Mesophilic and thermophilic anaerobic co-digestion of winery wastewater sludge and wine lees: an integrated approach for sustainable wine production

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Abstract

In this work, winery wastes generated by a cellar producing approximately 300,000 hectoliters of wine per year was monitored for a period of one year. On average, 196 L of wastewater, 0.1 kg of sludge (dry matter) and 1.6 kg of wine lees were produced per hectoliter of wine produced. Different winery wastes, deriving from different production steps, namely waste activated sludge from wastewater treatment and wine lees, were co-treated using an anaerobic digestion process. Testing was conducted on a pilot scale for both mesophilic and thermophilic conditions. The process was stable for a long period at 37°C, with an average biogas production of 0.386 m^3/kg COD_{fed}. However, for thermophilic conditions, volatile fatty acids accumulated in the reactor and the process failed after one hydraulic retention time. In order to fix the biological process, trace elements were added. Metals augmentation improved process stability and yields at 55°C. The pH ranged between 7.8 and 8.0, and specific gas production was 0.450 m^3 per kg COD fed, which corresponded to dry matter and COD removals of 34% and 88%, respectively. Although the observed performances in terms of biogas production were good, the thermophilic process exhibited some limitations related to both the necessity of metals addition and the worse dewaterability properties. In fact, while the mesophilic digestates reached a good dewatering quality via the addition of 6.5 g of polymer per kg of dry matter, the required dosage for the thermophilic sludge was greater than 10 g/kg of dry matter.

Keywords: Anaerobic digestion; dewatering; mesophilic; thermophilic; trace elements; winery wastes

1. Introduction

The winemaking process produces large volumes of waste streams, including solid organic waste, wastewater, greenhouse gases, and packaging waste (Lucas et al., 2010). Winery wastewater is a major waste stream resulting from a number of activities that include tank, floor and equipment washing; barrel cleaning; wine and product losses; bottling facilities; filtration units; and rainwater captured in the wastewater management system (Ioannou et al., 2014). The quantification of the produced wastewater is difficult, and it depends on the cellar dimensions and the technologies applied. In general, wastewater production ranges from 0.7 to 14 L per liter of wine produced (Andreottola et al., 2009), but specific studies conducted in South Africa (Walsdorff et al., 2005), Chile (Aybar et al., 2007), Portugal (Duarte et al., 2004), Italy (Berta et al., 2003), and Greece (Vlyssides et al., 2005), all demonstrated that typical values are approximately 2-6 L of wastewater per liter of wine produced.

This effluents generally presents a considerable level of COD, the major part of which is soluble (Beck et al., 2005) and highly biodegradable (Andreottola et al., 2005) due to the presence of ethanol, sugars, and organic acids (Malandra et al., 2003; Mosteo et al., 2008; Petruccioli et al., 2000; Vlyssides et al., 2005).

Because of its characteristics, this stream is generally treated using either aerobic or anaerobic processes (Ioannou et al., 2014). Among biological processes, the activated sludge process is the most commonly employed because of its high efficiency and simplicity. It can remove 98% of COD and cope with large variations in the hydraulic and pollution load (Beck et al., 2005; Fumi et al., 1995; Petruccioli et al., 2000).

The removal of organic material generates considerable quantities of excess sludge, normally in the range 0.21 - 0.28 kg MLVSS per kg of COD removed (Brucculeri et al., 2005; Torrijos and

Moletta, 1997). Ruggieri et al. (2009) estimated that dewatered wastewater sludge represents the 12% of the total organic solid waste produced by wineries and that its management via external companies is expensive and often difficult. An alternative to valorize this waste stream could be the use of an anaerobic digestion process. Anaerobic digestion (AD) is a mature technology and it is applied to treat different types of organic wastes (municipal solid wastes, sewage sludge, agro-industrial residues, livestock effluents, etc.) and to reduce their biodegradability while simultaneously recovering bio-energy. The combination of the conventional activated sludge process (CAS) and AD is a common practice in municipal wastewater treatment plants and limits the external management costs for sludge disposal thanks to a reduction in the sludge volume. Biogas is a renewable source of energy that is usable inside the same production process and/or wastewater treatment plant, which reduces the energy requirements (Shen et al., 2015). Moreover, digestate, the effluent from the anaerobic process, can be reused in vineyards because of the presence of nutrients such as N, P, and K together with stabilized C and humic substances. AD removes pathogens and polyphenolic compounds with different efficiencies based on the operating conditions used. Pathogen reduction is affected by temperature, retention time and fed substrates (Poudel et al., 2010; Sahlström et al., 2004), whereas the efficiency of polyphenol removal is mainly determined by the operational temperature (Cavinato et al., 2014; Levén and Schnürer, 2005).

Once AD is implemented for winery wastewater sludge, other winemaking process residues (e.g., wine pomace, pressed cake, or lees) should be co-treated to increase the biogas production, to improve the reactor utilization and to make the anaerobic process more economically advantageous.

Wine lees (WL) in particular are an interesting co-substrate because of their biodegradability and availability throughout the year. Like wastewater, WL contain a high organic content and their disposal requires the appropriate treatment. The composition of WL depends on the winemaking technology, although, according to de Bustamante and Temiño (1994), the main characteristics are an acidic pH (between 3 and 6), a COD greater than 30,000 mg/L, potassium in concentrations greater than 2,500 mg/L, and phenolic components in quantities up to 1,000 mg/L.

This paper reports the results obtained from a pilot scale study in which winery wastewater sludge and lees were co-digested under both mesophilic and thermophilic conditions. The study assesses the process feasibility and evaluates the effluent quality in terms of pollutant removal and dewatering capacity. The suggested approach is schematically represented in Figure 1.

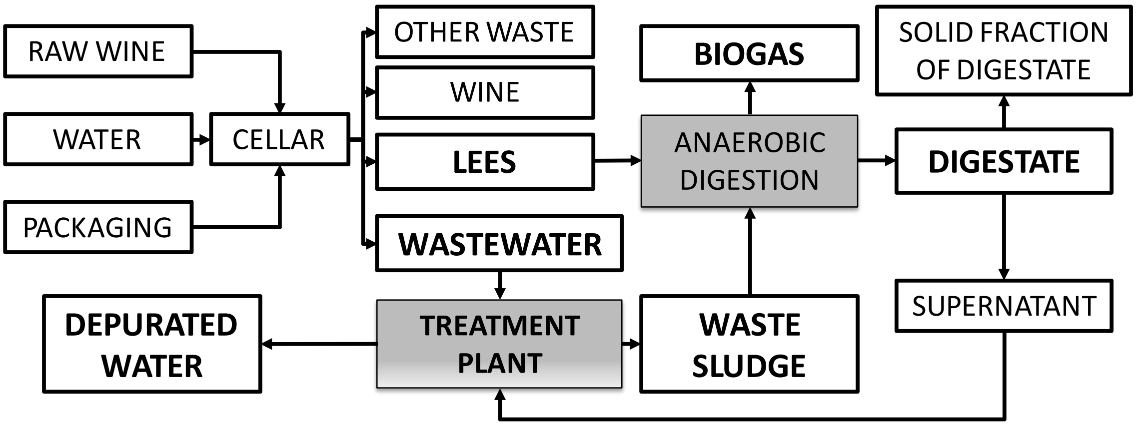


Figure 1 Integration of anaerobic digestion in the wine-making process

2. Materials and methods

2.1. Experimental set-up

2.1.1. Winery wastewater treatment plant

Waste activated sludge was collected in a cellar where a wastewater treatment plant was operating. The cellar was located in the northeast of Italy and produced approximately 300,000 hectoliters of wine per year. It processed and bottled both self-produced and bought wines; therefore, the working period is not restricted to the grape harvest, but rather, it is distributed throughout the year. Therefore, there is no real seasonal variation in the output. After pre-treatment (screening and primary sedimentation), the wastewater is sent to a 1,400 m^3 aerobic bioreactor. The biological process operated with average hydraulic and sludge retention times (HRT and SRT) of 6.7 d and 35 d, respectively. The mixer liquor volatile suspended solid (MLVSS) was 3,010 mg/L and the corresponding food to microorganisms ratio was 0.26 kg COD/g MLVSS per day. The treated water and sludge are separated in a secondary sedimentation tank. The treated water is eventually disinfected and filtrated using quartz sand. The sludge treatment process consists of a thickening section followed by a filter press. The sludge is not stabilized.

Influent and effluent streams for the wastewater treatment plant were monitored for one year to determine their characteristics. Additionally, dewatered sludge after filter pressing was collected and analyzed.

2.1.2. Pilot scale anaerobic reactors

Two parallel continuous stirred tank reactors (CSTRs) with working volumes of 230 liters were employed for anaerobic co-digestion tests. Mesophilic (37°C) and thermophilic (55°C) conditions were maintained by externally jacketed hot water recirculation systems. PT100 probes monitored the process temperatures and managed the water recirculation pumps. Biogas production was continuously monitored using a drum-type gas flow meter (Ritter, Bochum, Germany). The experimental design contemplated a start-up period, whereas the organic loading rate and wine lees content in the feed mixture increased stepwise. During this period, the anaerobic microorganisms acclimated to the substrates and to different readily biodegradable compounds present in the winery by-products. At the end of start-up, organic loading rate was 3.2 $kgCOD/m^3d$ and the HRT was 23 d. The distribution of the organic load between the two co-substrates considered the real waste flows: 80% was due to WL and the remaining 20% was due to WAS. Once the operational conditions were reached, the tests were conducted for several HRTs to obtain steady state biogas production, stability parameters, and digestate characteristics.

2.2. Analytical methods

The substrates and the digester effluents were collected and monitored once a week to determine the total and volatile solid content (TS and VS), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), and total phosphorus (P_{tot})(American Public Health Association et al., 1999). The process stability parameters, pH, volatile fatty acids (VFAs) content and composition, total and partial alkalinity, and ammonia concentration were checked two or three times per week. VFAs content was monitored using a gas chromatograph, as reported by Cavinato et al. [22]. At steady state conditions, the total polyphenols were analyzed spectrophotometrically using the Folin–Ciocalteu assay (Lafka et al., 2007). The concentration was reported in terms of Gallic acid equivalent per liter (mg GAE/L). Biogas composition (CO_2, CH_4, H_2, and O_2) was determined by a gas chromatograph (GC Agilent Technology 6890 N) equipped with a column HP-PLOT MOLESIEVE, 30 × 0.53 mm ID × 25 μm using a thermal conductivity detector and argon as the gas carrier.

The anaerobic process leads to changes in the structural matrix of the sludge flocs and particles, consequently affecting the particle size distribution and dewaterability(Yan et al., 1987). Filterability characteristics of the raw and conditioned effluents were determined by a capillary suction time (CST) test using a CST instrument (Triton, A304M model) according to the standard method (American Public Health Association et al., 1999) and by specific resistance to filtration (SRF), as reported by IRSA - CNR (1985).

3. Results and discussion

3.1. Winery wastewater treatment plant monitoring

Considering the wine production and winery wastewater flow in the monitored cellar during a one-year period, the specific wastewater generation was calculated to be 1.96 L of wastewater per liter of wine produced. Winery wastewater was treated in the internal WWTP and the wastewater and effluent characteristics are shown in Table 1.

Table 1 Influent and effluent characteristics

Parameter	**Unit**	**Influent**			**Effluent**		
		Average	St.Dev	Range	Average	St.Dev	Range
TSS	mg/L	148	144	30 - 760	19	21	<1 - 100
VSS	mg/L	94	141	0 - 640	9	20	<1 - 90
COD	mgCOD/L	3,747	2,478	518-12,731	33	27	1 - 148
rbCOD	mgCOD/L	2,668	1,682	446-6,602	-	-	-
TKN	mgN /L	25	13	9 - 57	11	4	3 - 19
$N\text{-}NH_4^+$	mgN/L	1.9	1.7	0.6 -10.1	0.5	0.4	<0.1-1.3
Ptot	mgP/L	7.5	4.0	2.0 - 19.3	3.3	2.3	0.3 - 9.6
$P\text{-}PO_4$	mgP /L	2.5	2.0	0.2 - 7.4	0.2	0.4	0.0 - 1.3

The total solids ranged from 30 to 328 mg/L, and contained 63% volatile solids. They were completely removed during the treatment process, and the treated water had a solid concentration that was usually lower than 50 mg/L and contained 66% inert material.

The influent COD concentration ranged between 518 and 12,731 mg/L (Table 1), with an average value of 3,747 mg/L. Approximately 71% of the COD was readily biodegradable COD (rbCOD) and contained sugars, ethanol, and other fermentation products, which were dominated by the presence of acetate. The determined rbCOD fraction was consistent with the value reported by Andreottola et al.. The remaining fraction was a readily hydrolysable fraction, whereas the amount of non-biodegradable COD was negligible. Thanks to the high biodegradability of the wastewater, COD removal was generally higher than 97%, with one exception (95%). The concentrations of 25 mg $N\text{-}NH_4^+$/L and 7.5 mg $P\text{-}PO_4^{3-}$/L in the influent were relatively low compared with the COD content and resulted in an unbalanced COD:N:P ratio (500:3:1). For this reason, urea and ammonium phosphate were added to the biological reactor to improve the activated sludge activity.

The wastewater treatment plant achieved a good efficiency and nutrient content in the effluent, which was sufficient to meet the legal threshold limits (Table 1).

Waste activated sludge was purged twice a week and dewatered by the addition of a chemical conditioner. On average, 3,858 kg of wet sludge, with a dry mass content between 15 and 20%, was produced per week. This corresponded to some 613 kg of dry matter per week, or 0.1 kg of dried sludge per hectoliter of wine produced. The dewatered sludge is usually managed by composting, which has an average cost of approximately 110 €/ton.

3.2. Anaerobic inoculum and substrates characterization

The pilot-scale reactors were filled with mesophilic and thermophilic inoculum digestates derived from previous experiments using the same temperature conditions. The inocula were well stabilized, with solid contents lower than 10 g TS/kg and stability parameters in the optimum ranges for AD (Table 2). The low content of COD in the inocula indicated the absence of organic matter that could affect the process, whereas the nitrogen and phosphorus concentrations were 41.6 and 33.1 mg N/gTS, and 27.7 and 26.8 mg P/g TS at 37°C and 55°C, respectively.

Table 2. Inocula characteristics

Parameter	Unit	37°C inoculum	55°C inoculum
TS	g TS/kg$_{ww}$	8.84	9.37
VS	g VS/kg$_{ww}$	5.92	4.69
VS/TS	%	67%	50%
COD	mg/g TS	552	751
sCOD	g/L	911	1,073
pH	-	7.53	8.33
TKN	mg N/g TS	41.63	33.09
Ammonium	mg N/L	193.4	539.4
Total phosphorus	mg P/g TS	27.7	26.8
Polyphenols	mg GAE/L	83.75	58.35

The substrates fed to the reactors were waste activated sludge from winery wastewater treatment and wine lees, both of which originated from the same cellar.

The solids in the dewatered sludge generally ranged from 129.0 to 193.7 g TS/kg. However, outliers were detected due to technical reasons (conditioner doses and filter press setting, Table 3). The volatile solids to total solids ratio (VS/TS) in the winery sludge was higher (88%) than for typical sludge from municipal wastewater, probably due to the high biodegradability of the raw wastewater. The high amount of volatile solids and COD concentration (868 mg/g TS) were indicative of the low biological stability of the sludge. However, the nutrients were well balanced for biological stabilization (Table 3) with a COD:N:P ratio of 124:7:1. A chemical analysis of the sludge showed limited contamination by metals (Cd <0.5 mg/kg TS, Cr^{6+} <0.5 mg/kg TS, Cr 46 mg/kg TS, Hg <0.1 mg/kg TS, Ni 18 mg/kg TS, Pb 7 mg/kg TS, Cu 280 mg/kg TS, and Zn 97 mg/kg TS). Therefore, it is possible to apply the sludge on land as an amendment (Directive 86/278/CEE, Italian Decree 99/1992).

Table 3 Waste activated sludge and wine lees characteristics

Parameter	Unit	Waste Activated Sludge			Wine Lees		
		average	**St.dev**	**Range**	**average**	**St.dev**	**range**
TS	**gTS/kg$_{ww}$**	158.9	49.3	22.7-267.8	62.0	27.9	12.3 -120.0
VS	**gVS/kg$_{ww}$**	143.5	41.6	20.7 – 237.3	33.6	15.1	10.3 -73.0
VS/TS	**%**	88%	3	79- 93%	57%	13%	29 - 86%
COD	**mg/g TS**	868	69.4	749-1008	559	151	312 – 919
sCOD	**g/l**	nd	nd	nd	167	45	111 -204
TKN	**mg N/g TS**	52.7	16.3	14.5 -80.3	30.3	12.7	9.7 -68.7
Ammonium	**mg N NH_4^+/L**	nd	nd	nd	33.9	22.7	6.7 – 95.3
Total phosphorus	**mg P/g TS**	7.3	2.0	2.5 -10.7	6.2	2.9	2.6 - 14.3
Polyphenols	**mg GAE/L**	nd	nd	nd	1537	1189	260-3,980

nd: not determined

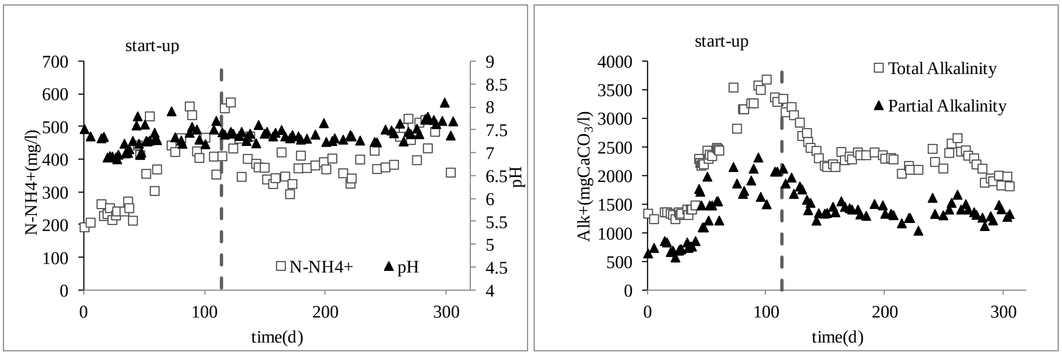
Wine lees were formed during the wine decanting step by adding bentonite (a fine clay used to remove suspended solids in the wine). Approximately 10 tons of lees were produced per week, corresponding to 1.6 kg/hl of wine produced. In this cellar, the typical wine lees production was lower than the reported average production from Italian wineries of 6 kg/hl (Laraia et al. 2001) because only part of the wine is processed within the cellar. The transformation into wine was not performed in the cellar considered in this work. Wine lees from both red and white wine processing were collected during experimentation to evaluate substrate variability and how it affects the process. Approximately 90% of wine lees samples had solid concentrations between 37.9 and 77.2 g TS/kg. However, extreme values were also detected (Table 3). Generally, these winery residues were characterized by a low content of volatile solids (57% of total solids) due to the presence of bentonite. For example, some samples of white WL had a low solid content and the VS/TS ratio was less than 50%. The COD was concentrated in the soluble form (sCOD was the 83% of total COD), whereas the particulate fraction was typically between 417 and 627 mg COD/g TS. The nitrogen and phosphorus levels were limiting factors for bacterial growth compared with the COD concentration. In fact, the COD:N:P ratio was 502:5:1, which is consistent with values reported by Moletta (2005). Polyphenols were also detected in samples. Polyphenolic compounds are a large and complex family of substances characterized by the presence of large, multiple phenol structural units (Battista et al., 2015). They are originally synthesized by plants as a defense against pathogens and are extracted from the grapes during the winemaking process. The concentration of polyphenolic compounds in WL varies greatly from 260 to 3,980 mg GAE/L.

Considering the typical concentrations of COD and nutrients in the waste activated sludge and wine lees, the anaerobic co-digestion of these two wastes may be considered an optimal option for their treatment. The process was tested at the pilot scale using continuous reactors.

3.3. Performance of the mesophilic anaerobic co-digestion process

The organic load was gradually increased during the start-up phase, from day 1 to day 114. In particular, a constant quantity of sludge was used (0.6 kg COD/m^{3}d), and the amount of wine lees fed to the reactor was increased gradually from 0 to 2.6 kg COD/m^{3}d. A prolonged start-up phase and progressive increase in the OLR promoted the biomass adaptation. Within this transient period, the stability parameters of the mesophilic reactor improved. The total alkalinity reached 3,690 mg $CaCO_3$/L because of the increase in ammonium, whereas the pH remained at 7.5. At the same time, the gas production rate rose from less than 0.1 m^3/m$^3_{reactor}$d to 1.2 m^3/m$^3_{reactor}$d.

At the end of the start-up phase, the organic loading rate was 3.2 kg COD/m^{3}d and HRT 23 d. These conditions were determined as optimal for the process. Once the operational conditions were stable, the alkalinity decreased until reaching a stable value of 2,248 mg$CaCO_3$/L. The pH did not change significantly and ranged between 7.2 and 7.8. The ammonium concentration was the most variable parameter (Fig. 2) because of high degree of variability in the substrate characteristics. The process stability was not affected by this fluctuation.



(a) (b)

Figure 2 Trend of pH and ammonium concentration (a), partial and total alkalinity (b)

Based on the stability parameters, the process did not exhibit any particular difficulties, and over a long period, the performance improved. The total solid and COD concentrations decreased with time to average values of 24 gTS/kg, 58% volatile, and 640 mg COD/g TS, respectively. The particulate COD removal was coupled with a slight increase in the soluble COD, which was lower than 1000 mg COD/L. The 40-50% of the soluble COD was due to the presence of volatile fatty acids, with acetic acid being the dominant volatile fatty acid (52% of the total VFAs) and propionic acid being the second most abundant (12% of the total VFAs).

The nutrient concentrations generally decreased until reaching steady values of 37 mgN/gTS and 9 mg P/gTS. The average COD:N:P ratio in the effluent digestate was 70:4:1. Thus, the solid digestate had a good fertilizer quality and a high phosphorus content.

The amount of polyphenolic compounds were in the range from 20 - 80 mg GAE/L during the start-up phase. Their concentration decreased to lower than 40 mg GAE/L during steady state conditions (Fig 3). The anaerobic microorganisms adapted to these compounds and were eventually able to degrade 94% of the influent polyphenols.

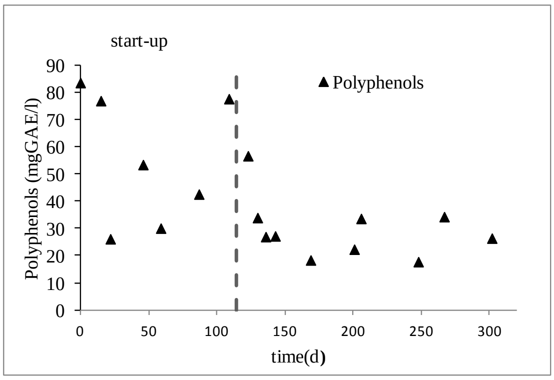


Figure 2 Trend of polyphenols concentration

Biogas production varied significantly depending on the characteristics of the wine lees in the influent. The average specific biogas production and gas production rates were 0.386 m^3/kg COD_{fed}, and 1.2 $m^3/m^3_{reactor}$ per day, respectively. Values as low as 0.30 m^3/kg COD_{fed} were also observed (Fig. 4), but they were not indicative of instability problems. The methane content ranged from 64 to 73% during the day due to degradation of the different types of compounds.

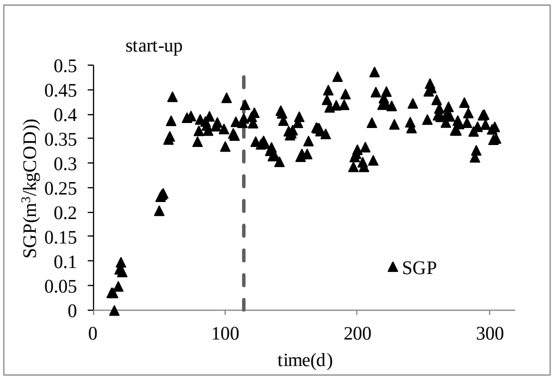


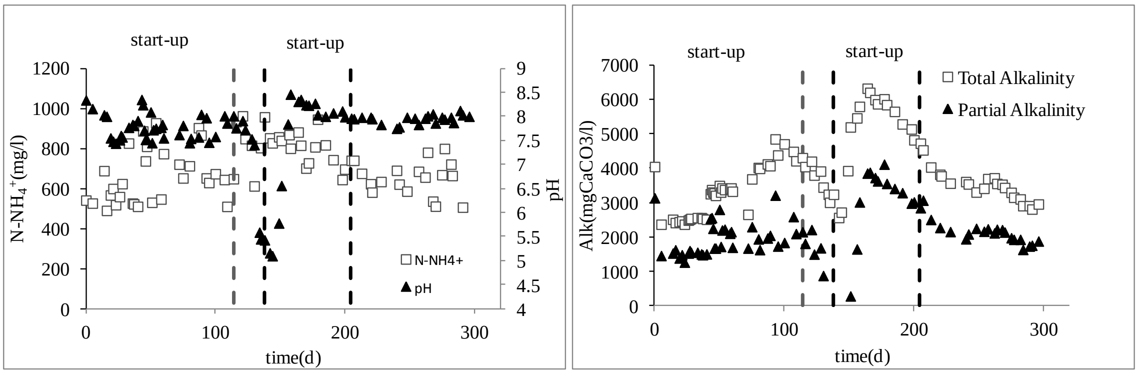
Figure 3 Trend of specific gas production

Based on the mass balances around the system, the solid conversion to biogas efficiency was 28%, whereas 79% of the COD in the influent was removed. Most of the biogas derived from the degradation of soluble COD (92%). This parameter was lower than 1 g/L in the effluent, whereas particulate substances were only partially degraded. Additionally, the ammonification of organic nitrogen, which represented approximately 99% of the total nitrogen in the influent, was limited by the hydrolysis process, and ammonium nitrogen in the effluent accounted for 32% of the total nitrogen. This value was consistent with protein degradation efficiencies after sludge anaerobic digestion (Bougrier et al., 2007; Pinnekamp, 1989; Yang et al., 2015). Digestate supernatant may be capable of acting as a source of nutrients for the wastewater treatment and to reduce the required urea and ammonium phosphate addition.

3.4. Performance of the thermophilic anaerobic codigestion process

For the thermophilic process, the start-up phase lasted 114 days, whereas the OLR was stepwise increased to obtain a final OLR of 3.2 kgCOD/m^{3}d and an HRT of 23 d. During the start-up phase, the pH stabilized above 7.5 due to the high buffer capacity in the inoculum (defined by the total alkalinity). However, at the end of this period, VFAs started to accumulate. In fact, at the end of the first HRT operating under constant condition VFAs reached a concentration of 6 gCOD/L, the pH fell down to 5 (Fig. 5a) and the specific biogas production decreased accordingly.

The different behaviors observed for mesophilic and thermophilic conditions could be due to the temperature effect on the microbial community (Cavinato et al., 2014; Yu et al., 2014).



(a) (b)

Fig. 4 Trend of pH and ammonium concentration (a), partial and total alkalinity (b)

Thermophilic bacteria are more susceptible to toxic compounds such as the polyphenols that accumulated in the reactor and reached 160 mg GAE/L (Figure 6). It is well documented that polyphenolic compounds have antioxidant, nutritional and anticancer properties. However, they are reported to be toxic to microorganisms because they inhibit microbial growth (Ramos-Cormenzana et al., 1996). Moreover, mesophilic and thermophilic bacteria have different polyphenol degrading capacities due to inactivation of the enzyme involved in the degradation pathway above 48°C (Levén and Schnürer, 2005). The first signal of instability is the polyphenol accumulation, followed by the VFAs concentration increase. It is not clear whether the high content of polyphenols caused the inhibition of methanogenesis or whether other inhibitors affected both the polyphenol and VFA degradation.

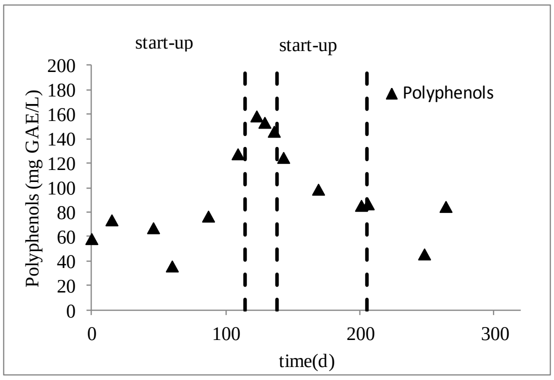


Figure 5 Trend of polyphenols concentration

VFAs in the bulk were composed mainly of 78% acetic and 10% propionic acids, whereas longer fatty acids accounted for less than 15%. This distribution indicated that the activity of the acetate-consuming microorganisms (acetoclastic methanogenesis or syntrophic acetate oxidizing microorganisms) was the rate-limiting step (Drake et al., 2013; Florencio et al., 1994; Nordell et al., 2015).

As reported in previous studies (Takashima et al., 2011; Uemura, 2010), elevated concentrations of VFAs, particularly propionate, at the thermophilic temperature could be caused by trace nutrients deficiencies. Trace elements additions affected the sulfide concentration and promoted the precipitation of insoluble metal sulfides and decreased the H_2S toxicity. At high doses, the trace elements could be more available for microorganisms and support the activity of fundamental enzymes (Demirel and Scherer, 2011; Jansen et al., 2002). Several authors (Moestedt et al., 2015; Nordell et al., 2015) have demonstrated the positive effect that the combined supplementation of Fe, Co and Ni when added into an anaerobic digester and have suggested different doses (Facchin et al., 2013).

To recover the biological process, trace metals were supplemented, and the pH adjusted by the addition of lime. Feeding was then stopped. From day 170, a new start-up phase was implemented with the addition of a metal solution (Demirel and Scherer, 2011). Metals were provided to the feed to obtain concentrations of 4.3 mg Fe-$FeCl_3$/L, 0.46 mg Ni- $NiCl_2$ $6H_2O$/L and 0.51 mg Co- $CoCl_2$ $6H_2O$/L in the feed, as suggested by Takashima et al.(2011).

A short start-up was conducted, and the process reached conditions similar to the period prior to failure event. The ammonium concentration stabilized approximately 630 mgN-NH_4^+/L, the alkalinity decreased to 3,360 mg$CaCO_3$/L, and pH ranged from 7.8 and 8.0 (Fig. 6). For these operational conditions, the effluent solid concentration slightly decreased to 21.3 g TS/kg and 57% volatile solids, whereas the particulate COD remained constant at 613 mg/g TS. The average concentration of soluble COD was 852 mgCOD/L, which contained approximately 30% VFAs. This value was likely due to the high hydrolysis rate at 55°C and not from the accumulation of intermediate metabolites.

The concentration of nutrients in the solid fraction was stable and the COD:N:P ratio was 60:3:1, indicating elevated biological stability.

The average polyphenol content reduced from 160 to 66 mg GAE/L (Fig. 6) because the removal efficiency increased from 67% to 78%. Degradation efficiencies observed during the entire experimental process were higher than those reported by Cavinato et al. (2014)at 55°C. Moreover, trace nutrient augmentation improved the microbial activity and allowed for better polyphenolic compound degradation.

The biogas production rate increased from 0.5 $m^3/m^3_{reactor}d$, at the beginning of second start-up, to 1.3 $m^3/m^3_{reactor}d$ during steady state. The average specific gas production for these conditions was 0.450 m^3/kg COD (Fig. 7) with 62% methane. Accordingly, with the increase in biogas production, solids and COD removal efficiencies increased to 34% and 88%, respectively. The high hydrolysis rate in the thermophilic range caused better solid reduction and organic matter stabilization.

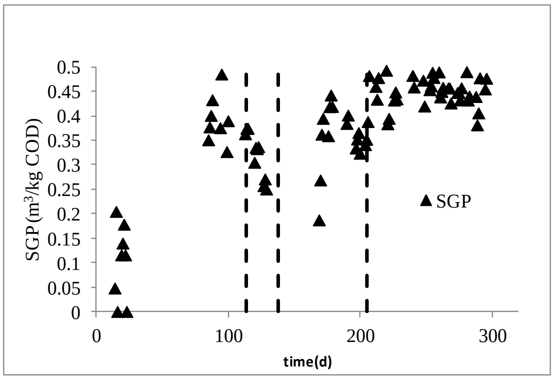


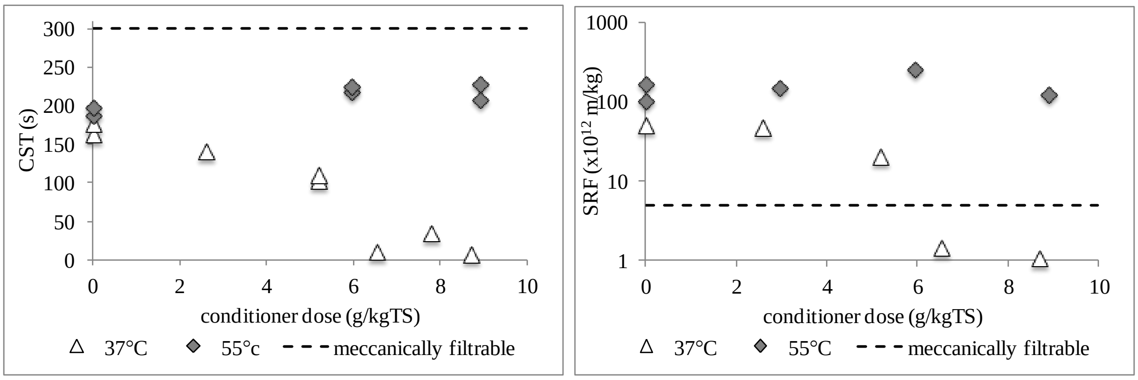
Figure 6 Trend specific gas production

3.5. Dewatering proprieties

Effluents from anaerobic digestion are usually dewatered to separate the liquid and solid fractions of the digestate for storage, transportation, post-treatment, and other purposes. Effective dewatering can significantly reduce the volume of digestate and the cost of further processing (Lü et al., 2015). The dewatering properties of digestate were determined by two indicators: the capillary suction time (CST) and the specific resistance to filtration (SRF). The tests were conducted on mesophilic and thermophilic digestates at steady state and for different doses of chemical conditioners (Tillflock 6480 –Tillmanns) to choose the optimal one for each digestate.

The mesophilic and thermophilic raw digestates had CST values of 171 and 193 s, whereas the SRF were 5.1 x 10^{13} and 1.3 x 10^{14}, respectively. The CST values were significantly lower than those reported by Da Ros et al. (2014), likely due to the presence of bentonite in the winery wastes (lees). Generally, the CST was strongly affected by the free water content in the sludge and, in this case, the bentonite improved the release of water on the filter paper (Jin et al., 2004). In fact, bentonite is a mineral conditioner that, thanks to its surface charges, reacts with suspended organic matter and releases the bound water (Alvarenga et al., 2015). However, the SRF values were consistent with the literature data (Schafer, 2001), which described slightly worse dewaterability at 55°C.

The application of a chemical conditioner to the mesophilic digestate improved the dewatering capability (Fig. 8). The SRF reached a value of 1.5 x 10^{12} with the addition of 6.5 g of chemical conditioner per kg of dry matter, indicating that the digestate can be dewatered mechanically. However, the thermophilic effluent required dosages higher than 10 g/kgTS. In the same way, the CST of the mesophilic digestate decreased to less than 10 s, whereas the CST of the thermophilic digestate possessed values greater than 200 s.



(a) (b)

Figure 7 Trends of CST (a) and SRF (b) increasing chemical conditioner dose

As reported by Alvarenga et al. (2015), the presence of a porous conditioner such as bentonite in the sludge can reduce the use of flocculants. In fact, the dosage used for mesophilic digestates was similar to the typical dosage for waste activated sludge dewatering.

3.6. Comparison of the performances of the mesophilic and thermophilic processes

One of the main advantages of using thermophilic conditions was a higher waste stream reduction due to the improvement of the hydrolysis rate. In fact, the solid concentration in the reactor reduced from 24.3 to 21.3 g TS/kg when the temperature increased from 37°C to 55°C. In agreement with the volatile solids and COD removal, the biogas production at 55°C improved by 18% compared with the mesophilic process by applying the same operational conditions. Instead, the biological stabilization of the substrates appeared similar in the two reactors, with a VS/TS ratio of 57-58% and a particulate COD concentration of 613-640 mg/g TS (Table 4). The hydrolysis also affected the ammonification, resulting in a higher ammonium concentration at 55°C (630 mg N-NH_4^+/L) than at 37°C (400 mg N-NH_4^+/L). In both reactors, the free ammonia content was far lower than the inhibiting level (Gallert and Winter, 1997) because of the low nitrogen concentration in the inlet mixture. The solid fraction of the digestates appeared to be an interesting fertilizer for its nitrogen and phosphorus level, in particular, the COD:N:P was 70:4:1 and 60:3:1 at 37°C and 55°C, respectively. The thermophilic process improved the effluent quality also in terms of pathogen removal. Polyphenols represented an important parameter for the reuse of the digestate because high concentrations of these compounds could reduce biological activity in the soils, affecting the fertility (Mosse et al., 2012). The process conducted at 37°C was more efficient at polyphenol removal and resulted in a lower content in the downstream flow (Table 4).

Table 4 Characteristics of mesophilic and thermophilic digestates, average values and standard deviations

Parameter	Unit	37°C		55°C (with metals)	
		Average	St.dev.	Average	St.dev.
pH	-	7.46	0.19	7.91	0.09
Partial alkalinity	mg $CaCO_3$/L	1,375	126	2,043	134
Total alkalinity	mg $CaCO_3$/L	2,248	200	3,390	193
$N\text{-}NH_4^+$	mg $N\text{-}NH_4^+$/L	400	56	630	73
TS	gTS/kg_{ww}	24.3	2.9	21.3	1.7
VS	gVS/kg_{ww}	14.2	1.7	12.1	1.5
VS/TS	%	58	4	57	7
COD	mg COD/gTS	640	46	613	34
sCOD	mg COD/L	360	152	852	223
TKN	mg N/gTS	36.3	4.5	32.8	5.4
P_{tot}	mg P gTS	8.8	1.6	10.2	1.3
Polyphenols	mg GAE/L	26	7	66	28
SGP	m^3/kgCOD	0.386	0.049	0.454	0.030
COD removal	%	76%		88%	

To evaluate the best operational condition, the characteristics of digestates are not the only aspect to consider. The feasibility of the process should also be analyzed. The thermophilic process with metals augmentation had a higher buffer capacity, as defined by the total alkalinity (3,560 mg$CaCO_3$/L) and, although the volatile fatty acid concentration in the bulk was higher, the pH remained between 7.8 and 8.0. In addition to the better efficiency for thermophilic anaerobic digestion, this operating temperature results in higher management costs due to the metal addition requirement and the greater energy required to maintain the reactor temperature. In fact, the cost of metal addition should be higher than 0.70 € per cubic meter of mixture fed into the digester. Consequently, this significantly reduces the economical sustainability of the process. Moreover, other inexpensive metals sources can be found in the surroundings: sewage sludge from municipal wastewater or livestock effluents are widely available co-substrates that can be co-treated to improve micronutrients content.

Both the processes could generate energy, which can be used inside the wastewater treatment plant, and the supernatant obtained by the dewatering of the digestates should have high concentrations of ammonium and orthophosphate. Often, management of digestate liquid fraction is considered a cost because it needs a specific reactor for nitrogen removal/recovery or it leads to an increased nitrogen load in the wastewater treatment plant. For a winery, recirculation of the supernatant in the wastewater line may represent a way to reduce management costs, as it could limit the need for chemical nutrient additions in the aerobic bioreactor.

4. Conclusions

The cellar monitored in this work, which produced approximately 300,000 hectoliters of wine per year, generated 196 L of wastewater, 0.1 kg of sludge (dry matter) and 1.6 kg of lees per hl of wine produced. Anaerobic co-digestion of wastewater sludge and lees was feasible, both in mesophilic and thermophilic conditions, when operating with an OLR of 3.2 kg COD/m^3d and an HRT of 23 d. The mesophilic process was stable over a long period in terms of the stability parameters (pH 7.46, 400 mg $N\text{-}NH_4^+$/L and 2,248 mg $CaCO_3$/L) and biogas production (0.386 m^3/kgCOD). The thermophilic digestion process accumulated VFAs, and after one HRT, the process failed. Metal augmentation (Fe, Co and Ni) improved the stability and biogas yields

(0.450 m^3/kgCOD) at 55°C. Solid removal increased by 18% compared with the mesophilic process and ammonification resulted in a higher ammonium concentration in the thermophilic digestate (630 mg $N\text{-}NH_4^+$/L). The dewaterability properties of the mesophilic process appeared to be better. A total of 6.5 g conditioner/kgTS was sufficient for mechanical dewatering of the digestate, whereas the thermophilic digestate dewaterability did not change over the dosage range of 0 to 10 g polymer/kg TS. Considering management costs, thermophilic conditions are economically competitive only if other co-substrates, derived from surrounding activities, are co-treated to supply the missing micronutrients.

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