

Investigation of the Effect of Compaction on the Anaerobic Digestion Process

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Research Article

Keywords: anaerobic digestion, compaction, rice straw, biogas production

Posted Date: August 4th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-743267/v1>

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1 **Investigation of the effect of compaction on the anaerobic**
2 **digestion process**

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7 **Abstract**

8 This paper aims to evaluate the effects of compaction on the anaerobic
9 biodegradability of straw. In the study, compaction tests were carried out at different
10 applied pressures, i.e., 0 (CK), 277 (T1), 555 (T2), and 1109 Pa (T3), respectively. The
11 changes in physicochemical indicators (i.e., pH, VFA, COD, and DHA) of the liquid
12 digestate were monitored. Factor analysis was adopted to analyze biogas production's
13 main factors in the batch Anaerobic digestion (AD) process. Changes in the surface
14 structures and composition of solid digestate were analyzed. The results showed that
15 the maximum gain in biogas production was $298.35\text{mL}\cdot\text{g}^{-1}\text{TS}$ for the T2 reactor,
16 significantly higher than that of CK and T3 reactors. The effect of compaction on the
17 physicochemical index of liquid digestate was not significant during the batch-type AD
18 process. The factor analysis results suggested that the major factors affecting biogas
19 production were influenced by the compaction and varied based on the different stages
20 of digestion. Scanning electron microscopy (SEM) showed that the straw surface was
21 damaged as the compaction increases; however, the degree of damage was not
22 significant. This research concluded that compaction on gas production via changing
23 the environment during the batch AD process and proper compaction could positively
24 affect biogas' yield, while excessive compaction will inhibit gas production.

25 **Keywords:** anaerobic digestion, compaction, rice straw, biogas production

26 **Introduction**

27 China has abundant agricultural straw resources, with about 560 million tons [1].
28 Anaerobic digestion (AD) is a proven and stable technology and primarily produces a
29 high energy recovery from agricultural waste and has been considered the leading
30 commercial option [2-3]. By converting straw into biogas, liquid, and solid digestate
31 through this technology, the recycling of straw carbon and nitrogen resources is
32 achieved, which is in line with the development requirements of circular agriculture.
33 Batch type AD process is a form of anaerobic digestion with the advantages of simple
34 operation and reduced labor management costs. Many centralized gas supply projects
35 with straw as the primary raw material have been built in rural areas of China, and most
36 of these projects are based on the batch-type AD process [4]

37 However, during the batch type AD process, the straw was subject to buoyancy,
38 gravity, the upward force of the generated biogas, coupled with many factors such as
39 the decline in mechanical strength of straw due to material decomposition, causing the
40 straw to squeeze each other. This will inevitably lead to straw porosity reduction,
41 tightness increase, and crusting more serious, ultimately affecting biogas production
42 from straw. Meanwhile, the leachate reflux will be limited during the straw batch type
43 AD process. However, it has to be noted that leachate circulation within the substrate
44 serves as a transport medium for heat, nutrients, and material exchange inside the
45 digester [5]. Furthermore, it is essential to enhance leachate reflux for biogas production
46 due to the difficulty of mixing in the batch-type AD process. Rocamora et al. [6] also
47 mentioned that batch AD was affected by factors like micro and macroporosity, the

48 degree of compaction or the material's permeability to be digested. On this note, it is
49 necessary to investigate the effect of compaction on the batch-type AD process.
50 Nevertheless, only very little research in the literature has been performed in connection
51 with this field. In a study conducted by Wedwitschka et al. [5], material compaction
52 occurred during the digestion process and can have a negative effect on substrate
53 permeability. André et al. [7] observed a slight decrease of the manure porosity, and
54 presumably, it was caused by compaction on the batch AD during the recirculation of
55 percolate. Wedwitschka et al. [8] found that compressive forces impacted the
56 permeability and resulted in compression of the material tested through matrix substrate
57 characterization experiments of an anaerobic batch digestion system. The above studies
58 mainly focused on the changes in the physical properties of the substrates by
59 compaction during the batch-type AD process. However, the exact effect of different
60 compaction levels on the batch type AD process is not apparent.

61 The objective of this study was to make a step forward to understanding the effect
62 of compaction during the batch-type AD process. Different compaction levels were set
63 based on batch AD experiments at the lab scale in the present study. The impacts of
64 compaction on the physicochemical indexes of the liquid digestate, structural and
65 compositional changes of solid digestate, and biogas yield were comprehensively
66 investigated and compared.

67 **Materials and methods**

68 **Feedstock and Inoculum**

69 Rice straw was harvested in local fields in Jiangsu, China. Rice straw was dry and

70 chopped manually to a size of about 2 to 3 centimeters, then set aside in a dry and cool
71 place. The inoculum was activated sludge taken from wastewater treatment plants
72 acclimated and cultured at (37 ± 1) °C with pig manure for 20 days. The inoculum stops
73 adding substrate one week before AD. The primary physicochemical properties of
74 straw and inoculum are shown in Table 1.

75 **Anaerobic digestion reactor**

76 A digestion experiment was conducted using the 5-L reactor. The reactor was made
77 of clear cylindrical acrylic with a diameter of 16cm and 28 cm in height. The hot water
78 in the thermostat water bath was pumped into the jacket of the reactor for heating. An
79 air vent was arranged at the top of the fermenter, and the biogas produced enters the gas
80 collecting bag through the outlet. The digestive fluid was collected through the outlet
81 at the bottom of the reactor. The anaerobic digestion unit is shown in Fig 1.

82 **Compaction experimental design**

83 Four different masses of weights were used, i.e., 0g, 500g, 1000g, and 2000g,
84 respectively, and the corresponding pressures are 0, 277, 555, and 1109 Pa. Weights
85 were placed at the top of the straw pile, which simulated compaction that occurs in the
86 batch type AD process. The treatment group with the pressure of 0, 277, 555, and 1109
87 Pa was denoted as CK, T1, T2, and T3 groups. A known weight of the porous round
88 plate, with a size and aperture of 14 diameters and 3 mm, respectively, was put on the
89 straw pile to bear different weights and ensure uniform force on the straw in the reactor.

90 Prepared 300 g of straw (based on the dry matter) was subjected to an anaerobic

91 test, and 1.5L inoculum was mixed into the reactors and then added 1.2L distilled water
92 to ensure TS concentration was 10%. The substrate/inoculum (S/I) ratio was 10.15
93 (based on volatile solids (VS)). During the experiment, the liquid digestate was returned
94 from the bottom to the top of the digester every day. To ensure that the digestive solution
95 flowed back into the straw pile evenly, two layers of gauze were placed between the
96 straw pile and the porous circular plate. Three replicates of each treatment group were
97 carried out in the batch-type AD process. The reactor was sealed at a controlled
98 temperature of (37 ± 1) °C using a thermostat water bath. The main performance
99 parameters, including pH value, chemical oxygen demand (COD), total volatile fatty
100 acids (VFA) content, and dehydrogenase activity (DHA), were monitored on the 0, 2,
101 5, 9, 14, 20, 30, and 45d of the experiment, respectively.

102 **Analytical methods**

103 The pH value was determined using a pH meter (Mettler Toledo, Switzerland);
104 total solids (TS), volatile solids (VS), total Kjeldahl nitrogen (TKN), total organic
105 carbon, and COD were determined according to APHA's Standard Methods [9]; Total
106 volatile fatty acids (TVFA) were measured according to Zou et al. [10]; The lignin,
107 cellulose, and hemicellulose contents were determined according to the procedures
108 proposed by Van Soest et al. [11]; The dehydrogenase activity (DHA) was determined
109 based on the reduction of 2,3,5-triphenyl tetrazolium chloride (TTC), as described by
110 Zhou et al. [12]. The external surface of straw with different compaction levels was
111 observed using a scanning electron microscope (SEM) (S3400, Hitachi, Japan) at an
112 acceleration voltage of 20 kV. The degradation of TS, cellulose, hemicellulose of

113 feedstock during AD was analyzed and calculated as earlier described [13], according
 114 to Eq. (1):

$$115 \text{ Degradation rate (\%)} = (Y-C)/Y \times 100\% \quad (1)$$

116 where Y (g) is the initial amount of a component in the raw straw and C (g) is the
 117 final amount of a component in the digestate at the end of the experiment.

118 **Factor analysis**

119 Factor analysis is a multivariate statistical technique used to finding theoretical
 120 concepts that underlie the association between observed variables. It explores the
 121 underlying structure in the observed data by examining the internal dependencies
 122 among many variables and representing their underlying data structure with a few
 123 dummy variables. These few dummy variables can reflect the primary information of
 124 the numerous original variables [10,14]. The mathematical model for factor analysis,
 125 as follows.

$$126 \begin{pmatrix} x_1 \\ x_2 \\ \vdots \\ x_p \end{pmatrix} = \begin{pmatrix} \mu_1 \\ \mu_2 \\ \vdots \\ \mu_p \end{pmatrix} + \begin{pmatrix} \alpha_{11} & \alpha_{12} & \dots & \alpha_{1m} \\ \alpha_{21} & \alpha_{22} & \dots & \alpha_{2m} \\ \dots & \dots & \ddots & \dots \\ \alpha_{p1} & \alpha_{p2} & \dots & \alpha_{pm} \end{pmatrix} \begin{pmatrix} F_1 \\ F_2 \\ \vdots \\ F_p \end{pmatrix} + \begin{pmatrix} \xi_1 \\ \xi_2 \\ \vdots \\ \xi_p \end{pmatrix} \quad (2)$$

127 Abbreviated as:

$$128 x_p = \mu_p + \alpha_{11}F_1 + \dots + \alpha_{pm}F_p + \xi_p \quad (3)$$

129 where the F_p , $p = 1, \dots, k$, are $k \geq 1$ random variables called factors, $X = (x_1,$
 130 $x_2, \dots, x_p)^T$ is an observable random variable, $F = (F_1, F_2, \dots, F_p)^T$ is a common factor
 131 (factors), $\xi = (\xi_1, \xi_2, \dots, \xi_p)^T$ is the special factor, F and ξ are not observed random
 132 variables, $\mu_p = (\mu_1, \mu_2, \dots, \mu_p)$ is the ensemble average of random variable X . Thus,
 133 X is standardized, and the ensemble average of the standard variable is '0,' and the

134 variance is '1'. This is represented as follows:

$$135 \begin{pmatrix} x_1 \\ x_2 \\ \vdots \\ x_p \end{pmatrix} = \begin{pmatrix} \alpha_{11} & \alpha_{12} & \dots & \alpha_{1m} \\ \alpha_{21} & \alpha_{22} & \dots & \alpha_{2m} \\ \dots & \dots & \ddots & \dots \\ \alpha_{p1} & \alpha_{p2} & \dots & \alpha_{pm} \end{pmatrix} \begin{pmatrix} F_1 \\ F_2 \\ \vdots \\ F_p \end{pmatrix} + \begin{pmatrix} \xi_1 \\ \xi_2 \\ \vdots \\ \xi_p \end{pmatrix} \quad (4)$$

136 Abbreviated as:

$$137 x_p = \alpha_{pm}F_p + \xi_p \quad (5)$$

138 If

$$139 (1) E(F_p) = 0, \delta(F_p) = 1.$$

$$140 (2) E(\xi_p) = 0, \delta(\xi_p) = \xi_p.$$

$$141 (3) F_p \text{ and } \xi_p \text{ mutual independence}$$

142 Thus, X is a factor model which has a common factor. There were four variables
143 in the model, namely pH, VFA, COD, and DHA content. The factor analysis was to
144 obtain the main factors influencing the biogas yield in different digestion stages and
145 compaction.

146 **Calculations and statistical analyses**

147 One-way ANOVA ($p < 0.05$) and factor analysis were carried out with the software
148 package SPSS, version 21. Origin Lab program was used for plotting. All data shown
149 are the average values of independent triplicates ($n = 3$) \pm SD.

150 **Results and discussion**

151 **Effect of compaction on the physicochemical index of liquid digestate**

152 **Influence on the pH value and VFA content**

153 The pH value is one of the indicators of the stability of the anaerobic digestion

154 system, reflecting the buffer capacity of the system. The changes in pH value under
155 different pressures during the experiment can easily be observed from the results
156 depicted in Fig. 2(a). For all runs, the trends of pH values change of experiments were
157 similar, but with some differences. For the initial stage of AD, the pH values had
158 dropped considerably. After that, it recovered and rose to reach the original level and
159 finally stabilized. There were some notable differences in pH changes as a result of
160 different pressures. The lowest pH values of CK and T1 reactor were observed on the
161 second day, while it can also be noted that the gain in lowest value of pH for T2 and T3
162 reactors during digestion on day five. The lowest pH over the whole anaerobic digestion
163 process at the different groups: 6.94, 6.78, 6.69, and 6.36 for CK, T1, T2, and T3 reactors.
164 pH had dropped considerably during the first five days can be ascribed that the rate of
165 acidogenesis, a conversion of soluble organics to VFAs, is typically much faster than
166 methanogenesis in an anaerobic environment. VFAs could not be utilized by
167 methanogens adapted to the environment slowly and weak metabolic capacity, resulting
168 in the accumulation of fatty acids [15]. Thus, this phenomenon is inevitable. It is also
169 noticed in Fig. 2(a) that the CK reactor obtained the highest pH value, followed by T1,
170 T2, and T3 reactors during the first nine days. It suggested a significant decrease in the
171 specific pH value within the more considerable pressure acting on the straw. This
172 phenomenon may be due to the floating of the straw in the CK group in this study,
173 resulting in insufficient contact with the inoculum. The pressure is a factor leading to
174 good contact with straw and inoculum, and the more organic matter that undergoes
175 hydrolysis, the more volatile fatty acids, which may lead to system acidification. pH

176 value, therefore, goes down as the pressure increases. The pH values of all digesters
177 were within an ideal range for AD, i.e., 4.0–8.5 for fermentative bacteria and 6.8–7.4
178 for methanogens [16-17].

179 In addition to pH measurement, VFA was determined since pH is not a sole
180 indicator of AD failure [18]. The analysis of VFA production provides valuable
181 information on the evolution of the AD process. VFA concentration is one of the most
182 critical parameters to indicate process stability during anaerobic digestion, and its initial
183 content is known to affect the CH₄ generation yield [19-20]. Acid-producing bacteria
184 degraded organic matter to produce VFA, resulting in a decrease of pH, and in this study,
185 pH changes corresponded well with the VFA concentration (Fig. 2b). VFA
186 concentration increased in the first five days and reached the highest level on the 5th
187 day. This phenomenon was typical for the start-up stage of AD because of the unbalance
188 among hydrolytic, acetogenic, and methanogenic functions during this period [21].
189 Meanwhile, peak values, starting time, and peak appearance times were similar during
190 AD. The highest concentrations of VFA were detected in digested slurry from all
191 reactors, with 11.62 g/L for T3, and with 9.28、 10.68、 10.64g/L for CK, T1, and T2
192 reactors, respectively. Furthermore, VFA concentration of CK, T1, T2, and T3 groups
193 on day 9 obtained significant deteriorations of 6.38, 6.33, 6.45, and 5.04 g/L,
194 respectively, compared with day five. The consumption of VFA in the T3 reactor was
195 the lowest. The results showed that acidification was most severe in the T3 reactor and
196 that methanogenic activity was easily inhibited during the AD process, which was
197 consistent with the biogas yield of the subsequent AD process. The stage of rapid

198 acidification in all reactors reached its peak need only five days, and then the VFA was
199 consumed heavily with the increase of biogas production. Thus, the VFA value
200 decreases rapidly.

201 **Influence on the COD and DHA content**

202 The utilization of COD was monitored in all anaerobic digester set-ups, reflecting
203 the utilization of organic matter in an anaerobic system. It was found that all reactors
204 under different pressure during the whole AD process for COD concentration showed
205 a noticeable trend of the first increase, then decrease, and finally stabilize, as shown in
206 Figure. 3(a). The refractory macromolecular organic matter was decomposed by
207 specific microorganisms, i.e., acid-forming bacteria, during the hydrolytic acidification
208 phase, reaching maximum COD concentrations of 33.39, 38.30, 37.54, and 39.12 g/L
209 in the reactor of CK, T1, T2, and T3, respectively. After then, small molecular organic
210 matter was decomposed into digestive juices, as a proper archaeal population (mainly
211 composed of Methanosarcina) was developed and became the dominant bacteria [22].
212 It also can be observed that COD content in this study remained between 14.56-
213 18.03g/L for each treatment during the stabilization phase (among the days 20 and 49).
214 Higher COD content was hardly obtained in this phase due to organic macromolecules
215 in digestive juices have been fully degraded. The COD concentrations of CK, T1, T2,
216 and T3 reactor were determined to be 15.93, 14.56, 16.69, and 16.91g/L, respectively,
217 at the end of AD. It was noteworthy that the COD concentration of the T3 reactor was
218 higher than the initial value, which was detected as 15.49g/L. This phenomenon may
219 be caused by higher compaction and acidification of the system, where microbial

220 activity was inhibited, and more soluble compounds were not utilized.

221 The DHA reflects the level of microbial activity during the AD process, which can
222 be used as an indirect indicator responding to the microbial quantity [22]. As an
223 intracellular enzyme, dehydrogenase is required by microorganisms to degrade organic
224 molecules and obtain energy [23]. The changes in the DHA levels are shown in Figure.
225 3(b), which demonstrates that the activity levels first increased and decreased gradually
226 during AD. The changing trend was consistent with that of VFA and COD concentration.
227 During the first nine days, the differences in DHA among all treatments were not
228 significant. The maximum values of DHA for the CK, T1, T2, and T3 reactors were
229 obtained on the second day with 26.11, 28.09, 29.94, and 31.97 ug TPF/ (h mL),
230 respectively. There was adequate preparation of the dehydrogenation reaction at the
231 beginning of AD, which reaches maximum value time was four days shorter than that
232 of [24]. As the organic matter in the digester was consumed and sufficient nutrients
233 cannot be provided for microbial growth and reproduction, the DHA began to decline.
234 Among the days 9 and 49, the main argument used for the higher DHA with increased
235 pressure may be because more straw was immersed under pressure into the digestive
236 juices, bringing it into contact with more microorganisms. Therefore, the T3 reactor
237 was the highest of all the digesters.

238 **Factor analysis**

239 Factor analysis can explore the relationship between daily gas production and
240 environmental factors in AD [10]. Thus, factor analysis was used to analyze the
241 influence of the pH value, COD, VFA, and DHA content on daily biogas production

242 between the CK and T3 groups. Factor analysis suitability test results showed that the
243 significant probability of Bartlett's spherical test was less than 0.01 for both CK and T3
244 groups, and the KMO test values were 0.581 and 0.545, respectively, which were
245 greater than 0.5, and hence factor analysis could be used.

246 The results of the total ANOVA in the factor analysis showed that all treatments
247 explained 91.06% and 97.15% of the information on pH value, COD, VFA, and DHA
248 content with two common factors (factor 1 and factor 2). The factor loading matrix
249 coefficients can be used to indicate the dependence of the factors on the indicators. The
250 larger the absolute value, indicated a higher determination coefficient of the factors on
251 the index., and also the importance of the factors on the indicators [14]. As shown in
252 Table 2, the absolute values of the loading coefficients for factor 1 of CK were 0.96
253 (COD), 0.76 (VFA), and 0.63 (DHA), and these were greater than the absolute values
254 of the corresponding loading coefficients for these variables for factor 2. The absolute
255 value of the loading coefficient on factor 2 of CK was 0.95 (pH), which was greater
256 than the absolute value of the corresponding loading coefficient of this variable for
257 factor 1. This means that factor 1 of CK was determined by COD, VFA, and DHA
258 content, and factor 2 of CK was determined by pH value. However, factor 1 of T3 was
259 determined by pH, COD, and VFA content, while factor 2 was determined by DHA.
260 Table 2 indicated that the primary factors and the daily biogas production were affected
261 by compaction. Research has shown that pH value [17], COD content [22], VFA content
262 [19], and DHA [23] affected biogas production, and these were consistent with the
263 results of the present study.

264 Then, the scores of factors were calculated. In CK, the scores of factor 1 in the
265 digestion process on days 0, 2, 5, 9, 14, 20, 30, and 49 were 0.39, 0.35, 1.65, 0.85, -
266 0.11, -0.93, -1.05, and -1.14, respectively, while the scores of factor 2 in CK were -1.73,
267 1.59, 0.73, -0.56, -0.66, 0.02, 0.30, and 0.31. The factor that scored higher also indicates
268 that a higher correlation at the same time with daily biogas production [10]. Thus,
269 combined with Table 2 (factor 1 of CK was determined by COD, VFA, and DHA
270 content and factor 2 was determined by pH), it was concluded that daily biogas
271 production was influenced greatly by COD, VFA, and DHA content on days 0, 5, 9 and
272 14 and that the daily biogas production was influenced greatly by pH value on days 2
273 and late in the reaction. This was in accordance with the findings of Zou et al. [25].
274 Similarly, in the T3 reactor (factor 1 was determined by pH value, COD, and VFA
275 content and factor 2 was determined by DHA), it was concluded that pH, COD, and
276 VFA content had the most influence on days 5, 9, and 49, while the DHA had the
277 maximum influence on days 0, 2, 14, 20 and 30. The combined analysis of CK and T3
278 results showed that the scores of factor 1 and factor 2 were different under different
279 compaction conditions in the same fermentation stage, so the compaction had an
280 impression on the main influencing factors of daily gas production during AD. And
281 finally, the combined factor scores were calculated (Table 3), the result revealed that
282 the top four of CK and T3 reactor scores were identical, all occurring on days 5, 2, 9,
283 and 14. It indicated that these days had the greatest effect on gas production and was
284 verified from the daily gas production graph.

285 **Effect of compaction on the straw structure and composition**

286 **The influence on the physical structure**

287 The effects of the compaction on the straw surface characterization changes during
288 batch AD process were studied by SEM, using the most severe pressure, i.e., T3 reactor
289 sample, compared with the uncompacted straw, as shown in Fig. 4. It was depicted that
290 the surface of straw was damaged during AD, and the texture was unordered and
291 fragmented. In general, however, the straw surface damage degree was not particularly
292 serious, and some holes also did not appear on the surface of solids; this is ascribed to
293 native straw is covered by a silica layer that prevents enzymatic hydrolysis [26]. After
294 AD, the available organic matter for microbes was completely utilized, and the rest of
295 the structure appeared rigid, which could not be degraded by microbes in AD [21].
296 Compared to the CK reactor, the straw surface damage increased in the T3 reactor;
297 however, the degree of damage was not significant. It indicated that compaction has
298 little effect on the extent of straw structure destruction during batch type AD process.

299 **The influence on the composition**

300 In terms of anaerobic biodegradability, straw can be divided into non-
301 biodegradable substances and biodegradable substances, which mainly consist of
302 cellulose, hemicellulose, lignin, and other non-structural components, called extractives
303 [2,27]. TS, cellulose, and hemicellulose content will be reduced accordingly during AD.
304 Thus, the removal rate of TS, cellulose, and hemicellulose can reflect the consumption
305 of substances in the AD for biogas production.

306 The percentage of cellulose, hemicellulose, and lignin together with the removal
307 rate of TS, cellulose, and hemicellulose during the 49-day AD of different pressure of

308 straw is presented in Fig. 5. As is shown, there was a significant decrease in the cellulose
309 and hemicellulose contents of the straw (Fig. 5a). The cellulose content of 28.64% in
310 raw straw was decreased to 15.58%, 9.65%, 11.68%, 13.92% for CK, T1, T2, T3 group,
311 respectively, the corresponding ranges for hemicellulose were from 24.47% in the raw
312 straw to 17.14%, 18.82%, 15.95%, 18.80%, respectively. It should be noticed that
313 hemicellulose can be utilized for methane production. Interestingly, it acts as a physical
314 barrier, preventing the accessibility of hydrolytic enzymes and microorganisms to
315 cellulose [26]. An increase in the percentage of lignin was observed; it was reasonably
316 due to a decrease in cellulose and hemicellulose content. During AD, the total lignin
317 had a slight change, and it being a practically non-biodegradable compound by
318 anaerobic microorganisms [28]. The highest decrease in cellulose percentage
319 composition was observed for the T1 reactor; accordingly, the highest cellulose
320 degradation of 69.40% was observed during AD (Fig. 5b). It is also noticed that the
321 higher the pressure applied, the lower the cellulose loss. The main argument may be
322 that the compaction production caused the straw to be more compacted and the
323 microorganisms to be more difficult to degrade. Upon inspection of Fig. 5b, we can also
324 clearly see that degradation of TS in the AD did correlate to biogas yield, the best
325 performance of TS loss rate was T2 (44.79%), followed by the T1 (43.61%) and T3
326 (41.91%), and the lowest was CK (37.64%).

327 **Effect of compaction on biogas production**

328 The daily biogas production and accumulative biogas yield over the 49-d's test
329 period at different reactors are displayed in Fig. 6. Fig. 6a shows that the daily biogas

330 production in all reactors experienced significant fluctuations, while the fluctuation of
331 daily biogas yield is a common phenomenon during the initial period of AD of
332 lignocellulosic materials due to acidification [29]. When most of the material was
333 consumed, the daily biogas production declines and maintained a low level after 30
334 days of incubation. Initially, as can be seen, biogas was generated from the day after
335 inoculation. However, the biogas yield was low at the start-up stage of the experiment
336 could be attributed to the inhibitory effects of by-products, e.g., VFAs, which can cause
337 an imbalance among hydrolysis, acidogenesis, acetogenesis, and methanogenesis
338 reactions, and the activity of methanogenic bacteria [30]. Methanogens then adapt
339 quickly, and biogas production gradually increased. Considering the daily biogas
340 production, T2 reactor, a maximum of $16.57 \text{ mL}\cdot\text{g}^{-1}\text{TS}$ was attained on day 5, while the
341 highest production yield ($11.17 \text{ mL}\cdot\text{g}^{-1}\text{TS}$) was observed at T3 digester until day 10,
342 which was five days late. For CK and T1 reactor, the maximum daily biogas production
343 was 15.35 and $15.07 \text{ mL}\cdot\text{g}^{-1}\text{TS}$ on days 6 and 8, respectively. Two obvious peaks of
344 daily biogas appeared during AD for all cases. Note that the peak values, starting time,
345 and peak appearance times were different. Peak one may be derived from the methane
346 conversion of pre-existing SCOD in the feedstock, while peak two represented the
347 further solubilization of tightly bound biodegradable substances and even some of the
348 hard-to-degrade biodegradable compounds [31].

349 Based on Fig. 6(b) information, it was significant that differences in biogas
350 accumulation existed between reactors. The effect of cumulation biogas was, as
351 expected, related to the external pressure applied. Again, the highest biogas

352 accumulation was for the T2 digester, followed by T1, T3, and CK reactor, with 298.35,
353 291.54, 249.14, 228.51 mL·g⁻¹TS, respectively. For T2 reactor, causing significant ($p <$
354 0.05) improvement of 23.41%, 16.49%, respectively, compared with CK, T3 reactors.
355 However, the cumulative biogas yield of the T2 reactor no significantly ($p > 0.05$)
356 improved 2.28%, compared with the T1 reactor. Further to this, no significant difference
357 ($p > 0.05$) was acquired between the cumulative biogas yields from the T3 reactor and
358 CK. The result indicated that biogas production could be increased when external
359 pressure was applied to the reactor. However, there was no remarkable effect when the
360 external pressure was too high. Further investigation on this issue was worth exploring
361 to evaluate the maximum compaction inside the digester that will restrict biogas
362 production.

363 Combined with Figure. 2, 3,4, and Figure. 6, it should also be worth mentioning
364 that, in this study, compared to other reactors, the concentration of COD and TVFA of
365 T3 reactor was the highest, same thing for DHA, while the peak time of daily gas
366 production was delayed and the cumulative biogas production was also significantly
367 lower. It can likely be ascribed to the following reasons: (1) The decomposition of
368 substances in rice straw led to a decrease in mechanical strength, causing the straws to
369 crush each other. Stress conditions of straw in digester were further investigated; The
370 straw was subjected to four forces inside the reactor, that is, gravity, the pressure
371 generated by the upper straw pile, support force, and thrust generated by biogas.
372 However, these four forces interact with each other in the AD process and, causing a
373 decrease in straw porosity and the straw surface to denser. As a result, the biogas

374 produced at the bottom of the reactor was difficult to be discharged, and thus, it was
375 clear that the volume of biogas collected in the T3 reactor was significantly lower
376 compared to other reactors; (2) As it can be appreciated, contact ratio between straw
377 and the percolate was greatly affected by the compaction, generally observing an
378 increasing trend in the TVFA yield when increasing the pressure. During the experiment,
379 it was found that the T3 treatment group was squeezed at the bottom of the fermenter
380 due to excessive compaction, and the digestive broth flooded the straw pile. This
381 resulted in blocked mass transfer during AD and accumulation of volatile fatty acids.
382 The reactor of T3 with the lowest biogas load had a significantly higher concentration
383 of TVFA than the others and, which may inhibit methanogen activity; (3) It has also
384 been confirmed by the fact that the substrate/inoculum (S/I) ratio (based on volatile
385 solids (VS)) is one of the most critical factors for the start of a balanced microbial
386 population in the anaerobic system [32]. In a single study by Li et al. [32], liquid
387 anaerobic digestion effluent, which can provide sufficient microbes and enough
388 buffering capacity to the reactor at a S/I ratio of 6; Cumulative methane production
389 decreased from 382 ± 22 to 232 ± 5 mL/g VS when the substrate to inoculum ratios
390 increased from 1:1 to 6:1, as also observed by other investigators [33]. In this study, the
391 substrate/inoculum (S/I) ratio was 10.15, far higher than the optimal value. The
392 inoculum was pretty insufficient, and the straw in the T3 reactor was wholly immersed
393 in the digestive, thus, therefore, easily acidified.

394 **Conclusions**

395 This paper investigated the effect of compaction on the AD of straw using different
396 pressure to simulate the compaction of the digester. The highest total biogas production
397 was for the T2 digester with 298.35 mL·g⁻¹TS, which was significantly higher than the
398 CK (68.55 L). The pH value, COD, VFA, and DHA content in the digester and their
399 maximum and the minimum value in the AD process were not significantly influenced
400 by compaction. The main factors affecting biogas production were different at varying
401 degrees of compaction; however, the main stages affecting gas production were the
402 same. Compaction has little effect on the extent of straw structure destruction during
403 batch type AD process.

404

405 **Funding** This work was supported by “Collaborative Innovation Project of Anhui
406 Universities” (GXXT-2019-010); “Natural Science Foundation of Anhui Province”
407 (1808085ME132).

408

409 **Data availability** The datasets generated during and/or analyzed during the current
410 study are available from the corresponding author on reasonable request.

411

412 **Author contribution**

413 1. Guang-Yin Chen: Conceptualization, Methodology, Supervision, Funding
414 acquisition.

415 2. Hai-Nan Cao: Formal analysis, Investigation, Writing- Original Draft, Visualization.

416 3. Xue-Qian Fan: Resources, Investigation.

417 4. Yi-Chen Sun: Resources, Investigation.

418 5. Jing-Wang: Resources, Investigation.

419 6. Jin-Zhu Dong: Writing - Review & Editing.

420 7. Pei-Wu: Writing - Review & Editing.

421 **Declarations**

422 **Ethics Approval** Not applicable.

423 **Consent to Participate** Written informed consent for publication was obtained from
424 all participants.

425 **Consent for Publication** All the authors consent to publication.

426 **Conflict of Interest** The authors declare no competing interests.

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Fig. 1. The batch anaerobic digestion unit

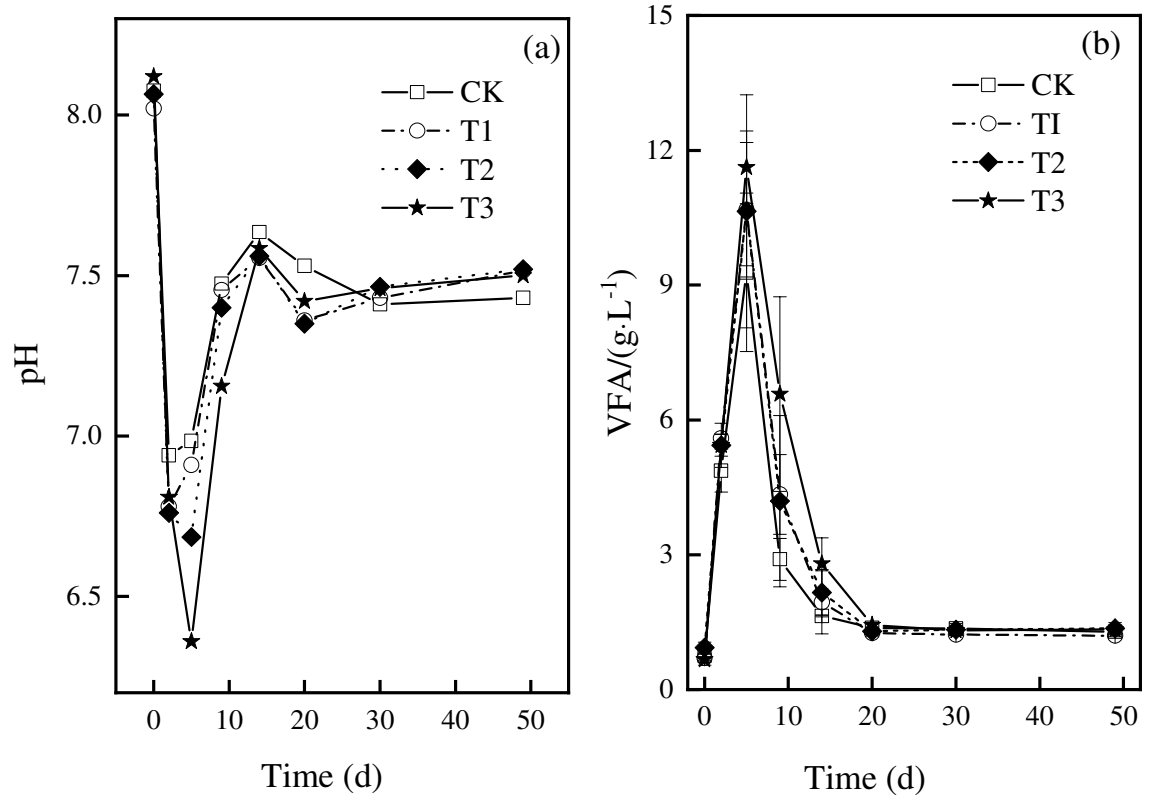


Fig. 2. Variation of pH value and VFA content of different external pressures during the biogas fermentation process.

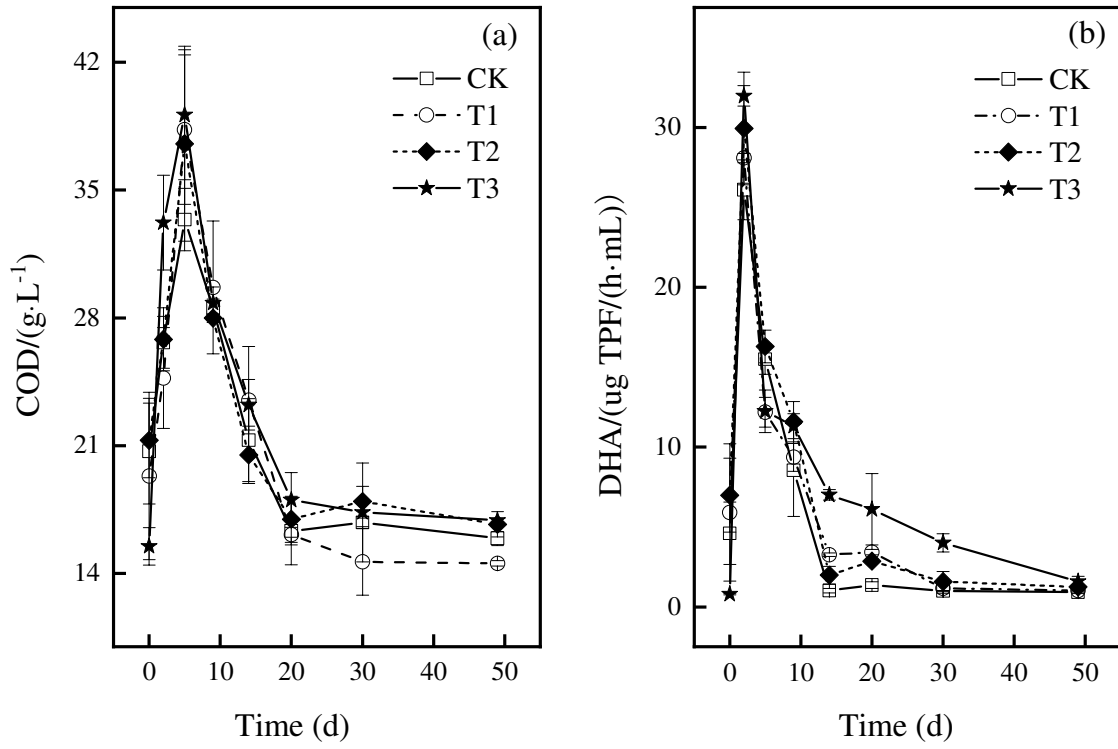


Fig. 3. Variation of COD and DHA concentration of different external pressures during biogas fermentation.

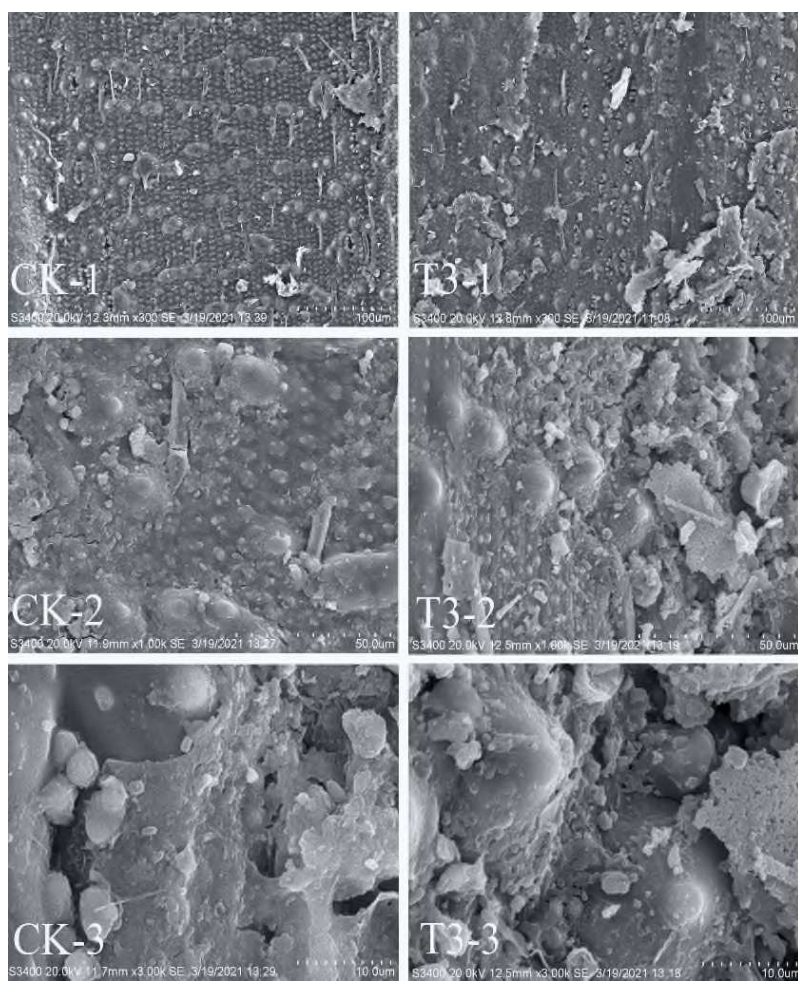


Fig. 4. Scanning electron micrographs of rice straw. CK: Untreated rice straw; T3: pressure was 2000 g. 1: $\times 300$; 2: $\times 1000$; 3: $\times 3000$

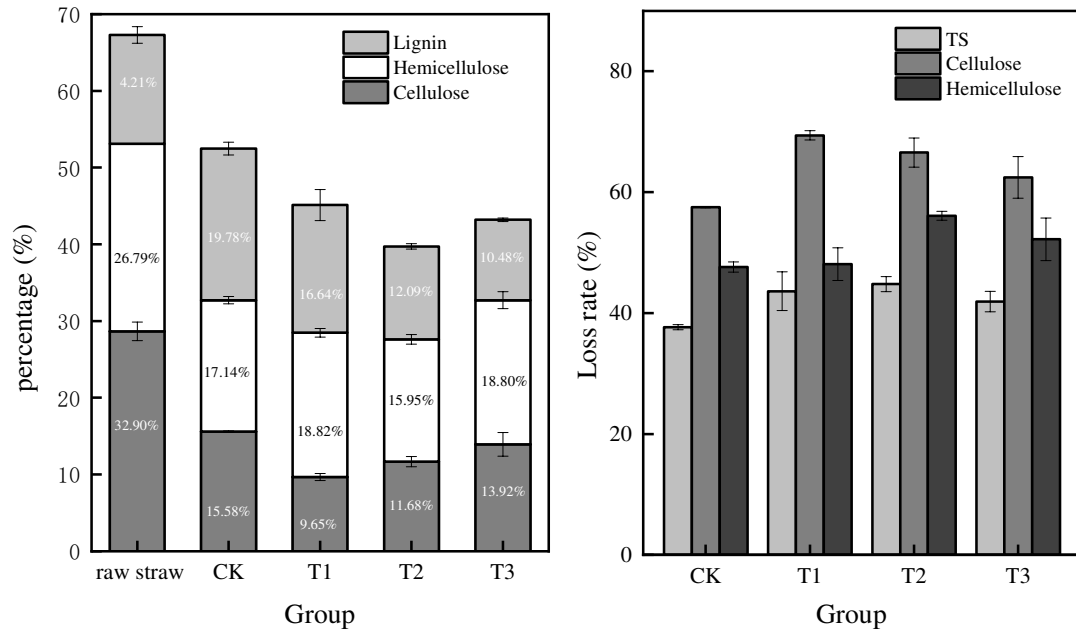


Fig. 5. The percentage lignocellulose content and the corresponding removal rate at the unused pressure

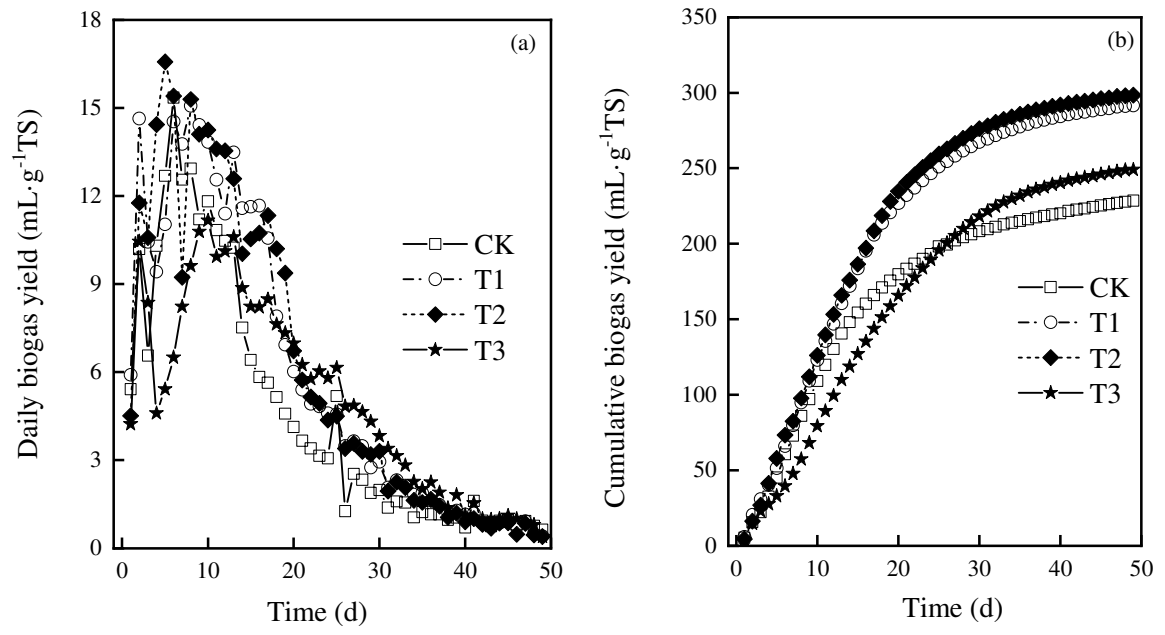


Fig. 6. Biogas production rate (a) and accumulative biogas yield (b) overtime under different external pressures.

Table 1 Basic characteristic of fermentable substrates and inoculum

	pH	TS(%)	VS(%)	Organic carbon(%)	Organic nitrogen(%)	Cellulose (%)	Hemicellulose (%)	Lignin (%)
straw	—	92.24	82.68	40.93	0.99	28.64	24.47	14.2
inoculum	7.35	2.85	47.22	—	—	—	—	—

Table 2 Factor load matrix coefficients

Reactors	Indexes	Loading	
		Factor1	Factor2
CK	pH	-0.28	-0.95
	COD	0.96	0.25
	VFA	0.76	0.56
	DHA	0.63	0.63
T3	pH	-0.87	-0.42
	COD	0.87	0.47
	VFA	0.97	0.21
	DHA	0.31	0.95

Table 3 scores of factors.

Reactor	Time (Day)	Scores			Overall Ranking	Reactor	Time (Day)	Scores			Overall Ranking
		Facto r1	Facto r2	Compre hensive				Facto r1	Facto r2	Compre hensive	
CK	0	0.39	1.73	-0.58	8	T3	0	0.96	0.64	-0.85	8
	2	0.35	1.59	0.92	2		2	0.10	2.41	0.77	2
	5	1.65	0.73	1.23	1		5	2.22	0.43	1.30	1
	9	0.85	0.56	0.21	3		9	0.59	0.04	0.37	3
	14	0.11	0.66	-0.37	4		14	0.28	0.15	-0.23	4
	20	0.93	0.02	-0.50	7		20	0.53	0.14	-0.39	5
	30	1.05	0.30	-0.43	5		30	0.50	0.37	-0.46	6
	49	1.14	0.31	-0.48	6		49	0.44	0.64	-0.51	7

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