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Kinetic study of the methanogenic step of a two-stage anaerobic digestion process treating olive mill solid residue

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ABSTRACT

A kinetic study of the methanogenic step of a two-stage anaerobic digestion process treating two-phase olive oil mill solid residue (OMSR) was conducted at mesophilic temperature (35 °C). The anaerobic digestion of OMSR was carried out in two different steps. After a hydrolytic–acidogenic stage, working at an organic loading rate (OLR) of 12.9 g COD L^{-1} day^{-1} (COD: chemical oxygen demand), the effluents or acidified OMSR obtained were employed for feeding a second or methanogenic step. For the methanogenic step, OLRs of between 0.8 and 22.0 g COD L^{-1} day^{-1} were studied (corresponding to hydraulic retention times (HRTs) of between 142.9 and 4.6 days).

The substrate treated in the second phase (acidified OMSR) had a high total concentration in volatile fatty acids (14.5 g CH_3COOH L^{-1}) and a high percentage of acetic acid as the main methane precursor (57.5% of the total concentration). As a consequence of the first step a high stability in the methanogenic stage was achieved.

A total chemical oxygen demand balance was developed over the methanogenic step. For this model two considerations were taken in account: (1) volumetric flow constant during the experiments (the volume of effluent that was taken from the methanogenic reactor every day was equal to the volume of acidified OMSR fed). (2) Constant concentration of methanogenic microorganisms during the experiments (the slow growing rate of the methanogenic microorganisms makes it possible for the concentration of microorganisms over the process to remain constant). The cellular maintenance coefficient (m) and methane yield coefficient ($Y_{G/S}$) were found to be 0.016 g COD removed g^{-1} VSS day^{-1} and 0.261 L CH_4 g^{-1} COD removed, respectively.

1. Introduction

The olive mill solid residue (OMSR) is the principal waste generated in the olive oil extraction process by using a two-phase decanter centrifugation system [1]. This waste is produced in a proportion of 800 kg ton^{-1} of olives processed. Its characteristics (high humidity, low pH, high content in solids/organic matter, presence of inhibitory compounds as poly-phenols, etc.) make it a very pollutant waste. At present, 90% of the olive mills in Spain use the two-phase decanter system because a great reduction in the water consumption of the milling process is aimed at. It means that between 2 and 4 million tons of this waste are generated annually [2]. These high quantities produced generate large-scale environmental problems for Spain and in particular for Andalucía, the region where most of the mills are located [1].

An extensive bibliography has detailed the benefits of two-stage anaerobic digestion as the separation of phases in anaerobic digestion processes providing good stability to the different groups of microorganisms and allowing a more specific control of the conditions required for each one of them [3] while very often yielding higher efficiencies. It is clear that the microorganisms that work in the anaerobic digestion processes (hydrolytics, acetogenics and methanogenics [4]) have different physiological and nutrient requirements, levels of sensitivity to the environmental conditions and growing kinetics. By phase separation, the action of these microorganisms is improved as a consequence of the enrichment of the different populations of microorganisms [5]. This separation prevents the accumulation of intermediate metabolic compounds like volatile fatty acids that could be very dangerous for the methanogenic step [6]. At the same time, the physical separation of both stages can improve the performance to be achieved in each one, helping the development of the limiting step (hydrolytic–acidogenic step [7] or the methanogenic step [8]).

The treatment by anaerobic digestion in one stage of this substrate gives a significant benefit as consequence of the obtained

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methane yield [9]. However, this is clearly higher when the process is separated into two stages [10].

The aim of this work was the development of a total chemical oxygen demand balance over the methanogenic step of a two-stage anaerobic digestion process treating two-phase OMSR. This balance allowed for the calculation of the cellular maintenance coefficient and methane yield coefficient.

2. Materials and methods

2.1. Feed characteristics

The waste used in the experiments was two-phase olive mill solid residue. The olives employed in the milling process were of the "Picual" variety with a low ripening level and were harvested at the beginning of the olive season. This substrate was collected from the experimental olive oil factory located at the "Instituto de la Grasa" (CSIC), Seville (Spain). After collection, the samples were stored at 4 °C to preserve the original characteristics of the residue.

The main characteristics and composition of the OMSR used in the experiments were: T-COD (total chemical oxygen demand): 162.0 g L^{-1}, S-COD (soluble chemical oxygen demand): 57.5 g L^{-1}, P-COD (particulate chemical oxygen demand): 104.5 g L^{-1}, S-OC (soluble organic carbon): 22.2 g L^{-1}, TS (total solids): 143.0 g L^{-1}, MS (total mineral solids): 17.0 g L^{-1}, VS (total volatile solids): 126.0 g L^{-1}, TSS (total suspended solids): 106.0 g L^{-1}, MSS (mineral suspended solids): 11.0 g L^{-1}, VSS (volatile suspended solids): 95.0 g L^{-1}, TVFA (total volatile fatty acids): 1.4 g L^{-1}, Palk (partial alkalinity): – g L^{-1}, Talk (total alkalinity): 1.1 g L^{-1}, total phenols (as caffeic acid): 15.0 g L^{-1} and phosphorous: 0.0035 g L^{-1}, oils and fats: 2.2%, moisture: 86.7% and pH: 5.3. Values are averages of six determinations; there was virtually no variation (less than 5%) between analyses [10].

Before the methanogenic step was carried out, the OMSR was acidified in a hydrolytic–acidogenic reactor. For this acidification process a 1.5 L reactor was used working at an OLR of 12.9 g COD L^{-1} day^{-1} (HRT = 12.4 days) (OLR: organic loading rate, HRT: hydraulic retention time). This previous phase, under controlled conditions, meant that a solubilised substrate was obtained [11]. The characteristics of the hydrolytic–acidogenic effluent (acidified OMSR) or influent used for feeding the methanogenic reactor were: pH = 6.0, Palk and Talk (as $CaCO_3$) = 0.4 and 7.7 g L^{-1}, T-COD = 99.4 g L^{-1}, S-COD = 45.0 g L^{-1}, P-COD = 54.4 g L^{-1}, S-OC = 12.5 g L^{-1}, VS = 66.0 g L^{-1} and total phenols (as caffeic acid) = 8.9 g L^{-1}. The acidified OMSR had a TVFA concentration of 14.5 g L^{-1} (expressed as CH_3COOH) with 57.5% acetic acid of the TVFA [10].

2.2. Inoculum

The inoculum used was an anaerobic sludge from an industrial reactor treating brewery wastewater. The characteristics of the inoculum used were: pH: 8.1; TSS: 34.9 g L^{-1}; MSS: 8.9 g L^{-1}; VSS: 26.0 g L^{-1}; TS: 37.4 g L^{-1}; MS: 11.0 g L^{-1} and VS: 26.4 g L^{-1} (all values were averages of triplicate samples with standard deviations lower than 5%).

At the beginning of the experiments 1 L of sludge, 0.4 L of a nutrient-trace element solution and 0.4 L of distilled water were used for starting up the reactor, keeping the effective reactor volume at 1.8 L. An inoculum/support media (saponite) ratio 1:1 was kept.

The nutrients were only added at the beginning of the experiments, and no additional nutrients were added to the reactor after the start-up. A detailed description of this nutrient-trace element solution is given elsewhere [12].

2.3. Equipment

The methanogenic reactor was fed with acidified OMSR (from a previous acidogenic step [11]). For the methanogenic step, an anaerobic stirred tank reactor with an effective working volume of 1.8 L was employed. The reactor was manually fed on a daily basis with the corresponding volume of acidified OMSR by means of an external feeder, and at the same time the same volume of effluent was removed. The temperature was kept at the mesophilic range (35 ± 2 °C). An adequate mass transfer between the inoculum and substrate was kept using a magnetic stirrer, keeping an appropriate stirring level (260 rpm).

The reactor was provided with a low density (0.8 g mL^{-1}) magnesium silicate support media called saponite ($(Mg,Fe)_3(Si,Al)_4O_{10}(OH)_2 \cdot 4H_2O$) and with a 0.5 L settler situated at the top. These devices prevent the loss of microorganisms with the reactor effluents [10].

The biogas produced was collected by a water displacement system (8 L Boyle–Mariotte reservoir) fitted to the reactor. CO_2 produced in the process was scrubbed by bubbling the gas mixture through a NaOH solution (3 M) before its entry into the reservoir; therefore, the volume of water collected was equivalent to the volume of methane produced [11].

2.4. Experimental procedure

Before starting the experiments, an adaptation or acclimatization of the inoculum to the substrate studied was carried out with different dilutions of the substrate [10]. Once the biomass of the reactor was acclimated, the experiment was started using acidified OMSR (100%) and an organic loading rate of 0.8 g COD L^{-1} day^{-1}.

OLRs from 0.8 g to 22.0 g COD L^{-1} day^{-1} corresponding to HRTs of between 142.9 and 4.6 days were assessed. The different OLRs, HRTs studied and the daily volume of acidified OMSR fed (q) to the methanogenic reactor throughout the experiments are shown in Table 1. During the experiments, an ammonia solution (15%) was used to keep the substrate pH (5.5–6.0) improving the consumption of acetic acid.

Once the steady-state conditions were achieved for each run studied (after at least 2–3 hydraulic retention times and when the deviations between the observed values of the consecutive measurements of a specific parameter were less than 5%) the samples were collected for analysis over a period of at least 5 consecutive days, constituting 5 different samples to ensure that representative data were obtained. The pH and the CH_4 volumes produced were determined daily.

Table 1
Organic loading rates (OLR), daily volume of acidified OMSR fed (q) to the methanogenic reactor and hydraulic retention times (HRT) studied at the methanogenic step.

OLR (g COD L^{-1} day^{-1})	q (L day^{-1})	HRT (days)
0.8	0.013	142.9
2.0	0.034	52.9
3.5	0.060	30.0
5.0	0.086	21.0
6.5	0.111	16.2
8.6	0.146	12.3
10.5	0.172	10.5
12.8	0.230	7.8
14.0	0.253	7.1
15.5	0.280	6.4
17.0	0.310	5.8
18.5	0.345	5.2
20.0	0.362	5.0
22.0	0.395	4.6

3. Analytical methods

The analyses were performed according to the recommendations of the Standard Methods of APHA [13].

Palk and Talk were determined using the 2320B method. The pH was analyzed with a pH-meter (Crison, model basic 20). T-COD, S-COD and P-COD were determined according to the method number 5220C. TS, MS, VS, TSS, MSS and VSS were analysed according to the method numbers 2540B and 2540E. S-OC was measured using a Dohrmann DC-190 analyser after filtrating the samples with a 0.45 μm acetate filter (Whatman).

Phosphorous was measured by spectrophotometry at 880 nm, using the normalized methods 4500-P, B and E. Finally, oils and fats were analysed by Soxhlet extraction with n-hexane using the official method of the EEC Nº2568/91 (European Community Official Diary, L248/1 of 05.09.1991).

Gas chromatographic analyses were carried out for determination of the total volatile fatty acids and partial volatile fatty acid species (acetic, propionic, butyric, isobutyric, valeric and isovaleric acids). A detailed description of the gas chromatograph used is given elsewhere [11].

Phenolic compounds were extracted beforehand [14] and measured at 725 nm by spectrophotometry using the Folin–Ciocalteau method [9].

4. Results and discussion

4.1. Process evolution

The existence of an initial hydrolytic–acidogenic stage made the methanogenic step a very stable process. It allowed the system to achieve OLRs as high as 20.0 g COD L^{-1} day^{-1} [10]. Similar OLRs were achieved in the methanogenic step of other two-stage anaerobic digestion processes [15–17].

For the methanogenic step all the parameters achieved had appropriate values up to HRTs of 5 days. The pH was very stable for OLRs in the range of 0.8–20.0 g COD L^{-1} day^{-1}, fluctuating around 7.0. Sufficient alkalinity levels were observed in the reactor, which aided in buffering the pH values during the experiment. Only after an OLR of 17.0 g COD L^{-1} day^{-1}, did the partial alkalinity (or bicarbonate alkalinity) start to decrease, as a consequence of the consumption of bicarbonates due to the increase in volatile fatty acids in the system. As the experiments progressed and the HRTs became shorter, the concentration of organic matter in the effluents taken from the methanogenic reactor was higher. In this way, T-COD, S-COD, P-COD and solid concentrations increased with decreased HRT. In the hydrolytic–acidogenic reactor, a large amount of the easily degradable matter of the OMSR was transformed into volatile fatty acids (14.5 g L^{-1} as CH_3COOH). However, the concentration of TVFA at the effluents of the methanogenic reactor was very low throughout the process (less than 1 g L^{-1}). This high conversion into methane did not allow for any accumulation of TVFA in the system. The TVFA concentration was only increased over 1 g L^{-1} (3 g L^{-1}) for the last and highest OLR studied (22.0 g COD L^{-1} day^{-1}, corresponding to a HRT of 4.6 days) where concentrations for acetic and valeric acid were higher than the inhibitory concentrations reported in the literature [10,18,19].

The methane yield coefficient obtained, measured at standard temperature and pressure conditions, was 0.268 ± 0.003 L CH_4 g^{-1} COD removed [10], which was higher than that observed in the one-stage anaerobic digestion process of this substrate (0.244 ± 0.005 L CH_4 g^{-1} COD removed) [20].

The fraction of organic matter (expressed as total chemical oxygen demand) transformed into methane can be calculated by this theoretical expression:

$$R_{CH_4}\ (\%) = \frac{r_{CH_4} \cdot 100}{0.350 \cdot \mathrm{OLR}} \quad (1)$$

where R_{CH_4}: methane yield percentage or fraction of organic matter transformed into methane (%); r_{CH_4}: methane production rate (at standard temperature and pressure conditions, L CH_4 $L^{-1}_{reactor}$ day^{-1}); 0.350: conversion theoretical factor of total chemical oxygen demand in methane (L CH_4 g^{-1} COD removed) [21].

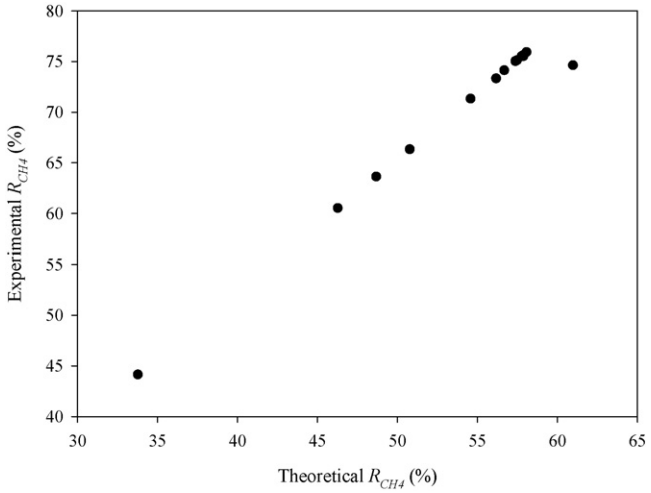

Fig. 1. Theoretical versus experimental R_{CH_4} values (%) obtained.

A first approximation of the values of the methane yield percentage using the Lawrence and McCarty coefficient [21] gave lower values of R_{CH_4}. Using the experimental methane yield obtained in the process, higher values for R_{CH_4} were obtained. Fig. 1 shows both theoretical and experimental values obtained.

The values of R_{CH_4} were kept between 74.6% and 75.6% for OLRs in the range of 0.8–12.8 g COD L^{-1} $days^{-1}$. The values started falling slightly at an OLR of 14.0 g COD L^{-1} day^{-1} (74.1%) and decreased to 60.5% at an OLR of 20.0 g COD L^{-1} day^{-1}, where the maximum methane production was achieved. For higher OLRs (≥22.0 g COD L^{-1} day^{-1}) the yield decreased to 44.1%. The yield obtained at the OLR of 20.0 g COD L^{-1} day^{-1} (OLR where the maximum methane generation is produced) was 60.5% which is quite high taking into account this high OLR.

4.2. Kinetic evaluation

By making a total chemical oxygen demand balance around the methanogenic reactor, the following equation is obtained:

$$q \cdot (\text{T-COD})_o = q \cdot (\text{T-COD})_e + \left(\frac{1}{Y_{G/S}}\right) \cdot q_{CH_4} + m \cdot V \cdot X \quad (2)$$

where q: daily volume or flow rate of acidified OMSR fed to the methanogenic reactor and of the effluent outgoing (L day^{-1}); q_{CH_4}: daily volume or flow rate of methane produced (L CH_4 day^{-1}); $(\text{T-COD})_o$: total chemical oxygen demand of the influent (g L^{-1}); $(\text{T-COD})_e$: total chemical oxygen demand of the effluent (g L^{-1}); $Y_{G/S}$: methane yield coefficient (L CH_4 g^{-1} COD removed); m: cellular maintenance coefficient (g COD consumed g^{-1} VSS in the reactor day^{-1}); X: concentration of microorganisms (g VSS L^{-1}); V: volume of the methanogenic reactor (L).

The following assumptions were made to obtain Eq. (2): (1) the volumetric flow was constant during the experiment. This means that the volume of effluent that was taken from the methanogenic reactor every day was the same volume as the acidified OMSR fed

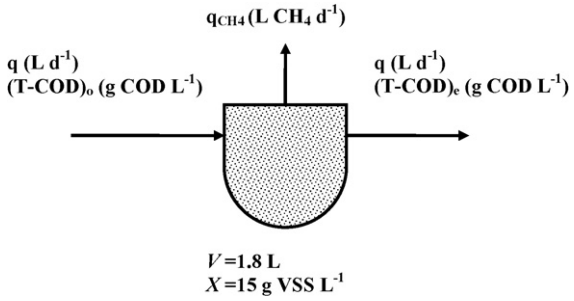

Fig. 2. Inlet and outlet flows taken into account in the total chemical oxygen demand balance around the methanogenic reactor.

to the reactor. (2) Taking into account the slow growing rate for the methanogenic microorganisms, no important variation in the concentration of microorganisms during the experiments was shown. For this reason the biomass concentration was assumed constant throughout the process.

A similar approach for separating the hydrolytic–acidogenic and methanogenic steps was developed by López and Borzacconi [22] to simulate an anaerobic digestion process in a full-scale upflow anaerobic sludge blanket reactor. These authors assumed that in the first step, the acidogenic bacteria consume the organic substrate and produce volatile fatty acids and CO_2 (and more bacteria) and next, methanogenic population consumes these acids and produce methane and more microorganisms. The biomass growth obtained by these authors for this reactor gave values as low as 0.01 day^{-1} [22].

The total chemical oxygen demand that went into the reactor was mainly used by the microorganisms for the generation of methane and cellular maintenance, but some of this total chemical oxygen demand came out of the reactor without suffering any transformation. In this T-COD that came out of the methanogenic reactor without any transformation, a small quantity of microorganisms can be included; those that came with the effluents and were lost with them when the effluents were removed from the reactor. Therefore, the first member of Eq. (2) represents the total chemical oxygen demand that goes into the reactor from the acidified OMSR while the second one is the sum of the total chemical oxygen demand which goes out without any transformation, the chemical oxygen demand that was transformed into methane and the chemical oxygen demand used for cellular maintenance.

Fig. 2 illustrates and schedules the acidified OMSR inlet and effluent flow and different T-COD concentrations used for calculating the mass balance around this reactor.

Eq. (2) can be converted into the following equation:

$$q \cdot [(\text{T-COD})_o - (\text{T-COD})_e] = \left(\frac{1}{Y_{G/S}}\right) \cdot q_{CH_4} + m \cdot V \cdot X \qquad (3)$$

Fig. 3 shows the different values obtained for T-COD of the effluents and the volume of methane obtained per day for each OLR studied until the process fail when the methane production started decreasing. With these values and the values of methane flow rate using Eq. (3) Fig. 4 was plotted. Fig. 4 plots the variation of the first member of Eq. (3) against the daily volume of methane produced. Using linear regression by the least-squares method, the points were adjusted to a straight line. The determination coefficient, R^2, obtained was 0.9785.

Using the values of the slope and the intercept obtained, the cellular maintenance coefficient (m) and methane yield coefficient ($Y_{G/S}$) were calculated. The inverse value of the line slope obtained was $Y_{G/S}$ = 0.261 L CH_4 g^{-1} COD removed. This value is very similar to that obtained from the experimental data, which was 0.268 ± 0.003 L CH_4 g^{-1} COD removed [10].

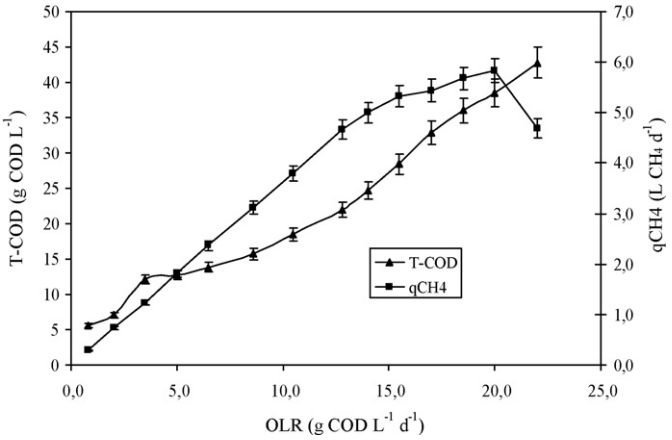

Fig. 3. Total chemical oxygen demand of the effluents of the methanogenic step and methane production (q_{CH_4}) at standard pressure and temperature conditions obtained for the different OLRs studied.

m was calculated from the value of the intercept, as the intercept was equal to $m{\cdot}V{\cdot}X$, where V or volume of the reactor was known (1.8 L) and X is the constant concentration of microorganism (15 g L^{-1}). In this case, the obtained value of m was 0.016 g COD removed g^{-1} VSS day^{-1}. This obtained value for m was low. This fact showed the low requirement of the microorganisms for their maintenance. However, the real value of m should be somewhat higher than the value calculated by the model, as X, or microorganisms' concentration during the experiments, took both active microorganisms and many of some non-biologic solids contained in the reactor into account. If the non-biologic solids contribution had been subtracted from the microorganisms' concentration, the real biomass fraction or X_{Real} ($X_{Real} = X$ – non-biologic solids concentration) would have been lower, with m higher. The value of m obtained in the methanogenic step of the two-phase anaerobic digestion process of OMSR is of the same order of magnitude as the values obtained in the one step anaerobic digestion of classical olive mill wastewaters (OMW) previously defenolized or fermented with pre-treatments with *Aspergillus terreus* (0.014 g COD g^{-1} VSS day^{-1}) or *Azotobacter chroococcum* (0.020 g COD g^{-1} VSS day^{-1}). The defenolized OMW (when 90–94% of the fenolic content is removed) is a non-inhibitor substrate which is easily degradable by anaerobic digestion [23].

The poly-phenolic compounds present in the OMSR were reduced to 40.7% of its initial value in the first stage

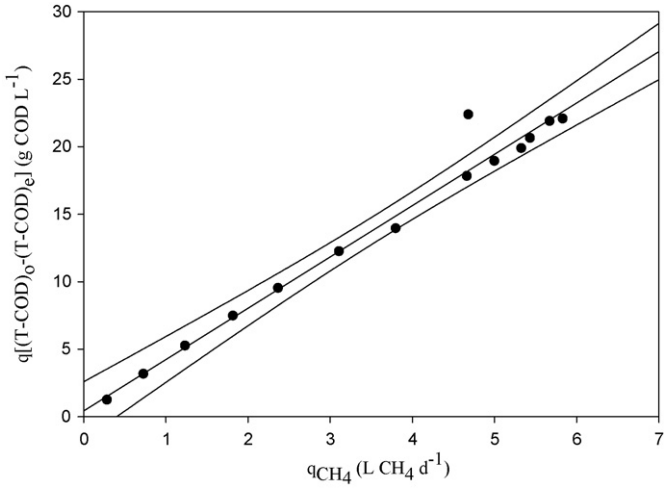

Fig. 4. Plot of the first member of Eq. (3) versus the methane produced per day.

(hydrolytic–acidogenic) with the initial concentration in the influent fed to the methanogenic reactor 8.89 g L^{-1} (expressed as caffeic acid). This previous elimination could also help to improve the performance of the methanogenic step, with the concentration of these compounds in the final effluents 5 g L^{-1} (at an OLR of 20.0 g COD L^{-1} day^{-1}) [10].

5. Conclusions

A total chemical oxygen demand balance around the methanogenic reactor meant that calculated values for methane yield coefficient ($Y_{G/S}$) and the cellular maintenance coefficient (m) were obtained. The calculated $Y_{G/S}$ value was very similar to that obtained from the experimental data. The coefficient m was of the same order of magnitude as others obtained for the anaerobic digestion in one step of OMW previously defenolized with pre-treatments with *A. terreus* and *A. chroococcum*. It was demonstrated that the methanogenic degradation of acidified olive mill solid residue from a previous hydrolytic–acidogenic reactor is very stable and effective as the elimination of phenolic compounds at the first stage (hydrolytic–acidogenic) improved the performance of the methanogenic step.

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