

Olive Pomace Protein Hydrolysate Waste Valorization Through Biogas Production: Evaluation of Energy Produced and Process Efficiency

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The biogas production from biomasses for energy production, must be considered as last step in the ideal biorefinery supply chain, since the extraction of valuable subproducts has greater importance and must be performed earlier than the final preparation of bio-digestates. This study aims to prove the technical feasibility of carrying out both these processes, or the initial extraction of valuable subproducts and the following production of biogas. Moreover, it aims to quantify the benefits introduced, in terms of biogas production, related to the addition of pomace to the digestate inoculation. For the scope, biogas was produced, in lab-scale apparatuses, starting from different digestate samples; among them, one was enriched with untreated pomace and another with the treated one, consisting of the residual of pomace used for sub-products extraction. Based on the measured quantity of biogas achieved, the energy produced was quantified; conversely, the energy consumed was evaluated according to the plant efficiencies declared in literature. The daily energy production and consumption were compared to define the optimal production period and, according to it, the quantity of energy lost, referred to the unextracted biogas from digestates, was quantified and the efficiency was re-defined. The benefits related to the addition of pomace and, in particular, treated pomace, were defined as a function of the overall energy production, the percentage of energy lost (or produced outside from the optimal production period) and the re-defined process efficiency. Finally, the energy obtainable per unit of dry matter and volatile solids was calculated.

1. Introduction

Nowadays, the industrial processing of agri-food products gives rise to large quantities of by-products that must be disposed of (Cesaretti et al., 2020). A considerable effort is currently underway to find use and utility for the waste inevitably produced and give new value to waste biomass by transforming it into products that can be used in other industrial fields and reintroduced into the market, thus responding to the Circular Economy challenge. Most agricultural and agri-food waste biomasses preserve a high content of potentially recoverable bioactive compounds that can be exploited to produce high-added-value products such as protein hydrolysates (PHs) or low-added-value products such as compost or digestate (Calzoni et al., 2021; Cesaretti et al., 2020; Puglia et al., 2021). The former have multiple applications in various industrial sectors, from the food industry, both human and animal, to the nutraceutical sector, the cosmetics industry, and the agricultural field; differently the low-added-value products mainly find applications in the agricultural sector (Cesaretti et al., 2020). Agricultural waste biomass has been identified as low-cost raw materials and represents a good portion of the biomass present in the world. It is estimated that globally 140 billion tons of biomass are generated from agricultural waste every year (Salisu et al., 2021). Among all the activities of the Mediterranean region, the olive industry produces tons of waste every year that could be valorized and reintroduced into the market, e.g. olive pomace (OP). Olive cultivation and olive oil making are in fact crucial activities in the Mediterranean basin of social and financial significance, as a matter of fact over 98% of the world's olive oil is produced in this area,

and Italy is one of the greatest manufacturers in this sector, alongside Spain, Greece, and Portugal (Dermeche et al., 2013; Espadas-Aldana et al., 2019). This large industry, however, generates large quantities of waste, which often have significant ecological implications due to their environmental impact, furthermore, they represent an economic problem for olive-oil-producing companies as they have to take charge of their disposal (Behera et al., 2021). Specifically, the olive industry produces large quantities of wet and solid by-products, such as OP and olive mill wastewater (Behera et al., 2021; Otero et al., 2021). The OP resulting from the processing of olives represents approximately 35-40% of the total weight of the olive and is made up of the solid part left after the milling process in the oil mill, *i.e.*, stone, and the pulp and peel (Gullón et al., 2020). It has been estimated that the annual global production of OP is around 400 million tons (Sánchez et al., 2021). In this scenario it becomes important to mitigate the impacts of olive oil production and find new ways of treating its potentially harmful by-products for the ecosystem which are aimed at obtaining new materials with added value, thus avoiding problems relating to the disposal of this waste. Therefore, by focusing on the reuse of waste, the olive oil production chain develops a potential yet to be explored in terms of Bioeconomy and Circular Economy activities. Depending on the variety of olives from which OP derive, the climate and cultivation techniques, they contain many molecules of important biological value such as proteins, fats, phenolic compounds, lignin, cellulose, hemicellulose, pectic polymers, minerals and other concentrations of other nutrients (Gullón et al., 2020; Rodrigues et al., 2015). From this perspective, by virtue of the considerable content of bioactive compounds that can be recovered and valorized, OP can be used as a raw material for the production of organic products with high added value by applying or developing appropriate technologies (Behera et al., 2021). The great potential of these waste materials lies in the opportunity to transform them into high-added-value products such as PHs, thus promoting the transition towards a Circular and Eco-compatible Economy. In order to fully valorize the waste biomass, the OP waste obtained following the production of a PH can be treated for anaerobic digestion aimed at producing biogas, from which it can be produced electricity, and biomethane that can be obtained through suitable purification processes and can be introduced in the natural gas network (Cucina et al., 2021). In this study, the waste of three-phase OP deriving from an alkaline hydrolysis, for producing high-added value PHs, was tested for anaerobic digestion. The controls were represented by an inoculum consisting of digestate, a mixture of inoculum and swine slurry, and a mixture of inoculum and untreated three-phase OP. The aim of this work was to develop an anaerobic digestion system starting from the three-phase OP wastes obtained from the production of PHs.

Taking into account the potential applications and the sustainability of the starting matrices, the biogas industry is an attractive answer to valorize agricultural and agroindustrial wastes that can satisfy the growing demand for an ecological transition and circularity.

2. Materials and Methods

2.1 Materials

The inoculum used in this study was represented by a digestate that was produced in our laboratory. The swine slurry was supplied by a local breeder in the Umbria region, Italy. The three-phase OP was supplied by a local olive mill in the Umbria region, Italy.

2.2 Recover of OP-PH waste

For the production of PH waste, three-phase OP was digested under alkaline conditions using mild temperatures (< 100 °C) for 12 hours. After the hydrolysis process, PHs were isolated from each sample and the waste produced following the treatment was recovered to be used for anaerobic digestion. This waste is what remains following the alkaline hydrolysis process of three-phase OP aimed at producing PHs, products with high added value. Its composition is linked to the hydrolysis process and the starting raw material. The hydrolysis process aimed at recovering the protein component as PHs will ensure that this waste will be mainly composed of a few amino acids, fatty acids, simple sugars, and the solid part residues of OP (*i.e.*, stone residues).

2.3 Anaerobic bioreactors

The production of biogas analysis was carried out in bioreactors of 50 mL kept in mesophilic conditions in a climatic chamber at a temperature of 37 °C for 30 days. The production of biogas was evaluated through the volumetric method (Figure 1).



Figure 1: Representation of the experimental system used in this study for the quantification of biogas produced.

In particular, 3 controls and 1 treated sample were set up in the anaerobic bioreactor as described in Table 1. A total of 37.5 g of each mixture was added to the bioreactors considering the percentage of each component specified in Table 1. Analyzes were performed in triplicate.

Table 1. Representation of the bioreactors used in this study: bioreactor 1 consisting of sole digestate representing the inoculum, bioreactor 2 consisting of inoculum and swine sludge, bioreactor 3 consisting of inoculum and untreated three-phase OP, and bioreactor 4 consisting of inoculum and waste of three-phase OP-PH.

	Inoculum [%]	Sample [%]
Bioreactor 1	100	-
Bioreactor 2	75	25
Bioreactor 3	75	25
Bioreactor 4	75	25

3. Results and Discussion

The waste of the hydrolysis process of three-phase OP aimed at valorizing the waste derived from the production of olive oil was tested for anaerobic digestion for the production of biogas. These residues were evaluated in laboratory-scale reactors maintained at 37 °C and followed for one month. The results showed how anaerobic bioreactors containing the waste of three-phase OP-PH show a greater production of biogas. The cumulative production of the biogas produced in the reactors inoculated with residues of OP-PHs was evaluated, and the results showed how the biogas was produced in greater quantities in this sample over the time of the examination (Figure 2). A 153.9 mL total volume of biogas was found in the three-phase OP-PH waste sample, followed by 77.2 mL in untreated three-phase OP, 62.5 mL in swine slurry sample, and 56.5 mL in the sole inoculum.

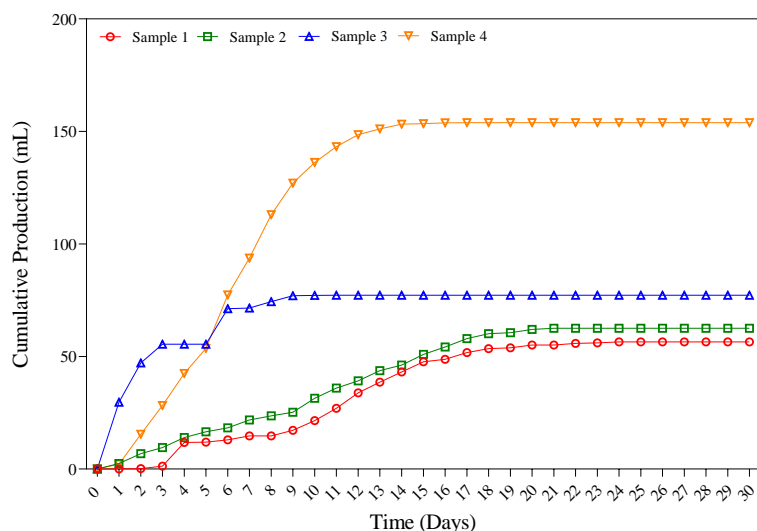


Figure 2. Cumulative (mL) biogas production of the anaerobic reactors containing sole inoculum (Sample 1), inoculum and swine sludge (Sample 2), inoculum and untreated three-phase OP (Sample 3), and inoculum and waste of three-phase OP-PH (Sample 4).

These results therefore highlight how the reactors treated with three-phase OP-PH waste showed a greater production of biogas, highlighting how the use of these wastes could have interesting potential industrial applications aimed at valorising waste from the olive oil supply chain.

Starting from biogas, the quantity of biomethane achieved was directly measured and the following results expressed as percentage of biogas produced were reached:

- 1) Sample 1 (sole inoculation): 47.52%;
- 2) Sample 2 (inoculation + $\frac{1}{4}$ swine slurry): 53.23%;
- 3) Sample 3 (inoculation + $\frac{1}{4}$ untreated three-phase OP): 66.30%;
- 4) Sample 4 (inoculation + $\frac{1}{4}$ three-phase OP-PH waste): 58.44 %.

While the energy produced was directly obtained from the experimental results, the energy spent was deduced from the literature.

The input energy consists of the sum of several different contributions; the most significant can be referred as follows: energy crop cultivation and feedstock pre-treatment; feedstock collection and transportation; biogas plant operation processes; biogas treatment and storage; digestate processing and handling.

The overall energy spent to carry out all these phases can be quantified as highly variable percentage of the energy contained in the produced biogas. To compare these two latter quantities, the Primary Energy Input Output ratio (PEIO) (Pöschl et al., 2010) was introduced. Based on the typology and composition of the feedstock used, the PEIO index was estimated to range from 10.5% to 64.0% (Pöschl et al., 2010). In this study, an average value of 33 % was used for PEIO taking into account values found in literature and available elsewhere (Berglund and Börjesson, 2006; Gkotsis et al. 2023; Prade et al. 2012).

Table 2 shows the energy consumed (quantity estimated according to what previously asserted) for each sample, to reach the final quantity of biogas produced. Such amount was then split out between the single day of production. The daily quantity is indicated in the last column of Table 2.

Table 2. Total and daily energy consumed, considering the effective days of production and PEIO=33%.

	Total energy consumed [kJ]	Days of production	Daily energy consumed [kJ]
Sample 1	0.29±0.005	24	0.01±0.005
Sample 2	0.36±0.005	21	0.02±0.005
Sample 3	0.52±0.005	11	0.05±0.005
Sample 4	0.97±0.005	17	0.06±0.005

Table 3 shows for each sample the total energy produced, the estimated time period having daily production higher than the energy consumption (or the optimal biogas production period), the portion of energy produced in this latter time range and, in the last column, the percentage of energy lost, corresponding to the portion of biogas not extracted from the digestate.

Table 3. From left to right and for each sample tested: overall quantity of energy produced; optimal production period, or the interval during which the energy production is higher than the energy consumption; quantity of energy produced during the so-defined time period and portion of energy unextracted from the digestate.

	Whole production of energy [kJ]	Optimal production period [day]	Energy produced in the optimal period [kJ]	Portion of energy lost [%]
Sample 1	0.88	18	0.80	9.09
Sample 2	1.09	18	1.05	3.67
Sample 3	1.67	6	1.54	7.78
Sample 4	2.94	12	2.84	3.40

Table 3 confirms the positive contribution of OP, both in terms of biogas extracted and time period duration. In particular, the OP-PH waste led to the best performance for biogas production: 2.94 kJ were produced, against 1.67 kJ, obtained with the presence of untreated OP, and 0.88 and 1.09 kJ were achieved with the other samples. The coupling of these two benefits, the higher production and the lower production period, led to a significantly better efficiency for biogas production from digestate containing OP. The efficiency value (η) was initially defined by considering the whole energy produced and the energy consumed during the whole production period. This latter parameter was considered different from the 30-day test initially fixed; it was assumed equal to the number of days during which the production of biogas was different from zero. In particular, from Sample 1 to Sample 4, it was respectively equal to 24, 21, 11, and 17 days. According to it and based to the efficiency values reported in the literature, the process efficiency was considered equal to 67%. The efficiency (η_{OPT}) was calculated by considering only the energy produced during the optimal production period, shown in Table 3, and the energy consumed during the same period. The results are shown in Table 4.

Table 4. Biogas production efficiency, calculated by comparing the energy consumption with the energy production, evaluated during the whole measured production period (on the left) and only during the optimal biogas production period (on the right).

	η [%]	η_{OPT} [%]
Sample 1	67.0	74.8
Sample 2	67.0	72.3
Sample 3	67.0	81.7
Sample 4	67.0	76.7

The re-evaluation of the process efficiency, limited to the optimal production period, clearly led to better results, especially in the presence of OP, where the efficiency was found to be equal to 81.7%, with untreated OP, and 76.7%, with the treated one. It must be remembered that the energy consumed was considered as percentage of the total energy produced and is therefore different between the different samples. As a consequence of it, the biogas production process of each sample must be evaluated by taking into consideration the quantity of energy produced and also the portion of energy lost if the production is limited to the optimal time period defined in Table 3. In this regard, Sample 4 (containing the OP-PH waste) showed higher performances than Sample 3 (containing untreated OP). The higher biogas production from reactors containing three-phase OP-PH waste could be again justified by the greater availability of simple and ready-available sugars, amino acids, and fatty acids in this raw material that are formed following the hydrolysis process, and that can ultimately lead to a more efficient methanogenic process over time (Li et al., 2011). On the contrary untreated OP being made up of olive peel and pulp mainly contains polysaccharides, proteins, and lipids, making the hydrolysis step necessary before the acetogenic and methanogenic process (Li et al., 2011). The analysis of these raw materials used for these experiments will be the subject of subsequent studies where these concepts will be explored in depth.

4. Conclusions

This research investigated the production of biogas from the waste biomass deriving from three three-phase OP-PH extraction process. The results achieved with this entering biomass were then compared with what obtained with different biomasses, for a total of four samples tested. The concentration of biomethane in the biogas mixture was detected and, considering the whole quantity of biogas produced from the samples, the quantity of energy producible for unit of mass was calculated. This latter quantity was then compared with the energy consumption, associated with the production of biogas and derived from the literature. Based on the energy spent/energy produced ratio, the optimal production period was defined for each sample and the process efficiency was re-defined within this time range.

The anaerobic bioreactors consisting of inoculum added with the waste of three-phase OP-PH showed a greater production of biogas compared to the controls consisting of sole inoculum, inoculum with the addition of swine slurry, and a mixture of inoculum and untreated three-phase OP. The largest production of biogas for the bioreactors inoculated with the three-phase OP-PH waste occurred in the first 12 days, reaching an optimal production yield of 76.7%. The quantity of energy potentially producible using this raw material accounts for 946.5 kJ/kg of TS or 1727.8 kJ/kg of VS. These results more than doubled those obtained by the other samples demonstrating how the energy values potentially obtainable from the waste of three-phase OP-PH are remarkable and should be taken into consideration in order to optimize processes aimed at valorising olive oil waste supply chain. The biogas obtained can be then used for electricity production, while through a purification process it is possible to obtain biomethane, which can instead be used by introducing it into the distribution network or for the transport sector.

These outcomes pave the way for possible applications of the waste from the olive supply chain in a multi-purpose biorefinery concept, aimed at valorising the olive by-products from different points of view. The potential industrial applications of this research lie then in the fact that these results can draw a biorefinery idea aimed at valorising waste from the olive oil supply chain, where each waste acts as a substrate for the subsequent targeted process to obtain value from substances that otherwise would not have it. In this way, the waste from a process of valorization of waste from the production of olive oil, *i.e.*, the waste derived from the production of PHs starting from three-phase OP, can also be reused to obtain an energy value in a Circular Economy perspective thus promoting the use of more sustainable raw materials for biogas production.

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