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Laboratory-scale anaerobic sequencing batch reactor for treatment of stillage from fruit distillation

Elena Cristina Rada, Marco Ragazzi and Vincenzo Torretta

ABSTRACT

This work describes batch anaerobic digestion tests carried out on stillages, the residue of the distillation process on fruit, in order to contribute to the setting of design parameters for a planned plant. The experimental apparatus was characterized by three reactors, each with a useful volume of 5 L. The different phases of the work carried out were: determining the basic components of the chemical oxygen demand (COD) of the stillages; determining the specific production of biogas; and estimating the rapidly biodegradable COD contained in the stillages. In particular, the main goal of the anaerobic digestion tests on stillages was to measure the parameters of specific gas production (SGP) and gas production rate (GPR) in reactors in which stillages were being digested using ASBR (anaerobic sequencing batch reactor) technology. Runs were developed with increasing concentrations of the feed. The optimal loads for obtaining the maximum SGP and GPR values were 8–9 gCOD L^{-1} and 0.9 gCOD g^{-1} volatile solids.

Key words | anaerobic digestion, ASBR, biogas, stillages

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INTRODUCTION

Stillage is a term used for some residues from distillery plants. These liquid residues are a big problem in the sector because of the high concentration of pollutants. However, distillery plants generate not only liquid discharges but also solid residues, the treatment of which is generally based on aerobic processes (Rada *et al.* 2009); in the case of liquid residues, both aerobic and anaerobic treatments are adopted (Deepak & Adholeya 2007).

The anaerobic process is widely used for treating wet residues to produce methane or bio-hydrogen (Bouallagui *et al.* 2005; Moletta 2005; Kaparaju *et al.* 2010; Luo *et al.* 2010a, b; Alkan-Ozkaynak & Karthikeyan 2011; Nasr *et al.* 2011; Wang *et al.* 2011). The aim of this process is to convert a large part of the chemical oxygen demand (COD) into biogas, thanks to the high efficiency of biochemical oxygen demand removal (Vlissidis & Zouboulis 1993; Wolmarans & de Villiers 2002). The anaerobic process has been studied over the years, from many points of view, and as a result, today, both the conventional (Fannin *et al.* 1982; Bories *et al.* 1998) and unconventional aspects (Rada & Ragazzi 2008; Braguglia *et al.* 2012; Tokumoto & Tanaka 2012) of this process are adequately known.

The work presented was developed on a laboratory scale as a first simplified step of an overall research aimed to set the design parameters for implementing the anaerobic digestion of stillage on a real scale. The aim of the present research was to measure the specific gas production (SGP) and gas production rate (GPR) from the anaerobic digestion of stillage under different conditions. The ASBR (anaerobic sequencing batch reactor) technology was used to develop the tests, as this process has demonstrated high stability when applied to stillage treatment (Farina *et al.* 2004). To this end, this paper contributes to the study of ASBR viability in the stillage treatment sector, which is often characterized by different kinds of anaerobic reactors (Melamane *et al.* 2007).

The properties of stillage can vary depending on the type of distillery substrate, local crop conditions, the distillery operation itself, etc. Thus, in general, results are greatly affected by particular conditions. The results obtained in this paper refer to stillage from grape distillation.

MATERIALS AND METHODS

The experimental apparatus (Figure 1) for batch anaerobic digestion tests included three reactors, each with a useful volume of 5 L, equipped with the following:
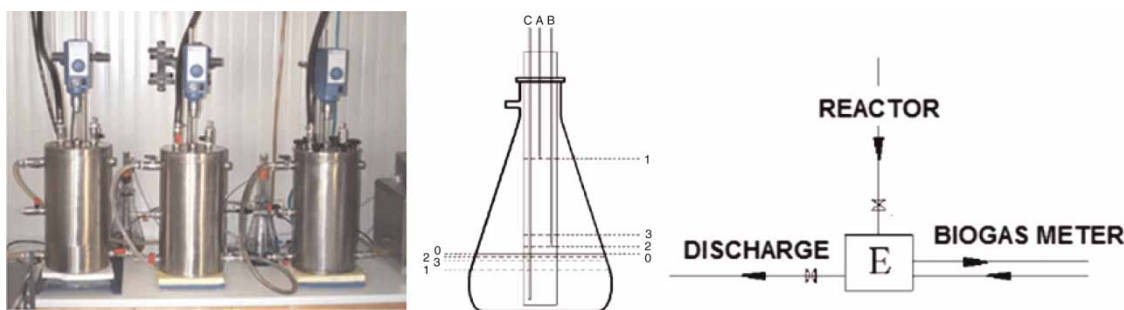
Figure 1 │ Anaerobic digestion reactors, biogas meter and electrovalve (E).

- **Digester:** a cylindrical steel container, that is resistant to corrosion, fitted with an external jacket. Two connection pipes were joined, and led to a thermostatic bath. Two lateral openings served to draw off sludge for sampling and for total emptying. Three openings on the cover allowed the mixer rod to be inserted, the biogas to be conveyed to the meter, and the substrate to be introduced.
- **Biogas meter:** a glass beaker with a double chamber, filled with 500 mL of water, in order to separate physically the interior and the exterior of the reactor. At the beginning of a test, under equilibrium conditions, the levels are at '0', as indicated in Figure 1. Once configuration '1' (maximum liquid level) is reached, the flow from the digester is interrupted and the connection valve to the outside is opened. Re-balancing the pressure brings the water column down to the position indicated by the number '2', the vent valve is closed and the connection to the reactor is reopened. The difference in level between '1' and '2' corresponds to a preset volume and is measured each time by an acquisition program. When the connection is reopened, the water column rises by about 1 cm, re-balancing the pressure of the meter with the pressure in the digester at the time of the interruption. This situation corresponds with the limit indicated by the number '3'. The volume discharged from time to time is set by calibrating the distance between the control electrodes, marked with the letters A (maximum level) and B (minimum level). A third electrode, marked C, serves as a common reference for the control processor, and therefore must always be immersed in the liquid. This approach kept the cost of the apparatus low. It would have been possible to prepare a NaOH solution in the gas meter to monitor the CH_4 production in the test. However, this option was not adopted for the presented runs, because a detailed methane generation analysis was planned for the second step of the overall research (on pilot scale).

- **A mixer:** the turns depend on the viscosity of the mixed sludge and on the friction that the mixer rods exert against the bronzes and the oil seals, which must be close-fitting to the rods in order to prevent any loss of biogas. The number of revs per minute is altered by electronically controlling the voltage output. Tests were carried out, with the sludge mixing speed kept constant.
- **Electrovalve:** it connects the digester with the biogas meter. The flow is interrupted when the liquid in the meter reaches the maximum level, when the known volume is sent outside; then it re-establishes the connection between the meter and the digester, by closing off the connection with the environment.

Each reactor was connected to an acquisition box, which allowed the process parameters to be continuously recorded and sent to a computer. The system was equipped with a thermostatic bath that kept the temperature inside the reactors constant ($35 \pm 1\,^{\circ}C$). The heating element was switched on automatically, whenever the temperature of the water went below a set value; it was switched off when it went above this limit. Finally, a pump kept the water recirculating, in order to maintain the temperature of the external jacket of the digesters steady.

The main characteristics of the simplified experimental apparatus presented is that the organic substance in a single reactor can be removed, which thereby avoids the need for a separate settler and for external recirculation of the biomass. The system entails a sequence of four phases: feeding, digestion, sedimentation and discharge.

In order to be able to check the development of the anaerobic digestion of stillage, the following parameters were determined:

- total solids (TS): with reference to the organic part and the inert part of the substrate;

- volatile solids (VS): with reference to the organic part of the dry substance;
- COD, through a $K_2Cr_2O_7$ solution in an acid environment;
- volumetric organic loading rate (OLR);
- OLR, with reference to the VS in the reactor (food/microorganism, F/M);
- GPR;
- SGP.

$$\text{OLR} = \frac{\text{VS}_{\text{in}}}{V_{\text{reactor}}} \text{ or OLR} = \frac{\text{COD}_{\text{in}}}{V_{\text{reactor}}} \qquad (1)$$

$$\frac{F}{M} = \frac{V_{\text{stillagein}}}{[\text{VS}_{\text{reactor}}]} \cdot \frac{[\text{COD}_{\text{stillage}}]}{V_{\text{reactor}}} \qquad (2)$$

$$\text{GPR} = \frac{Q_{\text{biogas}}}{V_{\text{reactor}}} \qquad (3)$$

$$\text{SGP} = \frac{V_{\text{biogas}}}{\text{VS}_{\text{in}}} \text{ or SGP} = \frac{V_{\text{biogas}}}{\text{COD}_{\text{in}}} \qquad (4)$$

where VS_{in}: total VS fed in (per cycle); COD_{in}: total COD fed in (per cycle); V_{reactor}: usable volume in the digester; $V_{\text{stillagein}}$: volume of stillage introduced into the reactor; $[\text{COD}_{\text{stillage}}]$: COD concentration of the stillages introduced; $[\text{VS}_{\text{reactor}}]$: concentration of the VS of the sludge present in the reactor; Q_{biogas}: biogas flow rate; V_{biogas}: volume of biogas produced in the time considered.

In the case of Equation (2), not all the VS concentration present in the inoculum (sludge) is directly microbial, so the microbial VS could be provided for a deeper analysis of the process. Another potential variation may be to due to the fact that both variables have the same unit of measure. Additional parameters measured were: ammonia and organic nitrogen, and filtered COD.

The primary aim of the tests was to measure the SGP and GPR. Three batch digestion tests were carried out with different organic volumetric loads, with a single feed and with different initial substrate concentrations; once the organic substance fed in was used up (reaching of the endogenous phase), the digesters were reloaded with an increasing target concentration of the organic substance (for several process cycles). One of the organic load values was applied for only one cycle:

- Reactor R1: load cycles of stillages with increasing concentrations: 1, 3, 5, 7, 9 gCOD L^{-1};

- Reactor R2: load cycles of stillages with increasing concentrations: 2, 4, 6, 8, 10 gCOD L^{-1};
- Reactor R3: load cycles of stillages with increasing concentrations: 10, 15, 20 gCOD L^{-1}.

These COD concentrations (COD L^{-1}) were assessed on the basis of the initial results obtained from the analysis of the stillages before a period of storage at low temperatures (2–5 °C). These values were corrected on the basis of the actual COD concentration in the stillages effectively loaded for each individual test, obtained using specific laboratory analyses. As the stillages before the test were kept at a temperature of 2–5 °C, a number of preliminary operations were carried out. The stillages were firstly warmed to a temperature of about 20 °C and treated, in order to obtain a homogeneous sample suitable for feeding into the reactor. In this way, thermal shock of the mesophilic biomass was avoided.

In order to carry out the anaerobic digestion tests, sludge was taken from a real anaerobic digester plant, which performed the anaerobic digestion of the excess activated sludge produced in the same wastewater treatment plant. This was used as inoculum for reactors R1, R2 and R3. This sludge was taken from time to time, and as it was not entirely stabilized, it was decided to stabilize it for 3–5 days. During this time, the sludge produced a non-negligible quantity of biogas, which could have interfered with the measurement of the biogas actually produced alone by the digestion of the stillages introduced into the reactors. Once the endogenous phase was reached, it was possible to proceed with the anaerobic digestion tests of the stillages, which were diluted according to the strategy described below. The stillages came from a grape distillery located in the North of Italy.

The subsequent tests were carried out, and the process parameters were corrected in accordance with the initial hypotheses:

- Reactor R1: load cycles of stillages with increasing concentrations: 0.7, 2.6, 4.9, 7.0, 8.8 gCOD L^{-1};
- Reactor R2: load cycles of stillages with increasing concentrations: 1.5, 3.4, 5.9, 8.0, 9.8 gCOD L^{-1};
- Reactor R3: load cycles of stillages with increasing concentrations: 8.0, 10.7, 15.7 gCOD L^{-1}.

The monitoring program recorded the biogas production as a function of time, and determined the accumulated biogas production curve and the production rate.

RESULTS AND DISCUSSION

Table 1 shows the results for the total COD (CODtot) and the filtered COD (CODf) for the stillage and sludge used. The low CODf/CODtot ratio may be explained as a consequence of organic matter entrapment. The values for ammonia, organic nitrogen, moisture and VS are also reported.

The characteristics of each individual test (one cycle each) are shown in Table 2, which highlights the fact that the assessed COD concentrations did not correspond exactly with the measured values.

All of the tests carried out followed a similar course: a quick initial production of biogas, because of the presence of rapidly biodegradable matter in the stillages; a following curve with a decreasing slope, corresponding to an ever decreasing production rate. The only exception to this

course was the curve for the anaerobic digestion of stillages with an initial concentration of 15.7 gCOD L^{-1}, in digester R3: after the initial degradation phase of the rapidly biodegradable matter, a slowing of the process was detected, followed by a slow recovery and an unstable phase. The 15.7 gCOD L^{-1} proved to be unfavorable to the biological anaerobic digestion reactions of the stillages, because of factors that inhibited this process. This may depend on an excessive concentration of toxic compounds, as a consequence of the limited dilution. Indeed, stillages contain phenolic compounds which have a high antibacterial activity (Melamane *et al.* 2007).

Figure 2, left, shows good alignment of the data, giving a SGP of 281 mL_{gas} g^{-1} COD_{add} ($R^2 = 0.99$). The differences are emphasized by analyzing the times necessary for the digestion of the substrate fed in. The GPR was calculated considering the time necessary for total consumption of

Table 1 | Characteristics of the anaerobic stillages and sludge used

Stillages

Code	COD_{tot} g L^{-1}	COD_f g L^{-1}	Ammonia mg L^{-1}	Organic N mg L^{-1}	Moisture %	VS %
B01	85.3	48.1	139	733	97.2	93.5
B02	98.8	52.7	140	841	–	93.9
B03	114.0	52.7	176	802	–	–
B04	98.0	54.5	135	776	91.7	94.6
B05	90.1	54.9	143	747	91.8	94.7
B06	86.8	52.1	160	673	90.7	94.6
B07	86.3	51.2	169	774	92.1	94.5

Sludge

Code	COD_{tot} g L^{-1}	COD_f g L^{-1}	Ammonia mg L^{-1}	Organic N mg L^{-1}	TS kg m^{-3}	VS kg m^{-3}
F01	11.4	2.1	713	457	24.0	19.1
F02	13.3	2.4	732	495	13.0	8.0
F03	12.4	1.9	665	492	13.5	9.3
F04	11.7	2.2	668	626	13.5	8.5
F05	11.3	3.8	626	509	11.4	7.8
F06	14.2	3.3	686	423	13.0	8.8
F07	14.1	3.3	673	297	13.3	8.7
F08	14.1	3.5	657	170	13.9	9.3
F09	13.1	3.1	644	30	14.0	9.5
F10	13.8	3.5	677	24	13.6	9.2
F11	15.4	4.2	687	102	11.9	7.5
F12	14.3	4.9	688	60	13.1	9.1
F13	16.4	4.7	647	60	14.7	10.2

Table 2 | Typical parameters of the sludge and stillages used

Notes	Stillage code	Sludge code	Reactor V L	F/M load gCOD$_{stillage}$/g^{-1}VS$_{sludge}$	Assessed COD load gCOD L^{-1}	Measured COD load gCOD L^{-1}
load 1 – R1	B01	F01	3.480	0.04	1	0.7
load 2 – R1	B02	F03	3.517	0.3	3	2.6
load 3 – R1	B03	F05	3.630	0.6	5	4.9
load 4 – R1	B04	F07	3.890	0.8	7	7.0
load 5 – R1	B04	F09	4.252	0.9	9	8.8
load 1 – R2	B01	F02	3.510	0.2	2	1.5
load 2 – R2	B02	F04	3.588	0.4	4	3.4
load 3 – R2	B03	F06	3.740	0.7	6	5.9
load 4 – R2	B04	F08	4.050	0.9	8	8.0
load 5 – R2	B04	F10	4.482	1.1	10	9.8
load 1 – R3	B05	F11	3.272	1.1	10	8.0
load 2 – R3	B06	F12	3.710	1.2	15	10.7
load 3 – R3	B07	F13	4.510	2.1	20	15.7
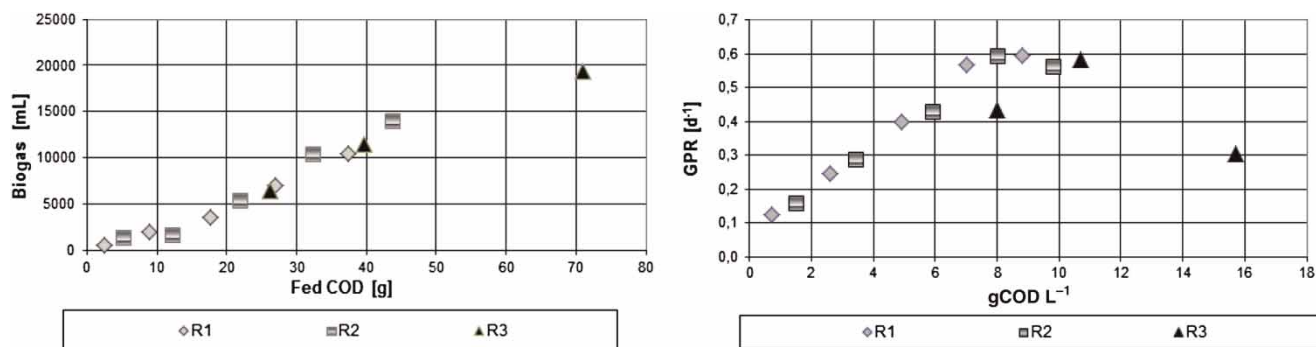
Figure 2 | Biogas production vs COD (left) and GPR values vs initial concentration of COD (right).

the COD introduced into the digester. There was an increase in the mean rate, up to values of 8–9 gCOD L^{-1} introduced, followed by a slight fall (Figure 2 right).

The GPR value of the first load into digester R3 was 8 gCOD L^{-1} and produced an anomalous result, possibly due to the fact that the first load suffered from incomplete acclimatization of the bacteria present in the sludge for the anaerobic digestion of the stillages.

The optimal loads for obtaining the maximum SGP and GPR values were 8–9 gCOD L^{-1} and 0.9 gCOD g^{-1}VS (load 5 R1 and load 4 R2). Under these conditions, the complete consumption of the stillages was achieved in about 4 days, as resulted from the dynamics of biogas production. By increasing the concentration of COD introduced, with similar levels of SGP and GPR, the consumption dynamics lasted longer. From these results, it is clear that dilution is a key factor for the optimization of the treatment of stillages.

Table 3 shows the accumulated production of biogas, and the bio GPR.

From an analysis of these parameters (particularly from the biogas generation slopes, which show a clear peak in the GPR curve for each run), it was found that about 30% of the COD introduced was consumed within 10–16 hours after the beginning of the tests (Table 4). For these analyses, the tests with a low incoming initial COD load were discarded. Low values of COD fed in did not enhance the change in the gradient of the biogas production curve as a function of time; this made identification of this variable difficult.

Table 3 | Table summarizing the SGP and GPR values for each test

Notes	Test duration Days	Biogas produced mL	SGP mL g^{-1}COD	GPR L V$_{reactor}^{-1}$ d^{-1}
load 1 – R1	1.142	490	191	0.123
load 2 – R1	2.283	1,960	218	0.244
load 3 – R1	2.495	3,621	205	0.400
load 4 – R1	3.205	7,050	261	0.565
load 5 – R1	4.106	10,416	279	0.597
load 1 – R2	2.638	1,462	286	0.158
load 2 – R2	3.159	3,248	267	0.287
load 3 – R2	3.356	5,380	246	0.429
load 4 – R2	4.343	10,454	323	0.594
load 5 – R2	5.575	14,048	321	0.562
load 1 – R3	4.486	6,365	242	0.434
load 2 – R3	5.278	11,404	288	0.582
load 3 – R3	13.972	19,298	272	0.306

Table 4 | Values of rapidly biodegradable COD and removal times (see also Appendix, available online at http://www.iwaponline.com/wst/067/611.pdf)

Notes	Rapidly biodegradable COD % on CODtot	Removal time Hours
load 3 – R1	31.4%	9.7
load 4 – R1	30.0%	12.6
load 5 – R1	34.6%	16.5
load 3 – R2	29.3%	10.2
load 4 – R2	30.0%	13.0
load 5 – R2	29.2%	11.6
load 2 – R3	30.9%	15.4

These tests also correspond with the initial tests, which might present problems of acclimatization of the biomass, and therefore underestimate the measurement of the biogas production, as seen in the load 1 test in digester R3. The load 3 test in digester R3 was discarded because it presented an entirely different course from that of the other tests.

CONCLUSIONS

The work presented here demonstrates that some design parameters for an ASBR plant intended to treat stillage from fruit distillation can be established by developing a simplified laboratory-scale study. With a limited number of experimental runs, the optimal loads for obtaining the maximum SGP and GPR values were found: 8–9 gCOD L^{-1} and 0.9 gCOD g^{-1}VS. Under these conditions the complete consumption of the stillages can be achieved in about 4 days, as demonstrated by the biogas generation curves. Higher concentrations of COD in the feed needed longer times for completion of the process (levels of SGP and GPR were similar). Dilution was a key factor in the optimization of the stillage treatment. More specifically, around 30% of the COD was rapidly biodegradable.

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