Development and application of an automatic feeding control to manage anaerobic co-digestion of winery wastes

Da Ros, C.[a,] *, Micolucci, F*[b], *Gottardo, M*[a]., *Cavinato, C*[a], *Bolzonella, D*[b], *Pavan, P*[a]

[a] University Ca' Foscari of Venice, Department of Environmental Sciences, Informatics and Statistics, Calle Larga Santa Marta, Dorsoduro 2137-30123, Venice, Italy.

[b] University of Verona, Department of Biotechnology, Strada le Grazie 15-37134, Verona, Italy.

*Corresponding author. E-mail address: cinzia.daros@unive.it (C. Da Ros)

Abstract

This paper deals with the optimization of anaerobic digestion of winery wastes considering the behavior of biogas production rate between two consecutive reactor feeds. Processes operating at different hydraulic retention times (23 and 40 d) were monitored and the specific biogas productions were comparable (0.386 and 0.378 $m^3/kgCOD_{fed}$ for retention time of 23 and 40 d, respectively). The biogas production rate reduced after 11-14 hours in both the processes, this time corresponds to the necessary period to consume readily biodegradable and easily hydrolysable COD. In order to maximize biogas production, a system able to increase feeding frequency was set-up. The system activated feeding pump when a reduction of biogas production rate (below 0.4 $m^3_{biogas}/(m^3_{reactor}d)$) was detected. Consequently, hydraulic retention time decreased to 21 d and organic loading rate reached 6.2 kg $COD/(m^3_{reactor}d)$. Moreover, these conditions favored the growth of microorganism involved into degradation of soluble COD fraction and faster kinetics were observed in this conditions. Finally, two kinetic models (first order and step-diffusional) were applied to manually and automatically fed processes, in order to understand how the retention time and automatic control affect the degradation rate of the different types of compounds and to confirm the results obtained from the preliminary kinetic study. Step-diffusional model better predicted the trend of degradation rates (R^2 0.97-1.00) because it considered three groups of compounds and the same number of parameters.

Keywords: Anaerobic digestion, Kinetics, Step-diffusional model, First order model, Winery wastes, Process control.

1. Introduction

Anaerobic digestion (AD[1]) of waste activated sludge (WAS) and wine lees (WL) is recently taken into consideration because of particular characteristics of substrates. WAS is often associated with poor methane yield because of low biodegradability of microorganisms cells; while WL had unbalanced COD:N:P ratio. The anaerobic co-digestion of these two wastes together should thus balances the nutrient content improving biogas conversion efficiency and consequently economical sustainability.

On the other hand, Da Ros et al. (2014) showed that this process had low efficiency when low hydraulic retention time (HRT) and high organic loading rate (OLR) were applied.

[1] *Abbreviations: 4a*, Kinetic constant representing the proportionality constant between degradation rate and time for the methanogenic step (mgCOD/L min^2); *4b*, Kinetic constant representing the proportionality constant between degradation rate and time for the acidogenic step (mgCOD/L min^2); *4c*, Kinetic constant representing the proportionality constant between degradation rate and time for the hydrolitic step (mgCOD/L min^2); *b*, Biomass decay coefficient; *GPR*, Biogas Production rate ($m^3/(m^3d)$); *HRT*, Hydraulic retention time (d); *k*, Hydrolysis constant (d^{-1}); *OLR*, Organic loading rate (kgCOD/(m^3d)); P_x, Biomass production (kgVSS/d); *Q*, Flow rate (l/d); *rbCOD*, Readily biodegradable COD; *rhCOD*, Readily hydrolysable COD; *S*, Concentration of substrate as COD in the digester (mgCOD/l); S_0, Substrate concentration (COD) in the digester after feeding (mgCOD/l); S_1,Residual organic matter content of the substrate, once the easily degradable compounds (acetate, methanol, etc.) have been removed (mgCOD/l); S_2, Residual organic matter content of the substrate, once the soluble degradable compounds have been removed (mgCOD/l); *sbCOD*, Slowly biodegradable COD; *SGPR*, Specific biogas production rate (m^3/(kg VSS d)); *SRT*, Solid retention time (d); *t*, Time (hour); $v_{0,}$ Maximum degradation rate for methanogenesis (mgCOD/L min); v_1, Maximum degradation rate for acidogenesis (mgCOD/L min); v_2, Maximum degradation rate for hydrolysis (mgCOD/L min); v_3, Maximum degradation rate for hydrolysis of recalcitrant complex biopolymers (mgCOD/L min); v_i, Maximum degradation rate (mgCOD/L min); V_r, Reactor volume (L); *x*, Generic kinetic constant in Step-diffusional model (mgCOD/L min^2); *X*, Biomass concentration (gVSS/l); *Y*, *S*pecific biomass yield (gVSS/gCOD).

Definition of the best conditions, able to optimize the process, is crucial when a new process is set-up but it could be difficult. Optimization has to maximize the biogas production and the organic matter removal, moreover it has to guarantee the stability in the long-term. All these aspectes depend on many factors and operational conditions such as the type of treated waste, the solid and nutrient contents, OLR, temperature and buffering capacity (Anjum et al., 2016; Krishnan et al., 2017).

The HRT is one of most important operational parameters for AD systems control (Zhang and Noike, 1994). It is defined as the average time that bacteria or solids spend inside the reactor, and affects yields and microbiological community involved in the process (Rincón et al., 2008). Usually, longer HRTs are applied to increase AD stability when drop in pH and the volatile fatty acids (VFA) accumulation are observed in absence of sufficient buffering capacity (Harsono et al., 2014). The HRT increases contact time between the substrate and microbial biomass, so it is associated with higher destruction of volatile solids (Appels et al., 2008) and specific biogas production.

On the other hand the biodegradability of the substrates fed to the reactor has a great importance and could change the effects of long HRT application. Ruile et al. (2015) showed how the volatile solids removal in biogas plant treating cattle manure increased from less than 20% to about 75% when HRT was extended from 21 to 127 days. On the other hand Nges and Liu (2010) studied AD of dewatered sewage sludge and reported that volatile solid removed increased from 48% to 56% when HRT varied from 20 d to 35 d, while longer HRT did not appear economically advantageous.

Considering the complexity of anaerobic co-digestion process, the best operational conditions have been usually chosen on basis of experimental results and using a preventive approach: the reactor is under-loaded in order to avoid stability problems. This paper proposed an innovative system to optimize the AD of winery wastes based on a simple kinetic study and modeling. In fact, one of the objectives of AD modeling is the prediction of reactors behavior,

in the future or under other similar circumstances, (Donoso-Bravo et al., 2011). Nowadays there are several models that differ for complexity and prediction capacity.

ADM1 and its modifications are promising models aimed at understanding the system's behavior and the interaction of components; however identifying all the parameters and coefficients is not easy and their application appears limited. Grey box models are simple and consider a sole limiting-rate step (hydrolysis or methanogenic), therefore they are defined by one kinetic parameter. Their parameters have a physical interpretation and are adjustable, for instance by a parameter estimation procedure (Lauwers et al., 2013). On the other hand, they can't describe the degradation of complex substrates, characterized by simultaneous presence of compounds with different degradability, because a higher number of parameters should be considered (Converti et al., 1999). For this reason, the most common grey-box models proposed for anaerobic digestion (Monod, first order, Contois, Singh) are inadequate to represent the actual situation inside the digester fed by complex substrates (Cecchi et al., 1990a; Converti et al., 1999). Step-diffusional model represents the evolution of grey-box models because takes into account the nature and chemical characteristics of compounds present in the substrates, including the extent to which they are removed. In fact, it considers four specific groups of compounds on basis of different utilization rate, clearly identified observing the plotting of biogas production rates (GPR) versus time during the semi-continuous degradation. The step-diffusional model was mainly applied to AD of organic fraction of municipal solid waste (Cecchi et al., 2013, 1997; 1991), but recently this model has been applied also to AD of pre-hydrolysed woody wastes (Converti et al., 1999) and to winery wastes (Da Ros et al., 2014).

The aim of the present work is to study the kinetics of the co-digestion process changing the HRT. The results were used to develop a feeding control system able to maximize the biogas production without affect the process stability. The novelty of the proposed system is to use the biogas production rate as the sole set-point. Finally, two kinetic models (first-order and step-diffusional models) were applied in order to confirm the advantages of the system and to evaluate their usefulness for the system improvement.

The study was carried out on anaerobic co-digestion of winery wastes because of its particular characteristics:

- the mixture of the substrates allows to have a complex substrates with different type of organic matter (soluble and particulate, sugar, proteins and lipids);
- absence of inhibitors under mesophilic condition in fact the low nitrogen content has never caused ammonia accumulation;
- overloading was the main cause of stability problems observed during the previous experimentation (Da Ros et al., 2014).

2. Material and Methods

2.1. Substrates characteristics

Dewatered WAS used during the experimentation was collected in a winery wastewater treatment plant located in the North-East of Italy: total solid concentration generally ranged from 129.0 to 193.7 g TS/kg but some outliers were detected because of some technical reasons (conditioner doses, filter press setting). Volatile Solids (VS) to Total Solid (TS) ratio was 88%, higher than the typical value of sludge from municipal wastewater (Collivignarelli et al., 2017), probably due to the high biodegradability of raw wastewater. Moreover, particulate COD concentration (pCOD 868 mg/g TS, Table 1) was indicative of low biological stability of the sludge. Sludge nutrients ratio is well balanced for AD stabilisation (Table 1), with COD:N:P ratio of 119:7:1. Chemical analysis of sludge showed limited contamination of 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, Zn 97 mg/kg TS), thus it is suitable for land application as amendment (D.Lgs. 99/1992; European Commission 2010).

Table 1 Average and standard deviation of waste activated sludge (25 samples) and wine lees characteristics (74 samples). nd: not detected

Wine lees were produced during wine decanting step. Both WL, from red and white wine, were used in the experimentation to evaluate substrate variability and how it affects the process.

About the 90% of WL samples had a solid concentration between 37.9 and 77.2 g TS/kg, but extreme values were also detected. Generally, these winery residues were characterized by low content of volatile solids (57% of TS) due to bentonite addition to make the solid removal easier. COD was concentrated in the soluble form (sCOD was the 83% of total COD) while pCOD was typically between 417 and 627 mg COD/g TS. Nitrogen and phosphorus levels were limiting for bacterial growth if compared with COD concentration, in fact, the COD:N:P ratio was 525:5:1.

2.2. Experimental setup

The AD experimentations were carried out at pilot scale. A completed stirred tank reactor of 0.23 m^3 working volume was employed. The reactor was made of stainless steel AISI-304 and the maximal mixing degree inside the reactor was ensured by mechanical anchor-bars agitator, thus avoiding the typical stratification of floating materials on the top and of sinking heaviest materials on the bottom of the reactor as reported by Micolucci et al. (2016). The operational temperature was 37 ± 2 °C and it was controlled by external jackets hot water recirculation systems. PT100 probe monitored process temperature and managed water recirculation pumps. Mixing and temperature were maintained constant during the whole study.

At the beginning of the trials, the reactor was filled up with digestate derived from anaerobic process treating winery wastes. Initial digestate has pH of 7.53 and low ammonium concentration (193.4 mg N-NH_4^+/l). The reactor was fed once a day with a mixture of winery wastes (WAS and WL) diluted with tap water to reach the desiderated flow rate and sludge solid concentration.

During the start-up, the organic loading rate was stepwise increased to 3.2 kg COD/($m^3_{reactor}$d) while HRT of 23d was maintained (RUN1). As reported by Da Ros et al. (2016), a constant quantity of sludge was used (0.6 kg COD/($m^3_{reactor}$d)), and the WL amount increased gradually from 0 to 2.6 kg COD/($m^3_{reactor}$d). Semi-steady state lasted 9 HRTs. After

that, HRT was set at 40 d reducing the water addition, in order to evaluate the HRT effect (RUN 2). This reactor operated until a steady-state condition.

Biogas production was measured by drum-type gas flow meter (Ritter, Bochum, Germany) connected to a real-time data recording system. GPRs were automatically calculated as the slope of biogas production curve over time. This approach was performed using cRIO™ hardware (National Instruments™) and a specific graphic interface (Virtual Instrument from LabVIEW™ software).

Considering the results obtained with these conditions, the automatic feeding system, interfaced with on-line biogas production monitoring, was set-up. The software automatically controlled a pump that feed the reactor as soon as the GPR decreases to value lower than a defined set-point. This approach minimized the time between two consecutive feeds then increases the feeding loading frequency. In order to avoid microbial biomass withdraw the substrates mixture was prepared reducing water dilution, corresponding with a higher solid concentration in the WAS.

The authors did not choose the operational conditions (not HRT neither OLR) in RUN3 but they were affected by the pump functioning and the process reaction rate. The average operational parameters observed in this third RUN were HRT of 21 d and OLR of 6.2 kg COD/($m^3_{reactor}$d).

2.3. Analytical methods

The substrates and the digesters effluents were collected and monitored once a week in terms of total and volatile solids 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 heavy metal contents in the WAS was analyzed as reported by IRSA - CNR (1985). 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. The VFAs content was monitored using a gas chromatograph as reported byMicolucci et al.

(2016). At steady state conditions, total phenols were spectrophotometrically analyzed by the Folin–Ciocalteu assay and concentration was reported in terms of Gallic acid equivalent per liter (mg GAE/L, Lafka et al., 2007). Biogas composition (CO_2, CH_4, H_2 and O_2) was determined by a gas chromatograph (GC Agilent Technology 6890 N) equipped with the column HP-PLOT MOLESIEVE, 30 × 0.53 mm ID ×25 μm film, using a thermal conductivity detector (TCD) and argon as gas carrier. The TCD was mantained at a temperature of 250 °C, while the injector temperature was 120°C and pressure in the injection port was 70 kPa. Samples were taken using a gastight syringe with an amount of 200 μL of biogas. Once vaporized the whole sample, the separation of the peaks takes place within the column with a constant temperature of 40 °C (8 min).

2.4. Kinetic models description

Seven profiles of biogas production versus the time were taken into consideration for each operational condition tested as soon as the steady state was reached The biogas production was converted into the corresponding removed COD and the trends of COD concentrations inside the reactor were determined. COD concentration over the time and consumption rate profiles were used in order to describe the kinetics of the processes by two linear kinetic models (first-order and step-diffusional).

First order model considers the microorganisms as ‘catalysts’ and represents an overall mass transfer kinetic model for a ‘catalyzed’ reaction (Cecchi et al., 1990a). Although this is not a sophisticated model, it can provide a single and useful kinetic constant called hydrolysis constant (*k*). The equation 1 describes the model where *S* is the substrates and *t* the elapsed time.

$$\frac{dS}{dt} = -k\,S \qquad (1)$$

The step diffusional model was introduced to overcome this restriction of a sole model parameter. Here, the anaerobic digester is described like a semi-continuous system, and the different substrates nature is taken into account. The degradation rate of each group of compounds can be therefore described by a differential kinetic equation (Cecchi et al., 1990a;

Cecchi et al., 1991). In general, given the assumptions reported above, the degradation equation for the use of a substrate, *S*, with time, *t*, is reported in Equation 2, where v_i is the maximum degradation rate and *x* is a generic kinetic parameter.

$$\frac{dS}{dt} = v_i \quad - \frac{4x(t-t_i)}{2} \tag{2}$$

This equation assumes a different form depending on the kind of substrate used in the process: in general at least three main groups of substrates can be detected and they are associated with the related equations.

The first group was mainly constituted by acetic acid, methanol and compounds directly convertible into biogas by methanogenic microorganisms (Cecchi et al., 1991). Its consumption can be described by the linear equation 3, where S_0 represents the overall organic matter content of the substrate, v_0 is the maximum degradation rate and *4a* the kinetic parameter representing the proportionality constant between degradation rate and time for the methanogenic step.

$$dS_0/dt = v_0 - 4at/2 \tag{3}$$

The second group, containing mainly volatile fatty acids with more than three carbon atoms and ethanol, is degraded more slowly of the easily digestible fraction, according to Eq. 4. S_1 represents the residual organic matter content of the substrate, once the easily degradable compounds have been removed. At this point, the degradation rate is v_1 which is indicated by an inflection in the plot of rate against time at $t = t_1$.

$$dS_1/dt = v_1 - 4b(t - t_1)/2 \tag{4}$$

The degradation of the last group constituted of recalcitrant complex biopolymers, is controlled by hydrolysis rate. The slowest degradation step is described by equation 5

$$\frac{dS_2}{dt} = v_2 \quad - \frac{4c(t-t_2)}{2} \tag{5}$$

The equations 5 is applied for $t_2 < t < t_3$ where S_2 represents the residual organic matter content of the substrate, once the soluble degradable compounds have been removed. At this point, the degradation rate is v_2 producing an inflection in the rate curve at $t = t_2$. At the end of

this period, the degradation rate (denoted as v_3) corresponds to the hydrolysis of most recalcitrant organic matter (equation 6).

$$\frac{dS}{dt} = v_3 \quad (6)$$

The parameters of the models were estimated using the linear regressions, minimizing sum of square differences between the predicted and experimental values in each point. For each data series were determined the parameter that minimize the error and, later, average value and standard deviation were calculated for each operational condition.

The regression coefficients (R^2), together with the average lack of fit, expressed as the average square differences between the real and the model predicted values, have been used to test the best fitting model considering the overall data

An analysis of variance (ANOVA) test was then applied on parameters of model considering the three operational conditions. The Duncan mean test was used to compare the means and evaluate if the observed differences are significant

3. Results and discussion

3.1 Anaerobic digestion processes performances

The first tested condition is HRT of 23 d (RUN1), corresponding to a hydraulic flow rate of 10 L/d. At steady state, the average pH was 7.46 and total alkalinity stabilized to 2,248 $mgCaCO_3/L$. Low level of sCOD (360 mg/L) indicated the complete removal of readily biodegradable fraction present in WL. About 40-50% of residual sCOD was due to volatile fatty acids: acetic acid was the dominant (52% of total VFAs) while propionic acid was the second most abundant with 12% of VFAs. The process, in this condition, was able to remove 76% of total COD. Total solids and pCOD concentrations reduced with time down to average values of 24 gTS/kg, with 58% volatile solids, and 640 mg COD/g TS (Table 2). Most of the biogas production derived from degradation of soluble compounds and the solid fraction was partially degraded. For this reason, total and volatile solids removal were 28% and 40%, respectively.

Table 2 Average and standard deviation of characteristics of digestates from process working with 23 (16 samples) and 40 days (10 samples) of HRT.

Nutrients concentrations generally reduced until steady values were reached (37 mgN/g TS and 9 mg P-PO_4^{3-}/g TS). Ammonium concentration stabilized around 400 mgN-NH_4^+/L: considering negligible ammonium nitrogen concentration in inlet substrates (less than 1% of total nitrogen in the feed is due to ammonium ion), it is possible to observe that about 30% of organic nitrogen was converted in soluble form. As reported by Owamah et al. (2014), digestate contains more readily available nutrients than the undigested products which make it better for crops fertilization. Biogas production scattered a lot, depending on the type of WL in the inlet mixture: average biogas production was 0.386 m^3/kg COD_{fed} with 64-73% of methane.

In the RUN2 (HRT 40 d) the flow rate has reduced to 5.75 L/d and HRT increased to 40 d. Flow rate decreased without changing the organic loading rate, in fact, only the amount of water reduced in the feeding mixture. The TS concentration of substrates mixture increased from 3% to 5%. Change in operational conditions caused a transient period of about one HRT, defined by an increased ammonium concentration and alkalinity. As reported in Table 2 the stability parameters were in optimal range for AD: pH was 7.51, similar than the previous period, while alkalinity increased to 3,332 mg$CaCO_3$/L because of higher concentration of ammonium (638 mgN-NH_4^+/L). The concentration of sCOD was around 349 mg/L and VFA contributed for less than 50 mg/L. Acetic acid was the only VFA detected during this period. The different VFAs concentration and distribution could be due to the effect of HRT. In fact, long HRT allows for higher content of acetoclastic methanogens bacteria, characterized by lower growth rate than bacteria degrading other organic compounds (Amani et al., 2012; Metcalf and Eddy, 1980). Moreover longer HRT increased the methanogenic population and promoted the efficient propionate and butyrate oxidation (Schmidt and Ahring, 1995), consequently reduced the total VFA concentration.

TS concentration increased from 24 to 33 gTS/kg due to higher solid concentration in the inlet mixture. Percentage of volatile to total solids was about 61% and pCOD was 752 mg/g TS. These values were slightly higher than those observed during RUN1, but the difference was not significant and it was probably due to substrates variation. In fact, also the mass balance on TS, VS and COD basis showed similar removal performances (22%, 36% and 78% respectively). Nitrogen fate didn't change increasing HRT, in fact, the same amount of organic nitrogen was converted into ammonium form (28% of total nitrogen) and the higher concentration in the second condition was due to minor dilution. Considering average and standard deviation of phosphorus concentration, no significant difference for this parameter can be detected in the two periods. Biogas production yield (0.378 m^3/kg COD) was like the previous trial, with a methane content of 65%.

3.2 Kinetic study

Comparing mass balances and SGP between RUN1 and RUN2(Table 2), it is evident that HRT did not affect the degradation efficiency but reduced the effluent sCOD concentration. The higher concentration of solids and minor flow rate could reduce the downstream volume to be disposed of and consequently the management costs.

On the other hand, some interesting differences were observed in cumulative biogas production curves (Fig.1). In both the conditions the curves could be separated into three parts, corresponding with different slopes and GPR values (Fig.2). These trends of GPR were comparable to those observed in several studies Cecchi et al. (1997) describing AD of organic fraction of municipal solid waste (Cecchi et al., 1997; Pavan et al., 2000).The higher GPR values correspond to the conversion of readily biodegradable carbon source (rbCOD) to biogas. These compounds were transformed into biogas in few hours and the complete consumption was highlighted by a sharp change in the slope. During the next 6-10 hours, degradation of ethanol and VFAs became important. This group of compounds could be defined as easily hydrolysed COD fraction (rhCOD), a term borrowed by wastewater COD fractionation (Cokgor

et al., 2009; Fall et al., 2012). It is composed by biodegradable sCOD that is quickly converted to volatile fatty acids. The amount of rhCOD fraction was the same in the two processes and contributed for the similar percentage to daily biogas production (37% and 42% in RUN1 and RUN2 respectively). Otherwise, the higher GPR observed in Run 2 caused faster consumption of rhCOD fraction.

Figure 1 Cumulative biogas production plotted against the time between two consecutive feeds in RUN1 and RUN2

Figure 2 Biogas production rates detected in RUN1 and RUN2 between two consecutive feeds

Another sharp change in gas production rates indicated consumption of rhCOD and start of slowly biodegradable substrate (sbCOD) degradation (Fig. 2). In RUN1 GPR suddenly reduced from 1.0 to less than 0.5 $m^3/(m^3_{reactor}d)$ after 14 hours from feeding, while in Run 2 biogas production rate decreased from 2.0 to 0.3 $m^3/(m^3_{reactor}d)$ around the 11^{th} hour from feeding. SbCOD was linked to particulate matter, then hydrolysis and solubilization steps controlled its degradation. Consumption of particulate organic matter was carried out during the whole day because the substrate for these biological reactions was never limiting but it became the bottleneck step of the whole process when the other groups of compounds were totally consumed.

The behaviors of the reactors during the two tested conditions were qualitatively similar and the different slopes were probably due to biomass concentration. The biomass production (P_x) was proportional to flow rate (Q) and COD removal (S_0 - S), where S_0 and S are influent and effluent COD concentrations (Eq. 7). In this equation parameters Y, b and SRT indicate respectively the specific biomass yield, the decay coefficient and the SRT, that in a system without recirculation corresponds to HRT.

$$P_x = \frac{Q\,Y\,(S_0 - S)}{1 + b\,(SRT)} \qquad (7)$$

The organic loading rate was the same in both processes hence also the biomass growth can be considered equal. However, the higher HRT determined minor withdrawn of microorganisms and higher biomass concentration in the reactor. The biomass concentration (X) can be calculated with equation 8, where V_r stands for reactor volume. The mass is expressed as concentration of suspended volatile solids (kgVSS/m^3).

$$X = \frac{P_x\, SRT}{\mathrm{V_r}} \qquad (8)$$

The calculated biomass concentrations were 2.60 and 3.82 kg VSS//m^3 and allowed to determine the maximum specific GPRs (m^3/(kg VSS d)) for the three group of compounds. As resumed in Table 3, the specific biogas production rate (sGPR) related to rbCOD was significantly higher in RUN2 than in RUN1 (at $p \leq 0.05$, Duncan test); while for rhCOD and sbCOD were similar. Longer HRT didn't promote the growth of biomass able to hydrolyse the substrates, neither didn't improve the process yields.

Table 3 Absolute and specific biogas production rate for rbCOD, rhCOD and sbCOD fractions, determined on basis of active biomass concentration (VSS). Average and standard deviation calculated on 7 replicates. Average value in a row followed by the same letter are not significantly different at p≤0.05 (Duncan test).

In both the processes appeared clear that biogas production was not continuous during the day and became negligible when rhCOD was totally consumed. Theoretically, a higher organic load could be treated by the AD process without accumulation of intermediate metabolites. The OLR can be increased feeding more substrate once a day or feeding the reactor more frequently: when the rhCOD was completely removed. In the first case the concentration of COD and VFAs in the reactor, just after the feeding, could reach inhibiting concentration for methanogens. Overloading due to higher OLR application was also reported by Da Ros et al. (2014) that worked with OLR of 4.5 kg COD/($m^3_{reactor}$d). Increase of feeding frequency allowed to high the organic load and reduce the flow rate. On the other hand, decrease of HRT could affect the microorganism concentration in the reactor and the degradative rates, as observed in Table 3.

The choice of the moment for the next feed is crucial and the kinetic study can help to decide the best moment for the feeding. In fact, the sharp GPR reduction, corresponding to rbCOD and rhCOD consumption, could be used as a signal to feed the reactor. Considering this approach biogas flow rate has been used as the control variable for the development of an automatic control system.

Several researchers have used methane or biogas production as an inner-loop variable for AD process control because it shows a faster response to perturbation compared with liquid phase variables. Otherwise, the existing studies considered the GPR as a stability parameter and combined this information with a secondary variable (Chynoweth et al., 1994; García-Diéguez et al., 2011; Liu et al., 2004). They have never considered the instantaneous biogas production rate as a consequence of process kinetics and as the sole control variable.

3.3 Automatic feeding system

An automatic feeding system was set-up on basis of GPR variable: the feeding pump was automatically activated when biogas production rate was lower than a fixed set point. Set point was chosen on basis of previous kinetic results (RUN1 and RUN2). In particular, it was observed that GPR decreased from 1.1 -2 $m^3/(m^3_{reactor}d)$ to 0.11-0.14 $m^3/(m^3_{reactor}d)$ after 11-14 hours. For this reason, biogas production rate of 0.4 $m^3/(m^3_{reactor}d)$, corresponding to production of 1 l every 15 min for the employed reactor, was chosen as a set point. The data-logger received a digital signal every liters of biogas produced and the pump activated if after 15 minutes no signal was detected.

In order to avoid excessive reduction of HRT and to make the comparison easier, in RUN3 the characteristics of substrates mixture and volume for each feed were similar to RUN2. The reactor worked for more than 90 d operating with this feeding mode. The automatic control fed 5.75 L of substrate every 14.4 h in average, corresponding to OLR of 6.2 kg COD/($m^3_{reactor}$ d) and HRT of 21 d. Some fluctuations of stability parameters were observed, but they were more affected by substrates variability than by the different feeding mode. pH values were quite

constant as reported in Table 4, while alkalinity increased according to ammonium concentration and biogas production (higher solubility of CO_2 in the liquid phase). Although the higher nitrogen load, the ammonium concentration did not reach inhibiting values (Table 4).

Table 4 Characteristics of digestates from automatic fed process (12 samples)

Lower influent dilution caused solid concentration of the effluent higher than previous trials (50 g TS/kg). VS/TS ratio (60%) and pCOD concentration (693 mg/g TS) indicated that stabilization of organic matter was at the same degree of those reached under other conditions. On the other hand, the sCOD was about 1,803 mg/L but the high value is not due to VFAs accumulation.

The biogas production reached a mean value of 0.457 $m^3/kgCOD_{fed}$ with 60% of methane. The TS and VS removal were respectively 24% and 35%, while the COD conversion was 69%, value lower than RUN1 and RUN2 because of low methane content in the biogas.

Also in this trial, the three fractions of COD were well identified by different biogas production rates. At steady state the degradation rates were constant: 7.61 ± 0.27, 3.21 ± 0.17 and 0.74 ± 0.98 $m^3/(m^3_{reactor}d)$ for rbCOD, rhCOD and sbCOD. The absolute degradation rates were higher than previous trials because greater biomass concentration (4.41 $kgVSS/m^3$). In fact, the biomass was proportional to consumed substrates (Eq. 7) that in RUN3 was almost the double of other RUNs.

Considering active biomass concentration, the specific biogas production rate associated with rbCOD and rhCOD were 1.99 ± 0.07 and 0.84± 0.05 $m^3/(kgVSS\ d)$, values significantly higher than the others Runs ($p \leq 0.05$, Duncan test). While the SGPR of sbCOD improved to 0.19 ± 0.26 but due to its high variance it was considered comparable to the others RUNs (Fig. 3). SGPR for rhCOD and sbCOD increased to 67% and 75% compared with the trial operating with HRT of 23d, of 35% and 93% compared with results of trials with 40 d of HRT.

Figure 3 Average and standard deviation of SGPR, related to rbCOD, rhCOD and sbCOD, determined in RUN1, RUN2 and RUN3.

3.4 Kinetic models application

In order to describe and compare the three RUNs, two different linear models were applied: first-order and step-diffusional models. Values of COD concentrations inside the reactor and degradation rates between one fed and the next one were considered for application of the models.

As can be seen from Table 5 the fit of the first-order model could be satisfactory (R^2 between 0.97 and 0.99) but this model cannot describe the sharp changes in biogas production rates because it had one sole parameter. Anyway the values of parameter model emphasized the increasing degradative capacity at longer HRT and higher OLR: kinetic constant increased from 1.33 to 2.28 d^{-1} with HRT of 23 and 40 d (OLR 3.2 kgCOD/($m^3_{reactor}$d)), respectively, and reached 2.85 d^{-1} with HRT of 21 d and OLR of 6.2 kgCOD/($m^3_{reactor}$d). As a final remark, it can be pointed out that biomass concentration became the limiting factor when the substrate concentration in the reactor was very high and the substrate to microorganism ratio was elevated. In fact, few hours after feed the curve reached a plateau corresponding to maximum degradation rate and this part should be described by zero-order reaction (Cecchi et al., 1997).

Table 5 Linear regression parameter applying first order model. Average and standard deviation calculated on 7 replicates. Average value in a row followed by the same letter are not significantly different at p≤0.05 (Duncan test).

The step-diffusional model introduced different kinetics parameters for three groups of compounds, in particular for each COD fraction the maximum degradation velocities (v_0, v_1 and v_2) and diffusional rate (*4a*, *4b* and *4c*) were identified (Cecchi et al., 1997, 1990b).

The velocities increased according to longer HRT and higher OLR applied (Table 6) and were positively affected by concentrations of active biomass involved into COD fraction degradation. ANOVA and Duncan tests indicated that all the velocities of RUN3 had significantly higher mean values ($p \leq 0.05$) than RUN1. RUN2 differed from RUN3 only for the velocities related to soluble fraction (v_0 and v_1) while v_2 values were comparable (Table 6). Comparison of velocity values determined in this study and those reported by Da Ros et al.

(2014) showed similar results operating with comparable operational conditions. Da Ros et al. (2014) reported that using HRT of 21 d the velocities v_0, v_1 and v_2 were 4.11, 1.17 and 0.45 mgCOD/(L min), and in RUN1, they are 4.62, 1.33 and 0.40mgCOD/(L min).

Table 6 Regression parameter applying the step-diffusional model. Average and standard deviation calculated on 7 replicates. Average value in a row followed by the same letter are not significantly different at p≤0.05 (Duncan test).

Comparing the values of v_0, v_1 and v_2 (Fig. 4) with SGPR reported in Fig. 3, the same trend could be observed and the step-diffusional model confirmed the results obtained by the preliminary kinetic study.

Increasing OLR from 3.2 to 6.2 kgCOD/($m^3_{reactor}$d) and maintaining similar HRT (23 and 21 d), all the velocity became higher. The proposed feeding system is promising because it should allow to increase OLR and consequently biogas production in the existing biogas plant; this means that the payback period could reduce. On the other hand design of new plants using this approach would reduce the digester volume and consequently the investment costs.

Figure 4 Degradation rates obtained applying step-diffusional model in the process operating with HRT of 23 and 40 d (RUN1 and RUN2) and HRT of 21 (RUN3)

The direct application of the model to the data caused the determination of the wrong value of the diffusional rate of acetate throughout the cell (*4a);* in fact, considering the physical meaning of this parameter, it should have positive value. Its calculation was affected by the slow increase of velocity just after the feed and the parameters in this condition assumed negative values (Table 6). On the other hand, if this initial period of time was not considered, the available points where velocity decreased due to consumption of rbCOD, were not enough for the parameter estimation. In RUN3 the maximum velocity was reached in minor time, probably due to the high concentration of biomass and to higher feeding frequency, and all the parameters *4a, 4b* and *4c* had positive values. These values had the same magnitude than those reported in the literature (Cecchi et al., 1991; Da Ros et al., 2014). The values are slightly higher than those derived from OFMSW anaerobic digestion indicating that diffusion of soluble

compounds and of extracellular enzymes was less limiting than with other substrates. Although the model limits described above, the correlation coefficients were higher than those of the first-order model, they ranged from 0.97 and >0.99.

4. Conclusions

Application of long HRT to AD of winery waste didn't affect the specific biogas production but increased microbial biomass concentration, and consequently improved the observed degradation rates. The behavior of biogas production rate between a reactor feed and the next one was considered and a negligible production after 11-14 hours was observed due to complete consumption of soluble biodegradable COD. Consequently, the feeding frequency was increased through a control system able to feed the reactor automatically when GPR reduced below 0.4 $m^3/(m^3_{reactor}d)$. The proposed approach allowed to increase the OLR from 3.2 to 6.2 $kgCOD/(m^3_{reactor}d)$, without any stability problems. The average GPR increased from to 1.1 to 2.8 $m^3/(m^3_{reactor}d)$, mainly due to a higher OLR and stimulation of degradation of all COD fractions. Two kinetic models were used to describe the process: first order and step-diffusional models. Step-diffusional model described better the trend of degradation rates (R^2 0.97-1.00) Step diffusional model showed how the diffusion of substrates and enzymes through the bacteria membrane has a minor effect in AD of winery waste than of OFMSW, because of high biodegradability of WL and to microbial community selection.

AD process, fed with WAS and WL, allowed to set-up a promising control system that has a local validity at the moment. Its general applicability has to be confirmed by experimentation on other processes.

Acknowledgements

The collaboration of Vinicola Serena s.r.l., the hospitality of the Treviso City Council and of Alto Trevigiano Servizi s.c.a.r.l. are kindly acknowledged. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Amani, T., Nosrati, M., Mousavi, S.M., 2012. Response surface methodology analysis of anaerobic syntrophic degradation of volatile fatty acids in an upflow anaerobic sludge bed reactor inoculated with enriched cultures. Biotechnol. Bioprocess Eng. 17, 133–144. doi:10.1007/s12257-011-0248-7

American Public Health Association, American Water Works Association, Water Environment Federation, 1999. Standard Methods for the Examination of Water and Wastewater. Stand. Methods 541.

Anjum, M., Khalid, A., Mahmood, T., Aziz, I., 2016. Anaerobic co-digestion of catering waste with partially pretreated lignocellulosic crop residues. J. Clean. Prod. 117, 56–63. doi:10.1016/j.jclepro.2015.11.061

Appels, L., Baeyens, J., Degrève, J., Dewil, R., 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. Prog. Energy Combust. Sci. 34, 755–781. doi:10.1016/j.pecs.2008.06.002

Cecchi, F., Alvarez, J.M., Traverso, P.G., Medici, F., Fazzini, G., 1990a. A new approach to the kinetic study of anaerobic degradation of the organic fraction of municipal solid waste. Biomass 23, 79–102. doi:10.1016/0144-4565(90)90028-I

Cecchi, F., Marcomini, A., Pavan, P., Fazzini, G., Mata-Alvarez, J., 1990b. Mesophilic digestion of the organic fraction of refuse: Performance and kinetic study. Waste Manag. Res. 8, 33–44. doi:10.1016/0734-242X(90)90050-W

Cecchi, F., Mata-Alvarez, J., Marcomini, A., Pavan, P., 1991. First order and step-diffusional kinetic models in simulating the mesophilic anaerobic digestion of complex substrates. Bioresour. Technol. 36, 261–269. doi:10.1016/0960-8524(91)90233-A

Cecchi, F., Pavan, P., Mata-Alvarez, J., Bassetti, A., Cozzolino, C., 1991. Anaerobic digestion of municipal solid waste: Thermophilic vs. mesophilic performance at high solids. Waste Manag. Res. 9, 305–315. doi:10.1016/0734-242X(91)90020-8

Cecchi, F., Pavan, P., MataAlvarez, J., 1997. Kinetic study of the thermophilic anaerobic digestion of the fresh and precomposted, mechanically selected, organic fraction of

municipal solid waste. J. Environ. Sci. Heal. Part a-Environmental Sci. Eng. Toxic Hazard. Subst. Control 32, 195–213. doi:10.1080/10934529709376536

Chynoweth, D.P., Svoronos, S.A., Lyberatos, G., Harman, J.L., Pullammanappallil, P., Owens, J.M., Peck, M.J., 1994. Real-time expert system control of anaerobic digestion, in: Water Science and Technology. pp. 21–29.

Cokgor, E.U., Insel, G., Aydin, E., Orhon, D., 2009. Respirometric evaluation of a mixture of organic chemicals with different biodegradation kinetics. J. Hazard. Mater. 161, 35–41. doi:10.1016/j.jhazmat.2008.03.051

Collivignarelli, M.C., Abbà, A., Castagnola, F., Bertanza, G., 2017. Minimization of municipal sewage sludge by means of a thermophilic membrane bioreactor with intermittent aeration. J. Clean. Prod. 143, 369–376. doi:10.1016/j.jclepro.2016.12.101

Converti, A., Del Borghi, A., Arni, S., Molinari, F., 1999. Linearized kinetic models for the simulation of the mesophilic anaerobic digestion of pre-hydrolyzed woody wastes. Chem. Eng. Technol. 22, 429–437. doi:10.1002/(SICI)1521-4125(199905)22:5<429::AID-CEAT429>3.0.CO;2-5

Da Ros, C., Cavinato, C., Cecchi, F., Bolzonella, D., 2014. Anaerobic co-digestion of winery waste and waste activated sludge: Assessment of process feasibility. Water Sci. Technol. 69, 269–277. doi:10.2166/wst.2013.692

Da Ros, C., Cavinato, C., Pavan, P., Bolzonella, D., 2016. Mesophilic and thermophilic anaerobic co-digestion of winery wastewater sludge and wine lees: An integrated approach for sustainable wine production. J. Environ. Manage. doi:10.1016/j.jenvman.2016.03.029

Donoso-Bravo, A., Mailier, J., Martin, C., Rodríguez, J., Aceves-Lara, C.A., Vande Wouwer, A., 2011. Model selection, identification and validation in anaerobic digestion: a review. Water Res. 45, 5347–64. doi:10.1016/j.watres.2011.08.059

European Commission, 2010. Environmental, economic and social impacts of the use of sewage sludge on land Final Report Part I: Overview Report.

Fall, C., Millán-Lagunas, E., Bâ, K.M., Gallego-Alarcón, I., García-Pulido, D., Díaz-Delgado,

C., Solís-Morelos, C., 2012. COD fractionation and biological treatability of mixed industrial wastewaters. J. Environ. Manage. 113, 71–7. doi:10.1016/j.jenvman.2012.08.032

García-Diéguez, C., Molina, F., Roca, E., 2011. Multi-objective cascade controller for an anaerobic digester. Process Biochem. 46, 900–909. doi:10.1016/j.procbio.2010.12.015

Harsono, S.S., Grundmann, P., Soebronto, S., 2014. Anaerobic treatment of palm oil mill effluents: Potential contribution to net energy yield and reduction of greenhouse gas emissions from biodiesel production. J. Clean. Prod. 64, 619–627. doi:10.1016/j.jclepro.2013.07.056

IRSA, CNR, 1985. Metodi Analitici per Fanghi, Quaderno 64.

Krishnan, S., Singh, L., Sakinah, M., Thakur, S., Wahid, Z.A., Ghrayeb, O.A., 2017. Role of organic loading rate in bioenergy generation from palm oil mill effluent in a two-stage up-flow anaerobic sludge blanket continuous-stirred tank reactor. J. Clean. Prod. 142, 3044–3049. doi:10.1016/j.jclepro.2016.10.165

Lafka, T.I., Sinanoglou, V., Lazos, E.S., 2007. On the extraction and antioxidant activity of phenolic compounds from winery wastes. Food Chem. 104, 1206–1214. doi:10.1016/j.foodchem.2007.01.068

Lauwers, J., Appels, L., Thompson, I.P., Degrève, J., Van Impe, J.F., Dewil, R., 2013. Mathematical modelling of anaerobic digestion of biomass and waste: Power and limitations. Prog. Energy Combust. Sci. 39, 383–402. doi:10.1016/j.pecs.2013.03.003

Liu, J., Olsson, G., Mattiasson, B., 2004. Monitoring and control of an anaerobic upflow fixed-bed reactor for high-loading-rate operation and rejection of disturbances. Biotechnol. Bioeng. 87, 43–53. doi:10.1002/bit.20088

Metcalf, E., Eddy, H., 1980. Wastewater engineering: Treatment, disposal and reuse. Adv. Water Resour. 3, 146. doi:10.1016/0309-1708(80)90067-6

Micolucci, F., Gottardo, M., Cavinato, C., Pavan, P., Bolzonella, D., 2016. Mesophilic and thermophilic anaerobic digestion of the liquid fraction of pressed biowaste for high energy

yields recovery. Waste Manag. 48, 227–235. doi:10.1016/j.wasman.2015.09.031

Nges, I.A., Liu, J., 2010. Effects of solid retention time on anaerobic digestion of dewatered-sewage sludge in mesophilic and thermophilic conditions. Renew. Energy 35, 2200–2206. doi:10.1016/j.renene.2010.02.022

Owamah, H.I., Dahunsi, S.O., Oranusi, U.S., Alfa, M.I., 2014. Fertilizer and sanitary quality of digestate biofertilizer from the co-digestion of food waste and human excreta. Waste Manag. 34, 747–752. doi:10.1016/j.wasman.2014.01.017

Pavan, P., Battistoni, P., Cecchi, F., Mata-Alvarez, J., 2000. Two-phase anaerobic digestion of source sorted OFMSW (organic fraction of municipal solid waste): Performance and kinetic study. Water Sci. Technol. 41, 111–118.

Rincón, B., Borja, R., González, J.M., Portillo, M.C., Sáiz-Jiménez, C., 2008. Influence of organic loading rate and hydraulic retention time on the performance, stability and microbial communities of one-stage anaerobic digestion of two-phase olive mill solid residue. Biochem. Eng. J. 40, 253–261. doi:10.1016/j.bej.2007.12.019

Ruile, S., Schmitz, S., Mönch-Tegeder, M., Oechsner, H., 2015. Degradation efficiency of agricultural biogas plants--a full-scale study. Bioresour. Technol. 178, 341–9. doi:10.1016/j.biortech.2014.10.053

Schmidt, J.E., Ahring, B.K., 1995. Interspecies electron transfer during propionate and butyrate degradation in mesophilic, granular sludge. Appl. Environ. Microbiol.

Zhang, T.C., Noike, T., 1994. Influence of retention time on reactor performance and bacterial trophic populations in anaerobic digestion processes. Water Res. 28, 27–36. doi:10.1016/0043-1354(94)90116-3

Figure

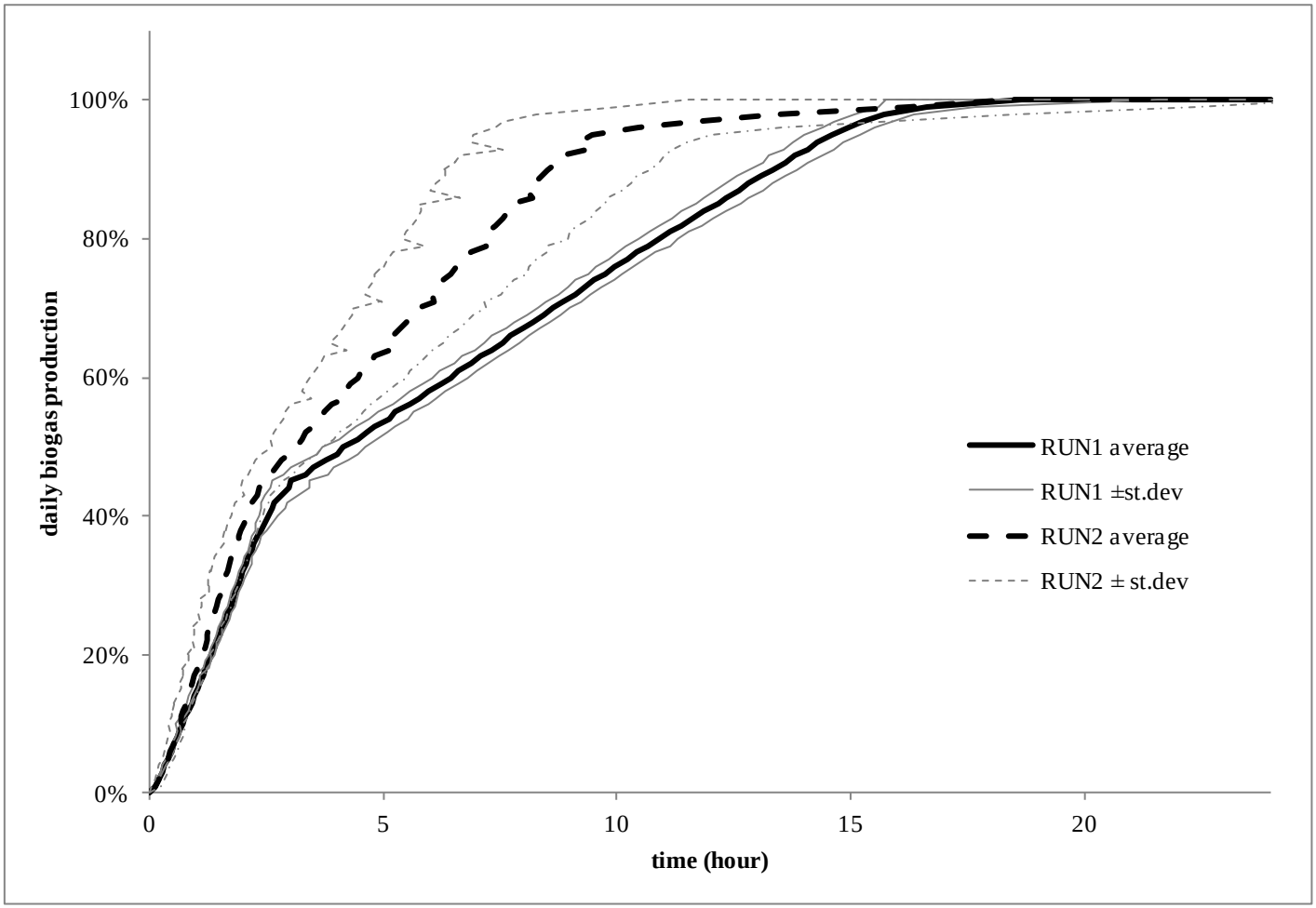

Figure 1 Cumulative biogas production plotted against the time between two consecutive feeds in RUN1 and RUN2

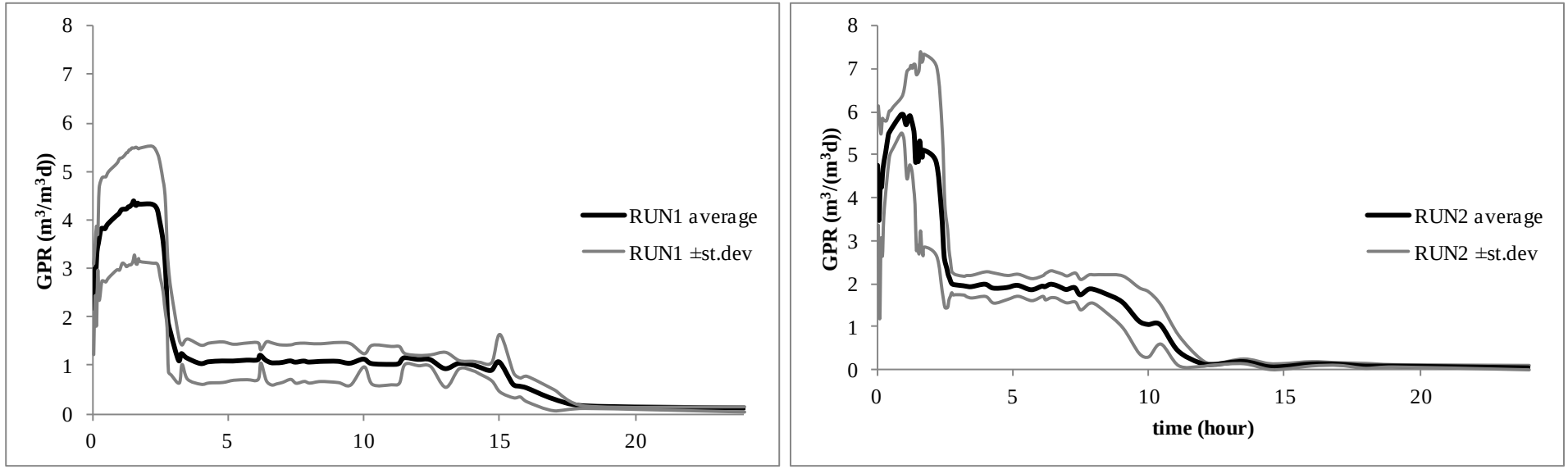

Figure 2 Biogas production rates detected in RUN1 and RUN2 between two consecutive feeds

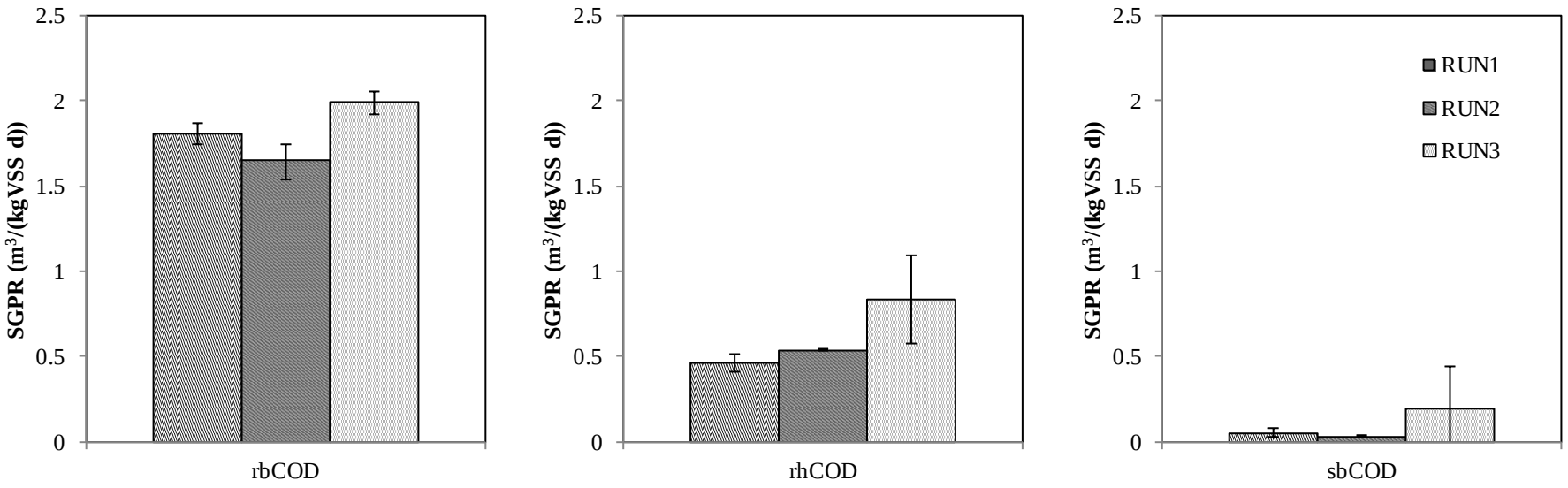

Figure 3 Average and standard deviation of SGPR, related to rbCOD, rhCOD and sbCOD, determined in RUN1, RUN2 and RUN3.

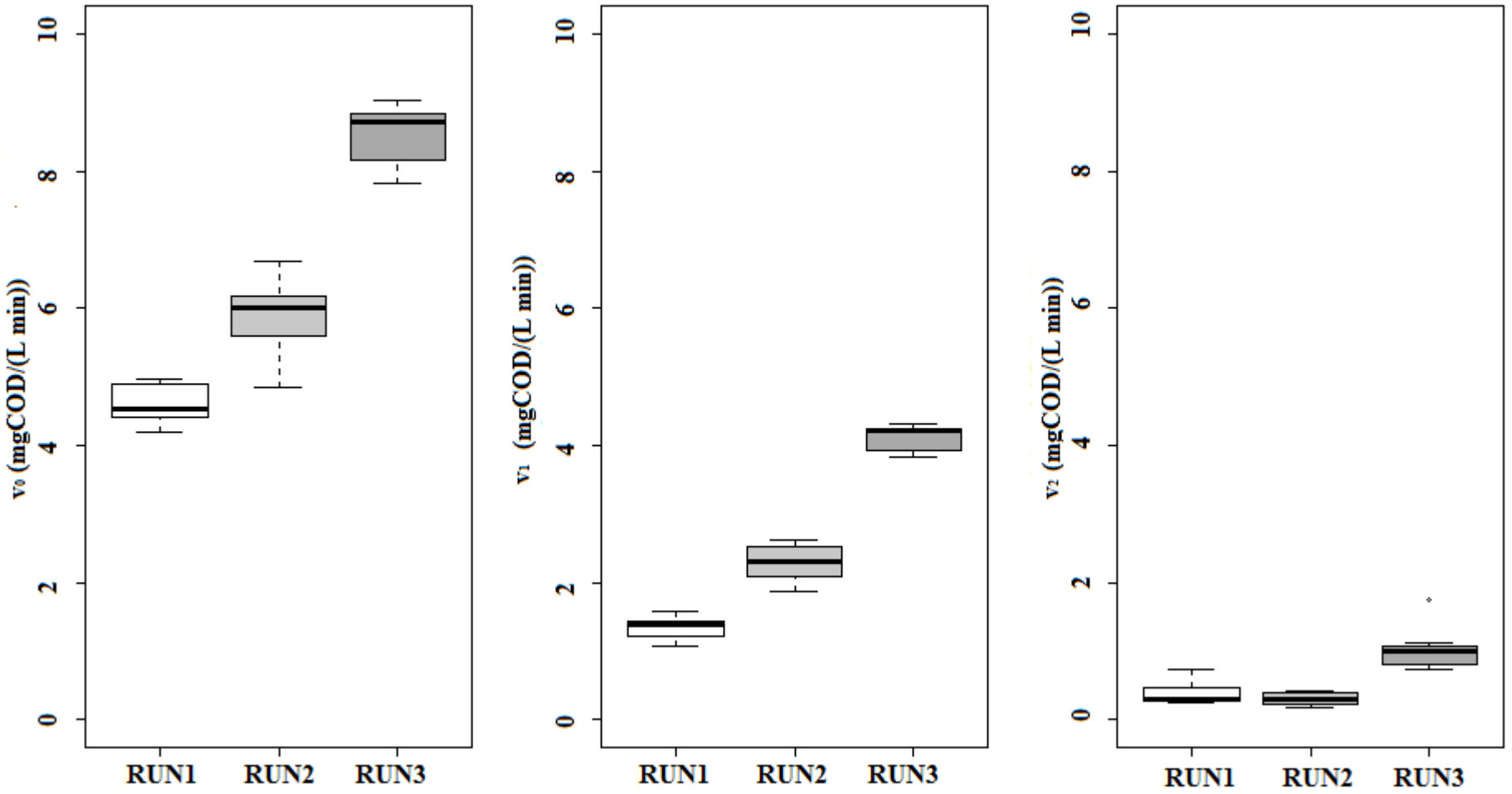

Figure 4 Degradation rates obtained applying step-diffusional model in the process operating with HRT of 23 and 40 d (RUN1 and RUN2) and HRT of 21 (RUN3)

Table 1 Average and standard deviation of waste activated sludge (25 samples) and wine lees characteristics (74 samples). nd: not detected

Parameter	Unit	Waste Activated Sludge	Wine Lees
TS	gTS/kg	158.9 ± 49.3	62.0 ±27.9
VS	gVS/kg	143.5 ±41.6	33.6 ± 15.1
VS/TS	%	88% ± 3%	57% ±13%
pCOD	mg/g TS	868 ± 69.4	559 ± 151
sCOD	g/l	nd	167 ± 45
TKN	mg N-NH_4^+/g TS	52.7±16.3	30.3 ±12.7
NH_4^+	mg N NH_4^+/L	nd	33.9±22.7
P_{tot}	mg P-PO_4^{3-}/g TS	7.3 ±2.0	6.2 ±2.9

Table 2 Average and standard deviation of characteristics of digestates from process working with 23 (16 samples) and 40 days (10 samples) of HRT

Parameter	Unit	Run 1	Run 2
HRT	d	23	40
TS	gTS/kg	24.3 ± 2.9	33.4 ± 2,43
VS	gVS/kg	14.2 ± 1.7	21.7 ± 1.1
pCOD	mg/gTS	640 ± 46	752 ± 73
sCOD	mg COD/L	360 ± 152	349 ± 74
TKN	mg N-NH_4^+/gTS	36.3 ± 4.5	52.8 ± 3.3
N-NH_4^+	mg N-NH_4^+/L	400 ± 56	638 ± 49
Ptot	mg P-PO_4^{3-}/gTS	8.8 ± 1.6	8.4 ± 0.8
pH	-	7.46 ± 0.19	7.51 ± 0.07
Total Alkalinity	mg $CaCO_3$/L	2248 ± 200	3,332 ± 124
Polyphenols	mgGAE/L	26 ± 7	56 ± 26
SGP	m^3/kgCOD	0.386± 0.049	0.378 ± 0.036
TS removal	%	28%	22%
VS removal	%	40%	36%
COD removal	%	76%	78%

Table 3 Absolute and specific biogas production rate for rbCOD, rhCOD and sbCOD fractions, determined on basis of active biomass concentration (VSS). Average and standard deviation calculated on 7 replicates. Average value in a row followed by the same letter are not significantly different at p≤0.05 (Duncan test).

Parameter		Unit	Run 1	Run 2
HRT		d	23	40
	rbCOD	$m^3/m^3_{reactor}d$	4.70^{a}±0.16	6.31^{b}±0.39
GPR	**rhCOD**	$m^3/m^3_{reactor}d$	1.21 [a] ±0.14	1.92 [a] ±0.25
	sbCOD	$m^3/m^3_{reactor}d$	0.14 [a] ±0.07	0.13 [a] ±0.03
VSS		g/l	2.60	3.82
	rbCOD	m^3/(kgVSS d)	1.81 [a] ± 0.06	1.65±0.10
SGPR	**rhCOD**	m^3/(kgVSS d)	0.47 [a] ±0.05	0.50 [a] ±0.07
	sbCOD	m^3/(kgVSS d)	0.05 [a] ± 0.03	0.03 [a] ±0.01

Table 4 Characteristics of digestates from automatic fed process (12 samples)

Parameter	Unit	Run 3
HRT	d	21
TS	gTS/kg	50.0 ± 5.1
VS	gVS/kg	30.0 ± 1.8
pCOD	mg/gTS	693 ± 73
sCOD	mg COD/L	1803 ± 931
TKN	mg $N\text{-}NH_4^+$/gTS	62.0 ± 7.3
$N\text{-}NH_4^+$	mg $N\text{-}NH_4^+$/L	818 ± 83
P_{tot}	mg $P\text{-}PO_4^{3-}$/gTS	8.8
pH	-	7.38± 0.14
Total Alkalinity	mg $CaCO_3$/L	3775 ± 202
Polyphenols	mgGAE/L	nd
SGP	m^3/kgCOD	0.457 ± 0.041
TS removal	%	24%
VS removal	%	35%
COD removal	%	69%
Polyphenols removal	%	nd

Table 5 Linear regression parameter applying first order model. Model parameters were calculated considering 7 replicates for each tested condition. Average and standard deviation calculated on 7 replicates. Average value in a row followed by the same letter are not significantly different at $p \leq 0.05$ (Duncan test).

		Run 1	Run 2	Run 3
First-order model				
k	d^{-1}	1.33 [a] ±0.11	2.28 [b] ±0.43	2.85 [c] ±0.14
R^2		0.98	0.97	0.89
Average lack of fit		19.98	16.65	5.82

Table 6 Regression parameter applying step-diffusional model. Average and standard deviation calculated on 7 replicates. Average value in a row followed by the same letter are not significantly different at $p \leq 0.05$ (Duncan test).

		Run 1	Run 2	Run 3
Step diffusional model				
v_0	mgCOD/L min	4.62 [a] ±0.30	5.87 [b] ±0.61	8.52 [c] ±0.47
v_1	mgCOD/L min	1.33 [a] ±0.18	2.30 [b] ±0.28	4.10 [c] ±0.20
v_2	mgCOD/L min	0.40 [a] ±0.18	0.31 [a] ±0.10	1.03 [b] ±0.35
4a	mgCOD/L min^2	-0.0163 ±0.0060	-0.0342±0.0130	0.0046±0.054
4b	mgCOD/L min^2	0.0005±0.0004	0.0022±0.0023	0.0064±0.0021
4c	mgCOD/L min^2	0.0045±0.0037	0.0006±0.0005	0.0116±0.0065
R^2		0.99	0.97	1.00*
Average lack of fit		6.60	11.70	0.56

*value > 0.995