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Influence of the microorganism support on the kinetics of anaerobic fermentation of condensation water from thermally concentrated olive mill wastewater

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Abstract

Two materials of different structure, sepiolite and bentonite, evaluated as supports for the microorganisms effecting anaerobic fermentation, behaved differently towards condensation water from thermally concentrated olive mill wastewater from a kinetic point of view. Assuming the overall anaerobic digestion process to conform to first-order kinetics, the apparent kinetic constant for the digester including sepiolite as support was 1.12 day^{-1}, while that of the digester using the bentonite support was 0.73 day^{-1}. Thus, the apparent kinetic constant of the process was increased by 35% with the use of sepiolite. The yield coefficient, $Y_{p/s}$, was 0.344 and 0.318 litres CH_4 STP/g COD for the sepiolite and bentonite supports respectively.

Introduction

The manufacturing process of olive oil usually yields an oily phase (20%), a solid residue (30%) and an aqueous phase (50%) formed from the water content of the fruit. Such water, when combined with that used in washing and processing the olives, makes up the so-called 'olive mill wastewater' (OMW) and also contains soft tissues from olive pulp and a very stable oil emulsion. The high polluting power and large volumes of OMW produced along the Mediterranean basin pose large-scale environmental problems (Fiestas 1984).

One of the physical processes used to treat this wastewater is evaporation or thermal concentration, which removes some of the water from the waste. Multiple-effect evaporators are used in order to avoid high energy consumption (Valenzuela, 1986).

Evaporation reduces the volume of OMW by 70–75%, leaving concentrated olive waste which can be used as fertilizer, since the concentrate is rich in organic material and minerals. The water obtained from the condensation of the vapor from the OMW, however, contains the most of the volatile components from the residue as well as small amounts of the waste itself, because of the sweeping and splashing during the evaporation process. Thus the water obtained through evaporation must be further purified before re-entry into the natural environment or re-use in agriculture.

Of the possible solutions and technologies available for the purification and re-use of this wastewater, anaerobic fermentation or biomethanization is among the best. Its most noteworthy advantages are (Fiestas 1984; Olthof et al. 1982): a) it demands little energy; b) anaerobic bacteria are capable of transforming most of the organic substances present into methane; c) sludge formation is minimal; d) nutrient demands are very low; and e) unpleasant odours are avoided.

Because of the low reproduction rate of the anaerobic microorganisms, a variety of methods have been developed to keep them inside the bioreactors and avoid their loss with the effluent, thereby slowing the fermentation process. Among the most widely used bioreactors for this process is the fluidized bed reactor, in which the bacteria colonize particles of certain materials which are used to increase the surface available for bacterial growth. These reactors also allow a higher volume load (over 50 kg $COD/m^3 \cdot d$), which makes them suitable for wastewater treatment either of high or low organic material content, the second of which concerns this work (Rozzi 1986).

The results obtained through previous research on the microbiology and biochemistry in anaerobic processes show the influences that different supports have on the immobilization of the microorganisms which carry out digestion (Huysman et al. 1983; Murray et al. 1981; Maestrojuan et al. 1986; Kida et al. 1990). The aim of this work was to carry out a kinetic study on the anaerobic purification of the condensation water from thermally concentrated OMW in two bioreactors containing microorganisms immobilized on sepiolite and bentonite supports respectively, in order to study the influence of these supports on the biokinetic parameters of digestion. Purifying procedures involving fluidized beds require sturdy supports of low apparent density in order to reduce power consumption, which is the case with the two assayed here.

Table 1. Composition and features of the supports.

	Sepiolite	Bentonite
SiO_2	62.0	60.3
Al_2O_3	1.7	16.8
Fe_2O_3	0.5	3.6
TiO_2	–	0.2
MgO	23.9	4.6
CaO	0.6	1.7
Na_2O	0.3	4.5
K_2O	0.6	1.3
Calcination loss	10.4	6.7
Average size of particles (μm)	2–5	2–5
Moisture content (%)	8.5	15
Apparent density (g/cm^3)	0.55	0.75
C.E.C. (Exchange capacity, mequ./100 g)	140	85

Typical chemical analysis (% sample dried at 105° C).

Materials and methods

Equipment

The equipment used consisted of 1-litre magnetically stirred batch anaerobic digestion units (ADU) immersed in a water bath at 37° C. The biogas produced throughout the process was measured daily in Boyle-Mariotte's bottles and the volume was measured indirectly from the amounts of water displaced by the gas.

Supports

The materials used as supports for the anaerobic bacteria were commercially available sepiolite and bentonite of micronized size (2–5 μm diameter), supplied by Tolsa S.A. (Madrid). Their chemical composition and features are summarized in Table 1.

Inoculum

The digestion process was started using previously diluted and neutralized anaerobic biomass from an olive mill wastewater (OMW) storage and evaporation pond as inoculum. Its composition is summarized in Table 2.

Table 2. Composition of the biomass used as inoculum.

TS	VS	MS	TSS	VSS	MSS	pH
32.6	25.8	6.8	26.0	20.8	5.2	7.0

TS, VS and MS: total, volatile and mineral solids (g/l).
TSS, VSS and MSS: total suspended, volatile suspended and mineral suspended solids (g/l).

Wastewater

The wastewater used was collected from an OMW thermal condensation line. Its COD, pH, total acidity (TA), volatile acidity (VA) and volatile acid composition are given in Table 3.

Condensation water from thermally concentrated olive mill wastewater is characterized by the absence of phenolic compounds, in contrast to the unmodified or natural OMW, which contains a large amount of these aromatic compounds.

Chemical analyses

The wastewater was analysed according to Standard Methods for the Examination of Water and Wastewater (APHA 1985).

Volatile organic acids were determined with a gas chromatograph equipped with a 2 m × 4 mm glass column packed with Supelcopor (100–120 mesh) coated with 10% Fluorad FC 431. The temperature of the column, the injection port and the flame ionization detector were 130, 220 and 240°C respectively. Nitrogen saturated with formic acid was used as the carrier gas at a flow rate of $50\,ml \cdot min^{-1}$.

Methane was determined by gas chromatography (Yanako G-80) with a stainless steel column (200 × 0,3 cm) packed with active carbon (30 to 60 mesh) using thermal conductivity detection.

The SEM micrographs were obtained using a Phillips SEM 501B microscope. The samples were dispersed in deionized water, dried and covered with electrodeposited gold for microscopic examination.

Specific surface area was determined by N_2 adsorption, using the BET equation. The adsorption and desorption curves were obtained with a Quantasorb Jr Surface Area Analyzer (Quantachrome Corporation Syosset, N.Y.).

Points of zero charge of the media were measured as follows. Medium powdered in a mixer was added stepwise to 20 ml distilled water, and the change of pH of the suspension was followed (Kida et al. 1990).

Experimental procedure

Experiments were conducted in two anaerobic digestion units (ADU) which included a sepiolite and a bentonite support, respectively. Each ADU contained 750 ml distilled water, 250 ml of the above-mentioned inoculum and 10 g of the support. While larger amounts of support allowed increased amounts of biomass to be processed, there is some evidence that they also increase the apparent viscosity of the medium and hence hinder mass transfer and decelerate the process (Martin et al. 1991).

Before the experiments were started, the biomass was conditioned by feeding it with gradually increasing volumes of the wastewater in question for 3 months. The added volume was modified every time methane production was finished. This period of acclimatization also allows the adhesion of the microorganisms to the supports. The experiments were conducted in batch fashion, using 40, 80, 120, 160, 200, 240, 280, 320 and 360 ml of the wastewater. In each experiment the methane volume produced per day, and the initial and final COD was determined. The duration of the experiment was the time required for complete biomethanation of each added volume. The wastewater volumes were added after separating the same volume of liquid from the bioreactor after settling (2 h) in

Table 3. Features of the wastewater used.

COD (g/l)	pH	TP (ppm caffeic a.)	TA (ppm acetic a.)	VA (ppm acetic a.)	VA (%)			
					C_2	C_3	C_4	C_5
6	3.5	0	2100	2050	37.5	18.7	3.1	0.7

TP = total polyphenols; TA = total acidity; VA = volatile acidity.

order to avoid biomass losses. All experiments were conducted in duplicate.

Results and discussion

Variation of the methane volume produced with time

Tables 4 and 5 list the net accumulated methane volumes at different times (days) for the different feed volumes used in the digesters containing the sepiolite and bentonite support respectively. Figure 1 shows the variation of the net accumulated methane volume as a function of time in both digesters. The variable is the volume of wastewater added to the digesters. As can be seen:

(a) The methane volume produced increased with increasing wastewater volume used.
(b) For a given time, the methane volume produced decreased in the order sepiolite > bentonite.
(c) The shape of the kinetic curves was very similar.

In order to characterize each experiment kinetically and thus facilitate comparisons, we developed the model described below. The anaerobic digester used can be considered to be a bioreactor where the nutrients, expressed as COD, react with microbial sludge of concentration X (Winkler 1986). The rate of nutrient removal will be given by:

$$- dS/dt = K \cdot S \cdot X \quad (1)$$

where t denotes time, S the substrate concentration and K a kinetic coefficient. Because of the small value of the cellular yield coefficient ($Y_{s/x}$) in the anaerobic digestion (0,02–0,06 g cells/g COD) (Gujer et al. 1983; Jeris 1983), and taking into account that the VSS was essentially constant throughout the experiment, X can be assumed to remain roughly constant. This hypothesis was supported by the data from all the digesters and in all experiments, since the values obtained were approximately the same in each case, ranging between 10,0 and 10,2 g VSS/l. Integration of Eq. (1) on this assumption yields:

$$S = S_o \cdot \exp(-K \cdot X \cdot t) \quad (2)$$

where S_o is the initial substrate concentration.

Defining a yield coefficient, $Y_{p/s}$, for the product formed (methane), such that

$$Y_{p/s} = - dG/dS \quad (3)$$

then gives:

$$G = G_m \cdot [1\text{-}\exp(-K_A \cdot t)] \quad (4)$$

Table 4. Net volume of methane accumulated (ml) as a function of time (days) for the different feed volumes used. Support: sepiolite.

Feed vol. (ml)	Time (days) 1	2	3	4	5	6	7
20	30	38	42	43	43	–	–
40	61	78	86	89	89	–	–
80	121	153	167	174	175	–	–
120	180	229	249	258	260	–	–
160	239	304	318	345	348	349	–
200	299	381	415	430	439	440	–
240	360	450	492	516	523	525	–
280	417	525	575	604	613	615	–
320	467	600	653	682	696	702	704
360	545	681	740	769	783	786	788

Table 5. Net volume of methane accumulated (ml) as a function of time (days) for the different feed volume used. Support: bentonite.

Feed vol. (ml)	Time (days) 1	2	3	4	5	6	7
20	21	31	36	39	39	–	–
40	42	62	73	78	79	–	–
80	98	122	146	159	161	–	–
120	127	187	219	234	236	–	–
160	168	252	292	312	321	322	–
200	210	321	366	390	399	401	–
240	248	372	428	457	475	476	–
280	294	435	507	546	559	561	–
320	328	487	573	621	638	641	643
360	362	543	635	683	709	719	722

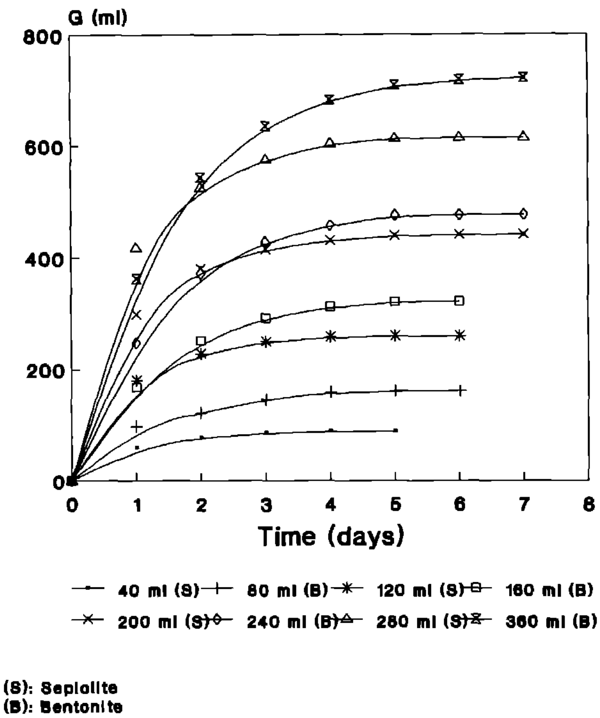


Fig. 1. Variation with time of the net methane volume produced for the sepiolite and bentonite digesters.

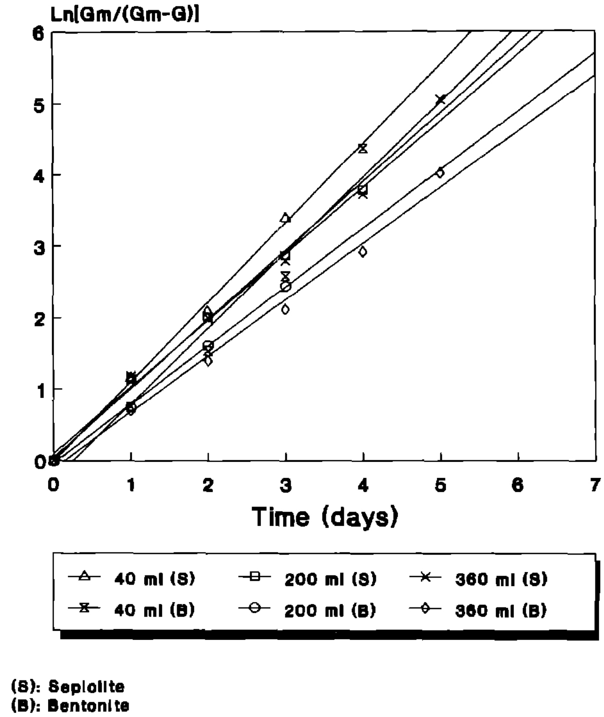


Fig. 2. Representation of the Ln $[G_m/(G_m–G)]$ values vs. time (days) for the sepiolite and bentonite reactors.

where G is the methane volume (ml) produced after a time t (days; G_m is the maximum volume accumulated at an infinite digestion time and is the product of the initial substrate concentration (S_o) and the yield coefficient of the product ($Y_{p/s}$); $G_m = S_o \cdot Y_{p/s}$; and K_A is an apparent kinetic constant that includes the biomass concentration: $K_A = K \cdot X$.

Equation (4) coincides with that established empirically by Roediger (Edeline 1980). According to Eq. (4), methane production conforms to a first-order kinetic model (Winkler 1986).

By plotting the experimental data listed in Tables 4 and 5 as G vs t, curves were obtained whose shape coincided with that predicted by Eq. (4) (Figs. 1 and 2). On the other hand, taking natural logarithms in equation (4), and ordering the terms, one obtains:

$$\mathrm{Ln}\,[G_m/(G_m–G)] = K_A \cdot t \qquad (5)$$

indicating that Ln $[G_m/(Gm\_G)]$ vs. t should give a straight line of slope equal to K_A with ordinate zero. As an example, Fig. 2 shows some of the experimental data. The value of G_m has been considered to be equal to the volume of methane accumulated at the end of each experiment. On representing the experimental data as indicated, equation 5 gives straight lines with ordinate almost at zero. Thus it is possible to fit the experimental data to the proposed model. Once it had been qualitatively checked that these results could be modelled analytically, parameters G_m and K_A were calculated numerically using standard software (Time Series Processor, TSP, International program version 4.0D, Stanford, CA, USA), using the (G,t) value pairs for each experiment (Tables 4 and 5) and equation (4) to which they must be fitted. The programme calculated G_m and K_A, as well as various statistical parameters required to evaluate goodness of fit.

Table 6 lists the K_A values with their bounds at 95% confidence level, obtained for each digester and feed volume.

The variation of K_A with the initial amount of

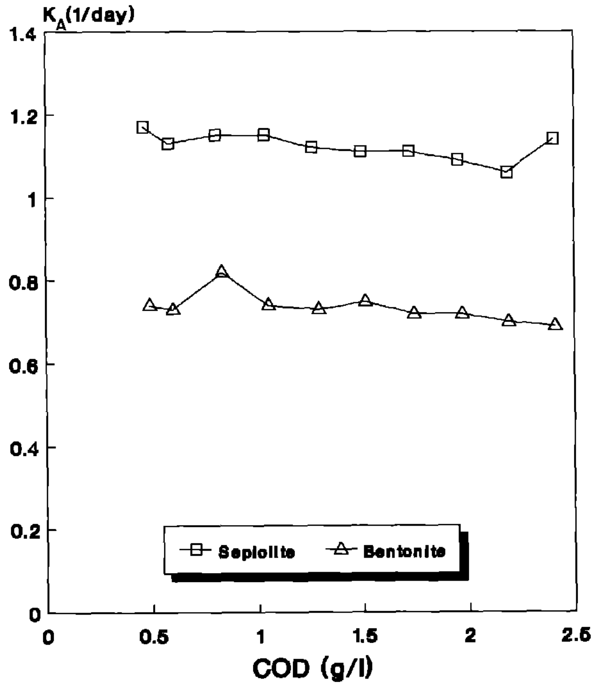


Fig. 3. Variation of the apparent kinetic constant, K_A, with the initial amount of soluble COD for the two digesters studied.

soluble COD added for each system is plotted in Fig. 3. From this figure and Table 6, sepiolite has the highest K_A values in the range of COD studied. Also, this kinetic constant is virtually independent of the substrate concentration in the two systems, up to COD = 2.0 g/l, above which it decreases very slightly in the bentonite digester. The mean values of K_A were 1.12 ± 0.11 and 0.73 ± 0.04 day^{-1} for the digesters containing sepiolite and bentonite support, respectively. Thus, the apparent kinetic constant of the anaerobic purification of this wastewater increased by 35% with sepiolite compared to bentonite. This is significant at the 95% confidence level.

The fact that parameter K_A is the product of the kinetic constant K and the concentration of biomass X may explain the difference observed, as there were different concentrations of biomass in the two digesters. However, monitoring of this parameter with time yields a practically constant value of 10.0–10.2 g VSS/l in the two digesters used, invalidating this hypothesis.

The specific surface of the supports is 283 m^2/g and 22 m^2/g for sepiolite and bentonite respectively. There appears to be a direct relationship between this value and K_A. However, the large size of the bacteria compared with the diameter of the micropores and mesopores (Huysman et al., 1983) means these can adhere only to the external surface of the particles. Given that the equivalent diameter of these is similar for both types of support, the external surface will also be so. Thus the values for specific surface cannot explain the observed difference.

Kida et al. (1990) indicate that the performance of a support depends more on the roughness and the possibility of surface attraction of the cells than on the specific surface. The SEM photographs (Fig. 4) demonstrate that sepiolite – the support showing greatest activity (K_o = 1.12 $days^{-1}$) – has the rougher surface.

Figure 5 shows points of zero charge of sepiolite and bentonite. Since they were pH 8.6 and 10.9 respectively, both supports were charged negatively at pH 7. Since the surface of microorganisms is generally negatively charged at pH 7 (Loder and Liss, 1985; Loosdrecht, Van et al. 1987a; Kawase et al. 1989), and may be considered as colloidal particles, their adhesion can be studied as a physicochemical phenomenon, applying colloid chemical principles (Absolom et al. 1983; Busscher et al. 1984; Loosdrecht, Van et al. 1987b). The DLVO theory for colloidal stability can be used to calcu-

Table 6. K_A, apparent kinetic constants values ($days^{-1}$) with 95% confidence level, obtained for each digester and feed volume (ml) of wastewater.

Feed vol. (ml)	K_A ($days^{-1}$)	
	Sepiolite	Bentonite
20	1.18 ± 0.11	0.74 ± 0.06
40	1.13 ± 0.11	0.73 ± 0.05
80	1.15 ± 0.13	0.82 ± 0.09
120	1.15 ± 0.10	0.74 ± 0.05
160	1.12 ± 0.10	0.73 ± 0.02
200	1.11 ± 0.11	0.75 ± 0.04
240	1.11 ± 0.15	0.72 ± 0.02
280	1.09 ± 0.14	0.72 ± 0.03
320	1.06 ± 0.10	0.70 ± 0.03
360	1.14 ± 0.12	0.69 ± 0.02

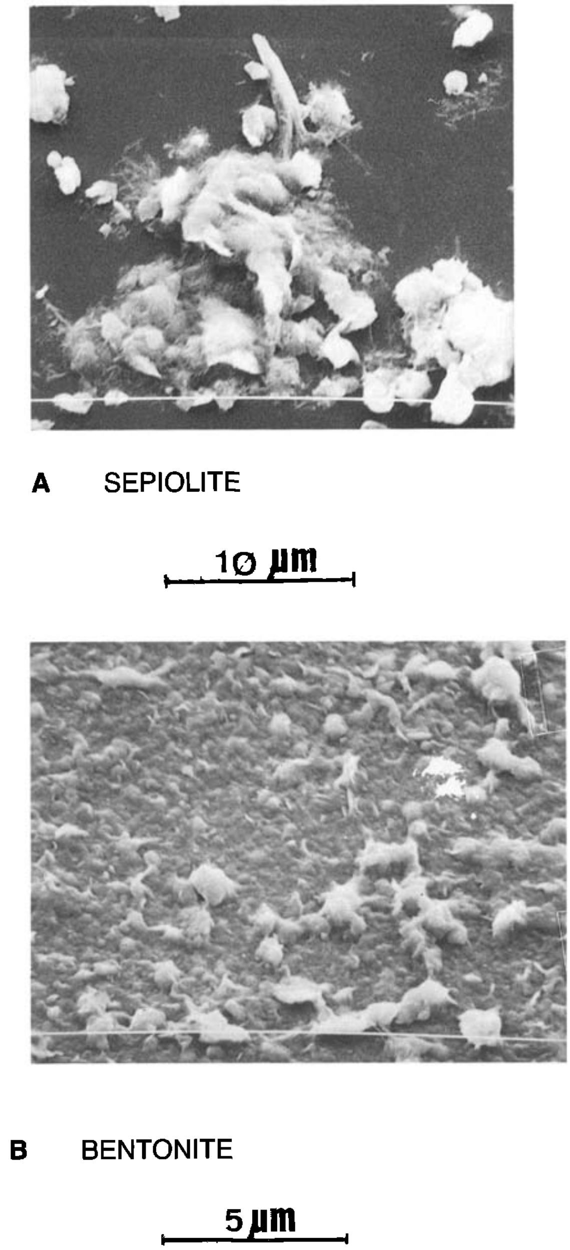


Fig. 4. Scanning electron micrographs of the surface of the two supports used. A: sepiolite; B: bentonite.

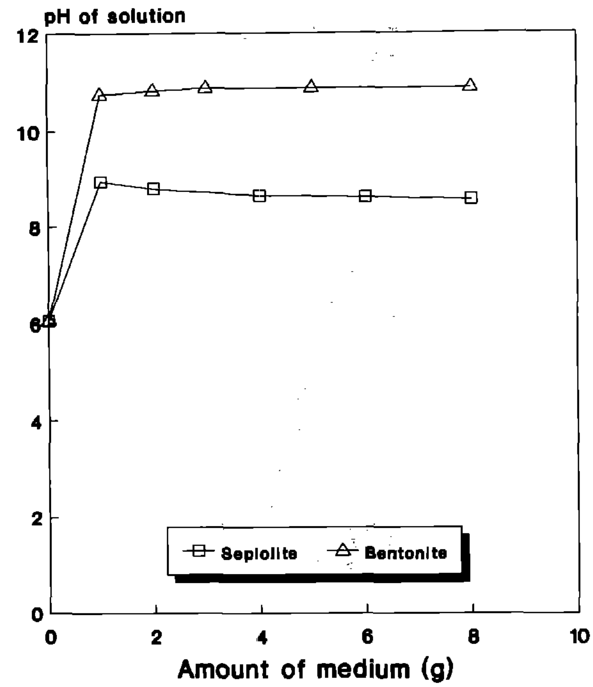


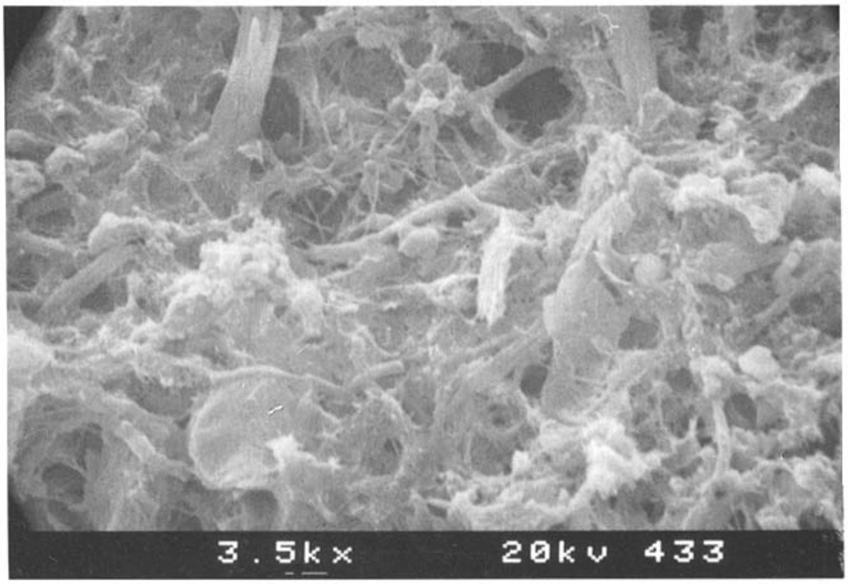
Fig. 5. Points of zero charge of sepiolite and bentonite.

late the interaction Gibbs energy between the support and bacteria (Loosdrecht Van et al. 1989), in terms of Van der Waals and electrostatic interactions. According to Rouxhet et al. (1984) and Stotzky (1985), some clays (e.g. sepiolite) favour these interactions and also the appearance of ionic bridges, which could explain better adhesion in sepiolite than in bentonite.

Another aspect to consider is the possibility that the materials used exchange cations with the medium, aiding the creation of appropriate microenvironments for efficient cell nutrition. In particular, the cation Mg – of great importance in the activity of methanogenic bacteria (Murray et al. 1981; Huysman et al. 1983) – can be transferred very easily in sepiolite and not at all in bentonite, where the Mg is replaced by Al, the activity of which does not seem important in anaerobic processes.

Sepiolite also shows a high capacity of microorganism adsorption, possibly interacting with specific components of the cell membrane. Thus sepiolite selectively adsorbs methanogenic bacteria (*Methanosarcina*) rather than competing bacteria such as the sulphate-reducers (Pérez et al. 1989).

The better adherence of the bacteria to the surface of sepiolite than to bentonite is shown by SEM (Fig. 6). To sum up, it seems that surface morphology, the support's capacity for microorganism adsorption, and the exchange of micronutrients play an important role in the behaviour of the supports.



A SEPIOLITE

5 µm

B BENTONITE

5 µm

Fig. 6. Scanning electron micrographs of the material in suspension from the two reactors used. A: sepiolite; B: bentonite.

Biodegradability

Table 7 lists the initial and final soluble COD values obtained by using different wastewater volumes for the two digesters studied. From these results, the fraction of biodegraded substrate was calculated. This ranged between 92 and 94%, and thus testifies to the effectiveness of anaerobic digestion as a purifying procedure for this wastewater.

The pH of the effluents remained virtually constant throughout the experiments at 7.2 and 7.1 for the sepiolite and bentonite digester, respectively. Also, the acidity of the effluents remained constant at 70 and 75 mg/l (acetic acid) for the two digesters used. The constancy and small value of this parameter show the efficient biomethanation of the condensation water from thermally concentrated OMW.

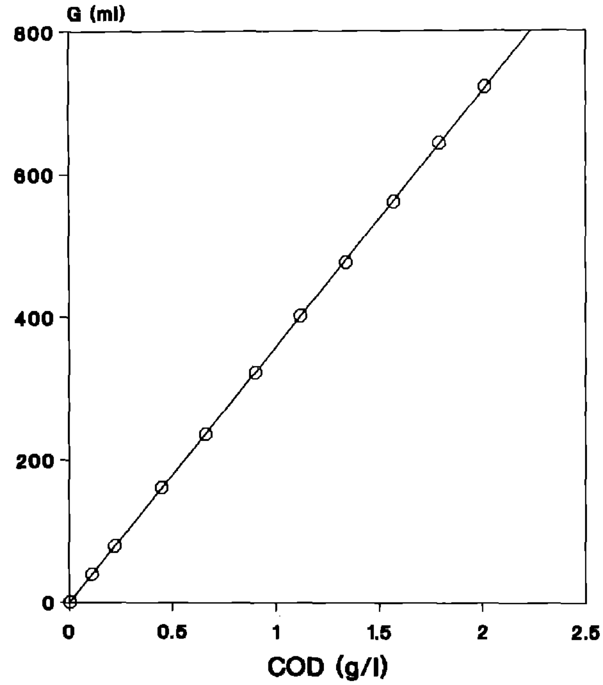


Fig. 7. Determination of the yield coefficient ($Y_{p/s}$) of the bentonite digester.

Yield coefficients

The yield coefficient of each system, $Y_{p/s}$, was determined from the methane volume produced and the initial and final COD. As can be seen in Fig. 7, the methane volume produced was proportional to the COD uptake for bentonite, so its yield coefficient was constant. The other digestor provided qualitatively analogous results. By fitting (G, COD uptake) value pairs to a straight line, the yield coefficients under standard temperature and pressure (STP) conditions in the absence of moisture were 345 ± 1 and 319 ± 2 ml CH_4 STP/g COD for the sepiolite and bentonite reactors, respectively (95% confidence level). Thus, the coefficient was larger (7.5%) for the reactors using sepiolite support than for bentonite.

Table 7. Initial and final soluble COD in the two digesters used.

Added volume (ml)	Sepiolite		Bentonite	
	$(COD)_i$	$(COD)_f$	$(COD)_i$	$(COD)_f$
20	0.46	0.35	0.49	0.38
40	0.58	0.35	0.60	0.38
80	0.80	0.35	0.83	0.38
120	1.03	0.36	1.05	0.39
160	1.26	0.36	1.29	0.39
200	1.49	0.36	1.51	0.39
240	1.72	0.37	1.74	0.40
280	1.95	0.37	1.97	0.40
320	2.18	0.37	2.19	0.40
360	2.40	0.37	2.41	0.40

In contrast to condensation water from thermally concentrated olive mill wastewater, unmodified or natural OMW has a large amount of phenolic compounds, which cause a high inhibition effect and high antibacterial activity (Ragazzi and Veronesse 1967; Sorlini et al. 1986; Rodriguez et al. 1988; Borja et al. 1990). Therefore, anaerobic digestion of this wastewater causes many problems, such as high toxicity, low biodegradability of the effluent and acidification of reactors (Fiestas et al. 1982; Boari et al. 1984). As a result, no significant influence of the support on the rate constants and on the yield coefficient of methane was observed in the anaerobic digestion process of this wastewater (Martin et al. 1991). On the other hand, the absence of phenolic compounds in the condensation water from thermally concentrated OMW allowed us to differentiate the behaviour of both immobilization supports in the anaerobic digestion process. This same behaviour has been observed in other wastewaters from food industries which do not contain this type of aromatic compound (Borja et al. 1992).

Conclusions

(1) Condensates from multi-effect OMW thermal concentration processes can be readily biodegraded by anaerobic digestion, independently of the support used, since over 90% of the initial COD is removed.
(2) At the COD levels assayed (COD ≤ 2.5 g/l), the use of sepiolite support significantly ($P < 0.05$) increases the magnitude of the kinetic constant of the anaerobic digestion process compared to bentonite.
(3) No inhibition phenomena were observed over the COD range studied. The values observed in the kinetic constants were virtually constant in this COD range.
(4) The COD uptake and the methane volume produced are linearly related. The slope of the COD-V_{CH4} plot coincides with the yield coefficient ($Y_{p/s}$) of each reactor, namely 345 and 319 ml CH_4 STP/g COD for the sepiolite and bentonite digester, respectively.

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