Production of biogas from remnants of olive mill waste following bioethanol production as a circular economy model

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Research Article

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Abstract

Lignocellulose biomass for bioethanol production is one way for solving the environmental problems of the agricultural residues, besides the production of renewable fuel for the transportation sector, however, the bioethanol production is still inefficient due to a large amount of remnant waste left. So the anaerobic digestion (AD) of this remnant could be an effective solution to increase the overall energy output from the biomass. This study aims to explore the overall energy output and sustainability from the olive mill solid waste (OMSW) through bioethanol production and the utilization of the remnant residues for biogas production by exploiting the organic waste-energy nexus as a circular economy model. The used remnant for biogas production was obtained following bioethanol production from OMSW subjected to microwave-assisted maleic acid, formic acid, or distilled water which were used for the pretreatment, and was followed with enzymatic hydrolysis and fermentation process. The biochemical methane potential results fitted the modified Gompertz model. No influence of pretreatment was observed on the maximum methane production. A clear difference was observed on the kinetic constant and on the lag phase. The maleic acid pretreatment showed the highest energy conversion efficiency of 71.0% and the energy yield for the combined ethanol production and biomethane production was 12.92 GJ fuel per ton of dry weight of substrate (10.3 MJ/kg from methane and 2.6 MJ/kg from ethanol). This study shows that the synergy between bioethanol production and AD process could exploit the OMSW – energy nexus.

Statement Of Novelty

The idea of this study become after conducting research for bioethanol production from OMSW and optimizing the pretreatment conditions. The results showed that bioethanol production is still inefficient due to a large amount of remnant waste left. The idea was to use this remnant for producing energy (biogas) and increasing the overall output energy, comparing different pretreatments that were done for assessing methane production. To our knowledge, the sequencing processes of bioethanol and biogas production from OMSW were not done before.

Introduction

Olive cultivation is an important agricultural practice in the Mediterranean region. The olive oil extraction results in a very large amount of waste, both olive mill wastewater (liquid fraction) and olive mill solid waste (OMSW) obtained during the traditional and 3-phase method, while it results in a solid/liquid mixture waste in the 2-phase extraction method [1–4]. The solid fraction consists of olive pulp, stone, and fruit skin and is rich in fibers, phenols, minerals, and oil remnants, with varying fractions of the three major structural components: lignin (20–43%), cellulose (16.6–37%), and hemicellulose (14.4–36.6%) (All as DW/DW) [5–7]. The high concentration of organic matter in the waste results in an elevated pollution load [8, 9], but the high phenol content makes composting process very complex and inefficient [7].

Previously we have demonstrated the valorization of OMSW to produce biochar [10] or to produce ethanol using enzymatic saccharification followed by yeast fermentation [1]. The process was especially effective when the OMSW was pretreated by microwave in the presence of formic acid followed by saccharification with

Novozyme CTec 2, releasing 85% of the cellulosic fraction as fermentable sugars and resulting in 15.9 $g \cdot L^{-1}$ ethanol (91 mg ethanol per g OMSW).

Although ethanol production was effective, even under the best conditions, only 20% of the OMSW was valorized to ethanol, leaving behind ~ 70% of the biomass, making the valorization of these remnants desirable. Our hypothesis is that this biomass remnant can be used as feedstock for the production of biomethane.

The process of obtaining biomethane is highly sustainable both environmentally and economically [9]. Bioethanol and further biomethane production have been investigated in several previous works, Kotarska et al. [11] and Rocha-Meneses et al. [12] investigated the option to obtain two types of fuel (bioethanol and biogas) in a sequential combination of biochemical processes from corn straw and Napier grass respectively. The results were promising, so the combination of processes for obtaining various biofuels enables the production of waste-less technology and it can be used as an alternative source of energy. Elsayed et al. [13] studied the potential of sequential fermentation and biogas production from rice straw. The authors showed that these strategies enhanced biomass degradation, where the maximum output bioenergy reached 10.58 GJ/ton [13].

Recently, several studies explored the valorization of OMSW by anaerobic digestion (AD) for biogas production. Donoso-Bravo et al. [14] investigated the impact of mechanical pretreatment (milling), enzyme maceration, direct enzyme addition on the methane production of olive pomace. The upstream enzymatic maceration lead to an ultimate methane production of 274 ml CH_4/ g VS, which represents an improvement of 32 and 71%, compared to the blank and control tests, respectively. Pellera et al. [9] evaluated the influence of alkaline (NaOH) pretreatment on AD of olive pomace. The highest methane yield (242 NmL CH_4/gVS) was obtained for the material pretreated at 90°C, using a NaOH dosage of 1 mmol/gVS. Rincón et al. [15] evaluated the effect of ultrasound pretreatment of two-phase OMSW at a power of 200 W. The best methane yield was obtained through biochemical methane potential (BMP) tests, where 393 mL CH_4/g VS was achieved for ultrasound pretreatment during 90 min; this yield was 5.6% higher than that obtained for OMSW without pretreatment. While Alagöz et al. [16] investigated the effect of ultrasonic and microwave pretreatment on biogas production from the anaerobic co-digestion of olive pomace and wastewater sludge. The maximum methane yield from the mono-digestion of olive pomace was 180 mL CH_4/g VS compared to co-digestion of olive pomace and the microwave pretreatment wastewater sludge which increased the methane production by 52%.

Therefore, the idea was to utilize the leftover OMSW after the ethanol production for biogas (methane) production using the AD process. AD is a biological conversion of organic matter into biogas in the absence of oxygen. The biogas consisted mainly of methane (CH_4), carbon dioxide (CO_2), and a trace amount of other gases such as hydrogen sulfite (H_2S), ammonia (NH_3), hydrogen (H_2), nitrogen (N_2), and carbon monoxide (CO). Biogas production was one of the supplementary processes added to the main experiment.

To our knowledge there are no previous studies in the literature about sequential ethanol fermentation and AD process of the OMSW, therefore, ethanol production followed by biogas production will support the circular economy model for the olive oil industry sector.

The main goal of this work is to increase the overall energy output and sustainability from the OMSW through bioethanol production and the utilization of the remnant residues for biogas production by exploiting the organic waste-energy nexus as a circular economy model. The remaining residue of the bioethanol fermentation process is the raw material that was utilized for the second product (methane) using anaerobic digestion.

Methods And Materials

The raw material of OMSW was collected from a 3-phase olive oil mill located in Reineh, Galilee region of Israel, in the fall of 2018. The OMSW was shade dried for a week, milled and screened through an 18 mesh screen (i.e. 1 mm), and stored in tightly closed plastic bags at ambient temperature until being used.

Experimental setup and procedure

This section is divided into two parts; part I, where the pretreatment, enzymatic hydrolysis, and fermentation processes were conducted and will be reported in a separate paper. Part II, where the setup and procedure of the BMP, from the solid remnant of Part I, is described in this paper.

Part I: Microwave pretreatment, enzymatic hydrolysis, and fermentation

Three main processes that were conducted for ethanol production were described and investigated in a previous study [5]. Briefly, using the methods of statistical design of experiments (DOE) in the design and analysis of the experiment, 5g dried ground OMSW was suspended in water in 10% (w/v). Maleic acid (MA) or formic acid (FA) was added to the designated concentrations (0.2, 0.4, 0.6 M). The suspended was subjected to microwave treatment (START D, Milestone S.r.l), for the different designated times (4, 7, 10 min), and temperatures (120°C, 150°C, 180°C). These four pretreatment factors of pretreatment temperature, pretreatment time, organic acid (OA) type, and OA concentration were varied for 64 runs as shown in Supplementary Table S1. Following the pretreatment, the solid phase was collected following filtration, washed with distilled water and dried (< 10% moisture), and kept for further process of enzymatic hydrolysis. The enzymatic hydrolysis was conducted with 16% solid, using Cellic CTec2 (Novozyme) (11–22 FPU per g; depending on the sample), tween 80 (0–0.09 g/g treated OMSW) for 24 h at 50°C and mixing at 190 rpm. Following the hydrolysis, the solids and hydrolysates were separated by centrifuge, where the hydrolysate was taken for fermentation for ethanol production [1]. The suspension was incubated for 24 h at 35°C under anaerobic conditions by flushing with N_2 for 3 min before closing the bottles (data not shown). The solid remnants were dried at 30°C till < 10% moisture, and each type of OA treated OMSW was gathered (sorted by pretreatment additive but different at microwave time and temperature, the enzyme load, and tween80 amount) and used for BMP. Figure 1 presents a simplified scheme of the pretreatment, enzymatic hydrolysis, and fermentation process used in advance of the BMP.

Part II: Biochemical methane potential (BMP) from the solid remnants

The remnant solids obtained after the enzymatic hydrolysis (sorted by pretreatment additive but different at microwave time and temperature, the enzyme load, and tween80 amount) were combined. The remnant solid

was used as the feedstock for the biogas production (i.e., three feedstock: MA, FA, distilled water (DW), in addition to a control of the OMSW, and a sludge control).

Anaerobic sludge (inoculum) was obtained from the wastewater treatment plant of Karmiel, the Galilee region, Israel. This sludge was kept at room temperature for about 30 days in a loosely capped bottle to exhaust any endogenous biogas production, and then analyzed using standard methods for moisture content (77.7%) and volatile solid (VS) (15.7%), and total Kjeldahl Nitrogen (TKN) (2,802 $mg \cdot L^{-1}$).

Small reactors consisting of 100 ml syringes, each connected to a three-way plastic valve were used for the biogas production process, with an active volume of 30 ml; consisting of 20 ml medium as previously described by Massalha et al. [17], and supplemented with 31 mg ammonium chloride, $1g \cdot L^{-1}$sodium bicarbonate buffer. The medium was adjusted to pH 7.0-7.2 with 1M NaOH solution, 2 g solid remnant after enzymatic hydrolysis (for the virgin OMSW control, only 1 g samples were used) was added, and in addition to 10 ml inoculum was added. The syringe was flushed with 99.99% N_2 for 2 min to remove remnant oxygen/air from the headspace. Substrate inoculum ratios (SIR) were 12:1 for each treatment of MA, FA, DW, and 6:1 for the virgin OMSW. The syringes (reactors) were incubated simultaneously at 37°C for 44–98 days, in the same incubation chamber, with the same inoculum. Three replicates were conducted for each feedstock.

The initial pH was ranged 6.9–7.4, and the final measured pH of 8.06–8.7 was recorded for different reactors of the AD process.

Biogas samples were collected using 50 mL plastic syringes for volume determination and gas analysis. Manual gentle shaking was conducted for each syringe before collecting the biogas.

Analytical methods

The samples for biogas production were analyzed for their composition: methane CH_4, carbon dioxide CO_2, hydrogen H_2, and trace gases using a gas chromatography GC (Agilent 7890B GC system) combined with thermal conductivity detector (TCD), equipped with Agilent DB-1ht GC Column, part number: 122–1111, 15 m length, 0.25 mm of inner diameter (ID), 0.10 µm film thickness, where nitrogen was used as a carrier gas. The column temperature was fixed at 150°C, while the injector and detector were set at 200°C. The volume of injected biogas sample was 0.2 ml.

The total solids (TS) and volatile solids (VS) of the investigated materials were determined by drying the solids to a constant weight at 105°C and 550°C, respectively. Cellulose, hemicellulose, and lignin were determined according to the standard analytical procedure as described in the two-stage acid hydrolysis protocol of the National Renewable Energy Laboratory (NREL) by Sluiter et al. [18].

Total Kjeldahl Nitrogen (TKN) was estimated in the inoculum using standard methods 4500-Norg B (APHA) [19]. The protein content was determined in all OMSW remnants using the Bio-Rad protein assay protocol.

Statistical methods

All experiments were performed using three replicates and the results are presented as means ± standard deviation (SD). Statistical analysis of the data was performed with JMP software using one-way analysis of

variance (ANOVA). $P < 0.05$ was considered significant.

Results And Discussion

Compositional analysis of OMSW

The content of cellulose, hemicelluloses, and lignin in the untreated and the three different treated OMSW samples are summarized in Table 1. The fiber analysis shows high lignin content, followed by cellulose, and finally hemicellulose, with a drastic decrease in cellulose content after treatment. The high cellulose content after the enzymatic hydrolysis is due to mixing remnants of multiple hydrolysis experiments (sorted by pretreatment additive but different at microwave time and temperature) some less efficient than others, leaving some cellulose.

The chemical composition of the remnants' feedstock is presented in Table 1. The VS content varied between 89.2% (untreated OMSW) and 93.9% (MA pretreated). This was an indicator of the potential for methane production since it represents the fraction of the solid material that can be converted into methane. The TS content varied between 92.1% (untreated) and 95.8% (MA).

Table 1 Characteristics of the OMSW after the different pretreatments followed by enzymatic hydrolysis, in addition to the inoculum used for the biogas production. Data are based on dry material. Each data point is an average of 2 replicates

Parameter	Virgin OMSW	Remnants after Maleic acid-MW and enzymatic hydrolysis	Remnants after formic acid-MW and enzymatic hydrolysis	Remnants after distilled water-MW and hydrolysis	One way ANOVA (F; *p*) df =3,8 in all cases	Inoculum
TS (%)	92.1 ± 0.39^{a}	95.8 ± 0.02^{b}	95.5 ± 0.01^{bc}	94.7 ± 0.61^{c}	64.7; *<0.001*	2.23
VS (%)	89.2 ± 0.16^{a}	93.9 ± 0.10^{b}	93.6 ± 0.11^{bc}	92.0 ± 1.08^{c}	45.8; *<0.001*	1.57
VS/TS (%)	96.9	98.0	98.0	97.1		70.4
Cellulose (%)	27.0 ± 1.9^{a}	17.7 ± 0.1^{b}	16.2 ± 0.3^{b}	16.1 ± 0.8^{b}	75.1; *<0.001*	NA
Hemicellulose (%)	11.1 ± 1.1^{a}	7.0 ± 1.0^{b}	8.8 ± 0.8^{bc}	9.3 ± 0.1^{ac}	11.9; *<0.005*	NA
Lignin (%)	42.9 ± 4.6^{a}	54.4 ± 2.0^{b}	47.5 ± 3.7^{ab}	1. ± 2.6^{ab}	5.93; *=0.02*	NA
VS/Lignin	2.08	1.73	2.0	1.94		NA
TKN (mg·L^{-1})	NA	NA	NA	NA		2802

Different superscript letters in each row mean statistically different results using Tukey HSD Post-hoc Test; NA – not available. TS - Total Solids, VS - Volatile solids, TKN - Total Kjeldahl Nitrogen

2.1 Methane (biogas) production potential test

Methane production from virgin OMSW calmed after 22 days, while such from remnants of enzymatic hydrolysis after pretreatment with DW, FA, and MA samples, calmed only after 53, 53, and 82 days respectively (Fig. 2). No methane was detected in the inoculum reactor (data not shown). There were no differences in the final level of methane production for all the samples, but there were differences in the rate and the lag time (Fig. 2). The methane production ranged from 252.2 - 289.5 mL CH_4/g VS (Table 3 and Fig. 2). The digestion time (DT_{80}) describes the time needed for producing 80% of potential biogas generation. The biogas yield rate and the bio-digestibility of the substrate can be determined based on DT_{80} [20]. In addition, concerning the continuous bio-digester, DT_{80} could be used as a hydraulic retention time [21]. The AD, in the current work, stayed up to 98 days, while DT_{80} for FA, DW, MA, and OMSW was only 42, 42, 66, and 16 days, respectively.

The main compositions of biogas produced during AD were methane, carbon dioxide, and some traces of oxygen. Fig. 3 shows the percent of the methane produced over the time of the tested samples. The methane

percentages ranged from approximately 68.5% - 72.3% for the samples MA, FA, DW, and OMSW. The lowest value was for DW while the highest value was for MA.

The carbon dioxide percentages ranged from 17.9 - 22.8. The lowest value was for MA while the highest value was for OMSW (control).

Due to the difference in the SIR (g VS substrate/g VS inoculum) in the AD tests between the untreated OMSW (SIR=6) and the OMSW bioethanol residues (SIR=12), it was difficult to compare kinetic constants. Many studies of AD tests showed that high hydrolysis rates were reached with low SIR for the same substrate [14, 22]. SIR influences were different for the various used substrates [22].

2.2 Kinetic model of methane production and fitting of Gompertz model parameters

Since the lag phase was reported in our results, the modified Gompertz model was selected to detect the cumulative methane production continuously and to perform an analysis of the effect of biomass solvent pretreatment on methanogenic activity. The modified Gompertz model was fitted to the experimental data as an important model for the study of methane production kinetics Eq. (1) [23]:

$$P(t) = P_{\max}\exp\left\{-\exp\left[\frac{2.7183\,R_{\max}}{P_{\max}}(\lambda - t) + 1\right]\right\} \qquad (1)$$

Where P(t) is the cumulative methane production (mL CH_4/g VS) at time t, P_{max} is the maximal potential of methane production (mL CH_4/g VS), R_{max} is the maximal specific methane production rate (mL CH_4/d g VS), λ is the lag phase duration (d), and 2.7183 is the exp(1). The parameters P_{max}, R_{max}, and λ were obtained by fitting the modified Gompertz equation to the experimental results through non-linear regression using the Solver tool of Microsoft Office Excel software. The suitability of fit was determined by taking into consideration, both, the Residual Sum of Squares (RSS) and the R-square (R^2) values.

Table 2 presented the calculated parameters (λ lag phase duration, R_{max} maximum specific methane production rate, and P_{max} methane production potential) which are based on the fitting model. Our results showed that the modified Gompertz model reflected well the methanogenesis of AD (all R^2 values were above 0.9). The λ value can reflect the rate of reaction of the substrate in the AD system, which shows the level of adaptation of the microbes to the substrates in the AD system. Low λ values reflect quick adaptation of the microbes to the new conditions almost without a lag phase, which means that the fermentation system produces methane faster which means with less fermentation time (short lag phase). Our results showed that MA had a large λ, indicating that the microbes were affected during the fermentation process, causing a delay in methane production. When FA and DW were used for the fermentation systems, the value of λ decreased significantly, which indicated that the AD process was not inhibited and the microbes were active and started to utilize the biomass and to produce biogas with a shorter lag phase.

The explanation of the large λ could be attributed to inhibition caused by sodium. Low concentration (~350 mg·L^{-1}) of sodium was previously demonstrated to be essential for mesophilic methanogenic bacteria [24], with 3.5 g·L^{-1} and higher having inhibitory effects on methane production at different temperatures [25–27]. Here we used 50 mM sodium citrate buffer for the enzymatic hydrolysis (i.e. 1.15 g·L^{-1} sodium), where some of it might have stuck to the remnants but this issue needs further study.

Another explanation could be that higher SIR delayed methane production, indicating a process inhibition [28]. The results of Pellera et al. [9] showed that lower SIR is more adequate for determining the methane potential of the agricultural waste. In our work, the SIR was relatively so high (SIR=12).

The highest R_{max} values of 29.7 were obtained for untreated OMSW (SIR=6) and the remnants of the FA pretreatment-hydrolysis (SIR=12) was R_{max} 20.9, where the lignin concentration was the lowest. When the lignin content increased, the value of R_{max} decreased, indicating that lignin has additional inhibitory effects, similar to the previous study of Fernández-Cegrí et al. [29].

Besides, the R_{max} and λ of DW and FA were similar with P_{max} of 249.1, 256.5 ml/g VS, respectively (Table 2). These results presented in Table 2 indicate that the fermentation potential of DW and FA based on R_{max} were similar. MA had a relatively high P_{max} of 289.8; however, the R_{max} was low, apparently due to the high λ value caused by some inhibitory factors during the biogas production.

Table 2 Kinetic parameters obtained from fitted modified Gompertz equation for untreated OMSW, and different pretreatments that followed by enzymatic hydrolysis. Each mean is an average of 3 replicates: the fitted modified curve was based on the average of the 3 replicates

	P_{max}* (mL CH_4/g VS)	R_{max} (mL CH_4/d g VS)	λ(d)	R^2
Virgin OMSW (1g)	272.5	29.7	7.3	0.974
DW	249.1	18.23	30.5	0.997
FA	256.5	20.88	31.2	0.9978
MA	289.8	12.6	47.5	0.982

***** P_{max} is the maximal potential of methane production, R_{max} is the maximal specific methane production rate, λ is the lag phase duration and R^2 is the correlation coefficient

2.3 The impact of pretreatment additive on methane production

The methane yields obtained through the BMP after the digestion process of untreated OMSW, MA, FA, DW, were 276 ± 15.5, 287.5 ± 20, 260 ± 17.5, 252.2 ± 20.2 mL/ gVS, respectively (Table 3). The obtained methane yield from the MA, FA, DW of treated OMSW and untreated OMSW was ca 34%, 29%, 27%, 20% (1.3, 1.3, 1.3, and 1.2fold) higher than the theoretical methane from the carbohydrate fraction, protein and lipid (Eq. 2), implying that portion of the lignin was also converted into methane and the dead bacterial remains constitute the digestive, which were similar to the results obtained by Mulat et al. [30]. The percentage of carbohydrates in treated OMSW, and after pretreatment followed by enzymatic hydrolysis was reduced to 34%, 40%, 40%, while the amount of lignin was increased to 26%, 11%, and 10% for MA, FA, and DW, respectively. The remaining carbohydrates of 66%, 60%, and 60% coming from MA, FA, DW of the pretreated OMSW, respectively, and a portion of the lignin fraction could be utilized as well as a substrate for methane production. High temperature and acidic conditions (released organic acids) during microwave pretreatment could catalyze the hydrolysis of hemicelluloses and further the degradation into lower molecular weight (LMW) compounds such as furfural. Other previous works have concluded that LMW compounds may undergo polymerization processes, forming lignin-like compounds termed “pseudo-lignin” [30, 31]. Thus, the formation of pseudo-lignin was probably the main reason for increasing the lignin fraction following pretreatment, in addition to the loss of volatiles during microwave pretreatment could also contribute to the increase in the lignin and cellulose fraction [30].

Table 3 Characteristics of the different biomass remnants and the raw material of OMSW used in the experiments. Each mean is an average of 2-3 replicates ± SD

Pretreatment additive / Parameter	OMSW	Maleic acid-OMSW	Formic acid-OMSW	Distilled water-OMSW
Lignin (%)	42.9 ± 4.6	54.4 ± 2.0	47.5 ± 3.7	47.4 ± 2.6
Carbohydrate (%) (cellulose+hemicellulose)	38.1	24.7	25	25.4
Protein (%)	1.56 ± 0.03	2.22 ± 0.07	1.43 ± 0.08	1.33 ± 0.04
Lipids (%)	9.2	11.35	10.2	10.2
BMP_{theo} (mL/ gVS)	231	215	201	199
$BMP_{exp\text{-}model}$ (mL/ gVS)	272.5	289.8	256.5	249.1
BMP_{exp} (mL/ gVS)	276 ± 15.5	287.5± 20.1	260.1 ± 17.5	252.2 ± 20.2
Difference (mL)	45	73	59	54
α*	1.2	1.3	1.3	1.3
Increasing (%)	20	34	29	27

***α = Experimental production/ Theoretical production

BMP_{theo}= theoretical biochemical methane potential

$BMP_{exp-model}$= fitted modified Gompertz model for the biochemical methane potential based on observed results

BMP_{exp}= observed biochemical methane potential.

The OMSW was pretreated using a microwave at various conditions to improve bioethanol production during the separate hydrolysis and fermentation process. It should be noted that the enzyme solutions used also contain carbon sources and could contribute as well to methane production.

According to the results in part I of this study (data not shown), that deal with optimization of the conditions of pretreatment and enzymatic hydrolysis processes for bioethanol production, the samples that were treated with MA had the highest glucose conversion in enzymatic hydrolysis and the highest hemicellulose conversion in the pretreatment process. Therefore, the MA raw material for AD in this paper contained less cellulose and hemicellulose and more lignin compared to the FA and DW raw material. In FA and DW there were more non-degradable cellulose and hemicellulose, while in MA there was more lignin, which is difficult to be broken down. So the results showed that the lag phase in MA was longer than FA and DW. The final value of BMP showed no statistical significant differences at ($p<0.05$).

The theoretical biochemical methane potential formula of feedstock is given as follows [32]:

BMP = 415 × % carbohydrates + 496 × % protein + 1014 × % lipids (2)

The energy conversion efficiency and the total energy outputs of the four feedstock used for AD are summarized in Fig. 4. The energy analysis of energy output from each raw material was determined by multiplying the amount of bio-ethanol (data not shown) or bio-methane which was produced with the corresponding calorific value. According to Ouazzane et al. [33] and Wang et al. [34], the calorific values for OMSW, ethanol, and methane are 18.2 MJ/kg, 26.7 MJ/kg, and 35.9 KJ/L, respectively. The output energy was considered in the energy analysis for this study where the input energy i.e. pretreatment, fermentation, and transport were not considered.

The energy output of methane production was calculated as:
[MJ/kg VS] = MP × 0.001× HHV (3)

Where the MP is methane production [L/Kg VS], 0.001 is the conversion factor from KJ to MJ, HHV is the high heating value of methane [KJ/L], where the high heating value is 35.9 KJ/L

The energy output of ethanol fermentation was calculated as:

[KJ/Kg VS] = EP × HHV (4)

Where the EP is the ethanol production [g/kg VS], HHV is the high heating value of ethanol [kJ/g], and the high heating value was 26.7 kJ/g.

The total energy output using the FA, DW, OMSW pretreatment were 11.57, 10.80, 9.91 GJ/ton, respectively. Using the MA pretreatment resulted in the maximum bioenergy output of 13.08 GJ/ton. The total energy

outputs obtained from the MA, FA, and DW were 32% and 16.75%, 9%, respectively, higher than that obtained from the OMSW. The MA showed the highest energy conversion efficiency of 71.88%.

The BMP values that were reported in the literature were about, 98-370 mL CH_4/gVS, for the traditional, two-phase, and three-phase process of OMSW (Table 4). The OMSW used in this work produced 276 mL CH_4/gVS. Various methane yields are due to the differences in the OMSW chemical composition, which depends on the geographical origins, olive cultivars, and the method of oil extraction processes (Table 4). The results of the combined biomethane production followed by bioethanol fermentation in the current work were compared with those of other reported values of biomethane production as a single process. Using residues of OMSW treated with microwave with MA for bioethanol production showed that the maximum fitted model methane value was 289.8 ml CH_4/g VS, by using the two-phase OMSW following ultrasound pretreatment reported by Rincón et al. [15] where 393 mL of CH_4/g VS was obtained. Using OMSW and olive mill wastewater mixture (1:2 ratio) with Fenton process pretreatment (H_2O_2/[Fe^{2+}]) Maamir et.al. [35] reported that 224 mL of CH_4/g VS was obtained.

In future research, it is recommended to operate large-scale reactors and calculate the energy balance of output/input energy.

Table 4 Comparison data of previous studies dealing with methane production from OMSW biomass

Reference	Substrate	Inoculum	Reactor type	Operating condition	Pretreatment	Methane yield
[36]	OMSW	Cattle Manure (CM)and Cattle Slurry (CS)	Batch stirred tank reactor	4.65% of CM, 72.10% of CS and 23.25% of OMSW ,mesophilic conditions (38°C), 55 days	None	103.4 mL/g VS
[37]	OMSW: Turkey manure 60:40	Turkey manure	Anaerobic batch operating reactors	8% total solid at mesophilic conditions, at 30°C for 40 days.	None	20.2 mL / g mix
	40:60					23.1 mL / g mix
[35]	OMSW and OMWW mixture was in a 1:2 ratio	Bovine manure	Batch digester of 300 mL as working volume.	Mesophilic conditions (37 °C) 30 days	Fenton process (FP) pretreatment (H_2O_2/[Fe^{2+}])	224 mL/ g VS
[15]	Two-phase OMSW	Industrial anaerobic reactor treating brewery wastewater and operating at mesophilic temperature	Reactors were continuously stirred at 500 rpm and placed in a thermostatic water bath	Mesophilic temperature (35 ± 2◦ C)	Ultrasound pretreatment	393 mL CH_4/g VS
[9]	Olive pomace (OP)	Anaerobic sludge sample originating from a mesophilic anaerobic digester of the Municipal Wastewater Treatment Facility	250 mL reactors, consisting of conical flasks covered with rubber stoppers	35° C	Alkaline (NaOH) pretreatment, 90°C	242 NmL CH_4/gVS
[14]	Olive pomace	Continuous lab-scale stirred tank reactor maintained in mesophilic conditions and feed with sewage sludge	Anaerobic batch	37° C	Upstream enzymatic maceration	274 mL CH_4/ g VS
[16]	Olive pomace	Mesophilically operated anaerobic digester of a yeast factory	2500 mL anaerobic reactors	37° C for 30 days	None	180 mL CH_4/ g VS
	wastewater sludge					160 mL CH_4/ g

						VS
	Olive pomace & wastewater sludge				Microwave pre-treatment was applied to the sludge samples for 30 min at 175 C and 2000 kPa	320 mL CH_4/ g VS
This work	OMSW	Wastewater Treatment Plant (WWTP)	100 mL syringe	mesophilic temperature (37°)	Microwave-assisted MA or FA followed by enzymatic hydrolysis	252 – 289 mL CH_4 / g VS

Abbreviations

AD Anaerobic digestion

BMP Biochemical methane potential

$BMP_{exp\text{-}model}$ Fitted modified Gompertz model for the biochemical methane potential based on observed results

BMP_{theo} Theoretical biochemical methane potential

BMP_{exp} Observed biochemical methane potential

DOE Design of experiments

DT_{80} Digestion time

DW Distilled water

DW Dry weight

EP Ethanol production [g/kg VS]

FA Formic acid

FPU Filter paper unit

GC Gas chromatography

HHV High heating value

ID	Inner diameter
Kg	Kilogram
KJ	Kilojoule
L	Liter
LMW	Lower molecular weight
MA	Maleic acid
MJ	Megajoule
MP	Methane production [L/Kg VS]
MW	Microwave
NREL	National Renewable Energy Laboratory
OMSW	Olive mill solid waste
OA	Organic acid
P_{max}	Maximal potential of methane production (mL CH_4/g VS)
P (t)	Cumulative methane production (mL CH_4/g VS) at time t
R_{max}	maximal specific methane production rate (mL CH_4/d g VS)
R^2	R-square
RSS	Residual Sum of Squares
SIR	Substrate inoculum ratios
TCD	Thermal conductivity detector
TKN	Total Kjeldahl Nitrogen
TS	Total solids
VS	Volatile solids
WWTP	Wastewater treatment plant
α	Experimental production/ Theoretical production
λ	Lag phase duration

Conclusion

This study explores the utilization of remnants of OMSW following bioethanol production for further AD for biogas production. The results show that the AD process of the remnant of bioethanol production of OMSW could be an effective solution to increase the overall energy output from the biomass by exploiting the OMSW-energy nexus approach. The results show that biomass remnant following MA treatment needs more retention time than FA or DW treatment, but with more total output energy. The MA residue shows the highest energy conversion efficiency of 71.0%. The OMSW used in our current work produces 276 mL CH_4/gVS. It is important to mention that the output of energy is considered in the energy analysis for this study, however, the input energy of pretreatment, fermentation, and transport is not considered.

Declarations

Supplementary Information: Table S1: Four pretreatment factors of temperature, time, organic acid (OA) type, and OA concentration, were varied for 64 runs as pretreatment process and 56 runs as saccharification process for ethanol production, by using the Design Of Experiments (DOE) approach

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Data Availability: All data generated or analyzed during this study are included in this published article.

Conflicts of Interest: The authors declare no conflict of interest.

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Figures

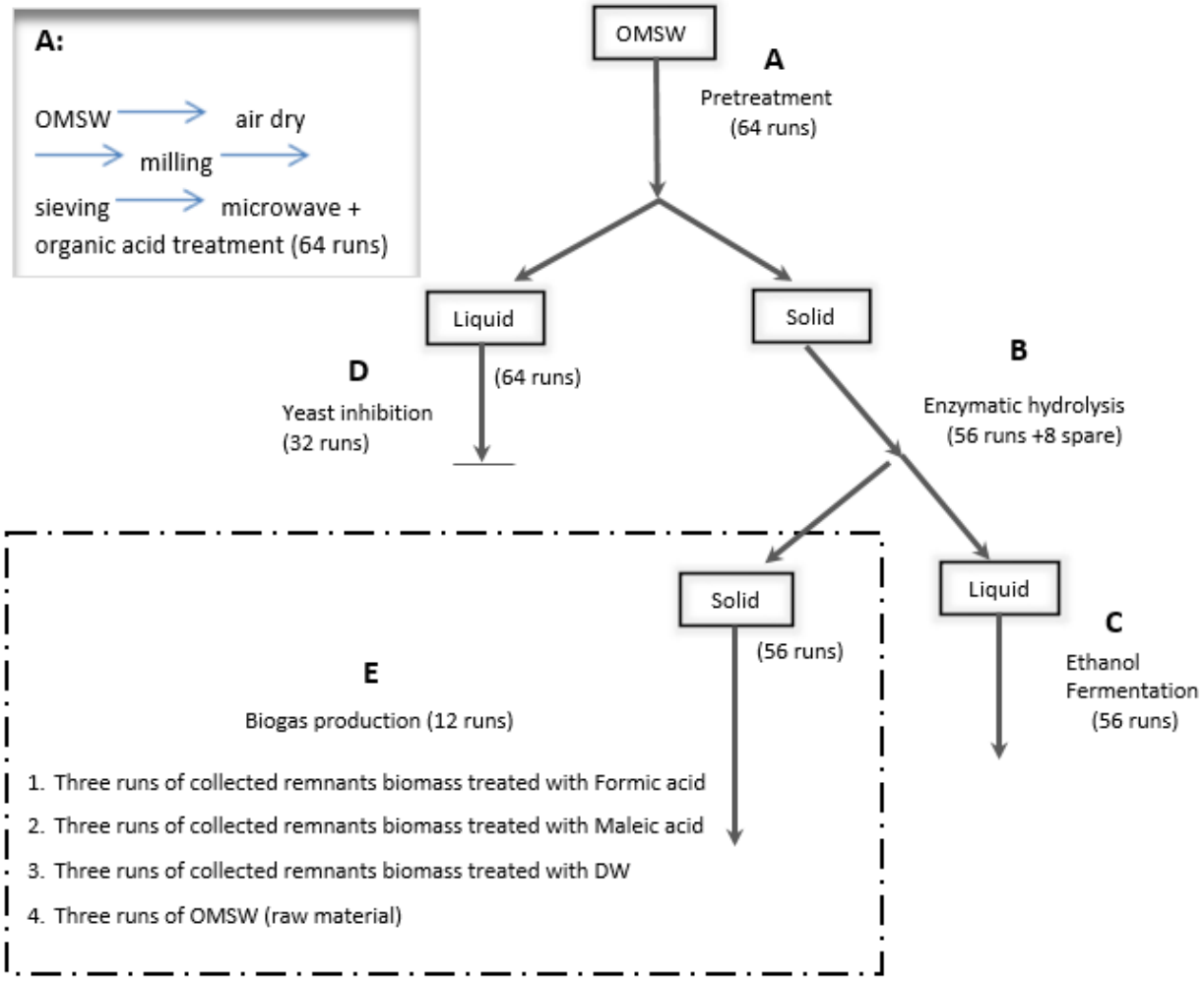

Figure 1

Schematic presentation of the whole course of the experiments conducted for the OMSW. The current results were obtained from stage **E** which is presented in this study. Stage A is based on different treatments of drying, milling, sieving, and microwave-assisted formic acid or maleic acid or distilled water (64 runs).

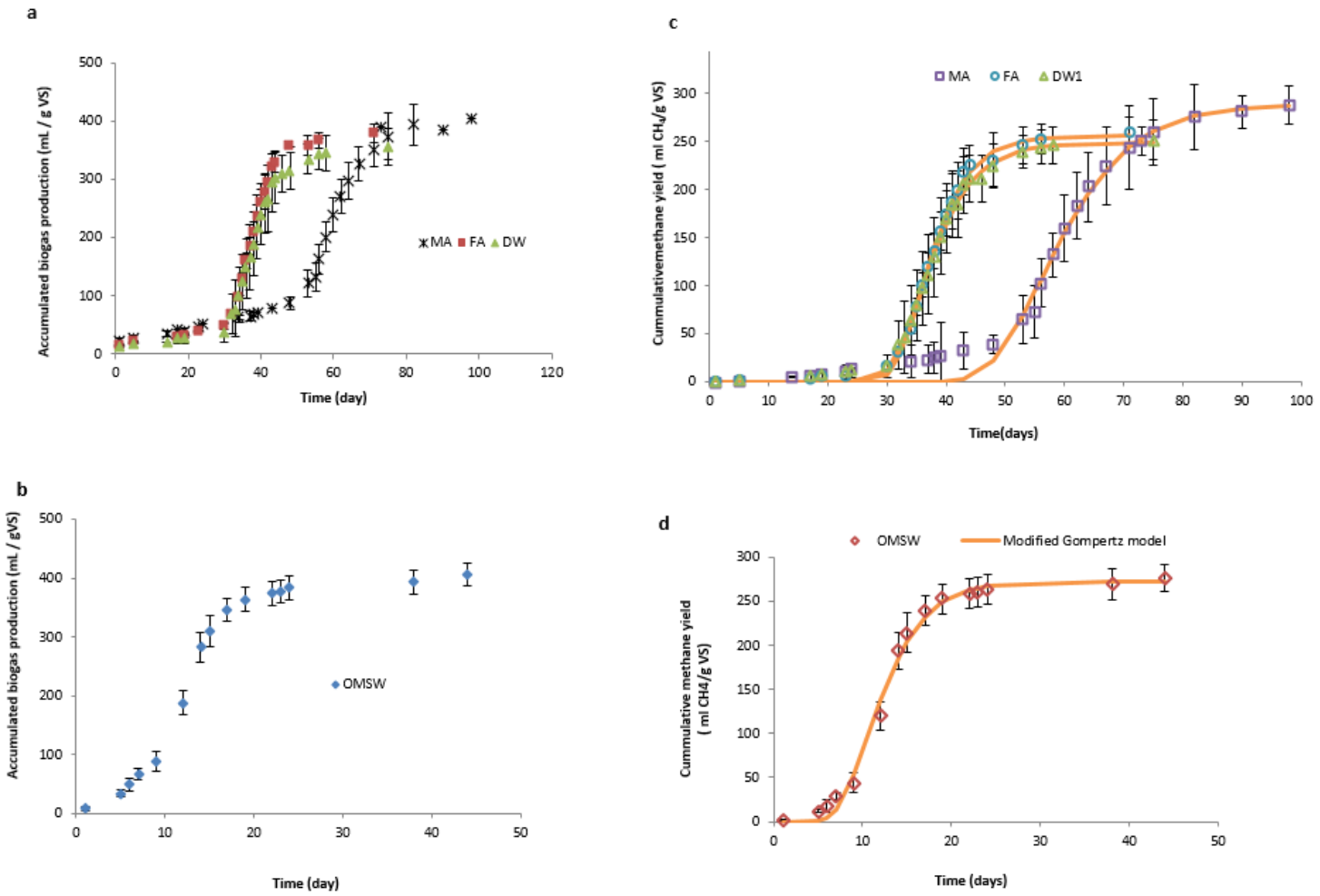

Figure 2

Cumulative biogas (a-b) and methane (c-d) production (ml/g VS) as a function of incubation time for the remnants of the enzymatic hydrolysis that was conducted on 2 g residues of different pretreatments (a, c) and for 1 g of raw OMSW (b, d). Experimental data (dots) and curve fitting are based on the Gompertz model (c-d)

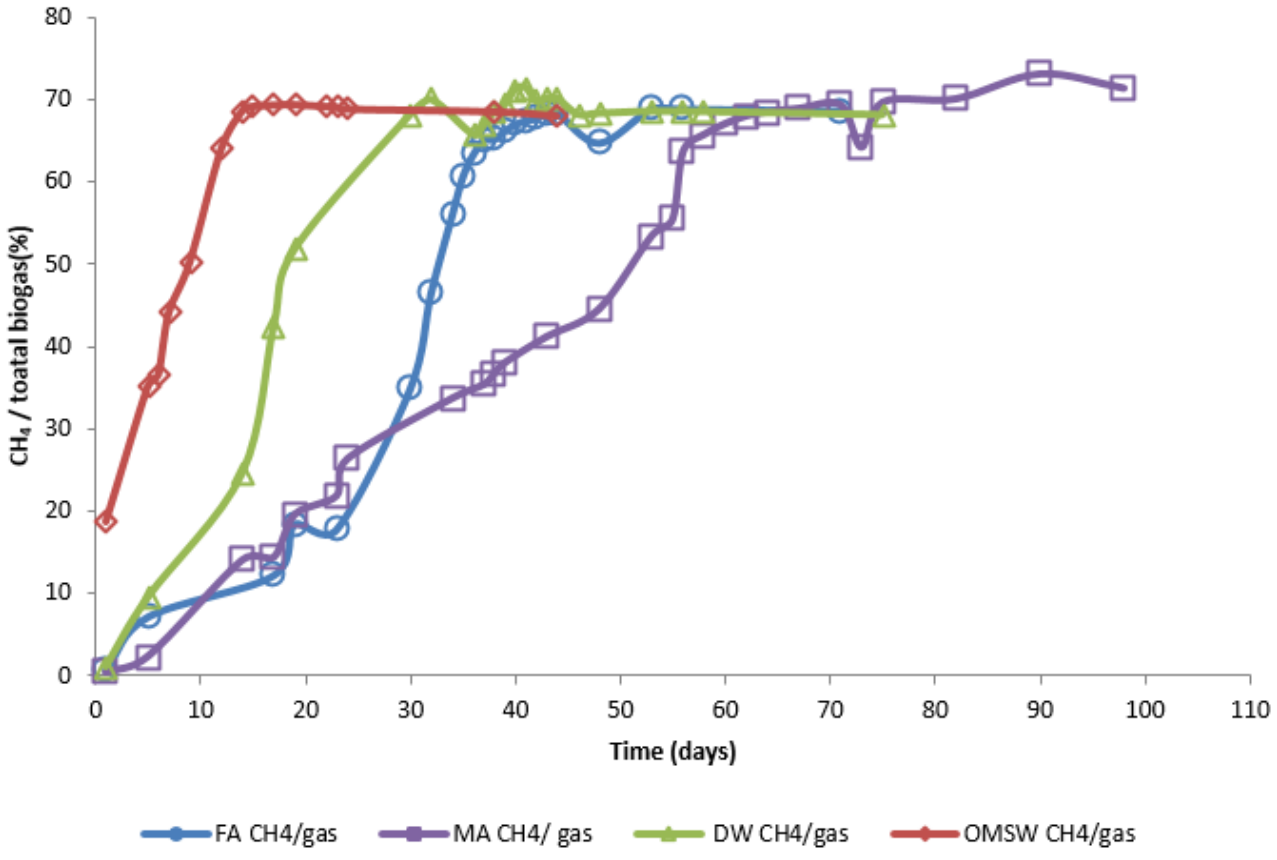

Figure 3

Methane proportion (%) of AD performances from BMP tests of residues of the different treated OMSW used for bioethanol production. Microwave pretreatment with formic acid (FA), maleic acid (MA), distilled water (DW), and control of no pretreatment (OMSW)

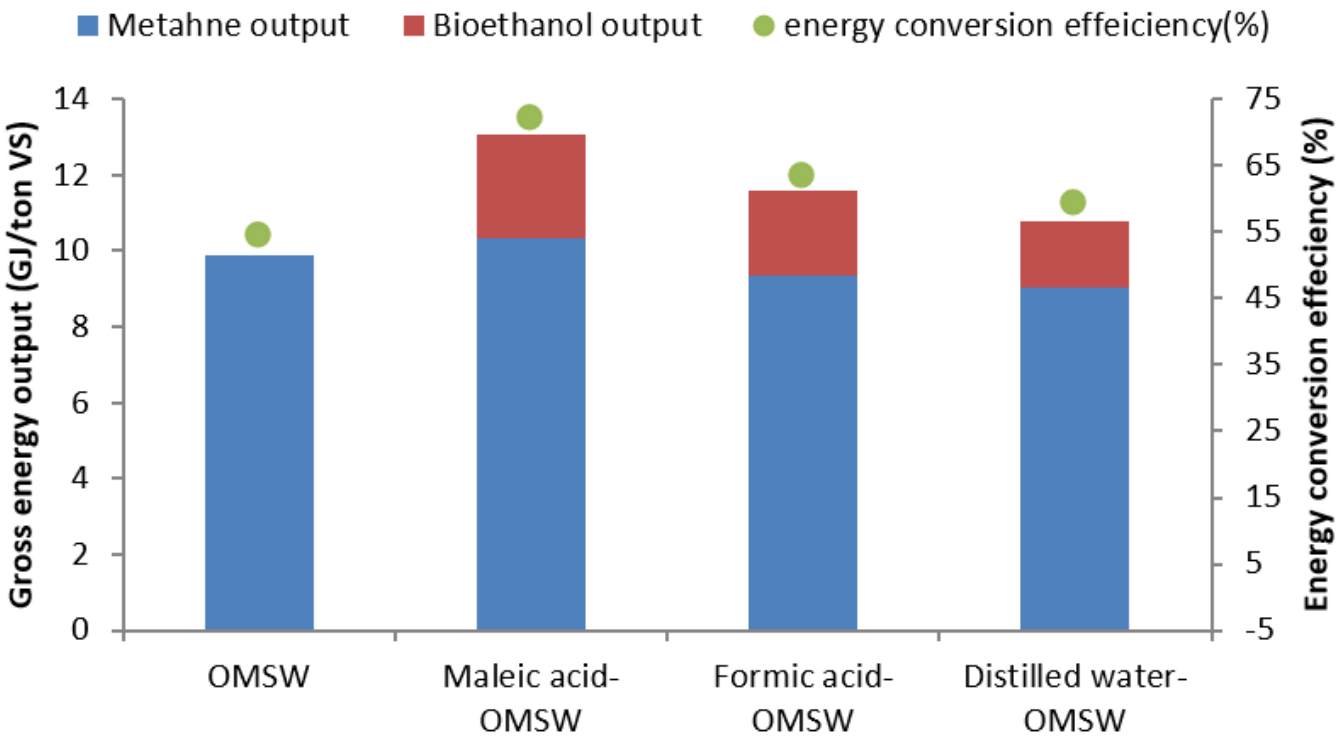

Figure 4

Energy outputs and energy conversion efficiency of the different OMSW biomass

Supplementary Files

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