Maize mono-digestion efficiency: results from laboratory tests
Elena Ficara and Francesca Malpei

ABSTRACT
A laboratory experimental campaign was carried out in order to assess the optimal configuration for the anaerobic digestion of a mixture of sweet corn and ensiled maize. Batch hydrolysis tests were conducted at 35 and 55 °C and at four different particle sizes (2, 5, 20 and 50 mm) obtained by manual chopping and sieving. Chemical pre-treatment by 24 h incubation at various acid and alkaline pH was also considered for its potential to increase the maize methane yield. Results suggest that the hydrolytic phase proceeds significantly faster under thermophilic conditions. Significant differences in the solubilization rate were also observed when comparing coarse (20–50 mm) with fine (2–5 mm) particles, while 2 and 5 mm particles were solubilized at similar rates. No advantages from the chemical pre-treatment, in terms of solubilization efficiency and biomethanization potential were observed. According to these preliminary results, a two-stage semi-continuous laboratory plant consisting of a thermophilic hydrolytic reactor followed by a mesophilic methanogenic reactor was operated for 110 days. Steady state loading parameters were: influent concentration (maize mixture diluted in tap water) of 46 g VS/L, hydraulic retention time of 31 d, organic loading rate of 1.5 g VS/L/d. Alkalinity was dosed to the methanogenic reactor to avoid pH drops. Collected data allowed the average biodegradation efficiency to be estimated at around 60–65%.

Key words | maize, mesophilic hydrolysis, pretreatment, thermophilic methanogenesis

INTRODUCTION
Anaerobic digestion (AD) of organic wastes and energy crops represents a sustainable way of generating renewable energy as bio-methane. Thanks to the introduction of economic incentivizing systems, AD has become an attractive technology to be implemented at farm level. Due to the high suspended solid content and the slow biodegradability of the majority of organic solid wastes, hydrolysis is the key process, limiting methanization kinetics (Vavilin et al. 1997). Therefore, to favour the overall process kinetics, two phase reactor configurations can be advantageously applied and are frequently encountered as a benchmark for full scale plants treating animal wastes and crops (Agrobiogas Project 2006; Al Seadi et al. 2008). Among other energy crops, maize is often co-digested with animal wastes to provide optimal organic solid content and to adjust the carbon to nitrogen ratio. Maize is known to be well degradable under anaerobic conditions (e.g. Schittenhelm 2008) with a methane yield between 200 and 400 L@273 K·CH4/kgVS and its mono-digestion appears as an attractive solution for energy production at farm level, since its availability and characteristics can be easily controlled, simplifying digester operation. However, optimal operational parameters are not discussed in any depth in the scientific literature, although specific issues, such as micronutrient limitations have been remarked (Hinken et al. 2008; Lebuhn et al. 2008). Adequate operational conditions for maize digestion are here studied at laboratory level to support a data-based reactor design and operation.

MATERIALS AND METHODS
Analytical determinations and maize characterization
TS, VS, COD, TKN and total VFA were determined according to the Italian analytical standard methods...
Single VFA were determined according to method 5560/D of the Standard methods (APHA, AWWA, WEF 2005), CH₄ in biogas by GC (Standard methods, 2720, 2005). Elementary composition was determined by an elementary analyzer (EA 1108 Fison). Finally, ADF–NDF–ADL, protein–fats–starch, fibers and ashes were performed by the NIR method (NIRSystem 5000 monochromator, Foss).

**BMP tests**

Biochemical methane potential tests were performed by measuring the overpressure growth by means of a manometric device (WTW, details on the apparatus in: Scaglione et al. 2008).

**Pretreatment tests**

A maize mixture consisting of 75 and 25% (w/w on a wet basis) of ensiled maize and of sweet corn, was used. Samples were prepared by soaking in tap water 25 g maize chopped at 5 mm particle size, corresponding to a concentration of 45 g VS/L. The pH of this suspension was then modified by adding a few mL of a concentrated acid (H₂SO₄ or HCl if BMP had to be measured on the sample) or base (NaOH). Samples were then left to incubate at room temperature, unshaken, for 24 h. Soluble COD release was measured and, on selected samples, BMP tests were performed after pH neutralization.

**Hydrolysis tests**

An experimental campaign was conducted in batch to assess the efficiency of maize hydrolysis at various particle sizes (2, 5, 20 and 50 mm) and operating temperatures (mesophilic = 35 °C and thermophilic = 55 °C).

**Table 1 | Initial and operating conditions for the experimental campaigns on maize hydrolysis**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
<th>M4</th>
<th>M5</th>
<th>M6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>55</td>
<td>55</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Particles size (mm)</td>
<td>5</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td>Initial concentrations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize (g COD/L)</td>
<td>4.4</td>
<td>5</td>
<td>4.4</td>
<td>5</td>
<td>5.8</td>
<td>5.8</td>
</tr>
<tr>
<td>Anaerobic sludge inoculum (g VS/L)</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Overall initial VS (g/L)</td>
<td>3.4</td>
<td>4.0</td>
<td>3.4</td>
<td>4.0</td>
<td>4.3</td>
<td>4.3</td>
</tr>
<tr>
<td>Reaction time (d)</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

**Semi-continuous maize digestion**

The experimental configuration (Figure 1) consisted of a pre-acidiﬁying reactor (operating volume = 2.9 L) kept at 55 °C, followed by a methanogenic reactor (operating volume =
9.0 L) kept at 35 °C, both featured with three openings: one at the top, for biogas collection and recirculation, one at the bottom, for digestate discharge, and a lateral one, for maize loading. A biogas recirculation line allowed for a mild mixing of the maize suspension. Biogas production was measured by a volumetric, water-displacement method. Loading and discharge was operated manually once a day and 5 days per week. As inoculum, two types of sludges were mixed, collected from a mesophilic digester fed on both maize and piggery wastewater and from a lab-scale digester fed on maize and pre-acclimated to thermophilic conditions for approximately one month; the previously cited mineral medium was used for dilution. As feed, the previously described mixture of sweet corn and ensiled maize was used after dilution in tap water to the desired volatile solid content. Feed concentration was initially set to 84 g VS/L and later reduced to 46 g VS/L to avoid cloggings of feeding and discharge ports. From day 25, alkalinity was also added to the methanogenic reactor. The bicarbonate alkalinity requested to compensate for the CO2 partial pressure and to maintain the VFA/alkalinity ratio below the threshold value of 0.3 mg HAc/mg CaCO3 was calculated to be around 50 mmol/d (corresponding to 0.14 g CaCO3/g VSin). During the last 20 days of experimentation, the mineral medium with micronutrients (again from OECD 311) was used for influent preparation instead of tap water to avoid long term nutrients limitation. Adopted loading parameters are reported in Figure 2. Temporary reductions in the organic loading rate (OLR) were operated in response to the build-up of VFA and/or the decrease in biogas production. Steady state values for the hydraulic retention time (HRT defined here as the ratio between the volume of influent suspension loaded daily and the maximum reactor volume, i.e., the liquid volume reached at the end of the feeding phase) and for the OLR were 31 d and 1.5 g VS/L/d, respectively. Reactors were monitored by daily measures of pH, biogas volume and, at variable intervals, COD, VFA and alkalinity.

RESULTS

Maize characterization

Results of maize characterization are summarized in Table 2. The TS content depends on the vegetation stage at which the plant is harvested and was consistently higher for silage maize (wax-maturity) than for sweet corn (milk-maturity). Both maize samples had similar VS/TS content and an average COD/VS of 1.3, which is situated in between the typical value for carbohydrates and proteins, that is in agreement with their chemical composition. The elementary composition was also similar with the exception only of the N-content, which was higher for the ensiled maize; however, the resulting COD/N ratio was similar for both maize samples and included within 114 and 119 g COD/gN; this value is adequate for anaerobic digestion (Speece 1996). Moreover, the ensiled maize had a higher ADL level and, therefore, a higher lignin content.

Available data for the ensiled maize allowed for the assessment of polysaccharides and fibers composition: starch = 4.3 g/100 g TS; hemicellulose (NDF–ADF) = 23.8 g/100 g TS; cellulose (ADF–ADL) = 40 g/100 g TS; lignin (ADL) = 7.9 g/100 g TS; total fibers = 71.5 g/100 g TS. These data are well in agreement with literature values (Schittenhelm 2008). According to the statistical correlation proposed by Amon et al. (2007) for various biomasses, this composition suggests a biomethanization potential of

![Figure 2](https://iwaponline.com/wst/article-pdf/64/10/2029/444050/2029.pdf)

**Figure 2** | Weekly average values for the loading parameters of the two-stage semi-continuous maize digestion.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ensilied maize</th>
<th>Sweet corn</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS (g/100 g)</td>
<td>26.8</td>
<td>15</td>
</tr>
<tr>
<td>VS/TS (g/100 g)</td>
<td>96</td>
<td>92</td>
</tr>
<tr>
<td>COD/VS (g/g)</td>
<td>1.33</td>
<td>1.32</td>
</tr>
<tr>
<td>TKN (gN/kg TS)</td>
<td>11.5</td>
<td>9.9</td>
</tr>
<tr>
<td>Elementary composition (g/100 g TS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C(48.25)</td>
<td>H(6.88)</td>
<td>N(0.63) S(&lt;0.5)</td>
</tr>
<tr>
<td>C(44.77)</td>
<td>H(6.47)</td>
<td>N(0.35) S(&lt;0.5)</td>
</tr>
<tr>
<td>ADF–NDF–ADL (g/100 g TS)</td>
<td>47.8–71.5–7.9</td>
<td>23–n.a.–5.0</td>
</tr>
<tr>
<td>Protein–fats–starch–fibres–ashes (g/100 g TS)</td>
<td>7.2–1.9–4.3–36.0–5.1</td>
<td>7.2–2.8–4.4–n.a.–n.a.</td>
</tr>
</tbody>
</table>
This expected biodegradability was then verified by experimental BMP determinations on a maize mixture consisting of 75 and 25% (w/w on a wet basis) of ensiled maize and of sweet corn, respectively. This maize mixture was manually chopped to 2 mm particles size before digestion. Biogas production resulted to be 612 L@273 K biogas/kg VS and 304 L@273 K CH4/kg VS (this last value was calculated from COD mass balance) corresponding to a 65% biodegradation. This value compares well with the prediction made from composition data and with literature values, e.g.: 320 L@273 K CH4/kg VS (Perez Lopez et al. 2006), 196–233 L@273 K CH4/kg VS (Raposo et al. 2006), 253–342 L@273 K CH4/kg VS (Nallathambi 1997), 359–422 L@273 K CH4/kg VS (Amon et al. 2007), 287–419 L@273 K CH4/kg VS (Schittenhelm 2008).

Maize pretreatment

The efficacy of the acid/alkaline chemical pre-treatment was tested on the maize mixture. First, COD solubilization in pH-modified samples ([COD]s,pH) was compared to that of the blank sample (soaked in tap water, [COD]s,blank) and their difference was referred to the initial total COD ([COD]mais) as indicator of the solubilization efficiency:

\[ \eta_{PT} = \frac{[\text{COD}]_{s,pH} - [\text{COD}]_{s,\text{blank}}}{[\text{COD}]_{\text{mais}}} \]

Results are summarized in Figure 3. These data suggest that no relevant release of soluble COD was observed for the acidified samples. On the contrary, the alkaline pre-treatment had a mild solubilization effect, which may be due to the saponification of esters of uronic acid which increases the pore size of the ligno-cellulosic structure (Datta 1981). However, the relevance of this hydrolytic effect was very limited since less than the 5% of the available COD in the treated maize sample was indeed solubilized.

To verify whether an improved maize degradability could be achieved by this chemical pre-treatment, two of the pre-treated samples (at pH 1.5 and 12) were neutralized and their BMP measured. A final lower biogas production was observed for both the acidified (500 L@273 K biogas/kg VS) and the alkalized sample (569 L@273 K biogas/kg VS) if compared with the blank (612 L@273 K biogas/kg VS). The degraded fraction, calculated from the initial and final COD values, was 69% for the acidified and 63% for the alkalized sample.

One can therefore conclude that no clear evidence of the efficacy of a mild acid or alkaline pre-treatment of maize could be obtained. This very poor result could be due to the low alkaline or acid dosage. Better results were obtained in previous attempts by adopting higher dosages; e.g., Neves et al. (2006) observed a 10 fold increase in biogas production by dosing 0.5 g NaOH/g TS to barley wastes, which is 4 times more than the highest dosage tested in this experimentation.

Hydrolysis tests

Results of the hydrolysis campaign performed on the maize mixture are reported in Figures 4 and 5. By comparing results from reactors M1 to M4, the following observations can be set forth. As for the soluble COD and total VFA concentrations, an initial increase to an approximately stable level, followed by a decreasing trend, starting from day 6–8, was observed. The initial COD and VFA increase, due to the hydrolytic activity, appears to be faster in the two thermophilic reactors (M1&2), while no significant differences were observed between reactors operating at different particle size. This evidence suggests that limited particle size variations (between 2 and 5 mm) do not have relevant effects on the hydrolysis rate.

However, thermophilic conditions appear to be more favorable than mesophilic ones. The concomitant increase in the concentration of VFA is an indication of an acidogenic activity, while the further VFA decrease is evidence of the start-up of the methanogenic activity. The VFAs composition allows for the assessment of their associated COD (mg CODVFA/L). The difference between the soluble COD content and the VFA-associated fraction accounts for soluble organics that have been produced by hydrolysis and have not yet undergone acidification (Figure 6). This unacidified soluble COD concentration increased slightly in the beginning, suggesting a somewhat faster solubilization than acidification, and decreased later on, with the exception of one anomalous value measured at day 3 in reactor M1, due to the very high VFA concentration. After 16 days, the residual unacidified CODs was higher under thermophilic conditions, which suggests a less complete acidification.
process. By contrast, a more complete methanization of VFA took place under thermophilic conditions as suggested by the lower final VFA concentration. According to the VFA composition, a temporary accumulation of butyric acid took place in all reactors, while an accumulation of propionic acid was observed under mesophilic conditions. This accumulation may be the cause of the incomplete methanogenesis in the mesophilic reactors suggested by the higher residual VFA.

Figure 4 | Monitored parameters during the batch hydrolysis of the maize mixture under mesophilic and thermophilic conditions and at various maize particles sizes. (M1: ●; M2: ■; M3: ◊; M4: ○; M5: □; M6: ⋄).

Figure 5 | Concentration of the C2–C4 VFA during the batch hydrolysis in reactors M1–4 (●: total VFA; ▲: acetic acid; △: propionic acid; ●: butyric acid).
The concentration of particulate COD ([COD]p) during time was hydrolysis when methanization was still negligible. The kinetic constant was calculated during the first days of hydrolysis when methanization was still negligible. The concentration of particulate COD ([COD]p) during time was estimated from its known initial value ([COD]p,0) and from soluble COD production data. These data were plotted in a semi-logarithmic graph and fitted by linear regression. The obtained kinetic constants are summarized in Table 3. In accordance with what was suggested by the trend of soluble COD production data. These data were plotted in a semi-logarithmic graph and fitted by linear regression. The observed kinetic advantage of the thermophilic maize disintegration/hydrolysis, the first order kinetic constant was calculated during the first days of hydrolysis when methanization was still negligible. The concentration of particulate COD ([COD]p) during time was estimated from its known initial value ([COD]p,0) and from soluble COD production data. These data were plotted in a semi-logarithmic graph and fitted by linear regression. The obtained kinetic constants are summarized in Table 3. In accordance with what was suggested by the trend of COD, VFA and biogas, the hydrolysis kinetic constant was 2.7 times higher at 55°C than at 35°C. Moreover, no significant differences were observed between M1 and M2 and between M3 and M4, confirming that the hydrolytic behavior of maize particles of 2 or 5 mm was the same.

As for reactors M5 and M6, i.e., those operated at 35°C and at higher average particle sizes (20-50 mm), their behavior was found to be very similar, but different from that of reactors M3&4 loaded with maize of 2 and 5 mm average size. This finding suggests that modifications in the maize particle size of one order of magnitude may affect the hydrolysis rate; however, smaller variations do not cause appreciable effects in the hydrolysis rate. More specifically, reactor M5 and M6 showed a lag phase of 2 days, during which no significant variations was observed in the concentrations of the monitored parameters. Later on, hydrolysis started, as suggested by the observed increase in the soluble COD and VFA concentration. However, the rate of hydrolysis (Table 3) resulted to be one half of that observed for 2–5 mm maize particles (M3 and M4). Previous experiments dealing with the particle size effect on maize digestion led to similar conclusions. The reduction from 10–20 to 0.35–2 mm was observed to have caused an increased biogas production of 10 to 20% (Angelidaki & Ahring 2000; Perez Lopez et al. 2006), while decreasing the particle size from 1 to 0.088 mm was not found to further improve biogas production (Sharma et al. 1988).

### Semi-continuous maize digestion

According to the hydrolysis kinetic constants reported in Table 3, the best hydrolysis performance was obtained at 55°C and for the 5 mm particle size and these conditions were selected for the semi-continuous experimentation. The sizing of the pre-acidiﬁying reactor was then obtained from the k₀, to achieve approximately a 60% solubilization efficiency of the inﬂuent volatile solids. Figure 7 reports the observed trends for pH, total VFA and single VFAs concentrations in both reactors (pre-acidiﬁer and methanogenic reactor). The pH in the thermophilic pre-acidiﬁer dropped to 4.5–5 as soon as the reactor HRT was reduced below 15 days and remained within this range for most of the experimental campaign. By contrast, the alkalinity addition in the methanogenic reactor, prevented the pH from dropping and its value remained steadily around 8.0. As for the total VFA concentration in the pre-acidiﬁer, it remained remarkably high during the ﬁrst month during which the maize concentration in the inﬂuent was 84 g VS/L. High concentrations of C2–C4 VFA was observed, with butyric levels as high as 1,800 mg/L. During this initial phase, the high concentration of maize in the pre-acidiﬁer made it difﬁcult to withdraw the acidified

![Figure 6](https://iwaponline.com/wst/article-pdf/64/10/2029/444050/2029.pdf)
mixture from the bottom opening, thus causing an undesired increase in the VS content in this reactor. After 28 days of operation, the VS influent content was reduced and the HRT increased (Figure 2) to ease reactor operation, and this resulted in the decrease in the VFA level in this reactor. After day 50, the level of butyric acid increased remarkably up to 2,000 mg/L. Apparently, maize acidification under thermophilic conditions may prompt temporary accumulations of butyric acid. This finding agrees with what was observed during the batch hydrolysis tests. Finally, during the last part of the experimentation, the VFA level in the pre-acidifier dropped to the same level as that observed in the methanogenic reactor. This suggests that VFA gasification was taking place in this reactor. This gasification may be associated mainly to H2, since acetoclastic methanogenesis is normally inhibited at pH levels below 6.0. Due to the presence of the gas recirculation line, individual assessment of the biogas composition in the two reactor headspaces could not be performed. In the methanogenic reactor, the VFA level stabilized around 1,200–2,000 mg HAc/L, while it decreased to a satisfactory concentration of 880 mg/L during the last 2 weeks of operation. A temporary accumulation of propionic acid was also observed and this finding, together with the accumulation of the same acid observed during the hydrolysis campaign, suggest that mesophilic conditions may prompt temporary propionic acid build-up.

The biogas production rate (BPR) is depicted in Figure 8. Up to day 55, its trend closely tracked that of the influent loading rate. Between day 62 and 70 a BPR reduction took place, possibly correlated with the increased propionic and butyric acid concentrations. Later on, the BPR kept increasing, well in accordance with the constant decrease in the VFA level in both reactors. The most relevant increase in biogas production was observed during the last week of operation and could be related to the dosage of micro- and macro-nutrients in the influent. This finding seems to confirm that nutrients limitation may occur when operating anaerobic digesters with maize as the sole substrate (Hinken et al. 2008; Lebuhn et al. 2008). The CO2 content in the biogas was assessed by measuring the volumetric...
reduction after bubbling a known volume of biogas through an alkaline (NaOH 3M) solution. Its average content during the last two months of operation was found to be quite low (24%). The remaining volumetric fraction was supposed to be methane. The final specific methane production rate resulted to be 282 L@273 K/CH4/kgVS, corresponding to the 93% of the BMP of the maize mixture and to an overall biomethanization of the 61% of the maize COD content. The overall biomethanisation was also assessed on the basis of the average concentration of total COD exiting the methanogenic reactor during the last 20 days of experimentation (14.2 ± 6.6 g COD/L) and resulted to be: 62.7 ± 16.2%, well in agreement with the value calculated from methane production.

CONCLUSIONS

Effective conditions for the degradation of a maize mixture made of 25% of sweet corn and of 75% of ensiled maize were studied. The biomethanization potential of this mixture under mesophilic conditions and after 45 days of incubation resulted to be 612 L@273 K/biogas/kgVS and 304 L@273 K/CH4/kgVS. This degradability could not be improved by a 24 h incubation at various pH. A mild solubilization effect was obtained by incubation at pH 11–12, nevertheless neither the batch biogas production trend nor the final degradability were significantly improved.

A maize mixture hydrolysis campaign was performed to assess the effect of the maize particle size (2, 5, 20 and 50 mm) and of the operating temperature (35 and 55 °C). Main results can be summarized as follows:

- temperature: the hydrolytic process of the maize mixture was found to be faster under thermophilic conditions, with a first order kinetic constant 2.7 times higher at 55 °C ($k_1 = 0.18$ d$^{-1}$) than at 35 °C ($k_1 = 0.07$ d$^{-1}$),
- particle size: reduction in the maize particle size of one order of magnitude (from 20–50 to 2–5 mm) made the hydrolytic process 2 times faster; by contrast, smaller variations in the particle size did not cause any appreciable effect in the hydrolysis rate.

According to these findings, a semi-continuous reactor configuration including a thermophilic acidifier (2.9 L) followed by a mesophilic methanization reactor was operated. Steady state loading parameters were the following: influent concentration (maize mixture diluted in tap water) = 46 g VS/L, hydraulic retention time = 51 d, organic loading rate 1.5 g VS/L/d. Alkalinity was dosed continuously to the methanogenic reactor to avoid pH drops (0.14 g CaCO$_3$/g VS$_{in}$). At an hydraulic retention time of 31 d, a satisfactory degradation was obtained (60–65%) which corresponded to a biogas specific production rate referred to the overall volume of 920 L biogas@273 K/L/d. Temporary accumulations of propionic acid were observed under mesophilic digestion, while butyric acid was found to accumulate temporarily under thermophilic conditions. Nevertheless, a slight load reduction was sufficient to overcome the problem. Final VFA concentrations in the effluent were low (880 mg HAc/L).

ACKNOWLEDGEMENTS

The authors thank Ing. Luca Migliorisi for his help in the experimentation and Veolia Water System Italia s.r.l., for funding this research.

REFERENCES


Enhancement of methane production from barley waste.  
OECD 2006 Test No. 311: Anaerobic biodegradability of  
organic compounds in digested sludge: by measurement  
of gas production, OECD Guidelines for the Testing of  
Chemicals, Section 3: Degradation and Accumulation.  
OECD Publishing.  
Effect of physical and chemical pre-treatments on methane  
yield from maize silage and grains. In: ADSW 2005  
Conference Proceedings, Volume 2 Poster Presentations, 4th  
International Symposium on Anaerobic Digestion of Solid  
Waste (B. K. Ahring & H. Hartmann, Eds.). August 31–  
September 2, 2005, Copenhagen, pp. 204–208.  
Influence of inoculum to substrate on the biochemical  
methane potential of maize in batch tests. Process Biochem.  
41, 1444–1450.  
The short-term biogas yield in anaerobic digestion of WAS and  
Schittenhelm, S. 2008 Chemical composition and methane yield of  
maize hybrids with contrasting maturity. Eur. J. Agronomy 29  
(2–3), 72–79.  
Sharma, S. K., Mishra, I. M., Sharma, M. P. & Saini, J. S. 1988  
Effect of particle size on biogas generation from biomass  
residues. Biomass 17, 251–263.  
Speece, R. E. 1996 Anaerobic Biotechnology for Industrial  
between hydrolysis and methanogenesis during the  
aerobic-digestion of organic-matter. Microbiology 66 (6),  
712–717.

First received 18 February 2011; accepted in revised form 15 April 2011