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Dissemination Level	
PU	Public
PP	Restricted to other programme participants (including the Commission Services)
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D26: Digestion and co-digestion mass balance for different operative conditions and process configurations for oily crops and agricultural market wastes

1 Introduction

The current deliverable concerns digestion and co-digestion mass balances for oily crops, as represented by the chosen substrate of extracted sunflower flour, and agricultural market wastes. The first section of the deliverable consists of summary information on the basic mass balances. In order to avoid omitting useful results, this summary is then followed by an appendix giving a more detailed description of the work carried out.

2 Oily wastes - extracted sunflower flower

Mass balances were carried out for semi-continuous anaerobic digestion tests run with the chosen test substrate of extracted sunflower flour (ESF). Four reactors with an initial biomass concentration of 15 g VS l^{-1} were used in the experiments. The reactors were fed every working day (normally 6 days a week) with ESF and different volumes of distilled water equivalent to HRTs of 30, 25, 20 and 15 days. Three sets of experiments were carried out using OLRs of 1.07, 2.14 and $3.21 \text{ g TS l}^{-1} \text{ d}^{-1}$. Each experiment had a duration equivalent to 1-2 times the corresponding HRT in order to ensure steady-state conditions. Tables 2.1, 2.2 and 2.3 summarize the TS, VS and COD mass balances obtained in the experiments.

HRT: hydraulic retention time (d).

OLR: organic loading rate ($\text{g TS l}^{-1} \text{ d}^{-1}$)

OL: organic load (g TS d^{-1})

Qo: influent flow-rate (ml d^{-1})

So: influent concentration (g TS l^{-1})

Xo: initial biomass concentration (g TS l^{-1})

Qe: effluent flow-rate (ml d^{-1})

Se: effluent concentration (g TS l^{-1})

E1: TS removal efficiency (%) taking into account the TS contribution of the biomass.

E2: Overall TS removal efficiency (%).

Table 2.1 Mass balance for total solids (TS) in semicontinuous experiments with extracted sunflower flour*

HRT (d)	OLR (g TS/l-d)	OL (g TS/d)	Qo (ml/d)	So (g TS/l)	Xo (g TS/l)	Qe (ml/d)	Se (g TS/l)	E1 (%)	E2 (%)
30	1,07	2,14	67	31,94	20	67	24,4	53,0	23,6
25	1,07	2,14	80	26,75	20	80	20,0	57,2	25,2
20	1,07	2,14	100	21,40	20	100	17,7	57,2	17,3
15	1,07	2,14	133	16,09	20	133	14,0	61,2	13,0
30	2,14	4,28	67	63,88	20	67	30,0	64,2	53,0
25	2,14	4,28	80	53,50	20	80	26,0	64,6	51,4
20	2,14	4,28	100	42,80	20	100	22,4	64,3	47,7
15	2,14	4,28	133	32,18	20	133	19,6	62,4	39,1
30	3,21	6,42	67	95,82	20	67	42,0	63,7	56,2
25	3,21	6,42	80	80,25	20	80	39,0	61,1	51,4
20	3,21	6,42	100	64,20	20	100	35,2	58,2	45,2
15	3,21	6,42	133	48,27	20	133	30,0	56,1	37,9

Table 2.2 Mass balance for volatile solids (VS) in semicontinuous experiments with extracted sunflower flour*

HRT (d)	OLR (g VS/l-d)	OL (g VS/d)	Qo (ml/d)	So (g VS/l)	Xo (g VS/l)	Qe (ml/d)	Se (g VS/l)	E1 (%)	E2 (%)
30	1	2	67	29,85	15	67	19,8	55,9	33,7
25	1	2	80	25,00	15	80	17,0	57,5	32,0
20	1	2	100	20,00	15	100	15,4	56,0	23,0
15	1	2	133	15,04	15	133	12,0	60,1	20,2
30	2	4	67	59,70	15	67	26,0	65,2	56,5
25	2	4	80	50,00	15	80	23,0	64,6	54,0
20	2	4	100	40,00	15	100	19,9	63,8	50,3
15	2	4	133	30,08	15	133	17,6	61,0	41,5
30	3	6	67	89,55	15	67	36,0	65,6	59,8
25	3	6	80	75,00	15	80	34,0	62,2	54,7
20	3	6	100	60,00	15	100	30,6	59,2	49,0
15	3	6	133	45,11	15	133	26,1	56,6	42,1

Table 2.3 Mass balance for chemical oxygen demand (COD) in semicontinuous experiments with extracted sunflower flour*

HRT (d)	OLR (g COD/l-d)	OL (g COD/d)	Qo (ml/d)	So (g COD/l)	Xo (g COD/l)	Qe (ml/d)	Se (g COD/l)	E1 (%)	E2 (%)
30	1,17	2,34	67	34,93	21,3	67	23,0	59,1	34,1
25	1,17	2,34	80	29,25	21,3	80	20,7	59,1	29,2
20	1,17	2,34	100	23,40	21,3	100	17,6	60,6	24,8
15	1,17	2,34	133	17,59	21,3	133	14,2	63,5	19,3
30	2,34	4,68	67	69,85	21,3	67	29,5	67,6	57,8
25	2,34	4,68	80	58,50	21,3	80	24,3	69,5	58,5
20	2,34	4,68	100	46,80	21,3	100	21,9	67,8	53,2
15	2,34	4,68	133	35,19	21,3	133	18,3	67,6	48,0
30	3,51	7,02	67	104,78	21,3	67	42,0	66,7	59,9
25	3,51	7,02	80	87,75	21,3	80	46,0	57,8	47,6
20	3,51	7,02	100	70,20	21,3	100	40,0	56,3	43,0
15	3,51	7,02	133	52,78	21,3	133	37,0	50,1	29,9

As can be seen, TS, VS and COD removal efficiencies (E1) were virtually constant with the HRT at the lowest OLR studied (1.07 and 2.14 g TS l⁻¹ d⁻¹). For example, VS removal efficiencies (E1) taking into account the VS contribution of the biomass ranged between 55.9% and 60.1% and 65.2% and 61.0% for the OLRs of 1.07 and 2.14 g TS l⁻¹ d⁻¹, respectively. In contrast, for the highest OLR studied (3.21 g TS l⁻¹ d⁻¹) a decrease in the VS removal efficiency from 65.6% to 56.6% was observed when the HRT decreased from 30 to 15 days. A higher decrease was observed for COD with values ranging from 66.7% to 50.1% when the HRT decreased from 30 to 15 days. In addition, the stability of the process at an OLR of 3.21 g TS l⁻¹ d⁻¹ was low due to the high values of the VFA (3017-8993 mg acetic acid l⁻¹) and VFA/alkalinity ratio observed, which exceeded considerably the failure limit value, especially for HRTs equal or lower than 25 days, as was previously reported in the WP 4. In the same way, the methane yields obtained at this OLR were considerably lower than those obtained for the lowest OLR studied.

3 Agricultural market wastes

Mass balances for agricultural market wastes were calculated for both single and two phase processes.

3.1 Single phase process

The experiments were carried out in a pilot-scale reactor (CSTR, 200 l working volume, mechanically stirred), fed once a day with sewage sludge and market waste. Operative conditions are shown in Table 3.1.

Table 3.1 Operative conditions used in single phase pilot scale tests

Run	Start-up	1	2	3
T, °C	55.0	54.8	54.5	55.1
HRT (days)	20	18	16	14
OLR (kgVS m ⁻³)	0.66	2.19	3.97	6.18

The mass balances for the single phase anaerobic codigestion process with market wastes were calculated considering the following flow rates (Figure 3.1):

- Inflow composed of sewage sludge from wastewater treatment plant and the organic fraction of market waste;
- Outflow as digested sludge and biogas flow, this last converted to mass flow using the perfect gas law.

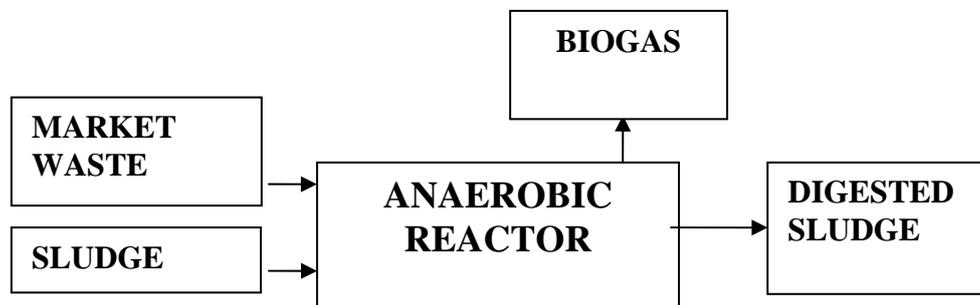


Figure 3.1 Mass balances of single phase anaerobic codigestion process

The amount of material loaded for each period is shown in Table 3.1 with reference to one ton of market waste:

Table 3.2 Inflow rates of market wastes and sludge for each period (Wet weight basis)

	kg d ⁻¹ market waste	l d ⁻¹ Sludge
Start-up	-	1000
Period I	1000	991
Period II	1000	984
Period III	1000	980

Considering the results obtained from daily analysis, the balances were carried out on TS, TVS, COD, P_{tot}, N_{tot} as illustrated in the following tables:

Table 3.3 Total solid mass balances for market waste codigestion

	TSin	TSout	Δ TS	%
Start-up	1000,0	1101,4	-101,4	-10,1
Period I	1000,0	945,2	54,8	5,5
Period II	1000,0	917,2	82,8	8,3
Period III	1000,0	997,4	2,6	0,3

Table 3.4 Total volatile solid mass balance for market waste codigestion

	TVSin	TVSout	Δ TVS	%
Start-up	1000,0	1071,9	-71,9	-7,2
Period I	1000,0	966,1	33,9	3,4
Period II	1000,0	940,5	59,5	6,0
Period III	1000,0	1027,1	-27,1	-2,7

Table 3.5 Total COD mass balance for market waste codigestion

	COD in	CODout	Δ COD	%
Start-up	1000,0	935,5	64,5	6,5
Period I	1000,0	900,6	99,4	9,9
Period II	1000,0	950,8	49,2	4,9
Period III	1000,0	988,1	11,9	1,2

Table 3.6 Total nitrogen mass balance for market waste codigestion

	N in	Nout	Δ N	%
Start-up	1000,0	910,4	89,6	9,0
Period I	1000,0	966,8	33,2	3,3
Period II	1000,0	933,9	66,1	6,6
Period III	1000,0	1081,9	-81,9	-8,2

Table 3.7 Total phosphorus mass balance for market waste codigestion

	Ptot in	Ptot out	Δ Ptot	%
Start-up	1000,0	1078,6	-78,6	-7,9
Period I	1000,0	894,3	105,7	10,6
Period II	1000,0	701,6	298,4	29,8
Period III	1000,0	677,8	322,2	32,2

In all cases the error in the mass balance was low, which confirm the high quality of the analysis and monitoring of the process. Only the phosphorus balance in lasts two periods was not in the acceptable range, probably due to analytical or sampling errors.

1.2 Two phase process

After the single phase process experiment, a second pilot-scale digester was prepared for the study. The first phase was the 200 l working volume digester used for the single phase trials,

while the second phase (methanogenic) was carried out in a 450 l working volume digester, electrically heated and mechanically stirred. Sludge transfers were carried out manually or using membrane pumps, in order to avoid problems with the suspended solids bulk waste components, which can cause pipe clogging at this scale. The thermophilic range was used for both the phases, with operative conditions as shown in Table 3.7.

Table 3.7 Operative conditions for two-phase codigestion test

Run	Start-up	1	2	3
1st phase HRT	24 hours	24 hours	24 hours	24 hours
2nd phase HRT	9,0 days	8,5 days	7,5 days	7,0 days
1st phase OLR kgVS m ⁻³ d ⁻¹	~14	~30	~50	~70
2nd phase OLR kgVS m ⁻³ d ⁻¹	1,5	3,0	7,0	10,5

The mass balances for the two-phase process were calculated in the same way, considering the inflow as composed of market waste and sewage sludge, and the outflow composed of digested sludge and biogas. Figure 3.2 shows the flow scheme of this process:

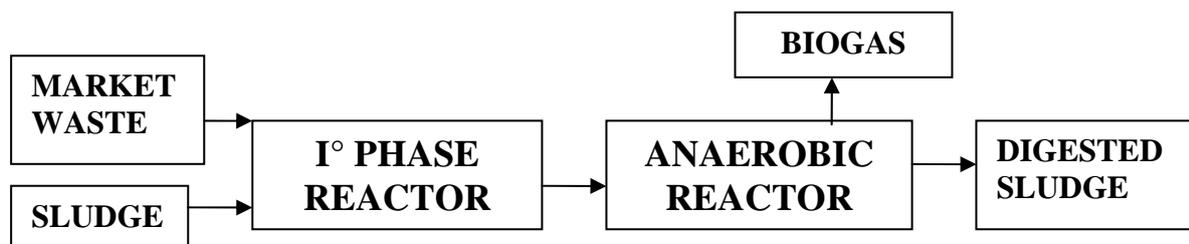


Figure 3.2 Mass balances for two-phase anaerobic codigestion process

The amount of waste feed in the first phase reactor is shown in Table 3.8 below. As in the single phase process, also in the two phase approach the amount of market waste was gradually increased during the experiment. In the last period the amount of solid waste was very high, and this caused some problems transferring the substrates from the first phase reactor to the digester.

Table 3.8 Inflow rate for each period (Wet weight basis).

	kg d ⁻¹ market waste	l d ⁻¹ Sludge
Start-up	-	1000
Period I	1000	12500
Period II	1000	5556
Period III	1000	3247

As in the single phase test, the mass balances were carried out on TS, TVS, COD, P_{tot}, N_{tot} for every period.

Table 3.9 Total solid mass balances for market waste codigestion

	TS _{in}	TS _{out}	Δ TS	%
Start-up	1000,0	1036,5	-36,5	-3,7
Period I	1000,0	948,2	51,8	5,2
Period II	1000,0	955,4	44,6	4,5
Period III	1000,0	791,5	208,5	20,9

Table 3.10 Total volatile solid mass balance for market waste codigestion

	TVSin	TVSout	Δ TVS	%
Start-up	1000,0	1014,6	-14,6	-1,5
Period I	1000,0	1069,0	-69,0	-6,9
Period II	1000,0	909,5	90,5	9,0
Period III	1000,0	807,3	192,7	19,3

Table 3.11 Total COD mass balance for market waste codigestion

	COD in	CODout	Δ COD	%
Start-up	1000,0	960,9	39,1	3,9
Period I	1000,0	927,3	72,7	7,3
Period II	1000,0	861,2	138,8	13,9
Period III	1000,0	734,6	265,4	26,5

Table 3.12 Total nitrogen mass balance for market waste codigestion

	N in	Nout	Δ N	%
Start-up	1000,0	996,0	4,0	0,4
Period I	1000,0	1034,2	-34,2	-3,4
Period II	1000,0	892,8	107,2	10,7
Period III	1000,0	849,3	150,7	15,1

Table 3.13 Total phosphorus mass balance for market waste codigestion

	Ptot in	Ptot out	Δ Ptot	%
Start-up	1000,0	930,4	69,6	7,0
Period I	1000,0	1033,8	-33,8	-3,4
Period II	1000,0	1022,3	-22,3	-2,2
Period III	1000,0	928,1	71,9	7,2

All the errors in the mass balances were less than 10%, but in the third period the error was a little higher; this is a consequence of the high quantity of market waste feed that affected the transition from one reactor to the other and caused sampling mistakes.

Appendix A: Detailed information on studies for mass balance deliverable

A1 Anaerobic digestion of sunflower flour (CSIC)

A1.1 Batch experiments

The biochemical methane potential (BMP) of sunflower flour was accurately determined in batch experiments. These were carried out in magnetically mixed 0.25 L Erlenmeyer flasks which comprised a multi-batch reactor system operating at 35°C. The effect of initial inoculum to substrate ratio (ISR) on methane production and methane yield was also investigated as part of the study. Each ISR was assayed using this system (9 reactors per run): 1 reactor control for time 0 days, 2 reactor control for time 7 days and 6 fed reactors. One reactor was sacrificed every day for analysis of chemical parameters. Controls containing only inoculum were used to measure the background methane production and this amount was subtracted from total methane production. The reactors were run for 7 days because no significant methane production was observed after this time. Table A1 shows the six different ISRs assayed, ranging from 3.0 to 0.5. The concentration of inoculum was constant, while the concentration of ESF ranged from 5 to 30 gVS l⁻¹.

Table A1. Amounts and concentrations of inoculum and substrate for every ISR.

Ratios (ISR)	Inoculum		Substrate	
	(gVS)	(gVS l ⁻¹)	(gVS)	(gVS l ⁻¹)
3.0	3.75	15	1.250	5.0
2.0	3.75	15	1.875	7.5
1.5	3.75	15	2.500	10.0
1.0	3.75	15	3.750	15.0
0.8	3.75	15	4.69	18.8
0.5	3.75	15	7.5	30.0

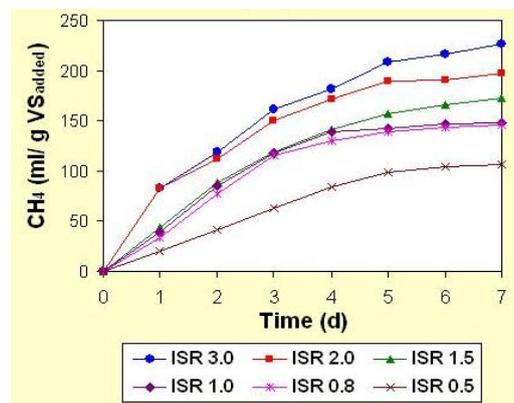


Figure A1 Cumulative methane yield for multi-batch reactors experiments.

As can be seen from Table A1 and Figure A1, the ISR had a big influence on the final values of methane yield. A first-order model based on the availability of substrate as the limiting factor was used to perform the kinetic studies of BMP tests. First-order kinetic models are applied to the AD of complex substrates as they provide a simple basis for comparing stable process performance under practical conditions. The first-order kinetic model which relates the cumulative methane production and digestion time is

$G = G_m \cdot [1 - \exp(-k_o \cdot t)]$ (1) where:

G= Cumulative Methane Production (ml CH₄)

G_m = Cumulative Maximum Methane Production (ml CH₄)

k_o = Methane Production Rate Constant (d⁻¹)

A similar first-order equation can also be used for correlating the cumulative methane yield and digestion time

$B = B_o \cdot [(1 - \exp(-k_o \cdot t))] \quad (2)$ where:

B = Methane yield (ml CH₄ g⁻¹ VS_{added})

B_o = Ultimate Methane Yield (ml CH₄ g⁻¹ VS_{added})

k_o = Methane Production Rate Constant (d⁻¹)

G_m , B_o and k_o were calculated using the SIGMA-PLOT software. Figures A2 and A3 show the variation of the experimental and theoretical B_o values with ISR. Except for the ISR of 0.5, a reasonable fit can be seen between the experimental values and theoretical values calculated using the model.

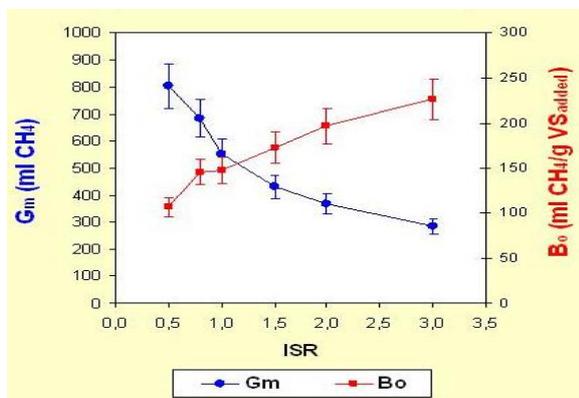


Figure A2 Variation of G_m and B_o

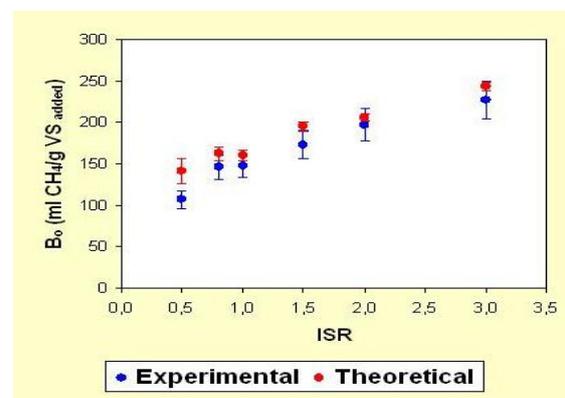


Figure A3 Variation of B_o values

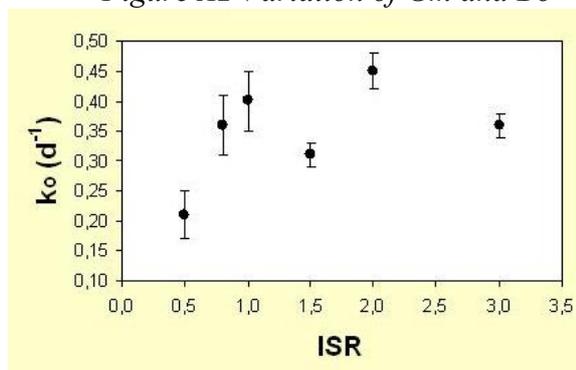


Figure A4 Variation of k_o with ISR.

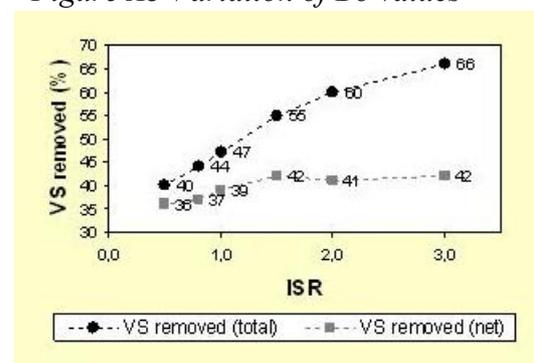


Figure A5 Total and net VS removed

Figure A4 shows the variation of the kinetic rate constant with the ISR. The values obtained were similar for ISR in the range of 3.0 – 0.8. Figure A5 shows the variation of the VS removal efficiency with ISRs. As can be seen, the total removal percentage decreased when the ISR decreased. However, when the substrate added is taken into account (subtracting the VS of the control reactors), there appears to be a constant value of VS removed of around 40 %.

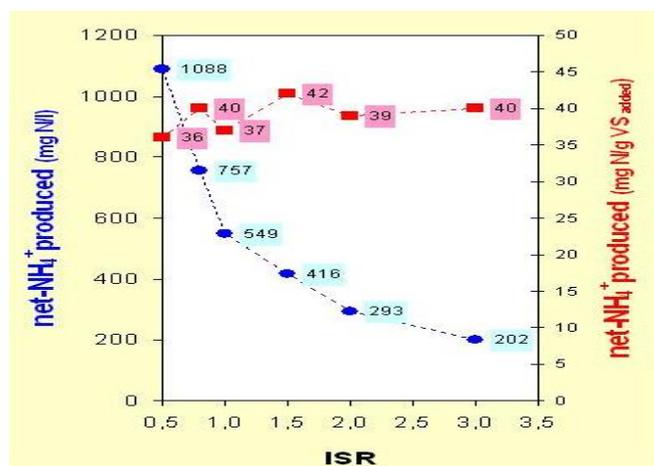


Figure A6. Ammonia produced for every ISR.

Figure A6 represents the net ammonia produced at the end of every experiment for each ISR studied. The net ammonia yield (subtracting the NH_4^+ of the control reactors) ranged from 36 to 42 with an average value of $39 \pm 2 \text{ mg N g}^{-1} \text{ VS added}$.

The pH ranged from 7.6 to 6.8, the lowest value corresponding to the ISR of 0.5 at 1 day of digestion time. These pH values were compatible with the normal growth of anaerobic microorganisms. This means that this parameter was always stable during the anaerobic digestion process. Furthermore, no imbalance was observed in the pH even at an ISR of 0.5. At ISR 0.5 the highest concentration of total volatile fatty acids (VFA) was produced which brought on an almost complete stop of methane production. Therefore, it can be concluded that this parameter is not a good tool for evaluating the stability of the process. In addition, the presence of low pH is the result of a well-developed imbalance and as such is not useful as an early warning indicator.

Table A2 Characteristics of reactors with digestion time

Time (days)	Evolution of chemical parameters: ISR 3.0					
	pH	TS (mg/l)	VS (mg/l)	CODs (mg/l)	TA (mg CaCO_3 /l)	NH_4^+ (mg N/l)
0	7.5	28300	19400	2600	3460	167
1	7.4	27300	18500	2800	4220	315
2	7.4	26400	17500	3400	4620	385
3	7.3	25400	16600	3400	4820	455
4	7.4	25700	16400	3300	5000	495
6	7.6	24800	15900	3300	5220	550
7	7.6	25100	16000	3400	5160	560

Time (days)	Evolution of chemical parameters: ISR 1.0					
	pH	TS (mg/l)	VS (mg/l)	CODs (mg/l)	TA (mg CaCO_3 /l)	NH_4^+ (mg N/l)
0	7.4	38700	29000	4200	3580	154
1	7.1	36600	27100	3800	4200	305
2	7.3	35400	25700	5700	5540	635
3	7.2	33700	24100	5600	5740	690
4	7.3	32500	22900	6100	6180	810
6	7.5	31600	22100	6100	6480	910
7	7.5	31300	21900	6200	6560	910

Time (days)	Evolution of chemical parameters: ISR 2.0					
	pH	TS (mg/l)	VS (mg/l)	CODs (mg/l)	TA (mg CaCO_3 /l)	NH_4^+ (mg N/l)
0	7.5	31300	21900	3000	3500	167
1	7.3	29300	20000	3800	4440	357
2	7.3	28600	19300	3700	4800	435
3	7.3	27600	18400	4100	5180	540
4	7.4	27000	17600	4200	5400	598
6	7.5	26600	17300	4000	6000	600
7	7.6	26600	17500	4000	6000	640

Time (days)	Evolution of chemical parameters: ISR 0.8					
	pH	TS (mg/l)	VS (mg/l)	CODs (mg/l)	TA (mg CaCO_3 /l)	NH_4^+ (mg N/l)
0	7.3	42400	33400	4500	3400	130
1	7.1	40100	31000	5200	4260	386
2	7.3	38000	29000	6200	5500	670
3	7.3	37000	27600	7100	6080	820
4	7.4	35600	26500	6800	6240	880
6	7.5	35000	25300	8200	6800	1030
7	7.5	34400	25200	7800	6800	1090

Time (days)	Evolution of chemical parameters: ISR 1.5					
	pH	TS (mg/l)	VS (mg/l)	CODs (mg/l)	TA (mg CaCO_3 /l)	NH_4^+ (mg N/l)
0	7.4	3340	0	3700	3.600	170
1	7.2	31700	22.700	3900	4.280	343
2	7.3	30400	21.400	3700	4.920	480
3	7.2	29500	20.500	4400	5.240	570
4	7.3	28900	19.900	4900	5.520	628
6	7.5	28400	19.200	5000	5.900	747
7	7.5	27800	18.800	5600	6.000	790

Time (days)	Evolution of chemical parameters: ISR 0.5					
	pH	TS (mg/l)	VS (mg/l)	CODs (mg/l)	TA (mg CaCO_3 /l)	NH_4^+ (mg N/l)
0	7.2	53700	43800	6000	3360	135
1	6.8	50500	40500	8300	3960	492
2	7.0	47800	37900	9800	4960	733
3	7.1	46000	35700	10500	5550	900
4	7.2	44000	34000	11600	6120	1100
6	7.4	42100	32200	11800	7240	1400
7	7.4	41600	31900	12100	7360	1410

During the anaerobic acid-phase stage of digestion of complex organic substrates, mainly constituted by carbohydrates, proteins and lipids, these are converted basically to VFA and to a lesser extent to other low molecular weight compounds. The COD is a parameter that represents the extent of solubilisation. In the present study, the initial and final values of COD were always proportional to the load added. The values of net COD (subtracting final and initial COD concentrations) obtained were: 780, 900, 1900, 1980, 3400 and 6100 mg O₂ l⁻¹ for ISR of 3.0, 2.0, 1.5, 1.0, 0.8 and 0.5, respectively.

The final values of VFA were proportional to the amount of ESF added, and no accumulation at the end of digestion time occurred at ISR 3.0 and 2.0. For ISR lower than 2.0, however, an imbalance of the process was observed.

The trend in the increase in the COD with digestion time observed (taking into account that the COD in the blank reactor was 1000 mg O₂ l⁻¹) was due mainly to the accumulation of VFA, which reflects a kinetic uncoupling between acid formers and consumers and is typical of a stress situation. This means that the hydrolytic-acidogenic stage was carried out satisfactorily and the imbalance of the process was due to the stress of methanogenic microorganisms.

From Table A2 the variation of net total alkalinity (TA) (calculated as the difference between final and initial concentrations, taking into account the blank contribution) and net TA yield as related to ISRs studied can be calculated. As can be seen, net TA increased in proportion to the amount of substrate added to the system. The sequence of variation is different from that observed for other substrates assayed such as maize, for which in the first days of batch digestion the TA dropped, due to the removal of the buffering capacity of the system. The increase in the net TA with decreasing ISR is due to the fact that TA is a non-specific determination that measures all the basic species present in the media, including the ammonia nitrogen generated from degradation of the high protein concentrations of ESF. However, when the net TA yield was evaluated, the values decreased with the load added and ranged from 164 ± 5 to 109 ± 3 mg CaCO₃ g⁻¹ VS_{added}. This is a normal sequence caused by the consumption of TA in the neutralisation of VFAs generated.

Table A3 summarises the variation in the concentration of individual VFA versus digestion time, for the different ISRs assayed. The VFA distribution showed the high influence of ISR on the fermentation process, and, therefore on the composition and concentration of the different VFA generated in the process. Identification of the individual VFA formed is important, since it may provide valuable information on the metabolic pathways involved in the process. On the other hand, it has been reported that the production of i-HBu, HVa and i-HVa are mainly associated with the fermentation of proteins and they can be formed via reductive deamination of single amino acids or by an oxidation-reduction reaction between pairs of amino acids known as the Stickland reaction. This same tendency was observed in the present study in which ESF with high protein content was digested, mainly at the lowest ISR assayed (0.8 and 0.5).

Table A3. Time course variations of individual VFAs in the digestates at different ISRs studied.

Table 2.2.4. Time course variations of individual VFAs in the digestates at different ISRs studied.

Time (days)	ISR 3.0					
	HAc (mg l ⁻¹)	HPr (mg l ⁻¹)	i-HBu (mg l ⁻¹)	Hbu (mg l ⁻¹)	i-HVa (mg l ⁻¹)	HVa (mg l ⁻¹)
0	306 (9)				4 (0)	
1	161 (5)		42 (1)	15 (1)	73 (2)	
2			56 (2)	6 (0)	88 (3)	
3			82 (3)	27 (1)	146 (4)	
4			80 (2)	8 (0)	115 (4)	
6			50 (1)			
7			74 (2)			

Time (days)	ISR 2.0					
	HAc (mg l ⁻¹)	HPr (mg l ⁻¹)	i-HBu (mg l ⁻¹)	HBu (mg l ⁻¹)	i-HVa (mg l ⁻¹)	HVa (mg l ⁻¹)
0	227 (7)	12 (0)				
1	26 (1)	18 (0)	56 (2)	9 (0)	96 (3)	
2	6 (0)		52 (2)		92 (3)	
3	16 (0)	19 (0)	100 (3)		163 (5)	
4			98 (3)		155 (5)	
6	5 (0)		97 (3)		78 (2)	
7	5 (0)		50 (2)			

Time (days)	ISR 1.5					
	HAc (mg l ⁻¹)	HPr (mg l ⁻¹)	i-HBu (mg l ⁻¹)	Hbu (mg l ⁻¹)	i-HVa (mg l ⁻¹)	HVa (mg l ⁻¹)
0	430 (13)		6 (0)		14 (0)	
1	430 (13)	151 (5)	49 (2)		95 (3)	
2			61 (2)		71 (2)	
3						
4	9 (0)		114 (3)		205 (6)	
6	7 (0)		116 (4)		197 (6)	
7	11 (0)		170 (5)		320 (10)	

Time (days)	ISR 1.0					
	HAc (mg l ⁻¹)	HPr (mg l ⁻¹)	i-HBu (mg l ⁻¹)	HBu (mg l ⁻¹)	i-HVa (mg l ⁻¹)	HVa (mg l ⁻¹)
0	320 (10)				6 (0)	
1	370 (11)	216 (7)	37 (1)		67 (2)	
2	55 (2)	262 (8)	129 (4)		240 (7)	
3	47 (1)	60 (2)	135 (4)		247 (7)	
4	11 (0)		176 (5)		305 (9)	
6	9 (0)		205 (6)		350 (10)	
7	12 (0)		189 (6)		296 (9)	

Time (days)	ISR 0.8					
	HAc (mg l ⁻¹)	HPr (mg l ⁻¹)	i-HBu (mg l ⁻¹)	HBu (mg l ⁻¹)	i-HVa (mg l ⁻¹)	HVa (mg l ⁻¹)
0	247 (7)					
1	620 (19)	440 (13)	58 (2)	7 (0)	115 (4)	
2	149 (5)	305 (9)	150 (5)	22 (1)	288 (9)	
3	70 (2)	210 (6)	200 (6)	38 (1)	400 (12)	
4			196 (6)	34 (1)	420 (13)	
6			297 (9)	27 (1)	610 (18)	
7		22 (1)	350 (10)		680 (21)	

Time (days)	ISR 0.5					
	HAc (mg l ⁻¹)	HPr (mg l ⁻¹)	i-HBu (mg l ⁻¹)	HBu (mg l ⁻¹)	i-HVa (mg l ⁻¹)	HVa (mg l ⁻¹)
0	390 (12)					
1	1460 (44)	1000 (30)	71 (2)		113 (3)	
2	1220 (37)	1520 (46)	146 (4)	9 (0)	293 (9)	
3						
4	650 (19)	1940 (58)	264 (8)	90 (3)	540 (16)	
6	94 (3)	1960 (59)	430 (13)	143 (4)	860 (26)	
7	185 (6)	1850 (55)	400 (12)	108 (3)	780 (23)	

The relevant data derived from these batch studies on sunflower flour are summarised as follows:

- ISRs of 3 and 2: the predominant VFA were i-HVa and i-HBu, but the residual compound was the latter. The absence of HAc and HPr demonstrates that the methanogenic stage was not disturbed and the formation of methane from these intermediates was quick.
- ISR of 1.5, 1.0, and 0.8: the predominant VFA during the first few days were HAc and HPr, followed by i-HVa and i-HBu, but the most residual VFA was i-HVa. The sequence obtained demonstrated that i-HVa and i-HBu acids could not be transformed into intermediates with a higher methane production rate. As can be seen, the lower the ISR the greater the accumulation of the longer chain VFA.
- ISR of 0.5: the predominant VFA were HAc and HPr during the first few days, followed by a decrease in HAc with time, with a significant residual concentration of HPr, i-HVa, i-HBu and HBu. The VFA profile obtained is a consequence of the imbalance in the methanogenic stage. Residual concentrations of HAc and HPr were 185 ± 6 and 1850 ± 55 mg l⁻¹, respectively.

In this study i-HBu and i-HVa accumulated to a greater extent than the respective n-isomers. Scarce accumulation of HBu was observed and HVa was not observed in the VFA profile,

whereas their respective iso-forms are difficult to convert and can remain in the medium for longer periods of time.

Finally, from the elemental composition of this substrate (C: 43.6%; H: 6.2%; N: 4.6%; and O: 45.6%), the empirical formula of the ESF was calculated, which was found to be:



The theoretical oxygen demand of this substrate was calculated from the above empirical equation being equal to 1.13 g of O₂ g⁻¹ of substrate. Additionally, the theoretical methane yield was calculated to be 0.323 litres of methane g⁻¹ substrate or 0.316 litres of methane g⁻¹ COD.

A mathematical model for substrate consumption has been proposed taking into account that a fraction of substrate is non-biodegradable. As consequence, the total volatile solids (VS_T) is the sum of the biodegradable (VS_B) and non-biodegradable volatile solids (VS_{NB}):

$$VS_T = VS_B + VS_{NB} \quad (3)$$

Taking into account this assumption, the following kinetic equation was obtained:

$$[VS_T]_t = VS_{NB} + \{[VS_T]_0 - VS_{NB}\} \cdot e^{-K_1 t} \quad (4)$$

where the subscripts 0 and t denote the volatile solids at time zero and time t, respectively and K₁ is an apparent kinetic constant that includes the biomass concentration. Equation (4) was solved for the different ISR studied by non-linear regression using the software SigmaPlot. The values of VS_{NB} and K₁ were summarised in Table A4.

Table A4. Values of the non-biodegradable volatile solids (VS_{NB}) and the apparent kinetic constants (K₁) obtained by equation (4) for the different ISR studied.

ISR	[VS _{NB}] (g l ⁻¹)	K ₁ (days ⁻¹)	Standard deviation (σ _{K₁})
3.0	15.5	0.36	0.08
2.0	17.1	0.40	0.07
1.5	18.2	0.32	0.02
1.0	20.6	0.29	0.05
0.8	24.1	0.33	0.02
0.5	29.8	0.29	0.02

As can be seen in Table A4, the VS_{NB} increased with decreasing ISR, while the apparent kinetic constant remained virtually independent of the ISR for ISR in the range of 3.0-0.8, decreasing slightly for the lowest ISR studied (0.5).

1.2 Semi-continuous experiments

Semi-continuous single phase anaerobic digestion experiments with extracted sunflower flour were carried out in laboratory-scale completely-mixed reactors operating at mesophilic temperature (35 °C). The chemical, solids and elemental composition of the extracted sunflower flour (ESF) used are summarised in Table A5.

The substrate used was cut in small particles, dried at 105 °C, sifted and homogenised at a size lower than 2 mm. The reactors used have a working volume of 2 litres. The biogas was evacuated in a continuous fashion; the volume of methane produced being measured after

removing CO₂ by adsorption into 2N NaOH solution. The operating temperature (35 °C) of the reactors was maintained constant by means of an external jacket through which water from a thermostatic bath circulated.

Table A5. Chemical composition of sunflower flour

Moisture, % w.w.	8.0	MS, g kg ⁻¹	66
Ash, % w.w.	5.9	TVS, g kg ⁻¹	934
Total protein, % w.w.	28.9	TVS/TS, %	93
Fat, % w.w.	1.6	C, %	43.6
Fibre, % w.w.	49.6	H, %	6.2
Soluble sugars, % w.w.	1.9	N, %	4.6
Polyphenols, % w.w.	0.6	S, %	0.27
TS, g kg ⁻¹	1000		

The reactors were initially inoculated with biomass from an industrial anaerobic reactor processing municipal sludge. The specific methanogenic activity (SMA) of this inoculum in presence of acetate and a mixture of acetate, propionate and butyrate in the proportion of 73:23:4 was 28-35 and 5-6 ml CH₄ gVSS⁻¹ day⁻¹, respectively. Due to the low specific methanogenic activity of this inoculum, a new set of experiments was carried out using a new inoculum from an industrial reactor treating brewery wastewater. The solids composition of the inoculum was TS 60 g L⁻¹, VS 45 g L⁻¹, and the specific methanogenic activity was 315-350 ml CH₄ gVSS⁻¹ day⁻¹, using acetic acid as feed.

The four reactors were filled-up with sludge to achieve a initial concentration of 15 gVS L⁻¹, a solution of sodium bicarbonate to increase the buffer capacity of the system and a solution of macro and microelements for obtain an optimum microbial growth and cell metabolism. The start-up of the reactors involved some steps: firstly, daily feed with 1g glucose in 50 ml H₂O during one week; secondly, daily feed with extracted sunflower flour (ESF) to a load of 1 g VS in 20 ml H₂O during 3 weeks. When the volume of the reactor achieved 2 litres, prior to feed the load of 1 g VS a daily volume of 20 ml was previously removed, during 3 weeks. Before starting the experiments corresponding to the first organic load rate (1 gVS l⁻¹ day⁻¹), the content of the 4 reactors was mixed and sub-divided into identical portions to have the same initial characteristics at the beginning of the experiments. The reactors are fed every working day (normally, 6 days a week) with ESF and different volumes of distilled water equivalent to the various HRT studied. Prior to feeding, an equivalent volume of reactor content was removed to maintain a constant working volume.

Table A6. HRT and flow-rates selected for the semi-continuous experiments.

Reactor (number)	Hydraulic Retention Time (days)	Flow-Rates (ml d ⁻¹)
1	30	67
2	25	80
3	20	100
4	15	133

The OLR of 1 g VS l⁻¹ d⁻¹ had a duration of 30 days, equivalent to 1-2 times the corresponding HRT in order to ensure steady-state conditions (SSC). When the reactors achieved the SSC, the average of various consecutive gas production measurements were used, also the sampling of the reactors and characterisation of the effluents were done. Table A7 summarizes the main parameters obtained for this OLR. As can be seen, no significant differences in the methane yield could be observed in the reactors for the first OLR, with values ranging slightly from 0.254 to 0.264 l CH₄ g⁻¹ VS_{added}. The rest of parameters followed a normal sequence, taking

into account the different volumes of water used every day to achieve the HRT. Therefore, the corresponding highest and lowest values were achieved in the reactors with HRT of 30 and 15 days, respectively.

Table A7. Experimental results obtained for the OLR of 1 g VS/L·d^(*).

REACTOR	R1	R2	R3	R4
HRT (d)	30	25	20	15
Organic Matter				
CODt (mg l ⁻¹)	23000 (1301)	20700 (758)	17600 (300)	14200 (752)
CODs (mg l ⁻¹)	3600 (160)	3500 (168)	2700 (135)	1900 (74)
TS (mg l ⁻¹)	24400 (342)	20000 (1249)	17700 (739)	14000 (1183)
VS (mg l ⁻¹)	19800 (619)	17000 (1160)	15400 (909)	12000 (1340)
Stability parameters				
pH	7.4 (0.1)	7.2 (0.1)	7.1 (0.1)	7.0 (0.1)
TA (mg CaCO ₃ l ⁻¹)	5720 (65)	4100 (165)	3440 (73)	2660 (52)
VFA (mg acetic acid l ⁻¹)	462 (21)	631 (31)	524 (25)	328 (16)
VFA/TA (equiv. acetic a./equiv. CaCO ₃)	0.06	0.12	0.12	0.10
AMMONIA (mg N l ⁻¹)	940 (28)	870 (14)	731 (6)	557 (6)
Yield				
MPR (L CH ₄ STP l ⁻¹ d ⁻¹)**	0.264 (0.008)	0.256 (0.009)	0.254 (0.007)	0.259 (0.012)
MY(L CH ₄ STPg VS ⁻¹)***	0.264 (0.008)	0.256 (0.009)	0.254 (0.007)	0.259 (0.012)

(*) the figures in brackets indicate the standard deviations of the mean values.

(**) methane production rate (L CH₄ STP l⁻¹·d)

(***) methane yield (L CH₄ STP g⁻¹ VS_{added})

It is significant that the pH values only decreased from 7.4 to 7.0 when the HRT decreased from 30 to 15 days, these values being very appropriate for the adequate operation and metabolism of methanogenic microorganisms. These stable pH values can be attributed to the appropriate TA values, which ranged between 5720 and 2660 mg CaCO₃ l⁻¹, as well as to the low values of volatile fatty acids (VFA) concentration, which ranged between 328 and 631 mg acetic acid l⁻¹.

As can be obtained from Table A7, the percentage of VS removed ranged between 80.3% and 91.8% for HRT of between 30 and 15 days. Additionally, both methane production rate and methane yield coefficient were independent on the HRT, achieving average values of 0.258 (± 0.004) l CH₄ STP l⁻¹ d⁻¹ and 0.258 (± 0.004) l CH₄ STP g⁻¹ VS added, respectively.

Tables A8 and A9 summarize the evolution of the different chemical parameters with the HRT for the organic loading rates of 2 and 3 g VS l⁻¹ d⁻¹, respectively.

The anaerobic process was also very stable at an OLR of 2 g VS l⁻¹ d⁻¹, with pH values ranging between 7.0 and 7.4. This high stability can be again attributed to the high values of total alkalinity (3770-7070 mg CaCO₃ l⁻¹) and low values of VFA concentration (252-486 mg acetic acid/L) observed for all HRT studied. In addition, the VFA/Alkalinity ratio can be used as a measure of process stability: 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 Table A8 these ratio values were lower than the suggested limit value for all HRTs studied.

For an OLR of 2 g VS l⁻¹ d⁻¹ the percentages of VS removal were somewhat lower than those obtained for an OLR of 1 g VS l⁻¹ d⁻¹, ranging between 76.7% and 88.8%. In addition, the methane production rate was maintained virtually constant for HRTs of 30, 25 and 20 days with values of 0.499, 0.499 and 0.492 L CH₄ STP l⁻¹ d⁻¹, decreasing slightly up to a value of 0.445 L CH₄ l⁻¹ d⁻¹ at a HRT of 15 days. A similar behaviour was observed for the methane

yield, whose values were practically constant for HRTs of 30, 25 and 20 days (0.250, 0.250 and 0.246 L CH₄ STP g⁻¹ VS added, respectively), achieving a minimum value of 0.223 L CH₄ STP g⁻¹ VS added for the minimum HRT studied (15 days).

Table A8. Experimental results obtained for the OLR of 2 g VS l⁻¹ d⁻¹ (*).

REACTOR	R1	R2	R3	R4
HRT (d)	30	25	20	15
Organic Matter				
CODt (mg l ⁻¹)	29500 (662)	24300 (562)	21900 (927)	18300 (731)
CODs (mg l ⁻¹)	4500 (125)	3800 (117)	2800 (166)	2300 (109)
TS (mg l ⁻¹)	30000 (1025)	26000 (1233)	22400 (365)	19600 (365)
VS (mg l ⁻¹)	26000 (1088)	23000 (1322)	19900 (383)	17600 (551)
Stability parameters				
pH	7.4 (0.1)	7.3 (0.1)	7.2 (0.1)	7.0 (0.1)
TA (mg CaCO ₃ l ⁻¹)	7070 (76)	5800 (180)	4900 (100)	3770 (76)
VFA (mg acetic acid l ⁻¹)	486 (23)	286 (14)	197 (9)	252 (12)
VFA/TA (equiv. acetic a./equiv. CaCO ₃)	0.06	0.04	0.03	0.06
AMMONIA (mg N l ⁻¹)	1470 (31)	1270 (21)	1090 (8)	840 (8)
Yield				
MPR (L CH ₄ STP l ⁻¹ d ⁻¹)**	0.499 (0.044)	0.499 (0.046)	0.492 (0.045)	0.445 (0.050)
MY(L CH ₄ STPg VS ⁻¹)***	0.250 (0.022)	0.250 (0.023)	0.246 (0.022)	0.223 (0.025)

(*) the figures in brackets indicate the standard deviations of the mean values.

(**) methane production rate (L CH₄ STP l⁻¹ d⁻¹)

(***) methane yield (L CH₄ STP/g VS_{added})

Table A9. Experimental results obtained for the OLR of 3 g VS/L·d (*).

REACTOR	R1	R2	R3	R4
HRT (d)	30	25	20	15
Organic Matter				
CODt (mg l ⁻¹)	42000 (611)	46000 (2081)	40000 (3679)	37000 (1028)
CODs (mg l ⁻¹)	9600 (628)	14000 (514)	13100 (704)	12000 (245)
TS (mg l ⁻¹)	42000 (1046)	39000 (1010)	35200 (312)	30000 (589)
VS (mg l ⁻¹)	36000 (1175)	34000 (1074)	30600 (596)	26100 (749)
Stability parameters				
pH	7.4 (0.1)	7.0 (0.1)	6.9 (0.1)	6.8 (0.1)
TA (mg CaCO ₃ l ⁻¹)	10400 (235)	8400 (104)	6900 (198)	5600 (251)
VFA (mg acetic acid l ⁻¹)	3017 (152)	8095 (405)	8993 (465)	5945 (293)
VFA/TA (equiv. Acetic a./equiv. CaCO ₃)	0.24	0.80	1.08	0.88
AMMONIA (mg N l ⁻¹)	2270 (80)	2170 (55)	1730 (24)	1340 (24)
Yield				
MPR (l CH ₄ STP l ⁻¹ d ⁻¹)**	0.556 (0.025)	0.394 (0.095)	0.424 (0.096)	0.381 (0.021)
MY(l CH ₄ STPg VS ⁻¹)***	0.185 (0.009)	0.131 (0.032)	0.141 (0.032)	0.127 (0.007)

(*) the figures in brackets indicate the standard deviations of the mean values.

(**) methane production rate (L CH₄ STP l⁻¹ d⁻¹)

(***) methane yield (L CH₄ STP g⁻¹ VS_{added})

The VS removal efficiencies decreased considerably with respect to previous values, ranging between 72.5% and 84.8% for HRTs of between 30 and 15 days. The pH values also decreased in comparison to the values observed in the previous OLR studied, with values of 6.9 and 6.8 for HRTs of 20 and 15 days, respectively. However, the decrease in pH values was not very marked due to the high values of TA (5600-10400 mg CaCO₃ l⁻¹) achieved in the four reactors studied.

In contrast with previous experiments, the stability of the process at an OLR of 3 g VS/L d was low due to the high values of the VFA (3017-8993 mg acetic acid l⁻¹) and VFA/Alkalinity

ratio observed, which exceeded considerably the failure limit value, especially for HRTs equal or lower than 25 days.

In the same way, the methane production rate was significantly influenced by the HRT, decreasing from 0.556 to 0.381 L CH₄ STP l⁻¹ d⁻¹ when the HRT diminished from 30 to 15 days. Similarly, the methane yield also decreased from 0.185 to 0.127 L CH₄ STP/g VS added when the HRT decreased between the above mentioned values. Moreover, these methane yield values were approximately 50% lower than those observed at an OLR of 1 g VS l⁻¹ d⁻¹. This decrease in the methane production at the highest OLR value might be attributed to an inhibition of the methanogenic microorganisms at high OLR values, which caused an increase in effluent VFA contents and VFA/Alkalinity ratio, as can be seen in Table A9.

On the other hand, the effluent substrate concentrations, expressed as total COD (COD_t), at the digester outlets are given in Tables A7, A8 and A9. A fraction of this was not biodegradable and was estimated, in order to perform the subsequent calculations, by a graphical extrapolation of the substrate concentrations (COD_t) to an infinite residence time. This non-biodegradable COD_t was found to be 7.63 g COD_t l⁻¹. Therefore, the experimental values of COD_t were corrected by subtracting the fraction of non-biodegradable substrate in order to obtain the biodegradable COD_t values (COD_t b). The observed methane production rates plotted as a function of the biodegradable COD concentration is illustrated in Figure A7. It can be seen from this Figure that methane production rates (R_{CH₄}) fit the Michaelis-menten kinetic model, which is a hyperbolic function, well. By using the SigmaPlot software, the following equation was obtained:

$$R_{CH_4} = 0.5(COD_t b)/(6.3 + (COD_t b))$$

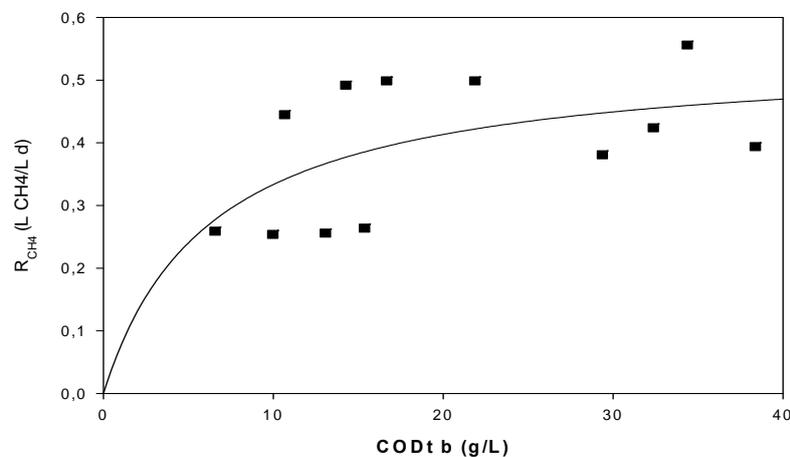


Figure A7. Variation of the methane production rate, R_{CH_4} (L CH₄ STP/L d), as a function of the biodegradable total COD concentration in the reactors (g/L) for all the experiments carried out.

2 Anaerobic digestion of market wastes (UNIVE-DSA and UNIVR-DST)

2.1 Batch tests

Tests were carried out to determine the ultimate BMP, as litre of biogas per gram of volatile solids added ($l\ g^{-1}VS$), and the hydrolysis constant (k , as day^{-1}). The tests were carried out in accordance with the working protocol developed in the first year of the project between Soton and WU, UNIVR-DST and UNIVE-DSA. Tests were carried out at both mesophilic and thermophilic temperatures.

It was found that operating at high initial inoculum:substrate (biomass:food) ratios, it was possible to define both the ultimate BMP and k ; By using mixtures in the range 1:3 to 7:1 as inoculum/substrate (expressed as volatile solids, VS) it was possible to determine both values.

The experimental results were introduced in the modified first order kinetic model to define the value of the hydrolysis constant once the BMP value was known. The model that allows for the description of the hydrolysis process is, in its simplest form, the first order kinetic model:

$$\frac{dS}{dt} = -kS$$

where S represents the substrate concentration, expressed in terms of gVS/L while k is the hydrolysis constant rate (d^{-1}).

On the other hand, in terms of product we have:

$$\frac{dP}{dt} = -\alpha \frac{dS}{dt}$$

that is, $dP = -\alpha dS$, so, solving the differential equation

$$S = \frac{P_0 - P}{\alpha} + S_0$$

Considering again the formula

$$\frac{dP}{dt} = -\alpha \frac{dS}{dt} = -\alpha kS$$

we can then write

$$\frac{dP}{dt} = -\alpha \frac{dS}{dt} = -\alpha kS = -\alpha k \left(\frac{P_0 - P}{\alpha} + S_0 \right)$$

$$-\frac{dP}{dt} = -k(P_0 - P + \alpha S_0)$$

$$-\frac{dP}{P_0 - P + \alpha S_0} = -k dt$$

$$\int_{P_0}^P \frac{dP}{P_0 - P + \alpha S_0} = -k \int_0^t dt$$

$$\ln\left(\frac{-P + P_0 + \alpha S_0}{P_0 - P(t=0) + \alpha S_0}\right) = -kt$$

$$\frac{-P + P_0 + \alpha S_0}{\alpha S_0} = e^{-kt}$$

(Note that this is equal to $\frac{-P + P_0 + P_{end}}{P_{end}} = e^{-kt}$ and for the bottles for BMP we have $P_0 = 0$,

so the relation becomes $\frac{P_{end} - P}{P_{end}} = e^{-kt}$)

$$\frac{-P + P_0}{\alpha S_0} + 1 = e^{-kt}$$

and then, finally:

$$P = P_0 + \alpha S(1 - e^{-kt})$$

From the mathematical point of view this equation can be solved in 2 equivalent modes:

$$P(t) = P_0 + \alpha \cdot S_0(1 - e^{-kt}) \text{ and } P(t) = P_0 + P_f(1 - e^{-kt})$$

where P represent the concentration of the analysed products (like biogas or methane, soluble COD, VFA, Acetate etc..). P_0 is the concentration at the beginning of the experiment, while P_f is the concentration of the product after the total conversion of the VS into the product and S_0 is the initial concentration of substrate (expressed in VSS/L)

So the 2 equations are the same just defining the parameter:

$$\alpha := \frac{P_f}{S_0}$$

that represents a conversion parameter (mg or ml product/ gVSS).

The problem about the definition of α or P_f originates when you have experimental data.

We have a equation with two parameters, for the solution of the mathematical problem:

1. we define two variable α and k
2. we calculate the “calculated data” at the same time of the “experimental” data
3. we evaluate the error in a balance way between the experimental and calculated data by:

$$Err = \left(\frac{C_{exp} - C_{calc}}{C_{exp}} \right)^2$$

4. we define a “objective function” as sum of the errors
5. we minimize the value of the objective function by changing the value of α and k through the MS-Excel “Solver”

In practical terms, we performed several BMP tests at different inoculum:substrate ratios, both in mesophilic and thermophilic conditions to define the ultimate biogas production and the hydrolysis constant. The typical profile of biogas production (as cumulative volume) is shown in Figure A8.

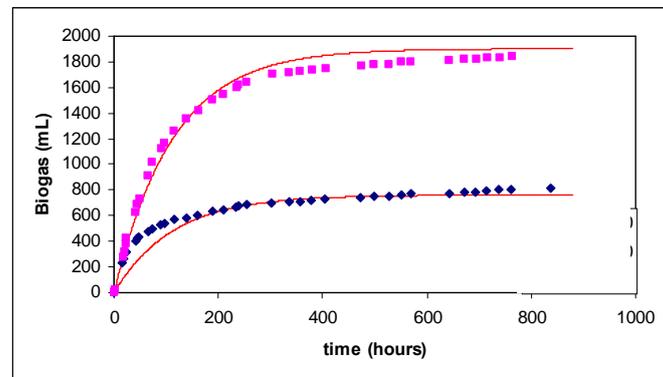


Figure A8. Cumulative biogas production of a batch test carried out at inoculum: substrate ratios of 1:9 (◆) and 3:1 (■)

According to the results obtained during the project the following parameters were calculated:

Table A10. Hydrolysis parameters

Temperature	Specific biogas, ml/gVS _{fed}	Hydrolysis constant, 1/d
Mesophilic (35 °C)	0.63 ± 0.19	0.212 ± 0.05
Thermophilic (55 °C)	0.81 ± 0.15	0.368 ± 0.06

These results for the hydrolysis constant are comparable with values reported in literature for this process: in fact reported results are in the range 0.2 - 0.4 day⁻¹ (Veeken and Hamelers, 1999; Bolzonella et al., 2005), while the specific biogas productions are higher than those found in CSTR trials by UNIVE-DST.

2.2 Semi-continuous single phase co-digestion trials

Experiments were carried out on the co-digestion of sewage sludge and market wastes, at thermophilic temperatures.

The digesters were operated as a single phase process using different loading conditions, to determine the optimal OLR for the substrate. The digesters were pilot-scale reactor (CSTR, 200 l working volume, mechanically stirred) and were fed once a day with sewage sludge and the market waste co-digestate. The main characteristics of the two substrate components used are reported in Table A11 for the 3 operative conditions as defined in Table A12.

The start-up of the digester was carried out using a non specific inoculum, coming from a full-scale mesophilic sewage sludge digester. The start-up time was about 60 days (more or less 3 HRT), in which the process reached a steady state condition, as shown in Figure A9.

The process reached SSC without any problems in terms of stability, due also to the low OLR applied. pH and alkalinity values were maintained in the normal ranges for these conditions and this kind of substrate, as reported in Table A12.

After the start-up, the reactor was fed using a mixture of sludge and market wastes, using three different OLRs, increasing the loading rate from about 2 up to 6 kgVS m⁻³ d⁻¹. During

all of this period, no evidence of system imbalance was found, also at the highest loading rate applied, as can be seen from trends in pH, VFA and alkalinity in figs. 9a -c and from the values reported in Table A12.

Table A11. Substrates characteristics

Run	Start-up	1	2	3
Sludge				
TS, g kg ⁻¹	20.9	24.8	30.4	34.6
TVS, g kg ⁻¹	13.3	13.9	20.5	21.7
COD, mgO ₂ l ⁻¹	21942	21734	20178	20742
pH	7.04	7.27	7.00	7.06
Alkalinity, mgCaCO ₃ l ⁻¹	416	315	361	350
TKN, mgN l ⁻¹	1335	865	1595	1873
Ptot, mgP gTS ⁻¹	20.4	17.5	17.3	8.4
Market wastes				
TS, g kg ⁻¹		242.5	241.6	276.4
TVS, g kg ⁻¹		192.8	198.4	218.7
COD, mgO ₂ l ⁻¹		912	920	841
TKN, mgN l ⁻¹		27.3	28.5	32.3
Ptot, mgP gTS ⁻¹		5.1	7.3	3.9

Table A12 Operative conditions used in this part of the programme

Run	Start-up	1	2	3
T, °C	55.0	54.8	54.5	55.1
HRT (days)	20	18	16	14
OLR (kgTVSa m ⁻³ r)	0.66	2.19	3.97	6.18

*Table A13. Effluent characteristics, stability parameters and yields**

Run	Start-up	1	2	3
Operational conditions				
T, °C	55.0	54.8	54.5	55.1
HRT (days)	20	18	16	14
OLR (kgV _S a m ⁻³ r)	0.66	2.19	3.97	6.18
Reactor sludge				
TS, g kg ⁻¹	21.3	27.0	42.0	62.1
TVS, g kg ⁻¹	12.4	17.0	28.9	41.3
COD, mg O' l ⁻¹	14600	17110	30930	40980
TKN, mgN l ⁻¹	643	839	1910	2580
Ptot, mgP gTS ⁻¹	23.0	16.5	12.8	6.0
Stability parameters				
pH	7.83	7.90	7.89	7.59
TA(6), mgCaCO ₃ l ⁻¹	2029	1952	2345	2947
TA(4), mgCaCO ₃ l ⁻¹	2678	2767	3505	7000
VFA, mgCOD l ⁻¹	156	70	200	321
NH ₃ /, mgN l ⁻¹	598	687	1265	1473
Yields				
GPR, m ³ m ⁻³ d ⁻¹	0.12	0.85	1.65	3.12
SGP, m ³ kgVS ⁻¹	0.16	0.41	0.42	0.51
SGP sludge, m ³ kgVS ⁻¹	0.16	0.16	0.16	0.16
SGP waste, m ³ kgVS ⁻¹	-	0.55	0.52	0.59
CH ₄ , %	-	-	-	67.0
TVS reduction, %	20	50	48	57

*Average value recorded over 2 HRT of SSC at least.

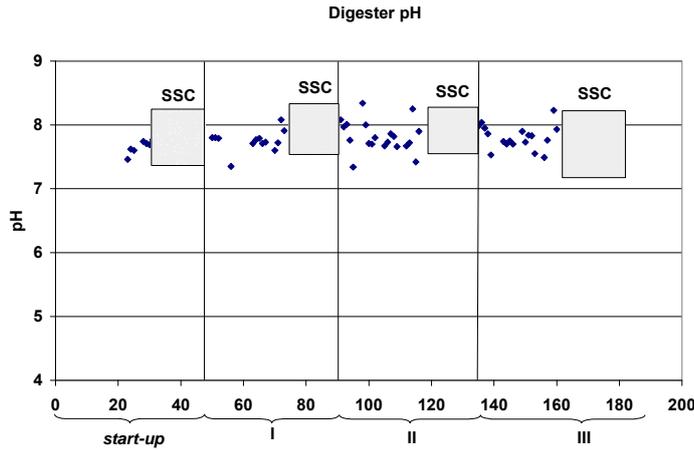


Figure A9a Trend of pH during the whole experiment (SSC=Steady state conditions).

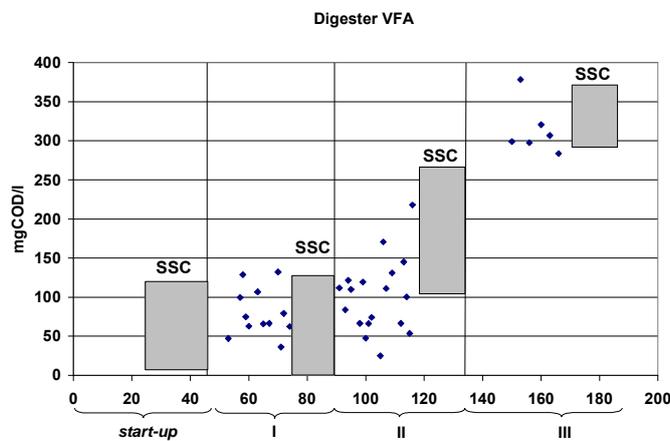


Figure A9b Trend of VFA during the whole experiment (SSC=Steady state conditions).

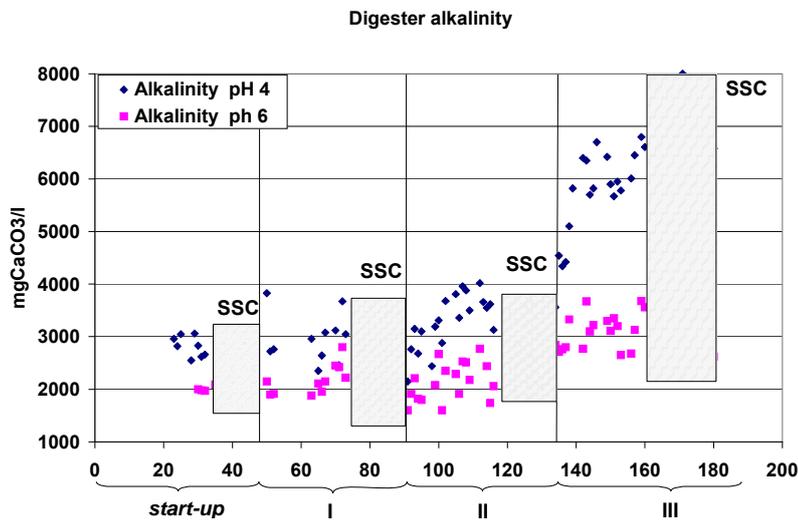


Figure A9c Trend of alkalinity during the whole experiment (SSC=Steady state conditions).

Considering the yields obtained, interesting results in terms of biogas production can be observed in GPR and SGP values (Table A13), which increase from 0.12 to $3.12 \text{ m}^3 \text{ m}^{-3} \text{ d}^{-1}$ and from 0.16 to $0.51 \text{ m}^3 \text{ kgVSf}^{-1}$ respectively. This means that normal digesters, normally operating with sewage sludge alone, can be converted in a co-digestion approach substantially increases the yields in terms of energy produced, up to 6 time larger (as GPR) to the normal sewage sludge production, always operating in single phase process. Further advantages, in

terms of specific yields, can be found separating the hydrolytic-acidogenic phase from methanogenic. In fact, the SGP found for the market wastes during these experiments was in the range of 0.52-0.59 m³ kgVS⁻¹ d⁻¹, which can be considered not the best yield achievable with these materials. Waste digestion, however, was shown to be stable at the highest loading rates tested (up to 6 kgVS m⁻³ d⁻¹). Stability parameters were in the normal ranges in all the conditions studied. In particular, the pH remains in the range 7.5-8, alkalinity (measured at pH 4) reached values over 7000 mgCaCO₃ l⁻¹, very high if compared to the digestion of sludge alone. This fact, together with the stable values of VFA during the conditions of highest OLR, seems to suggest that stability is not a problem for the process with this substrate with digestion in the thermophilic range.

Considering literature data and previous experiences, the value of 6 kgVS m⁻³ d⁻¹ can be regarded as the upper OLR that can be applied to a single phase reactor. After this value, the yields of the process may be lower and the system may be harder to control. Figs. 10 and 11 show the pattern found in terms of relation between yields and OLR.

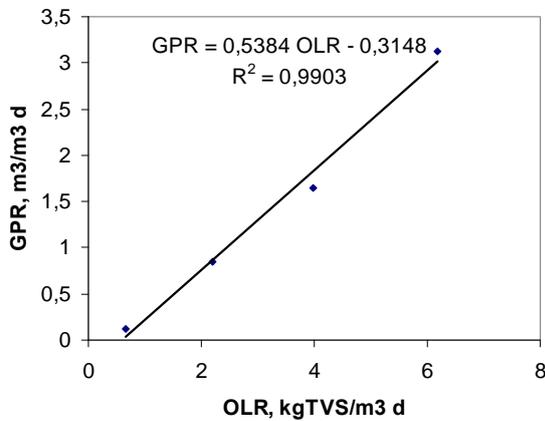


Figure A10. GPR vs. OLR

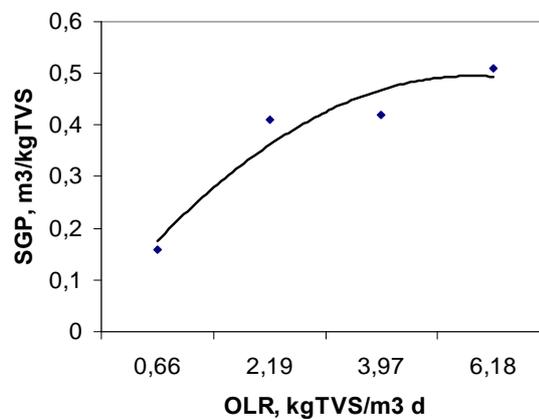


Figure A11 SGP vs. OLR

The pilot-scale on-line approach used during the experiments allows some considerations about the substrate biodegradability from a kinetic point of view. In particular, the semi-continuous way of feeding makes it possible to follow the substrate biodegradation using instant biogas production as degradation index. Figure A12 summarises the plots obtained throughout the single phase experiments. Each point of each plot is the average obtained from all the curves produced during each period, avoiding the situation in which problems in feeding occurred; thus the results can be considered very representative. As can be seen, a clear difference between one period and another is present. When the fraction of waste in the feeding mix increases, the first part of the curve changes slope significantly. This means a higher biodegradation rate, due to the presence of highly biodegradable compounds. When this part of substrate is consumed, the control change to slowly biodegradable compounds, which have a lower rate of degradation. The point of change is more or less after 10 hours of digestion.

Using these data, it is possible to define the first order kinetic constant for each period, and thus to each feed mixture used, considering the simple equation:

$$r_s = \frac{dS}{dt} = -kS, \quad \text{and so } \ln \frac{S}{S_0} = -kt,$$

where S is substrate concentration at time t , S_0 is the initial substrate concentration, r_s is the substrate utilisation rate, k is the first order kinetic constant.

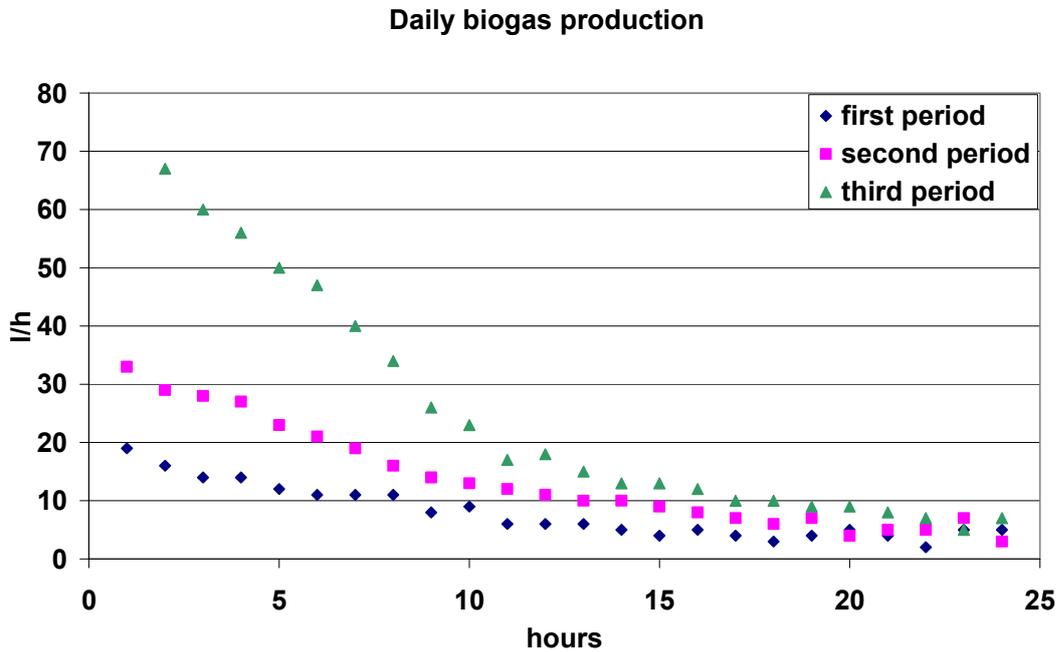


Figure A12. Average biogas trend during the experiments.

The biogas produced is directly linked with the substrate used, and thus to its value at time t . To convert the biogas production into substrate utilisation curves, the volume of gas produced (m^3) has to be converted into carbon equivalent, and this is possible using the perfect gas law ($PV = nRT$), considering an average molecular weight of 28.8 (60% CH_4 , 40 % CO_2). In this way, the curves in Figure A13 are produced.

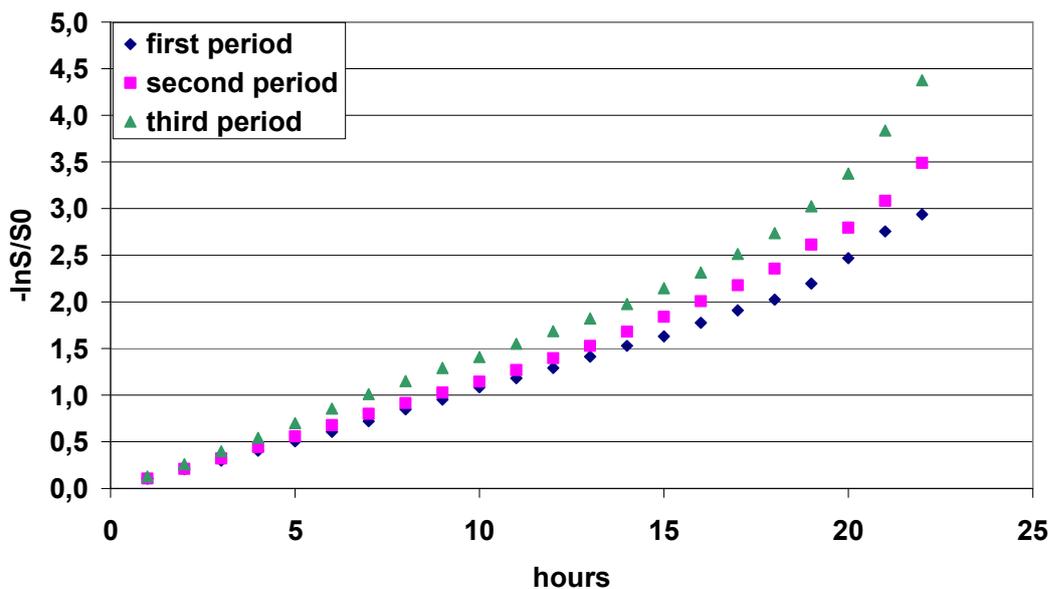


Figure A13. Biodegradation trends obtained in the experimental periods.

As can be seen, the first part of the plots (up to 18 hours, more or less) is always a straight line, thus the first order approach can be considered adequate as a first rough interpretation. A more appropriate approach can be given by the use of step-diffusional model (Cecchi et al., 1992), but this needs a more complex data treatment and a wider spectrum of data.

The evaluation of k using this data lead to the values reported in Table A14: as can be seen, the R^2 values can be considered always satisfactory (remembering that each plot is a result of 20-25 curves), ranging from 0.958 to 0.981.

Table A14. Values of k in the periods studied

	R^2	$K[h^{-1}]$	$k[d^{-1}]$
Period I	0,9814	0,1269	3,0456
Period II	0,9744	0,1479	3,5496
Period III	0,9583	0,1768	4,2432

A further confirmation of the effectiveness of the data treatment used is given by the linearisation of these values vs. the OLR used in each period. The best fit of this relation is given by the equation:

$$k = 0.0013 \times OLR + 2.5047$$

which has a R^2 of 0.995 and can be seen in Figure A14. This relation allows evaluation of the biodegradation rate of a mixture of SS/market waste in which the main part of the load (>70%) is given by market waste.

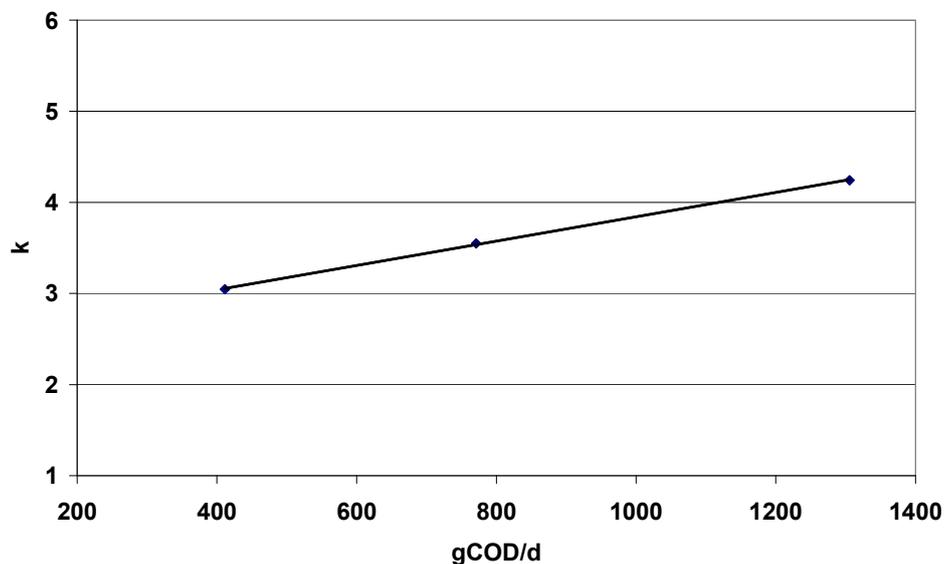


Figure A14. Relation between OLR and first order constant.

2.3 Two phase co-digestion of market waste at thermophilic temperatures

Although the process showed good operational stability as a single phase system using similar substrates in previous studies it has been possible to achieve biogas yields of up to 0.8-0.9 m³ kgVSf⁻¹, at mesophilic temperatures in a two-phase process. The second phase of the research looked at two phase digestion at thermophilic temperatures.

A second pilot-scale digester was added to the first digester to give a two phase anaerobic digester for co-digestion under thermophilic conditions. The first phase is the 200 l working volume digester used for the single phase trials, while the second phase (methanogenic) is carried out in an 450 l w.v. digester, electrically heated and mechanically stirred. Sludge transfers are carried out manually or using membrane pumps, in order to avoid problems with the suspended solids bulk waste components, which can cause pipe clogging at this scale. Thermophilic range was used for both the phases, while OLR and HRT conditions were tested following the scheme given in Table A15.

Table A15. Operative conditions for two-phase co-digestion test

Run	Start-up	1	2	3
1° phase HRT	24 hours	24 hours	24 hours	24 hours
2° phase HRT	9.0 days	8.5 days	7.5 days	7.0 days
1° phase OLR	~14 kgVS m ⁻³ d ⁻¹	~30 kgVS m ⁻³ d ⁻¹	~50 kgVS m ⁻³ d ⁻¹	~70 kgVS m ⁻³ d ⁻¹
2° phase OLR	1.5	3.0	7.0	10.5

(*) same mix SS/market waste ratio used in period 1 in single phase process

The start-up was carried out as follows: the second phase reactor was filled using the sludge from the previous single phase test for about half of the total volume (about 400 litres), and the rest using digested sludge from the full-scale plant of Treviso. The digester was maintained at 55 °C for 10 days without feeding, then the system was fed using sewage sludge alone for about three weeks.

Then an OLR of about 3 kgVS m⁻³ d⁻¹ was applied to the second phase, which means a load of about 30 kgVS m⁻³ d⁻¹ to the first one. The retention times applied to the reactors were respectively 24 hours and 9 days, and these were maintained constant during the whole experiment. After reaching steady state conditions (SSC), the OLR was increased to about 7 kgVS m⁻³ d⁻¹, as shown in Table A15. These conditions were maintained for a long time (150 days), due to a lot of problems in acquisition of the market waste, reactor maintenance, on-line probes malfunction etc. Also in the third condition with the higher OLR (10,5 kgVS m⁻³ d⁻¹) the problems were the same of the previous period, but the SSC was reached in 60 days.

Figures A15-A20 show the trends in monitoring parameters during all the experiments. It is possible to see that the first phase can be seen as a pretreatment of the mixture before the feeding of the second phase, obtaining a significant increase in VFA concentration and improvement of hydrolysis grade of the substrates. In Figure A15 and A16 the pattern of the organic loadings applied is shown: as can be seen, an average OLR of 26 kgVS m⁻³ d⁻¹ was used for the first phase in period 1, while 3,1 m³ kgVS⁻¹ d⁻¹ was used for the second. Using these conditions, no evidence of system imbalance was found in the process. In the next two periods the process was stressed with OLR of 7,2 and 10,5 kgVS m⁻³ d⁻¹. Observing VFA patterns of the two phases test, reported in figs. 17 and 18, it can be seen that the mixture reached values of 12.000 mgCOD l⁻¹ after the first phase.

This load, which can be considered high for the process, was treated without particular problems in the second phase, as can be seen from the pattern of VFA represented in Figure A18. A progressive decrease of VFA content in the second reactor is clearly evident in the first half of period 1, in which the process is in a transient phase, reaching the average value of more or less 100 mgCOD l⁻¹, which can be considered more than satisfactory for these conditions.

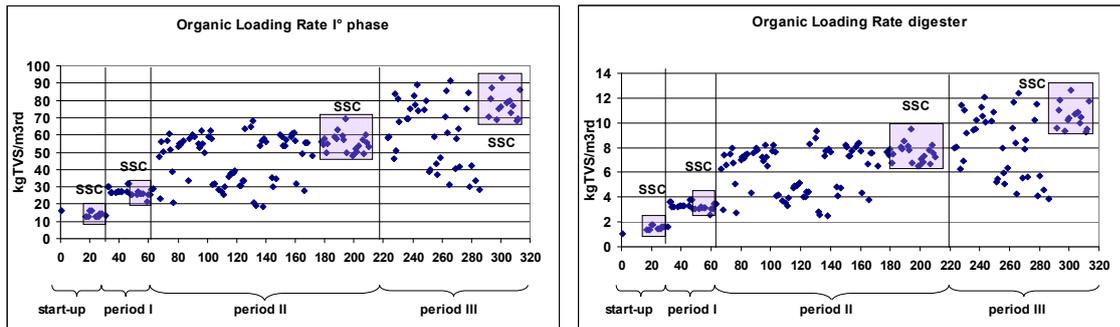


Figure A15 and A16 OLR trends during the experiments

The same thing happened in the transition between period 1 and 2, where the average value of the first 15 days reach 400 mg/l, decreasing up to 100 mg/l in the next days. However, the real SSC conditions for period 2 is given by the days 200-220, in which an higher VFA content is recorded (near to 200 mg/l). This increase, important if compared to the previous period, is in the normal range for these conditions.

The VFA production in the first phase, during period 2, reach values up to 12.000 mg/l, maintaining an average value of 7000 mg/l in SSC at the end of the period; in the third period the increase of OLR cause an higher production of VFA with an average of 12000 mgCOD/l. This high value didn't cause any kind of problems in the digester, in fact the VFA concentration in the SSC was ever less than 500 mg/l.

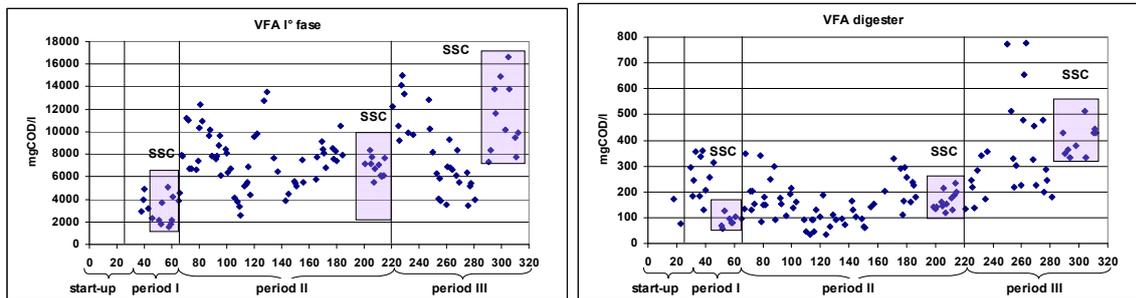


Figure A17 and A18 VFA trends during the experiments

Considering the yields, figs. 2.4.12 and 2.4.13 shows the GPR and SGP patterns recorded during the experiment. As can be seen, a GPR value of $1,27 \text{ m}^3 \text{ m}^{-3} \text{ d}^{-1}$ was reached after only 7-10 days in period 1, even if a complete stable situation is obtained after 15 days, according to the stability parameters patterns. The SGP showed the same trend.

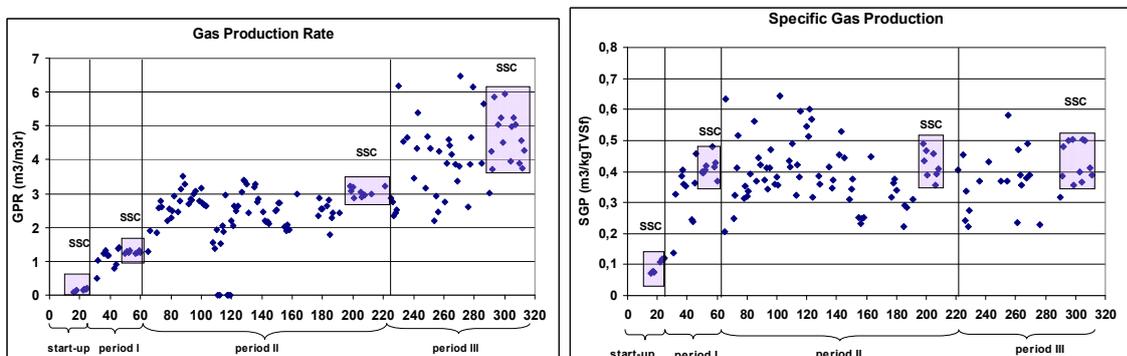


Figure A19 and A20 GPR and SGP trends.

The situation in period 2 is more complex, mainly due to inconstant feeding conditions during the first part of the period. A first an increase of GPR can be seen from day 65 to 90, more or less, but these values are not confirmed in the following days due to the decrease of OLR. After day 120, an average value of $2,8 \text{ m}^3/\text{m}^3 \text{ d}$ is reached, which is close to SSC conditions. Unfortunately, other problems with the feeding occurred, and a new decrease of yields can be observed. The real stable conditions can be considered after day 200, in which GPR reach and maintain a value of $3,0 \text{ m}^3/\text{m}^3 \text{ d}$. In the last period the system was stressed feeding an high amount of organic waste; as consequence the gas production rate rose up to $4,6 \text{ m}^3/\text{m}^3 \text{ d}$. This result shows that using high organic loading rate the hydrolysis reactor probably enable a better contact of substrate and biomass during the successive step. About SGP value evolution, the three period shows similar average value ($0,41$ vs. $0,42$ and $0,43 \text{ m}^3 \text{ kgVS}^{-1}$).

2.4 Comparison of single and two phase anaerobic co-digestion of sewage sludge and market waste.

Table A16 is a summary table which compares the single phase and two phase processes across the loading used, the figures shown the average values obtained for both experiments.

As can be seen, with this OLR (first period) the adoption of the two phases approach does not seems to lead to important improvement in terms of yields or stability parameters. GPR and SGP values are quite similar in the two trials although the OLR was little different, and also VFA contents in methanogenic phase are more or less the same in both the cases (70 vs. 87 mgCOD/l). The same can be seen about pH, ammonia and CH_4 content, which represent, together with VFA, the typical ‘panel’ of parameters to be considered for the stability of the reactor. At a first analysis, this behaviour can be linked to the low load for the codigestion process.

Increasing the stress on the system, the comparison between the two periods give similar consideration to the ones reported for the first period, as can be seen. In terms of yields, SGP maintain the same value. All values in both tests are clear indications of the stability of the system to the new load conditions. This could means that this loading range (applied during the second period) in both the experiments can be considered as a ‘low’ loading conditions, in which both process can be applied without any problem. This observation is very important, because this means, for example, that the existing digesters treating sewage sludge in WWTP can be overloaded ‘as it is’ up to $4 \text{ kgVS m}^{-3} \text{ d}^{-1}$ using agrowastes or similar substrates. This opens the way to a heavier utilisation of these reactors, greatly improving the energy balance of the system without any additional cost in terms of reactor investment. In the last period $6 \text{ kgVS m}^{-3} \text{ d}^{-1}$ in single phase and $10,5 \text{ kgVS m}^{-3} \text{ d}^{-1}$ in two phases process were applied in order to stress the system: this can be very significant to define the upper limit of the process.

Even if the OLR was higher in the two-phase approach the comparison between these data and the other obtained in single phase experiment give very important information about the best approach to be followed.

As can be seen, the two phase approach confirm the possibility to use high load condition ($10,5 \text{ kgTVS/m}^3\text{d}$) without particular problems for stability parameters that were the same values obtained at $6 \text{ kgTVS/m}^3\text{d}$ in single phase test, and also with an interesting biogas production ($4,59 \text{ m}^3/\text{m}^3\text{rd}$ in the last period).

The comparison between single and two-phases process shows that at similar load condition there aren’t significant differences, instead at high organic loading rate the hydrolysis reactor

probably enable a better contact of substrate and biomass during the successive step, with a great biogas production.

Table A16. Average values obtained in single and two-phase experiments.

	single-phase				two-phases			
	Start-up	I	II	III	Start-up	I	II	III
Fed parameters: Sludge								
pH	7.04	7.27	7.00	7.06	6.81	7.21	7.26	7.09
TS. %	2.17	2.48	3.04	3.46	2.19	1.69	3.67	3.95
TVS. %TS	62.0	60.3	67.0	60.8	62.7	64.3	60.6	60.9
Fed parameters: agrowaste								
TS. %		24.20	24.16	27.60		25.67	26.67	26.73
TVS.%TS		79.9	83.3	79.9		84.1	86.5	84.7
Operational condition: I° phase								
OLR. kgTVS/m ³ _{reactor} d					13.71	26.05	52.74	77.52
Temperature. °C					56±1	55±1	55±1	55±1
HRT. d					1	1	1	1
Operational condition: II° phase								
OLR. kgTVS/m ³ _{reactor} d	0.71	2.19	3.97	6.18	1.52	3.13	7.18	10.56
Temperature. °C	55±1	54±1	55±1	55±1	55±1	55±1	54±1	55±1
HRT. d	20.0	18.0	16.0	14.0	9.0	8.3	7.6	6.9
Digester parameters (*)								
pH	7.83	7.90	7.89	7.59	7.59	7.67	8.05	8.04
Alkalinity (@ pH 6). mgCaCO ₃ /l	2049	1952	2345	2946	833	1808	3191	3788
Alkalinity (@ pH 4). mgCaCO ₃ /l	2724	2767	3505	7000	2530	2921	5163	6241
Volatile fatty acids. mgCOD/l	156	70	200	321	124	87	151	399
Ammonia. mgN/l	598	687	1265	1473	416	640	1230	1334
TS reactor. %	2.15	2.70	4.20	6.21	2.13	2.15	4.37	4.04
TVS reactor. %TS	56.2	65.0	69.1	66.0	58.7	60.4	59.5	58.9
Yields								
GPR. m ³ _{biogas} /m ³ _{reactor} d	0.12	0.85	1.65	3.12	0.15	1.27	3.03	4.59
SGP. m ³ CH ₄ /kgTVS _{feed}	0.16	0.41	0.42	0.51	0.10	0.41	0.42	0.43
CH ₄ . %			70	67		73	72	70