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Toward the scale-up of agro-food feed mixture for biogas production

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ABSTRACT

Olive oil and dairy production are among the most important and widespread agro-food activities in southern Italy and particularly in the Puglia region. According to a territorial survey of the Puglia region, the related wastes, olive pomace (OP), olive mill wastewater (OMWW) and whey milk are very abundant (741,000 tons/year) and represent potential sources of contamination for the land and aquifers; however, these wastes also represent an interesting feedstock for biogas production through anaerobic digestion. OP, OMWW and whey milk are high in organic content (100 g/l, 65 g/l and 60 g/l, respectively), are acidic (with pH values between 3.5 and 5.5), and have high total solid percentage concentrations (30% (w/w), <5% (w/w) and 5% (w/w), respectively). In this study, the results of two experimental campaigns, both conducted at Asja Ambiente's research center in Ceglie Messapica (Italy) are reported. In the first campaign, different waste mixtures obtained from OP, OMWW and whey milk were fed into a 45-L anaerobic reactor to evaluate their biogas yields. In the second campaign, a combination of whey milk and OP was fed into an anaerobic pilot plant with a volume of 1.6 m^3. In the case of feed composed of 25% (w/w) OP and 75% (w/w) whey, a Chemical Oxygen Demand (COD) reduction of 64% and a biogas production of approximately 1.3 L/L day were obtained, which correspond to 0.013 L_{biogas}/gTS_{in}. This performance is potentially able to cover 0.015% of Puglia's yearly total demand for energy.

Introduction

Olive oil is used for food preparation in Mediterranean countries and has high economic value for many regions. Over 95% of the world's olive oil production is concentrated in the countries around the Mediterranean Sea, particularly Spain (36%), Italy (27%), Greece (15%), Tunisia (6%), Syria (6%), and Turkey (4%). With more than 3,353,200 tons of olives pressed per year (http// agri.istat.it, 2011), Italy is the second largest producer of olive oil in the world. The cultivation of olive trees is primarily located in the southern regions of the country, especially in Puglia (40% of the national olive oil production).

The main by-products of olive oil production are OMWW and OP. The features of the latter depend on the technology used during olive oil production processes, which could involve a three-phase centrifugation (oil phase 20% (w/w) olive, aqueous phase 50% (w/w) olive and 30% (w/w) as solid phase) [1], or a two-phase centrifugation of oil and "wet OP" with a water content up to 65% (w/w) [2].

All these wastes have a significant environmental impact. They have a high organic concentration (COD >250 g/l), high electrical conductivity, a high concentration of polyphenols (0.5–5 g/l), and low pH (4–6), and they produce bad odors as well [3].

For these reasons, the amount of olive mill effluent that can be applied to agricultural soil is limited and regulated by national laws (e.g., Legge n. 574 (1996) for the Italian State [4]) that differ among the various Mediterranean countries. In some regions (Puglia, Calabria, Campania), these effluents are collected in lagoons to evaporate the water they contain. The remaining solid part of these wastes can be used as either fertilizer or fuels [5]. However, a considerable amount of energy is required to evaporate the water, so the process is not economically attractive, and it is time- and land-consuming.

The dairy industry in Puglia, which produces more than 150,000 tons of milk products per year [6], is another industrial sector of great importance to the regional economy. The main waste product of the dairy industry is cheese whey. Olive oil production wastewater and cheese whey are very difficult to dispose of because of their low pH, their high protein and lactose contents and their COD (>90 mg/l) [7]. Anaerobic digestion, which ensures, with low nutrient addition, a high degree of abatement of the organic load and the production of stabilized sludge and a

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biogas with high energy value, may be a feasible method for treating these wastes [8].

During the last decade, many experiments have been carried out to evaluate the operating conditions that optimize the anaerobic digestion of olive oil production's sludge. Yu et al. [9] have conducted some tests of anaerobic digestion at different temperatures, keeping the hydraulic retention time (HRT) constant. Their main finding was that the hydrogen concentration, the most important precursor of methane, increases with temperature, as does the VFA concentration. In a later study, Wang and Banks [10] showed that the optimum temperature range for the anaerobic process of olive productions wastes is mesophilic conditions between 37 and 42 °C, while the best pH for the input sludge is 5.5–6.5. This means that it is necessary to operate at mesophilic conditions with a slightly acidity to the feed to obtain a stable bioreaction. Another study, conducted by Rincon et al. [11], analyzed the influence of the organic loading rate (OLR) on the anaerobic digestion of OP and showed that OLR affects the VFA concentration, which must be controlled to avoid a reversal of the system toward the acidogenesis step. In addition, Rincon et al. [11] demonstrated that the optimal OLR value produced the maximum hydrogen concentration in the biogas. Lastly, Borja et al. [1] conducted several anaerobic digestion tests on olive sludge mixed with other food wastes, testing different concentrations of the mix. Their study proved that at the beginning of a change of the feed mix, the production of biogas decreases, indicating that the collection of microorganisms needs some time to adapt to the new feed conditions.

The number of research studies conducted on the anaerobic digestion of cheese whey is limited [12]. This substrate, in fact, is too acidic for the anaerobic process. To mitigate the effect of the acidic pH of cheese whey, Comino et al. [7] conducted some codigestion tests with manure at different concentrations. They demonstrated that increasing the whey concentration in the feed to 50% (w/w), increases the methane content in the biogas. However, at higher levels of whey, biogas production shut down. Other studies have confirmed the difficulty of conducting the anaerobic process using cheese whey as the substrate [13].

The present study is based on a survey of the Puglia region concerning the residues from agriculture and farming activities. The residues were considered potentially interesting because of their abundance and availability throughout the year. In the survey, conducted by Asja Ambiente in 2011 (Asja, 2011, private communication), the following categories were considered: (a) wastes from the dairy industry such as whey, buttermilk, dairy waste water and by-products from cheese farms (121,000 tons/year); (b) wastes from the olive oil industry, such as OP and OMWW (620,000 tons/year); and (c) manure and sludge from animal farms (bovine, ovine and poultry farms) (7900 tons/year). The analysis of the region's agro-industrial production yielded very encouraging results in terms of abundance (especially the abundance of the residues of olive oil production) and availability throughout the year (Fig. 1).

The objective of this study, conducted by Asja Ambiente's research center with economic funding from the regional government of Puglia, was to examine the production of biogas and its methane yield by codigestion of different types of wastes from food, agricultural and livestock activities typical of the Puglian economy.

Materials and methods

Based on the results of the territorial survey, two experimental campaigns were conducted to evaluate biogas and methane production from various wastes of Apulia's agricultural and dairy activities. The first campaign was conducted using a laboratory-scale reactor, while the second campaign was conducted using a larger-scale bioreactor with a working volume of 1.6 m^3.

Lab-scale digester

Equipment

The anaerobic digestion tests were carried out in a 45 l CSTR (Fig. 2a). The bioreactor's temperature was maintained at 38 °C by means of an external jacket. The bioreactor was operated in continuous mode, with a HRT in the range of 30–40 days. A stirrer keeps the content inside the reactor approximately homogeneous. Two sampling ports were located alongside the two reactor outlets devoted to digestate and biogas.

Inoculum

The anaerobic digester was initially fed with an anaerobic sludge, used as an inoculum, consisting of a mixture of cow and chicken manure and anaerobic sludge obtained from a domestic wastewater treatment system. This inoculum–water mixture was 3.4% (w/w) total solids (TS) and 89% (w/w) volatile solids VS per TS.

Experimental procedure

Various mixtures intended to represent the availability of local wastes were prepared to feed the bioreactor. Table 1 shows some chemical parameters of the biomasses used as feed for the anaerobic digestion tests. The full set of bioreactor inlet conditions,

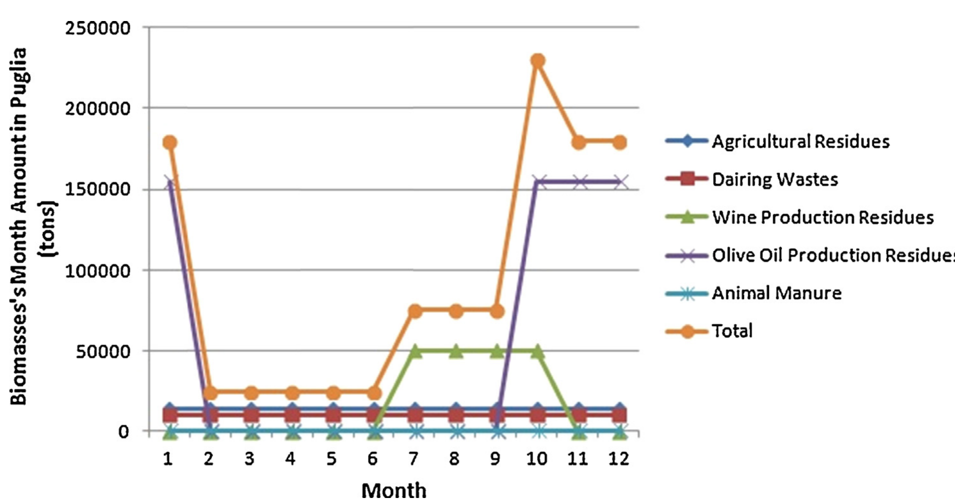

Fig. 1. Monthly available waste amounts in Puglia.

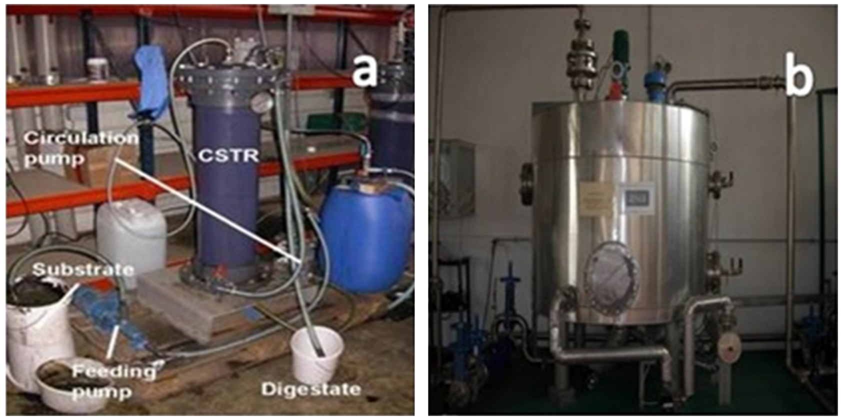

Fig. 2. Experimental apparatus: (a) laboratory-scale reactor and (b) scaled-up plant.

over an experimental campaign lasting 75 days, is detailed in Table 2. Various mixes of wastes were tested for various lengths of time. A feed mixture of 50% (w/w) OP and 50% (w/w) OMWW was diluted with water over time to ratios of 1:10, 1:6 and 1:3 (Table 2). Starting on the 40th day of the experimental campaign, water first and later OMWW were gradually substituted with whey. At the end of this first experimental campaign, the mixture consisted of 25% (w/w) OP and 75% (w/w) whey milk.

Before being fed into the reactor, the feed mixture was first milled to reduce the particle size and thereby increase the mass transfer and better disperse organic substances in the reaction medium. The test campaign required several adjustments to the mixture feed and the operating parameters. First, a COD:N:P ratio of 350:5:1 was maintained inside the reactor, which required adding nitrogen ($NaNO_3$) and phosphorous (Na_3PO_4) salts to the reactor at times. The nutrient deficiency was slightly reduced when whey milk was introduced to replace water and then OMWW (Table 2).

Another relevant parameter is the polyphenol content, the mean value of which was approximately 500 mg/l for OMWW and 163 mg/l for whey. The polyphenol content determines the dilution factor (DF) to be employed in the anaerobic digester to limit the polyphenol biostatics effect on microbial activity [14]. DF is a factor used to evaluate the amount of water present in the mixture. It has been defined as the ratio between the total weight of the feed and the weight of olive waste (OP + OMWW). Table 2 shows that DF was controlled either by water or by whey. The use of whey as a means of controlling DF with OMWW could be very convenient because whey contributes to an optimal C/N ratio (20–30) for anaerobic digestion and dilutes the polyphenols content of OMWW.

The pH was adjusted using 12 ml of a 2 N NaOH solution per liter of bioreactor when whey was added to the feed (on day 40). The pH of the feed was approximately 7.5 at the beginning of the campaign, with the 1:10 diluted feed. The pH then decreased to 7 when the proportion of olive-based wastes in the feed was progressively increased. Subsequently, when whey was added, the pH decreased further to a mean of 6.7 because of the combination of the whey acidity and the NaOH added to control the pH.

Analytical methods

Several parameters were measured daily using samples taken at the outlet stream: pH, total COD, TS, VS, and the concentrations of total nitrogen compounds (N) and phosphorus (P). TS, VS, COD, N and P were determined using the standard methods described in the scientific literature [15]. In addition, the biogas produced was measured both qualitatively and quantitatively, using a TG1PP gas meter (RITTER, Germany) and a DRÄGER X-AM 7000 (Dräger, Germany), respectively. Offline biogas samples for the latter were collected using GSR sample sacs (RITTER, Germany).

Table 1
Chemical parameters characterizing OP, OMWW and whey used in the tests.

Organic wastes	Lab-scale reactor			Scaled-up reactor	
	Olive pomace	OMWW	Whey milk	Olive pomace	Whey milk
pH	5.6 ± 0.5	5.3 ± 0.3	3.3 ± 1.1	5.6 ± 0.4	3.8 ± 0.9
COD (g/l)	123 ± 21	50 ± 5.7	65 ± 11.3	106 ± 21.5	63 ± 16.2
Total solids (%, w/w)	28.5 ± 4.6	1.7 ± 0.3	6.1 ± 2.7	29.3 ± 5.2	5.8 ± 1.9
Volatile solids (%, w/w, VS/TS)	92.4 ± 2.6	67.7 ± 4.8	91.8 ± 3.1	79.1 ± 4.5	90.6 ± 3.4
Total N (g/l)	1.58 ± 0.3	1.25 ± 0.20	1.85 ± 0.15	1.13 ± 0.41	1.33 ± 0.36
NH_4^+ (g/l)	0.12 ± 0.1	0.27 ± 0.12	0.26 ± 0.06	0.15 ± 0.2	0.29 ± 0.1
Total P (g/l)	0.113 ± 0.02	0.07 ± 0.01	0.47 ± 0.02	0.11 ± 0.06	0.45 ± 0.08

Table 2
Different inlet reactor feed mixtures over time for the laboratory-scale digester.

Feed type	A	B	C	D	E
Inlet reactor composition (%, w/w)	5% pomace 5% OMWW 90% water	8% pomace 8% OMWW 84% water	16% pomace 16% OMWW 68% water	25% pomace 25% OMWW 50% whey	25% pomace 75% whey
Feeding period	Days 0–9	Days 10–25	Days 26–39	Days 40–50	Days 51–75
Dilution factor	10	6.25	3.13	2	4

Scaled-up digester

Equipment

At the end of the experimental campaign conducted with the 45-L reactor, the wastes from olive oil, milk and cheese production were tested using a larger-scale anaerobic digestion plant consisting of a pulper, into which organic refuse is fed, and a reactor (Fig. 2b). The pulper was used to physically pre-treat the feed to grind and mix the waste stream. The anaerobic reactor had a total volume of 2 m^3 and a working volume of 1.6 m^3. The pulper (with a total volume of 196 L and a working volume of 120 L) was equipped with a level control and a stirring system able to work at 50 or 100 rpm. Organic wastes from the pulper tank were pumped by a centrifugal pump in the anaerobic reactor that was equipped with a thermostatic jacket and operated at 38 °C and a gentle rate of agitation (50 rpm). The biogas produced flowed into the collection line equipped with a safety device break-flame, a water condenser to eliminate water vapor and a gas counter up to reach a flexible storage balloon, where it was stored at ambient atmosphere for safety reasons.

Inoculum

At the beginning of the experimental campaign, the reactor was inoculated with 200 kg of methanogenically active biomass consisting of a mixture of cow manure (20%, w/w) and chicken manure (20%, w/w) and an anaerobic sludge from a domestic wastewater treatment plant (60%, w/w). The characteristics of this seed microorganism mixture were the same as that used with the laboratory-scale digester.

Organic substrates used

The whey milk and OP used to feed the reactor were obtained from two production plants located near Brindisi, in the southern part of the Puglia region. The bovine and chicken manure were also obtained from this area. The characteristics of the whey and two-phase OP are shown in Table 1. They were slightly different from the materials used in the laboratory-scale digester tests due to the variation in time and origin of production manufacturing (Table 3).

Experimental procedure

Table 4 shows the different mixtures fed into the reactor during the experimental campaign with the pilot plant. The anaerobic reactor was initially charged (on day 1) with 200 kg of inoculum. Between the 2nd and the 10th day, the reactor was gradually brought up to a working volume of 1.6 m^3 by feeding in the mixture at a rate of 175 kg/day of a mixture. When the working volume was reached, the plant was operated with approximately 30 days of HRT. The reactor was fed using two-phase OP and whey milk, with the influent substrate concentration gradually increased until it reached, at the end of the 20th day, a daily feed composition of 12 kg of two-phase OP, 20 kg of whey milk, 2 kg of chicken manure and 5 kg of tap water. Except during the transition period (days 1–10), at the end of every working day, approximately 40 L of digestate was discharged to keep the volume of the reaction medium in the tank constant.

Table 3
Feed mixture over the time for the scaled-up reactor.

Feeding plan	Batch mode		Continuous mode	
	Day 1	Days 2–10	Days 11–20	Days 21 –75
Two phase olive pomace (kg/day)	0	25	5.5	12
Whey milk (kg/day)	0	25	10	20
Bovine manure (kg/day)	0	70	5	0
Chicken manure (kg/day)	0	5	1	2
Water (kg/day)	0	50	15	5
Inoculum (kg/day)	200	0	0	0

Before being conveyed to the reactor, the feed mixture was pre-treated in the pulper with the stirring system set at 100 rpm. This mechanical treatment was tailored to increase the material surface area of the substrates exposed to active biomass and to mix the feed. The experimental campaign was conducted over a period of 75 days.

Analytical methods

The daily parameters that were measured were the pH, total COD, TS, VS, and the concentrations of total nitrogen compounds (N) and phosphorus compounds (P), using the methodology mentioned above.

Another important parameter that was assessed in the test with the 2-m^3 bioreactor was the ratio between the concentration of volatile fatty acids (VFA) and alkalinity (ALK). This parameter provides information on the stability of the system. It is important to control the VFA content because in high concentrations, VFA can inhibit methane-producing bacteria. The methods used to measure the volatile acidity and alkalinity are reported in Chen et al. [16]. The concentration of methane in the biogas produced was measured using the DRÄEGER X-AM 7000 by Dräger (Germany), a hydrocarbon gas analyzer that uses infrared, electrochemical and catalytic sensors to evaluate the concentrations of CO_2, O_2, H_2S, H_2 and CO. The analyzer is equipped with a pump that is continuously controlled by diagnostic software. The pump draws the biogas from the collection line through a sampling port. The quantity of biogas produced was measured using a TG1PP gas meter (RITTER, Germany).

Results and discussion

Lab-scale digester

Fig. 3 reports the quantity of daily biogas productivity, the pH of the outlet stream and the mean CH_4 concentration in the biogas for each feed type. The biogas production was quite low when the substrate was initially diluted (DF = 10) but increased progressively until a stable production level of approximately 0.4 L/L day was reached with a dilution factor of 3.13 and a CH_4 concentration of 60% (v/v) when the water content was reduced (Table 2). The addition of whey increased the daily production of biogas even though some relevant changes were recorded. In particular, after whey was added, the amount of biogas produced increased and the methane concentration decreased. This can be explained by the

Table 4
Main results of the experimental campaign with the laboratory-scale reactor.

Feed type	A	B	C	D	E
HRT (days)	40	30	30	40	40
OLR (g COD/L day)	1	2	2.4	4.5	3.4
VFA/ALK	0.17 ± 0.05	0.13 ± 0.06	0.31 ± 0.1	0.4 ± 0.16	0.35 ± 0.11
VS reduction (%)	25.5 ± 3.2	61.4 ± 6.5	72.4 ± 3.7	68.6 ± 5.2	64.1 ± 3.9
COD reduction (%)	46.8 ± 2.1	49.6 ± 5.4	46.7 ± 4.5	56.5 ± 3.9	63.9 ± 2.8
Specific biogas production (L/kg ΔVS)	345 ± 15	190 ± 21	141 ± 23	336 ± 42	311 ± 35
Biogas productivity (L/L day)	0.26 ± 0.08	0.32 ± 0.09	0.43 ± 0.12	0.66 ± 0.25	0.78 ± 0.2

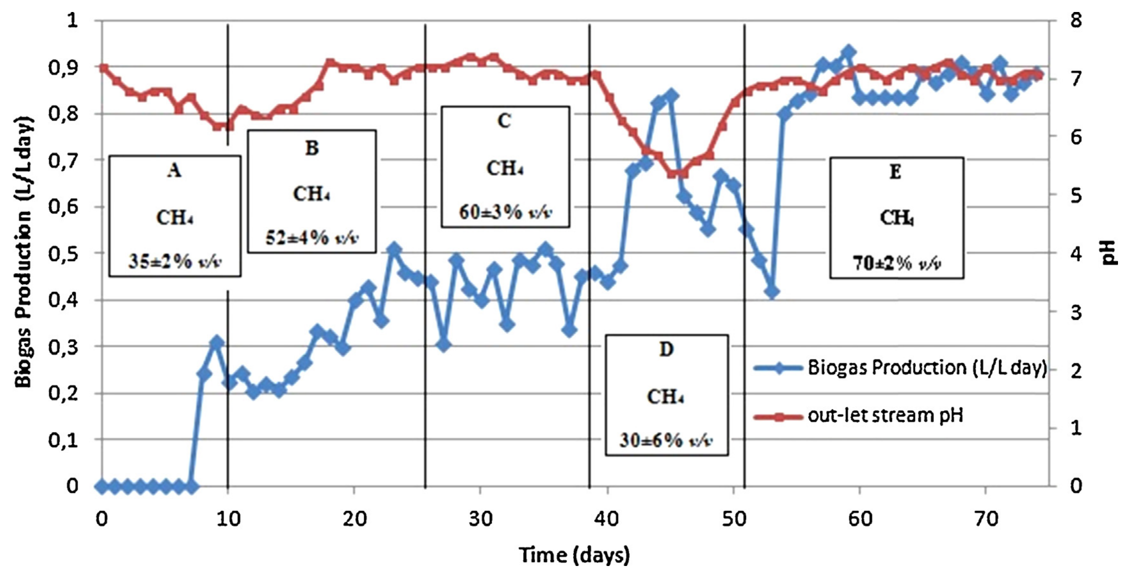

Fig. 3. Daily biogas production for the laboratory-scale reactor, mean methane concentration and outlet pH for each feed composition tested. A, B, C, D, E refer to feed mixture of Table 2.

very complex microbiological activity of anaerobic bacteria [17]. As a consequence of the whey addition, the pH decreased (Fig. 3). In this manner, the activity of acid-forming bacteria such as *Clostridium* spp. was able to prevail over that of methane-forming bacteria, provoking an increase in the total biogas production as a consequence of replacement of 50% of the water with whey (Table 2). The decrease in the CH_4 content (Fig. 3) could be the consequence of an H_2 increase that accompanied the VFA production [18]. The decrease in biogas production could be explained by the partial inhibition of the activity of methane-forming bacteria at low pH levels [19]. As the pH increased toward the neutral range, due to the buffering action of the carbon dioxide–hydrogencarbonate–carbonate system, which at low pH levels forms CO_2, as well as the direct reduction of acetic acid to methane by the *acetoclastic methanogens* microorganisms [20], the activity of both microbial populations entered a stable regime in which a synergism of microbes occurred. The H_2 produced by the acetogenic bacteria was consumed by methanogens in symbiotic action with *Acetogenic* spp., which are able to reduce CO_2 to CH_4 using H_2, by a so-called interspecies hydrogen transfer mechanism [21]. As the pH increases, the production of CH_4 increases (Fig. 3) for many reasons, including direct reduction of CO_2, accounting for 25–30% of the total methane produced [22] and for the acetoclastic pathway above recalled. In the last phase of the test, from the 51st to the 75th day, when the feed constituted pomace and whey, a stable biogas production of 0.8 ± 0.2 L/L day was reached, with a high content of methane. The mean methane content in the biogas data are reported for each feed type in Fig. 3.

The set of parameters reported in Table 4 were estimated throughout the experimental campaign to provide useful data for full-scale plant design. The trends in VS reduction and their averaged values during each feeding period demonstrate the VS abatement capacity of the microorganism's consortium. In the first phase, with a substrate based on OP and OMWW, the average VS inlet concentration was 30 g/l, with a dilution factor of 10. This concentration increased to 50 g/l and then to 100 g/l when the dilution factor was reduced to 6.25 and then to 3.15, respectively. The mean VS reductions were 25.5%, 61.4% and 72.4%, respectively, and it were strongly correlated to the dilution factor. The addition of whey stabilized the VS at approximately 75 g/l and then 90 g/l when whey totally replaced OMWW. In this case, the VS abatement was also quite stable at approximately 68.6% and then 64.1% for feed type D and E respectively. The introduction of whey was particularly relevant to the COD abatement, considering the organic load rates of 4.5 and 3.5 g COD/L day. These results confirm that whey is an ideal substitute for water (Table 4) as the dilution agent that keeps the polyphenols content of the mixture low. At the same time, even if the whey added increased the COD in the feed from 65 g/l to 165 g/l, the COD reduction was greater than 60% (Table 5). This is most likely because whey furnishes nutrients and vitamins vital to the activity of bacteria.

Lastly, the pH level is also of great importance because it affects the growth of the microorganisms and the production of metabolites, as demonstrated when the whey was added, as discussed above. During the campaign, the outlet pH was always close to neutral, except for feed type D in Table 2, but the microbiological system was able to react to absorb the variation in the pH as a consequence of the whey addition as is evident from Fig. 3.

Table 5
Comparison of results obtained with data reported in the literature.

Reference	Test description	Methane yield (L/kg ΔVS)	COD reduction (%)
[8]	Olive pomace + water	135	71.4
[11]	Two phase olive pomace	632	87
[1]	Two phase olive pomace	480	–
[7]	Whey and cattle slurry	621	82
[13]	Whey	560	55
This work	Olive pomace, whey and chicken	864 ± 51	71.6 ± 3.2
	Olive pomace + whey	218 ± 35	63.9 ± 2.8

Scaled-up digester

Fig. 4 illustrates the biogas production obtained with the scaled-up reactor as consequence of variation of the feed composition, as reported in Table 3. Throughout the feeding period, the biogas production increased constantly, reaching a mean of 0.25 L/L day during the batch mode of the first 15 days, when the hydrolysis and acidogenesis of the substrates started. In fact, if anaerobic digestion's first two phases for carbohydrates begin within a few hours of the reactor feeding, the degradation of lipids and proteins occurs in a few days. Cellulose and lignin, the most important components of OP, are degraded incompletely, and their degradation takes a long time [23]. The biogas production

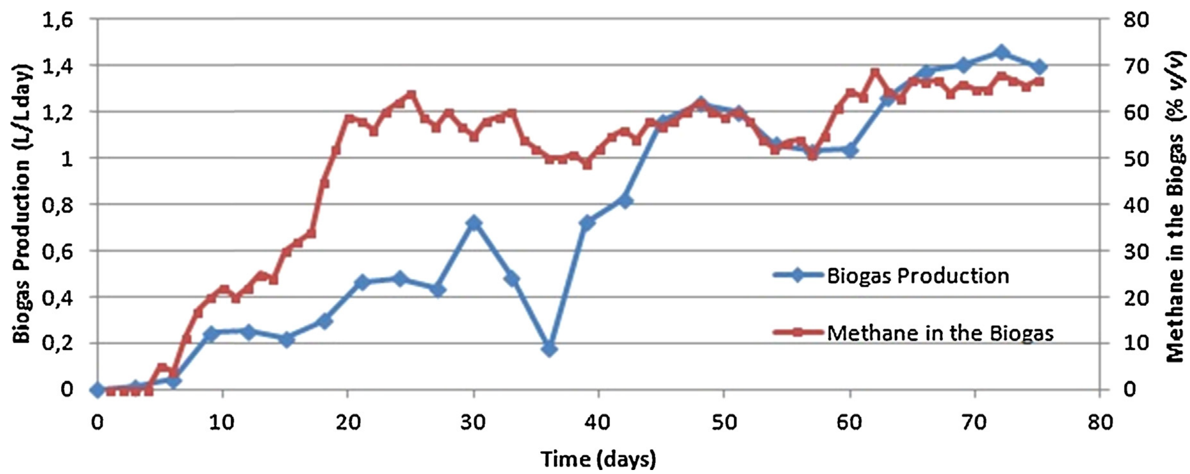

Fig. 4. Daily biogas production and methane content for the scaled-up reactor.

increased to 0.55–0.76 L/L day between the 16th and 30th days, when the formation of methane started, with sufficient COD content available for the bioreaction when the reactor was fed in continuous mode. In addition, the contribution of such a high COD made it possible to increase the ratios between the organic matter content and the contents of the two main nutrients, nitrogen and phosphorous. The need for these nutrients is very low, however, because during anaerobic digestion, as consequence of the low rate of biomass growth, the nutrient ratio needed is C:N:P:S of (500–1000):(15–20):(5–3):(1–0.5), equivalent to an organic matter ratio of COD:N:P:S = 350:5:1:0.5 [22]. On day 36th, small quantities of $NaNO_3$ and Na_3PO_4 (5 g/l and 1 g/l, respectively) were added, and the biogas production started again.

Between the 30th and 36th days, a sharp drop in the biogas production was recorded. During this period, the feed mixture, before being charged in the reactor, was subjected to a further pre-treatment intended to remove the solid snippets from the OP seed, which during the previous days had caused obstructions of the inlet pipes. However, this operation caused the loss of a large amount of organic matter, as verified by chemical analyses of the COD content (data not reported). This problem was solved by barely crushing the solid snippets before re-adding them to the inlet flow stream. After this operation, biogas production restarted in 2–3 days. Between days 52nd and 56th, a slight decrease in biogas production occurred as a consequence of an increase in the viscosity of the sludge in the tank, which hampered mixing of the organic matter substrates into the reaction medium because it was circulated at a low rate (100 rpm) to prevent shear stress on the methanogenic microorganisms. Lastly, when the problems described were resolved, biogas production reached a quasi-steady state, with values in the range of 1.25–1.45 L/L day.

The volumetric percentage of methane measured in the biogas produced during the 75 days of the second experimental campaign with scaled-up reactor is shown in Fig. 4. As a consequence of the mixing problem described above, the methane content was only in 52–54%, indicating the strong effects of the mixing problem on the whole bioreaction producing biogas, especially on the methanogenic microorganisms. After the problem was resolved, the methane concentration remained constant through the end of the campaign in the range of 65–70% (v/v).

The pH is the first and the most immediate control parameter because it indicates whether the substrates in the reactor are degrading through a well-balanced microbiological process of anaerobic digestion. As Fig. 5 shows, the pH of the feed mixture was initially slightly alkaline because of the alkalinity of the inoculums. The pH then became more acidic at the beginning of the addition of acidic substrates, such as the two-phase OP and whey milk, to the feed. The pH of the digestate remained near

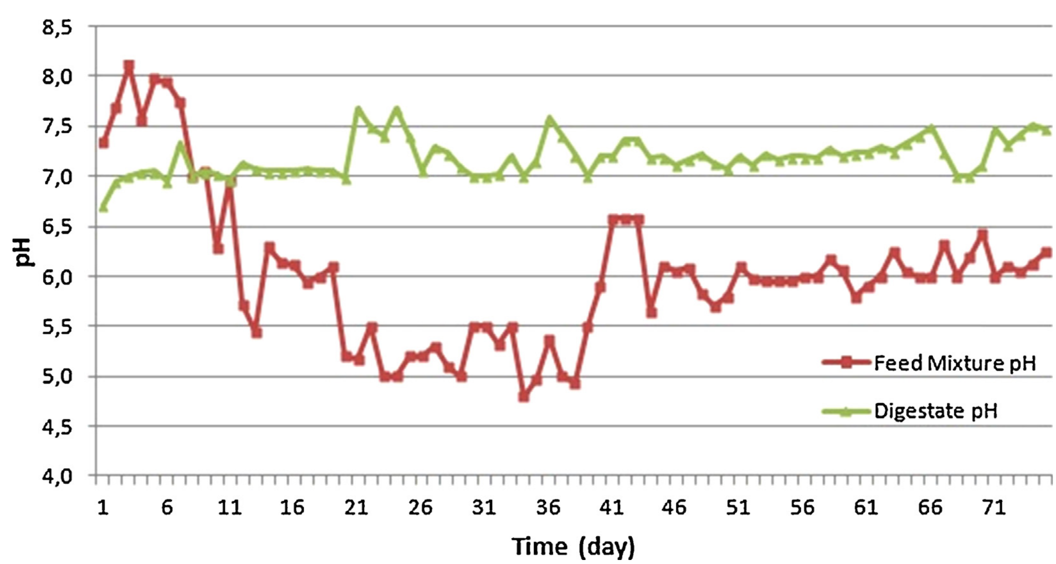

Fig. 5. Inlet and outlet pH variations *vs.* time for the scaled-up reactor.

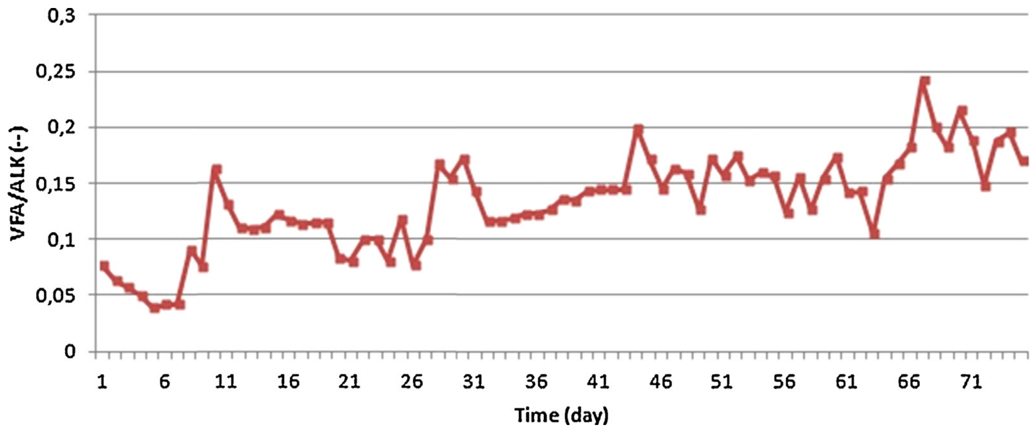

Fig. 6. VFA/ALK ratio *vs.* time for the scaled-up reactor.

7–7.5 throughout the experimental campaign. This is the first indication that the anaerobic digestion process is yielding a stabilized sludge and good methane production. Examination of the acidity–alkalinity ratio *vs.* time, illustrated in Fig. 6, confirms that VFA produced in the second and third stages of anaerobic digestion are subsequently degraded and converted into methane. The ratio was always less than 0.3, which is a limiting value for stable anaerobic digestion [22]. The ratio of acidity to alkalinity shown in Fig. 6 confirms that the management of the feed and its composition were effective in controlling the bioreaction. In particular, the addition of a small quantity of chicken manure (Table 4) had a beneficial effect in supplying the necessary nitrogen to balance the acidity of the OP and whey. As Fig. 6 shows, the addition of a slightly basic inoculum in the first days of the experimental campaign contributed to a low ratio of the volatile fatty acid concentration to alkalinity. With the beginning of the hydrolysis and acidogenesis processes, and especially with the beginning of the production of VFA in the reactor on day 11, the VFA concentration increased, and the acidity–alkalinity ratio increased to values between 0.1 and 0.2, but always remained below the critical value of 0.3. Organic acids exist in un-dissociated form and partly in dissociated form, depending on the pH value. Un-dissociated acids have a greater inhibiting effect because they penetrate as lipophilics into cells, where they denaturize cell proteins. The alkalinity needed to balance this effect reduces the amount of un-dissociated VFA.

Fig. 7 shows the percentage abatement of TS, VS and COD. The graph illustrates the good performance of the process in terms of reduction of TS, VS and COD. The average percentage values are 62.1, 67.8 and 71.6, respectively. However, the use on the 55th day of a mechanical pre-treatment to crush the solid residues of olive seeds in the feed mixture increased the mixing of the broth without the speed (in rpm) of the reactor being increased, to avoid increasing the shear stress on methanogenic microorganisms. Moreover, from the 45th day on, the mixing problem described reduced the mass transfer of organic matter in the reaction medium and was likely, responsible for the poorer performance of the bioreactor illustrated in Fig. 7. Because of the bad mixing, the microorganisms were not able to degrade all of the organic matter, and biogas production decreased as a result. This problem, which occurred during the second experimental campaign with the scaled-up bioreactor, was the cause of the sharp decline in the performance of the bioreactor, particularly in terms of abatement of VS and COD associated with the mixing problem. In fact, the VS and COD concentrations constitute two important parameters for the control of the anaerobic digestion process: they indicate the amount of organic matter present in the feed mixture that contributes to the production of biogas. This means that the greater the loss of VS is over the loss of TS, the more biogas is produced.

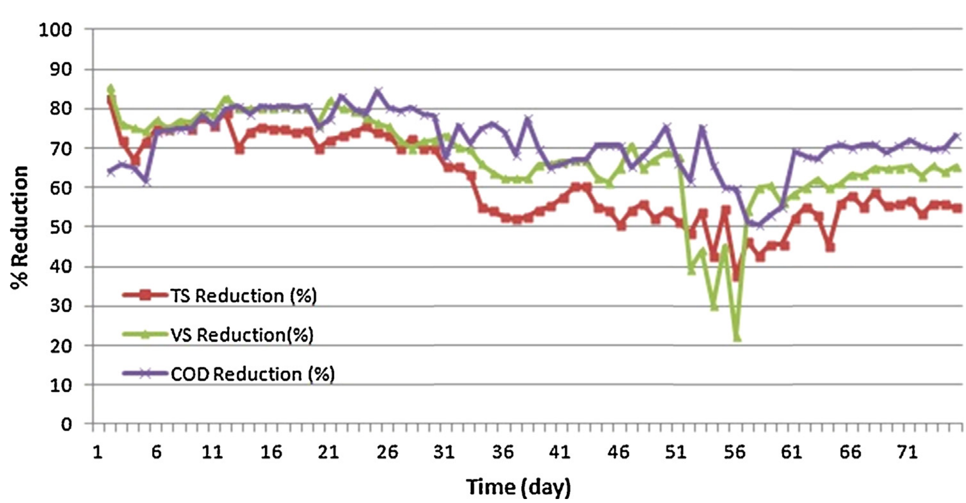

Fig. 7. Process performance *vs.* time for the scaled-up reactor.

Lastly, Table 5 presents some figures for the purpose of comparing the results of the present study with results reported in the literature data. It is important to note the difficulties in making such comparisons because different researchers have tested different type of wastes with different types of organic substrates. To facilitate a comparison, we expressed the results in terms of methane yield, i.e., the methane produced per unit of VS destroyed. This measure, in our opinion, overcomes the difficulty of comparing the results of studies conducted using different materials because it reflects all of the parameters that affect the production of methane in the bioreaction. Table 5 shows that the combinations of different organic wastes tested rank well as being suitable for methane production, as well as COD reduction, via anaerobic digestion. These results indicate a different use for olive mill sludge than that suggested by Hytiris et al. [24].

Conclusions

The results of the present study demonstrate the advantages of anaerobic digestion of organic wastes, mainly OP and whey, either for pollution control or energy production. The various tests conducted highlight the good performance of the anaerobic digestion process in terms of abatement of COD, TS and VS: 70%, 57% and 80%, respectively. The process yielded very encouraging results in terms of the production of biogas (0.8–1.4 L/L day), as well as the production of methane, the concentration of which was in the range of 65–70%. The process also produced a stabilized sludge with a pH between 7 and 7.5, which is suitable for use as a fertilizer.

Tests with the scaled-up reactor under mechanical agitation demonstrated that mixing is a potential problem in the process that deserves particular attention. The experience gained suggests that careful management of the dilution of the reaction medium is necessary to avoid increasing the viscosity of the mixture and controlling the mixing process. It is also important to control the dimensions of the solid particles that can settle to the bottom of the reactor. Nevertheless, it is possible to achieve codigestion of OP and whey with the addition of a small quantity of chicken manure as a substrate for energy production via anaerobic digestion. Particularly a feed constituted by 25% (w/w) and 75% (w/w) of OP and whey respectively is resulted to be the optimum composition for biogas production. The biogas production intensity of 1.4 L/L day of such a bioreactor, considering the potential of wastes produced in Puglia's region, corresponds to an annual energy production potential of 55 GJ. Taking into account that Puglia' s annual energy consumption is approximately 375,000 GJ (Piano Energetico Ambientale Regionale–Regione Puglia, 2004), the anaerobic digestion of wastes from olive oil and dairy production could provide approximately 0.015% of the region's energy demand.

These encouraging results pave the way to design of the process on an industrial scale. In October 2012, Asja Ambiente started construction of the first full industrial plant to be totally fed with agro-food wastes (Asja, private communication).

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References

[1] R. Borja, B. Rincon, F. Raposo, Anaerobic biodegradation of two phase olive mill solid wastes and liquid effluents: kinetic studies and process performance, Journal of Chemical Technology and Biotechnology 81 (2006) 1450–1462.
[2] A. Roig, M.L. Cayuela, M.A. Sanchez Monedero, An overview on olive mill wastes and their valorisation methods, Waste Management 26 (2006) 960–969.
[3] A. Saviozzi, R. Levi Minzi, R. Cardelli, A. Biasci, R. Riffaldi, Suitability of moist olive pomace as soil amendment, Water, Air, and Soil Pollution 128 (2001) 13–22.
[4] Legge 574, 11 November, 1996, Nuove norme in materia di utilizzazione agronomica delle acque di vegetazione e di scarichi dei reflui oleari.
[5] M. Jeguirim, A. Chouchène, A.F. Réguillon, G. Trouvé, G. Le Buzit, A new valorization strategy of olive mill wastewater: impregnation on sawdust and combustion, Resources, Conservation and Recycling 59 (2012) 4–8, 48.
[6] L. Ercoli, E. Bonari, F. Barresi, Acque reflue dei caseifici, in: Linee guida per l'utilizzazione agronomica delle acque di vegetazione e delle acque reflue da aziende agroalimentari, APAT – Agenzia per la protezione dell'ambiente e per i servizi tecnici, 2007, pp. 81–100.
[7] E. Comino, V. Riggio, M. Rosso, Biogas production by anaerobic co-digestion of cattle slurry and cheese whey, Bioresource Technology 114 (2012) 46–53.
[8] A.R. Tekin, A.C. Dalgic, Biogas production from olive pomace, Resources, Conservation and Recycling 30 (2000) 301–313.
[9] H.Q. Yu, H.H.P. Fang, G.W. Gu, Comparative performance of mesophilic and thermophilic acidogenic upflow reactors, Process Biochemistry 38 (2002) 447–454.
[10] Z. Wang, C.J. Banks, Evaluation of a two stage anaerobic digester for the treatment of mixed abattoir wastes, Process Biochemistry 38 (2003) 1267–1273.
[11] B. Rincon, E. Sanchez, F. Raposo, R. Borja, L. Travieso, M.A. Martin, A. Martin, Effect of the organic loading rate on the performance of anaerobic acidogenic fermentation of two phase olive mill solid residue, Waste Management 28 (2008) 870–877.
[12] A.S.E. Yay, H.V. Oral, T.T. Onay, O. Yenigun, A study on olive oil mill wastewater management in Turkey: a questionnaire and experimental approach, Resources, Conservation and Recycling 60 (2012) 64–71.
[13] A.E. Ghaly, A comparative study of anaerobic digestion of acid cheese whey and dairy manure in a two stage reactor, Bioresource Technology 58 (1996) 61–72.
[14] H.K. Obied, M.S. Allen, D.R. Bedgood, P.D. Prensler, K. Robords, R. Stockmann, Bioactivity and analysis of biophenols recovered from olive mill waste, Journal of Agricultural and Food Chemistry 53 (2005) 823–837.
[15] APHA/AWWA/WEF, Standards Methods for the Examination of Water and Wastewater, 20th ed., United Book Press, Inc., Baltimore, Maryland, 1998.
[16] Y. Chen, A.A. Randall, T. McCue, The efficiency of enhanced biological phosphorus removal from real wastewater affected by different ratios of acetic to propionic acid, Water Resource 38 (2004) 27–36.
[17] B. Demiral, P. Scherer, The role of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane: a review, Environmental Science and Biotechnology 7 (2) (2008) 173–190.
[18] Q. Nathao, U. Sirisukpoka, M. Pisutpaisol, Production of hydrogen and methane by one and two stage fermentation of food waste, International Journal Hydrogen Energy (2013), http://dx.doi.org/10.1016/j.ijhydene.2013.05.047.
[19] I.S. Kim, M.H. Hwang, N.J. Jang, S.H. Hyun, S.T. Lee, Effect of low pH on the activity of hydrogen utilizing methanogens in bio-hydrogen process, International Journal Hydrogen Energy 29 (11) (2004) 1133–1140.
[20] P.E. Galant, H. Fritze, R. Courad, K. Yrjale, Pathways for methanogenesis and diversity of methanogenic archea in three boreal peat land ecosystems, Applied and Environmental Microbiology 71 (4) (2005) 2195–2198.
[21] T. Shimada, E. Morgenroth, M. Tandukor, S.G. Pavlostathis, A. Smith, L. Raskin, R.E. Kilian, Syntrophic acetate oxidation in two phase (acid–methane) anaerobic digesters, Water Science & Technology 64 (9) (2011) 1812–1820.
[22] D. Deublein, A. Steinhauser, Biogas From Waste and Renewable Resources, Wiley-VCH Verlag GmbH & Co., Weinheim, 2011.
[23] B. Ruggeri, M. Bernardi, T. Tommasi, On the pre-treatment of municipal organic waste towards fuel production: a review, International Journal of Environment and Pollution 49 (2012) 226–250.
[24] N. Hytiris, I.E. Kapellakis, R. La Roij, K.P. Tsagarakis, The potential use of olive mill sludge in solidification process, Resources, Conservation and Recycling 40 (2004) 129–139.

Web references

http://agri.istat.it/jsp/dawinci.jsp?q=plc270000010000012000&an=2011&ig=1&ct=311&id=21a|15a|32a. Tavola C27 – Superficie (ettari) e produzione (quintali): olivo,olive da tavola,olive da olio,olio di pressione. Dettaglio per regione – Anno 2011 (last accessed 03.13). http://www.regione.puglia.it/index.php?page=progetti&opz=downfile&id=273. Piano Energetico Ambientale Regionale–Regione Puglia (last accessed 04.13).