

# Valorization of agro-industrial waste through anaerobic digestion to enhance energy balance in Lesvos Island

Maria Botzori<sup>1</sup>, Ioannis Daskaloudis<sup>1</sup>, Bernhard Wern<sup>2</sup>, Eleftheria Klontza<sup>1,3</sup>, Stergios Vakalis<sup>4</sup>, Antonis A. Zorpas<sup>5</sup> and Demetris Francis Lekkas<sup>1\*</sup>

<sup>1</sup>Department of Environment University of the Aegean, University Hill, GR-81100 Mytilene, Greece

<sup>2</sup>IZES gGmbH Albrechtstraße 22, 10117 Berlin, Germany

<sup>3</sup>Department of Tourism Economics and Management, University of the Aegean, GR-82132, Chios, Greece

<sup>4</sup>Thermodynamics and Transport Phenomena Laboratory, School of Chemical Engineering, National Technical University of Athens, 9 Heroon Polytechniou, GR-15780, Athens, Greece

<sup>5</sup>Open University of Cyprus. Giannou Kranidioti 89, Latsia, Nicosia, 2231, Cyprus, Laboratory of Chemical Engineering and Engineering Sustainability, Faculty of Pure and Applied Sciences

\*Correspondence e-mail: dlekkas@aegean.gr

## Abstract

This study investigates the potential of several types of agro-industrial waste and residues for biogas production and the feasibility of generating electricity from different renewable energy sources such as biomass in Lesvos Island (Greece). There are significant quantities of agro-industrial waste that could significantly enhance the island's energy balance and security. Biomethane Potential (BMP) assays indicated olive mill wastewater (OMWW) as a residue with very high methane potential. The mono-digestion yielded 1060, 77 ml methane/gVS while the increasing proportion of OMWW in the co-digestion essays resulted in higher yields proportional comparable to mono-digestion. An inclusive analysis is provided to support a large-scale application and contribute towards the decarbonization of the island of Lesvos through the reduction of fossil fuel utilization. It has been estimated that the total power production from biomass in Lesvos could reach up to 295.45GWh, however there is a strict margin for the electricity produced from renewable sources for the non-interconnected islands in Greece.

**Keywords:** Anaerobic Digestion; agro-industrial waste; Biogas, Olive Mill Wastewater; Cheese Whey; Sheep Manure; Sewage Sludge; Electricity generation; Circular economy.



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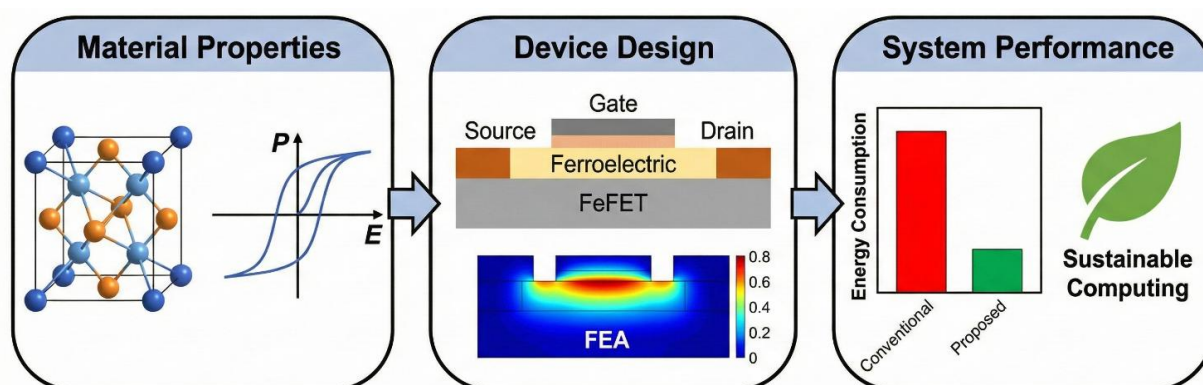
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## Graphical abstract



## 1. Introduction

The World Energy Council (WEC) emphasizes that energy plays a vital role in all aspects of modern life (World Energy Council, 2019). The transition to sustainable energy supplies is a global challenge, given concerns both about climate change and the depletion of fossil fuel resources (Ugwu *et al.*, 2022; Longo *et al.*, 2020). The Circular Economy (CE) framework can enhance resource utilization and waste reduction as waste and by-products can be used as secondary sources of materials and energy (Arora *et al.*, 2022). Biogas, a mixture of methane, CO<sub>2</sub>, and other gases, is considered an important sustainable energy source since it can be produced by several bio-based materials (Huboyo *et al.*, 2025; Assia, 2025; Jijai 2024). Biogas can be upgraded to biomethane (CH<sub>4</sub> - methane content over 95%) and can be considered Renewable Natural Gas (RNG) (Khan *et al.*, 2021). The REPowerEU-Plan aims to produce 35 billion cubic meters (bcm) of biomethane by 2030, replacing Russian natural gas with a €37 billion EU investment. In Germany, biomethane could replace 8% of natural gas consumption, while in Europe there has been a significant increase by almost 20% in 2022 (European Commission, 2022).

Anaerobic digestion (AD) is an effective and established technology for bioenergy production (Aboelfetoh *et al.*, 2022) and agro-industrial waste and residues, rich in cellulose, hemicellulose, lipids, proteins, and carbohydrates, are widely used. Biogas production in AD plants has several limitations (e.g., seasonality of biomass) which consequently affect the energy production. Therefore, optimizing anaerobic digestion is crucial both in terms of specific methane production (volume of produced methane with respect to the substrate used) as well as in enhancing its quality when reduced methane yields and efficiency are low. The anaerobic co-digestion system is an efficient solution where waste from two or more different sources are mixed together in an appropriate ratio and has process efficacy through balancing nutrients, diversifying the microbial population, reducing toxins, balancing alkalinity, and other mechanisms (Esposito *et al.*, 2012; Rabii *et al.*, 2019; Karki *et al.*, 2021; Orangun *et al.*, 2021;

Prithivira *et al.*, 2023; Li *et al.*, 2023). The co-digestion of various substrates has been studied and shown to maintain stable performance over extended periods in previous investigations (Sagani *et al.*, 2019; González *et al.*, 2022).

Olive Mill Wastewater (OMWW) is a typical agricultural residue in the Mediterranean region which accounts for over 95% of global olive oil production and its management presents several challenges due to the presence of phenols and furans, which are known to inhibit the anaerobic digestion process. High concentration of phenolic compounds, which are the main inhibitory agents suppressing hydrolysis, acidogenesis, and methanogenesis, is the primary reason for low degradation of OMWW during anaerobic digestion (Alrawashdeh and Al-Essa, 2020; Benaddi *et al.*, 2023; Lenzuni *et al.*, 2024). Nevertheless, some researchers have investigated that the combination of these inhibitory compounds, the interaction of bacteria, individual compound concentration, and other factors may be more complex and influence their toxicity (Caroca *et al.*, 2021; Trujillo-Reyes *et al.*, 2019). A further issue with OMWW is that it contains a significant number of solids and includes a variety of complex components (lipids and tannins) that cannot be easily degraded. This composition inhibits biological breakdown processes, particularly when combined with a high C/N ratio and low pH values (Bernardi *et al.*, 2017). The AD of OMWW is further influenced by fast acidification (Inan *et al.*, 2004; Al-Bkoor Alrawashdeh *et al.*, 2019).

On the other hand, the Cheese Whey (CW) due to its characteristics has several advantages that are mentioned above. Cheese Whey is either considered a valuable resource with high proteins, carbohydrates, and minerals that could be valorised or as concentrated effluent that needs to be treated (Dereli *et al.*, 2019, Escalante *et al.*, 2018). The type of milk that is used in cheese production (goat, cow, sheep, and buffalo) depends on the animal's breed, diet, state of health, and stage of lactation, and these characteristics can alter the quality of the resulting CW (de Wit, 2001). Cheese whey contains volatile solids (VS) ranging from 45 to 65 g/kg, while COD values range

from 68 to 94 g/L (Dareioti and Kornaros, 2015; Riggio *et al.*, 2015). Its residues can be valorized with AD and produce methane (Mostafa *et al.*, 2019).

Animal manure is a substrate often used for biogas production due to its high moisture content and low C:N ratios (González *et al.*, 2022). However, the AD process can be hindered by protein degradation or ammonia-rich wastewater, which has the potential to impede methanogens (Rajagopal *et al.*, 2013).

Greece significantly increased its renewable energy share, nearly doubling it from 6.9% in 2004 to 15.5% in 2017 (Eurostat, 2022). The ambitious European Green Deal (COM (2019) 640 final) aims to make Europe climate-neutral by 2050. Greece has covered 20% of its total energy demand from renewables recently in 2021, with electricity consumption at 51.9 TWh in 2020 and a 21.7% share of electricity from renewables in 2020 (IEA, 2021). The new EU renewable energy target of at least 27% by 2030 is adopted by EU Member States, but Greece aspires to exceed it, driven by EU laws and the Kyoto Protocol (Eurostat, 2018).

To achieve these goals, Greece is implementing extensive energy sector reforms, including the shutdown of lignite-operated power plants. In 2019, Greece exceeded the EU's 2020 renewable energy production target. In response to current energy sector challenges, alternative energy sources like biogas from organic waste are becoming increasingly important (Huang & Fooladi, 2021).

The EU's Renewable Energy Directive (RED II) sets a target of 32% renewable energy by 2030, defining sustainability criteria and GHG standards for biomass fuels (European Commission, 2023). Biomass, the world's fourth-largest energy source, offers a solution for waste reduction and rural efficiency (Mekhilef *et al.*, 2011). Greece's agricultural sector, contributing 5.2% to GDP compared to the EU average of 1.8%, can contribute to energy production by by-products and residues (Energypedia, 2020). Additionally, the European Commission's circular economy action plan, part of the European Green Deal, aims to alleviate resource pressure, promote long-term growth, and create jobs while aligning with the EU's 2050 climate neutrality goal and biodiversity conservation (European Commission, 2020). According to the REPower plan, there must be an investigation of the possibilities of utilizing local renewable energy sources (RES) and more. The number of biogas plants in Greece in 2022 was 69. In the last revision of National Energy and Climate Plan (NECP, 2024), which serves as the Greek strategic plan for the achievement of specific energy and climate goals until the year 2030 and 2050 in the long term, is estimated that biomethane production the year 2030 would be 2.1 TWh. The objective mentioned above could be achieved by introducing a legislative and regulatory framework and fostering a competitive biomass market.

In Lesvos, the installed power of fossil-fuel-based units is 102.60 MW and of RES units (wind and solar) 22.89 MW. The maximum annual peak demand is 60.66 MW while the monthly energy requirements account for about 25 GWh

to 30 GWh and about 18.94% come from renewable energy sources (RES) (HEDNO S.A., 2026). Lesvos is a sizable island renowned for its thriving agricultural and stock farming sectors. The by-products of agro-industrial practices and agricultural activities have reached a critical level, resulting in a significant amount of waste, frequently disposed untreated, leading to environmental degradation. As such, it serves as an ideal case study for investigating several treatment and valorisation options with anaerobic digestion providing several advantages. Unfortunately, inadequate investment and restrictions on RES integration have led to the underutilization of available RES potential in autonomous insular grids. The valorization of waste from Lesvos Island's agribusiness and stock farming sectors remains largely unexplored (Vasileiadou *et al.*, 2022). This becomes particularly intriguing when considering the substantial dairy and olive oil sectors in relation to the island's size (Chatzimaliakas *et al.*, 2021). Dairy waste, known as Cheese Whey (CW), is a notable by-product, constituting 85%-95% of the total milk volume used for cheese making and containing roughly 50% of milk nutrients (Charalambous *et al.*, 2020).

The focus also lies on connecting the numerous Greek islands with the mainland, as many rely on imported fossil fuels, impacting energy costs and output negatively (Katsaprakakis *et al.*, 2022). Biogas/Biomass Stations (BBS) for unconnected islands, along with solar PV, wind, hydropower, and hybrid plants is promoted in Greece (Law 3468/2006), and the island of Lesvos with abundant agro-industrial waste, can leverage livestock dung for biogas and other sources like slurry for methane production (Trypolska *et al.*, 2021). Biomethane potential from various sources is substantial in Lesvos, making power generation from agro-industrial waste an attractive alternative option due to its sustainability, economic benefits, and positive environmental impact.

The main target of this study is to investigate and optimise biogas production by valorizing agro-industrial waste and residues generated in Lesvos Island through anaerobic digestion. Mixtures of cheese whey, manure and olive mill wastewater from local factories were used. Specific objectives of the study include the investigation of the use of the produced biomethane for power generation and therefore estimate the capacities of AD plants that could allow the penetration of RES in the power mix of the island as well as the key issues that would support the transition from the current mixed production of RES (wind and solar) to 100% RES. This is challenging since Lesvos is a non-interconnected island and covers most of the energy needs via the installed diesel engines.

## 2. Materials and Methods

### 2.1. Cheese whey, manure and olive mill wastewater

#### 2.1.1. Data collection

In Lesvos, milk production, as it is indicated in the data of the institute ELGO Dimitra, for 2023 is 45.083 tons of sheep's milk and 3.520 tons of goat's milk. Cheese production can generate between 41.313,31 and 46.173,70 tons of CW, with peak production between

January and July. Significant numbers of sheep and goats are farmed on the island; 400.966 sheep and 32.227 goats, with manure production rates of approximately 1.44 kg per day per sheep and 1.55 kg per day per goat, based on research by Ogejo *et al.* (2010). Even though most sheep are farmed outdoors in extensive systems, with less than 1% kept in intensive systems, it's possible to collect about 50% of the annual manure production (Katsaprakakis *et al.*, 2022). When considering biogas production from sheep manure, an estimated rate of 150 L per kg can be

**Table 1.** Manure production in Lesvos from sheep and goats (ELGO Dimitra, 2019).

| Manure Production in Lesvos                 |         |        |
|---------------------------------------------|---------|--------|
|                                             | Sheep   | Goats  |
| Animal feedstock (number of animals)        | 400,966 | 32,227 |
| Daily manure production per animal (kg/day) | 1.44    | 1.55   |
| Total daily Manure production (kg/day)      | 577,391 | 49,952 |
| Annual Manure production (tons/year)        | 210,747 | 18,231 |

The data for the olive oil mills were collected by the Ministry of Agriculture for 2021-2022 season. There are twenty-one (21) two-phase olive mills and thirty-one (31) three-phase olive mills - resulting in differences in the residues, mainly on the water content of the effluents. Particularly the three-phase mills produce the Olive mill wastewater fraction (OMWW), whereas the two-phase uses less water during the process therefore an effluent has reduced water and more solids. There are also 33 olive mills that haven't reported clearly their operational status. The olive oil production in Lesvos for the 2021- 2022 season was 5.286,67 tons resulting from the treatment of approximately. 25.000 tons of olives as shown in **Table 2**. Olives are collected in the winter months, especially from

**Table 2.** Available biomass from agricultural waste in Lesvos Island.

| Type of waste    | Minimum Biomass Estimate (tons) | Maximum Biomass Estimate (tons) |
|------------------|---------------------------------|---------------------------------|
| Three-phase OMWW | 9,209                           | 14,734                          |
| Two-phase OMWW   | 2,432                           | 5,536                           |
| Cheese Whey      | 41,313                          | 46,173                          |
| Sheep Manure*    | 105,374                         |                                 |
| Goat Manure      | 9,116                           |                                 |

\*sheep and goat manure feasibility of manure collection is 50% of the total production

### 2.1.2. Collection of substrates and inoculum

Olive mill wastewater (OMWW) was collected from a three-phase olive mill, cheese whey (CW) was collected from a cheese factory, and sheep manure (SM) was obtained from a farm, all located near Mytilene, Lesvos. The anaerobic sludge (AS) used as the inoculum to start the experiments was provided by a commercial biogas plant, Biogas Lagada SA, situated in Thessaloniki, Greece. The total solids (TS), volatile solids, and pH of the inoculum were 24.9 g/L, 12.9 g/L wet weight (ww), and 7.9, respectively. The specific plant treats agricultural waste at 44 °C. The inoculum was maintained at ambient temperature (about 20°C) for a couple of days for transportation reasons and then it was incubated in a water bath at 35 °C for 10 days to gas out the remaining methane. Substrates were preserved in a fridge at 4-6 °C for almost one week until the preparation of the BMP test.

considered (Eurostat, 2023). The calculated daily production of sheep manure (SM) is 577.39 tons, and for goat manure (GM), it is 49.95 tons. Annually, Lesvos has the potential to produce 210,747 tons of sheep manure and 18,231 tons of goat manure, as shown in **Table 1**. This study evaluates and calculates livestock manure production solely based on annual statistics, reflecting the trend of sheep and goat manure output in Lesvos.

October to March. Diverse production methods for olive oil produce different yields of OMWW. As presented by Messineo *et al.*, 2020, the processing of 12.682.100 kg of fresh olives will result in 2.536,4 m<sup>3</sup> OMWW when treated in a two- phases mill and 9.208,67 m<sup>3</sup> OMWW when treated in a three-phases mill.

**Table 2** gives an overview on existing organic residues in Lesvos and their variabilities related to annual agricultural production. The quantities of waste produced were provided by the Ministry of Agriculture. In this study the density of liquid waste is assumed as 1:1 (1000 kg/m<sup>3</sup>).

### 2.2. Experimental design for anaerobic digestion

Several sets of lab experiments have been conducted in order to estimate the biogas production using different substrates from the organic materials available on Lesvos Island as presented in the previous section. The interest has been focused on the characteristics of the materials, the seasonal availability as well as the mixtures that could be representative - with respect to the agricultural activities - and could allow a constant biogas annual production.

The BMP vessels had a volume of 100ml with a working volume of 65ml which was maintained constant for all trials by adding deionized water whenever was needed. Vials containing only the inoculum were used as blanks to subtract the produced methane from the other trials. Three different ratios of OMWW and CW (20%, 40%, 60%) were used for the test, and there were trials of each substrate alone with inoculum. The set-up of the trials is



presented in detail in **Table 3**. Every trial was performed in triplicate. SM had a fixed contribution of 20% to all co-digestion mixtures. The reason that the percentage of SM was maintained low was because of its low BMP (Achinas *et al.*, 2018) also indicated in the current research. The inoculum-to-substrate ratio used was 3:1 based on VS as Angelidaki *et al.*, (2016) recommend. After the end of the mixing preparations the headspace of the vials was flushed

**Table 3.** Experimental design based on the proportion of the different substrates in the mixture.

| Trial No. | Substrate | Proportion of each substrate in the test mixture (% of substrate volume) |     |     |
|-----------|-----------|--------------------------------------------------------------------------|-----|-----|
|           |           | OMWW                                                                     | CW  | SM  |
| 1         | OMWW      | 100                                                                      | 0   | 0   |
| 2         | CW        | 0                                                                        | 100 | 0   |
| 3         | SM        | 0                                                                        | 0   | 100 |
| 4         | OMWW 20   | 20                                                                       | 60  | 20  |
| 5         | OMWW 40   | 40                                                                       | 40  | 20  |
| 6         | OMWW 60   | 60                                                                       | 20  | 20  |

For methane determination the volumetric technique was used. A syringe of 100ml was used for the measurement. 10ml of NaOH (6N) was contained inside the syringe during the measurement as a carbon dioxide trap and was replaced after each measurement. In case of methane volumes higher than the syringe's volume a second similar one was used. The frequency of methane measurements was every two or three days. The presented values are the average of the triplicate vials. On a regular basis an evaluation was carried out in order to validate the presence of CO<sub>2</sub> in the collected methane by gas chromatography. The BMP assays were stopped when daily methane production was less than 2-3% of the total production.

### 2.3. Analytical Methods

The physicochemical parameters determined in the experiments are: total solids (TS) after 24 hours at 105 °C, volatile solids (VS) at 550 °C for 2 hours and pH according to APHA (2017), chemical oxygen demand (COD) with LCK 514 kits (Hach, USA), organic carbon (OC) by dividing VS by 1.8 (Daskaloudis & Lekkas, 2021), and total nitrogen (Hach TNT827 kits, USA), in the beginning and in the end of the BMP test. The samples were tested in triplicate, and the results are the average values. For the gas chromatography it was used an Agilent 6890N supplied with a Thermal Conductivity Detector (TCD) and a capillary column (HP-Plot Q, Agilent, USA) with helium serving as carrier gas. The oven temperature was 40 °C for the first 1.5 min and then increased gradually (50 °C/min) to 220 °C. The temperature of the injector and the detector was set at 200 °C and 230 °C, respectively. The analysis of measured parameters, especially VS and COD, indicates the dynamics of methane production. Moreover, these parameters are also used to validate the stability of a continuous anaerobic digester (Mákadi *et al.* 2012; Möller and Müller 2012; Guilayn *et al.* 2020b; Fernandez - Dominguez *et al.* 2020). The mechanism of anaerobic digestion as outlined in previous studies, is better understood with continuous monitoring of these parameters (Huang *et al.* 2021; Xu *et al.* 2021). As discussed, the experimental testing aims to evaluate the TS and VS decrease in AD when mixtures of agrowaste,

for three minutes using nitrogen (N<sub>2</sub>) to ensure anaerobic conditions before incubation and then capped with butyl rubber stoppers, sealed with aluminium caps and placed in a water bath, in which the temperature was kept constant to 35°C. The BMP assay lasted 40 days and the vials were stirred daily.

including sheep manure, olive wastewater, and cheese whey, are co-digested.

### 2.4. Methodology for estimating electricity after combustion of biogas

The technical analysis is based on several elements including substrate diversification, substrate utilization, wastewater treatment, producing high-quality products, operational costs and environmental impact (Devi *et al.*, 2022). The paper provides a broad view that investigates possibilities for the utilization of biomass availability in Lesvos for electricity generation from renewable resources. The calorific value of 1m<sup>3</sup> methane is 36 MJ. The conversion coefficient of methane to electricity depends on the power plants' technology and the assumption that the efficiency for the conversion is 35%, hence 1m<sup>3</sup> of methane has the potential to produce 10kWh (Suhartini *et al.*, 2019, Khalil *et al.*, 2019). Therefore, the electrical power that can be generated from the produced methane can be estimated by the following equation [1]

$$E_{el} = e_{methane} * mp \quad (1)$$

where  $E_{el}$  is the total estimated production of electric energy (kWh),  $e_{methane}$  is the converted electrical output per cubic meter of methane, which is equal to 10kWh assuming that electrical conversion efficiency is 35%, and  $mp$  is the amount of the produced methane.

## 3. Results and discussion

### 3.1. Physicochemical characteristics of the collected samples

The experiments were conducted without any pretreatment of the inoculum or substrates, such as pH adjustment, trace element supplementation, or the application of chemical or physical pretreatment methods. **Table 4** presents the physicochemical characteristics of inoculum and substrates.

### 3.2. Anaerobic digestion (AD) process

In total, 7 triplicate sets of different substrates were tested in this study. Maximizing the substrate performance in AD

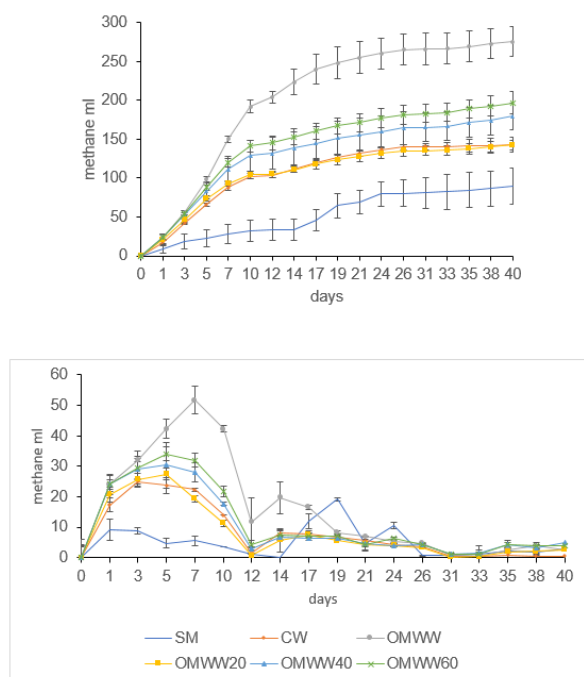
is critical for increasing biogas yield and consequently improving the process's potential economic performance (Ghimire *et al.*, 2015; Raja *et al.*, 2017). The anaerobic digestion experiments presented a quick start with the corresponding biogas generation, and by the 22nd day of digestion, the process had reached the 90% of the maximum cumulative methane production (Sandhu *et al.*, 2022). SM presented the lowest total methane production while OMWW the highest. The other trials had almost similar production profile (Figure 1) and the one with the higher OMWW content (OMWW60) presented the highest methane production among the co-digestion trials. The rate at which the methane is produced could be negatively affected mostly by volatile fatty acids (VFA) balance. The

**Table 4.** Physicochemical characteristics of inoculum and substrates.

|          | TS %  | VS % (ww)* | C %   | N (mg/L) | COD mg /L |
|----------|-------|------------|-------|----------|-----------|
| inoculum | 2.49  | 1.29       | 28.9  | 3,200    | 20,250    |
| OMWW     | 8.15  | 6.55       | 43.3  | 901      | 140,500   |
| CW       | 7.77  | 6.9        | 45.6  | 830      | 78,200    |
| SM       | 85.47 | 73.95      | 47.25 | 30,600** | *nd       |

\*ww: wet weight

\*\* Gonzalez *et al.*, 2021. Measurement unit mg/Kg.



**Figure 1.** Cumulative (up), and day of sampling (down) methane production.

The highest methane production was obtained in the mono-digestion assay, where OMWW was used as the sole substrate. In the present study, the specific methane production of OMWW reached 1060.77 mL CH<sub>4</sub>/g VS, which is substantially higher than values commonly reported for OMWW mono-digestion. A study with values comparable to our results investigated the mono-digestion of OMWW along with CW and liquid cow manure and stated that the highest biomethane potential was from OMWW reaching 820 Nml/gVS (Manthos *et al.*, 2023). However, the highest methane yield found in the rest literature for raw OMWW under BMP conditions was 472 mL CH<sub>4</sub>/g VS, reported by Tsigkou *et al.* (2019). The more than twofold difference observed in the present

anaerobic digestion of readily biodegradable waste frequently causes the accumulation of these intermediaries - i.e. VFAs. Food processing waste, like cheese whey or fruit waste, has low nitrogen contents which makes the fermentation liquor's buffering capacities insufficient (González *et al.*, 2022). **Table 5** presents the average results of methane production from anaerobic digested residues. Daily biogas measurements were performed using the water displacement method (Salam *et al.*, 2015). The methane production levels vary significantly among individual samples and inoculum productions. **Table 5** displays the results, which help identify the most effective substrate.

study may be associated with inoculum origin, previous acclimation, and the diversity of the microbial community. This interpretation is supported by Kamal and Alkarimiah *et al.* (2026), who reported that inoculum from a food-waste digester more than doubled the methane yield of chicken-manure-leachate mono-digestion compared with inoculum from a digester treating palm-oil-mill waste. In the study of Tsigkou *et al.* (2019), the inoculum originated from a lab-scale UASB reactor treating OMW, whereas the inoculum used in the study of Manthos *et al.* (2023) and the present study was obtained from a wastewater treatment plant and a full-scale digester treating OMWW together with other agro-industrial residues, respectively. Inoculum comes from mono digestion can inherently have disadvantages because of the limitations in this type of digestion like lack of micronutrients and low microbial diversity (le Rose and Caro, 2026). Therefore, the inoculum used here has combined prior exposure to OMWW with a broader microbial and nutritional background. The high methane potential of OMWW is also supported by earlier work of Ergüder *et al.* (2000), who reported 1339 ml methane/gVSS. The high methane production may also be partly related to the composition of the OMWW used, particularly its lipid fraction, since lipids have a high theoretical methane potential, generally reported in the range of 850–1050 mL CH<sub>4</sub>/g VS (Bedoić *et al.*, 2020). The co-digestion assays in the present study further supported the strong methanogenic potential of this OMWW, as methane yield increased with increasing OMWW proportion. More specifically, the OMWW60 trial, containing 60% OMWW, produced 756.92 mL CH<sub>4</sub>/g VS, a value approaching the yield obtained during OMWW mono-digestion. In a similar study containing 60% OMWW, 20% CW, and 20% poultry manure methane yield was 447.5±20.7 ml (Daskaloudis *et al.*, 2026). On the contrary, in the study of Manthos *et al.* (2023) that used co-digestion of 50% OMWW, 25% CW, and 25% liquid cow manure reported almost 1100 Nml methane/gVS. Overall, these

results indicate an unusually high methane potential for the investigated OMWW and suggest that further

characterization of its composition, lipid content, and biodegradability is required.

**Table 5.** Comparison of methane production using different substrates.

| Substrate | VS content added in BMP vial (g) | Total Methane Production (ml) | SMP (ml Methane/gVS) | m <sup>3</sup> Methane/ t |
|-----------|----------------------------------|-------------------------------|----------------------|---------------------------|
| OMWW      | 0.26                             | 275.8 ±19.52                  | 1060.77±75.07        | 69.48±4.92                |
| CW        | 0.26                             | 142.8 ±9.17                   | 549.23±35.25         | 37.9±2.43                 |
| SM        | 0.26                             | 89.33 ±23.53                  | 343.58±90.5          | 254.08±66.92              |
| OMWW 20   | 0.26                             | 142.46 ±6.43                  | 547.92±24.73         | -                         |
| OMWW 40   | 0.26                             | 179.46 ±17.62                 | 690.23±67.75         | -                         |
| OMWW 60   | 0.26                             | 196.8 ±14.18                  | 756.92±54.53         | -                         |

The existing literature provides some interesting findings that can assist the assessment of the results that are presented in this study. The total solids (TS) concentration significantly influences anaerobic digestion (AD) by affecting the availability of organic materials, energy use, environmental impact, and profitability of the AD process (Aboudi *et al.*, 2017; Li *et al.*, 2018b; 2018c). The volatile solids (VS) concentration is crucial for estimating the effectively degradable ratio, indicating the amount of organic matter in the substrate (Carotenuto *et al.*, 2016). Wet AD systems often deal with substrates having TS content below 10%, requiring the addition of substantial amounts of water to the digester (Wang *et al.*, 2020). The presented experiments allow the evaluation of the influence of TS and VS content before and after anaerobic digestion.

The carbon-to-nitrogen (C:N) ratio plays a pivotal role in lipid accumulation (Guitierrez *et al.*, 2022). While some studies suggest that increasing the C:N ratio promotes bacterial growth and solid removal in co-digestion (Anjum *et al.*, 2012; Ghaleb *et al.*, 2020), an appropriate C:N ratio of around 30 is recommended to avoid limitations on microbial metabolism and excessive ammonia production (Hakimi *et al.*, 2021). Certain waste types, like agro-industrial waste, have higher carbon and nitrogen concentrations, enhancing biogas production (Oladejo *et al.*, 2020). However, a low C:N ratio may result in ammonia production, which is hazardous for the environment

(Caldeira *et al.*, 2020) and significantly reduces AD. Co-digestion helps maintain the ideal C:N ratio, boosting biogas production (Devi *et al.*, 2022).

Biogas yield, measuring the conversion of organic pollutants to biogas during AD, is typically calculated as biogas generation per unit COD digested, with COD content serving as the basis (Xu *et al.*, 2021). BOD and COD are used to assess oxygen demand in wastewater, important for determining aeration times (Nourani *et al.*, 2018). Microorganisms, especially methanogens, are sensitive to changes in pH, therefore its levels are crucial during AD (Hassan *et al.*, 2016). In co-digestion, pH reduction can occur during the initial phases due to VFA accumulation and insufficient self-buffering mechanisms (Potdukhe *et al.*, 2021). The results presented in **Table 6** suggest that the energy potential of agrowaste in Lesvos is high; nevertheless, the information at hand indicates that this is only a theoretical assumption. There is potential, and this presents the technical feasibility of managing it, but an extensive assessment of the total available rates of biomass is required to give a complete picture of current availability.

### 3.3. Biomass potential and energy content

In **Table 6**, the theoretical biomass availability in Lesvos and the energy content from olive oil mill wastes, cheese whey, and livestock manure is presented. The energy content for the island ranges from 289.79 to 295.45 GWh.

**Table 6.** Theoretical biomass potential and energy content of methane.

| Usable biomass   | Lowest Biomass Estimate (tons) | Highest Biomass Estimate (tons) | kWh / t | Lowest Production Electricity (GWh) | Highest Production Electricity (GWh) |
|------------------|--------------------------------|---------------------------------|---------|-------------------------------------|--------------------------------------|
| OMWW Three Phase | 9,208.69                       | 14,734                          | 694.8   | 6.39                                | 10.23                                |
| CW               | 41,313                         | 46,173                          | 378.97  | 15.66                               | 17.49                                |
| SM               | 105,374                        | 2,540.8                         | 267.73  |                                     |                                      |
| Total            | 155,896                        | 166,281                         | -       | 289.79                              | 295.45                               |

### 3.4. Electricity production from anaerobic digestion

The analysis has been applied using a simplified approach. Specifically, the energy content of 1 ton of OMWW, CW, and SM is equal to 694.8; 379; and 2540.8 m<sup>3</sup>, respectively (Table 5). From an economic point of view, there are relevant parameters that may have an impact on the outcomes. Among other parameters like the type of substrate, its volume for each scenario and the current electricity price, the amount of biogas production has high significance (Imeni *et al.*, 2019). As of 2021, electricity

generation per capita in Greece was reported to be 5.133 KWh. In this study, the electric energy consumption per resident unit on the island of Lesvos, which has a population of 114,880, is investigated. Additionally, the study delves into the feasibility of utilizing renewable energy sources, specifically biogas production, to meet the energy demand in remote places.

### 3.5. Energy needs in Lesvos

In Greece, the number of non-interconnected islands (NII) is considered because of their different sizes and high-RES

potential. NIs are the islands that are not connected to the national distribution network and as expected have significant differences from mainland regions. The increasing penetration of RES technologies other than wind mainly causes a violation of the minimum load criterion of the mandatory integrated conventional units. Following the determination of the acceptable penetration limits of uncontrolled RES technologies, the possibilities of receiving distributed RES stations were investigated concurrently with the acceptable penetration of unregulated ones. The categories of plants examined are hybrid plants with wind units and pumped storage or accumulators, parabolic trough solar plants, and solar panels with various storage sizes, as well as biomass and biogas plants with the ability to control their power. In terms of BBSs, their installed capacity was parametrically estimated with a penetration range of 3–3.5% of the yearly peak. In Lesvos, the maximum annual peak demand in 2021 was 68.36 MW and the amount of the total annual peak from BBS could be maximum 2.39 MW, as result mostly to the restricted primary potential on the non-interconnected islands (RAE, 616/2016). The non-integrated energy exhibits a correlation with the discarded primary source energy, which, however, depends on the storage capacity and internal dimensioning of each RES.

For the assessment of the scenario approach in Lesvos, we used the estimate of sustainable potential per feedstock and converted the feedstock potential to anaerobic digestion and methane production. The maximum theoretical biomass potential in Lesvos from livestock manure, cheese whey, and olive oil wastewater has been estimated at about 166,281 tons per year (table 2) while the energy content at about 295.45 GWh (table 5). Regarding the limitation for the installed power for BBSs (2.39 MW) and assuming an average of 8,000 operating hours per year (Lantz, 2012), the maximum energy production permitted is 19.1 GWh/year. The total monthly energy needs in Lesvos are in the range of 25 to 30 GWh depending on the month as Lesvos is a touristic destination during the summer (HEDNO, 2026). It is apparent that the high energy potential can cover the energy needs of Lesvos island in electricity instead of been wasted unexploited. The evaluation of biomass distribution, the analysis of energy potential, the modernization of the grid, the ending of the limitations, and the scenario development for Lesvos can provide valuable help towards the creation of suitable implementation strategies for boosting the contribution of biomass to the energy system.

### 3.6. Limitations of the study

The present study has some limitations that should be considered when interpreting the results. First, in the context of the BMP assays, they were performed using specific locally collected substrates, and therefore the reported methane yields may reflect the particular composition of the investigated OMWW, CW, and SM. This is especially relevant for OMWW, whose composition may vary according to olive variety, extraction system, storage, and seasonal conditions. Although the OMWW showed exceptionally high methane production, further

characterization of lipid content, phenolic compounds, volatile fatty acids, and COD fractions would be required to explain the mechanisms responsible for this performance. In addition, because only one inoculum source was used; therefore, the relative contribution of inoculum adaptation and substrate composition cannot be fully separated. Finally, the experimental results were obtained under BMP conditions and should be considered as methane-potential data. Pilot- or full-scale continuous trials are required to confirm process stability, achievable organic loading rates, and practical applicability.

As it concerns the energy production the biomass-to-electricity assessment also represents a theoretical potential. The actual implementation of anaerobic digestion on Lesvos would require a detailed evaluation of seasonal biomass availability, collection efficiency, transport distance, storage requirements, competing uses of residues, plant location, economic feasibility, and the electricity-integration limits that apply to non-interconnected islands.

## 4. Conclusion and Perspectives

In Lesvos Island there are high quantities of biomass generated from waste that could be used for power production. The mono-digestion of the specific OMWW used in this study presented an exceptional methane yield equal to 1060.77 ml methane/gVS and the reasons would be further investigated. In the assays with co-digestion the increasing of the OMWW content led to increasing methane production, comparable to mono-digestion, justifying its high methane potential. A total power production of approximately 295.45 GWh is theoretically possible through the valorization of the identified residues. The optimum approach for saving energy will be the transition from fossil-fuel-based power to high-efficiency cogeneration using Renewable Energy Sources (RES). It should be pointed out that it is necessary to ensure a sustainable scenario for saving measures and create smart grids with high feasibility and secure RES penetration levels. EU directives are important, and the member states should harmonize their national legislation based on EU lines for green energy sources. For this reason, it is necessary to involve new technical solutions for green energy like anaerobic digestion capable of treating the available biomass on the island and recalcitrant wastes like OMWW, logistics of biomass collections in extensive farming systems and scattered waste producers (olive mills), storage facilities, and sustainable system management.

### Author Contributions

Conceptualization, Antonis A. Zorpas; Methodology, Ioannis Daskaloudis; Formal analysis, Antonis A. Zorpas; Investigation, Maria Botzori; Data curation, Maria Botzori and Bernhard Wern; Writing – original draft, Ioannis Daskaloudis; Writing – review & editing, Maria Botzori and Stergios Vakalis; Project administration, Eleftheria Klontza and Demetris Francis Lekkas.

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## Conflicts of Interest

The authors declare no conflict of interest.

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