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Abstract

Biogas yield of mixtures of cattle slurry and cheese whey, rates of production of methane, removal efficiencies of chemical oxygen demand (COD) and biological oxygen demand (BOD) from the mixtures were investigated at 35°C. Four feed regimens (by volume) were studied. Stable biogas production of 621 l/kg Volatile Solids at an Hydraulic Retention Time of 42 days in a mixture containing 50% slurry and whey was obtained. The concentration of methane in the biogas was around 55%. Maximum removal efficiencies for COD and BOD5 were 82 and 90%, respectively. A maximum biogas production increase of 79% with respect to the start-up phase was achieved. The result of this study show that co-digestion of a high volume of whey (up to 65% in volume) is possible without the use of chemicals for pH correction, but also that this kind of mix has a similar energetic potential of Anaerobic Digestion as energy crops such as maize.

Keywords: Anaerobic digestion; Cattle slurry; Cheese whey; Methane yield; COD reduction; Digestate yield test; Energy production.

1. Introduction

Anaerobic digestion is a technology for wastewater treatment, but also for energy production (electricity and heat) (Tchobanolous et al., 2006). Biogas production from agricultural biomass is of growing importance as it offers considerable environmental benefits and is an additional source of income for farmers (Amon et al., 2007). Currently the most-used feedstock for the anaerobic digester are crops. Maize is the dominating crop for biogas production and more than 40 tons of maize per hectare can be produced in Europe with a biogas yield is up to 350 l-CH_4/kg-VS (after ensilage) at a low cost of 20-40 € per ton. Beside crops, other agro-wastes can be of interest for anaerobic co-digestion with livestock effluents in the form of manures (generally semi solid with a high straw content) or slurry (only cattle excrement that is generally liquid) because of their high energy potential (Angeliadaki and Ellegaard, 2003).
Anaerobic co-digestion of livestock effluents and agricultural waste is widely applied in Europe (Weiland, 2010, Murto et al., 2004). Cheese whey is a by-product of cheese production rich in proteins and lactose with a high organic matter content (up to 70,000 mg/l chemical oxygen demand COD), very high biodegradability (approximately 99%), and relatively high alkalinity (about 2500 mg/l $CaCO_3$) (Mawson, 1994; Erguder et al., 2001). Several studies found that treatment of raw whey was a concern due to the tendency for rapid acidification (Kalyuzhnyi et al., 1997). Other problems associated with direct anaerobic treatment of whey include instability of the reactor, difficulty to obtain granulation, and reduced sludge settling due to the tendency to produce an excess of viscous expolymeric materials, probably of bacterial origin (Malaspina,1995). Low biogas productivity and methane yields have been associated with the low pH of

whey (Ghaly, 1996; Lo, 1989; Yan et al., 1990). Gelengis et al. (2007), Comino et al.(2009), and Kavacik et al. (2010) examined the co-digestion of whey with different types of manure and concluded that whey was quantitatively degraded to biogas but not in an efficient way. In the present study, the feasibility of co-digestion of raw cheese whey and cattle slurry was investigated n a dairy farm anaerobic digestion treatment plant. Anaerobic digestion was initiated without the use of inoculums and tests with untreated substrates at different ratios were conducted. A digestate methane yield test was also carried out and economic aspects of the technology were analyzed.

2. Methods

2.1 Experimental device

The anaerobic reactor used in the tests (Fig. 1) has a total volume of 128 L. The reactor was heated with 15 m of electrical resistance and insulated to maintain a constant temperature of 35 °C ± 0.5. The system can be divided into control panel, feeding system, digester and agitation system and gasometer.

The control panel was located in a closed box and included the electric system controls required for the functioning of the digester and collection of analytical data. The pH was monitored with a pH probe (Endress Hauser CPS-11D) and the mixer system speed was controlled by Altivar ATV11 regulator. Data were accessible remotely through a GSM modem. Temperature probes (Endress Hauser TR10) were placed inside the digester and in the gasometer. A pressure probe (Endress Hauser PMC41) was placed inside the gasometer.

Biomass was loaded from the top of the reactor through a 3” (76.19 mm) hole. The feed entered through a pipe with three subsequent valves that allowed feeding air entering the digester. The different valves had configurations/functions. A 3.4” diameter hatch (85 mm) and a V4 valve were utilized for purging the reactor with nitrogen gas. The reactor was loaded in three ways; a single load for each cycle, batch cycle or staged loads. Loading and unloading operations were carried out during the same cycle of test batch or fed-batch.

The digester was a cylindrical tank made from 316 stainless steel with a height of 94 cm and a diameter of 40.3 cm and closed at the ends with domes 35 cm in diameter and 40.3 mm of height, with a total volume of 128 l, of which 102.8 l were the maximum filling volume. The mixing system consisted of two 316 stainless steel propellers, whose rotation was provided by an electric three-phase motor (380V) and managed by an inverter through the control panel . An additional pH sensor was manually inserted in the reactor, through a hole which was linked to a series of external valves. In this way, it was very easy to operate scheduled calibration and provide precise measurements inside the substrate. In the tests, the probe was used to take random measurements to avoid that the numerous mechanical agents obstruct the sensor . Samples were collected through a valve located on the side of the digester. Unloading of the digester was done through a 3" (76.19 mm) valve located at the bottom of the digester. Biogas was captured by a ½ "(12.7 mm) diameter pipe entering at the bottom of the gasometer. A condensate catcher was placed in the lower part of this pipe. The catcher can be emptied manually or automatically through a small. The influx of biogas can be stopped through a valve located between the digester and the tank.

The gasometer had a fixed and a mobile section. It was built of 316 stainless steel with a height of 90 cm, a diameter of 40.3 cm, and a total volume of 122.8 L. The collection of biogas inside the gasometer was done by a hydraulic closure formed by a saturated aqueous solution of sodium chloride and 10% sulphuric acid. At the top, sensors enabled real-time monitoring of temperature and pressure. In the external part, a water column indicated the level of the aqueous solution inside the gasometer. Biogas production was measured automatically by a slide-wired potentiometer. The data collected was analyzed with Readwin 2000 software and post-processed with a self-made database.

2.2.1 Feed material

About 400 L of cattle slurry were collected in two different sessions at the exit of the stable grid from the livestock farm, Fontanacervo, located in Villastellone (Turin, Italy). Part of it was used to fill the digester, and part was stored at 4°C for feeding the system. The farm also has a diary and a cheese production factory nearby for the collection of fresh whey. The whey was placed in 10-L plastic bags transported and stored at 4°C. Prior to experiments the whey was warm to room temperature. The influent and effluent details are listed in Tables 1 and 2.

2.2.2 Start-up phase

The reactor was initially filled with only cattle slurry to avoid a process collapse, due to the whey tendency to acidify very rapidly. It was operated until the anaerobic digestion reaction started and the system reached a steady state of biogas production (Comino et al., 2009). The digester was fed with a total of 75.5 kg of cattle slurry. This first part of the test lasted 62 days. The substrate was stirred every 2 days at 28 rpm (50 Hz) for 30-45 min, at the same time biogas analysis was performed.

2.2.3 Co-digestion phases

At the end of the start-up phase (62nd day) co-digestion of cattle slurry and raw cheese whey was started. The test was divided into four subsequent phases with different feeding ratios. Each experiment lasted 34 days of fed-batch feeding, plus 7 days of anaerobic rest (no feeding, batch condition). In the first experiment, the digester was fed with a total of 75.5 kg of mix (80% cattle slurry, 20% whey). Feeding was done 3 times a week for a total of 15 times. Each time, 5 kg of substrate was removed and a 5-kg mix (4 kg of slurry plus 1 kg of whey) was loaded. The second experiment started on day 103 when 75.5 kg biomass inside the reactor was substituted with an equivalent mass of mix (65% slurry, 35% whey). The feeding operations were performed 3 times a week for a total of 15 times by replacing 5 kg of substrate with 5 kg of mix (3.25 kg of slurry plus 1.75 kg of whey) loading. The third phase started on day 145. The biomass inside the reactor was replaced with an equal mass of mix (50% slurry and 50% whey). The feeding operations were conducted three times a week for a total of 15 times by replacing 5 kg of substrate with 5 kg of mix (2.5 kg of slurry plus 2.5 kg of whey) loading. The fourth and last phase of the experiment started on day 187 with a feeding mix of 75.5 kg (35% slurry,65% whey). The feeding operations were conducted three times a week for 15 times by replacing 5 kg of substrate with 5 kg of mix (1.75 kg of slurry plus 3.25 of whey). No purging with nitrogen was done since it was observed that less than 1% in the reactor volume did not adversely affect the anaerobic reaction. The substrate was stirred every time a feeding operation was performed for 30-45 min at 28 rpm. The pH probe and the gas analyzer were calibrated at every starting phase. Gas production was checked at least twice a day via remote control. The gasometer was emptied when it reached a pre-established value through the opening of the discharge electro valve (V9b, Fig. 1); pH and temperature were monitored at 5-min intervals. After the last charge, the system was left undisturbed for 7 days, starting on day 222, for biogas production stabilization. The test was stopped on day 229. At the end of every phase, substrate samples for the chemical analysis were collected (Table 2).

2.2.4 Digestate methane yield test (DMY).

A DMY test was performed after the last phase on day 229. The biomass was from the digestion of the last test where a mix of 35% cattle slurry and 65% whey was used. The substrate was stirred every two days at 28 rpm for a duration of 45 min when biogas analysis was performed. Main control parameters were constantly monitored, as was the methane concentration inside the biogas. The test system remained sealed during the test duration. On day 270, after 41 days of HRT, the test was terminated and samples collected for chemical analysis.

2.3 Chemical analysis and procedures

Biogas analyses were conducted using a GA-2000 gas analyzer. The feed materials and digestates were stored at 4°C immediately after sampling, and chemical analyses were performed within 48 h by an independent laboratory. BOD5 was analyzed with the IRSA – CNR n. 5100 A/94 method; COD with the IRSA – CNR n. 5110/94 method; pH with IRSA – CNR Quad 100 met. 2080/94 and directly inside the reactor with the pH probe. Density was calculated with the EMRO/012/1999 method; 105°C residual, and the 550°C residual as the Total Volatile Solids were obtained with the IRSA – CNR Quad. 64 n. 2.4.2/84 method. Ammonia (NH_4^+) was measured following the IRSA/APAT guidelines 29/2003 met. N. 4030C, and the volatile fatty acids (C1-C6) were measured with the EMGC 003/1999 method. Biogas sampling was conducted in real time and the samples were analyzed for CH_4, CO_2, O_2, CO and H_2S as well as barometric pressure relative pressure inside the gasometer. The organic loading rate (OLR) and the hydraulic retention time (HRT) were calculated on the basis of the regular additions. The objective was to follow with accuracy the different phases of each test and evaluating the reactor behavior under different mix ratios.

3. Results and discussion

3.1 Start-up phase

In the first 35 days, very limited biogas production was observed (Fig. 2). The pH value increased during days 22 to 26 from 6 to 7.8 (Fig. 2). This behavior predicted an increase in biogas production which then reached a maximum on day 50. A total of 1647 L of biogas was produced (Fig. 3) and a COD reduction equal to 62% was achieved. Considering a CH_4 proportion of 45%, 747 L of methane were produced. A total of 5.62 kg of VS was obtained and the methane potential was equal to 108.74 l-CH_4/kg-VS for 62 days of total test and 35 days of active anaerobic digestion. The digestion followed the expected steps and the trend of biogas production was similar to those observed previously (Comino et al., 2010) when a specific methane yield of 119.17 l-CH_4/kg-VS was obtained. Similar values were found by Amon et al. (2007) specific methane yields between 125.5 and 166.3 l-CH_4/kg-VS; Brachtl (2000) and Thomè-Kozmiensky (1995) found biogas yields between 200 and 300L kg-VS, and Braun (1982) reported a range between 140 and 266 L biogas/kg-VS. These ranges corresponded with the current start-up phase that gave a production of 239.83L biogas/kg-VS.

3.2 Co-digestion phases

The OLR of the different phases ranged from 3.3 to 2.4 g-VS/l-d (Table 4) in conjunction with the decrease of cattle slurry portion in the feeding mix. The pH values always remained near 7.7, fully compatible with the optimal working range after the stabilization occurred in the start-up phase. In the first two trials, the pH remained at a constant value of 7.65. In the third and fourth tests, the values were 7.47 and 7.49, respectively. Gelegensis et al. (2007) recorded a strong pH variation when they used a mix of 50% diluted poultry manure and 50% raw whey. The only fluctuation recorded during the present tests was due to the periodic unloading/loading operations of the 65% whey mix. The temperature inside the anaerobic reactor was always maintained at 35.5 °C ±0.5. Biogas production is presented in Fig. 4. In the cumulative curve, it is possible to recognize the phases of the experiment from the start-up, fed in batch, to the four phase of fed-batch feeding. It can also be observed that the final rest period of 7 days was very smoothly and progressive reduction of the biogas production rate is clearly visible. At the beginning of every new feeding phase, a reduction in biogas quality occurred (Fig. 4) until the microbiota had adapted to the new mix. Near day 212, and before the start of the digestate methane yield test, the biogas production curve became almost horizontal, mostly due to the replacement of slurry with whey in the influent mixture and/or to the variation of VS with the different whey fractions.

The methane proportion in the produced biogas was stable around 50-55% during most of the experiment (Table 3), but increased at the end of the second phase (65% slurry and 35% whey) with an average value of 56% and a maximum value of 61% and continued in the first part of the third phase (50% slurry and 50% whey) with an average value of 55% and a maximum value of 64%. The methane proportion stabilized at the end of the experiment, while during the most part of the experiment it followed a course related to the feeding operation (Fig. 5). Accumulation of undegraded material was observed inside the digester, mostly due to the liquid phase of the substrate, as a crust on the upper part of the liquid phase. The stirring period strategy adopted worked well for the entire duration of the test.

In Table 4 are summarized the different phases main process parameters. The obtained yields were high, especially in the last two phases. The best methane yield corresponded to 249 l-CH4/kg-VS in a previous experiment conducted with a mix of pre-treated crop silage and dairy manure (Comino et al., 2010). Lehtomäki et al. (2007), during trials with several energy crops and cow manure mix, found methane yields between 149 and 268 l-CH4/kg-VS. Also Lindorfer et al. (2008), in a trial with co-digestion of energy crops and cow manure under higher OLR, found methane yields between 360 and 400 l-CH4/kg-VS. Few experiments were conducted on co-digestion of slurry and/or manure with whey mostly because in the past anaerobic digestion was mainly used as a wastewater treatment technology and the production of energy was not a prior consideration. Lo and Liao (1989) tested a similar mix of whey and cow manure with a 2:1 ratio and obtained a methane yield of 222 l-CH4/kg-VS, and Ghaly (1996) recorded a whey-based methane yield of about 240 l-CH4/kg-VS using chemical pH control. The present results show that anaerobic digestion of cattle slurry and raw cheese whey could be achieved without the use of chemicals with a 50:50 mix as long as this ratio is approached. The benefits of optimizing the proportion of whey and loading rate in co-digestion were shown by the fact that during feeding with 50% of whey in the feedstock, an up to 300% higher specific methane yield was obtained than during the start-up phase (only cattle slurry). Further increasing the proportion of whey up (to 65%) led to a higher production of methane, but the process started to be unstable and less efficient. The good efficiency of the reactor was confirmed during the start-up and the first three feeding phases. During the last feeding phase the anaerobic process started to be lightly unstable (Fig. 5). The above mentioned test started with an influent mix containing a slurry fraction of 35% and a whey fraction of 65% in terms of COD with a value in the feed equal to 81.8 g/l, OLR = 145 g, COD/l_R and HRT = 42 days. For almost half of the test, the digester continued to produce steadily, but after the 212nd day, it became less productive, the total biogas volume produced was equal to 2897.1 L (a 76% increase compared to the start-up, but 2% less compared to the previous feeding phase) and a COD reduction of 60%. All the COD reductions are visible in Table 5. In quantitative terms, the substrate that allowed to obtain very high biogas production was the one used in the third feeding phase, where cattle slurry and raw cheese whey were both at 50% v/v present. Even if the methane yield of the fourth phase was a little higher, the process stability was greater in the third one. Very high rates of BOD5 (90%) and COD (82%) removal can be achieved.

With the present results, it would be possible to obtain electricity production equal to 13.5 kW per 1 t/d with a CHP technology with an efficiency of 36%. This mix represents a valid alternative to the co-digestion of cow manure and crop silage that was tested in the past (Comino et al., 2010) where the electricity production at the optimal mix ratio was equal to 14.8 kW.

3.3 Digestate methane yield (DMY) test.

During this experiment, the digester followed a batch-fed regime as described in section 2.2.2. With an estimated OLR of 1.6 g-VS/l-d, the total quantity of produced biogas was equal to 628.5 L (Fig. 6). Considering a CH4 proportion of 58%, this amount corresponded to 364.53 L of methane. With a quantity of VS (2.34 kg) calculated from the chemical analysis of the initial digestate, the methane yield was 155.75 l-CH4/kg-VS. Digestate tested showed a good biogas and methane production when compared to results obtained in similar studies using different co-digested substrates (Hensen et al., 2006; Lehtomäki et al., 2008). It must be taken into account that chemical characteristics of the substrate and operating conditions were optimal for hydrolyses and digestion. Longer HRT or lower OLR could bring to minor biogas/methane yields. Digestate can yield an important amount of biogas that could be transformed into electricity. With the above values it is possible to obtain an electricity production equal to 2.8 kW per t/d. This value is obtained by digestate batch digestion, a CHP technology with an efficiency of 36% (Table 4).

4. Conclusions

The results of this study show that the production of methane by co-digestion of cheese whey and cattle slurry without pH chemical correction is possible. A mix of 50% cattle slurry and 50% whey achieved and the OLR of 2.65 g-VS/l-d with a methane yield of 343.43 l-CH4/kg-VS. This kind of mix has the energy potential typical of energy crop and livestock waste co-digestion. Even the digestate has a valuable methane yield since with an OLR of 1.6 g-VS/l-d, a methane yield of 155.75 l-CH4/kg-VS was achieved. For this reason, it will be a must to cover the store tank to catch the emissions.

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Table 1: Physical and chemical parameters of start-up phase inlet and outlet.

Table 2: Physical and chemical parameters of co-digestion tests (subdivided chronologically).

Table 3: Biogas average parameters for the different phases of the experiment.

Table 4: Comparison of main process parameters.

Table 5: COD behavior during the entire experiment.

Fig. 1: Details of experimental anaerobic digester, gasometer and different equipment used to perform the tests.

Fig. 2: pH behavior during the start-up phase. This initial test was performed with slurry only, and was used as reference for the following co-digestion tests.

Fig. 3: Process performance during anaerobic digestion of cattle slurry phase at ca. 35°C.

Fig. 4: Comparison of biogas production for the four tested proportion: first phase with 80% cattle slurry – 20% whey, second phase with 65% cattle slurry – 35% whey, third phase with 50% slurry – 50% whey and fourth phase with 35% slurry – 65% whey.

Fig. 5: Process performance during anaerobic co-digestion of the entire experiment at ca. 35°C. Start-up (only cattle slurry), four different feeding phases (with variable cattle slurry and whey percentage) and digestate yield test (no feed).

Fig. 6: Process performance during Digestate Methane Yeld test conducted in batch condition at ca. 35°C.

Tab. 1

Parameter	Cattle Slurry 1st day	Outlet mix 62nd day
pH	6.94	8.71
BOD 5 (mgO2/l)	39,000	34,000
COD (mgO2/l)	120,000	45,700
Density (g/cm3)	0.975	1.015
105° Residual (%)	11.6	4.22
550° Residual (%)	2.51	1.34
Total Volatile Solid (%)	9.1	2.88
NH4 (mg/l)	1,400	1,200
VFA	< 10	< 10
Sulfides (H2S) (mg/l)	0.5	0
Alkalinity (meq/l)	140	240

Tab. 2

Parameter	Whey 62nd day	Digestate 103rd day	Slurry 103rd day	Digestate 145th day	Digestate 187th day	Digestate 229th day
pH	4.12	7.65	6.97	7.65	7.47	7.49
BOD 5 (mgO2/l)	59,000	14,000	72,000	9,500	6,200	20,200
COD (mgO2/l)	74,400	23,300	95,600	20,000	14,600	32,900
Density (g/cm3)	1.012	1.015	1.014	1.017	1.016	1.015
105° Residual (%)	5.08	2.74	10.6	2.76	1.92	4.71
550° Residual (%)	0.559	0.909	2.42	1.44	1.04	1.61
Total Volatile Solid (%)	4.521	1.831	8.18	1.32	0.88	3.1
NH4 (mg/l)	78	870	2,000	1,900	1,800	2,100
VFA	<10	< 10	< 10	< 10	< 10	<10
Sulfides (H2S) (mg/l)	0	0	0	0	0	0
Alkalinity (meq/l)	NA	160	310	240	200	220

Tab. 3

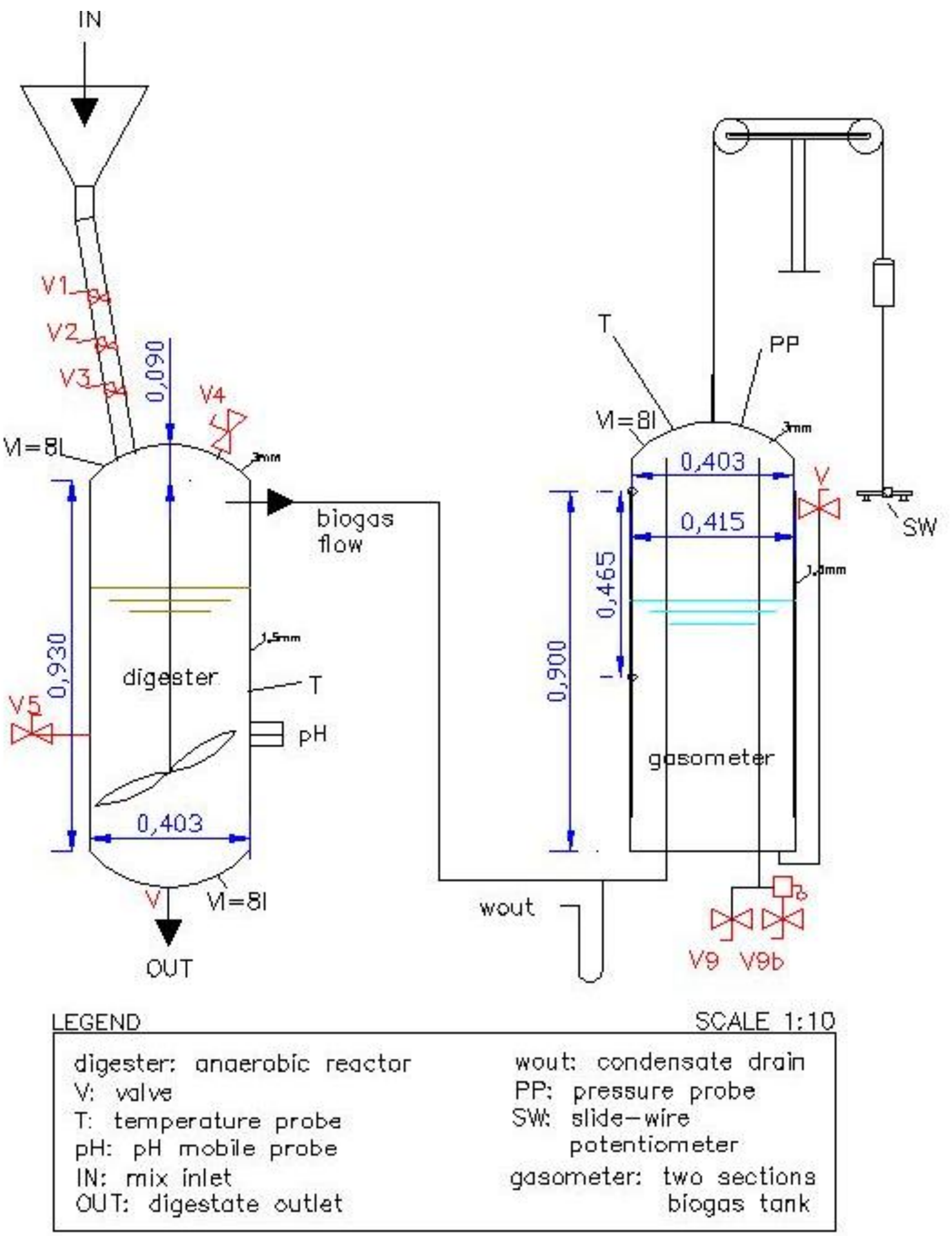
Parameter	Start up phase biogas sample (Average)	1st phase biogas sample (Average)	2nd phase biogas sample (Average)	3th phase biogas sample (Average)	4th phase biogas sample (Average)	DMY Test
CH_4 (%V/V)	45.3	53.1	56.1	55.2	57.4	58.1
CO_2 (%V/V)	54.7	46.9	43.9	44.8	42.6	39.7
O_2 (%V/V)	0.5	0	0	0	0	0
CO (ppm)	14	450	>1000	>1000	>1000	827
H_2S (ppm)	<10	380	>600	>600	>600	531

Tab. 4

Parameter	Start up phase (only slurry)	1st phase (80% - 20%)	2nd phase (65% - 35%)	3th phase (50% - 50%)	4th phase (35% - 65%)
HRT (d)	62	41	42	42	42
OLR (g VS/(l*d))	5.64	3.33	3.12	2.65	2.42
Biogas produced (l)	1647	2250.5	2435.8	2959	2897.1
VS feeded (kg)	5.62	6.14	5.6	4.7	4.3
Biogas yield (l/kg VS)	239.83	366.63	433.18	621.26	665.82
Biogas quality (%)	45.3	53.18	56.1	55.28	57.46
Methane yield (l/kg VS)	108.74	194.98	243.01	343.43	382.58
Electricity (kW)	3.39	10.23	11.07	13.45	13.17

Tab. 5

Parameter	Start up phase (only slurry)	1st phase (80% - 20%)	2nd phase (65% - 35%)	3th phase (50% - 50%)	4th phase (35% - 65%)
HRT	62	41	42	42	42
COD feeded (g/l)	110	110	104	85	81.8
OLR (g)		202	186	152	145
COD reduction (%)	62	78.8	81	82	60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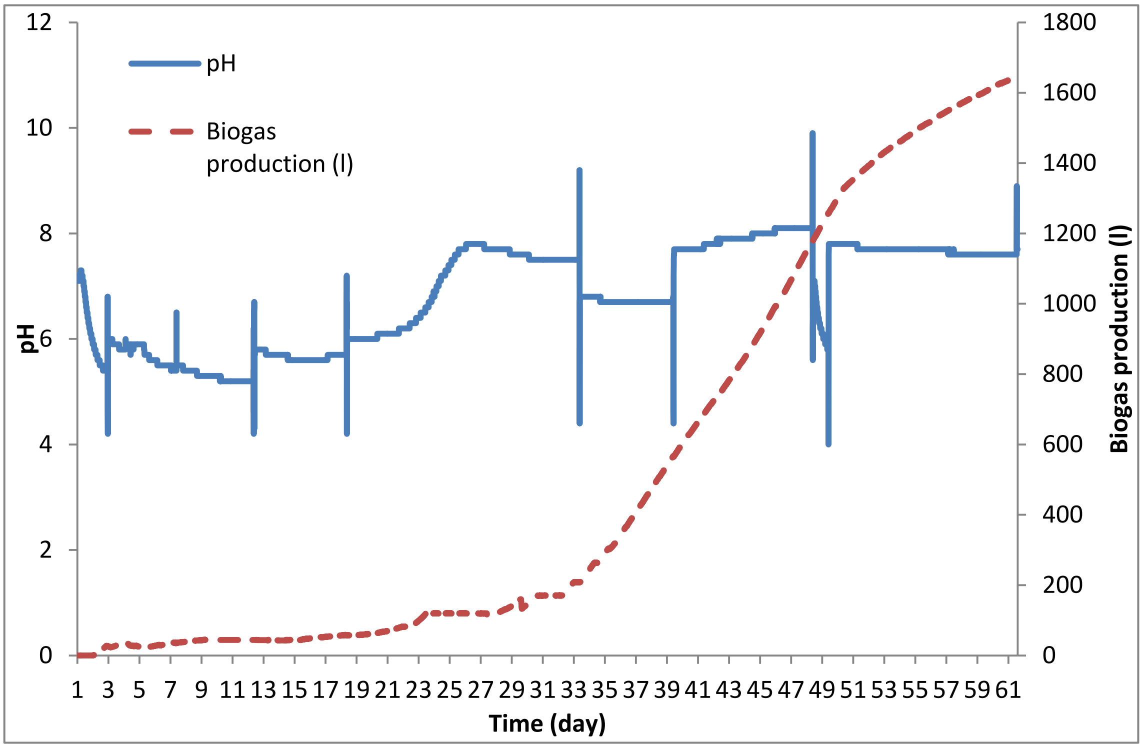
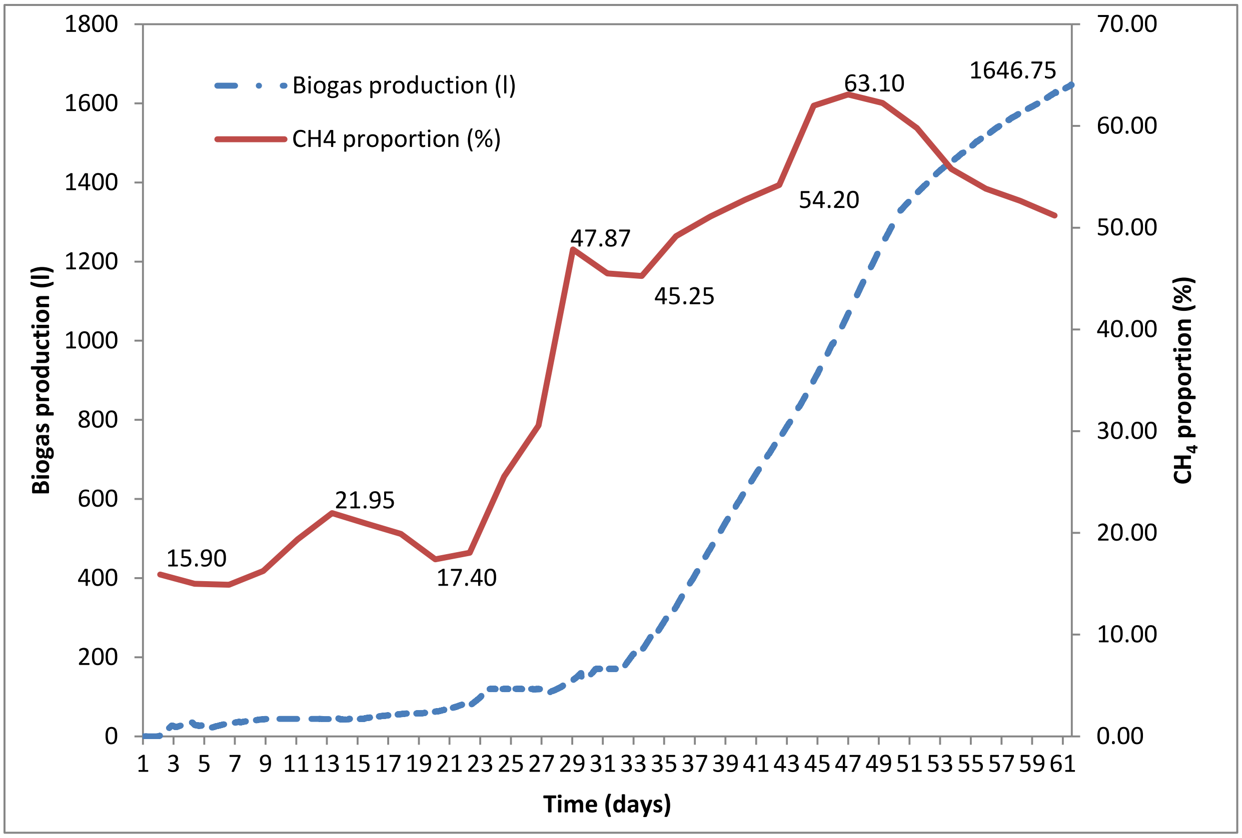
pH
V5
0,403
V
Vl=8l
OUT
biogas flow
wout
T
PP
Vl=8l
3mm
0,403
0,415
V
SW
0,465
0,900
gasometer
V9
V9b
LEGEND
SCALE 1:10
digester: anaerobic reactor
V: valve
T: temperature probe
pH: pH mobile probe
IN: mix inlet
OUT: digestate outlet
wout: condensate drain
PP: pressure probe
SW: slide-wire potentiometer
gasometer: two sections biogas tank

Fig. 1



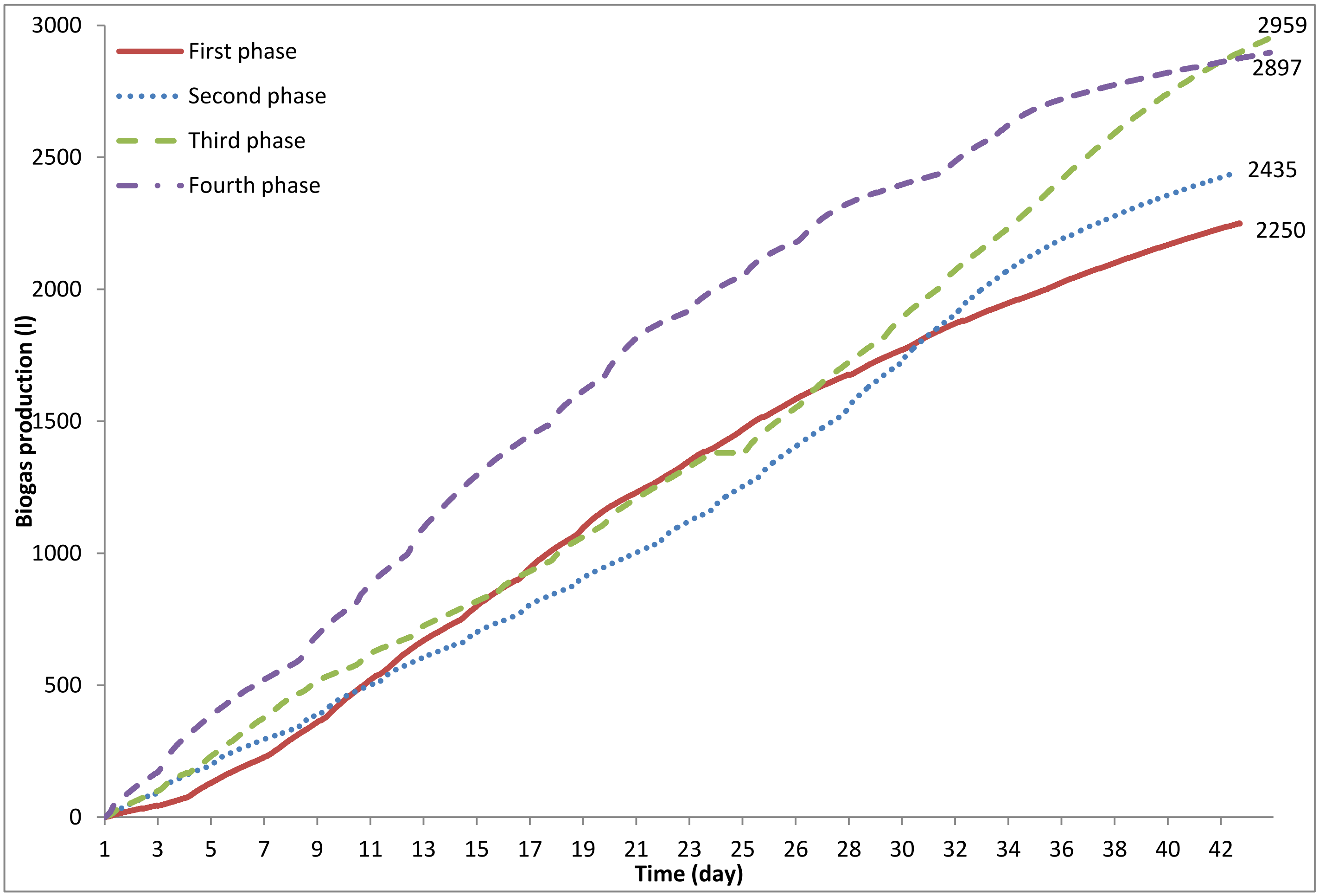
12
10
8
6
4
2
0
pH
pH
Biogas production (l)
1800
1600
1400
1200
1000
800
600
400
200
0
Biogas production (l)
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55 57 59 61
Time (day)

Fig. 2



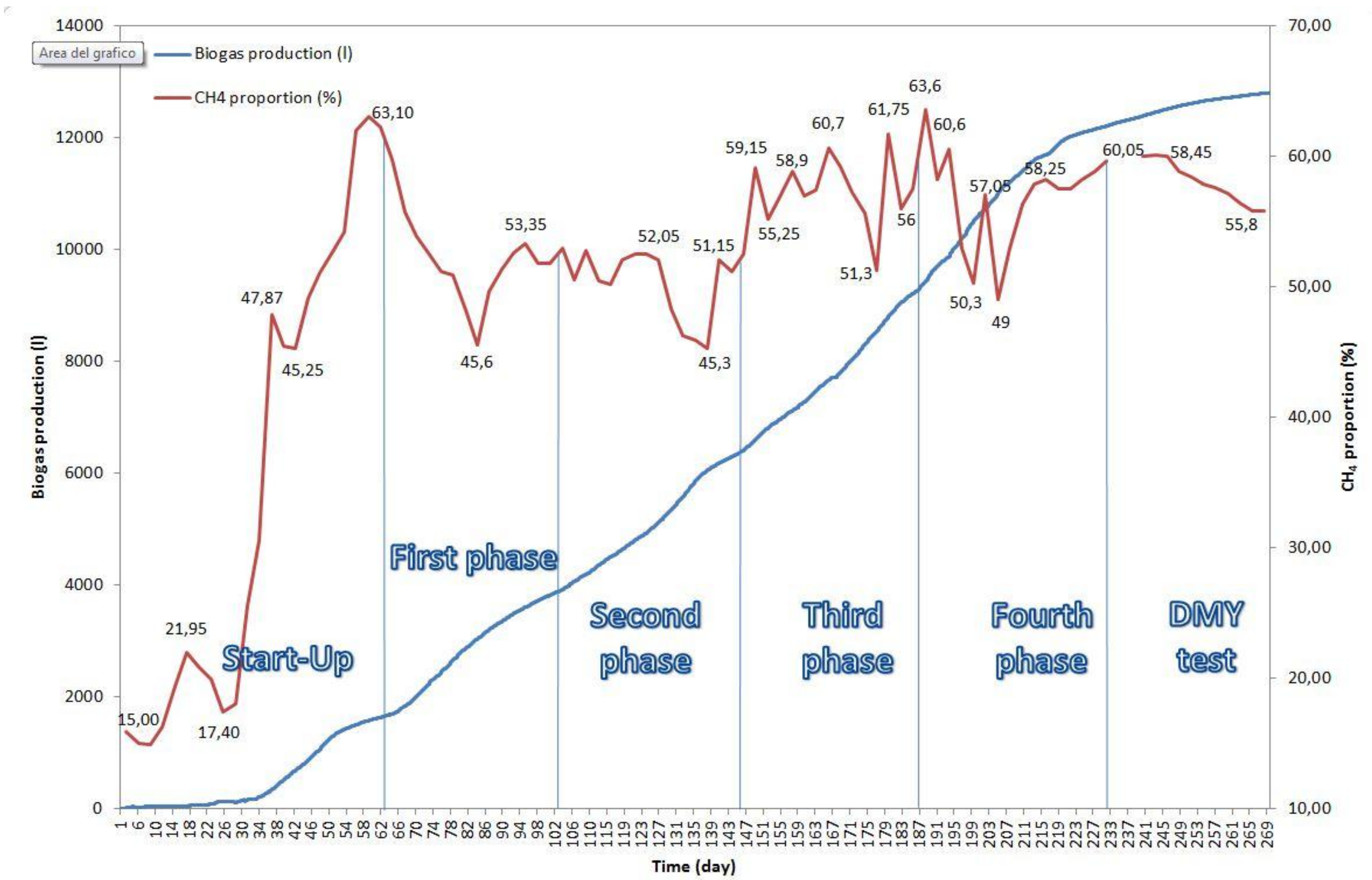
Biogas production (l)
CH4 proportion (%)
15.90
21.95
17.40
47.87
45.25
54.20
63.10
1646.75
Biogas production (l)
CH4 proportion (%)
Time (days)

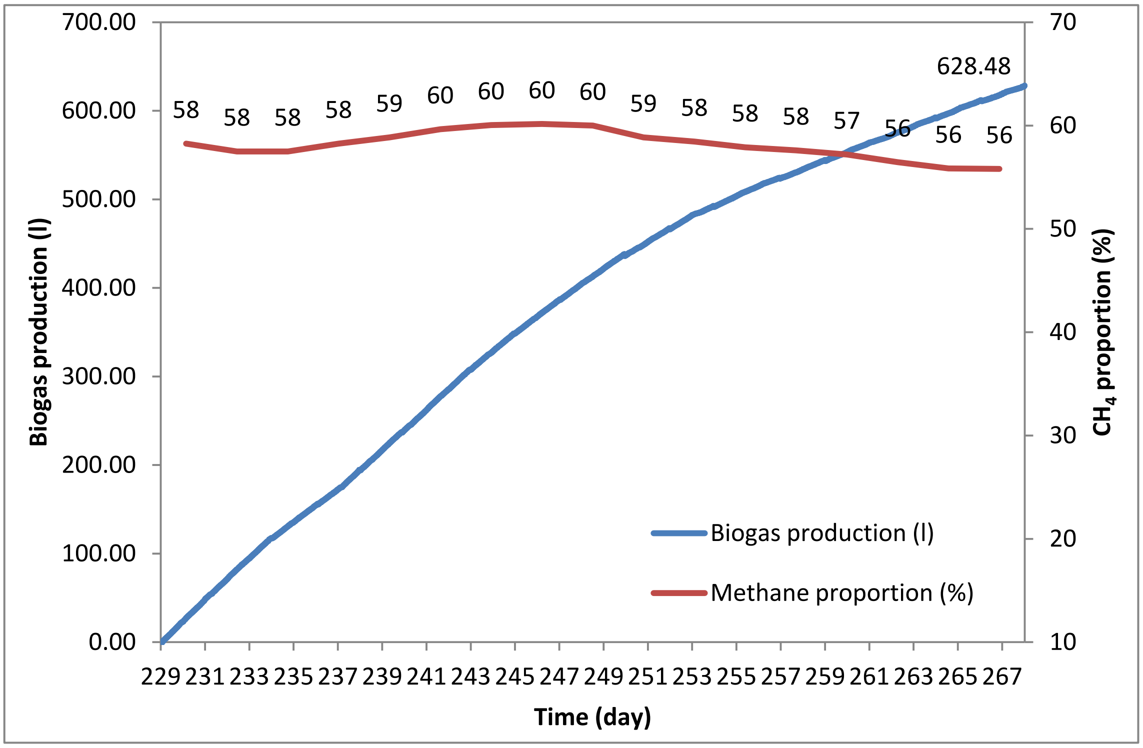
Fig. 3



3000
2500
2000
1500
1000
500
0
First phase
Second phase
Third phase
Fourth phase
2959
2897
2435
2250
Biogas production (l)
1
3
5
7
9
11
13
15
17
19
21
23
25
26
28
30
32
34
36
38
40
42
Time (day)

Fig. 4


Area del grafico
Biogas production (l)
CH4 proportion (%)
Biogas production (l)
CH4 proportion (%)
Time (day)
Start-Up
First phase
Second phase
Third phase
Fourth phase
DMY test
15,00
21,95
17,40
47,87
45,25
63,10
45,6
53,35
52,05
51,15
45,3
59,15
55,25
58,9
60,7
51,3
61,75
56
63,6
60,6
50,3
57,05
49
58,25
60,05
58,45
55,8
Fig. 5



700.00
600.00
500.00
400.00
300.00
200.00
100.00
0.00
Biogas production (l)
70
60
50
40
30
20
10
CH4 proportion (%)
628.48
58
58
58
58
59
60
60
60
60
59
58
58
58
57
56
56
56
Biogas production (l)
Methane proportion (%)
229 231 233 235 237 239 241 243 245 247 249 251 253 255 257 259 261 263 265 267
Time (day)

Fig. 6