Performance evaluation and substrate removal kinetics in the semi-continuous anaerobic digestion of thermally pretreated two-phase olive pomace or “Alperujo”

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ABSTRACT

A study of the semi-continuous anaerobic digestion process of thermally pretreated two-phase olive pomace (TPOP) at 120 °C during 180 minutes was carried out in laboratory-scale completely stirred tank reactors at mesophilic temperature. Organic loading rates (OLR) in the range of 2.0-7.0 g VS/(L·d) were tested after the pretreatment and the breakage of complex organic matter. Total and soluble chemical oxygen demand (COD_t and COD_s) removal efficiencies decreased slightly between 77.9-71.7% and 92.3-85.0% when the OLR increased from 2 to 5 g VS/(L·d). However, when the OLR increased to 7.0, a sudden decrease in process performance was observed. The maximum methane production rate (1.72 L CH₄/(L·d)) was achieved at an OLR of 4.5 g VS/(L·d). This value was 39.8% higher than that obtained in the semi-continuous anaerobic digestion of

untreated TPOP at the same OLR. The modified Stover-Kicannon model and the Grau second-order kinetic model were found to be adequate to fit the experimental results of COD_t and COD_s, respectively.

Keywords: two-phase olive pomace; thermal pretreatment; semi-continuous anaerobic digestion

1. INTRODUCTION

Over the last 20 years the manufacture of olive oil has undergone important evolutionary changes in the equipment used for the separation of olive oil from the remaining components. The latest development has been the introduction of a two-phase centrifugation process in which a horizontally-mounted centrifuge is used for a primary separation of the olive oil fraction from the vegetable solid material and vegetation water. Therefore, the new two-phase olive oil mills produce three identifiable and separate waste streams. These are: 1) the wash waters from the initial cleansing of the fruit; 2) the wash waters from the secondary centrifuge and 3) the aqueous solid residues from the primary centrifugation. As well as offering process advantages they also reduce the water consumption of the mill. The introduction of this technology was carried out in 90% of Spanish olive oil factories. Therefore, the new two-phase olive mill effluents (TPOME) are made up of the mixture of effluents (1) and (2). The total volume of TPOME generated being around 0.25 L/kg of olives processed. In addition, the solid residue, the two-phase olive mill solid waste (OMSW) or two-phase olive pomace (TPOP) has a high organic matter concentration giving an elevated polluting load and it cannot be easily handled by traditional technology which deals with the conventional three-phase olive cake (Borja et al., 2002; Rincón et al., 2008a).

Spain was the first country where the two-phase system was used and from there this new technology was installed around the world. The two-phase decanting reduces the water requirements. Nevertheless, it has created the new solid residue, TPOP, which has high organic matter content, high humidity, low pH, presence of inhibitory compounds as poly-phenols, etc., which make it a very pollutant waste. The high polluting power and large volumes of this solid waste (around 2-2.5 millions of tons per year in Spain) can pose large-scale environmental problems, taking into account the 2000 Spanish olive oil factories, most of them located in the south of the country (Borja et al., 2006; Rincón et al., 2009).

Anaerobic digestion (AD) of solid wastes is an efficient, attractive and well-established option for solid waste treatment because of its excellent waste stabilization and high energy recovery (Page et al., 2014). AD of particulate materials and complex compounds is carried out by anaerobic microorganisms (Rincón et al., 2013) and it occurs in four biological steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Hall & Howe, 2012).

The results obtained previously in the anaerobic digestion of different concentrations of TPOP were evaluated using the Chen-Hashimoto methane production model to determine the maximum microorganisms specific growth rate and kinetic constants of the process (Borja et al., 2003). The above-mentioned kinetic constants were affected by the influent substrate concentration and decreased by 63% and 65%, respectively, when the feed concentration increased from 34.5 to 113.1 g COD/L (COD: chemical oxygen demand). This behaviour is believed to be due to the higher levels of phenolic compounds and biotoxicity present in the most concentrated substrate used. Methane production was reproducible within deviations of up to 10% (Borja et al., 2003). Another simultaneous study of anaerobic biodegradation of TPOP carried out with different concentrations,

included 100% TPOP, at hydraulic retention times (HRTs) of between 40.0 and 8.3 days, revealed that the increase in influent substrate concentration favoured the process failure reducing the pH and increasing the ratio of total volatile fatty acids (VFA) to total alkalinity (TA) (Borja et al., 2004). This ratio was found to be proportional to the substrate concentration (S), as follows: $VFA/TA = 0.04 (S)$. The inhibition kinetic Andrews model was used to describe the relationship between anaerobic degradation of COD and volatile solids (VS) and the formation of methane. The values of the kinetic constants for COD_t removal were determined to be 28 g COD_t/(L·d), 27 g COD_t/L and 352 g COD_t/L, respectively, for maximum substrate utilization rate (R_{Smax}), saturation constant (K_S) and inhibition constant (K_i). Process inhibition started at COD_t and VS concentrations of around 20 and 18 g/L, respectively. The rates of COD_t removal were lower than those observed for VS removal and inhibition of VS removal occurred at a lower concentration compared to that for COD_t. Inhibition of methane formation started at a substrate concentration of around 17 g COD_t/L. COD_t and VS removal rates were higher than the rates of methane formation and these differences increased when the substrate concentration increased. This fact was underlined by the decrease of pH, the increase of VFA/TA ratio and the reduction of methane production rate.

Hydrolysis is generally the rate-limiting step when bacteria release extracellular enzymes that break down and further solubilise organic particulate matter to be used as substrate in subsequent reactions (Jackowiak et al., 2011). Therefore, to improve digestion efficiency, the most productive approach is to disrupt the chemical bonds in the material to be subjected to hydrolysis. In fact, the structure and composition of lignocellulosic compounds makes its AD especially difficult (Carrere et al., 2010 and 2016; Hendriks and Zeeman, 2009). In addition, the presence of lignin in this structure acts as a physical barrier that induces a non-productive adsorption of the enzyme. Thus, with a view to disrupting

the lignocellulose structure of the wastes in order to increase its anaerobic digestibility, pretreatment would appear to be not only ideal but also necessary in some cases. The efficiency of a pretreatment can be evaluated by the generated matter solubilization, the increase of anaerobic biodegradability and its cost.

Thermal pretreatments are those where biomass or complex substrate is solubilized by applying heat. Their performance may be influenced by both temperature and exposure time. However, temperature seems to be the most influencing factor on biomass disintegration and anaerobic biodegradability.

Thermal pretreatments have long been used for enhancing particulate organic matter disintegration at temperatures from 50 to 270 °C (Carrère et al., 2016; Hendriks and Zeeman, 2009). However, the optimal temperature range depends on the substrate characteristics. In the case of waste activated sludge (WAS), temperatures above 180 °C may lead to the formation of recalcitrant compounds, reducing biomass anaerobic biodegradability. On the other hand, lignocellulosic biomass starts solubilizing at temperatures above 120–180 °C, and only temperatures above 250 °C are to be avoided (Hendriks and Zeeman, 2009). There are studies that with the same purpose, organic matter disintegration, combine temperature and pressure (Rincón et al., 2016).

On the other hand, a key point defining the relevance of thermal pretreatment is the energy balance. Heat integration in the whole plant is essential (Perez-Elvira and Fdz-Polanco, 2012). For example, heat from pretreated sludge can be recovered to heat the digester or to preheat influent substrate. In addition, the initial solids concentration of sludge is a key parameter. In the case of combined heat and power generation from biogas, heat is often produced in excess and thermal treatment, using the heat released from biogas combustion is more energy effective than technologies that make use of electrical power (e.g. microwave heating) (Carrere et al., 2010). Thermal pretreatment of sewage sludge has

been studied with good results to reduce the quantity of sludges and improve the biogas yields obtained. The treatment at 170°C gave better performance than without pretreatment, with about 80% improvement in organic matter removal and biogas yield (Bougner et al., 2006).

Thermal pretreatment of WAS has been heavily investigated, while other complex substrates such as lignocellulosic wastes have received minor attention. A previous paper showed the influence of a thermal pretreatment on TPOP solubilization and methane production in biochemical methane production (BMP) tests carried out in batch mode (Rincón et al., 2013). Temperatures in the range of 100-180 °C were tested during 60, 120, and 180 minutes. The highest COD solubilization after pretreatment (42%) was found for 120 °C and 180 °C during 180 minutes in both cases. BMP tests of the pretreated TPOP at the above-mentioned conditions showed two different stages: a first exponential stage and a sigmoidal zone after a lag step. During this second stage the pretreated TPOP at 120 °C during 180 minutes showed an increase in the maximum methane production of 22.2% and 25.1% than those obtained for untreated TPOP and thermally pretreated TPOP at 180 °C during 180 minutes, respectively.

The aim of the present research study was to assess the influence of a thermal pretreatment of TPOP at 120 °C during 180 minutes on the semi-continuous anaerobic digestion of this pretreated waste. A previous study detailed the biochemical methane potential (discontinuous mode) of TPOP in optimized conditions (Rincón et al., 2013), the current study goes further working in semicontinuous mode. The semicontinuous operation mode allow us to know more about the characteristics of the anaerobic digestion that would be carried out in an industrial exploitation of this waste. The effect of the organic loading rate (OLR) and HRT on methane yield, methane production rate and biodegradability of the substrate was evaluated. The modified Stover-Kincannon model

and Grau second order model were used to determine the substrate removal kinetics of the anaerobic reactor for total and soluble COD, respectively.

2. MATERIALS AND METHODS

2.1 Equipment

Seven 2 L reactors with effective volumes of 1.7 L were used to carry out the semi-continuous experiments. The reactors were placed in a thermostated water bath, which was kept at 35 ± 2 °C during the experiments. A stirring rate of 450 rpm was kept during the assays to favor a good transfer inoculum/substrate.

The biogas produced was passed through a 3N NaOH solution to capture CO₂. The remaining gas was assumed methane. The CH₄ produced after the anaerobic digestion was measured by liquid displacement in gasometers of 5 L of volume.

2.2 Inoculum

The inoculum for the anaerobic digestion process was collected from a full-scale UASB reactor placed in a brewery in Seville (Spain). The main characteristics of this methanogenically active inoculum were: pH: 7.05, TKN: 0.5 ± 0.4 g TKN/kg, TS (total solids): 68.7 ± 0.7 g TS/kg and VS: 24.7 ± 1.8 g VS/kg.

2.3 Two-phase Olive Pomace (TPOP)

The TPOP used in the present study was collected from the Experimental Olive Oil Mill located in the “Instituto de la Grasa (Consejo Superior de Investigaciones Científicas)” in Sevilla, Spain. The TPOP was sieved (2 mm mesh) to remove olive stone

pieces before the pretreatment. The main characteristics of the TPOP are summarized in Table 1.

2.4 Thermal pretreatment

The conditions for the thermal pretreatment of the TPOP were chosen taking into account a previous study carried out in batch mode by Rincón et al., 2013. In that study different temperatures (100 °C, 120 °C, 160 °C and 180 °C) and heating times (100 °C, 120 °C, 160 °C and 180 °C) were assayed in the TPOP to study the effect of these pretreatments on the methane yields of subsequent biochemical methane potential tests. Rincón et al., 2013 found that the best methane yield after the batch anaerobic tests was obtained at a temperature of 120°C during 180 minutes. These conditions were selected for the thermal pretreatment before the semi-continuous anaerobic digestion process.

The thermal pretreatment was carried out adding the TPOP to open Pyrex glass cylinders which were connected with glass refrigerants to avoid losses of moisture. These cylinders were placed in a thermo-block at a temperature of 120°C during 180 min. After the pretreatment the samples were cooled to ambient temperature before feeding.

The main characteristics of the pretreated TPOP were: TS= 272.4 ± 0.2 g TS/kg , VS: 229.8 ± 2.6 g VS/kg, MS= 42.6 ± 0.1 g MS/kg (mineral solids), CODt: 360.8 ± 0.3 g O₂/kg and CODs: 143.4 ± 0.1 g O₂/kg.

2.5 Experimental procedure

The 2 L reactors were charged with methanogenic anaerobic sludge at a concentration of 25 g VS/L. The effective work volume of the reactors was 1.7 L. A nutrient solution was added in at 10%. The composition of this nutrient solution is given in detail elsewhere

(Borja et al., 2003). The headspace of each flask was flushed with N₂ at the beginning of the experiment.

After the thermal pretreatment, the pretreated TPOP was used to feed the reactors. Seven different OLRs were studied: 2.0, 3.5, 4.5, 5.0, 5.5, 6.0, and 7.0 g VS/(L·d), which corresponded to seven HRTs of: 116, 95, 74, 66, 60, 55 and 47 days, respectively. The reactors were fed once per day with the quantity corresponded to each OLR and HRT studied. Initially, an acclimation stage was carried out with different dilutions of TPOP. When the methane production was stable the different experiments started. Once steady-state conditions were achieved at each OLR, the daily volume of methane produced and COD_t, COD_s, pH, VFA, TA and AN of the different effluents were determined. The steady-state value of a given parameter was taken as the average of these consecutive measurements for that parameter when the deviations of the observed values were less than 5% in all cases.

2.6 Analytical methods

Most of the parameters were analysed according to the APHA recommendations (APHA et al., 1998). TS and VS: Standard Methods 2540B and 2540E, respectively, COD_s: Standard Method 5220D, TKN: a method based on the 4500-N_{org} B of Standard Methods, AN. Standard Method 4500-NH₃E. pH was analysed using a pH-meter model Crison 20 Basic. TA was determined by pH titration to 4.3 (APHA, 1998).

COD_t was determined by the method described by Raposo et al. (2008). Hemicellulose, cellulose and lignin were determined according to van Soest method (van Soest et al. 1991).

3. RESULTS AND DISCUSSION

3.1. Influence of OLR and HRT on the operational parameters and COD_t removal efficiency

Table 2 shows the results obtained for steady-state conditions during reactor operation at seven different OLRs and HRTs, including COD_t and COD_s, TA, VFA, AN (NH₄⁺) and methane production. The variations of COD_t and COD_s with OLR are plotted in Figure 1. As can be seen, the percentages of COD_t and COD_s removed decreased slightly between 77.9%-71.7% and 92.3%-85.0% respectively, when the OLR increased between 2.0 and 5.0 g VS/(L·d). For OLRs higher than these values a marked decrease in efficiency was observed. For example, the COD_t and COD_s removal efficiency decreased to 62.4% and 71.1% respectively, at an OLR of 5.5 g VS/(L·d), which is equivalent to an HRT of 60 days. However, despite this decrease in the COD_t and COD_s removal efficiencies, more than 71% of feed CODs could be removed up to OLRs of 6.0 g VS/(L·d), which demonstrates that the anaerobic process was effective.

The VS removal rates observed at different OLRs are showed in Figure 2. As can be seen, the rate of VS removal increased linearly with OLR up to OLR values up to 5.5 g VS/(L·d), the slope of the fitted line being 0.63 (±0.02) with a determination coefficient of 0.994. A decrease in the sequence was observed for OLRs of 6.0 and 7.0 g VS/(L·d), equivalent to HRTs of 55 and 47 days, respectively. Therefore, the sensitivity and metabolism of anaerobic microorganisms was impaired for the highest OLR studied, resulting in a negative response of the anaerobic system. Although the pH remained in the optimal working range (6.5-7.5) for anaerobic processes (Isik and Sponza, 2005), the VFA values increased from 3220 to 7400 mg acetic acid/L when the OLR augmented from 2 to 7. These optimal pH values observed for all OLRs studied could be explained by the neutralization of the hydrogen ions released from the volatile fatty acids together with the

carbonates dissociated from the carbonic acid with the bicarbonate alkalinity inside the anaerobic reactors (Isik and Sponza, 2005). Buffering in anaerobic processes is normally due to bicarbonate, as carbonate is, generally, negligible if compared with the bicarbonate (Fannin, 1987). The buffering guards against a possible acidification of the reactors giving a pH of the same order as the optimal for methanogenic microorganisms (Fannin, 1987).

The VFA/TA ratio can be used as an indicator of the stability of the process. When this ratio is lower than 0.4-0.5 (equivalents acetic acid/equivalents CaCO_3) the process is considered to be operating favorably without risk of acidification. As can be calculated from Table 2, these ratio values were in the range of 0.29-0.48 (equiv. acetic acid/equiv. CaCO_3), which were lower than the suggested limit value for OLRs in the range of 2-6 g VS/(L·d). This was due to the moderate increase in the VFA values and the high values of total alkalinity present for all OLRs tested.

3.2. *Methane production rates and methane yield coefficient*

Figure 3 illustrates the variation of the volumetric methane production rate with OLR for all experiments carried out. As can be seen, the volume of methane produced per day and per reactor volume (L) increased linearly with increased OLR up to an OLR value of 4.5 g VS/(L·d), after which a marked decrease was observed over the OLR range tested. Thus, seemingly, the activity of methanogens was not hindered up to OLR values higher than 5.0 g VS/(L·d) because of the appropriate stability and buffering capacities provided in the anaerobic reactors. However, the methane production rate decreased sharply from 1.720 to 0.294 L CH_4 /(L·d) when the OLR was increased from 4.5 to 7.0 VS/(L·d). This reduction in the methane production rate at the highest OLR value tested might be attributed to an inhibition of the methanogenic microorganisms at high OLR values, which entailed an increase in the effluent VFA concentrations and VFA/TA ratios (Table 2). To

be specific, VFA content increased from 3060 to 7400 mg acetic acid/L when the OLR was increased from 4.5 to 7.0 VS/(L·d).

Moreover, it is interesting to note that the maximum methane production rate (1.72 L CH₄/(L·d)) achieved in the present work treating thermally-pretreated TPOP was obtained at an OLR of 4.5 VS/(L·d). This methane production rate value was 39.8% higher than the methane production rate reached in the anaerobic digestion of untreated TPOP (1.23 L CH₄/(L·d)) at the same OLR (4.5 VS/(L·d)) (Borja et al., 2002; Rincón et al., 2008b).

The experimental data summarized in Table 2 were used to determine the methane yield coefficient, Y_p . Taking into account that the volume of methane produced per day, V_{CH_4} (L CH₄/d) is assumed to be proportional to the amount of substrate consumed, then

$$V_{CH_4} = Y_p \cdot q \cdot (S_0 - S) \quad (1)$$

where S_0 and S are the influent and effluent substrate concentrations (g VS/L) respectively, and q is the feed flow-rate. By plotting the equation (1) in the form V_{CH_4} against $q \cdot (S_0 - S)$ and taking into account the working volume of the reactor (1.7 L), a virtually constant value of 0.45 ± 0.04 L CH₄/g VS_{consumed} (95% confidence limits) was obtained within the OLR range 2.0-5.0 g VS/(L·d). This methane yield value was 45% higher than that obtained in the anaerobic digestion process of untreated TPOP (Rincón et al., 2008b) and 12.5% higher than that achieved in the methanogenic step of the two-stage anaerobic digestion process of untreated TPOP (Rincón et al., 2009). Therefore, the highest methane yield was found for the TPOP pretreated at 120 °C during 180 min, which showed the lowest lignin and hemicellulose contents after pretreatment (Rincón et al., 2013). By contrast, the untreated TPOP, with the highest lignin and hemicellulose concentrations, gave the lowest methane yield. In the same way, Monlau et al. (2012) and Li et al. (2012) also found that the methane yield was negatively correlated with the amount of lignin present in different lignocellulosic substrates such as sunflower stalks, grass materials, etc.

In addition, during thermal degradation of hemicellulose, acids are formed and these compounds are assumed to catalyze the further hydrolysis of hemicelluloses (Hendriks and Zeeman, 2009; Rincón et al., 2013).

Finally, an important point to consider the pertinence and relevance of thermal pretreatment is the energy balance. Although it might seem that the energy consumption to pretreat the TPOP at 120 °C is high, a heat integration in the whole system and an energy balance demonstrates that the energy expense can be balanced due to the increase in waste biodegradability and to the use of residual heat in the maintenance of digester temperature or to preheat the influent TPOP (Carrère et al., 2016; Rincón et al., 2013).

3.3 Evaluation of substrate removal kinetic models

Kinetic models are usually used in anaerobic digestion processes to check initial hypothesis, to evaluate the experimental results and to control and predict the process performance, optimizing the plant design (Isik and Sponza, 2005).

With the aim of characterize kinetically the semi-continuous anaerobic digestion process studied and assessing the influence of OLR on effluent substrate concentration and given the complexity of the substrate studied two different kinetic models were evaluated. Firstly, the modified Stover–Kicannon model was used to study the COD_t degradation, while the Grau second-order kinetic model was assayed to determine the kinetics of COD_s removal.

Modified Stover-Kicannon kinetic model

Stover–Kincannon is one of the most widely used mathematical models to determine the kinetic constants for anaerobic processes, both in suspended and immobilized biomass systems. This model has been applied to continuously operated mesophilic and

thermophilic anaerobic reactors treating several high-strength wastewaters such as petrochemical wastes, saline wastewaters, textile wastewaters, industrial wastes, etc. (Jafarzadeh et al., 2009; Kapdan and Erten, 2007; Kosinska and Miskiewicz, 2009; Sandhya and Swaminathan, 2006). The modified Stover–Kincannon model considers the organic substance removal rate as a function of organic loading rate at steady state as described in Eq. (1):

$$\frac{dS}{dt} = \frac{q(S_o - S_e)}{V} = \frac{R_{max} \left(\frac{qS_o}{V} \right)}{K_b + \left(\frac{qS_o}{V} \right)} \quad (1)$$

where dS/dt is the substrate removal rate ($\text{g COD}_t/(\text{L}\cdot\text{d})$), S_o and S_e the substrate concentrations in the influent and reactor effluent ($\text{g COD}_t/\text{L}$), respectively, q the flow-rate, R_{max} the maximum substrate removal rate ($\text{g COD}_t/(\text{L}\cdot\text{d})$) and K_b is a saturation value constant ($\text{g COD}_t/(\text{L}\cdot\text{d})$).

Taking into account that the HRT is equal to the quotient V/q , an arrangement of equation (1) gives the following relationship:

$$\frac{HRT}{(S_o - S_e)} = \frac{K_b}{R_{max} \cdot S_o} HRT + \frac{1}{R_{max}} \quad (2)$$

Equation (2) coincides with the modified Stover-Kicannon model. According to this model a plot of the quotient $\frac{HRT}{(S_o - S_e)}$ versus HRT should result in a straight line with slope equal to $\frac{K_b}{R_{max} \cdot S_o}$ and intercept equal to $\frac{1}{R_{max}}$. Figure 5 shows a plot of the above-mentioned pair values. By using the least squares method, a straight line with a slope = 0.0033 and an intercept = 0.098 was obtained, the linear regression coefficient being 0.986. From these values, the kinetic parameters were calculated as $K_b = 11.15 \text{ g COD}_t/(\text{L}\cdot\text{d})$ and $R_{max} = 10.2 \text{ g COD}_t/(\text{L}\cdot\text{d})$. These values were higher than those obtained in the anaerobic digestion of saline wastewater at different initial COD_t concentration (1900-6300 $\text{mg COD}_t/\text{L}$) in an upflow packed bed reactor, for which the K_b and R_{max} values were 5.3 $\text{g COD}_t/(\text{L}\cdot\text{d})$ and 10.2 $\text{g COD}_t/(\text{L}\cdot\text{d})$, respectively.

COD_t/(L·d) and 7.05 g COD_t/(L·d), respectively (Kapdan and Erten, 2007). However, the kinetic parameters of the present work were lower than those achieved in an hybrid column upflow anaerobic fixed bed reactor processing textile wastewater ($Kb = 45.37$ g COD_t/(L·d) and $R_{max} = 31.69$ g COD_t/(L·d)) (Sandhya and Swaminathan, 2006) and those reached in the anaerobic digestion of industrial wastes (effluents from an industrial pig fattening farm) ($Kb = 91.58$ g COD_t/(L·d) and $R_{max} = 80.9$ g COD_t/(L·d)) (Kosinska and Miskiewicz, 2009).

Finally, the effluent COD_t concentration can be predicted by using the following equation (3), which is easily deducible from equation (2):

$$Se = So - \frac{R_{max} \cdot So}{Kb + So/HRT} \quad (3)$$

With the values of the kinetic parameters R_{max} and Kb and using the equation (3), the theoretical values of effluent substrate concentrations (g COD_t/L) were calculated. Deviations lower than 15% between the theoretical and experimental values were reached in all cases. These slow deviations demonstrate the suitability of the modified Stover-Kicannon model to predict the effluent total COD concentration and ultimately the process performance.

Grau Second-order kinetic model

Complete mixed hydraulic conditions were considered as occurring in the anaerobic reactors operating with thermally-pretreated TPOP. In the case of multicomponent substrate kinetics, the substrate removal rate can be expressed according to equation (4):

$$-dS/dt = k_{n(s)} \cdot X \cdot (S/So)^n \quad (4)$$

where $-dS/dt$ is the substrate removal rate (g COD_s/(L·d)), $k_{n(s)}$ is the reaction constant (g COD_s/(g VS·d)), X is the concentration of microorganisms (g VS/L), which can be virtually

assumed as constant, S is the substrate concentration at any time (g COD_s/L) and S_o is the initial substrate concentration (g COD_s/L).

Integrating the equation (4) for $n = 2$, one obtains the following second order kinetic equation:

$$Se = S_o / (1 + k_{2(s)} \cdot X_o \cdot HRT / S_o) \quad (5)$$

Given the complexity of the substrate studied, a second order was finally selected to describe the kinetics of COD_s removal. Second-order models were also used to obtain the kinetic parameters of anaerobic processes when inhibition by substrate concentration or by presence of inhibitory compounds was detected such as occurs for piggery wastewater, chicken manure, untreated two-phase olive pomace, etc. (Ayol and Filibeli, 2003; Borja et al., 2004; Sanchez et al., 2001).

For the determination of the kinetic parameter $k_{2(s)}$, equation (5) can be transformed into the following linear expression, equation (6):

$$HRT [S_o / (S_o - Se)] = S_o / k_{2(s)} \cdot X_o + HRT \quad (6)$$

According to equation (6), the value of the second-order reaction constant can be obtained by the plot of the term $HRT [S_o / (S_o - Se)]$ versus HRT . The validity of the application of this model was corroborated when plotting the experimental data and a straight line was obtained with a slope near unity (0.982), the linear regression coefficient being 0.992. The experimental points were fitted to a straight line using linear regression. Figure 6 shows this plot, expressing the substrate concentration as COD_s. Once the value of the intercept is known, the value of the kinetic parameter, $k_{2(s)}$, was easily determined to be 0.25 g COD_s/(g VS·d).

This kinetic constant value is higher than that obtained in the anaerobic digestion process of sunflower oil cake pretreated with ultrasounds (0.09 g COD_s/(g VS·d)) (Fernández-Cegrí et al., 2013) and that achieved in the anaerobic digestion of

petrochemical waste in a hybrid reactor ($0.14 \text{ g COD}_s/(\text{g VS}\cdot\text{d})$) (Jafarzadeh et al., 2009). However, higher values of the second-order kinetic constant (2.32, 2.57 and $10.81 \text{ g COD}_s/(\text{g VS}\cdot\text{d})$ for the sludge bed region, fixed bed region and overall reactor, respectively) were obtained in the anaerobic digestion process of synthetic wastewater based on diluted molasses with a COD/N/P ratio of 100/5/1 carried out in a hybrid reactor (Buyukkamaci and Filibeli, 2002).

With the value of the kinetic constant obtained with this model and applying Equation (5), the theoretical values of the effluent soluble COD concentration were determined. Deviations lower than 18% between the experimental and calculated values obtained, which demonstrates the appropriateness of the Grau second-order kinetic model in predicting the behavior of the reactor accurately.

Conclusions

The experimental results obtained demonstrate the stability and high performance of the semi-continuous anaerobic digestion of thermally-pretreated TPOP. COD_t and COD_s removal efficiencies in the ranges of 77.9-71.7% and 92.3-85.0% were achieved when the OLR increased from 2.0 to $5.0 \text{ g VS}/(\text{L}\cdot\text{d})$. However for OLRs higher than $5.0 \text{ g VS}/(\text{L}\cdot\text{d})$ a sudden decrease in the process performance was observed. The maximum methane production rate achieved was 39.8% higher than that obtained in the semi-continuous anaerobic digestion of untreated TPOP. The modified Stover-Kicannon model and the Grau second order kinetic model fitted adequately the experimental results of COD_t and COD_s , respectively.

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Table 1. Main characteristics of the two-phase olive pomace (TPOP).

Parameter	Composition
pH	4.9 ± 0.2
CODt (g O ₂ /kg)	331.1 ± 0.7
CODs (g O ₂ /kg)	143.4 ± 3.2
TA (g CaCO ₃ /kg)	2.5 ± 0.1
TS (g/kg)	265.0 ± 2.6
VS (g/kg)	228.4 ± 2.3
Hemicellulose (%)	11.3 ± 0.2
Cellulose (%)	5.2 ± 0.1
Lignin (%)	19.7 ± 0.4
VFA (%)	3.8 ± 0.3
TKN (g /kg)	3.6 ± 0.1
Ammonia (g N/kg)	0.3 ± 0.0

TS: total solids; VS: volatile solids; CODt: total chemical oxygen demand; CODs: soluble chemical oxygen demand; VFA: total volatile fatty acid; TKN: total Kjeldahl nitrogen; AN: ammoniacal nitrogen, and TA: total alkalinity. Units are expressed per kg of fresh and pitted TPOP. Percentages are expressed as: weight: weight of wet sample.

Table 2. Experimental results obtained for the different organic loading rates (OLRs) and (HRTs) studied.*

OLR (g VS/(L d))	HRT (d)	pH	CODt (g/kg)	CODs (g/kg)	TA (mg CaCO ₃ /L)	VFA (mg acetic a./L)	NH ₄ ⁺ (mg/kg)	Methane Production (mL/d)
2.0	166	7.5	79.72	11.49	7230	3220	450	1410
3.5	95	7.5	90.22	19.94	7160	4160	450	2050
4.5	74	7.4	98.20	22.91	7230	2560	695	2920
5.0	66	7.4	101.90	22.52	7210	3060	670	2880
5.5	60	7.2	135.66	43.54	8930	3550	680	1660
6.0	55	7.1	143.94	43.32	8370	2955	594	1550
7.0	47	6.6	164.32	45.67		7400		500

*Values are the averages of 4 determinations taken over 4 days after the steady-state conditions had been achieved. The differences between the observed values were lower than 5% in all cases.

OLR: organic loading rate, HRT: hydraulic retention time; CODt: total chemical oxygen demand; CODs: soluble chemical oxygen demand; TA: total alkalinity; VFA: total volatile fatty acid; NH₄⁺: ammoniacal nitrogen. Units are expressed per kg of fresh and pitted TPOP. Percentages are expressed as: weight: weight of wet sample.

Figure Captions

Figure 1. Variation of the percentages of COD_t and COD_s removed with the OLR (g VS/(L·d)) in the anaerobic digestion process of thermally-treated TPOP.

Figure 2. Variation of the VS removal rate (g VS_{removed}/(L·d)) with OLR (g VS/(L·d)) of the reactor.

Figure 3. Variation of the methane production rate (L CH₄/(L·d)) with the OLR (g VS/(L·d)) of the reactor.

Figure 4. Variation of the methane production rate (L CH₄/(L·d)) as a function of the product of the differences of substrate concentrations at the reactor inlet (S_o) and outlet (S_e) and the feed flow-rate (q).

Figure 5. Modified Stover-Kicannon model plot.

Figure 6. Linearization of the Grau second-order kinetic model to calculate the kinetic constant value $k_{2(s)}$ for COD_s.

Fig. 1

Fig. 2

Fig. 3

Fig.4

Modified Stover-Kicannon model

Fig. 5

Grau second order kinetic model

Fig. 6