4

Influence of Substrate Concentration on the Anaerobic Degradability of Two-Phase Olive Mill Solid Waste: A Kinetic Evaluation

Bárbara Rincón and Rafael Borja
Instituto de la Grasa (CSIC), Avda. Padre García Tejero, Sevilla,
Spain

1. Introduction

The evolution of modern technology for olive oil extraction has affected the industrial sector depending directly on the by-products obtained. The traditional three-phase continuous centrifugation process for olive oil extraction was introduced in the 1970s, notably to increase the processing capacity and extraction yield and to reduce labour. This three-phase manufacturing process of olive oil usually yields an oily phase (20%), a solid residue (30%) and an aqueous phase (50%), the latter coming from the water content of the fruit, which is usually defined as vegetation water. Such water, combined with that used to wash and process the olives, make up the so-called "olive mill wastewater" (OMW) and also contains soft tissues from olive pulp and a very stable oil emulsion (Borja et al., 2006). This process generates a total volume of traditional OMW of around 1.25 litres per kg of olives processed. Consequently, the three-phase centrifugation process caused an increase in the average mill size, a decrease in the total number of mills, increased water consumption and increased production of wastewaters.

The OMW composition is not constant either qualitatively or quantitatively and it varies according to cultivation soil, harvesting time, the degree of ripening, olive variety, climatic conditions, the use of pesticides and fertilizers and the duration of aging. The three-phase OMW is characterized by the following special features and components: intensive violet-dark brown to black in colour; specific strong olive oil smell; high degree of organic pollution (chemical oxygen demand –COD– values up to 220 g/L); pH between 3 and 6 (slightly acidic); high electrical conductivity; high content of poly-phenols (0.5-24 g/L) and high content of solid matter (Niaounakis and Halvadakis, 2004).

The annual OMW production of Mediterranean olive-growing countries is estimated to ranging from 7 million to over 30 million m³. This huge divergence of results can partly be explained by the fact that the production of olives varies from one year to another due to weather conditions and plagues that can affect the olive trees. The average total production amounts approximately to 10-12x10⁶ m³ per year and occurs over a brief period of the year (November-March). Spain produced 20% of the OMW of the Mediterranean basin (2-3x10⁶

m^3/year) before the implantation of the two-phase extraction process in most of the Spanish olive oil factories, which represented an equivalent pollution of 10-16x10^6 inhabitants in the short milling period (Nioaunakis and Halvadakis, 2004).

The efforts to find a solution to the OMW problem are more than 50 years old (Borja et al., 2006). There are many different types of processes that have been tested: detoxification processes (such as physical, thermal, physicochemical, biological and combination of processes), recyclying and recovery of valuable components, production system modification, etc. However, none of the detoxification techniques on an individual basis allow the problem of disposal of OMW to be solved to a complete and exhaustive extent, effectively and in an ecologically satisfactory way. At the present state of OMW treatment technology, industry has shown little interest in supporting any traditional process (physical, chemical, thermal or biological) on a wide scale. This is because of the high investment and operational costs, the short duration of the production period (3-5 months) and the small size of the olive mills (Borja et al., 2006).

2. The two-phase olive oil manufacturing process

The failure to develop a suitable and economical effluent wastewater treatment technology for OMW has lead manufacturers of technology to develop the "ecological" two-phase process, which delivers oil as the liquid phase and a very wet olive cake (**two-phase olive mill solid waste -OMSW-**) as the solid residue. This technology has attracted special interest where water supplies are restricted and/or aqueous effluent must be reduced (Borja et al., 2006).

In the two-phase process a horizontally mounted centrifuge is used for primary separation of the olive oil fraction from the vegetable solid material and vegetation water. The resultant olive oil is further washed to remove residual impurities before finally being separated from this wash water in a vertical centrifuge. Therefore, the two-phase olive mills produce three identifiable and separate waste streams. These are:

1. The wash waters generated during the initial cleansing of the fruit.
2. The aqueous solid residues generated during the primary centrifugation (two-phase OMSW).
3. The wash waters from the secondary centrifuge generated during the washing and purification of virgin olive oil.

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 a new solid residue, two-phase OMSW, which requires further investigation to find out how it must be handled.

The two-phase olive oil extraction process has several advantages over the three-phase centrifugation process (Alba et al., 2001; Di Giovacchino et al., 2001 and 2002):

- The construction of the two-phase scroll centrifuge is less complicated and thus is more reliable in operation and less expensive than the three-phase decanter.
- During operation of the three-phase scroll centrifuge the separated oil and water may be remixed; volatile compounds from the vegetation water may cause a sticky deposit on the centrifuge.

- The throughput of the two-phase centrifuge in relation to the oil quantity is higher because no additional water is required to produce the pulp. Energy consumption is also reduced as a result of the lower processing quantity.
- Oil produced by the two-phase centrifuge is of higher quality; in particular, it has higher oxidation stability and better organoleptic characteristics.
- The operating costs are lower. Water utilization in the olive mill decreases considerably.

In addition, the disadvantages of two-phase manufacturing process are:

- The two-phase process, although it produces no olive mill wastewater as such, generates the wash waters derived from the initial cleansing of the fruit and from the purification of virgin olive oil. In addition, it combines the olive vegetation water that is generated with the solid waste to produce a single effluent stream in semi-solid form. This doubles the amount of "solid" waste (OMSW or 'alperujo') requiring disposal, and it cannot be composted or burned without some form of expensive pre-treatment.
- Two-phase OMSW has a moisture content significantly higher than that of traditional cake from three-phase centrifuges. This increased amount of moisture, together with the sugars and fine solids that in the three-phase system were contained in OMW give two-phase OMSW a doughy consistency and makes transport, storage and handling difficult –it can not be piled and must be kept in large ponds.
- Two-phase OMSW is characterized by higher values of the pulp/stone ratio, as well as the greater weight produced.
- This two-phase technology transfers the problem of disposing of the olive-mill waste from the mill to the seed-oil refineries. Two-phase OMSW, prior to oil solvent extraction, must be dried with considerably higher energy requirements than in the three-phase continuous oil production process, making the industrial recovery of the residual oil difficult and expensive.

2.1 The two-phase Olive Mill Solid Waste (OMSW)

The characteristics of two-phase OMSW are obviously very different from the characteristics of olive cake resulting from three-phase centrifuge systems. Two-phase OMSW is a thick sludge that contains pieces of stone and pulp of the olive fruit as well as vegetation water. It has a moisture content in the range of 60-70% while olive cake from a three-phase extraction process has only around 40-45% moisture. It also contains some residual olive oil (2-4%), 2% ash with a 30% potassium content (Alba et al., 2001).

The average composition of the two-phase OMSW is: water (60-70%), lignine (13-15%), cellulose and hemicellulose (18-20%), olive oil retained in the pulp (2.5-3%), mineral solids (2.5%). Among their organic components, the major ingredients are as follows: sugars (3%), volatile fatty acids (C2-C7) (1%), poly-alcohols (0.2%), proteins (1.5%), poly-phenols (0.2%) and other pigments (0.5%) (Borja et al., 2002).

As it can be seen, the two-phase OMSW has a high organic matter concentration giving an elevated polluting load. The high polluting power and large volumes of solid waste generated (around 2 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 Andalusia Community (Borja et al., 2002).

3. Anaerobic digestion as an alternative for treatment of two-phase OMSW

Anaerobic digestion (AD) is an attractive treatment for this waste of difficult disposal. AD processes transform the organic matter contained in a certain waste in biogas as main product. This process is carried out for different kind of microorganisms which work in a coordinate and interdependent chain until biogas obtaining.

Anaerobic treatment of moderate and high strength wastes with high biodegradable content presents a number of advantages in comparison to the classical aerobic processes: a) quite a high degree of purification with high-organic load feeds can be achieved; b) low nutrient requirements are necessary; c) small quantities of excess sludge are usually produced and finally, d) a combustible biogas is generated. The production of biogas enables the process to generate or recover energy instead of just energy-saving; this can reduce operational costs as compared with other processes such as physical, physico-chemical or biological aerobic treatments (Borja et al., 2006).

Previous works carried out at pilot-scale have shown that most of agro-industrial residues, such as sugar beet pulp, potato pulp, potato thick stillage and brewer´s grains, can be treated anaerobically with an efficient solids stabilisation and energy recovery, if the applied process-type (one or two stages) is selected according to the C:N ratio of the residues. These works demonstrated that at hydraulic retention times (HRT) of between 10 and 20 days, normally, the 50-60% of the organic matter was degraded. The ultimate anaerobic biodegradability was higher and lied between 76% (brewer´s grain) and 88% (potato pulp), which demonstrated that more than 60% of the available energy potential could be used in the industrial processes. The gas production varied between 300 and 500 m^3 biogas per ton of dry matter with a methane content of 60-70%. The undigested solids, which were separated from the effluent of the reactors could be completely stabilised after a short aerobic post-treatment to be used as a soil conditioner (Borja et al., 2006).

A number of kinetic models have been proposed for the process of anaerobic digestion. Early models were based on a single-culture system and used the Monod equation or variations. More recently, several dynamic simulation models have been developed based on a continuous multi-culture system; these correspond to the major bioconversion steps in anaerobic digestion but again make the assumption that culture growth obeys Monod type kinetics. Doubt has been expressed by several investigators on the validity of applying the Monod equation to waste treatment as the specific growth rate is expressed only as a function of the concentration of the limiting substrate in the reactor. The Monod equation contains no term relating to input substrate concentration; this implies that the effluent substrate concentration is independent of the input concentration. Experimental results do not always agree with this implication; for example the anaerobic digestion of dairy manure, beef cattle manure at mesophilic and thermophilic temperatures, rice straw or poultry litter (Borja et al., 2003).

Deviation from the Monod relationship in many digestion systems may be due to their complexity. This complexity has necessitated the use of generalized measures of feed and effluent strength, namely total Chemical Oxygen Demand (COD) and volatile solids (VS), which may not truly reflect the nature of the growth-limiting substrate. Utilizable carbon in the digester is derived from the hydrolysis of polymeric compounds, constituting the waste, by exo-enzymes in the extracellular medium or on the surface / vicinity of the

Influence of Substrate Concentration on the Anaerobic
Degradability of Two-Phase Olive Mill Solid Waste: A Kinetic Evaluation

81

microorganisms: only these hydrolysed, assimilable compounds can be considered as the growth-limiting substrate in terms of the Monod relationship. Extra-cellular hydrolysis is often considered the rate-limiting step in anaerobic digestion of organic wastes (Borja et al., 2003) and for a model to be truly valid this must be taken into account.

Multi-culture system kinetics may be desirable in view of the heterogeneous nature of the microbial population performing the various bioconversion steps involved. However, the kinetic models based on this premise necessarily involve a number of kinetic equations and coefficients making them highly complex, as shown by the reported models (Borja et al., 2003). Complexity does not necessarily equate to accuracy and there is still a strong case in favour of a simpler kinetic treatment based on a single culture system. Methanogenesis is particularly suited to this approach as there is a strong holistic characteristic in the process. Various cultures and bioconversion steps in digestion are interdependent and the whole process has certain self-regulatory characteristics within the process limits.

Kincannon and Stover (1982) proposed a widely used mathematical model to determine the kinetic constants for immobilized systems and high-rate reactors. In this model the substrate utilization rate is expressed as a function of the organic loading rate by monomolecular kinetics for biofilm reactors such as rotating biological contactors and biological filters (Kapdan and Erten, 2007). A special feature of the modified Stover-Kincannon model is the utilization of the concept of organic loading rate as the major parameter to describe the kinetics of an anaerobic filter in terms of organic matter removal and methane production (Büyükkamaci and Filibeli, 2002; Kapdan and Erten, 2007).

The modified Stover-Kincannon model allows to calculate the maximum substrate utilization rate by the microorganisms (R_{max}) and the saturation constant (K_B) in anaerobic digestion processes (Yu et al., 1998). Therefore, this model allows determining the effluent substrate concentration for a known volume of reactor and an initial concentration of the substrate. The modified Stover-Kincannon model has been used for different substrates and reactor configurations: anaerobic hybrid reactors treating petrochemical waste (Jafarzadeh et al., 2009), anaerobic treatment of synthetic saline wastewater by *Halanaerobium lacusrosei* (Kapdan and Erten, 2007), anaerobic digestion of soybean wastewaters (Yu et al., 1998) and molasses (Büyükkamaci and Filibeli, 2002) in a filter and in a hybrid reactor, respectively.

The aim of the present study was focused on the AD of two-phase OMSW at two different influent substrate concentrations and on the determination of kinetics constants of the system using the above-mentioned modified Stover-Kincannon model.

4. Materials and methods

4.1 Equipment

An anaerobic reactor with a working volume of 1 litre equipped with magnetic stirring and placed in a thermostatic chamber at 35 ºC was used. The reactor had an upper settling zone designed to minimize loss of the biomass responsible for the process. The reactor was fed daily by means of an external feeder and liquid effluent removed daily through a hydraulic seal, comprising 25 cm liquid column, designed to prevent air from entering the reactor and biogas from leaving. This reactor has been described in detail elsewhere (Martín et al., 1991).

The methane volume produced in the process was measured using a 5 litre Mariotte reservoir fitted to the reactor. A tightly closed bubbler containing a NaOH solution (3 M) to collect the CO_2 produced in the process was intercalated between the two elements. The methane produced displaced a given volume of water from the reservoir, allowing ready determination of the gas (Martín et al., 1991).

4.2 Inoculum

The reactor was inoculated with methanogenically active biomass from a laboratory-scale anaerobic reactor processing olive mill wastewater. The composition and features of the biomass used were: pH, 7.2; total solids (TS), 60.3 g/L; mineral solids (MS), 19.3 g/L; volatile solids (VS), 41.0 g/L; total suspended solids (TSS), 59.9 g/L; mineral suspended solids (MSS), 18.8 g/L; volatile suspended solids (VSS), 41.1 g/L.

4.3 Two-phase Olive Mill Solid Waste (OMSW)

The OMSW used for the experiments was collected from a two-phase technology mill. The OMSW was derived from olives with a high ripening index (6.5) and an intense purple colour. Before use the small stone pieces were removed by sieving the OMSW through a 3.15 and 2.00 mm sieve. Two influent substrate concentrations were used for the experiments: 35 g COD/L (OMSW 1) and 150 g COD/L (OMSW 2). These concentrations were obtained by dilution of the collected waste. The features and composition of these two-phase OMSWs are summarised in Table 1.

	Units	OMSW 1	OMSW 2
pH	*	5.6	5.8
COD	g O_2/L	35	150
SCOD	g O_2/L	15	67
TVFA	g acetic acid/L	0.70	2.90
Alkalinity	g $CaCO_3$/L	0.74	2.20
TS	g/L	40.2	165.3
MS	g/L	5.6	21.1
VS	g/ L	34.6	144.2
TSS	g/ L	35.2	142.2
MSS	g/ L	4.1	15.7
VSS	g/ L	31.1	126.5
Total phenolic compounds	g caffeic acid/L	0.61	2.44

COD: total chemical oxygen demand; SCOD: soluble chemical oxygen demand; TVFA: total volatile fatty acids (as acetic acid); Alkalinity (as $CaCO_3$). Values are averages of five determinations; there was virtually no variation (less than 3 %) between analyses.

Table 1. Composition and features of the OMSWs.

4.4 Experimental procedure

The anaerobic reactor was initially charged with 300 mL of distilled water, 500 mL of the inoculum and 200 mL of a nutrient-trace element solution. The composition of this nutrient-trace element solution is given in detail elsewhere (Borja et al., 2001).

The start-up of the reactor involved stepped increases in COD loading using an influent substrate concentration of 17.2 g COD/L. During this period the organic loading rate (OLR) was gradually increased from 0.25 to 0.50 g COD/(L d) between 1 and 15 d, 0.75 g COD/(L d) between 16 and 30 d, 1.00 g COD/(L d) between 31 and 45 d and finally 1.25 g COD/(L d) between 46 and 60 d.

After the preliminary step, the reactor was fed in series of semicontinuous experiments using OLRs of 0.9, 1.2, 1.4, 1.7, 2.1, 2.8, 3.5, 4.1 L COD/(L d) for the OWSW1, which correspond to hydraulic retention times (HRTs) of 40.0, 28.6, 25.0, 20.0, 16.6, 12.5, 10.0 and 8.3 d, respectively. After these experiments with OMSW 1 five different OLRs were assessed for the OMSW 2, 3.0, 6.0, 9.05, 12.0 and 15.0 g COD/(L d), these OLRs corresponded to HRTs of 50.0, 25.0, 16.6, 12.5 and 10.0 d, respectively.

Once steady-state conditions were achieved at each feed flow-rate, the daily volume of methane produced, and total and soluble COD, pH, total volatile fatty acids (TVFA) and volatile solids (VS) of the different effluents were determined. The samples were collected and analysed for at least 5 consecutive days. The steady-state value of a given parameter was taken as the average of these consecutive measurements for that parameter when the deviations between the observed values were less than 3% in all cases. Each experiment had a duration of 2-3 times the corresponding HRT.

The organic loadings applied in this work were increased in a stepwise fashion in order to minimise the transient impact on the reactor that might be induced by a sudden increase in loadings.

4.5 Chemical analyses

The following parameters were determined: total and soluble COD, pH, total solids, mineral solids, volatile solids, total suspended solids, mineral suspended solids, volatile suspended solids, total volatile fatty acids (TVFA), alkalinity and total phenolic compounds. All analyses were carried out according to the recommendations of the Standard Methods of American Public Health Association (APHA, 1989).

In each steady-state experiment, samples were collected and the above parameters analysed. The pH and gas volume were determined daily, whilst the remaining parameters were measured at least five times per week on five different samples taken on different days to ensure that representative data were obtained.

5. Results and discussion

5.1 Influence of substrate concentration and OLR on the COD removal efficiency and operational parameters

The anaerobic degradability studies were carried out using two different two-phase OMSWs with COD concentrations of 35 g COD/L (OMSW 1) and 150 g COD/L (OMSW 2). The

experiments were performed using progressive influent substrate concentrations, those corresponding to the OMSW 1 being the first ones and those corresponding to the OMSW 2 carried out at the end of the study.

Tables 2 and 3 summarize the steady-state operating results including HRT, OLR, methane production rates (r_{CH4}), total and soluble CODs, VS, TVFA, alkalinity and TVFA/alkalinity ratio for the OMSW 1 and OMSW 2, respectively (Borja et al., 2002).

Figure 1 shows the variation of the COD removal efficiency with the OLR for the two OMSWs used.
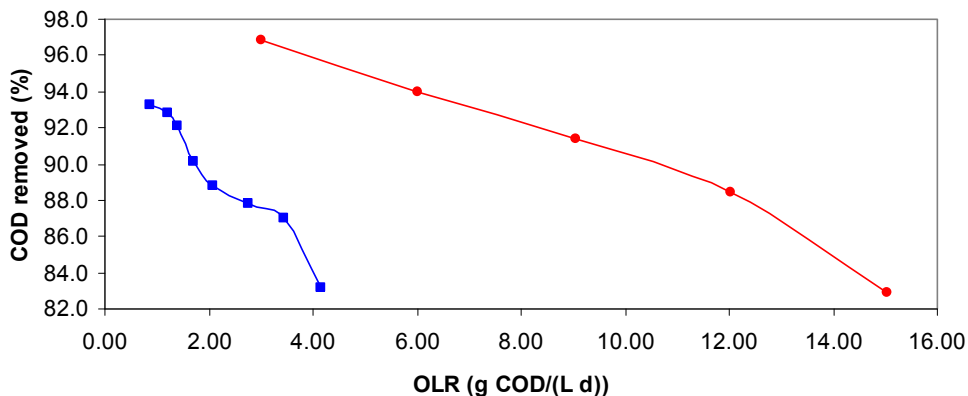
Fig. 1. Variation of the percentage of COD removed with the OLR for the two OMSWs used (■: OMSW 1; ●: OMSW 2).

OLR (g COD/(L d))	0.86	1.21	1.38	1.72	2.08	2.76	3.45	4.14
HRT (d)	40.0	28.6	25.0	20.0	16.6	12.5	10.0	8.3
pH	7.9	7.8	8.0	7.9	8.0	7.9	7.8	7.1
* r_{CH4} (L CH$_4$/(L d))	0.24	0.34	0.38	0.47	0.56	0.73	0.91	0.85
COD (g/L)	2.30	2.50	2.74	3.40	3.85	4.20	4.50	5.80
Soluble COD	0.72	1.20	1.40	1.65	1.90	2.15	2.35	3.80
VS (g/L)	1.70	1.88	2.07	2.40	2.75	3.10	3.40	4.50
TVFA (g acetic acid/L)	0.105	0.155	0.180	0.205	0.215	0.260	0.310	0.495
Alkalinity (g CaCO$_3$/L)	1.950	1.850	1.715	1.690	1.640	1.690	1.670	1.410
TVFA/Alkalinity	0.04	0.07	0.09	0.10	0.11	0.13	0.15	0.29

Values are the averages of 5 determinations taken over 5 days after the steady-state conditions had been reached. The differences between the observed values were less than 3 % in all cases. (* r_{CH4}: methane production rates)

Table 2. Steady-state results under different experimental conditions for the OMSW 1 with a COD of 35 g/L.

OLR (g COD/(L d))	3.00	6.01	9.05	12.02	15.03
HRT (d)	50.0	25.0	16.6	12.5	10.0
pH	7.2	7.0	7.0	6.9	6.5
* r_{CH4} (L CH$_4$/(L d))	0.59	1.13	1.64	2.12	2.05
COD (g/L)	4.80	9.05	12.95	17.50	25.70
Soluble COD	3.05	6.00	8.25	11.30	15.05
VS (g/L)	3.60	6.80	9.70	13.10	19.30
TVFA (g acetic acid/L)	0.56	0.81	1.08	1.25	1.57
Alkalinity (g CaCO$_3$/L)	1.98	1.90	1.81	1.70	1.32
TVFA/Alkalinity	0.23	0.35	0.40	0.61	0.95

Values are the averages of 5 determinations taken over 5 days after the steady-state conditions had been reached. The differences between the observed values were less than 3 % in all cases. (* r_{CH4}: methane production rates)

Table 3. Steady-state results under different experimental conditions for the OMSW 2 with a COD of 150 g/L.

As can be seen in Figure 1 the percentage of COD removed decreased with increased OLR for the two influent substrate concentrations studied. The percentage of COD removal decreased from 93.3% to 83.2% when OLR increased from 0.86 to 4.14 g COD/(L d) for the most diluted substrate (OMSW 1). For the most concentrated influent (OMSW 2) OLRs were varied from 3.00 to 15.03 g COD/(L d) and COD removal efficiencies higher than 88% were obtained at an OLR of 12.02 g COD/(L d). Even under a higher OLR of 15.03 g COD/(L d), corresponding to an HRT of 10 days, COD removal was 82.9%.

The total effluent CODs of the anaerobic reactor increased with increased OLR for the two influent substrate concentrations studied, as summarized in Tables 2 and 3. Such an increase in the effluent COD was paralleled by a similar increase in the effluent total volatile fatty acids (TVFA). This seems to indicate that, at higher OLR, the effluent total COD and mainly soluble COD is largely composed of the unused volatile acids produced in the reactor.

Given that the buffering capacity of the experimental system was found to be at favourable levels with excessive total alkalinity present at virtually all loadings, the efficiency of the process and the rate of methanogenesis was not very affected. The experimental data obtained in this work indicate that a total alkalinity of about 1.7 g/L as CaCO$_3$ is sufficient to prevent the pH from dropping to below 7.0 at an OLR of 9.05 g COD/(L d) for the most concentrated substrate used (OMSW 2).

The pH in the reactor was always higher that 7.0 for all the HRTs and OLRs studied corresponding to the most diluted OMSW studied. In addition, pH values equal or higher than 6.9 were observed for OLRs lower than 12.02 g COD/(L d) and HRTs higher than 12.5 d when the most concentrated influent was processed, with pH of 7.2 as a maximum value achieved. This high stability can be attributed to carbonate/bicarbonate buffering. This is produced by the generation of CO_2 in the digestion process which is not completely removed from the reactor as gas. Buffering in anaerobic digestion is normally due to bicarbonate, as carbonate is, generally, negligible if compared to the bicarbonate (carbonate/bicarbonate ratio is equal to 0.01 for pH 8.2) (Speece, 1983). The buffering guards

against possible acidification of the reactor giving a pH of the same order as the optimal for methanogenic bacteria (Wheatley, 1990).

The TVFA/Alkalinity ratio can be used as a measure of process stability (Wheatley, 1990): when this ratio is less than 0.3-0.4 the process is considered to be operating favourably without acidification risk. As was observed in Tables 2 and 3 the ratio values were lower than the suggested limit value for OLRs lower than 9.05 g COD/(L d) in the experiments corresponding to the highest influent substrate concentrations studied (OMSW 2). For this substrate, between HRTs of 50.0 and 16.6 days, the TVFA/Alkalinity ratio was always lower than the above-mentioned failure limit and the TVFA values were always lower than 1,08 g/L (as acetic acid). However, at a HRT of 10.0 days, a considerable increase of the TVFA/Alkalinity ratio until a value of 0.95 was observed in the reactor, which was mainly due to a considerable increase in the TVFA concentration (1.57 g/L as acetic acid) with simultaneous decrease in alkalinity (1.32 g/L, as $CaCO_3$).

5.2 Influence of substrate concentration on the methane production rates and methane yield coefficients

The volumetric methane production rates as a function of OLR are illustrated in Figure 2. As can be seen the volume of methane produced per day increased linearly with increased OLR up to OLR values of 3.45 and 12.02 g COD/(L d) for the influents OMSW 1 and OMSW 2, respectively. After these values a slight decrease was observed in the cases studied over the different ranges tested. Apparently, the activity of methanogenic bacteria was not impaired up to OLR values of 12.02 g COD/(L d) for the most concentrated influent (OMSW 2) used because of the appropriate stability and adequate buffering capacities provided in the experimental system. Nevertheless, the methane production rate decreased slightly from 2.12 to 2.05 L CH_4/(L d) when the OLR was increased from 12.02 to 15.03 g COD/(L d).

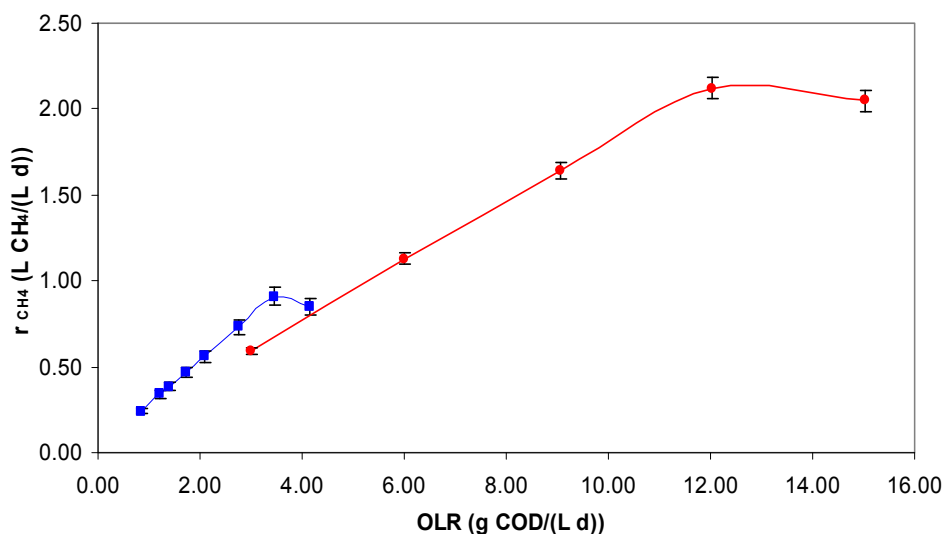Fig. 2. Variation of the methane production rate, r_{CH4}, with the OLR (g COD/(L d)) of the reactor for the two OMSWs used as influents (■: OMSW 1; ●: OMSW 2).

This decrease in the methane production at the highest OLR values might be attributed to an inhibition of the methanogenic bacteria at high OLR values, which caused an increase in effluent TVFA contents and TVFA/Alkalinity ratio, as can be seen in Table 3. Specifically, TVFA content increased from 1.25 to 1.57 g/L (as acetic acid) when the OLR was increased from 12.02 to 15.03 g COD/(L d).

The experimental data listed in Tables 2 and 3 were used to determine the methane yield coefficient, Y_p. As the volume of gas produced per day, r_{CH4}, is assumed to be proportional to the amount of substrate consumed, then:

$$r_{CH4} = Y_p q (S_0 - S) \tag{1}$$

where S_0 and S are the substrate concentrations (expressed as g COD/L) at the digester inlet and effluent, respectively, and q is the feed flow-rate. By plotting Eq (1) in the form r_{CH4} against $q (S_0 - S)$ (Figure 3), the following values of the methane yield coefficients with their 95% confidence limits were obtained for the two substrate concentrations used: 0.300 (± 0.001) and 0.200 (± 0.006) L methane STP/g COD removed when the OMSW 1 and OMSW 2, respectively, were processed. These values agree with the data reported in the literature for anaerobic treatment of food industry wastewaters (Borja et al., 1995; Maqueda et al., 1998; Martín et al., 1993). Taking into account that, theoretically, 0.35 L of methane is produced per gram of COD removed when the starting compound is glucose (Wheatley, 1990), the effectiveness of the anaerobic process in converting OMSW into methane at mesophilic temperature is demonstrated.
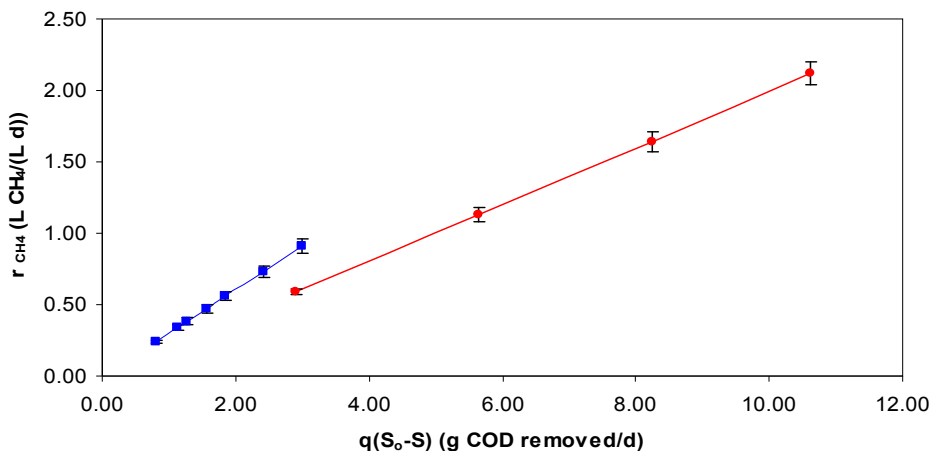

Fig. 3. Variation of the volume of methane produced per day, r_{CH4}, as a function of the product of the differences of substrate concentrations at the reactor inlet (S_0 in g COD/L) and outlet (S in g COD/L) and the feed flow-rate (q in L/day) for the two OMSWs used as influent. (■: OMSW 1; ●: OMSW 2).

5.3 Kinetic evaluation

Since the early 1980's, Stover and Kincannon have proposed a design concept of total organic loading rate and established a kinetic model for biofilm reactors. In this model the

substrate utilization rate is expressed as a function of the organic loading rate by monomolecular kinetics for biofilm reactors such as rotating biological contactors and biological filters (Yu et al., 1998). This kinetic model can be used to describe carbonaceous removal in terms of BOD (biochemical oxygen demand), COD (chemical oxygen demand) and TOC (total organic carbon) as well as for nitrification.

The original Stover-Kincannon model (Kincannon and Stover, 1982) (Equation 2) was initially proposed for rotating biological contactor (RBC) systems and can be expressed by the following equation:

$$\mathrm{d}S/\mathrm{d}t = [R_{max}\,(qS_o/A)]/[K_B+(qS_o/A)] \tag{2}$$

where: A is the disc surface area where the active biomass is attached; S is the substrate concentration in the reactor (in COD units) for a time (t); S_o is the initial substrate concentration; q is the flow rate; R_{max} is the maximum removal rate constant and K_B is the saturation value constant (in g COD/(L d)).

In the modified Stover-Kincannon model the substrate utilization rate is expressed as function of the organic loading rate as follows (Yu et al., 1998).

$$\mathrm{d}S/\mathrm{d}t = [R_{max}\,(qS_o/V)]/[K_B+(qS_o/V)] \tag{3}$$

where V is the volume of the anaerobic reactor. The term $\mathrm{d}S/\mathrm{d}t$ is defined for a steady-state relationship for different authors as:

$$\mathrm{d}S/\mathrm{d}t = q\,(S_o - S)/V \tag{4}$$

Linearization of equation (3) gives:

$$V/[q\,(S_o - S)] = [K_B\,V/(R_{max}\,q\,S_o)] + [1/R_{max}] \tag{5}$$

In continuously stirred tank reactors the hydraulic retention time (HRT) can be defined as: $HRT=V/q$, so equation (5) can be written as follows:

$$(HRT)/(S_o - S) = [K_B\,(HRT)/(R_{max}\,S_o)] + [1/R_{max}] \tag{6}$$

According to this model a plot of $(HRT)/(S_o - S)$ versus HRT should give a straight line of intercept $[1/R_{max}]$ and slope equal to $K_B/(R_{max}\,S_o)$.

As can be seen in Figure 4 the experimental data fitted to a straight line with $R^2= 0.9992$ for OMSW 1 and $R^2= 0.9999$ for OMSW 2. The maximum removal rate constant (R_{max}) increased from 26.6 to 83.3 g COD/(L d) when the OMSW concentration changed from 35 to 150 g COD/L, indicating a good adaptation of the initial inoculum to the OMSW treated and to increasing concentrations of organic matter fed. The saturation value constants (K_B) were 27.7 g COD/(L d) and 82.7 g COD/(L d) for OMSW 1 and OMSW 2, respectively. The values of R_{max} and K_B obtained for the concentrated OMSW were similar to those obtained by other authors for the anaerobic digestion of soybean wastewaters (Yu et al., 1998) and molasses (Büyükkamaci & Filibeli, 2002). Stover and Campana (2003) have shown that in the model R_{max} is reduced by refractory organics and toxicity. Moreover, the refractory compounds change K_B significantly from R_{max}. These affirmations are in agreement with the data obtained in these experiments, where the higher organic concentration of OMSW 2 gave R_{max} values higher than for OMSW 1.
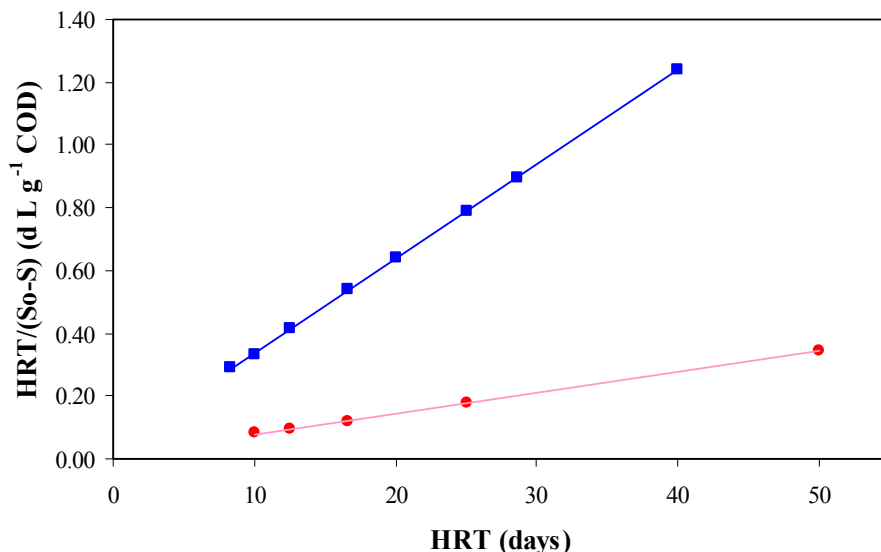
Fig. 4. Determination of the kinetic parameters using the modified Stover-Kincannon model for the two-phase OMSW 1 and OMSW 2. (■: OMSW 1; ●: OMSW 2).

6. Conclusions

The kinetic constants obtained define the bio-treatability of the two-phase olive mill solid waste. The values obtained for R_{max} and K_B were similar to those obtained for other substrates of high organic content. The increase in the maximum methane removal rate for the most concentrated two-phase olive mill solid waste used demonstrated the good adaptation of the bacterial inoculum used to the increase in the substrate concentration. This adaptation allowed the microorganisms to work with high stability even with high organic matter concentrations in the fed substrate. These results can be used to estimate the treatment efficiency of industrial-scale reactors working with similar operational conditions.

7. References

Alba, J.; Hidalgo, F.; Ruiz, M.A.; Martínez, F.; Moyano, M.J.; Borja, R.; Graciani, E. & Ruiz, M.V. (2001). Elaboración de aceite de oliva virgen. In: *El Cultivo del Olivo*, D. Barranco, R. Fernández-Escobar, L. Rallo (Ed.), 551-588, Mundi-Prensa, Madrid, Spain, ISBN 84-7114-983-4.

APHA (American Public Health Association). (1989). Standard Methods for the Examination of Water and Wastewater, 17th ed., APHA, Washington DC, USA, ISBN 087553161X.

Borja, R.; Banks, C.J. & Wang, Z. (1995). Effect of organic loading rate on anaerobic treatment of slaughterhouse wastewater in a fluidised-bed reactor. *Bioresource Technology*, Vol. 52, No. 2, pp. 157-162, ISSN 0960-8524.

Borja, R.; González, E.; Raposo, F.; Millán, F. & Martín, A. (2001). Performance evaluation of a mesophilic anaerobic fluidized bed reactor treating wastewater derived from the production of proteins from extracted sunflower flour. *Bioresource Technology*, Vol. 76, No. 1, pp. 45-52, ISSN 0960-8524.

Borja, R.; Rincón, B.; Raposo, F.; Alba, J. & Martín, A. (2002). A study of anaerobic digestibility of two-phases olive mill solid waste (OMSW) at mesophilic temperature. *Process Biochemistry,* Vol. 38, No. 5, pp. 733-742, ISSN 0032-9592.

Borja, R.; Martín, A.; Rincón, B. & Raposo, F. (2003). Kinetics for substrate utilization and methane production during the mesophilic anaerobic digestion of two-phases olive pomace (TPOP). *Journal of Agricultural and Food Chemistry,* Vol. 51, No. 11, pp. 3390-3395, ISSN 0021-8561.

Borja, R.; Rincón, B. & Raposo, F. (2006). Review: Anaerobic biodegradation of two-phase olive mill solid wastes and liquid effluents: Kinetic studies and process performance. *Journal of Chemical Technology and Biotechnology,* Vol. 81, No. 9, pp. 1450-1462, ISSN 0268-2575.

Büyükkamaci, N. & Filibeli, A. (2002). Concentrated wastewater treatment studies using an anaerobic hybrid reactor. *Process Biochemistry,* Vol. 38, No. 5, pp. 771-775, ISSN 0032-9592.

Di Giovacchino, L.; Costantini, N.; Serraiocco, A.; Surrichio, G. & Basti C. (2001). Natural antioxidants and volatile compounds of virgin olive oils obtained by the two or three-phase centrifugal decanters. *European Journal of Lipid Science and Technology,* Vol. 103, pp. 279-285, ISSN 1438-7697.

Di Giovacchino, L.; Sestili, S. & Di Vencenzo, D. (2002). Influence of olive processing on virgin olive quality. *European Journal of Lipid Science and Technology,* Vol. 104, pp. 587-601, ISSN 1438-7697.

Jafarzadeh, M.T.; Mehrdadi, N. & Hashemian, S.J. (2009). Kinetic constants of anaerobic hybrid reactor treating petrochemical wastes. *Asian Journal of Chemistry,* Vol. 21, No. 3, pp. 1672-1684, ISSN 0970-7077.

Kapdan, I.K. & Erten, B. (2007). Anaerobic treatment of saline wastewater by *Halanaerobium lacusrosei*. *Process Biochemistry,* Vol. 42, No. 3, pp. 449-453, ISSN 1359-5113.

Kincannon, D.F.; Stover, E. L. (1982). Design methodology for fixed-film reactors-RBCs and biological towers. Civil Engineering for Practicing and Design Engineers 2: 107.

Maqueda, C.; Pérez-Rodríguez, J.L. & Lebrato, J. (1998). An evaluation of clay minerals as support materials in anaerobic digesters. *Environmental Technology*, Vol. 19, No. 8, pp. 811-819, ISSN 0959-3330.

Martín, A.; Borja, R.; García, I. & Fiestas, J.A. (1991). Kinetics of methane production from olive mill wastewater. *Process Biochemistry*, Vol. 26, No. 2, pp. 101-107, ISSN 0032-9592.

Martín, A.; Borja, R. & Chica, A. (1993). Kinetic study of an anaerobic fluidized-bed system used for the purification of fermented olive mill wastewater. *Journal of Chemical Technology and Biotechnology*, Vol. 56, No. 2, pp. 155-162, ISSN 1097-4660.

Niaounakis, M. & Halvadakis, C.P. (2004). *Olive-Mill Waste Management: Literature review and Patent Survey.* Typothito – George Dardanos Publications, ISBN 0-08-044851-8, Athens, Greece.

Speece, R.E. (1983). Anaerobic biotechnology for industrial wastewater treatment. *Environmental Science and Technology*, Vol. 17, pp. 416-427, ISSN 0013-936X.

Stover, E. L.; Campana, C.K. (2003). Effluents from food processing/On-site Processing of waste, In: *Encyclopedia of Food Sciences and Nutrition*. Caballero, B., Trugo, L.C., Finglas, P.M. Editors, pp. 1975-1976, ISBN:0-12-227055-X, 978-0-12-227055-0.

Wheatley, A. (1990). *Anaerobic digestion: a waste treatment technology,* published for SCI, ISBN 1-85166-526-9, London, UK, Elsevier Applied Science.

Yu, H.; Wilson, F. & Tay, J.H. (1998). Kinetic analysis of an anaerobic filter treating soybean wastewater. *Water Research,* Vol. 32, No. 11, pp. 3341-3352, ISSN 0043-1354.
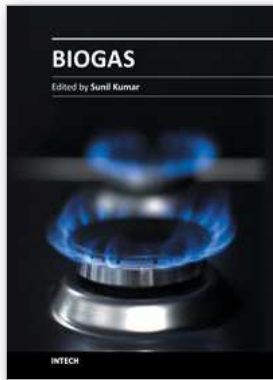
Biogas

Edited by Dr. Sunil Kumar

ISBN 978-953-51-0204-5

Hard cover, 408 pages

Publisher InTech

Published online 14, March, 2012

Published in print edition March, 2012

This book contains research on the chemistry of each step of biogas generation, along with engineering principles and practices, feasibility of biogas production in processing technologies, especially anaerobic digestion of waste and gas production system, its modeling, kinetics along with other associated aspects, utilization and purification of biogas, economy and energy issues, pipe design for biogas energy, microbiological aspects, phyto-fermentation, biogas plant constructions, assessment of ecological potential, biogas generation from sludge, rheological characterization, etc.

INTECH
open science | open minds