Biochemical methane potential of various promising agricultural residues in Southern and Northern Greece

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ABSTRACT
Purpose: Greece produces significant amount of residual biomass due to its intense agricultural and agro-industrial sector. The anaerobic digestion process has been frequently considered as the best environmental and economic solution for energy recovery from different biodegradable waste such as agricultural waste, livestock manure, agro-industrial waste, as well as for their co-digestion. The aim of this study was the assessment of biochemical methane potential (BMP) of biomass feedstocks representative of Northern and Southern Greece, most specifically focusing on the Southern Greece, which are available during the spring and summer season, through the implementation of BMP assays.
Methods: The raw residues that were evaluated in the current work included (a) crop residues (corn silage and unsuitable for human consumption watermelon), (b) agro-industrial residues (tomato processing residues) and (c) livestock (cattle) manure. BMP assays testing the single substrates and various mixtures were conducted, for the evaluation of the methane yields.
Results: Concerning the results of the BMP assays, the highest methane yield was exhibited by watermelon as mono-substrate (421 ml CH₄/g VS₅ads). After the evaluation of the mixtures and mono-substrates results, one of the most promising mixtures seemed to be the case of 12.5% corn silage-12.5% cattle manure-62.5% watermelon-12.5% tomato processing residues (w/w).
Conclusions: The proposed feedstock mixture not only exhibited high yield (almost 90% of the maximum experimental BMP value), but is also characterized by high moisture due to the increased ratio in watermelon, leading to its valorization in wet anaerobic digestion systems. Additionally, most of the mixtures indicated synergistic effects during co-digestion compared to the single substrates’ efficiency.
Keywords: crop residues, agro-industrial residues, cattle manure, BMP assays, Northern and Southern Greece

INTRODUCTION

Biomass as a renewable source of bioenergy, is the most important contributor to the global future energy supply [1, 2]. Bioenergy can be produced from various sources that can limit the dependency on conventional fuels, reduce greenhouse gases emissions and cover the local energy needs [1]. However, countries such as Greece (with high amounts of produced biomass nationwide) still depend their energy production on fossil fuels [2]. Such dependence threatens human health and additionally leads to ecological degradation [2, 3].

The most widespread activities in the Mediterranean union are the agricultural and agro-industrial activities, contributing significantly to national economies [4]. Greece, as a country of Mediterranean region, is characterized by significant agricultural and livestock production [2, 5]. Greece’s agricultural residues include: a) the remaining matter after harvesting such as cereal straw, shoots from tobacco plant, cotton, sunflower, etc., b) materials collected during the pruning of fruit trees, olive trees and vineyards, c) the by-products of agricultural industries such as nut shells, kernels, tomato peels etc., and d) wastes generated in animal breeding farms such as manure, wastewater etc. [2]. Huge quantities of agricultural and animal waste are dumped uncontrollably in the environment or in landfills, while farmers continue to burn the residual biomass in the fields. Uncontrolled combustion leads not only to significant impacts on both environment and human health, but also to loss of large amounts of energy and unexploited biomass [6].

Anaerobic digestion (AD) has been frequently considered as the best environmental and economic solution for energy recovery from different biodegradable waste such as agricultural waste, livestock manure, agro-industrial waste, as well as for their co-digestion [2]. More specifically, anaerobic co-digestion has proven to be an effective, low-cost and promising solution for overcoming process inhibition problems due to the presence of toxic substances such as heavy metals, phenolic compounds etc. contained in single waste streams [7, 8]. The Biochemical Methane Potential (BMP) is the key...
parameter for the methanogenic potential assessment of any substrate or substrates mixture. In fact, this parameter is utilized for the techno-economic analysis and the design optimization and operation of the AD process [9–11], while it is also indicative of the stability of stored solid wastes in disposal areas [10].

AD is a well-known biological process that has been investigated for few decades. However, in Greece there is lack of experimental knowledge on the valorization of locally available substrates. For the needs of this study, Greece was divided into a Northern and a Southern part and relevant plans were made for residual biomass valorization through energy production via AD. The aim of this study was the assessment of biochemical methane potential of biomass feedstocks representative of Northern and Southern Greece, most specifically focusing on the Southern Greece, which are available during the spring and summer season, through the implementation of BMP assays. The current study evaluates the potential of such feedstocks for biogas production through AD, determines the most favourable and promising mixtures as well as their synergistic effects. Regarding the experiments for the evaluation of Northern Greece most abundant and available feedstocks, a detailed report has been included in a previous work by the same research team [12], thus the present study builds on and extends our previous work on the most promising Greek feedstocks for AD.

MATERIALS AND METHODS

Materials
As mentioned above, Greece was divided into a Northern and Southern part and relevant proposals were made for the residual biomass valorization through energy production via AD process. More specifically, the most abundant residues for Northern Greece, which are also suitable for anaerobic digestion, are those of corn silage, cattle manure, and malt (the main by-product of breweries). For Southern Greece, the most abundant residues suitable for anaerobic digestion are those of corn silage, cattle manure, unsuitable for human consumption watermelon, and residues of tomato processing, orange and olive processing. The choice of residues was also based on their characteristics and ease of collection. The information of the biomass abundance was extracted from the data of the Hellenic Statistical Authority [13].

The raw residues used in this study for conducting the BMP assays were collected from local plants in the region of Western Greece. Due to their seasonal availability and high tendency to fermentation, the feedstocks were minced and homogenized (when needed) and subsequent stored in the freezer at -18 °C. Concerning the crop residues, i.e. corn silage, which was spoiled animal feed and the unsuitable for human consumption watermelons were collected directly from the field, while the cattle manure referred to the liquid part obtained after the separation of the solid residue. Finally, the agro-industrial residues i.e. tomato processing residues, malt, orange peels, and olive pomace were collected from the corresponding processing plants.

Anaerobic inoculum
Anaerobic sludge obtained from a biogas plant in Messolonghi (Western Greece), was used as inoculum. Concerning the solids content of the anaerobic inoculum, the total solids (TS) were measured at 22.8 ± 0.3 g/L, 54.9% of which were volatile solids (VS).

Biochemical Methane Potential Assay
BMP assay is a well-established and widely used protocol, followed in order to assess the methane potential and biodegradability of wastewater and waste biomass [14]. In the present work, the BMP assays were carried out concerning Northern and Southern Greece residual biomass. More specifically, the current study focuses on the BMP assays for Southern Greece feedstocks, which are available during the spring and summer seasons. The biomass feedstocks tested in this study are corn silage, tomato processing residues, unsuitable for human consumption watermelons and cattle manure. DOE (Design of Experiments) for mixtures with a design of degree 3 (Minitab 2019) was used as a tool for the experimental design of BMP assays. For mixtures, the proportions of the ingredients were variable (0-100% w/w), while their total quantity remains unchanged [7].

The BMP method followed was based on the protocol of Angelidaki et al. [15]. Batch anaerobic digestion of the single substrates and their mixtures were performed in 160 mL closed serum vials. Each vial was filled with known amounts of substrate (2 g VS/L), active anaerobic inoculum (20% v/v) and nutrient medium (80% v/v) consisting of vitamins, trace elements etc. There were two serum vials of blank (control), containing only anaerobic inoculum and nutrient medium. Cellulose was used as control substrate for the BMP assays in order to verify the activity of the inoculum. The vials were purged with N2/CO2 (80/20%) gas mixture for 5 min and sealed immediately to ensure anaerobic conditions. All the BMP tests were carried out in duplicate and the vials (after sealing) were placed at a shaking water bath
at 37 °C for 98 days. The first 6 days of the experiment, the produced biogas was measured daily, while the measurements decreased during the rest experimentation period (every 2-7 days), depending on the cumulative biogas production. The quantification of the produced biogas was carried out using a precise plastic gas syringe of 50 ml volume, while 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. [16]. The methane produced in the blank vial was also analyzed and subtracted from the total volume of produced methane of each sample. The measured biogas was converted to dry biogas at STP conditions. The expected BMP of the mixtures was calculated using the following equation (which has been adapted to the data and needs of the present experiment), according to Tsikou et al. [17]:

$$\text{Expected BMP (ml CH}_4 \text{ g VS}_\text{added}^{-1}) = \frac{(V_{S1} \cdot VS_{S1} \cdot BMP_{S1}) + (V_{S2} \cdot VS_{S2} \cdot BMP_{S2}) + (V_{S3} \cdot VS_{S3} \cdot BMP_{S3}) + (V_{S4} \cdot VS_{S4} \cdot BMP_{S4})}{V_{S1} + V_{S2} + V_{S3} + V_{S4}}$$

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

**RESULTS AND DISCUSSION**

Concerning the BMP assays for the available feedstocks during fall/winter and spring/summer seasons for the Northern part of Greece (i.e. corn silage, cattle manure, and malt), the experimental results of the mono-substrates were in agreement with other published studies. Specifically, for corn silage the value was $262.4 \pm 34.4$ ml CH$_4$/g VS$_\text{added}$, for cattle manure $236.1 \pm 3.4$ ml CH$_4$/g VS$_\text{added}$ and for malt $255.2 \pm 16.4$ ml CH$_4$/g VS$_\text{added}$. In addition, neither statistically significant differences were noticed, nor synergistic effects related to the methane yields of the tested mixtures.

Table 1 and Figure 1 present the results of BMP assays concerning the available feedstocks of Southern Greece during the spring/summer season, i.e. corn silage, cattle manure, watermelon, and tomato processing residues. In Table 1, the mixture ratios, the standard deviation of the experimental BMP values, the expected BMP values as well as the factor ‘desirability’ (defined below), are also presented. Concerning the experimental BMP values, it is obvious from both Table 1 and Figure 1 that the highest methane yield is presented in case of watermelon as single substrate. Such a high yield was in fact anticipated due to the physicochemical characteristics of the specific substrate. In particular, the high concentration of sugars combined not only with the negligible content of solids [18] but also the absence of toxic and/or non-easily biodegradable compounds indicate a promising substrate with high methane potential. However, values close to the maximum were also presented by other feedstock mixtures, such as 50% cattle manure-50% watermelon, 33.3% corn silage-33.3% cattle manure-33.3% watermelon or 62.5% corn silage-12.5% cattle manure-12.5% watermelon-12.5% tomato processing residues. Concerning the co-digestion effect and the possible synergistic or antagonistic phenomena, the results strongly depend on the mixtures composition. As can be seen in Table 1, the expected BMP value is presenting not only higher but also lower values compared to the experimental BMP results. Synergistic phenomena are presented in all cases except in the mixtures of 50% watermelon-50% tomato processing residues, 50% cattle manure-50% tomato processing residues, 50% corn silage-50% tomato processing residues and 33.3% cattle manure-33.3% watermelon-33.3% tomato processing residues.

Regarding the mono-substrates, namely corn silage, cattle manure, watermelon, and tomato processing residues, their BMP values are in accordance with the existing literature. More specifically, for corn silage the values range from 204 to 410 ml CH$_4$/g VS$_\text{added}$ [19–23]. In addition, the experimental BMP value of 294.1 ml CH$_4$/g VS$_\text{added}$ for cattle manure is included in the literature range of 100-350 ml CH$_4$/g VS$_\text{added}$ [24–29]. Moreover, the literature values for tomato processing residues typically range from 199 to 384 ml CH$_4$/g VS [30–33], depending mainly on the physicochemical characteristics of the substrate. Concerning the experimental BMP value of 421 ml CH$_4$/g VS$_\text{added}$ for watermelon, it is also in accordance to other references [34].
Table 1: The mixture ratios, experimental and expected values of BMP expressed as ml CH₄ g/VS_{added} and desirability factor.

<table>
<thead>
<tr>
<th>Corn silage (%)</th>
<th>Cattle manure (%)</th>
<th>Watermelon</th>
<th>Tomato processing residues (%)</th>
<th>BMP (mL CH₄/g VS ± SD)</th>
<th>Expected BMP (mL CH₄/g VS)</th>
<th>Desirability (%)</th>
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<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>305.53 ± 27.82</td>
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<td>0</td>
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<tr>
<td>0</td>
<td>100</td>
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<tr>
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<td>12.5</td>
<td>367.45 ± 13.36</td>
<td>341.49</td>
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Figure 1: BMP results according to component amounts (% w/w).
The following cubic equation resulted through fitting to the obtained experimental BMP values. Such equation predicts the methane productivity for every possible combination of the four tested substrates.

\[
Y (\text{ml} \text{CH}_4/\text{g VS}_{\text{added}}) \\
= 3.36016 \times \text{Corn silage} + 2.96731 \times \text{Manure} + 4.1688 \times \text{Watermelon} \\
+ 3.08904 \times \text{Tomato Processing} + 0.02449 \times \text{Corn silage} \times \text{Manure} - 0.00454 \\
\times \text{Corn silage} \times \text{Watermelon} - 0.00295 \times \text{Corn silage} \times \text{Tomato processing} \\
+ 0.01226 \times \text{Manure} \times \text{Watermelon} - 0.00825 \times \text{Manure} \times \text{Tomato processing} \\
- 0.03257 \times \text{Watermelon} \times \text{Tomato processing} + 0.00039 \times \text{Corn silage} \\
\times \text{Manure} \times \text{Watermelon} + 0.00038 \times \text{Corn silage} \times \text{Manure} \\
\times \text{Tomato processing} + 0.0013 \times \text{Corn silage} \times \text{Watermelon} \\
\times \text{Tomato processing} - 0.00019 \times \text{Manure} \times \text{Watermelon} \\
\times \text{Tomato processing}
\]

where \(Y\) is the calculated BMP (ml CH\(_4\)/g VS\(_{\text{added}}\)) referring to any proportional mixture (% w/w fresh matter) of Corn silage, Manure, Watermelon and Tomato processing residues.

The coefficient of each term indicates synergistic effects or antagonistic phenomena, depending on its value. More specifically, when the coefficient is higher than zero, synergistic effects are exhibited, otherwise, antagonistic phenomena are indicated. Taking into consideration the higher expected BMP values compared to the experimental ones and the negative coefficients of the equation, it is obvious that the proposed equation satisfactorily predicts not only the BMP yields, but also the possible synergistic or antagonistic phenomena. According to literature, anaerobic co-digestion usually enhances the methane yields. For example, cattle waste presents poor biogas yields due to its intrinsic lack of carbon, i.e. low C/N ratio [35]. Thus, co-digestion of cattle waste with another agricultural waste such as corn silage could improve the biogas yield by enhancing the buffering capacity and the C/N ratio, as described by Arici et al. [35], while the risk of ammonia inhibition is limited [35, 36]. The antagonistic phenomena noticed in the current study, might be due to the absence of easily biodegradable fractions at the corresponding mixtures [37].

In Table 1 the factor of desirability (%) is presented. This factor originated after the use of the equation described previously and the response optimizer option (Minitab, 19), targeting at maximum values within the range of 0-420 ml CH\(_4\)/g VS\(_{\text{added}}\), namely the range of the minimum and maximum BMP values of the present study. Desirability indicates the assessment of the defined target by combining the available variables, namely the substrates. The most promising 4-ingredient mixtures with desirability over 85% are: 25% corn silage-25% cattle manure-25% watermelon-25% tomato processing residues, 62.5% corn silage-12.5% cattle manure-62.5% watermelon-12.5% tomato processing residues and 12.5% corn silage-12.5% cattle manure-62.5% watermelon-12.5% tomato processing residues. The latter seems to be the most promising one, with high potential due to its composition (high watermelon content and thus high moisture) and 90% desirability, indicating successful treatment in wet anaerobic digestion systems.

CONCLUSIONS

Greece, due to its strong agro-industrial sector, generates annually significant amounts of agricultural and livestock waste. Even though anaerobic digestion is one of the most environmentally friendly and low-cost methods, in Greece there is still lack of experimental knowledge on the processing of locally available substrates. Regarding the BMP results obtained in this study, the most promising mixture of the tested substrates namely corn silage, cattle manure, watermelon and tomato processing residues is 12.5% corn silage-12.5% cattle manure-62.5% watermelon-12.5% tomato processing residues. Moreover, the values of the mono-substrates are in agreement with the existing literature, while the highest methane yield was presented by watermelon. In this work, synergistic and antagonistic phenomena were also exhibited, depending on the mixture composition and the physicochemical characteristics of the substrates.

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