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14 **The use of co-digested solid fraction as feedstock for biogas plants**

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20 Keywords: anaerobic digestion, biogas, mechanical separation, solid fraction

21

22 **Abstract**

23 A comparative study was set up in order to assess the technical feasibility of the long-term
24 reuse of the mechanically separated co-digested solid fraction as a feedstock for anaerobic
25 digestion plants (ADP). The biogas yields of two feedstock mixtures (A and B) were
26 assessed in mesophilic conditions ($40\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$) using 6 lab-scale continuous stirred-tank
27 reactors (CSRT). Feedstock mixture A (control) consisted of pig slurry (70%), farmyard
28 manure (4%), sorghum silage (12%) and maize silage (14%). Feedstock mixture B was the
29 same as the control plus the solid fraction derived from the mechanical separation of the
30 output raw co-digestate collected from the reactors. All reactors were fed simultaneously,
31 three times a week, over a period of nine month. According to the study results, the reuse
32 of the co-digested solid fraction as feedstock for ADP could increase the methane yield by
33 approximately 4%. However, ADP efficiency evaluation (e.g., daily yield of methane per
34 m^3 of digester) suggest to limit this practice to a maximum time period of 120 days.

35 **Introduction**

36 Anaerobic digestion of organic substrates for the production and transformation of biogas
37 into electric and thermal energy is experiencing a period of strong growth in Italy.
38 According to a recent survey (Fabbri *et al.*, 2013), approximately 1000 agricultural
39 anaerobic digestion plants (ADP) are currently running on the national territory with a total

40 installed electrical capacity of 756 MW. These ADP are generally installed at livestock
41 farms and are mostly fed with animal manure, energy crops and agricultural by-products.
42 Co-digestate is the main final product of ADP. It contains mostly water, undigested
43 organic matter and readily available inorganic compounds (e.g., nitrogen, phosphorus,
44 potash) to crops. Due to the construction of the ADP often inside intensive livestock
45 production units with insufficient arable land for nutrient recycling, export of nutrients to
46 outside farm areas may be necessary to avoid excess load of nutrients, with special regards
47 to nitrogen (N). For such a reason, in many Italian anaerobic digestion plants, co-digestate
48 is mechanically separated in order to obtain a liquid and a solid fraction (Dinuccio *et al.*,
49 2010). In the liquid phase the greater amount of potassium and inorganic nitrogen is
50 concentrated, whereas the solid fraction mainly contains organic compounds and
51 phosphorus (Dinuccio *et al.*, 2010). The liquid fraction is generally land applied near the
52 ADP while the solid fraction is exported to outside farm areas or sold to other farmers.
53 Nevertheless, the co-digested solid fraction can still contain a high biogas and methane
54 (CH₄) potential (Balsari *et al.*, 2010), due to the presence of residual and undigested
55 volatile solids (VS). Thus, it can be reused as ADP feedstock. Balsari *et al.* (2010), in a
56 work carried out at a national level through batch trials, found specific CH₄ yields of co-
57 digested solid fraction ranging between 0.07 and 0.16 Nm³/kgVS. According to these
58 figures they estimated that the reuse of the mechanically separated co-digested solid
59 fraction into the digester has the potential to improve the total CH₄ production of the ADP
60 by between 4% and 8%, depending on ADP operating parameters (e.g., feedstock type and
61 quality, organic loading rate - OLR, hydraulic retention time - HRT) and the type of
62 separator (e.g., screw press, one stage rotating separator) used to separate the raw co-
63 digested slurry. Moreover, utilizing the co-digested solid fraction in this manner could
64 reduce greenhouse gases (GHG) and ammonia (NH₃) normally released (Dinuccio *et al.*,

65 2013) during its storage. However, specific studies assessing the applicability of such an
66 option in a continuous fed anaerobic digestion system are lacking. This paper presents the
67 results of a laboratory scale experiment carried out with the objective to assess the
68 technical feasibility of the long-term reuse of the mechanically separated co-digested solid
69 fraction as a feedstock for ADP.

70

71 **Material and methods**

72 Biomasses collection and characterization

73 Fresh samples of pig slurry, farmyard manure, sorghum silage and maize silage were
74 collected at a selected full scale ADP operating in the Piemonte region (north western
75 Italy), on the first working day of each month for the duration of the experimental period
76 (270 days). The selected full scale ADP is a mesophilic (40 °C), completely stirred tank
77 reactor (CSRT) with 0.5 MW of installed electric power. It is fed with a mixture of pig
78 slurry (70%), farmyard manure (4%), sorghum silage (12%), maize silage (14%). The OLR
79 of the plant is 2.20 kgVS/ m³ dig. day, and the HRT is approximately 40 days. Collected
80 samples were stored at 5°C prior to the anaerobic digestion tests. All biomasses were
81 analysed in triplicate for pH, total solids (TS), VS, total nitrogen (TN), total ammoniacal
82 nitrogen (TAN), hemicelluloses (HC), celluloses (CE) and lignin (ADL). The pH was
83 measured by a portable pH meter (Hanna Instruments HI 9026) using a glass electrode
84 combined with a thermal automatic compensation system. TS were determined after 24 h
85 at 105 °C. VS were determined after 4 h at 550 °C in a muffle furnace (AOAC, 2000). TN
86 and TAN were analysed by the Kjeldahl standard method (AOAC, 2000). HC, CE and
87 ADL were determined by the Van Soest methods (Van Soest *et al.*, 1991).

88 Continuous anaerobic digestion experiment

89 The biogas yields of two different feedstock mixtures were compared:

- 90 - mixture A (control – the same of the selected full scale ADP): pig slurry (70%)
91 farmyard manure (4%), sorghum silage (12%), maize silage (14%)
- 92 - mixture B: the same mixture as the control plus all (100%) the solid fraction
93 obtained after mechanical separation of the output co-digestate collected from the
94 digester.

95 The experiment was carried out under mesophilic conditions ($40\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$), within a
96 temperature-controlled chamber, by using 6 identical lab-scale continuous fed stirred-tank
97 reactors. Each reactor (Figure 1), cylindrical in shape, is made up of plexiglass, with a total
98 volume of 6.5L. The biomass within the reactor is continuously mixed at a constant rate of
99 about 4 rpm by a vertical mixer connected to a geared motor installed on the top of reactor.
100 The reactors are equipped with inlet and outlet ports for feeding and effluent discharge. A
101 pipe situated at the top of the reactors is connected to Tedlar[®] gas bags by means of
102 Tygon[®] tubing to collect the produced biogas.

103 The experiment lasted 270 days. At the beginning of the experiment (day 0), the reactors
104 were inoculated with 5.5 L of co-digested slurry coming from the selected full scale ADP.
105 Thereafter all reactors (named R1-R6) were fed simultaneously, three times a week, with a
106 determined amount of tested biomasses, throughout the experimental period (270 days).
107 Prior to feeding, an equivalent volume of digester content (raw co-digestate) was
108 discharged.

109 Startup phase

110 In the first part of the experiment all reactors were run with feedstock mixture A for 60
111 days in order to establish a stable digestion process and to ensure steady state conditions.
112 During this period the reactors were operated with an OLR of $2.2\text{ kgVS}/\text{m}^3\text{ dig. day}$ and a
113 HRT of 40 days, in order to reproduce the same conditions of the selected full scale ADP.

114 Assessment of reuse of the co-digested solid fraction as a feedstock on the performances of
115 anaerobic digesters

116 In the second part of the experiment (days 61 - 270), a set of three reactors (named R1-R3)
117 continued to be fed with feedstock mixture A (control) and operated as during the startup
118 period (i.e., OLR= 2.2 kgVS/m³ dig. day; HRT= 40 days) while the others three reactors
119 (named R4-R6) were fed using feedstock mixture B (i.e., the same mixture as the control
120 plus all the solid fraction obtained by mechanical separation of the output raw co-digestate
121 collected three times a week from reactors R4-R6; Figure 2).

122 Mechanical separation of the raw co-digestate was performed by using a lab scale
123 mechanical separator as described by Dinuccio *et al.* (2008). The total amount of separated
124 raw co-digestate, as well as the amount of solid fraction recovered, were weighed and
125 recorded.

126 Biogas and CH₄ yields were measured three times a week throughout the experimental
127 period. Biogas volume was determined connecting the Tedlar[®] bags to a Ritter drum-type
128 gas meter type TG05/5 instrument, while the biogas composition was determined using a
129 Draeger XAM 7000 analyzer with infrared sensors. The recorded data were normalized at
130 standard temperature and pressure (0 °C and 1013 hPa) according to German Standard
131 Procedure (VDI 4630, 2006). The specific yields of biogas and CH₄ were subsequently
132 expressed as normal m³ per m³ digester and day (Nm³/m³ dig. day) or as normal m³ per kg
133 of volatile solids daily fed into the digester (Nm³/kgVS day). In order to assess the effect
134 of the long-term reuse of the co-digested solid fraction as a feedstock on the performances
135 of anaerobic digesters, the second part of the experiment (days 61 - 270) was divided into 7
136 periods of 30 days. During the experimental period the pH, TS and VS of raw co-digestate
137 and co-digested solid fraction were monitored monthly, while TN, TAN and fibres (HC,
138 CE, ADL) were analysed two times: at the end of startup phase (day 60), and at the end of

139 the trial (day 270). All parameters were analysed in triplicate using the same procedures as
140 previously described for fresh biomasses. Data were analysed by analysis of variance
141 procedure (ANOVA) followed by Tukey's means grouping tests. The level of significance
142 was defined as a p-value below 0.05.

143

144 **Results and discussion**

145 Characterisation of fresh biomasses

146 The main characteristics of fresh biomasses used for the trial are summarized in Table 1.
147 The TS content ranged from 1.13% in pig slurry to about 30% in maize silage, whereas the
148 VS/TS ratio ranged from 0.68 to 0.96. The TAN/TN ratio ranged from 7.43% (sorghum
149 silage) to 78.8% (Farmyard manure). Maize silage had the lowest ADL content, whereas
150 that of farmyard manure was the highest. The average amount of feedstock mixture A and
151 feedstock mixture B used to feed the reactors during the investigation period resulted,
152 respectively, 149 ± 8.16 and 162 ± 10.7 g/reactor day.

153 Continuous anaerobic digestion experiment

154 Startup phase

155 During the startup phase (60 days) the average percentage of CH₄ in biogas (Figure 3)
156 gradually increased up to the greatest value (53.8%) at day 13; then it stabilized around an
157 average value of 52.4% (range 50.6 - 53.9%). The average biogas yield followed a similar
158 trend; this trend showed a peak ($1.49 \text{ Nm}^3/\text{m}^3 \text{ dig. day}$) at day 18 followed by a steady
159 state period (days 19-60) during which the biogas yield averaged 1.40 (range 1.32 -1.48)
160 $\text{Nm}^3/\text{m}^3 \text{ dig. day}$. During the 41 days steady state period, the average daily CH₄ produced
161 by reactors R1-R6 ranged between 0.313 and 0.353 Nm^3/kgVS , comparable to values
162 measured by Gioelli *et al.* (2012) during a 12 months period of monitoring of the selected

163 full scale ADP; the degree of VS degraded during the anaerobic digestion process resulted
164 64%. Investigations of 41 biogas plants in Austria by Hopfner-Sixt and Amon (2007)
165 found CH₄ yields from co-fermentation of animal manure and energy crops up to 0.39
166 Nm³/kgVS, with VS degradation rates of 78–84%. The lower degree of degradation found
167 in this study can be explained by the shorter HRT (~ 40 days) of the reactors, which is
168 similar to that of the selected full scale ADP but lesser than the minimum HRT of 45 – 60
169 days recommended in the literature (e.g., Öchsner and Helffrich, 2005) for an optimal
170 degradation of VS content in energy crops.

171 Assessment of reuse of the co-digested solid fraction as a feedstock on the performances of
172 anaerobic digesters

173 In Table 2 are shown the main chemical and physical characteristics of the co-digested
174 solid fraction obtained by mechanical separation of raw co-digestate from reactors R4-R6,
175 and used as feedstock for the reactors during the test. Total solids content of co-digested
176 solid fraction ranged from 16.4 to 18.1; VS and TS ratio resulted to be always higher than
177 0.85 suggesting a residual availability of undigested organic matter. However, the
178 concentrations of HC and CE in co-digested solid fraction tended to decrease over time
179 (Table 2), while, in contrast, the concentration of ADL increased, resulting 1.43% at day
180 60 (end of startup phase) and 5.03% at day 270 (end of the experiment). The average
181 amount of raw co-digestate recorded from reactors R1-R3 (feedstock mixture A) and from
182 reactors R4-R6 (feedstock mixture B) over the investigation period resulted, respectively,
183 135 ± 7.43 and 145 ± 9.03 g/reactor day. The separation efficiency in terms of mass (i.e., the
184 relative amount of co-digested solid fraction obtained by mechanical separation of the raw
185 co-digestate) of the used lab-scale mechanical separator resulted, on average, 9.70% (range
186 8.70-10.6%).

187 Figure 4 depicts the average CH₄ yields recorded from each feedstock mixture (A and B)
188 during the second part (days 61-270) of the experiment. During this 210 days period, the
189 average volumetric CH₄ produced by mixture A (control, reactors R1-R3) ranged between
190 0.674 and 0.802 Nm³/m³ dig. day, reflecting the variability of the characteristics of fresh
191 biomasses (Table 1) collected at the selected ADP during the experiment. The specific CH₄
192 yields, expressed as Nm³/kgVS (Table 3), obtained over the experimental period by
193 feedstock mixture B (reactors R4-R6) were, on average, 17% lower than those recorded
194 from the control (feedstock mixture A reactors R1-R3). However, the average daily
195 volumetric CH₄ yields by mixture B (reactors R4-R6) (Figure 4) were generally higher
196 than those obtained by mixture A for most of the experimental period. The reuse of the co-
197 digested solid fraction in reactors R4-R6 gradually increased the average volumetric CH₄
198 production rate from 0.728 (days 61-90) to 0.791 (days 151-180) Nm³/m³ dig. day (Table
199 4). The latter value corresponds to a significant (p<0.05) increase of 4.36% when
200 compared to the average volumetric CH₄ production rate (0.758 Nm³/m³ dig. day) recorded
201 from reactors R1-R3 (control). After this period such differences did, however, start to
202 decrease, dropping to a value of +0.28% during the last 30 days of trial (Table 4).

203 The pH values of the raw co-digestate recorded over time (Figure 5A) suggests a regular
204 course of the anaerobic digestion process within all the reactors. The average pH values of
205 co-digestate from reactors R4-R6 ranged between 7.4 and 7.7, within the optimum range
206 (6.5–7.8) for the adequate growth of anaerobic microorganisms (Liu *et al.*, 2008). This
207 observation indicates that the process adapted well to the introduction of the co-digested
208 solid fraction as co-substrate, as pH fluctuation is a widely used indicator of process stress
209 in anaerobic reactors (Ward *et al.*, 2008). However, the concentration of TAN (Table 5) in
210 raw co-digestate from reactors R4-R6 has shown the tendency to increase, resulting 0.14%
211 at day 60 (end of start up phase) and 0.20% at day 270 (end of the experiment), indicating

212 the possibility of inhibition on the activity of microorganisms. Free ammonia has been
213 suggested to be the main cause of inhibition in anaerobic digesters due to its high
214 membrane permeability (Kroeker *et al.*, 1979; de Baere *et al.*, 1984). Ammonia inhibition
215 was reported to occur above pH 7.4 in the range of 1500–3000 mgTAN/L, whereas at
216 concentrations in excess of 3000 mgTAN/L, ammonia was claimed to be toxic irrespective
217 of pH (Van Velsen, 1979; Koster and Lettinga, 1984). A remarkable increase over time of
218 hemicelluloses, celluloses and lignin content of raw co-digestate from reactors R4-R6 was
219 also observed (Table 5). Lignin is not degradable under anaerobic conditions and may
220 prevent microbial access to hemicelluloses and celluloses (Mussatto *et al.*, 2008). On
221 average, the concentration of TS (Figure 5B) and VS (Figure 5C) in raw co-digestate from
222 reactors R4-R6 resulted, respectively, 15.5% and 18.9% higher than the concentration in
223 raw co-digestate from reactors (R1-R3). An average VS removal efficiency (Figure 5D) of
224 66.0% and 63.6%, respectively, for reactors R1-R3 and reactors R4-R6 was calculated.

225

226 **Conclusions**

227 The results obtained in this laboratory-scale study confirm that the co-digested solid
228 fraction can still contain a high biogas and methane potential. The reuse of the co-digested
229 solid fraction as feedstock for ADP seems to be an interesting option. Under the specific
230 laboratory conditions adopted in this study, the long-term reuse of the co-digested solid
231 fraction into the digester improved the total CH₄ production by approximately 4%.
232 However, after 120 days of continuous recirculation of the co-digested solid fraction the
233 volumetric CH₄ yield of the reactors started to decline, mainly due to the accumulation of
234 recalcitrant organic fibres (e.g., lignin) which are compounds minimally digestible by
235 anaerobic microorganisms. Therefore it is suggested to restrict this practice for limited
236 periods of time, monitoring regularly the productivity of the ADP (e.g., daily yield of

237 biogas and methane per m³ of digester) and the key process parameters (e.g., pH and TAN
238 concentration in raw co-digestate) in order to maintain such variables steady and within the
239 optimal ranges for the adequate growth of anaerobic microorganisms.

240

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297

298 TABLES

299 Table 1. Main chemical and physical characteristics of the fresh biomasses used in the trial
300 (standard deviation in parentheses, n=27)

301 Table 2. Main chemical and physical characteristics of the co-digested solid fraction
302 obtained by mechanical separation of raw co-digestate from reactors R4-R6

303 Table 3. Average specific methane yields recorded during the experiment from reactors
304 R1-R3 (feedstock mixture A, control) and from reactors R4-R6 (feedstock mixture B).
305 Standard deviation in parentheses (n=36).

306 Table 4. Average volumetric methane production rates recorded during the experiment
307 from reactors R1-R3 (feedstock mixture A, control) and from reactors R4-R6 (feedstock
308 mixture B). Standard deviation in parentheses (n=36).

309 Table 5. Main chemical and physical characteristics of the raw co-digestate recorded at day
310 60 (end of the startup phase) and at day 270 (end of the trial) from reactors R1-R3
311 (feedstock mixture A, control) and from reactors R4-R6 (feedstock mixture B). Standard
312 deviation in parentheses (n=3).

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Table 1.

	Maize silage	Sorghum silage	Farmyard manure	Pig slurry
pH	3.75 (0.22)	3.96 (0.19)	8.49 (0.17)	7.26 (0.25)
TS (%)	30.5 (2.96)	27.6 (2.46)	22.3 (2.37)	1.13 (0.52)
VS (%TS)	95.5 (0.96)	91.2 (1.05)	78.7 (4.01)	67.6 (4.38)
TN (%)	0.34 (0.10)	0.37 (0.09)	0.48 (0.09)	0.15 (0.07)
TAN (%)	0.03 (0.02)	0.03 (0.01)	0.38 (0.06)	0.11 (0.04)
HC (%)	7.82 (0.74)	6.24 (0.12)	4.20 (0.94)	n.d.
CE (%)	8.16 (0.63)	9.15 (0.72)	7.01 (0.41)	n.d.
ADL (%)	0.99 (0.18)	1.41 (0.32)	2.67 (0.84)	n.d.

321

322 Table 2.

Days from the beginning of the experiment	pH	TS (%)	VS (%TS)	TN (%)	TAN (%)	HC (%)	CE (%)	ADL (%)
60 (end of start up phase)	8.26	17.2	85.7	0.43	0.14	5.86	8.51	1.43
90	8.30	18.1	86.6	-	-	-	-	-
120	8.18	17.6	88.8	-	-	-	-	-
150	8.30	16.5	86.9	-	-	-	-	-
180	8.21	16.6	87.6	-	-	-	-	-
210	8.14	16.4	86.9	-	-	-	-	-
240	8.23	17.2	87.5	-	-	-	-	-
270 (end of the experiment)	8.18	17.9	86.5	0.53	0.15	3.75	5.65	5.03

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331 Table 3.

Experimental period (days)	Reactors		Significance (p)
	R1-R3 (Nm ³ /kgVS)	R4-R6 (Nm ³ /kgVS)	
61-90	0.320a (0.008)	0.281b (0.007)	< 0.00
91-120	0.325a (0.007)	0.287b (0.006)	< 0.00
121-150	0.344a (0.008)	0.304b (0.008)	< 0.00
151-180	0.345a (0.010)	0.303b (0.006)	< 0.00
181-210	0.343a (0.009)	0.295b (0.007)	< 0.00
211-240	0.344a (0.014)	0.293b (0.007)	< 0.00
241-270	0.342a (0.009)	0.284b (0.003)	< 0.00

332 a–b: data in a row followed by a different letter differ significantly (p < 0.05)

333 Table 4.

Experimental period (days)	Reactors		Significance (p)
	R1-R3 (Nm ³ /m ³ dig. day)	R4-R6 (Nm ³ /m ³ dig. day)	
61-90	0.704b (0.018)	0.728a (0.019)	0.002
91-120	0.715b (0.015)	0.743a (0.016)	< 0.000
121-150	0.756b (0.017)	0.789a (0.021)	< 0.000
151-180	0.758b (0.022)	0.791a (0.016)	< 0.000
181-210	0.755a (0.020)	0.771a (0.018)	0.066
211-240	0.757a (0.030)	0.774a (0.018)	0.101
241-270	0.752a (0.020)	0.754a (0.009)	0.737

334 a–b: data in a row followed by a different letter differ significantly (p < 0.05)

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338 Table 5.

Days from the start of the experiment	Reactors	pH	TS (%)	VS (%TS)	TN (%)	TAN (%)	HC (%)	CE (%)	ADL (%)
60 (end of start up phase)	R1-R6	7.68 (0.07)	4.66 (0.10)	70.0 (0.76)	0.23	0.14	0.47	0.26	0.21
270 (end of experiment)	R1-R3	7.53 (0.07)	5.09 (0.19)	72.8 (1.03)	0.24 (0.03)	0.15 (0.01)	0.84 (0.14)	0.73 (0.12)	1.13 (0.34)
	R4-R6	7.45 (0.07)	6.33 (0.25)	75.4 (0.89)	0.24 (0.02)	0.20 (0.01)	1.90 (0.11)	0.93 (0.09)	2.23 (0.15)

339

340 FIGURES

341 Figure 1. The lab-scale continuous fed stirred-tank reactors (CSTR) used for the trial.

342 Figure 2. Feeding scheme of the reactors R4-R6.

343 Figure 3. Specific biogas yield and methane concentration recorded from reactors R1-R6
344 during the startup phase (days 0 – 60). Error bars indicate standard deviation (N = 6).

345 Figure 4. Average volumetric methane yields recorded from day 60 (end of the startup
346 phase) to day 270 (end of the trial) from reactors R1-R3 (feedstock mixture A, control) and
347 from reactors R4-R6 (feedstock mixture B). N = 3; standard deviation removed for clarity.

348 Figure 5. Evolution of pH (A), total solids (B) and volatile solids (C) content in raw co-
349 digestate and volatile solids removal efficiencies (D) measured from reactors R1-R3 and
350 reactors R4-R6.

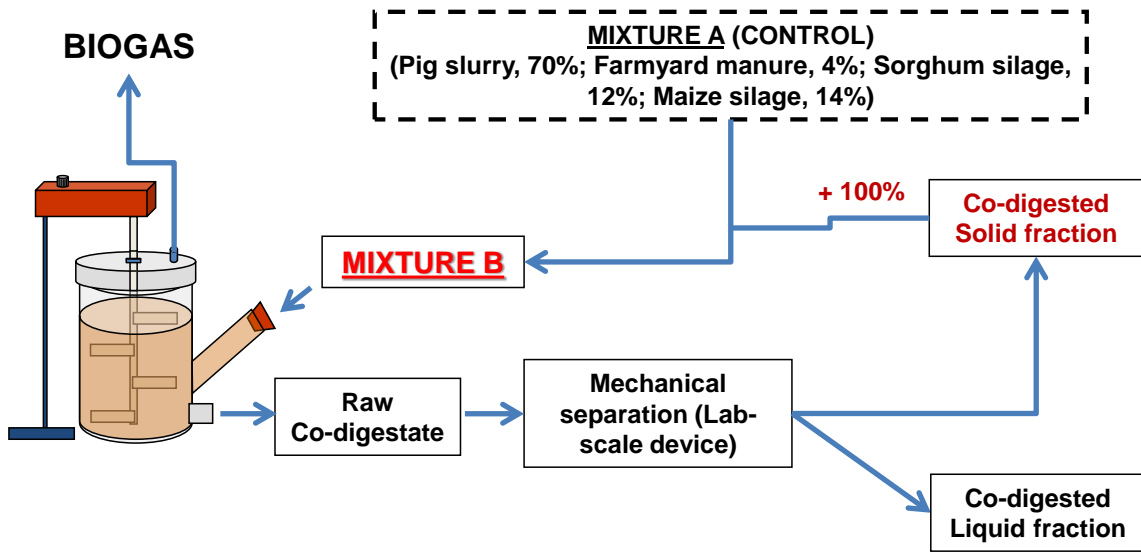
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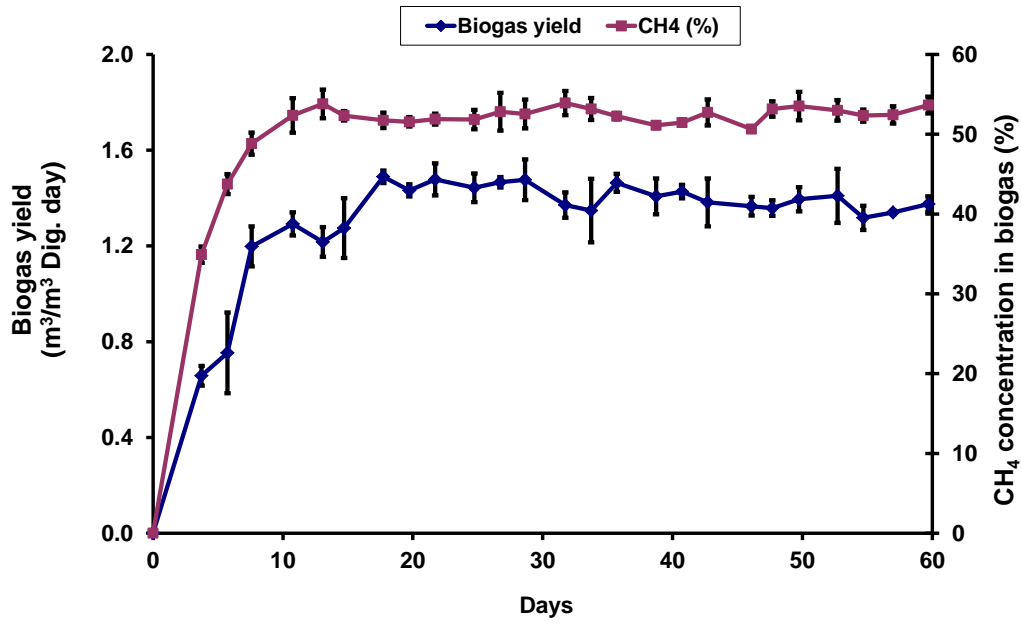
Figure 1.

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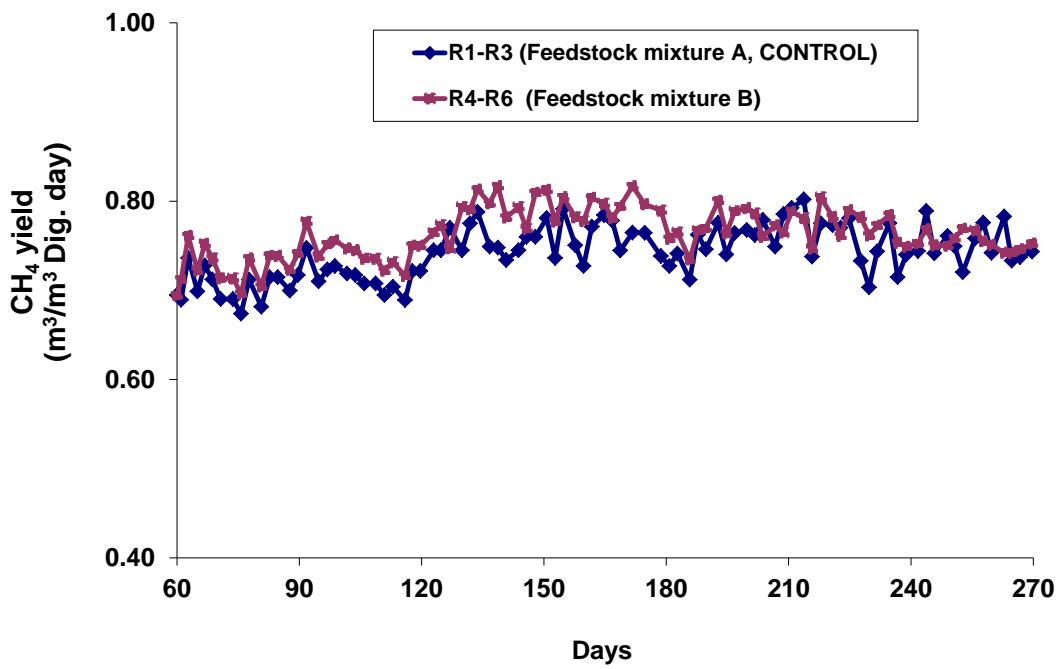
Figure 2.



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361 Figure 3.

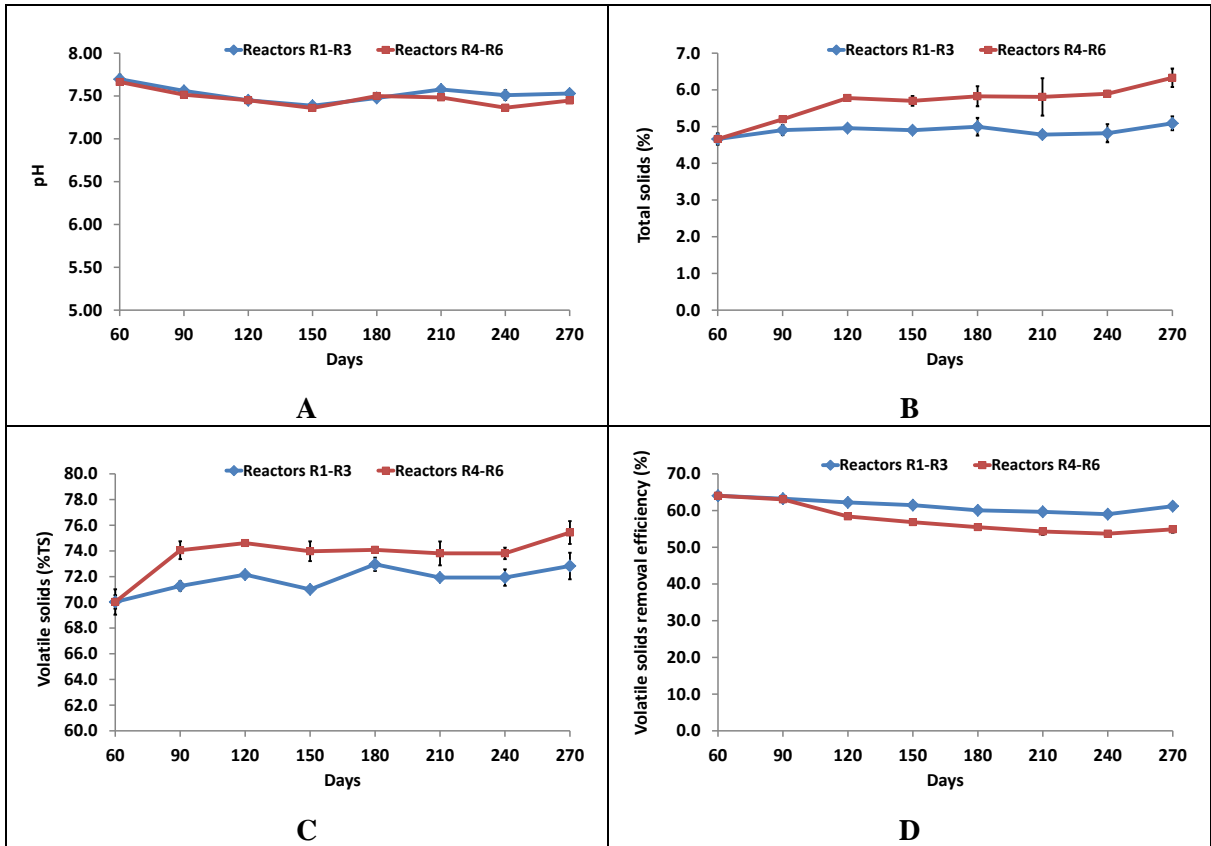
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364 Figure 4.

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366 Figure 5.
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