Renewable energy from thermophilic anaerobic digestion of winery residues: batch and CSTR lab-scale study

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Abstract

Winemaking process generates many by-products, mainly grape marcs, grape stalks and wine lees. Large amounts of wastewater are also produced.

Anaerobic digestion is considered particularly suitable to treat winery wastes because of their high organic matter content with respect of nutrients and for the interesting energetic potential, but to date only mesophilic process was investigated. In this study potential methane production and kinetic constants were determined by batch trials at thermophilic condition, and compared with mesophilic ones. Grape marcs appeared the most interesting substrates with an estimated potential higher than 0.3 $Nm^3CH_4/kgVS_{fed}$. In order to assess the feasibility of the continuous process a lab-scale semi-continuous reactor was setup. Because of the consumption of buffer capacity, the biological process was hard to control, on the other hand, an interesting production was obtained with HRT of 40 days and with previous fermentation of grape marcs (0.29 $Nm^3/kgVS_{fed}$). The results were used to calculate the potential energy recovery from grape marcs in a full-scale application, in terms of heat and electricity: 245 GWh/y of heat and 201 GWh/y of electricity in different scenarios.

Keywords

Anaerobic digestion; winery waste; renewable energy; BMP test; grape marcs.

1. Introduction

The International Organization of Vine and Wine (OIV) estimated a global wine production of 278.6 MHl in 2013 (OIV, 2014). Italy, France and Spain are the countries with the highest productions (131.6 MHl), and together with others European countries account for about 59% of world production.

In Europe wineries have a long tradition and high economic relevance in the agricultural sector, on the other hand, with their activity they also determine a considerable environmental footprint: intensive use of soil, introduction on the environment of pesticides and heavy metals, water consumption, production of high quantities of co-products and wastes (Bolzonella and Rosso, 2009).

In early 2000s the Italian Agency for Environmental Protection (ANPA, 2001) evaluated the amount of winery waste (WW) for each hectolitre of wine produced, considering different type of cellars in terms of dimensions and location. The production of wastewater is typically 2hl for each hl of wine produced, but it depends on process technology and can reach 6 hl per hl of wine (Berta et al., 2003). The wastewater is usually treated by conventional activated sludge processes and the sludge obtained is about 1kg of dry matter per hl of wine (ANPA, 2001). Sludge, generally, could not be spread on land for agricultural purpose cause of its high heavy metals content and low biological stability: this means high management and disposal costs (110€/ton).

Grape stalks and marcs, wine lees, exhausted grape marcs and vinasses are the main by-products generated by the wine and alcohol-producing industries (Bustamante et al., 2007). The productions of solid by-products and wastes were estimated of 4 kg of grape stalks, 18 kg of grape marcs and 6 kg of wine lees per hl of wine produced. The European Council Regulation (EC) 1493/1999 of the common organization of the wine market introduced compulsory distillation of wine by-products, including grape marcs and lees, to produce spirits, alcohol, tartaric acid, exhausted grape marcs and liquid waste (vinasses). The aim of this practice was to avoid over-pressing of marcs and to maintain high quality wine markets, but not to reduce the polluting hazard of winery waste disposal. Distilleries received a subsidy to distil the alcohol for industrial and energy uses but, on the other hand, disposal of marcs via distillation was becoming a considerable financial and bureaucratic burden for wine producers. In fact, although the distilleries paid a small amount for the marcs, the producers had to pay for the cost of transport. As a result, producers were not only being constrained to waste potentially useful by-products but also the transport cost was higher than price paid by the distillery.

Recently the market-protective legislation (EC 479/2008 and EC 555/2008) allowed alternative uses of grape marcs in the wineries (Krzywoszynska, 2013) and granted more autonomy for marcs management strategy. In Italy, since 2011 (DM 27/11/08) marcs could be distilled or utilized by producers in other ways (under supervision of environmental control bodies) such as anaerobic digestion, pyrolysis or incineration, spreading on agricultural land (raw or composted) according to local legal framework of course, or as a raw material for cosmetics and pharmaceuticals production. Alternative technologies to reuse WW are the extraction and recovery of polyphenolic compounds, the production of grape seed oil, fermentation to produce lactic acid and laccase (Arvatoyannis et al., 2008).

The use of raw solid winery waste for agricultural purpose is an hazardous practice because the high organic content could consume high quantity of oxygen in the soil, then establishes anoxic conditions. As reported by Moraes et al. (2014), the impacts caused by potential greenhouse gas emissions resulting from organic matter degradation on soil, the unpleasant smells generated and possibility attracting insects should be considered. The presence of polyphenols, compounds related to phytotoxic and antimicrobial effect, and low pH due to high concentration of organic acids such as tartaric, citric and malic acids, make their treatment and disposal indispensable and their management really difficult. Biological treatment could be a solution, increasing the biological stability and improving COD/N/P ratio. Among the available technologies, composting of winery solid organic waste can produce a good quality fertilizer (Bertan et al., 2004) but it must be taken into account the costs related to the energy required for pile handling and venting (Bonifazi et al., 2013). Moreover winery wastes had nutrients ratio unbalanced for aerobic degradation, caused by a low nitrogen and phosphorus content.

Another biological treatment is Anaerobic Digestion (AD), a particularly suitable treatment to stabilize winery waste (Moletta et al., 2005) even at low level of nitrogen and phosphorus and it can remove organic pollutant such as polyphenols. This technology is considered an important future contributor to the energy supply of Europe (Appels et al., 2008) and the WW represents a consistent raw material. The appropriate use of biogas for energy generation may also reduce GHG emissions because it would avoid the release of biogenic CH_4 in the atmosphere (Moraes et al., 2014).

~~Several authors reported positive cases of anaerobic treatment of winery wastewater, using process with micro-organisms in biofilm structures and low influent suspended solids concentration such as UASB, MBBR, hybrid USBF (Andreottola et al. 2009; Sheli et al.,2007; Molina et al, 2007).~~ Completed stirred tank reactors (CSTR) were preferred when the semi-solid waste, such as grape marcs (GM), grape stalk (GS) and wine lees (WL), were treated. The OLR applied to AD was lower than in above-mentioned technologies, the HRT usually was higher than 12 days to avoid wash-out of methanogens microorganisms and to increase the hydrolysis rate.

Lots of examples of AD of WW were reported in literature but all trials were carried out in mesophilic condition, with temperature from 37°C to 40°C. In the most cases batch mode was applied, then the results were the potential methane production obtained from best operational conditions. The range of results was wide due to several factors: inoculum characteristics and substrate to inoculum ratio (VS basis) determined the answer of microorganisms to new substrates (Rebecchi et al., 2013), in fact winery waste can contained substances, such as lignin, ethanol and polyphenols, that are not present in the animal effluents, sewage sludge and bio-waste; also variability of substrates characteristics determined different biogas productions (Fabbri et al.,2014; Lo et Liao, 1986) and was usually linked to the type and the origin place of grape and to the wine-making processes that generate them. For example Fabbri et al. (2014) reported that best biomethane potential was achieved by a white grape marc (0.273 $m^3CH_4/kgVS$) while the red one reached 0.101 m^3 $CH_4/kgVS$. Anaerobic digestion of grape marcs is the more investigated process: the specific production ranges from 0.076 to 0.283 $m^3CH_4/kgVS_{fed}$ (Failla et al., 2009; Dinuccio et al., 2010; Gunaseelan et al., 2003). Specific methane production of grape stalk (0.098 - 0.180 $m^3CH_4/kgVS_{fed}$) is lower than other agro-industrial biomasses probably due to high lignin content (23.3% of solids), that cannot be degraded in anaerobic conditions (Angelidaki et al., 2000; Ward et al.,2008). Few authors studied the wine lees (WL), although the potential methane is very high in terms of biogas produced and methane content (Lo and Liao, 1986; Da Ros et al., 2014); this probably because of the low quantity of WL that could be recovered (6 kg each hl of wine produced). Moreover there are many typologies of lees, different for kind of fining agents, such as bentonite, gelatine, albumin or activated carbon, added in order to settle suspended solid and colloidal particles. Sometimes wineries recovery must or wine in the lees by centrifugation or filtration through filter medium consist of diatomaceous earth or perlite (Setti et al., 2009). In these cases at the end of recovery operation the lees are composed by the filter medium plus the lees itself, so the final lees have high concentration of inert material.

Generally the greater difficulty found in AD of WW was the control of pH in the reactor, in fact the abundance of organic acids caused the rapid formation of volatile fatty acids (VFA) and the drop in pH (Lo and Liao, 1986). Limited buffer capacity was partially due to low nitrogen content in substrates and it was usually supplied by adding NaOH solution (Sheli et al., 2007, Melamane et al., 2007) but the use of chemicals reduces the economic revenues of the process. A solution to increase ammonium content and alkalinity in the anaerobic reactors is to introduce organic waste from others agro-industrial sectors. Anaerobic co-digestion (AcoD) with manure or waste activated sludge allows to reach C/N ratio more favourable, and consequently the biogas production and removal efficiency increase (Riaño et al.,2011; Rodriguez et al., 2007; Soldano et al., 2009).

The results of cited studies were mainly obtained by mesophilic process (35-40°C), while thermophilic condition was less applied (Da Ros et al., 2014, Rebecchi et al., 2013). Increasing temperature at 55°C has several benefits: improves solubility of the organic compounds and depletion of pathogens (Sahlström, 2003), enhances biological and chemical reaction rates (Cavinato et al. 2013, Cecchi et al. 1991). However, the application of high temperatures has some disadvantages: the higher free ammonia fraction, which plays an inhibiting role for the microorganisms, and the pK_a change of the VFAs that makes the process more susceptible to inhibition (Appels and al., 2008). In both the above cited thermophilic anaerobic digestion of winery waste studies, process instability was observed with VFAs accumulation and pH drop. In particular Rebecchi et al. (2013) found results lower than mesophilic ones and continuous process was not investigates, on the other hand some studies (Da Ros et al., 2014; Riaño et al., 2011) described trials carried out using a co-substrate and didn't evaluate potential biogas production of sole winery waste in the optimal conditions.

The aim of this work was to supply the information on thermophilic anaerobic digestion of winery solid waste and in particular of grape marcs, both in batch and continuous mode. Batch test were carried out according with guidelines of Angelidaki et al. (2009) with different wastes, obtaining preliminary results about biogas productions and they were compared with mesophilic methane production and few existing data at 55°C. Empirical linear and non-linear regression models were developed to evaluate digestion performance and to compare mesophilic and thermophilic process in terms of kinetics parameters. However, it is well known as biogas yields, under semi-continuous flux condition can be different from those observed under batch conditions, then semi-continuous trials in lab-scale were carried out to determine best operational conditions and to highlight criticisms treating grape marcs. Economical and energy aspects are than evaluated considering a potential full-scale application.

2. Materials and Methods

2.1 Winery wastes

The winery by-products considered in this study were grape marcs (GM), grape stalks (GS) and wine lees (WL). Two type of GM were considered: virgin grape marc (VGM) and grape marc after a natural fermentation (FGM). Samples were collected in a cellar in the north-east of Italy and each waste stream were labelled and stored in a freezer at -20°C until required. These substrates were analyzed in triplicate in terms of total and volatile solids, chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN) and total phosphorus, according to the Standard Methods (2012).

2.2 BMP tests

The biogas production was determined using biochemical methane potential tests (BMP) at 55°C. The inoculum digestate was collected from a full-scale digester (Treviso WWTP) treating organic fraction of municipal solid waste and waste activated sludge at mesophilic temperature. Inoculum had an average solid concentration of 43.1 gTS/kg, with 67% volatile solids. The particulate COD was 928 mg/gTS, while nitrogen and phosphorus were present respectively with 16.1 and 7.8 mg/gTS. For each waste considered different substrate to inoculum ratio were tested, ranged from 0.04 to 0.40 $gVS_{substrate}/gVS_{inoculum}$ in order to evaluate possible inhibition effect. BMP tests of the inoculum alone (blank) were also conducted. The results were expressed at standard conditions for temperature and pressure (0°C and 1 atm), and the unit of specific methane production (SMP) was $Nm^3CH_4/kgVS_{fed}$. The average methane yield from the blanks was subtracted from the yields of tested samples, in order to accurately assess the BMP yields.

The COD content of winery wastes and the theoretical methane production on COD basis (0.350 m^3 CH_4/gCOD by stoichiometric conversion reaction) were used to calculate the theoretical maximum methane potential from each substrate, and the biodegradability index was calculated considering the ratio between the measured and the theoretical methane production.

Two first order kinetic models were used to fit the cumulative methane production data from the BMP tests. Assuming first-order kinetics for the hydrolysis of particulate organic matter, the cumulative methane production can be described by the kinetic equation (1):

$$BMP = BMP_{\infty}(1 - \exp(-kt)) \tag{1}$$

where BMP is the cumulative methane yield at digestion time t days (ml CH_4/ g VS_{fed}), BMP_{∞} methane potential of the substrate (ml CH_4/g VS_{fed}), k is methane production rate constant (first order disintegration rate constant) (d^{-1}), t is the time (d). The duration of the lag phase is also an important factor in determining the efficiency of anaerobic digestion. The lag phase can be considered with the modified Gompertz model as described in Eq. (2):

$$BMP = BMP_{\infty}\, exp\left\{-\exp\left[\frac{R_{max}e}{BMP_{\infty}}(\lambda - t) + 1\right]\right\} \tag{2}$$

where R_{max} is the maximum methane production rate (l CH_4/kg VS d), e = 2.7183 and λ is the lag phase for methane production to begin (d).

As Browne et al (2014) suggested, a nonlinear least-square regression analysis was performed using Excel to determine the equations parameters (k, R_{max} , λ) and the predicted methane yield. The predicted methane yield obtained from the regression analysis was plotted with the measured methane yield. The statistical indicators, correlation coefficient (R^2) and root mean square error (RMSE) were calculated to assess the goodness of fit and evaluate the best kinetic equation to describe WW biogas production.

2.3 Continuous AD trials

The BMP tests estimated the potential methane production while semi-continuous tests allowed to evaluate the feasibility of the process. Continuous trials were carried out in a lab-scale CSTR maintained at temperature of 55°C in a controlled oven. The system was inoculated with the same mesophilic digestate sludge used for BMP tests. The temperature was increased by one temperature step strategy, passing in a very short time from mesophilic to thermophilic condition as suggested by Cecchi et al. (1993). Biogas flow was measured using a MilliGascounter® (Ritter) and the gas was collected in a Tedlar gas bag to analysed its composition (CH_4, CO_2) by gas chromatography equipped with HP-Molesieve column (30m×0.3mm×0.25μm film thickness) and a thermal conductivity detector (TCD).

The grape marcs were used as main substrate because of its availability and potential BMP. The reactor was fed once a day with substrate and tap water to reach the correct flow rate. Different operational conditions were tested in terms of feeding substrate, HRT and OLR, dividing the tests in four RUNs, as reported in Table 1.

Table 1 Operational conditions of semi-continuous trials

	Feed	Organic loading rate ($kgVS/m^3d$)	HRT (d)
RUN1	Grape marcs	1.0	20
RUN 2	Grape marcs	2.0	20
RUN 3	Grape marcs + Activated Sludge	4.0	20
RUN 4	Fermented Grape marcs	1.2	40

Partial and total alkalinity (PA and TA), pH, VFA, ammonium nitrogen were measured twice a week to monitor the process stability, while the characteristics of the effluent in terms of total and volatile solids, COD, TKN and total phosphorus (P_{tot}) were analyzed weekly. All the analyses, except for VFA, were carried out in accordance with the Standard Methods (APHA, 2012). Volatile fatty acids content was monitored using a gas chromatograph (Carlo Erba instruments) with hydrogen as gas carrier, equipped with a Fused Silica Capillary Column (Supelco NUKOLTM, 15m×0.53mm×0.5 µm film thickness) and with a flame ionization detector (200°C). The temperature during the analysis started from 80°C and reaches 200°C trough two other steps at 140 and 160°C, with a rate of 10°C/min. The analyzed samples were centrifuged and filtrated on a 0.45 µm membrane.

3. Results and Discussion

3.1 Characterization of winery waste samples.

The characteristics of substrates tested in trials are reported in Table 2. The results reflected typical characteristics of WW: total solids ranging from 225 to 430 gTS/kg, of which about 90% were volatile solids. WL had low volatile solids content, because during the winemaking process diatomaceous earth, that is an inert material, was used in order to remove suspended solids and colloidal particles. The COD in the WW was higher than 1,000 mgO_2/l, except for WL, but part of organic matter was lignin and cellulose (Dinuccio et al., 2010).

During the experimentation waste activated sludge (WAS), collected in cellar wastewater treatment plant , was used. The sludge was derived from biological nutrient removal process working with a solid retention time of 15 d and food to microorganisms ratio of 0.15 kg COD/kg MLVSS d. The WAS had 45.4 gTS/kg with 60% of volatile solids. The concentrations of COD, TKN and P_{tot} were 921 mgCOD/gTS, 31.4 mgN/gTS and 7.0 mgP/gTS respectively, corresponding with COD:N:P ratio of 132:4:1.

Table 2 Chemical-Physical characteristics of analyzed winery wastes

Parameters	Unit	GS	Virgin GM	Fermented GM	WL
TS	g/kg_{ww}	226.8± 40.8	371.0 ± 51.9	425.2 ± 21.3	289.5 ± 5.8
VS	g/kg_{ww}	201.7 ± 54.5	334.1 ± 30.1	383.4 ± 30.7	114.6 ± 4.6
COD	mg O_2/gTS	1,306 ± 131	1,078 ± 32	1,132 ± 68	352 ± 46
TKN	mgN-NH_4^+/gTS	6.1 ± 1.0	20.4 ± 0.8	16.8 ± 4.5	15.0 ± 2.4
Ptot	mgP-PO_4^{3-}/gTS	3.6 ± 1.8	5.5 ± 4.1	1.2 ± 0.3	0.8 ± 0.4

3.2 Methane production in batch trials

The specific methane production (SMP) values were measured for each substrate (WL, GS, virgin GM and fermented GM) using the BMP tests in thermophilic condition. The Figure 1 shows the cumulative average SMP from the batch experimentations. The hydraulic retention time of 30 days was enough to convert the organic matter in anaerobic condition, in fact within this time the daily variation of methane was lower than 5% of cumulative production.

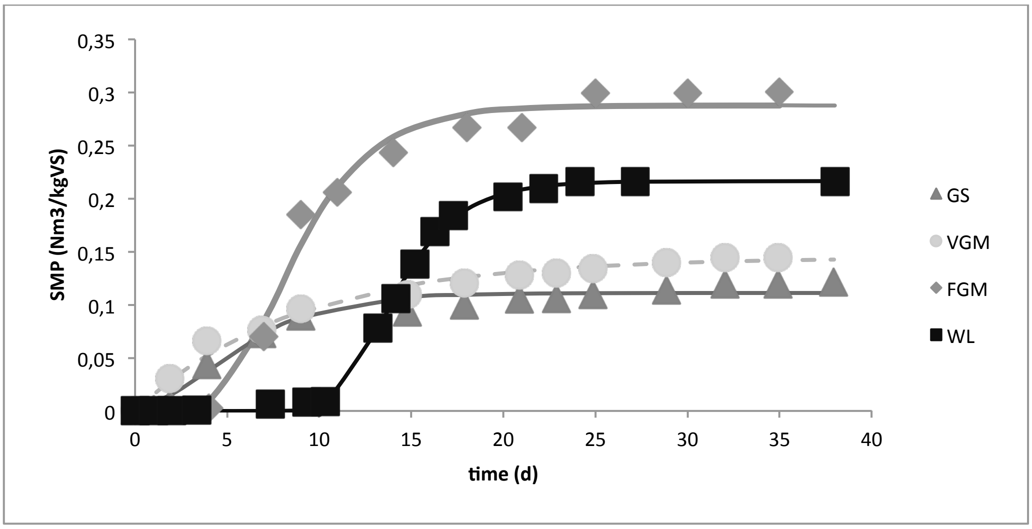


Figure 1 Cumulative specific methane production of wine lees, grape stalk, virgin and fermented grape marcs at thermophilic temperature. Continuous lines indicate modified Gompertz model predictions, while dotted line indicates first order model prediction.

Specific methane production of GS was 0.121 Nm^3/kgVSfed the value was far from theoretical methane production deriving from COD concentration (0.514 $m^3/kgVS_{fed}$), consequently only 24% of organic matter was converted into methane. The low biodegradability index was justified by data reported by Dinuccio et al (2011) about high content of lignin (23% TS) that was not biodegradable in anaerobic condition, and of cellulose and hemicellulose (15.9 and 23.5 %TS respectively) that were partially degraded.

Significant differences were observed in virgin and fermented GM. The average SMP of virgin grape marcs were 0.150 $Nm^3/kgVS_{fed}$ with 36% of COD conversion into methane. The production value was consistent with those reported by Colussi et al. (2009). The composition in terms of lignin, hemicellulose and cellulose was similar to GS and explained the low gas production. The natural fermentation of GM was a pre-treatment that allow to obtain, from complex molecules, rapidly biodegradable compounds, and improved methane production, in fact it reached 0.301 $Nm^3/kgVS_{fed}$ corresponding to biodegradability index of 68%.

The WL had similar conversion efficiency (70%) but SMP reached 0.216 Nm^3/kgVSfed; the presence of readily biodegradable compounds partially inhibited the process and the methanogenesis started after about 9 days.

Considering winery wastes biogas production reported in literature, the most evident aspect was the variability in specific methane production. As demonstrated by Fabbri et al. (2014) the grape type determined different biogas production also operating in same operational conditions, in fact the variation coefficient calculated for available mesophilic values for GM was about 40%, while for GS lowered to 25%. Existing result of thermophilic digestion of GM (Rebecchi et al., 2013) was discouraging (0.04 m^{3}CH$_4$/kgTVS), instead this study determined methane yields in same magnitude order of mesophilic one (Table 3). Considering many available figures, comparison between mesophilic and thermophilic potentials was not easy. Average values for GM and GS, 0.196 (metti anche range e riferimento alla tabella sotto) and 0.139 m^{3}CH$_4$/kgVS respectively, were considered for mesophilic process. Process at 55°C reduced methane production of GS of 13%, value consistent with intrinsic variability and difficulty associable to operational temperature. The behaviour of GM depends on pre-treatment, virgin substrate was characterized by lowering of methane production of 23%, while fermented one increased of 53%. These preliminary results didn't justify possible inhibition at thermophilic temperature but probably indicated the low biodegradability of used pomace; anyway they are inside the range of literature values. The wine lees BMP appeared significantly lower than value reported by some authors (Lo and Liao, 1986; Danieli and Aldeovandi, 2011) mainly due to different origin process. Overall, the thermophilic AD in batch reactor didn't significantly increase biogas production compared to mesophilic test.

Table 3 Comparison of biogas and methane production reported in literature and in this study (* indicates value obtained by continuous process, bolt data refer to thermophilic process)

Substrate	**Temperature**	**Specific production**			**CH$_4$**	**References**
	°C	m^{3}biogas/ kgTVS	m^{3}CH$_4$/ kgTVS	m^{3}CH$_4$/ kgCOD	%	
GS	40	0.225	0.098		44	Dinuccio et al., 2010
	35		0.18			Gunaseelan, 2003
	35-37	0.297	0.14			Fabbri et al 2014
	55		**0.121**			**This study**
GM	40	0.25	0.116		46	Dinuccio et al., 2010
	35		0.283			Gunaseelan, 2003
	40	0.120-0.159	0.096-0.128		80-81	Failla and Restuccia., 2009
	35		0.15			Colussi et al., 2009
	35			0.147		Fountolakis et al., 2008 *
	35-37	0.406	0.273		67.32	Fabbri et al 2014
	35-37	0.322	0.157		48.71	Fabbri et al 2014
	55		**0.04**			**Rebecchi et al., 2013**
	55		**0.150-0.301**			**This study**
WL	35		0.367-1.048			Lo and Liao, 1986
	36		0.488		60	Danieli and Aldeovandi, 2011
	55		**0.216**			**This study**

3.3 Kinetic study results

The results of the kinetics analysis using the first order kinetic model and the modified Gompertz model are summarised in Table 4. The first order kinetic model could not be applied to test WL because the result was far from real data. In the other cases the model gave similar *k* values for GS and VGM, and the *k* value of FGM was lower probably due to inhibition effect that was not consider in the model. In fact GS and GM had lower lag times determined by modified Gompertz model, while the VGM and WL had 4.7 and 10.8 days g lag phase, respectively.

Both models exhibited a good fit when plotted against the measured data, with a determination correlation coefficients (R^2) ranging from 0.97 to 0.99 for the first order model and from 0.98 to 1.00 for the Gompertz model. The RMSE ranged from 7 to 35 mlCH$_4$/g VS$_{fed}$ for the first order model while the Gompertz model gave lower values of over 8–14 mlCH$_4$/g VS$_{fed}$. The modified Gompertz model gave slightly lower predicted maximum BMP yields than the measured data ranging from -4% to -8% while the first order model generally gave higher predicted methane. The model Gompertz gave a more accurate predicted max methane yield than the first order equation, especially when lag time was observed. While if there wasn't lag phase, as in test with VGM, this model introduced a significant error with negative value of λ. Correlation coefficient and root mean square demonstrated that modified Gompertz model fitted better than first order model for the trials characterized by lag phase. On the other hand the model that better described the production of VGM was first order kinetic one.

Table 4. Results of the kinetics analysis using the first order kinetic model and the modified Gompertz model

Substrate	**Unit**	**GS**	**VGM**	**FGM**	**WL**
BMP	m^{3}CH$_4$/kgVS$_{fed}$	0.121±0.023	0.150±0.088	0.301±0.087	0.216±0.137
Biodegradabilty index	%	24	36	68	70
First order kinetic model					
BMP predicted	m^{3}CH$_4$/kgVS$_{fed}$	0.119	0.144	0.372	-
Δ BMP	%	-2%	-4%	24%	-
k	d-1	0.117	0.114	0.059	-
RMSE	m^{3}CH$_4$/kgVS$_{fed}$	0.007	0.006	0.035	-
R^2		0.99	0.99	0.97	-
Modified Gompertz kinetic model					
BMP predicted	m^{3}CH$_4$/kgVS$_{fed}$	0.111	0.141	0.288	0.217
Δ BMP	%	-8%	-6%	-4%	0%
λ	d	0.9	1.8	4.7	10.9
Rmax	m^{3}CH$_4$/kgVS$_{fed}$d	0.012	0.009	0.036	0.034
RMSE	m^{3}CH$_4$/kgVS$_{fed}$	0.008	0.009	0.014	0.004
R^2		0.99	0.98	0.99	1.00

Fabbri et al.(2014) applied same models to digestion performances and observed lag phases due to presence of phenol, alkyl phenols and alcohols, commonly present in winery wastes. Consequently, also at mesophilic temperature the best results were obtained by application of Gompertz model.
Comparing kinetic constants calculated in this study with those reported by Fabbri et al. (2014) some interesting observations were made. Considering grape stalks digestion had different methane production but equal kinetic constants, in fact the previous study reported hydrolysis rate of 0.11 d-1, 0.011 $m^3CH_4/kgVS_{fed}d$ and no lag phase was detected. By these values thermophilic digestion of lignocelluloses substrates had similar behaviour than mesophilic one.
GM had larger variability range, in terms of kinetic parameters both in mesophilic and thermophilic studies.

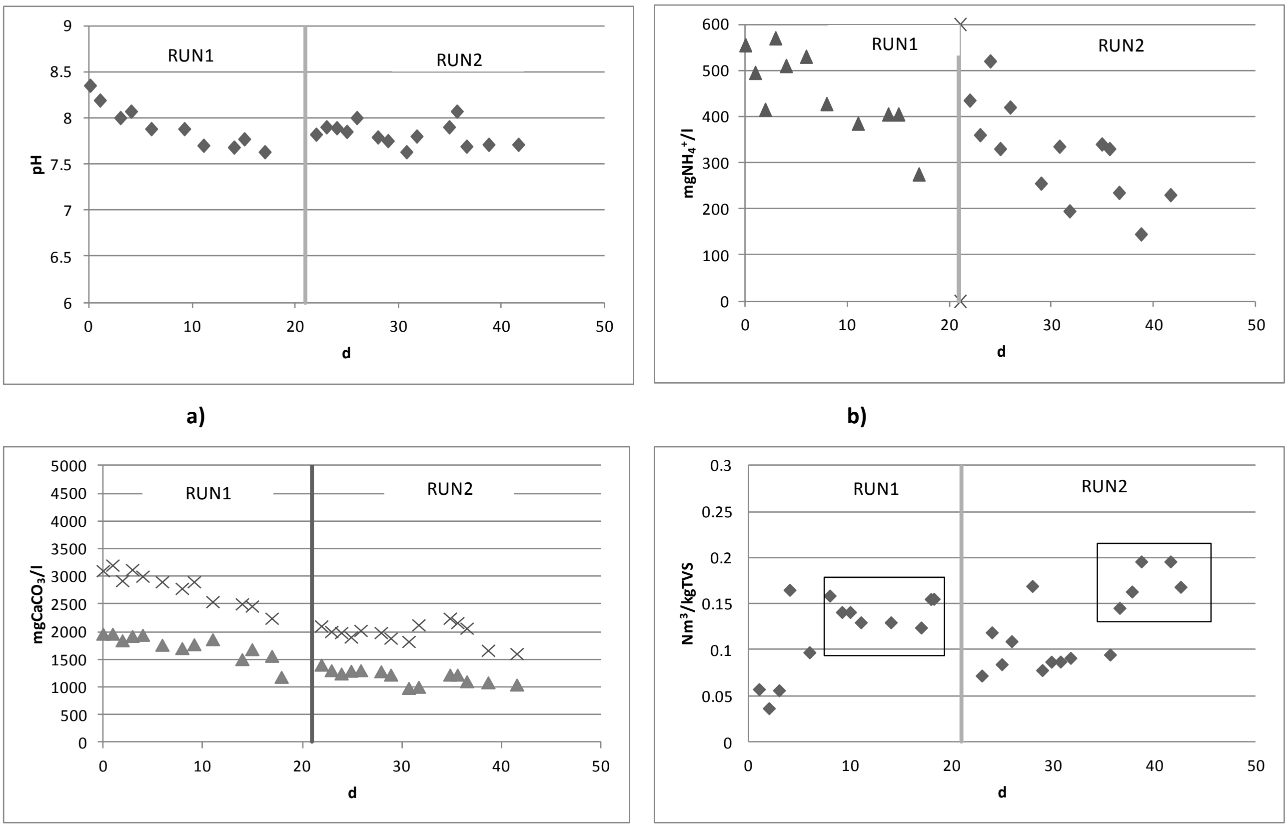
Comparing the mesophilic results obtained by Fabbri with thermophilic of this study, it is possible to observe that the higher methane production was also characterized by a lower lag phase and by a greater maximum methane production rate. In particular …confronta nelle due condizioni separatamente .(0.9 d and 1.8d, at 37°C and 55°C respectively) (0.033 and 0.036 $m^3CH_4/kgVS_{fed}d$, at 37°C and 55°C).

On basis of these comparisons, thermophilic digestion in batch reactor appeared quite similar to mesophilic one, and no inhibition effects or metabolites accumulation were observed.

3.4 Biogas production in lab-scale CSTR

GM appeared the be more interesting by-product of winemaking process in terms of available quantity and biogas production, so the semi-continuous tests were carried out using this substrate as feeding.
Two trials were conducted with virgin GM alone, with organic loading rate (OLR) of 1 and 2 kg VS/m^3d and 20 day of HRT respectively (RUN1 and RUN2). Considering the low OLR applied the process didn't need start-up and acclimatisation period. By the end of the first HRTs the system had reached a steady state of methane production. The biogas production was 0.11 and 0.15 $Nm^3/kgVS_{fed}$ in RUN1 and RUN2 respectively, both with 75% of methane content. Considering the mass balance, the volatile solid removals were of 12% and 15% respectively for OLR 1 and 2 $kgVS/m^3d$.
Both partial and total alkalinity decreased during experimentation (Figure 2) determining a net reduction of buffer capacity of the system. This eventually results in the failure of the biological process although the pH value was upper 7.5. The low biodegradability index (Table 3) affected the concentration of VFA that was lower than 100 mgCOD/l. The good percentage of methane and the stability parameters suggested that methanogenesis was not inhibited and that hydrolysis was the

limiting step of the process. It was not clear if inhibition was due only to low biodegradability of substrate or if unbalance COD:N:P ratio limited the biomass growth and consequently its activity.



a)

b)

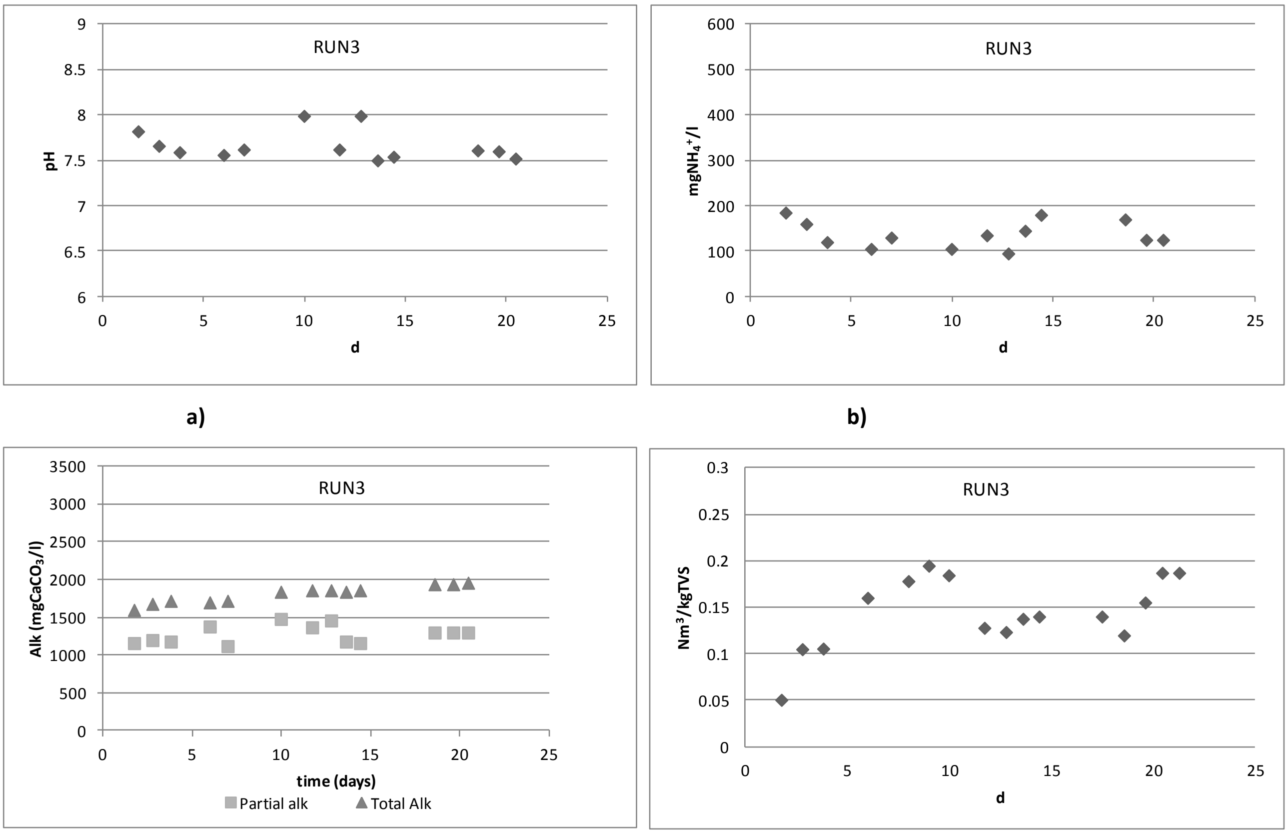
c)

d)

Figure 2 Trends of pH a), ammonia b), total and partial alkalinity c) SGP d), at OLR 1 $kgVS/m^3d$ (RUN1) and 2 $kgVS/m^3d$ (RUN2)

In order to supply to missing nutrients and to improve stability of the process waste activated sludge from winery wastewater treatment was added as co-substrate (RUN3) because it is widely available in wine industry (Brucculeri et al., 2005). The feed was composed by 66% of virgin GM (in terms of volatile solids) and the rest derived from sludge. The total OLR applied was 4 $kgVS/m^3d$.

As illustrated in Figure 3(b), the average biogas production was 0.146 $Nm^3/kgVS_{fed}$ with 50% of methane. The volatile solids removed by biogas conversion corresponded to 20%. The pH value range from 7.5 to 8, without considerable variation during the test, while the alkalinity increased slightly and reached 1,952 $mgCaCO_3/l$ in the last period (Figure 3). The ammonia concentration was constant at value of 143 $mgN\text{-}NH_4^+/l$. The volatile fatty acids were completely consumed by methanogens and the concentration in the effluent was lower than 100 mgCOD/l. The utilisation of a co-substrate improved the stability of the process (pH, alkalinity and ammonium concentration), increased the volatile solids removal and, as confirmed by Fountoulakis et al. (2008) increased the methane production of 10-30%.



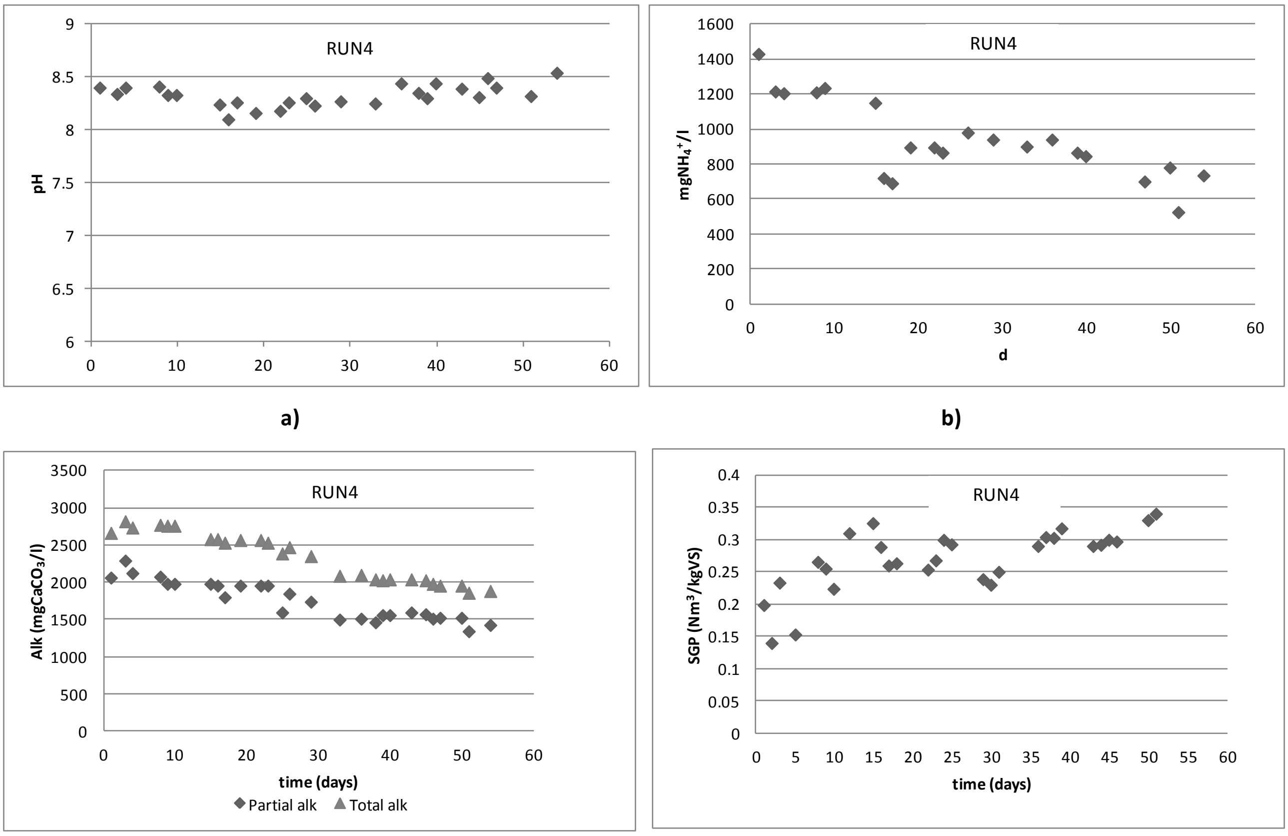
a) b)

c) d)

Figure 3. Trends of pH a), ammonia b), total and partial alkalinity c) SGP d), at OLR 4 kgVS/m3d (RUN3).

Anyway the specific biogas production remained lower than value obtained by BMP tests, in order to improve the degradation efficiency longer HRT was applied (RUN 4). Also a pre-treatment such as fermentation could improve the biogas yield as reported by BMP of fermented GM. The effects of these aspects were evaluated (RUN 4) with a reactor working with 1.2 gVS/ld of OLR and 40 days of HRT, fed with fermented GM. Increasing the HRT, the flow rate was reduced from 250 ml/d to 125ml/d. Consequently the organic load decreased in order to maintain solid concentration in the feed mixture comparable with RUN1 and RUN2.

The process was monitored for 55 days and reached stability after 35 days (Figure 4). The SGP was 0.290 $Nm^3/kgVS_{fed}$ with 61% of methane. The corresponding VS conversion into biogas was of 35%, higher than previous continuous trials because the long retention time and previous fermentation promoted the hydrolysis of organic matter, however it was half of biodegradability index obtained by batch test because of semi-continuous feeding mode. The biogas production agrees with higher stability of the process. pH remained higher than 8 during the whole trial, this value was explained by the higher concentration of ammonium in the digestate. In fact the concentration at steady state was 734 $mgN\text{-}NH_4^+/l$, significant major than in others examined digestates, because of higher protein degradation. At the beginning the trend of alkalinity was similar of those of the tests with VGM alone (Figure 3), but it stabilized on values 1,992 $mgCaCO_3/l$ at steady state.



a) **b)**

c) **d)**

Figure 4 Trends of pH a), ammonia b), total and partial alkalinity c) and SGP d), at OLR 1.2 kgVS/m3d (RUN4).

In Table 5 the operational conditions, effluents characteristics and process yields are summarized. The effluents solids concentrations were affected by operational conditions, in particular the TS concentration increase with OLR. The VS/TS ratio and COD concentration reflected the substrates stabilisation: the reactor working with long HRT was characterized by lowest VS/TS percentage and lower COD content, due to higher organic matter conversion.

Comparing the SGP recorded during the continuous trials with VGM alone or with WAS, similar biogas production appeared but the percentage of methane was quite higher when only one substrate was used. The reason of different biogas composition could be due to organic matter composition of the feed, in particular grape marcs contained oil, compounds with higher theoretical methane production. Fermentation of GM duplicated the biogas production in agreement with BMP tests.

Thermophilic digestion didn't seem to be more sensitive than mesophilic process, in fact no inhibition effects were detected. Anyway biogas production was comparable in the two operational temperature, consequently the higher cost of maintaining reactor at 55°C could be only justified by better hygienisation of effluent of co-digestion process.

Table 5 Comparison of operational conditions, effluents characteristics and specific yields of continuous trials

		RUN1	RUN2	RUN3	RUN4
Operational conditions					
V_r	l	5	5	5	5
HRT	d	20	20	20	40
OLR	gVS/ld	1	2	4	1.2
Q	l/d	0.25	0.25	0.25	0.125
Effluent characteristics					
TS		35.6	41.0	55.1	55.0
VS		27.8	33.6	47.8	37.6
VS/TS	%	78	82	87	68
COD	mg/gTS	920.7	1083	981.2	889
pH	-	7.7	7.8	7.5	8.4
N-NH4	$mgN\text{-}NH_4^+/l$	367.5	258.6	142.5	734
PA	$mgCaCO_3/l$	1,556	1,091	1,217	1,509
TA	$mgCaCO_3/l$	2,435	1,951	1,918	1,992
Yields					
SGP	$Nm^3/kgVS_{fed}$	0.114	0.145	0.146	0.290
CH_4	%	75%	76%	50%	61%
VS_{rem}	%	12%	15%	20%	35%

3.5 Potential Energy recovery from Winery Waste production

The Italian, European and worldwide winery wastes production was calculated considering the wine production of 2013 and the specific production factor reported by ANPA (2001). The GM represented the 65% of whole organic waste production by winery sector (Table 5) and the recent legislation encourages its use to energy production.

Considering this biogas yield and the production of GM in Italy, in Europe and around the World, the evaluation of potential energy from this by-product was calculated. If the biogas produced is exploited using CHP units, with thermal efficiency of 50% and electrical efficiency of 35%, the energy produced would be a great deal (Table 6). The heat energy reported in Table 6 is a net value, subtracting the thermal energy used for biomass and digesters heating, then the process could be energetically self-sustaining. Assuming same biogas yields operating at 37°C the available heat increased of 8%.

Table 6 Production of grape marcs, grape stalks and wine lees in 2011

	Wine	GM	GS	WL
	MHl	Thousands of Tons		
Italy	44.9	808.2	179.6	269.4
Europe	164.2	2,955.6	656.8	985.2
World	278.6	5,014.8	1,114.4	1,671.6

Table 7 Production of grape marcs and corresponding heat and electricity production

	GM	Thermal Energy	Electricity
	10^3 tons	GWh/y	GWh/y
Italy	808.2	245	201
Europe	2,955.6	896	734
World	5,014.8	1,520	1,245

The gain obtained from selling the electric energy depends by the incentives applied, but even considering average plant size smaller than 1MWh the minimum price for 2014 is 174.4 €/MWh (DM July 6th, 2012), it is possible to obtain about 35,054,400 €/y just in Italy.

4. Conclusions

Thermophilic batch test were carried out with different winery waste and the results of BMP tests showed specific methane production of 0.150-0.301 $Nm^3CH_4/kgVS_{fed}$ for virgin and fermented grape marcs, 0.121 $Nm^3/kgVS_{fed}$ for grape stalks and 0.216 $Nm^3CH_4/kgVS_{fed}$ for wine lees. The specific methane production were similar with those reported in literature at 37°C. The kinetic study demonstrated that modified Gompertz model well fitted (R^2 0.99-1.00) the methane production cumulative curve in case of presence of lag phase at the beginning of the test. While the anaerobic degradation of virgin grape marcs was better described by first order equation (R^2 0.99). The determined specific yields and kinetic parameters were comparable with one at 37°C.

AD semi-continuous trials of GM were characterized by low biogas production (0.114-0.145 $Nm^3/kgVS_{fed}$). The addition of waste activated sludge improve process stability but didn't increased biodegradation of substrate. The biogas production increased with fermented grape marcs and applying longer HRT (40 d) obtaining 0.29 $Nm^3/kgVS_{fed}$; the effluent had lower COD content due to higher organic matter degradation (35% of volatile solids).

Considering best biogas yields, the potential electricity and heat were calculated using a typical CHP unit efficiency. The combustion of biogas generated by GM could generate, in whole world, 1520 GWh/year of heat and 1245 GWh/year of electricity that could be used in cellar facilities.

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