

## **Laboratory analyses for assessing the potential for biogas production of various agricultural residues in Greece**

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**Abstract.** Greece produces significant amounts of agricultural and livestock waste. For the needs of this study, Greece was divided into a Northern and a Southern part and relevant proposals were made for residues that can be used for energy production, through anaerobic digestion. For Northern Greece, this study concluded that the most abundant residues and potential substrates for anaerobic digestion valorisation are those of maize, inedible vegetables (including greenhouse vegetables), cattle manure, as well as the residues of beer and wine industry. For Southern Greece, the corresponding substrates are those of maize, inedible vegetables, sheep/goat manure and residues of wine, tomato, orange and olive processing, respectively. Based on the physicochemical characterization of individual feedstocks, corn silage, tomato husks, watermelon, malt, cattle manure, orange, and olive processing residues (olive pomace) were considered as the most suitable feedstocks for anaerobic digestion. Biochemical Methane Potential (BMP) assays for Northern Greece were also performed, testing the most abundant and appropriate residues for anaerobic digestion (of this area), namely corn silage, cattle manure and malt, in order to define their BMP yield as well as their prospective optimum mixtures. It was concluded that the BMP of the mono-substrates is in accordance with literature, while there were no statistically significant differences in the methane yield of all tested mixtures. The residual biomass originating from the three main categories of the agricultural sector (crop residues, agro-industrial residues, and animal manure) in Northern Greece can be efficiently valorised via anaerobic co-digestion, without observing, though, any synergistic effects on methane production.

**Key words:** agricultural residues, anaerobic digestion, biogas, BMP assays, Greece, residue characterization

### **INTRODUCTION**

Greece is an agricultural country and considerable proportion of its residual biomass consists of crop residues and livestock manure (Lais et al., 2017). Almost 70% of the total area of Greece is used for agricultural activities. This is the reason why the country exhibits a high biomass potential for renewable energy production (Sagani et al.,

2019). Most European countries use residual biomass as a significant resource for electricity and thermal energy production. However, in Greece, only a small percentage of it is used to meet the electrical energy needs, as the country still largely depends on fossil fuels. In general, 61% of national energy needs are met by fuel imports and the rest (39%) through national energy sources such as lignite (77%) and renewable energy systems (22%) (Moustakas et al., 2020). Despite their vast availability, huge amounts of agricultural residues and animal manures are disposed of uncontrollably in the environment or in landfills, while farmers usually proceed to open burning of residual biomass in their fields, even though these solutions lead to deterioration of the Greek environment (Alatzas et al., 2019).

Residual biomass from the agricultural sector usually appears in the form of crop residues, agro-industrial residues, and animal manure. Vlyssides et al. (2015) have recently estimated the total annual residues production in Greece. According to their study, the production amounts to 45,957,990 t year<sup>-1</sup>, taking into consideration the estimations for the annual production of agro-industrial residues and livestock manure. The theoretical annual potential of agro-industrial residues (such as wheat products, potatoes, olives, fruits, dairy products etc.) was found to be 19,005,490 t year<sup>-1</sup>, while the corresponding amount of livestock manure (cattle, chicken, pigs, sheep etc.) was 26,952,500 t year<sup>-1</sup>. As the estimated residual biomass could be an energy source with essential contribution to the Greek energy balance, studying the energy generation potential of these waste streams is of great importance (Vlyssides et al., 2015). The choice of the energy conversion process depends on the physicochemical properties of biomass, such as its moisture content and the stoichiometric ratio C:N. According to the literature, the described residues are characterized by high moisture content (above 50–55%) and low C:N ratio (below 30), (Skoulou & Zabaniotou, 2007; Vlyssides et al., 2015).

Anaerobic digestion (AD) is a biological process through which different organic wastes can be converted to biogas (methane and carbon dioxide), under anaerobic conditions (Vlyssides et al., 2015; Zhao et al., 2016). AD process can be considered as one of the best environmental and low-cost solutions for the treatment of different biodegradable wastes, which are generated due to anthropogenic activities (Moustakas et al., 2020). The application of AD on single waste streams sometimes implies undesirable inhibitory effects due to toxic compounds or metals' presence. A solution to this problem could be the anaerobic co-digestion of two or more waste streams. This perspective is considered promising enough for overcoming the inhibition effects and thus enhancing the efficiency of the AD process (Dareioti & Kornaros, 2015; Kashi et al., 2017). Even if AD has been extensively studied for several years, in Greece there is still lack of experience concerning biomass residues' valorisation from the three main categories that are produced in Greece, i.e. crop residues, agro-industrial residues and animal manure.

A reliable and simple method to obtain the extent and the rate of organic wastes conversion into methane is the Biochemical Methane Potential (BMP) assays, by which information can be derived about the methane potential of each substrate, the operation conditions and the anaerobic inoculum efficiency. As BMP assays are characterized as a tool for the design optimization and operation of anaerobic digesters, their application is mandatory before any large-scale reactor performance (Tsigkou et al., 2019).

Concerning the aim of this study, Greece was divided into a Northern and a Southern part and relevant proposals were made for residues that can be exploited for energy production through AD. Following our estimations and detailed survey, samples were collected from all promising feedstocks (Northern and Southern Greece) for AD to conduct physicochemical characterization. BMP assays of Northern Greece feedstocks followed, in order to assess the wastes' BMP values and define thus the most promising mixtures as well as their potential synergistic effects, based on detailed design of experiment (DOE).

## **MATERIALS AND METHODS**

### **Materials**

As Greece was divided into the Northern and the Southern part, relevant proposals were made for residues that can be used for energy production through the AD process. Briefly, for Northern Greece the most abundant and suitable residues for AD are those of maize, inedible vegetables (vegetables characterized by low quality, including greenhouse vegetables, as well as their crop residues such as stalks), cattle manure, beer and wine industry residues, while for Southern Greece are those of maize, inedible vegetables, sheep/goat manure and residues of wine, tomato, orange and olive processing (two-phase decanter residues). This information is extracted from the data of the Hellenic Statistical Authority (<https://www.statistics.gr/en/statistics/agr>). The areas where the main crops are produced and the main categories of livestock are bred, were determined according to the abovementioned statistical data. The areas with the highest crop production and breeding activity lead respectively to the highest amount of biomass residues.

Samples were collected from all promising feedstocks for physicochemical characterization, followed by BMP assays. Nevertheless, sheep and goat manure presents serious difficulties for its collection due to inaccessibility to the livestock farms and thus it was not considered as potential feedstock, in this study. The raw residues that were evaluated in the current work included (a) crop residues (corn silage, tomato stalks, and watermelon), (b) agro-industrial residues (tomato husks, wine marcs, malt, olive pomace, and orange processing residues), and (c) animal manure (cattle manure). Regarding the crop residues, the corn silage which was used in the current study was spoiled animal feed, while the tomato stalks and the unsuitable for human consumption watermelons were collected directly from the field. The agro-industrial residues, namely tomato husks, wine marcs and malt were collected from the corresponding processing plants. Finally, the cattle manure (liquid part) was collected after the separation of the solid residue.

All samples were collected from small local plants in the area of Patras, Western Greece. Because of their seasonal availability and high tendency to fermentation, all samples were collected fresh and stored in the freezer at -18 °C, before further treatment.

### **Anaerobic inoculum**

The anaerobic sludge, which was used as inoculum for the BMP assays, was obtained from a pilot-scale mesophilic anaerobic digester with working volume of 15 L operating at the Laboratory of Biochemical Engineering and Environmental Technology, Department of Chemical Engineering, University of Patras (Greece). The digester was

fed with a mixture of bread, meat and fruits/vegetables (which does not meet the quality and safety standards for human consumption) at an Organic Loading Rate of  $0.2 \text{ g VS} \cdot \text{L}_{\text{Reactor}}^{-1} \cdot \text{d}^{-1}$ . Concerning the characteristics of the anaerobic inoculum, TS were measured at  $44.02 \pm 0.31 \text{ g L}^{-1}$ , 53.4 % of which were VS.

### **Analytical Methods**

Physicochemical parameters such as pH, humidity, total solids (TS), volatile solids (VS), total suspended solids (TSS), and volatile suspended solids (VSS), total and soluble chemical oxygen demand (t-COD and d-COD), total and soluble carbohydrates (t-CHO and d-CHO), soluble phenolic compounds (d-phenols), total Kjeldahl nitrogen (TKN), proteins, ammonia/ammonium nitrogen ( $\text{NH}_3\text{-N}$ ), fats/oils, total and soluble phosphorus (t-P and d-P) were measured according to the Standard Methods for the Examination of Water and Wastewater (APHA AWWA WEF, 2012). The lignin, cellulose, and hemicellulose content was determined using freeze-dried material from each feedstock according to the protocol of NREL (Sluiter et al., 2011). The biogas composition analysis was conducted using a gas chromatograph (Agilent Technologies 7890A) with a thermal conductivity detector (TCD), as described in detail by Dareioti et al. (2014).

### **Biochemical Methane Potential Assay**

BMP refers to the experimental procedure developed to determine the maximum methane potential of a given organic substrate during its anaerobic decomposition (Owen et al., 1979; Chynoweth et al., 1993). In this study, the BMP assays were carried out concerning Northern Greece residual biomass and more specifically the most abundant residues that are produced during winter and summer, in this area. Both seasons, share the same feedstocks, such as corn silage, malt (main by-product of brewery), and cattle manure.

The experimental design of BMP assays was realized according to DOE (Design of Experiments) for mixtures with a design degree 3 in Minitab, 2019. In DOE for mixtures, the proportions of the constituents are variable, while their total amount remains unchanged. Such DOEs, try to model the blending surface with mathematical equation forms based on data that are procured for the set of mixture compositions. Afterwards the system's response can be estimated for any other desired mixture composition by the developed model (Kashi et al., 2017).

The biogas volume produced is usually expressed in standard pressure (1 atm) and temperature ( $0 \text{ }^\circ\text{C}$ ) conditions (STP conditions) (Dareioti, 2015). The BMP protocol followed in this study was based on the principles described by Angelidaki et al. (2009). Each test was conducted in duplicate. Known amounts of substrate ( $2 \text{ g VS L}^{-1}$ ) and active anaerobic inoculum ( $20\% \text{ v v}^{-1}$ ) were added to 160-mL closed serum vials. Nutrient medium containing macro-micronutrients, vitamins etc., as described in detail by Angelidaki et al. (2009), was also added to the vessels in order to ensure the optimal function of the anaerobic inoculum. Two serum vials with the abovementioned composition (without substrate addition) were used as blank (control) in order to subtract the endogenous methane production of the inoculum. The assay vessels were flushed continuously with  $\text{N}_2/\text{CO}_2$  (80/20% as volume) for about 5 min, after transferring the substrate, the medium, and the inoculum followed by the sealing of the vessels with a thick butyl-rubber stopper and an aluminum crimp (Angelidaki et al., 2009). Once

sealed, the bottles were placed in an orbital shaking water bath at approximately 90 rpm and maintained at a constant mesophilic temperature at 37 °C. The produced biogas was measured daily for the first 6 days of the experiment, while for the remaining experimentation period (34 days) measurements were taken every 2–7 days, depending on the produced biogas volume. A precision gas syringe (50 mL volume) was used for the collection and quantification of the produced biogas. Concerning the final biogas composition, corrections were occurred for the conversion to dry biogas (STP conditions) as well as for water loss during biogas quantification, according to Tsigkou et al. (2019). The final BMP of each sample represents the sum of the total methane produced and released via the gas syringe, as well as the methane contained in the headspace volume. Concerning the evaluation of possible synergistic effects during co-digestion of tested feedstocks, the equation (1) was used, as described in detail by Tsigkou et al. (2021).

$$\text{Expected BMP (ml CH}_4\text{ g VS}_{\text{added}}^{-1}\text{)} = \frac{(V_{S1} * VS_{S1} * BMP_{S1}) + (V_{S2} * VS_{S2} * BMP_{S2}) + (V_{S3} * VS_{S3} * BMP_{S3})}{V_{S1} * VS_{S1} + V_{S2} * VS_{S2} + V_{S3} * VS_{S3}} \quad (1)$$

where  $V$ ,  $VS$  and  $BMP$  are the volume, volatile solids and experimental BMP, respectively for each tested mono-substrate.

## RESULTS AND DISCUSSION

### Physicochemical characterization

The results obtained from the physicochemical characterization of raw feedstocks are presented in Table 1. The characterization of samples showed that almost all raw residues have high moisture content, which is above 50%, except from wine marc residues (30%). In addition, the fats/oils content (especially for the case of olive pomace, wine marcs and tomato husks) are quite high, while the protein content is of the same order of magnitude for all tested substrates. The aforementioned characteristics indicate the potential suitability of these substrates for treatment via anaerobic digestion (Zhao et al., 2016). However, taking into account both the physicochemical analysis as well as visual observation of tomato stalks, it was realized that these crop residues are very fibrous, which could cause serious operating problems (blocking of pumps, valves etc.) in the subsequent anaerobic digestion experiments.

Even though wine marcs are characterized by sufficient amounts of carbohydrates, proteins and lipids, low anaerobic biodegradability is reported in literature (compared to other agro-industrial materials) (Nikolaidou et al., 2016), probably due to the extremely high percentage of lignin (42.64% in our study), which is a limiting factor for the anaerobic digestion process (Zhao et al., 2016). Therefore, corn silage, tomato husks, watermelon, malt, cattle manure, orange and olive pomace were chosen for the anaerobic digestion process due to various limitations of the non-selected substrates (such as high fiber content, low biodegradability etc.).

**Table 1.** Physicochemical characteristics of raw feedstocks

Parameters	Animal manure				Crop residues				
	Cattle manure		Corn silage		Tomato stalks		Watermelon		
	(g L <sup>-1</sup> )	(g kg <sup>-1</sup> )	Malt	Olive pomace					
	Average ± SD				min-max				
pH	7.02 ± 0.01	4.95 ± 0.01	6.37 ± 0.03	4.55 ± 0.01	4.44 ± 0.01	4.09 ± 0.03	6.50 ± 0.03	4.86–6.45	3.42–4.47
Alkalinity <sup>1</sup>	11.18 ± 0.01	n.d. ±	n.d.	0.18 ± 0.01	n.d.	n.d.	n.d.	n.d.	1,950–2,062
t-COD	60.80 ± 2.25	332.96 ± 30.93	125.04 ± 36.31	75.64 ± 0.21	90.63 ± 25.58	184.21 ± 17.44	60.74 ± 0.99	n.d.	1,030–1,140
d-COD	20.97 ± 0.74	n.d.	n.d.	67.93 ± 0.94	n.d.	n.d. ±	n.d.	n.d.	n.d.
TS	45.83 ± 3.87	413.95 ± 7.78	274.56 ± 15.65	50.12 ± 5.49	320.82 ± 2.00	693.48 ± 3.43	250.11 ± 2.01	n.d.	151–281.60
VS	33.20 ± 2.92	394.29 ± 7.40	216.31 ± 16.37	44.17 ± 3.21	309.65 ± 1.77	619.06 ± 11.73	240.22 ± 1.65	n.d.	146–203.90
TSS	17.18 ± 1.09	n.d.	n.d.	7.56 ± 0.50	n.d.	n.d.	n.d.	n.d.	n.d.
VSS	14.09 ± 0.75	n.d.	n.d.	7.27 ± 0.37	n.d.	n.d.	n.d.	n.d.	n.d.
Humidity <sup>2</sup>	95.42 ± 0.39	58.60 ± 0.78	72.54 ± 1.57	94.99 ± 0.55	67.92 ± 0.20	30.65 ± 0.34	74.99 ± 0.20	48.20–64.50	71.84–84.90
t-CHO <sup>3</sup>	9.23 ± 1.41	221.96 ± 36.75	25.99 ± 2.40	58.05 ± 5.52	39.62 ± 1.16	55.04 ± 5.65	18.77 ± 2.97	46–146	n.d.
d-CHO <sup>3</sup>	0.55 ± 0.01	n.d.	n.d.	54.83 ± 2.56	n.d.	n.d.	n.d.	n.d.	n.d.
d-phenols <sup>4</sup>	1.01 ± 0.00	n.d.	n.d.	0.31 ± 0.02	n.d.	n.d.	n.d.	0.5–12.20	n.d.
TKN	3.21 ± 0.23	3.80 ± 0.06	3.85 ± 0.20	1.11 ± 0.18	10.45 ± 0.74	8.19 ± 0.85	6.53 ± 0.03	n.d.	11.67–13
Proteins	20.07 ± 1.46	23.76 ± 0.35	24.06 ± 1.23	6.91 ± 1.11	65.31 ± 4.62	51.20 ± 5.30	40.79 ± 0.17	n.d.	65.40–69.40
NH <sub>3</sub> -N	1.97 ± 0.02	0.58 ± 0.10	0.49 ± 0.03	0.07 ± 0.00	0.65 ± 0.02	1.04 ± 0.15	0.71 ± 0.03	n.d.	1.49–1.87
Fats and oils	1.67 ± 0.01	6.60 ± 0.49	5.47 ± 0.12	0.51 ± 0.11	13.98 ± 0.44	32.63 ± 0.29	5.96 ± 2.36	142–262	n.d.
t-P	4.05 ± 0.09	6.01 ± 0.09	6.85 ± 0.22	1.12 ± 0.07	6.79 ± 0.02	12.92 ± 0.18	6.57 ± 0.15	0.50–2.75	1.15–1.21
d-P	0.22 ± 0.01	n.d.	n.d.	1.12 ± 0.05	n.d.	n.d.	n.d.	n.d.	n.d.
Cellulose <sup>2</sup>	n.d.	19.48 ± 1.73	11.11 ± 0.31	n.d.	7.45 ± 0.80	16.51 ± 0.01	7.34 ± 0.68	16.50–22.80	20.03–22.43
Hemicellulose <sup>2</sup>	n.d.	5.16 ± 0.94	8.93 ± 0.49	n.d.	4.67 ± 2.02	16.72 ± 0.01	5.76 ± 0.29	19.10–38.70	11.65–12.50
Lignin <sup>2</sup>	n.d.	4.97 ± 0.08	6.49 ± 0.93	n.d.	12.53 ± 1.42	42.64 ± 0.69	3.35 ± 0.09	19.60–47.50	13.02–15.52

n.d.: not determined; <sup>1</sup>g CaCO<sub>3</sub> L<sup>-1</sup> or g CaCO<sub>3</sub> kg<sup>-1</sup>; <sup>2</sup>expressed as percentage; <sup>3</sup>measured as equivalent glucose; <sup>4</sup>measured as equivalent syringic acid; <sup>5</sup>values from Cayuela et al. (2006); López-Piñeiro et al. (2008); Ntougias et al. (2013); <sup>6</sup>values from Martin et al. (2018); Zema et al. (2018); Rokaya et al. (2019); Jiménez-Castro et al. (2020).

## Biochemical Methane Potential assays

The results of the BMP assays (expressed as mL CH<sub>4</sub> g VS<sub>added</sub><sup>-1</sup>) concerning the Northern part of Greece (namely the feedstocks of corn silage, cattle manure and malt) are presented in Table 2. The ratios of fresh matter and the corresponding VS ratios of the tested substrates, which were added to the vials after the DOE, are also presented in the same Table. In addition, the results of the BMP assays are illustrated in Fig. 1.

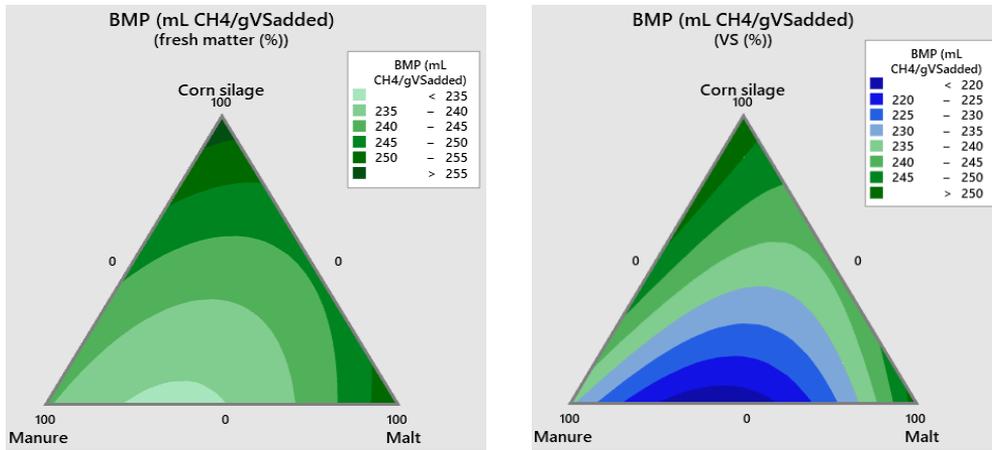
**Table 2.** Volume and VS ratios of mixtures, experimental and expected BMP values, expressed as mL CH<sub>4</sub> g VS<sub>added</sub><sup>-1</sup>

Corn silage	Cattle manure	Malt	Corn silage	Cattle manure	Malt	Measured BMP	Expected BMP
(% w/w fresh matter)			(% VS)			(mL CH <sub>4</sub> g VS <sub>added</sub> <sup>-1</sup> ± SD)	(mL CH <sub>4</sub> g VS <sub>added</sub> <sup>-1</sup> )
0	0	100	0	0	100	255.23 ± 16.40	-
100	0	0	100	0	0	262.40 ± 34.37	-
0	100	0	0	100	0	236.08 ± 3.36	-
0	50	50	0	12.14	87.86	239.35 ± 11.17	252.90
50	0	50	62.14	0	37.86	259.25 ± 10.04	259.68
50	50	0	92.23	7.77	0	254.60 ± 7.28	260.36
33.33	33.33	33.33	59.05	4.97	35.98	238.60 ± 5.87	258.51
16.67	16.67	66.67	28.40	2.39	69.21	232.65 ± 10.96	256.81
66.67	16.67	16.67	85.22	1.79	12.98	226.70 ± 32.53	260.99
16.67	66.67	16.67	51.39	17.31	31.31	243.03 ± 8.94	255.60

The BMP assays for Northern Greece using residues of corn silage, malt and cattle manure indicate that the BMP of the mono-substrates are in agreement to other published works in literature. Specifically for corn silage, other values found in literature range from 204 to 410 mL CH<sub>4</sub> g VS<sub>added</sub><sup>-1</sup> (Bruni et al., 2010; Labatut et al., 2011; Mayer et al., 2014; Menardo et al., 2015; Roj-Rojewski et al., 2018), with the highest value being presented for experiments in which whole corn grains are used. Concerning the results of the current study, the spoilage of the corn silage combined with its long storage duration might have led to partial degradation of the easily biodegradable organic compounds, resulting thus in a decreased BMP yield. In addition, the literature values for malt varied from 64 mL CH<sub>4</sub> g VS<sub>removed</sub><sup>-1</sup> to 366 mL CH<sub>4</sub> g VS<sub>added</sub><sup>-1</sup> (Peces et al., 2015; Diego- Díaz et al., 2018), with the values of the present study being close enough to the aforementioned yields (approximately 255 mL CH<sub>4</sub> g VS<sub>added</sub><sup>-1</sup>). Regarding the experimental yield of cattle manure, the value of 236 mL CH<sub>4</sub> g VS<sub>added</sub><sup>-1</sup>, obtained in this study, is in accordance with other references (Krishania et al., 2013; Strömberg et al., 2014; Thygesen et al., 2014; Cu et al., 2015; Diaz et al., 2016; Wijaya et al., 2020).

As can be seen in Table 2 and Fig. 1, corn silage and malt exhibited the maximum BMP value. However, there are no statistically significant differences in the produced methane between the tested substrates, as described by the *P* values (*P* > 0.05) in Table 3. For this reason, these residues can be used in practice, either as mono-substrates or in mixtures, taking into account only their regional availability and the reactor's operating conditions, such as wet, semi-dry or dry anaerobic digestion, high-rate or conventional operation etc. These results further indicate that co-digestion of residues from the three main agro-waste categories produced in Northern Greece, i.e. crop and

industrial residues as well as animal manure, can be performed and be beneficial for the process performance in terms of process parameter adjustment (such as moisture content or nutrients ratio), without, however, observing any synergistic effects as the expected BMP values of the feedstock mixtures were equal or less to the mono-substrates' performance (Table 2); moreover, the highest BMP values were observed nearly to 100% malt or corn silage (Fig. 1).



**Figure 1.** Results of the BMP assays according to dry matter (left) and volatile solids percentage (right).

**Table 3.** ANOVA table for the cumulative methane yields

Source	DF	Seq SS	Adj SS	Adj MS	F-Value	P-Value
Regression	5	499.22	499.22	99.84	0.47	0.78
Linear	2	265.55	192.40	96.20	0.45	0.66
Quadratic	3	233.67	233.67	77.89	0.37	0.78
Corn silage*Manure	1	7.41	8.13	8.13	0.04	0.85
Corn silage*Malt	1	89.41	90.76	90.76	0.43	0.55
Manure*Malt	1	136.85	136.85	136.85	0.65	0.47
Residual Error	4	846.63	846.63	211.66		
Total	9	1345.85				

According to literature, feedstocks such as manure or maize silage have already been co-digested with other waste streams like food wastes, whey or other agro-industrial residues, exhibiting various results (Hidalgo & Martín-Marroquín, 2015; Cárdenas-Cleves et al., 2018; Valenti et al., 2018; Vivekanand et al., 2018). It is widely hypothesized that co-digestion could lead to higher methane yields and thus synergistic effects, if higher buffering capacity, more balanced C/N ratio or higher readily biodegradable organic fraction could be achieved (Xie et al., 2017). In our study, even if the buffering capacity or the C/N ratio are improved due to the presence of manure, the absence of easily biodegradable compounds, such as monomers, is considered to be equally important for the lack of synergistic effects. The recalcitrant lignocellulosic content combined with the monomers limitation of the tested mixtures can strongly affect the bacterial community functions. The most possible explanation is that, as the hydrolytic bacteria exhibit

notably higher growth rates comparing to the methanogens (Lim et al., 2020), the presence of easily biodegradable monomers could lead to the hydrolytic microbial community enrichment and therefore to prospective increased yields.

The quadratic equation fitting to the experimental methane yields resulted in the regression equation described in Eq. 2. This equation simulates the productivity of methane for any proportion of the aforementioned substrates (fresh matter). The nonlinear terms in this equation indicate whether a substrate is affected by the other. Since these terms present low values, no statistically significant differences between the substrates were obtained.

$$Y \text{ (ml CH}_4\text{/g VS}_{added}) = 2.58\text{Corn silage} + 2.41\text{Manure} + 2.55\text{Malt} - 0.001\text{Corn silage} * \text{Manure} - 0.004\text{Corn silage} * \text{Malt} - 0.005\text{Manure} * \text{Malt} \quad (2)$$

where  $Y$  is the expected BMP (mL CH<sub>4</sub> g VS<sub>added</sub><sup>-1</sup>) and *Corn silage*, *Manure*, *Malt* refer to the percentage (% of fresh mater) of corn silage, manure, and malt, respectively.

## CONCLUSIONS

Greece generates significant amounts of agricultural residues. Even if there are many promising valorisation methods, the anaerobic digestion process has been proven not only an environmentally friendly, but also a feasible renewable energy production method, over the recent years. Concerning the available residues examined, corn silage, malt and cattle manure seemed to be the most suitable feedstocks for anaerobic digestion, in the case of Northern Greece. The BMP results obtained in this study are in agreement with the methane potential of the tested mono-substrates reported in the existing literature. There were no statistically significant differences in the produced methane nor any synergistic effects on methane production during co-digestion of the tested feedstocks, in various ratios; as a result, the decision for energy valorisation of these residues, via anaerobic co-digestion, has to be based on their regional availability and the digester's operating conditions. More studies are yet required to unravel the potential of all available feedstocks, remaining still unexploited in Greek fields and agro-industries, for energy production through anaerobic co-digestion.

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