ten brown glass serum bottles (each of 240 mL) were placed in a thermostatic water bath and coupled with gas collecting bags.

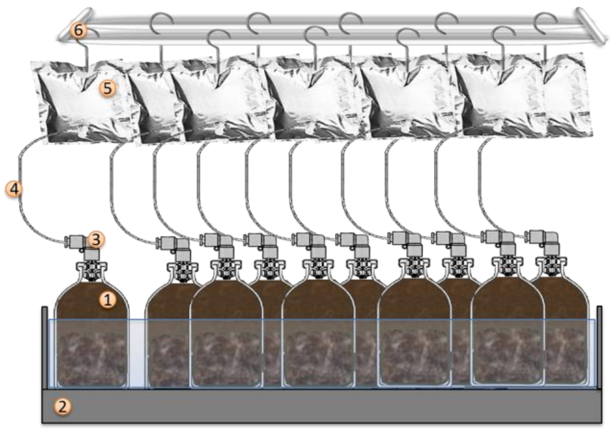

Fig. 1. Experimental set-up: (1) glass serum bottles; (2) thermostatic water bath; (3) connection system; (4) connection tube; (5) gas collecting bag; (6) hanging system for gas bags.

The tests were performed in duplicate for each substrate sample (S1-S4), while two bottles were used to determine the residual biomethane production of inoculum (I). Substrate to inoculum ratio was 2 on volatile solids (VS) basis and total solids (TS) content of substrate in the fermentation suspension was 8% (w/v). To complete the volume of the fermentation mass to a total of 120 mL, distilled water was added, then the bottles headspace was flushed with pure nitrogen to provide anaerobic conditions in the system and gas bags were quickly connected. Bottles necks were pre-adapted to guarantee a leak-free gas transfer to the collection bags which were connected to the bottles by PTFE tubes. The working temperature of the water bath was set within the mesophilic range at 37 ºC. No temperature variations higher than ±0.5 ºC were noticed during the experiment. Homogenization of the reaction mixtures was manually done by slightly shaking the bottles twice a day. All BMP tests lasted 74 days (d), after this period the daily biogas production dropping to less than 2% from the cumulative gas volume. The methane concentration of collected biogas was analysed by gas chromatography as described elsewhere [23]. The biogas volume was determined by water displacement method [24-26]. The specific production of inoculum was subtracted from the production of each sample.

3. Results and discussions

3.1. Physico-chemical parameters of substrate and inoculum

Physico-chemical analysis of untreated SuSC revealed 93.3% TS, 93.6% VS, 10.88% oil content, and a C/N ratio of 18.04, while the inoculum had a pH of 8.3 and contained 8.54% TS and 53.75% VS [22].

3.1. BMP experimental data

Experimental dynamics of biogas (B) and biomethane (M) yields, $Y_{B,ex}(t)$ and $Y_{M,ex}(t)$, where t (d) is the digestion time, are shown in Fig. 2.

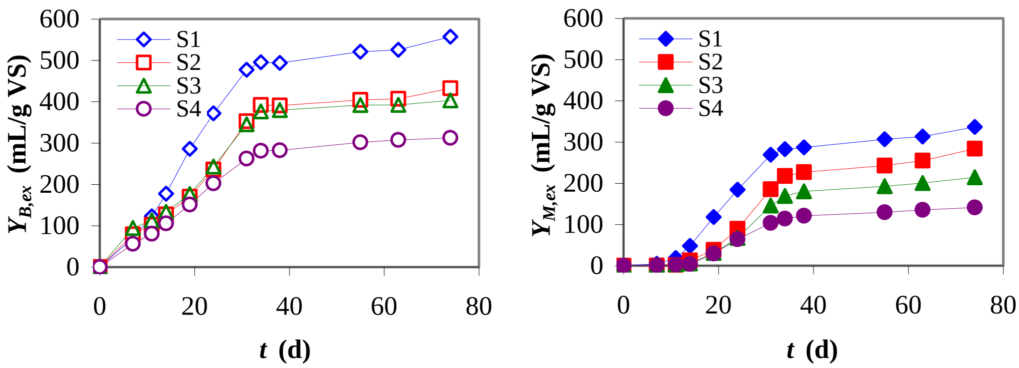

Fig. 2. Time variation of experimental biogas (B) and biomethane (M) yields for untreated (S1) and pretreated (S2-S4) substrates.

Depicted data reveal the following issues: (i) the values of $Y_{B,ex}$ and $Y_{M,ex}$ as well as the process rate were lower for the pretreated material (S2-S4) than for the untreated sample (S1) and they decreased with an increase in the irradiation dose (D=0-150 kGy); (ii) the values of experimental maximum (ultimate) biogas/biomethane yield, $Y_{B,m,ex}$ and $Y_{M,m,ex}$, were of 312.4-557.2 mL/g VS and of 141.2-336.5 mL/g VS, respectively; they were lower (1.3-1.8 times and 1.2-2.4 times) for the pretreated material and decreased linearly (R^2>0.950) with an increase in D from 0 to 150 kGy (Fig. 3a); (iii) the levels of methane concentration in the biogas, $c_{M,ex}$, were of 45.20-65.68% and they decreased linearly (R^2=0.985) with an increase in D from 50 to 150 kGy (Fig. 3b).

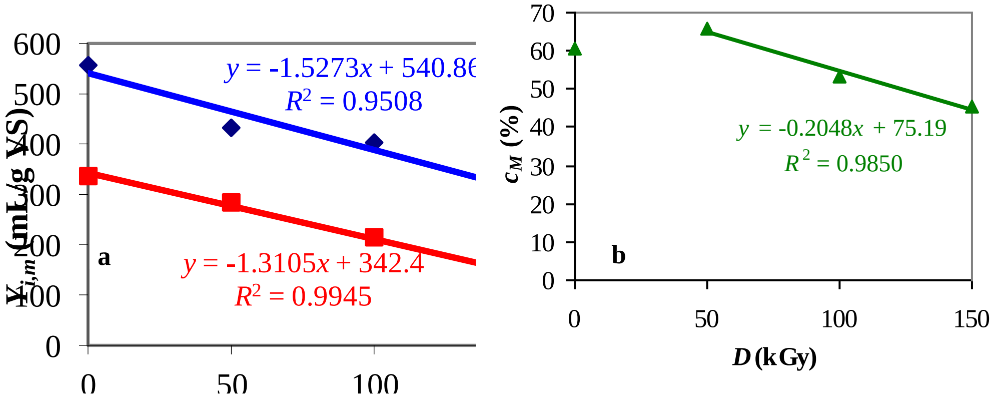

Fig. 3. Maximum (ultimate) biogas (♦) and biomethane (■) yields (a) and methane concentration in the biogas (▲) (b) *vs.* irradiation dose (bullets: experimental data, lines: linear model).

3.2. Kinetic modelling

Cone and modified Gompertz models described by Eqs. 1 and 2, respectively, were applied to predict the dynamics of i product (biogas and biomethane) yield, $Y_i(t)$. Parameters in Eqs. 1 and 2 are as follows: Y_i (mL/g VS) is the product yield at a digestion time t (d), $Y_{i\infty}$ (mL/g VS) the maximum product yield predicted by the kinetic model at $t\to\infty$, k_i (d^{-1}) the rate constant, n_i the shape factor, $r_{i,m}$ (mL/g VS/d) the maximum production rate, and λ_i (d) the lag-phase period [22,23].

Adjustable parameters of Cone and modified Gompertz models in terms of $Y_{i\infty}$, k_i, n_i, r_{im}, and λ_i were estimated based on experimental data using Solver program (Microsoft Excel) by minimizing the root mean square error (*RMSE*) defined by Eq. (3), where N_{ex}=12 represents the number of experimental points. The values of adjustable kinetic parameters, which are summarized in Tables 1 and 2, are within the ranges reported in the literature [27-29].

Table 1

Values of adjustable parameters of kinetic models, root mean square error, and coefficient of variation for biogas production

Model	Substrate / Parameter	S1	S2	S3	S4
Cone	$Y_{B\infty}$ (mL/g VS)	561.8	458.6	442.6	334.1
	k_B (d^{-1})	0.055	0.049	0.051	0.053
	n_B	2.67	2.33	2.10	2.23
	RMSE (mL/g VS)	15.43	25.99	25.75	11.38
	CV (%)	4.52	10.08	10.13	5.83
Modified	$Y_{B\infty}$ (mL/g VS)	560.8	435.2	415.8	314.6

Gompertz	$r_{B,m}$ (mL/g VS/d)	20.70	14.06	13.34	10.81
	λ_B (d)	5.04	4.63	4.31	3.81
	RMSE (mL/g VS)	15.16	21.92	22.61	7.94
	CV (%)	4.44	8.50	8.90	4.07

Table 2

Values of adjustable parameters of kinetic models, root mean square error, and coefficient of variation for biomethane production

Model	Substrate / Parameter	S1	S2	S3	S4
Cone	$Y_{M\infty}$ (mL/g VS)	339.9	288.0	217.7	144.9
	k_M (d^{-1})	0.044	0.036	0.037	0.039
	n_M	3.60	4.35	4.99	4.37
	RMSE (mL/g VS)	9.19	13.11	7.42	4.16
	CV (%)	5.08	10.12	7.34	5.88
Modified Gompertz	$Y_{M\infty}$ (mL/g VS)	339.7	287.2	217.2	143.9
	$r_{M,m}$ (mL/g VS/d)	14.13	12.02	10.35	6.58
	λ_M (d)	10.81	15.87	15.34	14.50
	RMSE (mL/g VS)	11.67	14.42	10.63	4.46
	CV (%)	6.45	11.13	10.52	6.31

The levels of *RMSE* as well as those of coefficient of variation (*CV*) defined by Eq. (4), where $Y_{i,ex,mn}$ is the mean value of $Y_{i,ex}(t)$, are also specified in Tables 1 and 2. Tabulated results indicate an acceptable agreement between experimental and predicted data (*CV*=4.07-11.13%). The values of maximum biogas and methane yields predicted by Cone model at $t\rightarrow\infty$, $Y_{B\infty}$ and $Y_{M\infty}$, were 334.1-561.8 mL/g VS and 144.9-339.9 mL/g VS, whereas those of rate constants, k_B and k_M, were of 0.049-0.055 d^{-1} and of 0.036-0.044 d^{-1}.

$$Y_i(t)=\frac{Y_{i\infty}}{1+(k_i t)^{-n_i}} \tag{1}$$

$$Y_i(t)=Y_{i\infty}\exp\left\{-\exp\left[\frac{r_{i,m}\exp(1)}{Y_{i\infty}}(\lambda_i-t)+1\right]\right\} \tag{2}$$

$$RMSE=\sqrt{\frac{\sum_{j=1}^{N_{ex}}\left(Y_i(t_j)-Y_{i,ex}(t_j)\right)^2}{N_{ex}}} \tag{3}$$

$$CV=\frac{RMSE}{Y_{i,ex,mn}}\times 100 \tag{4}$$

The values of $Y_{B\infty}$ and $Y_{M\infty}$ were lower (1.2-1.7 times and 1.2-2.4 times) for the pretreated material (S2-S4) and they decreased linearly (R^2>0.937) with an increase in *D* from 0 to 150 kGy, as shown in Fig. 4a. The values of k_B and k_M

were lower (up to 1.2 times) for the pretreated material and they presented a slight linear increase (R^2>0.964) with an increase in D from 50 to 150 kGy (Fig. 4b)

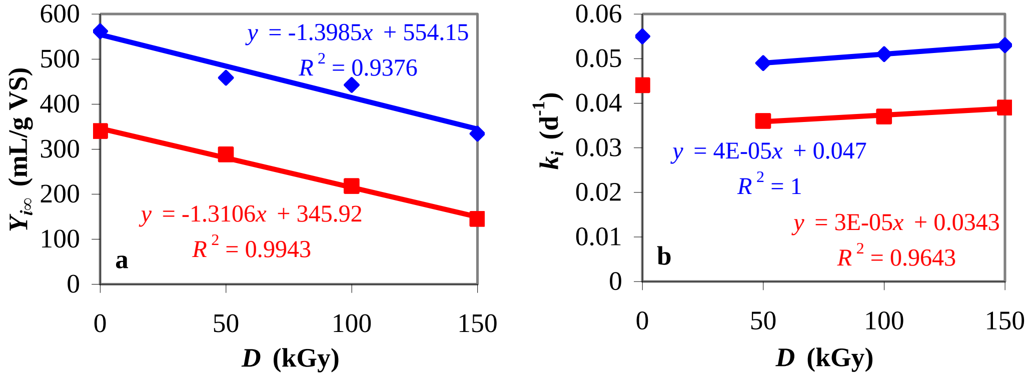

Fig. 4. Maximum product yields at $t \to \infty$ (a) and rate constants (b) for biogas (♦) and biomethane (■) production *vs.* irradiation dose
(bullets: data predicted by Cone model (Eq. (1)), lines: linear model).

The values of characteristic kinetic parameters of modified Gompertz model were as follows: $Y_{B\infty}$=314.6-560.8 mL/g VS, $Y_{M\infty}$=143.9-339.7 mL/g VS, $r_{B,m}$=10.81-20.70 mL/g VS/d, $r_{M,m}$=6.58-14.13 mL/g VS/d, λ_B=3.81-5.04 d, and λ_M=10.81-15.87 d. The values of $Y_{B\infty}$ and $Y_{M\infty}$ were lower (1.3-1.8 times and 1.2-2.4 times) for the pretreated material (S2-S4) and they decreased linearly (R^2>0.937) with an increase in D from 0 to 150 kGy, as shown in Fig. 5a.

Moreover, the levels of $Y_{B\infty}$ and $Y_{M\infty}$ for Cone and modified Gompertz models were almost the same. The values of $r_{B,m}$ and $r_{M,m}$ were lower (1.5-1.9 times and 1.2-2.2 times) for the pretreated sample and they decreased linearly (R^2>0.865) with an increase in D from 0 to 150 kGy (Fig. 5b). The levels of λ_B were 1.1-1.3 times lower for the pretreated samples and decreased linearly (R^2=0.993) with an increase in D from 0 to 150 kGy, whereas those of λ_M were 1.3-1.5 times higher for the pretreated material and decreased linearly (R^2=0.984) with an increase in D from 50 to 150 kGy (Fig. 5c).

Experimental and predicted dynamics of biogas and biomethane yields are shown in Figs. 6 and 7. Depicted data reveal that results predicted by both Cone and modified Gompertz models are almost identical. It can be therefore noted that the Cone and Gompertz models were both suitable for predicting the dynamics of biogas and biomethane production when untreated and irradiated SuSC were used as substrates for anaerobic digestion. 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 [17].

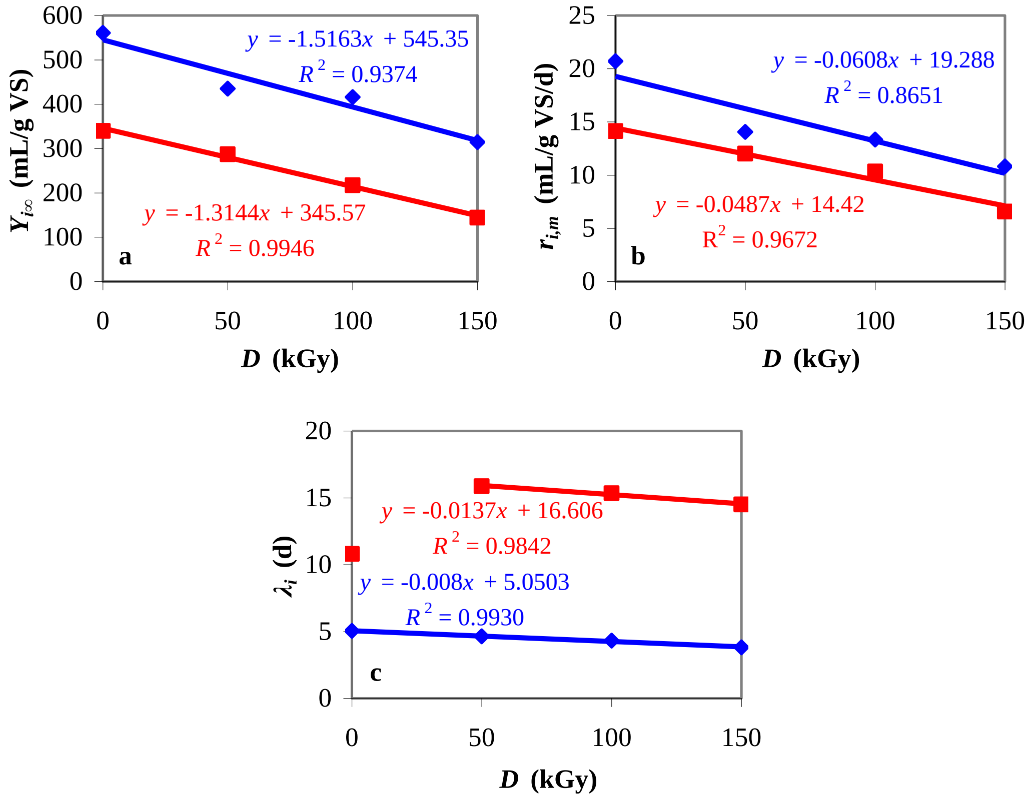

Fig. 5. Maximum product yields at $t \rightarrow \infty$ (c) for biogas (♦) and biomethane (■) production *vs.* irradiation dose (bullets: data predicted by modified Gompertz model (Eq. (2)), lines: linear model)

A possible explanation of the obtained results might be the over-acidification of the fermentation medium, as an effect of the excessive accumulation of acid-precursors as biodegradation products into the system. For example, Liu et al. obtained high levels of sugars after they exposed various types of lignocellulosic biomass to γ-ray irradiation and enzymatic hydrolysis, assuming this behaviour was due to effective modifications in the cellulose surface and degradation induced by γ-radiation [30]. The radiations affected the degree of polymerization and crystallinity of cellulose from the sunflower seed hulls, which are directly linked to increasing enzymatic hydrolysis rate [31,32]. Accordingly, an excessive increase of fermentable sugar concentration and a fast release of organic acids may induce a disruption of acid-base equilibrium in the digester by a pH drop in the fermentation medium, causing inhibition of methanogenic activity and switch of biochemical reactions towards carbon dioxide generation [33].

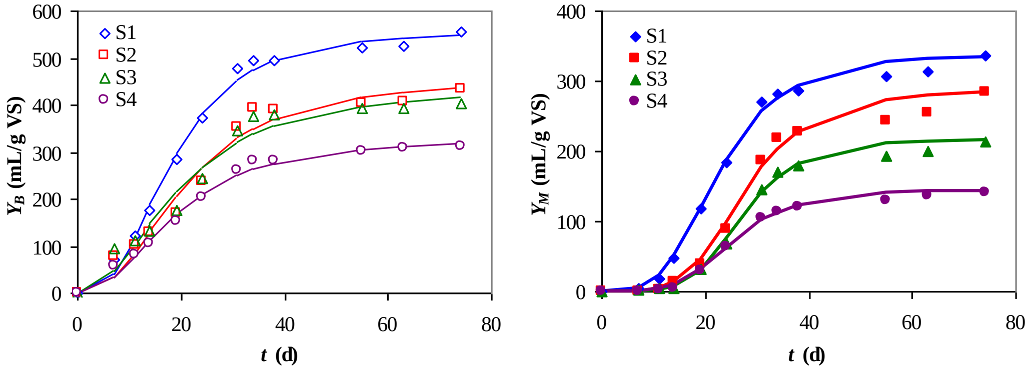

Fig. 6. Time variation of biogas and biomethane yields for untreated (S1) and pretreated (S2-S4) substrates (bullets: experimental data, lines: data predicted by Cone model (Eq. (1)).

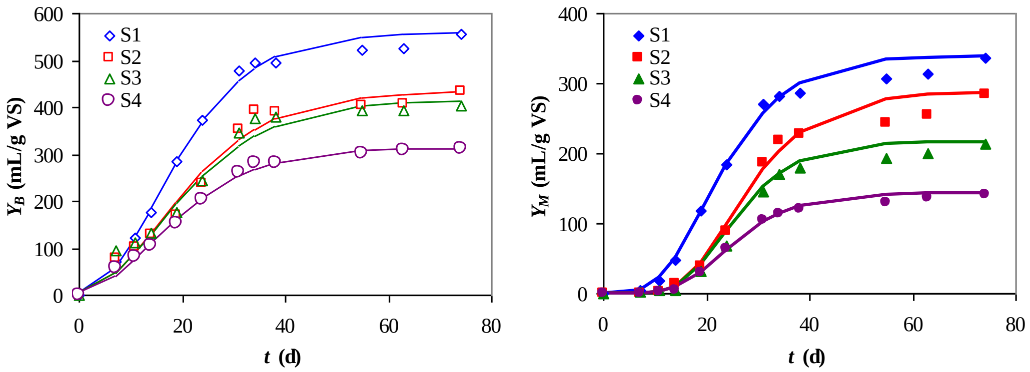

Fig. 7. Time variation of biogas and methane yields for untreated (S1) and pretreated (S2-S4) substrates (bullets: experimental data, lines: data predicted by modified Gompertz model (Eq. (2)).

Faster degradation of triglycerides from the feedstock material could also contribute to the pH decrease, particularly that chemical analysis showed high oil content (10.88%) in the SuSC samples. Niyas et al. reported that γ-rays altered the lipid content of nutmeg at doses higher than 5 kGy and led to free fatty acids release, which are easier to be consumed by lipases [34]. In the anaerobic digestion process, fats are hydrolysed to fatty acids and glycerol, followed by degradation to organic acids, which induce pH reduction and inhibition of methanogenic activity [33]. The negative effect of ionizing γ-radiation on the methane production of SuSC could also be explained considering that ionizing radiations may not only cause the degradation of biomass, but could also result in chemical bond formation, depending on the irradiation conditions and the chemical structure of the exposed material. In other words, during material irradiation, two competing processes may occur simultaneously: scission of the main polymer chains (accompanied by a decrease in molecular weight) and cross-linking (process of formation of chemical bonds between two or more macro-molecular chains, which leads to the increase in the molecular weight and, under certain conditions, to the formation of a 3D network) [35,36]. Both processes are initiated by radio-induced free radicals. Depending on the irradiation conditions (*e.g.*, irradiation dose, oxygen content of the environment) and the nature of the irradiated material, one of the two processes is predominant. The material suffers degradation only if the chain scission reactions prevail [37-39]. In case of low oxygen content, the cross-linking process is predominant [38,40]. The SuSC samples were irradiated in quasi-sealed bags and oxygen existing in the medium or dissolved in the sample was limited, being quickly consumed by reactions with free radicals, therefore the crosslinking of biopolymer chains such as those of hemicellulose and cellulose is most likely predominant. Hence, high molecular weight compounds that make the enzymatic hydrolysis difficult are formed. Irradiated proteins, for instance, generate protein radicals that often have a long half-life and undergo cross-linking reactions forming polymers that are hardly accessible to enzymes or delaying the hydrolysis stage of anaerobic digestion [36]. Besides lipids and fibres, SuSC also contains a high amount of crude proteins (about 28-40%) [20].

Another hypothesis to consider is the chemical structure of lignin which is a highly branched phenolic polymer [41]. Rao et al. studied the effect of γ-ray irradiation on lignin and observed that it had a strong scavenging free radicals effect, leading to peroxyl radical reduction [42]. These species are promoters of radio-oxidative degradation of the material [39,43,44]. Thus, the lignin could prevent the degradation of the most radiation sensitive biopolymers, mainly of cellulose and hemicellulose from SuSC [41]. To overcome these effects, the γ-ray irradiation in the presence of water [42,44] and investigation of a larger range of irradiation dose should be considered.

4. Conclusions

Results indicate that besides its current recovery options, SuSC may also be a promising substrate for biomethane production by anaerobic digestion. The values of experimental maximum (ultimate) biogas and biomethane yields, $Y_{B,m,ex}$ and $Y_{M,m,ex}$, obtained after 74 d in batch mesophilic anaerobic digestion tests using untreated SuSC 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 SuSC 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. The values of $Y_{B\infty}$ (314.6-561.8 mL/g VS) and $Y_{M\infty}$ (143.9-339.9 mL/g VS) predicted by Cone and modified Gompertz models were almost the same and up to 2.4 times lower for the pretreated samples (S2-S4) than for the untreated sample (S1). Moreover, they decreased linearly (R^2>0.937) with an increase in D. The values of rate constants, k_B (0.049-0.055 d^{-1}) and k_M (0.036-0.044 d^{-1}), were lower (up to 1.2 times) for the pretreated material and they increased slightly with an increase in D from 50 to 150 kGy. The levels of lag-phase period, λ_B (3.81-5.04 d) and λ_M (10.81-15.87 d), were up to 1.3 times lower and 1.5 times higher for the pretreated samples, respectively, as well as they decreased linearly (R^2>0.984) with an increase in D from 50 to 150 kGy.

Acknowledgements

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