Anaerobic digestion of the vegetable fraction of municipal refuses: mesophilic versus thermophilic conditions

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Abstract The phenomena limiting the anaerobic digestion of vegetable refuses are studied through batch tests carried out using anaerobic sludge previously selected under either mesophilic (37 °C) or thermophilic (55 °C) conditions. The compositions of the hydrolysed cellulosic and hemicellulosic fractions of these materials are simulated by starch and hemicellulose hydrolysates, respectively. Non-hydrolysed mixtures of vegetable waste with sewage sludge are used to ascertain whether the hydrolysis of these polymeric materials is the limiting step of the digestion process or not. The experimental data of methane production are then worked out by a first-order equation derived from the Monod's model to estimate the kinetic rate constant and methane production yield for each material. Comparison of these results shows that passing from mesophilic to thermophilic conditions is responsible for a slight deceleration of methane production but remarkably enhances both methanation yield and methane content of biogas. The final part of the study deals with the fed-batch digestion of the same residues in static digester. Working under thermophilic conditions at a loading rate threshold of 6.0 $g_{COD}/l \cdot d$, the hemicellulose hydrolysate ensures the highest methane productivity ($60 \, mmol_{CH_4}/l \cdot d$) and methane content of biogas (60%), while unbalance towards the acidogenic phase takes place under the same conditions for the starch hydrolysate. The intermediate behaviour of the non-hydrolysed mixture of vegetable waste with sewage sludge demonstrates that hemicellulose hydrolysis is the limiting step of digestion and suggests the occurrence of ligninic by products inhibition on methane productivity.

1
Introduction

One of the most promising alternative to dumping, incinerating and composting of municipal solid waste (MSW) is to digest its organic fraction (MSWOF) in an anaerobic reactor. The main advantages of this process are the production of biogas which can be used to produce electricity for own consumption and the obtaining of a valuable sludge which can eventually be used as a soil amendant after minor treatments. Therefore, using this technology for the treatment of MSW can reduce the environmental impact and can allow us to recover energy and materials.

It is well known that the digester performance is highly sensitive to the quality of the feed, the yield and kinetics of the biological reactions involved in anaerobic digestion being strongly dependent upon waste composition. For example, the biodegradation kinetic constant for the source-selected MSWOF is about 5–10 times larger than that for fresh mechanically-selected MSWOF, while the ultimate biogas yield is 2.5 times larger [1].

Experiments carried out to obtain information about yields and kinetics of the anaerobic digestion of the organic waste of a food market showed a very high yield of biomethanation. The very high ultimate methane yields of both soluble and non-soluble fractions confirm the excellent biodegradability of this type of waste [2].

The anaerobic digestion of putrescible wastes has been studied extensively in relation to the organic fraction of municipal solid wastes [3]. Because of the similar composition, the satisfactory results obtained with this type of substrate, that are the direct consequence of the development of anaerobic digestion for MSWOF, have then been successfully transferred to the anaerobic digestion of vegetable and food residue as well as of the putrescible fraction of agroindustrial wastes [4].

The good results obtained for the anaerobic digestion of vegetable and agroindustrial wastes are valid, in general, when the lignin content of the waste is not too high. In fact, it was undoubtedly demonstrated by Pfeffer and Khan that more than 15% by weight of lignin greatly inhibits digestion [5].

In this study we simulate the hydrolysed hemicellulosic and cellulosic fractions of these refuses using hemicellulose and starch hydrolysates after ligninic fraction removal. In fact, the final aim of this work is to make a separate kinetic study on the digestion of the various components of the vegetable fraction of waste (lignin, cellulose and hemicellulose), in order to better understand the complete digestion process. Pre-hydrolysis of these separate components (except for lignin) has been used to increase the fraction of soluble organic substances as well as to investigate the effects of this pre-treatment on biogas production and conversion yields.

Among the various techniques available to pretreat lignocellulosics [6], all of which are drastic, we have selected the dilute acid hydrolysis at high pressure and temperature [7]. This technique allows for the most effi-

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cient solubilisation of hemicellulosic sugars, but both lig-
ninic and cellulosic fractions remain nearly intact in the
residue under these conditions. In addition, some unde-
sired phenolic and furanic inhibitors are formed [8]. It was
demonstrated that simultaneous lignin and cellulose sol-
ubilisation from the resulting solid residue could be
performed by alkaline hydrolysis at high temperature, but
lignin and its decomposition products are generally con-
sidered to be non-biodegradable under any condition [5].
So, in order to avoid the formation of lignin by-products
and the consequent negative effects on digestion, the en-
zymatic hydrolysis of the cellulose fraction has been taken
into consideration in this study.

Previous work showed that preliminary hydrolysis of
these fractions is able to nearly redouble the organic
strength of domestic sewage sludge and that an even
greater effect takes place for MSWOF [9]. This is due to
higher content of organic hydrolysable polymers in
MSWOF, including proteins, polysaccharides, etc. (400–
600 $g \cdot kg^{-1}$) [10, 11], than in municipal sewage sludge
(24–26 $g \cdot kg^{-1}$) [12] because of the high water content
(92–94%). Such a low content of organic polymers in do-
mestic sewage sludge suggests that the performance of
anaerobic digestion of the material under consideration
could not be significantly influenced by separate sludge
hydrolysis.

Batch digestion tests have been carried out in the
present work under both mesophilic and thermophilic
conditions, using as feeds either pre-hydrolysed (hemi-
cellulose and cellulose hydrolysates) or non-hydrolysed
substrates (mixture of vegetable waste and sludge). This
has allowed to study the digestion kinetics and to get one
general model able to describe the behaviour of all the
different components, either separately or simultaneously
present in mixture.

At last, the same residues have been submitted to fed-
batch digestion to verify the results obtained with the
batch tests.

2
Materials and methods

2.1
Experimental set-up
Digestion tests were carried out simultaneously using four
3-litres glass digesters partially submerged in water baths
kept at the selected temperature (37 ± 0.1 °C for me-
sophilic and 55 ± 0.1 °C for thermophilic tests). The re-
actors were fed for both batch or fed-batch runs with one
of the different feeds selected in this study, namely
hemicellulose hydrolysate, starch hydrolysate, and mix-
tures of vegetable waste and sludge.

Batch tests were carried out following the time varia-
tions of biogas volume, CH_4 content of biogas, soluble
COD, and volatile suspended solids.

Fed-batch operation was performed by replacing daily
a fixed volume of anaerobic sludge with an equivalent
amount of feed, in order to keep the volume constant.
Subsequently, the reactors were gently mixed for 2 min.
The time interval between two consecutive feeds was
24 h.

2.2
Hydrolysis of the residues
The hydrolysed hemicellulosic fraction of food wastes was
simulated using a hardwood hemicellulose hydrolysate
prepared according to the dilute acid Tennessee Valley
Authority (TVA) procedure [7, 13], whose sugar concen-
tration was subsequently increased by washing successive
batches of residues through a countercurrent scheme. The
resulting hydrolysate mainly consisted of hemicellulosic
sugars (pentoses), while the remaining residue, consisting
primarily of cellulose and lignin, was not recovered. In fact,
a previous study demonstrated that its caustic solubilisat-
ion, releasing ligninic by-products, unacceptably inhibited
the subsequent digestion [14]. So, the cellulose fraction has
been simulated with a starch hydrolysate obtained by en-
zymatic hydrolysis of starchy residues coming from both
corn overproduction and cultivation waste [15].

2.3
Feed preparation
Starting COD varied between 193.7 and 200.2 g_{COD}/l for
hemicellulose hydrolysate (HH) and between 680 and
740 g_{COD}/l for cellulose hydrolysate (CH), while COD of
domestic sludge was approximately 2.72 g_{COD}/l. Total dry
solids concentration varied between 135 and 140 g_{TDS}/l for
HH and between 550 and 600 g_{TDS}/l for CH.

Little volumes of these concentrated solutions were
added into the reactors to reach the starting COD levels
selected for this study, which ranged from 1.4 to 2.8 g_{COD}/l
for HH and from 1.4 and 3.0 g_{COD}/l for CH, respectively.

A mixture of primary and excess sludge, supplied by the
municipal wastewater treatment plant of Punta Vagno
(Genoa), was also used to prepare the third feed consisting
of a non-hydrolysed mixture of vegetable waste and
sludge. For convenience, the sludge was previously cen-
trifuged for 5 min at 2000 rpm and then mixed in different
proportions with a finely grounded mixture of different
vegetables up to the selected starting COD level, which
varied from 1.5 to 5.0 g_{COD}/l.

In the absence of any preliminary information about the
digestibility of these preparations, the ranges of starting
COD have been selected on the basis of the average com-
position of several completely hydrolysed municipal and
agroindustrial solid wastes [4, 8, 16].

2.4
Measuring methods
COD, total dry solids and volatile solids concentrations
were determined as described in APHA Standard Methods
[17]. The volume of biogas produced was measured by
means of liquid level displacement. The methane fraction
of biogas was determined by a gas chromatograph, Frac-
tovap model M, Carlo Erba, Milan, equipped with a column
packed with Cromosorb 1102, using helium as carrier gas.

3
Theory
As well known, substrate consumption in a bioprocess can
be described, neglecting the maintenance contribution, by
the Monod-type [18]:

$$dC_s/dt = -r_{max}C_sC_x/(K_s + C_s) \ , \qquad (1)$$

where C_s is the substrate concentration (g_{COD}/l), C_x the biomass concentration (g_{VSS}/l), r_{max} the maximum value of the specific degradation rate (g_{COD}/g_{VSS}.h), and K_s the saturation constant (g_{COD}/l).

In the particular case of anaerobic digestion of difficult materials, rich in polymeric substances, which need preliminary hydrolysis to be digested, it is convenient to indicate with C_s the concentration only of the rapidly digestible organic substances. In addition, because of the peculiar slowness of anaerobic digestion, the saturation constant is so high that C_s can be neglected with respect to it, thus Eq. (1) simplifies as follows:

$$dC_s/dt = -kC_sC_x \ , \qquad (2)$$

where k can be considered as a sort of kinetic constant. A further simplification comes from the very long duplication time of methanogenic bacteria ($t_d = 1$-3d for *Methanothrix hungatei* and *Methanosarcina barkeri*, as examples) [19]; in fact, under very slow growth conditions, biomass concentration can be assumed to keep practically constant during a batch run started at relatively high C_x°. As such Eq. (2) can easily be integrated, giving:

$$C_s = C_s^\circ \exp(-kC_xt) \ , \qquad (3)$$

where C_s° is obviously the starting level of the rapidly digestible substances in the feed.

Alternatively, it is possible to express methane production rather than substrate concentration versus time. To this purpose, it is sufficient to substitute into Eq. (3) the following material balance:

$$n_{CH_4} = n'_{CH_4}(C_s^\circ - C_s) \ , \qquad (4)$$

where n'_{CH_4} ($mmol_{CH_4}/g_{COD}$) is the methane yield on substrate. This substitution gives:

$$n_{CH_4} = n'_{CH_4}C_s^\circ[1 - \exp(-kC_xt)] \ . \qquad (5)$$

This equation has been used to study the kinetics of anaerobic digestion of the different materials tested.

4
Results and discussion

4.1
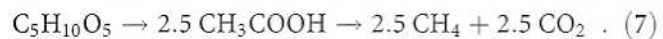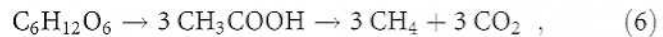## Batch digestion

4.1.1
Estimation of the kinetic parameters of anaerobic digestion

In order to ensure a sufficiently high initial biomass concentration, the set of batch tests selected for each feeding material has been carried out at the end of a preceding fed-batch run only after waiting for the completion of biogas development. So, we have supposed as negligible the contribution to methane production due to the organic substance remained in the digester before start.

The value of C_x to use in Eq. (5) for each test was experimentally determined as volatile suspended solids during the previous fed-batch run, while that of C_s° was estimated case by case considering both the COD value of

the feed and the dilution consequent to feed addition into the reactor. The experimental values of the progressive methane production were then used in a common programme for error minimisation (Table Curve Jandel for Windows) to estimate for each test the values of both the kinetic constant, k, and the methane yield on substrate, n'_{CH_4}.

The theoretical behaviours described by Eq. (5) are compared in Figs. 1–3 with the experimental data of methane production for the anaerobic digestion of the hemicellulose hydrolysate, the starch hydrolysate, and the mixture of vegetable waste and sludge, respectively. The main results of these tests, which are summarised in Tables 1–3 respectively, can be compared under either mesophilic or thermophilic conditions to provide useful information on the digestion kinetics of the various materials. More information on the organic load to employ in fed-batch run can also be obtained by comparison of the experimental and the theoretical methanation yields. The latter ($Y_{th} = 100\%$), corresponding to a theoretical methane yield on substrate of $15.6 \ mmol_{CH_4}/g_{COD}$, is the yield which should be obtained from both hexose- and pentose-based substrates, hypothesising the following simplified gasification reactions:

$$C_6H_{12}O_6 \rightarrow 3\ CH_3COOH \rightarrow 3\ CH_4 + 3\ CO_2 \ , \qquad (6)$$

$$C_5H_{10}O_5 \rightarrow 2.5\ CH_3COOH \rightarrow 2.5\ CH_4 + 2.5\ CO_2 \ . \qquad (7)$$

It is evident that Eqs. (6) and (7) provide a good approximation of the real phenomena occurring during anaerobic digestion only when the substrates are present in the form of monosaccharides and when methane content of biogas is almost 50%.

4.1.2
Kinetic comparison among different feeds

A comparison of the values listed in Tables 1–3 points out that, under thermophilic conditions, hemicellulose hydrolysate is the material more quickly digestible (tests C and D), whereas the mixture of vegetable waste and sludge (tests H and I) the more recalcitrant one; in fact, average values of the first-order kinetic constant around
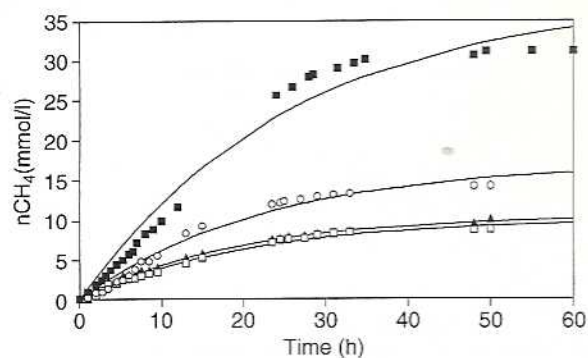

Fig. 1. Methane production during the anaerobic digestion of hemicellulose hydrolysate. Mesophilic conditions (37 °C): (▲) $C_s^\circ = 1.4 \ g_{COD}$/l, test A; (□) $C_s^\circ = 2.8 \ g_{COD}$/l, test B. Thermophilic conditions (55 °C): (○) $C_s^\circ = 1.4 \ g_{COD}$/l, test C; (■) $C_s^\circ = 2.8 \ g_{COD}$/l, test D
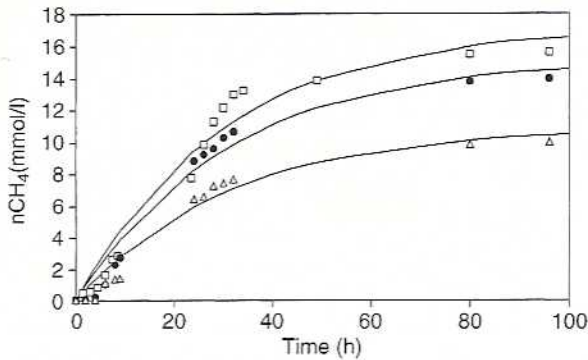
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Fig. 2. Methane production during the thermophilic (55 °C) anaerobic digestion of starch hydrolysate: ($\square$) $C_s^o = 1.4$ g_{COD}/l, test E; ($\bullet$) $C_s^o = 2.0$ g_{COD}/l, test F; ($\triangle$) $C_s^o = 3.0$ g_{COD}/l, test G

$8.4 \cdot 10^{-3}$ l/h $\cdot$ g_{VSS} and $4.1 \cdot 10^{-3}$ l/h $\cdot$ g_{VSS} have been estimated for these two materials, respectively. This result is consistent with what expected from the abundant presence of non-hydrolysed polymeric substances in vegetable waste, whose hydrolysis can become the limiting step for the whole digestion process.

The low acidifying power of hemicellulose hydrolysate, which could be ascribed to the buffering capacity of sulphate, seems to be responsible, on the other hand, for its quicker biomethanation with respect to starch hydrolysate. In fact, it does not seem reasonable to associate this unexpected result to a rate of the pentose-phosphate pathway higher than that of the Embden-Meyerhof pathway in acetogenic bacteria belonging to the metabolic type of *Ruminococcus albus* [20]. Support to the former hypothesis is provided by the fact that the thermophilic digestion of hemicellulose hydrolysate was not affected by an increase in C_s^o from 1.4 to about 3.0 g_{COD}/l (Table 1), while methane content of biogas from starch hydrolysate decreased from about 50% up to 30% (Table 2), thus evidencing a progressive unbalance of the process towards the acidogenic phase. The same kind of process deterioration can obviously be met for all the materials above a critical threshold, whose value is characteristic for any substrate.
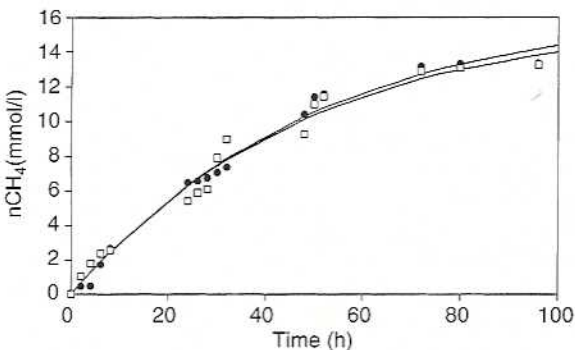

Fig. 3. Methane production during the thermophilic (55 °C) anaerobic digestion of mixtures of vegetable waste and sludge: ($\bullet$) $C_s^o = 1.5$ g_{COD}/l, test H; ($\square$) $C_s^o = 5.0$ g_{COD}/l, test I

4.1.3
Effect of temperature on both methanation kinetics and yields

Comparing in Table 1 the results obtained for hemicellulose hydrolysate from tests A and B on the one hand and tests C and D on the other hand put in evidence that the change from mesophilic to thermophilic conditions is responsible for a decrease by almost 20% in the biomethanation rate, k, which is likely due to the typically slow metabolism of thermophilic archeobacteria. On the contrary, the parallel increase by no less than 60% observed in the biomethanation yield, Y, should be ascribed to a more effective hydrolysis of polymeric materials at higher temperature rather than to the unlikely capability of the thermophilic microflora of utilising a wider number of carbon sources with respect to the mesophilic one. It should also be noticed that even under the most favourable conditions (tests C, D, E, and H) this yield hardly exceeds 80% of the theoretical value. This could be the consequence not only of the possible presence of recalcitrant substances even in completely hydrolysed feeds (such as toxic hydrolysis by-products, etc., which can either act as inhibitors or be degraded only with much difficulty), but also of the simplification introduced by Eqs. (6) and (7), that is assuming all the carbon source present only in the form of simple directly digestible monosaccharides.

The gradual change from mesophilic to thermophilic conditions also seems to be responsible for an increase by more than 15% in the methane content of biogas. This is probably due to a hydrogenotrophic methanogenic activity (methane production from CO_2 and H_2), characteristic of a wide variety of thermophilic archeobacteria of the metabolic type of *Methanobacterium thermoautotrophicum* [20], which would be much more intense than what expected from the simple sequences described by Eqs. (6) and (7).

4.1.4
First-order model verification

Notwithstanding the typically high intrinsic errors introduced by the laboratory method for methane production measurement, the values of the determination coefficient estimated for all the batch tests are always sufficiently close to 1.000, ranging from 0.957 to 0.997 (Tables 1–3). This allows us to conclude that the first-order equation derived from the Monod model can successfully be used to describe the progressive development of methane also from recalcitrant materials, provided that only the fraction of quickly digestible organic substances is considered in the material balance for carbon [21]. The best accordance between experimental and theoretical values estimated by the model has been observed for the hemicellulose hydrolysate digestion (from 0.972 to 0.997), whereas the worst for the starch hydrolysate (from 0.957 to 0.966). This result is consistent with the well-known fact that, because of volatile acid accumulation, the digestion of the more acidifying material (the latter) shows the wider deviations from the behaviour depicted by Eqs. (6) and (7).

4.2

Fed-batch digestion

The dependence of the biomethanation yield on the starting level of digestible organic substances observed in batch digestion tests suggested the following operating conditions for the fed-batch digestion of the materials under consideration. Notwithstanding the high rate constant, hemicellulose hydrolysate could separately be digested only under thermophilic conditions in order to prevent unsatisfactory biomethanation yields and methane contents of biogas. The batch results collected in this work also suggested that, apart from the necessity of using adequate precautionary factors to take into account bulk unhomogeneity, very short residence times (2 days or higher) and organic loads not exceeding 1.5 $g_{COD}/l \cdot d$ could be sufficient in static digesters like those used in this study to ensure a nearly complete transformation of organic substances into methane. More attention should be paid, on the other hand, during the digestion of the other two materials for which residence times no less than 3.5 days and organic loads not exceeding 0.5 $g_{COD}/l \cdot d$ should be used to avoid dangerous situations of organic overloads, which could unbalance the process towards the acidogenic phase. In this case, besides the use of precautionary factors, mixing with less acidifying materials is recommended to shift the organic load threshold towards more acceptable values.

In order to verify whether the suggestions coming from the batch tests would actually be applicable for fed-batch operation or not, a set of semi-continuous tests has been carried out by daily substituting a known amount of digesting material with fresh feed at variable COD but keeping constant the residence time at 20 days and the temperature at 55 °C. The main results of these tests, summarised in Fig. 4 in terms of methane productivity versus the organic load, show that the hemicellulose hydrolysate is the only material, among those tested, which can effectively be digested at organic load as high as 6.0 $g_{COD}/l \cdot d$, whereas starch hydrolysate provokes acidogenesis already at values higher than 2.8 $g_{COD}/l \cdot d$. These results are consistent with the above-mentioned acidifying power of starch hydrolysate and the buffering capacity of

Table 1. Main results of batch tests of hemicellulose hydrolysate anaerobic digestion carried out under both mesophilic and thermophilic conditions

Test	A	B	C	D
T (°C)	37	37	55	55
C_s^o (g_{COD}/l)	1.4	2.8	1.4	2.8
C_x (g_{SS}/l)	5.1	5.0	5.2	4.8
$k\ 10^3$ (l/h g_{VSS})	10.45	10.10	8.79	8.02
a ($mmol_{CH_4}/l$)	10.50	10.06	16.99	37.92
Y (%)	48.1	23.0	77.8	86.8
$\%_{CH_4}$	46	43	53	60
r^2	0.997	0.990	0.978	0.972

Table 2. Main results of batch tests of starch hydrolysate anaerobic digestion carried out under thermophilic conditions

Test	E	F	G
T (°C)	55	55	55
C_s^o (g_{COD}/l)	1.4	2.0	3.0
C_x (g_{SS}/l)	5.0	4.9	5.0
$k\ 10^3$ (l/h g_{VSS})	6.76	6.76	6.60
a ($mmol_{CH_4}/l$)	17.11	15.13	10.92
Y (%)	78.3	48.5	23.3
$\%_{CH_4}$	50	35	30
r^2	0.963	0.966	0.957

Table 3. Main results of batch tests of anaerobic digestion of mixture of vegetable waste and sludge carried out under thermophilic conditions

Test	H	I
T (°C)	55	55
C_s^o (g_{COD}/l)	1.5	5.0
C_x (g_{SS}/l)	5.0	5.1
$k\ 10^3$ (l/h g_{VSS})	4.06	4.14
a ($mmol_{CH_4}/l$)	16.54	15.94
Y (%)	69.3	20.4
$\%_{CH_4}$	61	46
r^2	0.990	0.978

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sulphates in hemicellulose hydrolysate, early evidenced from batch results. Comparing these results with those obtained under mesophilic conditions [22] proves that average methane content of biogas increases from 50 to 60% while residence times about 25–35% shorter could be applied to ensure the same digestion performances.

Although characterised by lower methane productivity at a given organic load, both pre-hydrolysed and non-hydrolysed mixtures of vegetable waste and sludge showed an intermediate maximum organic load threshold (5.0 $g_{COD}/l \cdot h$) with respect the above hydrolysates. The pre-hydrolysis of this mixture, however, did not show any significant effect on the organic load threshold, while ensured a methane productivity increase of no more than 10% as well as a reduction of the time necessary to reach the steady state from about 15 to 9 days [22]. However, this last improvement, which denotes a better ability of the
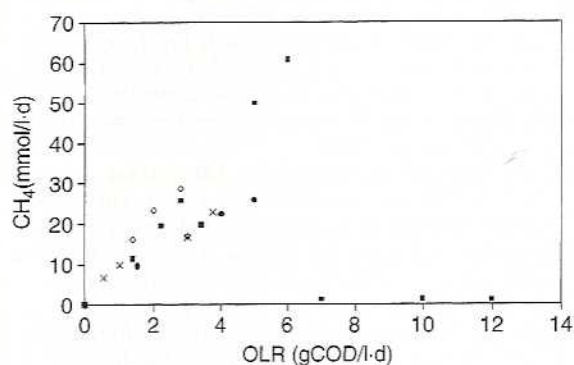

Fig. 4. Results of fed-batch tests of thermophilic anaerobic digestion of different feeds, carried out at variable organic load. ($\diamond$) Starch hydrolysate; ($\bullet$) hemicellulose hydrolysate; ($\blacksquare$) non-hydrolysed mixtures of vegetable waste and sludge; ($\times$) hydrolysed mixtures of vegetable waste and sludge

thermophilic biomass to adapt itself to a substrate already partially decomposed, is not sufficient to justify the additional hydrolytic treatment of the substrate during the whole process. Nevertheless, the combination of thermophilic conditions to hydrolysis of the feed could constitute an interesting way to accelerate the start-up operation.

5
Conclusions
Batch digestion tests have been carried out under both mesophilic (37 °C) and thermophilic (55 °C) conditions for starch and hemicellulose hydrolysates as well as for non-hydrolysed mixtures of vegetable waste with sewage sludge. A comparison of the kinetic results obtained for these materials has demonstrated that: (a) the hydrolysis of the lignocellulosic fraction of vegetable residues is always the limiting step of the process; (b) a possible inhibition is exerted by ligninic by-products; (c) each material behaves differently from the others. In particular, starch hydrolysate showed a certain tendency to unbalance the process towards the acidogenic phase, the hemicellulose hydrolysate was digested even at very high starting COD levels, while the mixture of vegetable waste and sludge showed an intermediate behaviour.

Passing from mesophilic to thermophilic conditions was responsible for a slight deceleration of methane production but remarkably enhanced both methanation yield and methane content of biogas from these materials.

Fed-batch tests carried out in static digester working under thermophilic condition confirmed the preliminary results of batch tests, i.e. the hemicellulose hydrolysate ensured the highest methane productivity (60 $mmol_{CH_4}/l \cdot d$) and methane content of biogas (60%) up to a loading rate threshold of 6.0 $g_{COD}/l \cdot d$), whereas unbalance towards the acidogenic phase already took place at much lower organic load (2.8 $g_{COD}/l \cdot d$) with starch hydrolysate. An intermediate threshold was finally observed for the non-hydrolysed mixture of vegetable waste with sewage sludge.

The relatively high values of the organic loading rate tested in this study, if compared with those usually employed in traditional plants, demonstrate the actual feasibility of such a process to treat the vegetable residues coming from food market activities, once mixed with municipal sewage sludge. Under thermophilic conditions, the steady-state can be reached within 9 days at organic loading rate of 0.5 $g_{COD}/l \cdot d$, ensuring a biogas production around 1.5 $m^3/m^3 \cdot d$ and a methane content of biogas of 60%. In addition, residence times about 30% shorter than those employed under mesophilic conditions are sufficient to ensure the same performance.

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