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OPTIMIZATION OF THERMOPHILIC ANAEROBIC DIGESTION OF WINERY BIO-WASTE BY MICRO-NUTRIENTS AUGMENTATION

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Abstract

Thermophilic anaerobic digestion is a suitable technology to treat agricultural waste because of its higher biogas production, hygienisation effect and solids removal efficiency. Although these benefits, poor effluent quality and instability are encountered in some cases. The anaerobic co-digestion of winery waste and waste activated sludge at 55°C, operating at 23 days HRT and with an organic loading rate of 3.2 kg COD/m^3d, was characterized by accumulation of volatile fatty acids, pH fall and reduction of biogas production, while mesophilic process was steady at long term. The study evaluated the effect of trace elements (iron, cobalt and nickel) augmentation in the thermophilic reactor at different concentration of micro-nutrients. The addition improved the process stability: pH became constant and average volatile fatty acids concentration was below 1,000 mg COD/L. The biogas production increased from 0.38 to 0.45 m^3/kg COD, corresponding to 90% of COD removal, while mesophilic reactor removed the 78% of total COD. Digestate had interesting characteristics as fertilizer in fact the higher solids removal (28%) allowed to concentrate the phosphorus in the particulate fraction and nitrogen was transformed into more available form for plants growth.

Key words: mesophilic anaerobic digestion, micro-nutrients, minimum requirement, thermophilic anaerobic digestion, winery waste

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1. Introduction

Agro-industrial activities represent important sources of environmental impact because fruit and vegetable processing generates high amount of organic wastes. Among these bio-wastes, the winery residues have great relevance in terms of quantity and of potential pollution. In fact their uncontrolled field spreading could cause relevant environmental problems due to their high biodegradability, expressed by high level of chemical oxygen demand (COD). The raw wastes disposal on soils can consume large quantity of oxygen consequently they create anoxic conditions, reducing soil fertility. Moreover winery residues content antibacterial compounds produced by plants such as polyphenolic compounds, and toxic compounds like pesticides and heavy metals that can reduce crops growth (Moldes et al., 2008).

For these reasons winery wastes need a treatment before the disposal, the most diffused process is composting but it is economically and energetically expensive. Considering the costs of the composting and of disposal, the production companies are seeking alternative biological treatments with a low environmental impact and economically sustainable for winery residues valorisation, consisting in the recovery or transformation of the present components into high value-added resources.

Anaerobic digestion represents a suitable technology to reduce pollutant load of winery residues and to produce biogas, a renewable energy source. This technology is commercially proven and

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is widely used for treating high moisture content organic wastes (Ciubota-Rosie et al., 2008).

Mesophilic processes, with a working temperature of 35-37°C, are the most applied conditions but the interest for thermophilic condition (55°C) is increasing for its several advantages. Kafle et al. (2014) demonstrated, in terms of digestate characteristics and kinetic constant, that VS removal was significantly higher under thermophilic temperature conditions than under mesophilic temperature conditions. Since reaction rates increase with temperature, significantly higher organic loads and considerably shorter hydrolysis retention times are expected at 55°C (Van Lier et al., 1997). Therefore, a smaller reactor volume will be sufficient at thermophilic temperatures compared to mesophilic conditions (Lens and Verstraete, 2001). Usually higher temperature improves solid and organic matter removals due to faster hydrolysis rates, and consequently biogas productions increased and effluent stream quantity reduced. Pathogens removal is guaranteed by maintaining 55°C for few days (Sahlström et al., 2004) and also the phytotoxicity of thermophilic digestate seems lower than mesophilic one (Vallini et al., 1993).

Although these benefits few full scale plants operate in thermophilic range, this is due to the higher heating costs and less stable operation (De Baere, 2000). The causes of instability are several: with regard to manure digestion, high ammonia concentrations may limit the thermophilic anaerobic treatability, owing to toxicity problems (Angelidaki and Ahring, 1993; Zeeman et al., 1985). As the free ammonia fraction increases with temperature and pH, the ammonia concentration tolerated at high pH and at thermophilic temperatures would be expected to be low (Ahring, 1994). Another drawback is the often-found high effluent volatile fatty acids concentration but the etiology is not clear. Variation of temperature determined a change in methanogenic population (Van Lier et al., 1992) explained by a rapid die-off of mesophilic organisms at temperature exceeding the maximum growth temperature of these bacteria. Thermophilic anaerobic population is composed by a lower numbers of species and probably has different nutrients requirements.

The treatment of industrial substrates emphasizes these aspects because usually these wastes have low concentration of micro-nutrients and an inadequate amounts of bio-available trace elements, metals in particular (Rittmann and McCarty, 2001; Speece, 1983; Zandvoort et al., 2006). In fact some metals are involved into biochemistry of anaerobic microorganisms and play important roles as co-factors of various enzymes involved in anaerobic reactions and transformations as reported by Fermoso et al. (2009).

Depending on the pathway, metal requirements may differ, but the general trends remain the same: Fe is the most abundant metal, followed by Ni and Co, and smaller amounts of Mo (and/or W) and Zn. The function of these metals on the growth and metabolism of anaerobic bacteria is well documented in the literature (Agler et al., 2008; Fermoso et al., 2009; Oleszkiewicz et al., 1990) but the effect of missing nutrients occasionally could be not evident. In fact the anaerobic microorganisms respond quite slowly and have long lag phase time after the supplementation of micronutrients, therefore batch tests results usually are not sufficient to understand the real nutrients necessity.

The experiments have to be carried out in continuous mode for quite long period as made by Takashima et al. (2011). That work reported a complete study on continuous reactor working at 55-57°C with metals supply and demonstrated that trace elements requirements in thermophilic digestion is greater than in mesophilic one, implying more requirements for biomass growth and activity and/or less bioavailability of those trace metals at higher temperature. The experiment was carried out using a solution of sole glucose as feed, then the effect of the substrate was not considered in that study. In general the variations in optimal concentration of trace elements at mesophilic and thermophilic temperature ranges are explained by the variety of methanogens, each having a unique trace metal requirement, which also depends on the type of substrate utilized (Paulo et al., 2004). For example Qiang et al. (2013) determined the metals requirements in thermophilic digestion of solid food waste and the values were lower than ones reported by Takashima et al. (2011).

In present study the anaerobic digestion of winery wastes, originated from wine making process, was carried out in thermophilic condition with different supplementation of trace elements. In order to evaluate the process, the stability parameters and the specific biogas production were compared with the same parameters of stable mesophilic reactor working with the same operational conditions but without necessity of metals supply. Two parallel experiments, with different temperature conditions, were carried out in order to verify that the operational conditions were not extreme and that inhibition of thermophilic reactor was not due to overloading of the reactor.

2. Materials and methods

2.1. Analytical methods

Substrates and effluents were monitored once a week in terms of total and volatile solids content (TS and VS), COD on particulate and soluble fraction (pCOD and sCOD respectively), total Kjeldahl nitrogen (TKN) and total phosphorus (P_{tot}). The process stability parameters, namely pH, volatile fatty acids (VFAs) content and speciation, total and partial alkalinity (TA and PA) and ammonium ion, were checked twice a week. All the analyses, except for VFAs, were carried out in accordance with the Standard Methods (APHA–AWWA–WEF, 2011). 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. The concentration of total polyphenols was measured using a modified version of the Folin–Ciocalteu reaction as reported in Laftka et al. (2007) and converted into Gallic acid equivalent (mg HGal/L).

Gas productions were monitored continuously by two gas flow meters (Ritter Company, drum-type wet-test volumetric gas meters), and their composition (CH_4, CO_2 and O_2) were monitored by portable biogas analyzer (Geotechnical Instruments, GA 2000). This instrument was calibrated using air with content of oxygen of 21%, and a certificated mixture of CH_4 and CO_2 (60% and 40% respectively).

2.2. Experimental setup

The experimental trial was carried out by two identical continuous stirred pilot-scale bioreactors each with working volume of 230 L. The reactors included a water jacket connected to a heating recirculation system to maintain a constant temperature of 37°C and 55°C. The digesters were fed once a day with a mixture of waste activated sludge and wine lee to reach HRT of 23 d and organic loading rate (OLR) of 3.2 kg COD/m^{3}d. The organic loading rate was chosen considering the results obtained by Da Ros et al. (2014), that demonstrate this OLR could inhibit the thermophilic process. The composition of feeding mixture derived from the real availability of winery wastes in a cellar able to receive about 300 thousand liters of wine per year: the mixture was composed by 0.6 kg COD/m^{3}d of waste activated sludge (WAS), and the remaining fraction (2.6 kg COD/m^{3}d) of wine lees (WL). Both the reactors started without metal elements addition, but after the failure of thermophilic process, a solution of Iron, Cobalt and Nickel was added to the reactor working at 55°C to reach the concentration suggested by Takashima et al. (2011) of 4.3 mg Fe-$FeCl_3$/L, 0.46 mg Ni-$NiCl_2$ $6H_2O$/L and 0.51 mg Co-$CoCl_2$ $6H_2O$. In order to evaluate the best concentration of metals, the doses were lowered step-by-step and maintained for at least a HRT for each dosage. In Table 1 tested doses in thermophilic reactor were reported, while the stable mesophilic system worked without metals addition and was used as control.

2.3. Inoculum and substrates characteristics

The reactors were initially filled-up with mesophilic and thermophilic digestates deriving from previous experimentation. The inocula were well stabilized, solids content was lower than 10 gTS/kg and stability parameters were in the optimum ranges of anaerobic digestion (Table 2).

Biowastes from wine-making process were used as feeding in this experimentation because of their low concentration of trace elements. Waste activated sludge (WAS) derived from a wastewater treatment plant working mainly with winery wastewater; the plant treated about 170 m^3/d of wastewater with average concentration of 3,747 mg COD/L. The treatment process was characterized by food to microorganisms ratio of 0.26 g COD/g MLVSS and long sludge retention time (35 d).

The WAS had high VS/TS ratio (88%) probably due to characteristics of raw wastewater, and well balanced nutrients ratio for biological treatment (Table 3). The wine lees were collected in same cellar that produced the WAS and it was formed by wine decanting after addition of bentonite.

Table 1. Tested metals doses in thermophilic reactor

	RUN 0	*RUN 1*	*RUN 2*	*RUN 3*	*RUN 4*
Fe (mg/L)	0	4.3	3.01	2.15	0.86
Ni (mg/L)	0	0.46	0.32	0.23	0.09
Co (mg/L)	0	0.51	0.36	0.25	0.10

Table 2. Mesophilic and thermophilic seed digestates characteristics (TS: total solids, VS: volatile solids on wet and dry weight, pCOD: COD on particulate fraction, sCOD: COD on soluble fraction, pH, TKN: total Kjeldahl nitrogen on particulate fraction, NH_4^+: ammonium concentration in soluble fraction; P_{tot}: total phosphorus, Polyphenols)

Parameter	*Unit*	*37°C*	*55°C*
TS	gTS/kg$_{ww}$	8.84	9.37
VS	gVS/kg$_{ww}$	5.92	4.69
VS	% TS	67%	50%
pCOD	mg/g TS	552	751
sCOD	g/L	910.7	1072.5
pH	-	7.53	8.33
TKN	mg N-NH_4^+/g TS	41.63	33.09
NH_4^+	mg N NH_4^+/L	193.4	539.4
P_{tot}	mg P-PO_4^{3-}/g TS	47.0	26.8
Polyphenols	mg HGal/L	83.75	58.35

The presence of this inert material determined low content of volatile solids (57% of total solids) and the COD was concentrated in the soluble form (sCOD was the 83% of total COD). The levels of nitrogen and phosphorus were limiting for bacterial growth if compared with pCOD concentration. Considering WL (Table 3), variability ranges of total and volatile solids were larger than ones of WAS, because of the variability of produced wine. Both substrates were poor in micro-nutrients because of their origin.

3. Results and discussion

The initial start-up period, in both reactors, consisted with stepwise increases of organic load maintaining constant the contribution of WAS (0.6 kg COD/m^3d) and increasing the amount of WL. In the same time the HRT was lowered from 46 d to 23d. During this period the specific gas production increased agree with OLR (Fig. 1), in fact supplied sCOD from WL, which was easily biodegradable COD, was completely converted to biogas. This transient period lasted 114 days.

3.1. Comparison of mesophilic and thermophilic processes without trace-elements

Mesophilic process reached steady state after two HRTs at constant conditions and was characterized by good stability parameters for the monitored period (9 HRTs). In particular pH ranged from 7.2 to 8.1, the soluble COD concentration was around 360 mg/L with less than half due to VFAs, and the ammonium content was about 400 mg/L. The process guaranteed the complete soluble COD removal but only a part of the particulate COD was converted into biogas. The 81% of COD was converted into biogas while solids were reduced of 19%. The average biogas production was 0.386 m^3/kgCOD$_{fed}$ with 78% of methane.

On the other hand thermophilic process did not show instability problems during the start-up period but started to accumulate VFAs after reaching the fixed conditions. In particular the VFAs concentration ranged from 476 mgCOD/L, at the end of start-up, to 6,825 mgCOD/L after 23 days of regular feeding.

The dominant volatile fatty acids were acetic and propionic acids, corresponding to the 66 and 15% of total COD, respectively. Consequently partial alkalinity was consumed, pH dropped down to 5 and methanogenesis was totally inhibited. The biogas production was reduced in this period from 0.39 to 0.25 m^3/kgCOD$_{fed}$, and later stopped.

Fig. 1 highlights the process instability in RUN0, detected by VFAs increase, and consequently specific biogas production (SGP) reduction. After feed suspension the hydrolysis of organic matter continued, in fact the VFAs concentration increased and degradation of proteins enhanced ammonium content.

The possible inhibitors were examined (free ammonia, polyphenols, sulphide). The free ammonia (FA) at 55°C was about 177 mg N-NH_3/L and concentrations below 200 mg N-NH_3/L are generally believed beneficial to anaerobic process since nitrogen is an essential nutrient for anaerobic microorganisms (Liu and Sung, 2002). Polyphenols were present in the winery waste at concentration of 1,496 mg HGal/L and, although their degradation was more difficult at thermophilic temperature than at mesophilic one (Levén and Schnürer, 2005), the measured concentration in the thermophilic effluent was 152.8 mg HGal/L, far lower than inhibiting level (Melamane et al., 2007). Utilization of $CuSO_4$ and SO_2 during winemaking process can cause high concentration of sulphates in WL. During the anaerobic digestion the sulphates were reduced to sulphides and H_2S was formed.

The H_2S is the most toxic sulphide form for the microorganisms involved into methanisation, and the inhibiting concentration range was 50–400 mg H_2S /L (Parkin et al., 1990). The content of H_2S was monitored in the biogas of both the reactors and resulted similar at different temperature (800 ppm). Considering the Henry's law the concentration in liquid phase of H_2S was lower at 55°C than at 37°C, and mesophilic reactor did not show inhibition effects. Moreover the pH > 7 determined the dominance of HS^- specie, less toxic than unionized sulphide.

Table 3. Waste activated sludge and wine lees characteristics (TS: total solids, VS: volatile solids on wet and dry weight, pCOD: COD on particulate fraction, sCOD: COD on soluble fraction, pH, TKN: total Kjeldahl nitrogen on particulate fraction, NH_4^+: ammonium concentration in soluble fraction; P_{tot}: total phosphorus)

Parameter	***Unit***	***Waste Activated Sludge***				***Wine Lees***			
		average	CV %	min	max	average	CV %	min	max
TS	gTS/kg$_{ww}$	158.9	31%	22.7	267.8	62.0	45%	12.3	120.0
VS	gVS/kg$_{ww}$	143.5	29%	20.7	237.3	33.6	45%	10.3	73.0
VS/TS	%	88%	3%	79%	93%	57%	23%	29%	86%
pCOD	mg/g TS	868	8%	749	1008	559	27%	312	919
sCOD	g/L	-				167	27%	111	204
TKN	mg N-NH_4^+/g TS	52.7	31%	14.5	80.3	30.3	42%	9.7	68.7
NH_4^+	mg N NH_4^+/L	-				33.9	67%	6.7	95.3
P_{tot}	mg P-PO_4^{3-}/g TS	7.3	27%	2.5	10.7	6.2	46%	2.6	14.3

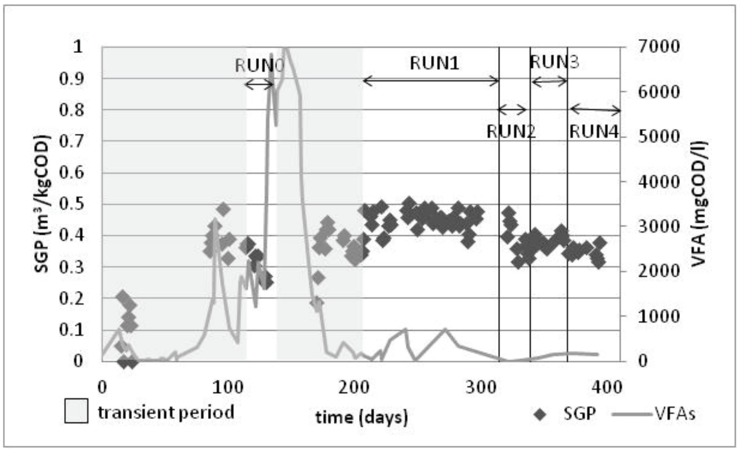

Fig. 1. Trend of biogas production in thermophilic reactor, comparison with mesophilic process (continuous line)

3.2. Thermophilic anaerobic digestion with trace-elements augmentation

High concentrations of VFAs and mainly of propionate were indicative of less bioavailability of trace elements at thermophilic operating temperature as suggested by Takashima et al. (2011). In order to verify the effect of trace elements supply, the thermophilic reactor was recovered and the second start-up was designed with trace elements addition to obtain concentration of 4.3 mg Fe /L, 0.46 mg Ni/L and 0.51 mg Co/L. The same metals augmentation was tested in the feed during RUN1 from day 205. The thermophilic process with these dosages was carried out for 4 HRT and it appeared steady in terms of stability parameters, effluents characteristics and biogas production. The pH ranged from 7.7 to 8.0, average ammonium concentration was 664 mg N-NH_4^+/L and the corresponding free ammonia value was 158 mg N-NH_3/L. Comparing VFAs in RUN0 and RUN1 (Fig. 1), it is clear the VFA concentration reduction and the corresponding increase of biogas production to 0.45 m^3/kgCOD_{fed} with 77% of methane. On the other hand, comparison of mesophilic and thermophilic process (RUN1) showed an improved biogas production of 18% and increase of solid removal from 17% to 28%. In fact the average solid concentration was 20.6 g TS/kg_{ww} because of greater hydrolysis of particulate matter at this temperature. The higher hydrolysis rate explained also the value of sCOD (995 mg COD/L) that was higher than in mesophilic effluent. Just the 30% of soluble COD was due to VFA, but they did not accumulate and the buffer capacity (total alkalinity 3,390 mg $CaCO_3$/L) was enough to maintain optimum pH value for anaerobic digestion.

The addition of trace elements also improved polyphenols degradation, in fact the concentration has been reduced to 66 mg HGal/L, slightly higher than in mesophilic effluents. The difference in degradation efficiencies between the operational temperatures were due to presence of the different microbial populations in the two environments and to partial inactivation of enzyme involved into phthalate-degrading pathways at 55°C (Levén and Schnürer, 2005).

In order to evaluate the best dosage, the amount of added metals were reduced to 70% of initial quantities (3.01 mgFe/L, 0.32 mgNi/L and 0.36 mgCo/L) in the RUN2. The stability parameters remained in the suggested range for anaerobic digestion during whole HRT. The monitoring results showed a slightly reduction of ammonium concentration (590 mg/L), alkalinity and pH, also the solid concentration was lower. These changes were not due to process but to wine lees variability. In fact the WL at the beginning of this period had low solid concentration (20.6 mg/g) and consequently the inlet nitrogen into reactor has been reduced. The results showed that the process could support nitrogen load variation and macro-nutrients were not limiting. During RUN2 the biogas production reduced of 14% and reached values equal of mesophilic process (0.39 m^3/kgCOD) with 71% of methane. Also the COD removal (73%) was quite similar to process at 37°C. Beside the reduction of biogas production the process remained stable and metabolites remains at low concentration, in fact average soluble COD was 740 mg/L. Instead, in terms of energetic and economical balance, thermophilic process became not advantageous compared with mesophilic one, even considering the major cost of metals supplementation.

In RUN3 metals addition had been reduced again to 50% of initial dose (2.15 mgFe/L, 0.23 mgNi/L and 0.25 mgCo/L) and the process did not change significantly its performances if compared with RUN2. The soluble fraction in the effluent remained below than 900 mgCOD/L, pH values ranged from 7.6 to 8 and ammonium stabilized around 650 mgN/L, corresponding to about 150 mgN/L of free ammonia. Total alkalinity was strongly affected by ammonium and increased from 2,440 mg$CaCO_3$/L, of previous condition, to about 3,160 mg$CaCO_3$/L in RUN3. The biogas production slightly reduced to 0.381 m^3/kgCOD with 69% of methane. Considering these results, significant differences between RUN2 and RUN3 were not detected, but anaerobic digestion is really complex process and is affected by many factors such as variability of substrates.

Finally trials with 20% of initial dose were carried out (RUN4) and the main variation was in terms of biogas production that reduced to 0.347 m^3/kgCOD, but the methane percentage remained good (70%). Although the decrease in biogas production was a sign of instability, the other parameters were consistent with anaerobic digestion range. pH was 7.9, total alkalinity stayed around 3,023 mg$CaCO_3$/L and ammonium concentration was 644 mg/L. Soluble COD was similar to the value obtained in RUN3 and indicated that methanogenic bacteria were not inhibited but probably the biological activity slowed with low metals addition. Considering performances of this condition the failure of process was expected with further reduction in metals dose.

3.3. Comparison of anaerobic digestion operational conditions

Monitored parameters of all tested conditions were reported in Table 4. Thermophilic process with metals augmentation had suitable stability parameters for anaerobic digestion without any significant differences among the tested dosages. Comparing with mesophilic process, ammonium concentration was higher, because of greater hydrolysis rate, and the free ammonia was one magnitude order different. Ammonium and volatile fatty acid concentration determined values of alkalinity greater than 3,000 mg $CaCO_3$/L and pH around of 7.9. The degradation of solid particles also affected the solid content in the digestates, which reduced at least of 10% respect to mesophilic effluent, and the nitrogen distribution. In fact the nitrogen is for 39-49% in soluble form at 55°C, while at 37°C less than 30% was ammonium nitrogen.

Greater solids removal efficiency also determined concentration of phosphorus into digestate, in fact the content in thermophilic effluent was always higher than in mesophilic one. The nutrients concentration in thermophilic digestate became it more interesting in terms of fertilization capacity.

Comparing metals requirement obtained by linear correlation between removed COD and metals addition, reported in Fig. 2 (0.352 $mgFe_{added}/gCOD_{rem}$, 0.042 $mgCo_{added}/gCOD_{rem}$ and 0.038 $mgNi_{added}/gCOD_{rem}$), with those reported by Takashima et al. (2011), they were in the same magnitude order but slightly lower probably because the metals content of the substrates increased the available metals concentration in the reactor feed.

The addition of metals, also in low concentration, allowed better degradation of polyphenols probably because trace elements were involved into polyphenolic degradation pathway. The process yields were the most interesting results of this study, the specific gas production and COD removed at 55°C went over mesophilic yields only with maximum tested dose, while in the other cases the productions were comparable or minor. Relationships between metals addition and COD removal were showed in Fig. 2. Considering the trials with lower additions of metals (RUN2, RUN3 and RUN4), micro-nutrients augmentation was well correlated with COD removal (R^2 99%), while in RUN1 the COD removal was higher than expected value from linear correlation.

Table 4. Comparison of stability parameters, digestate characteristics and yield at different operational conditions (pH, PA: partial alkalinity, TA: total alkalinity, NH_4^+: ammonium concentration in soluble fraction, FA: free ammonia, TS: total solids, VS: volatile solids on wet and dry weight, VS/TS percentage, pCOD: COD on particulate fraction, sCOD: COD on soluble fraction, TKN: total Kjeldahl nitrogen on particulate fraction, P_{tot}: total phosphorus, Polyphenols, SGP: specific gas prouction, percentage of CH_4, COD removal)

Parameter	***Unit***	***37°C***	***55°C***				
			RUN0	***RUN1***	***RUN2***	***RUN3***	***RUN4***
Stability parameters							
pH	-	7.38	6.7	7.91	7.78	7.82	7.9
PA	mg $CaCO_3$/L	1370	1688	2043	1678	1941	1944
TA	mg $CaCO_3$/L	2287	3673	3390	2439	3062	3023
$N\text{-}NH_4^+$	mg $N\text{-}NH_4^+$/L	373	820	630	455	665	644
FA	mg $N\text{-}NH_3$/L	11.8	110	154	90	145	155
Digestate characteristics							
TS	gTS/kg_{ww}	24.7	31.9	20.6	19.8	22.1	20.3
VS	gVS/kg_{ww}	14.3	19.5	12.1	13.3	11.6	11.5
VS/TS	%	58	61	59	67	52	61
pCOD	mg COD/gTS	614	671	615	680	602	556
sCOD	mg COD/L	391	5394	995	740	870	882
TKN	mg $N\text{-}NH_4^+$/gTS	37.9	40.4	33.1	35.9	37.6	33.1
P_{tot}	mg $P\text{-}PO_4^{3-}$/gTS	8	11.1	10.6	11.3	9.8	8.5
Polyphenols	mg HGal/L	26	153	66	61	57	-
Yields							
SGP	m^3/kgCOD	0.386	0.390	0.450	0.386	0.381	0.347
CH_4	%	78%	72%	77%	71%	69%	70%
COD removal	%	79%	-	92%	73%	70%	65%

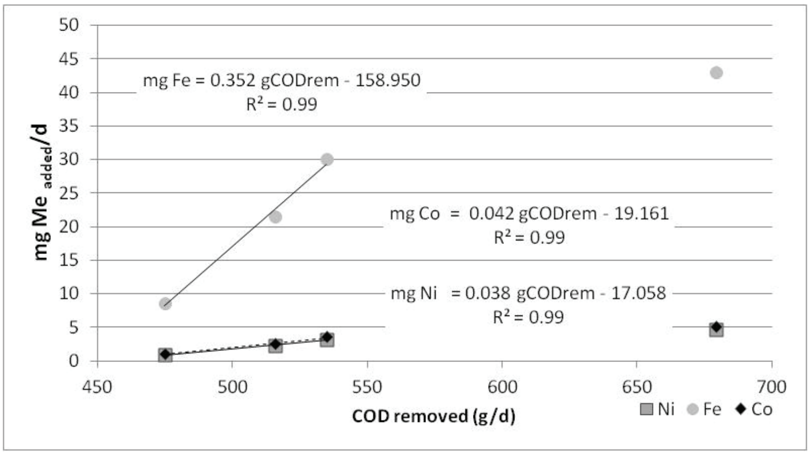

Fig. 2. Metals requirements in different tested conditions

It is clear that micro-nutrients have positive effect on anaerobic degradation of organic matter but they were also involved in complex chemical reaction in the reactor. Probably the metals at high concentration react with potential inhibiting agents as reported by Gustavsson et al. (2013) and have synergic effect on anaerobic process.

Naturally the metals dosages and the consume of energy to maintain a higher operational temperature should increase management cost, then the economical aspect has to be evaluated deeply in order to apply thermophilic process at full-scale. Metal salts costs depend on location of the treatment plant and transportation cost, quality of salts and quantity purchase. Quoted prizes range from 0.29 to 7.10 \$/kg $FeCl_3$ (Schafer, 2001) and are 147 \$/kg $NiCl_2$ $6H_2O$ and 1440 \$/kg $CoCl_2$ $6H_2O$ (Pfluger, 2010). More attractive nutrient sources could be some wastes with good content of metals such as livestock effluents or waste activated sludge from civil or industrial wastewater treatment. In fact sludge, deriving from wastewater treatment of fruit and vegetable processing, has low concentrations of metals. It is important to note that both sludge and manure have characteristics other than nutrients which may aid digestion: they increase bacteria population by continuous system inoculum, add alkalinity to the system and are a source of degradable organic matter. It is more effective to mix two or three organic wastes to prepare a nutrient sufficient feed-stock for a high-solids anaerobic digestion process (Kayhanian et al., 1995). Hinken et al. (2008) reported that anaerobic digestion of silage failed after the removal of manure in the feeding of reactor and demonstrated that trace elements concentration in biomasses depends on amount of manure in the substrate for digestion plants.

On the other hand thermophilic process could reduce effluent disposal costs because of better hygienisation effect. Several studies reported the greater pathogens depletion were reached at 55°C because the *E.coli* and *Salmonellae spp.* were significantly removed (Da Ros et al., 2014; Sahlstrom et al., 2004).

4. Conclusions

Mesophilic anaerobic digestion fed with winery wastes was steady and SGP reached 0.386 m^3/kgCOD, while thermophilic one failed because VFAs accumulated. The cause of instability was the different requirement of thermophilic bacteria. The augmentation of iron, cobalt and nickel in thermophilic process at different concentrations was carried out. Higher trace-elements augmentation (4.3 mg Fe /L, 0.46 mg Ni/L and 0.51 mg Co/L) increased biogas production to 0.450 $m^3/kgCOD_{fed}$ and COD removal reached 92%. While reducing metals addition, stability process remained in the optimum ranges for anaerobic digestion but yield reduced to value equal or lower than mesophilic one.

Relationship between metals addition and COD removal was linear only for the lowest three doses: 0.352 $mgFe_{added}/gCOD_{rem}$, 0.042 $mgCo_{added}/gCOD_{rem}$ and 0.038 $mgNi_{added}/gCOD_{rem}$. In the case of highest addition maybe other chemical equilibria, not considered in this study, interact with trace-elements availability.

Thermophilic anaerobic digestion had several benefits, but metals and heat costs should be kept into account.

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