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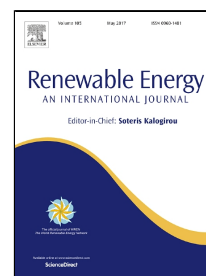
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Extrusion and enzymatic hydrolysis as pretreatments on corn cob for biogas production

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**Highlights**

Extrusion and enzymatic hydrolysis treatments enhanced methane produced from corn cob.

Sequential alkali extrusion and enzymatic hydrolysis achieved the major improvement.

Fungal extract was as suitable as Ultraflo® L for the corn cob enzymatic treatment.

# Extrusion and enzymatic hydrolysis as pretreatments on corn cob for biogas production

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## Abstract

Although agricultural wastes such as corn cob could be employed as substrates for biogas production, their lignocellulosic nature entails difficulties for their use. For this reason, a pretreatment of the biomass is recommended. Considering that there is limited information about the effects of extrusion as pretreatment on biogas production from biomass, this pretreatment alone or in combination with alkali and/or with the enzymatic hydrolysis was evaluated to improve the production of methane by anaerobic digestion of corn cob. The lignocellulosic composition, methane yields and biogas composition of the corn cob before and after pretreatments were compared. Among all the pretreatments studied, the sequential alkali extrusion and enzymatic hydrolysis (A+FE+H) pretreatment accomplished the major improvement with an increase of the methane volume produced of 22.3% in regards to the anaerobic digestion of the raw corn cob.

*Keywords: methane, biogas, pretreatment, extrusion, enzymatic hydrolysis, corncob.*

Abbreviations: AD, anaerobic digestion; A+FE, alkali and fast extrusion; A+FE+H, alkali and fast extrusion followed by enzymatic hydrolysis; BMP, biochemical methane potential; FE, fast extrusion; FE+H, fast extrusion followed by enzymatic hydrolysis; H, enzymatic hydrolysis; NDS, neutral detergent soluble compounds; SE, slow extrusion; SE+H, slow extrusion followed by enzymatic hydrolysis; VS, volatile solids.

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## 31 1 Introduction

32 Biogas, a mixture formed mainly by methane and carbon dioxide [1], is a  
33 renewable and cleaner form of energy that could partially substitute the conventional  
34 petroleum-based sources of energy [2].

35 Anaerobic digestion of lignocellulosic agricultural residues represent a good  
36 alternative for biogas generation because of the large availability of this kind of  
37 biomass, the lack of competition with food or feed chains, and the conversion of these  
38 wastes into feedstocks taking advantage of its composition while mitigating the problem  
39 of the management of these wastes [3]. Despite the many advantages offered by the use  
40 of agricultural wastes, its degradation is complex due to the inherent properties and  
41 structure of lignocellulosic materials which limit their digestibility [4,5]. To overcome  
42 those limitations, pretreatment of materials is recommended in order to make them more  
43 amendable for anaerobic digestion [5–7]. In this regard, de-polymerization of  
44 lignocellulose into monomers accomplished by an enzymatic hydrolysis can help to  
45 promote biogas production [8–10].

46 However, previous pretreatments, to disturb and change the highly ordered  
47 structure of native lignocellulose are positive to increase the accessibility of enzymes  
48 [11]. Among the various pretreatment processes available for lignocellulosic materials,  
49 extrusion is simple, cheap, and has proven to be viable. Moreover, this technology is  
50 applicable to a wide range of biomass and has great flexibility and adaptability with  
51 respect to scale-up and process modifications [12]. Extrusion is a process where  
52 uniformly moistened biomass material is passed through a extruder barrel applying  
53 pressure with a screw [7]. Extruded lignocellulosic materials result in larger specific  
54 areas [11], a lower bulk density and a higher water-holding capacity which favors the

55 access for microorganisms and enzymes [11,13]. This technology has been reported as  
56 an effective physical method for biomass-size-reduction that enhance anaerobic  
57 digestion of lignocellulosic materials, and consequently the biogas production [11,14].

58 Although extrusion can effectively enhance the anaerobic digestion performance,  
59 its effect is still limited [13]. To improve the extrusion process, chemical solutions such  
60 as alkalis can be incorporated to increase the severity of the pretreatment to the  
61 lignocellulosic biomass [7,11]. Alkalis (e.g. sodium hydroxide) are delignification  
62 agents [11] with a marked role in de-esterification of plant cell walls [15], and in  
63 solubilization of some hemicelluloses and lignin [11]. Moreover, the combination of  
64 alkali and extrusion processes produces more pores [11].

65 Most researches have focused on the extrusion effects on bioethanol production.  
66 However, there is limited information about the effects of extrusion as pretreatment on  
67 biogas production from biomass [12]. For this reason, the objective of this study was to  
68 analyze the effect of extrusion, the combination of extrusion with alkali, and the  
69 sequential of extrusion or extrusion combined with alkali and enzymatic hydrolysis, as  
70 pretreatments of corn cob for the anaerobic production of methane.

## 71 **2 Materials and methods**

### 72 *2.1 Substrate*

73 Corn cob is the elongated woody core of maize resulting from the removal of  
74 embedded grains, and it is considered a lignocellulosic agricultural waste. Corn cob was  
75 collected from Mondariz (Pontevedra, Spain). Corn cob was dried at room temperature,  
76 grinded in an electric shredder MTD 220E (Saarbrücken, Germany), then milled in an

77 IKA<sup>®</sup> Werke universal mill model M 20 (Staufen, Germany) and sieved to get a particle  
78 size below 2 mm.

## 79 2.2 *Van Soest fiber analysis*

80 Total solids (TS) and volatile solids (VS) of the substrate and the inoculum were  
81 determined gravimetrically following standard methods.

82 The Van Soest fiber analysis allows the organic matter fractionation into soluble  
83 components, hemicellulose, cellulose and lignin by sequential extraction with neutral  
84 and acid detergents, followed by strong acid extraction [16]. Therefore, substrates  
85 composition was determined with the help of a Gerhardt Fibrebag system (Les Essart Le  
86 Roi, France). Changes in fiber composition induced by enzymatic hydrolysis, extrusion,  
87 alkaline extrusion and combined pretreatments were investigated by analyzing the  
88 separated solid fraction as described Pérez-Rodríguez et al. [17].

## 89 2.3 *Pretreatments*

### 90 2.3.1 *Extrusion pretreatment of corn cob*

91 A twin-screw extruder (HAAKE Rheomex<sup>™</sup> PTW OS, Fisher, France) was  
92 employed to pretreat the milled corn cob.

93 Two different extrusion conditions were assayed: a slow feed rate extrusion  
94 using 200 g corn cob/h and 179 g distilled water/h for 55 s (SE), and a faster feed rate  
95 extrusion using 400 g corn cob/h and 360 g distilled water/h for 35 s (FE).

96 Additionally, the best condition (FE) was also assayed in combination with an  
97 alkali (sodium hydroxide) as pretreatment (A+FE) by including a solution of 0.4%  
98 NaOH (w/v) to the extruder using a volumetric pump.

99 Extrusion was carried out mixing equal masses of corn cob and distilled water or  
100 sodium hydroxide solution (1:1 (w/w)). After alkali extrusion pretreatment, samples  
101 were washed until neutrality.

102 Extrusion was carried out at room temperature. As consequence to the friction  
103 between the screw and the biomass during the extrusion operation a slightly elevation in  
104 temperature was detected but no extra heating was applied.

### 105 2.3.2 Enzymatic hydrolysis

106 Raw or pretreated samples were submitted to enzymatic hydrolysis using the  
107 commercial enzymatic preparation Ultraflo<sup>®</sup> L or *Aspergillus* enzymatic extract  
108 produced by solid-state fermentation of corn cob.

109 Ultraflo<sup>®</sup> L, an enzymatic cocktail with endo-1,3(4)- $\beta$ -glucanase, and collateral  
110 xylanase, cellobiase, cellulase, and feruloyl esterase activities among others, was  
111 graciously provided by Novozymes (Bagsvaerd, Denmark).

112 Obtainment of *Aspergillus* enzymatic extract used in this work, including fungal  
113 production and enzyme extraction, was described in Pérez-Rodríguez et al. [18].  
114 Briefly, solid state fermentation (SSF) was carried out in 250 mL Erlenmeyer flasks  
115 containing 5 g (dry weight) of corn cob moistened (1:3.6 (w/v)) with a concentrated  
116 nutrients salts solution. After being sterilized, each flask was inoculated with one  
117 milliliter of  $1 \times 10^6$  spores of *Aspergillus terreus* CECT 2808/g dry corn cob. Cultures  
118 were incubated statically at 30°C for 7 days. Crude enzymatic extracts were obtained by  
119 adding distilled water (10 mL/g dry corn cob) to each flask and incubated for 1 h, 200  
120 rpm at 30 °C. After incubation, the flaks content was filtered, and the permeate obtained  
121 was centrifuged at 2755×g for 15 min. The supernatant was recovered and constitute the  
122 hereinafter denominated *Aspergillus* enzymatic extract.



123 The feruloyl esterase, xylanase, cellulose and cellobiase activities of both  
124 Ultraflo® L and *Aspergillus* enzymatic extracts were determined in a previously work  
125 [18].

126 Enzymatic hydrolyses were performed as it was previously described by Pérez-  
127 Rodríguez et al. [17] by the addition of a volume of Ultraflo® L or of the *Aspergillus*  
128 enzymatic extract, which mean 0.2 U of feruloyl esterase per gram of dry substrate.  
129 After hydrolysis, samples were boiled (100°C for 10 min) for thermal inactivation of  
130 enzymes, and centrifuged in a Sorvall Legend X1 centrifuge from Fisher Scientific at  
131 11250 x g for 15 min.

132 Then, the amount of carbohydrate reducing ends (Dinitrosalicylic acid method  
133 described by [19]) of the supernatant (liquid fraction) was determined.

134 Finally, supernatants were separated and the solid residues were used as  
135 substrate for anaerobic digestion to produce biogas.

#### 136 2.4 Anaerobic digestion of corn cob samples

137 Raw or pretreated corn cob (1 g dry substrate) were anaerobically digested by a  
138 mesophilic anaerobic sludge in 575 mL serum bottles with a working volume of 400  
139 mL as it was previously described by Pérez-Rodríguez et al. [17].

140 Two control tests were done under the same conditions. Controls without  
141 substrate were done to determine the endogen gas production of inoculum and make the  
142 appropriate correction of results. Other controls with ethanol as substrate were done to  
143 verify the aptitude of inoculum for biomass degradation.

## 144 2.5 Biochemical Methane Potential (BMP) test

145 Bottles were periodically submitted to Biochemical Methane Potential (BMP)  
146 test. The BMP value can be used as an index of the anaerobic biodegradation potential  
147 [20], measuring the methane or biogas produced by a known amount of substrate in  
148 batch anaerobic conditions.

149 Biogas volume produced by the anaerobic digestion of corn cob samples was  
150 measured volumetrically using the displacement method with acidified water as  
151 solution. The biogas was introduced into the liquid column directly from the bottle and  
152 the biogas volume produced was calculated from the measurement of change in barrier  
153 solution height. All volumes were expressed under normal conditions (273 K  
154 temperature and pressure 1013 mbar).

155 The gas composition ( $\text{CH}_4$ ,  $\text{CO}_2$ ,  $\text{O}_2$ ,  $\text{N}_2$  and  $\text{H}_2\text{S}$ ) was analyzed employing a  
156 Varian CP 4900 micro-gas chromatograph (Middelburg, Netherlands) under the  
157 conditions and using the method previously described by Pérez-Rodríguez et al. [17].

158 The methane produced by a known mass of volatile solids of corn con sample  
159 ( $\text{VS}_{\text{substrate}}$ ) was calculated as the difference between the volume of methane produced  
160 from the corn cob sample ( $V \text{CH}_{4\text{substrate}}$ ) minus the volume of methane produced by the  
161 control without substrate ( $V \text{CH}_{4\text{blank}}$ ):

$$162 \quad \text{L CH}_4/\text{kg VS} = (\text{L CH}_{4\text{substrate}} - \text{L CH}_{4\text{blank}})/\text{VS}_{\text{substrate}}$$

163 The methane yield was expressed as the volumetric methane production (liter)  
164 under standard conditions per unit of substrate fed expressed as volatile solids (VS).

165 Results are presented as the average of at least two replicas along with their  
166 standard deviation.

### 167 3 Results & Discussion

#### 168 3.1 *Effect of slow or fast extrusion, alkali extrusion and enzymatic hydrolysis on corn* 169 *cob composition*

170 Untreated corn cob is composed by  $42.0 \pm 0.15\%$  of cellulose,  $45.9 \pm 0.90\%$  of  
171 hemicellulose,  $2.8 \pm 0.20$  of lignin, and  $9.3 \pm 0.95\%$  of neutral detergent soluble  
172 compounds (NDS).

173 The use of pretreatments involved modifications in the main lignocellulosic  
174 fractions (cellulose, hemicellulose, lignin and NDS) of corn cob in comparison with  
175 untreated material as it can be seen in [Figure 1](#) and [Figure 2](#).

176 The pretreatments of extrusion (slow (SE) or fast (FE) extrusion and the alkali  
177 fast extrusion (A+FE)) involved similar variations in the composition of corn cob  
178 ([Figure 1](#)). These modifications were characterized by a decrease in the percentage of  
179 hemicellulose (from  $45.9 \pm 0.90\%$  in the raw corn cob to  $38.9 \pm 0.52\%$  (SE),  $40.6 \pm$   
180  $0.19\%$  (FE), and  $39.4 \pm 1.20\%$  (A+FE)) as well as in the percentage of cellulose (from  
181  $42.0 \pm 0.15\%$  in the raw corn cob to  $34.8 \pm 0.23\%$  (SE),  $34.8 \pm 1.24\%$  (FE),  $37.6$   
182  $\pm 1.22\%$  (A+FE)). The changes in these fractions were accompanied by an increment in  
183 the NDS whose percentages doubled the value quantified in the raw corn cob (from  $9.3$   
184  $\pm 0.95\%$  in the raw corn cob to  $19.0 \pm 0.60\%$  (SE),  $18.7 \pm 1.39\%$  (FE), and  $17.1 \pm$   
185  $0.48\%$  (A+FE)). Thus, extrusion pretreatments caused depolymerization of cellulose,  
186 hemicellulose, and lignin of corn cob which is in accordance with the analysis reported  
187 by Panepinto [21].

188 Regarding the samples submitted to the enzymatic hydrolysis (H) as sole  
189 pretreatment or after the extrusion pretreatments (SE+H, FE+ H and A+FE+H) ([Figure](#)

190 2), the reduction of the percentage of cellulose was remarkable comprising between  
191 43.3 (H) to 57.5% (SE+H). Moreover, the NDS percentages of the samples  
192 enzymatically hydrolyzed ( $41.9 \pm 0.73\%$  (H),  $41.7 \pm 0.86\%$  (SE+H),  $42.9 \pm 0.84\%$   
193 (FE+H), and  $43.5 \pm 0.04\%$  (A+FE+H)) were 4.5 times higher than the percentage  
194 represented by the same fraction in the raw corn cob composition ( $9.3 \pm 0.95\%$ ). Due to  
195 the term NDS includes soluble carbohydrates, proteins, lipids, minerals and vitamins  
196 [16], which are easily assimilable compounds for methanogenic microorganisms, higher  
197 NDS availability in samples should increase the methane production rate. In summary,  
198 the results shown a de-polymerization of the samples as consequence of the  
199 pretreatments applied and it could be assumed that their anaerobic digestion would lead  
200 to higher methane productions.

201 Additionally, the carbohydrate reducing ends were also analyzed in the liquid  
202 phase from enzymatic hydrolyses, obtaining the following amounts:  $8.39 \pm 0.05$  g  
203 carbohydrate reducing ends/L (H),  $8.86 \pm 0.04$  g carbohydrate reducing ends/L (SE+H),  
204  $8.95 \pm 0.06$  g carbohydrate reducing ends/L (FE+H) and  $9.34 \pm 0.02$  g carbohydrate  
205 reducing ends/L (A+FE+H). According to [22] an increment in sugar availability due to  
206 extrusion was one of the causes to explain the increment observed in methane  
207 production. Therefore, higher sugars concentration can be related to higher volumes of  
208 methane produced. Consequently, in our case, the highest methane level could be  
209 expected to be achieved by the anaerobic digestion of alkali fast-extruded corn cob  
210 (A+FE+H).

### 211 3.2 *Impact of the extrusion pretreatments on the production of methane*

212 The evolution of methane production from raw or extrusion pretreated corn cob  
213 during 32 days of anaerobic digestion is shown in [Figure 3](#).

214 All the extrusion pretreatments enhanced the volume and rate of methane  
215 production from the anaerobic digestion of corn cob (Figure 3a). However the extrusion  
216 feed rate hardly influenced the process, since a methane yield of  $269.6 \pm 1.31$  L CH<sub>4</sub>/kg  
217 VS ( $6.54 \pm 0.03\%$  more than the raw corn cob) was obtained during the lower speed rate  
218 (SE) and  $270.6 \pm 3.11$  L CH<sub>4</sub>/kg VS ( $6.94 \pm 0.08\%$  more than the raw corn cob) during  
219 the faster speed rate (FE). There is a correspondence between the production of methane  
220 and the composition of the extruded samples since no compositional differences  
221 appeared between fast or lower speed rate pretreatments (Figure 1). Moreover, our  
222 results are in agreement with Khor et al. (2015), which lend support to the claim that  
223 different extrusion speeds do not cause important variations in the volume of methane  
224 released, since these authors did not observe notable differences in the percentage of  
225 improvement between fast and slow extrusion neither for maize straw ( $36.4 \pm 6.9\%$   
226 from fast extruded and  $36.3 \pm 4.7\%$  from slow extruded) nor for sprout stem ( $29.6 \pm$   
227  $3.2\%$  from fast extruded and  $26.2 \pm 5.9\%$  from slow extruded). However, from an  
228 energy point of view fast extrusion is more favorable as biomass pretreatment [23].  
229 Thus, the higher feed rate extrusion (FE) was selected for the assay of extrusion  
230 combined with sodium hydroxide solution (A+FE). The anaerobic digestion of the  
231 A+FE corn cob resulted in  $275.6 \pm 1.14$  L CH<sub>4</sub>/kg VS, which implicated an additional  
232 surplus in the methane produced versus the FE of 1.28 time. This enhancement in  
233 methane respect to FE is analogous to those obtained from other alkali pretreated and  
234 extruded biomass such as maize straw (1.20 times), grass (1.15 times) and sprout stem  
235 (1.08 times) [23].

236 Higher percentages of amelioration were described for the methane production  
237 from other extruded biomass involving 72.2% for rice straw [24], 70 % for maize straw,  
238 62% for grass [14], between 26–49% for grass, maize straw and sprout stem [23], or

239 between 12–29% for wheat straw [22]). Similarly, the combination of extrusion and  
240 alkali pretreatment resulted in an overall increase of 54.0% for rice straw (Zhang et al.,  
241 2015), 53.8% for grass, 47.6% for maize straw and 38.6% for sprout stem [23].  
242 Therefore, despite being positive, the extrusion pretreatments of corn cob did not reach  
243 a high percentage of improvement in regards to those reported by other authors.

### 244 3.3 *Impact of the enzymatic hydrolysis of raw or pretreated corn cob on the* 245 *production of methane*

246 The influence of enzymatic hydrolysis was studied independently or in addition  
247 to the previous extrusion pretreatments. Figure 3b summarizes the results obtained by  
248 anaerobic digestion after 32 days.

249 Raw corn cob was enzymatically hydrolyzed by Ultraflo® L. The amount of  
250 methane generated from the hydrolyzed corn cob was  $289.8 \pm 0.79$  L CH<sub>4</sub>/kg VS,  
251 proving a modest enhancement in regard to the anaerobic digestion of the fast extruded  
252 (5.2%) and to the raw (14.0%) corn cob. Consequently, the enzymatic hydrolysis  
253 accomplished a higher volume of methane than the extrusion.

254 The samples submitted to the extrusion pretreatments (SE, FE and A + FE) were  
255 also hydrolyzed by Ultraflo® L. The initial methane production rate from the extruded  
256 and hydrolyzed samples were higher than from the hydrolyzed samples. However,  
257 similar levels of methane were attained from the samples hydrolyzed or extruded and  
258 hydrolyzed (series H, SE+H and FE+H) in the long term of the anaerobic digestion.

259 The volume of methane generated only increased appreciably in the A+FE+H  
260 samples where  $309.4 \pm 5.11$  L CH<sub>4</sub>/kg VS were achieved. Nevertheless, although the  
261 anaerobic digestion of the corn cob pretreated with the combination A+FE+H exceed

262 the volume of methane obtained by the fermentation of the other pretreated samples,  
263 some researchers point that the additional methane produced as a result of the sum of  
264 pretreatments could be insufficient to offset the additional costs [25]. Therefore,  
265 economic studies should be assess to evaluate the convenience of submitting corn cob to  
266 the combination of pretreatments or maybe to just one of the types of pretreatment  
267 (extrusion or enzymatic hydrolysis).

### 268 3.4 Comparison of Ultraflo<sup>®</sup> L and *Aspergillus* extracts for the enzymatic hydrolysis 269 of raw corn cob on the production of methane

270 From an economic point of view, reducing the price of the enzymes used in the  
271 enzymatic processes is a key research priority [1]. In this way, Singhanian et al.[26] and  
272 Thomas et al. [27] support that production of enzymes by solid-state fermentation using  
273 low-cost agro-industrial residues as substrates, offers potential benefits for  
274 bioprocessing because of the high production of the enzymes generated as well as for  
275 the reduction in the costs derived from the sources employed. Thereby, solid-state  
276 fermentation of corn cob was performed using the strain *Aspergillus terreus* CECT  
277 2808 [18] and it was compared the anaerobic digestion after hydrolysis using the  
278 *Aspergillus* enzymatic extract or the commercial Ultraflo<sup>®</sup> L (Figure 3c).

279 The volume of methane produced from the corn cob hydrolyzed by *Aspergillus*  
280 enzymatic extract was below that produced from the raw corn cob until day 9<sup>th</sup> of  
281 anaerobic digestion. Then, the volume of methane produced from the sample  
282 hydrolyzed by the *Aspergillus* extract gradually increased reaching the methane levels  
283 obtained from the anaerobic digestion of the sample hydrolyzed by Ultraflo<sup>®</sup> L.  
284 Therefore, despite of the corn cob hydrolyzed by Ultraflo<sup>®</sup> L resulted in an initial faster  
285 methane production rate than that attained by the *Aspergillus* enzymatic extract, the

286 methane accumulated during 25-26 days was the same ( $264.85 \pm 7.70$  L CH<sub>4</sub>/kg VS  
287 (Ultraflo® L) and  $262.9 \pm 8.50$  L CH<sub>4</sub>/kg VS (*Aspergillus* enzymatic extract)), showing  
288 the potential of this extract to be used as an alternative to the commercial enzymatic  
289 cocktail.

### 290 3.5 Methane yield in biogas produced

291 Biogas comprises primarily methane and carbon dioxide, and other minor  
292 components (water, hydrogen sulfide, nitrogen, ammonia and oxygen). However, the  
293 substrate employed and the digestion conditions influence strongly on the composition  
294 of the biogas [28].

295 Generally, the main final use of the biogas is for energy [29]. For this  
296 application, the methane is considered to be useful, while carbon dioxide and the minor  
297 components are not [29]. In this sense, the presence of carbon dioxide reduces  
298 considerably the calorific value of the biogas, and the minor components may lead to  
299 critical operational problems [28,29]. Moreover, the avoidance of the transport of the  
300 carbon dioxide reduce the volume and the density of the biogas, thus reducing transport  
301 costs [29]. Therefore, a widespread practice is the biogas upgrading to a higher quality  
302 combustible gas, the so-called biomethane, requires removing most of that carbon  
303 dioxide and of the minor components [28].

304 Considering that, the higher percentage of methane present in biogas involves a  
305 closer composition of the biogas to biomethane target. Consequently, in addition to the  
306 volume of methane generated by the anaerobic digestion, it is important to measure the  
307 composition of biogas. The main fractions of biogas, including methane and carbon  
308 dioxide, were described in Table 1. Comparing to the biogas produced by the digestion



309 of raw corn cob, all the pretreatments assayed had a positive effect rising the percentage  
310 of methane in biogas produced (Table 1).

#### 311 **4 Conclusions**

312 Although corn cob proved to be an adequate substrate to produce biogas by  
313 anaerobic digestion under the studied conditions, the pretreatments assayed  
314 demonstrated to be effective in order to increase the methane production. There were  
315 not considerable differences between the extrusion feed rate but the addition of an alkali  
316 solution during extrusion involved an improvement in the methane yield. In regards to  
317 the enzymatic hydrolysis, although both the commercial Ultraflo<sup>®</sup> L and the *Aspergillus*  
318 enzymatic extract were efficient pretreatments increasing the volume of methane  
319 obtained from the corn cob compared with the raw material, Ultraflo<sup>®</sup> L achieved the  
320 highest methane production rate. Despite the combination of extrusion and hydrolysis  
321 reached similar levels of methane production than the extruded samples, the  
322 combination of pretreatments (sequential alkali extrusion and enzymatic hydrolysis,  
323 A+FE+H) accomplished the major improvement in both production rate and final  
324 volume of methane with an overall increase of 22.3% regarding the anaerobic digestion  
325 of raw corn cob.

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418 **Table 1: Main components of biogas produced by the anaerobic digestion of raw**  
 419 **and pretreated corn cob.**

Corn cob samples/main components of biogas	CH <sub>4</sub> (%)	CO <sub>2</sub> (%)
Raw	57.5±0.04	42.4±0.03
Slow extrusion (SE)	60.3±0.37	39.9±0.24
Fast extrusion (FE)	60.6±0.18	39.6±0.19
Alkali+fast extrusion (A + FE)	60.5±0.46	39.5±0.28
Ultraflo L hydrolysis	60.2±0.26	40.2±0.38
<i>Aspergillus</i> extract hydrolysis	61.4±0.44	38.5±0.42
Slow extrusion+hydrolysis (SE + H)	65.6±0.94	37.9±0.26
Fast extrusion+hydrolysis (FE + H)	65.4±0.84	36.5±0.11
Alkali+fast extrusion+hydrolysis (A+ FE + H)	65.4±0.41	37.8±0.66

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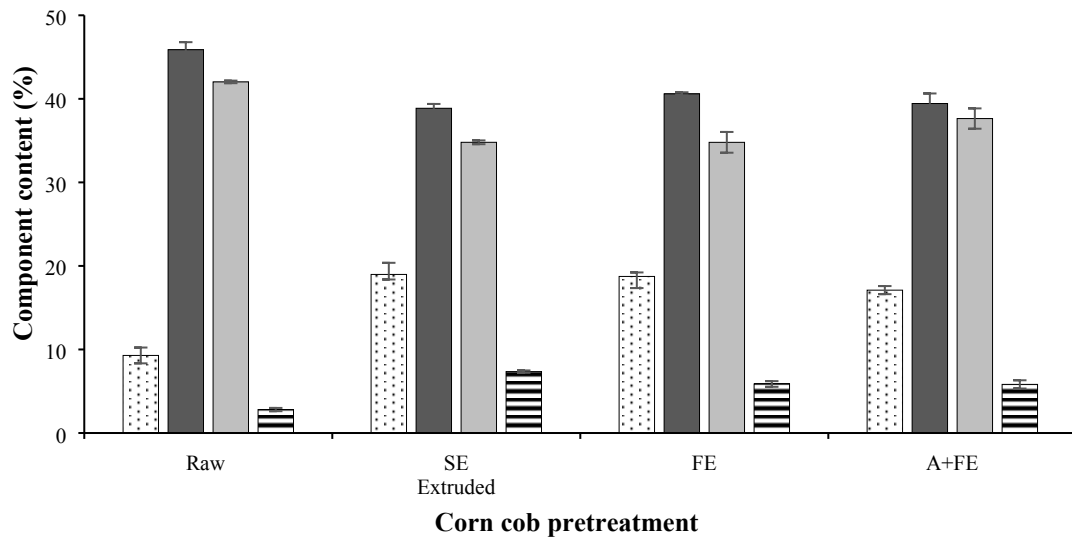
454 **Figure Legends**

455 Figure 1: Comparison between contents of neutral detergent soluble compounds  
456 (dotted), hemicellulose (dark grey), cellulose (light grey), and lignin (striped) of  
457 untreated corn cob (raw) or submitted to different extrusion pretreatments: slow  
458 extrusion (SE), fast extrusion (FE), and alkali and fast extrusion (A+FE).

459 Figure 2: Comparison between contents of neutral detergent soluble compounds  
460 (dotted), hemicellulose (dark grey), cellulose (light grey), and lignin (striped) of  
461 untreated corncob (raw) or submitted to hydrolysis (hydrolyzed) or to the different  
462 extrusion pretreatments followed by enzymatic hydrolysis: hydrolysis of the slow  
463 extruded corn cob (SE+H), hydrolysis of fast extruded corn cob (FE+H), and hydrolysis  
464 of the alkali and fast extruded corn cob (A+FE+H).

465 Figure 3: Cumulative methane productions as a function of anaerobic digestion time  
466 obtained from raw corn cob (\*), (a) after the different extrusion pretreatments (slow  
467 extrusion (SE) (○), fast extrusion (FE) (△), alkali and fast extrusion (A+FE) (□)); (b)  
468 after the hydrolysis by Ultraflo<sup>®</sup> L (◆), after the combination of extrusion and  
469 hydrolysis by Ultraflo<sup>®</sup> L (slow extrusion followed by enzymatic hydrolysis (SE+H)  
470 (●); fast extrusion followed by enzymatic hydrolysis (FE+H) (▲); alkali and fast  
471 extrusion followed by enzymatic hydrolysis (A+FE+H) (■)), and (c) after the  
472 hydrolysis by Ultraflo<sup>®</sup> L (◆) or by the *Aspergillus* enzymatic extract (▪). In some  
473 cases, the error bars in that scale are smaller than the symbols and cannot be seen.

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Figure 1

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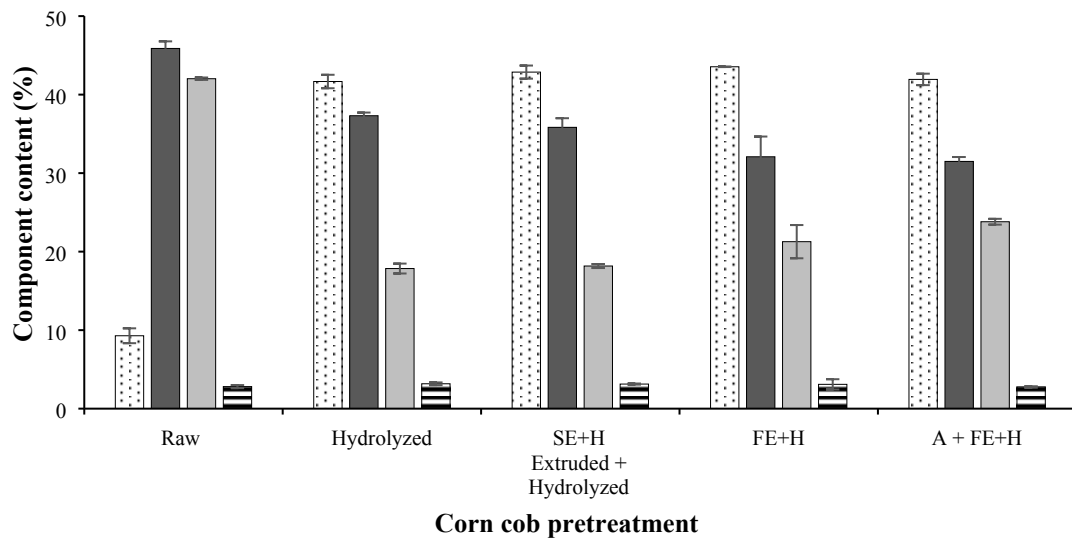
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Figure 2

495 a)

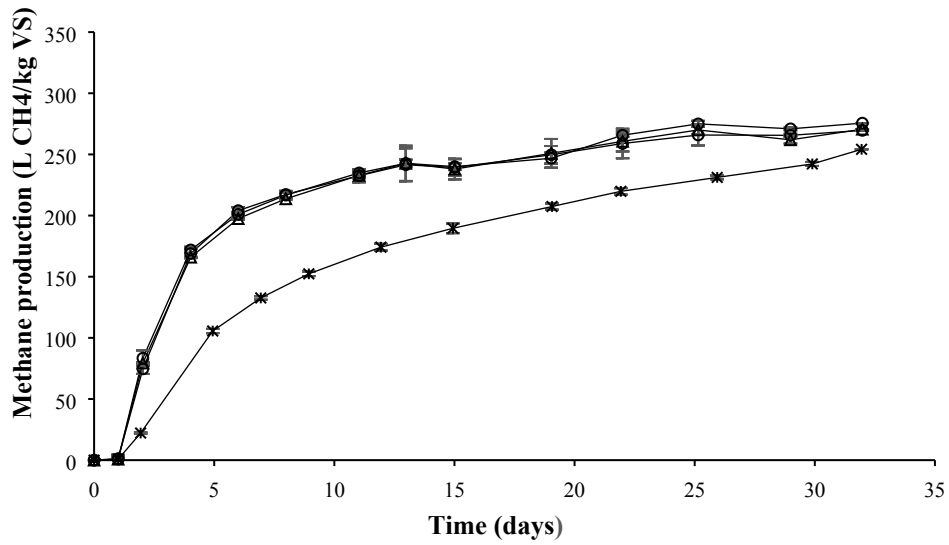
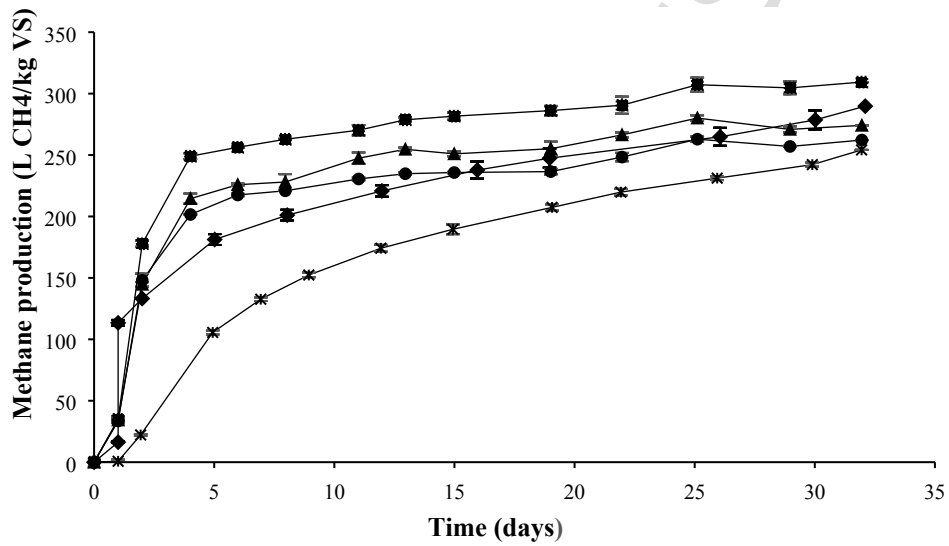
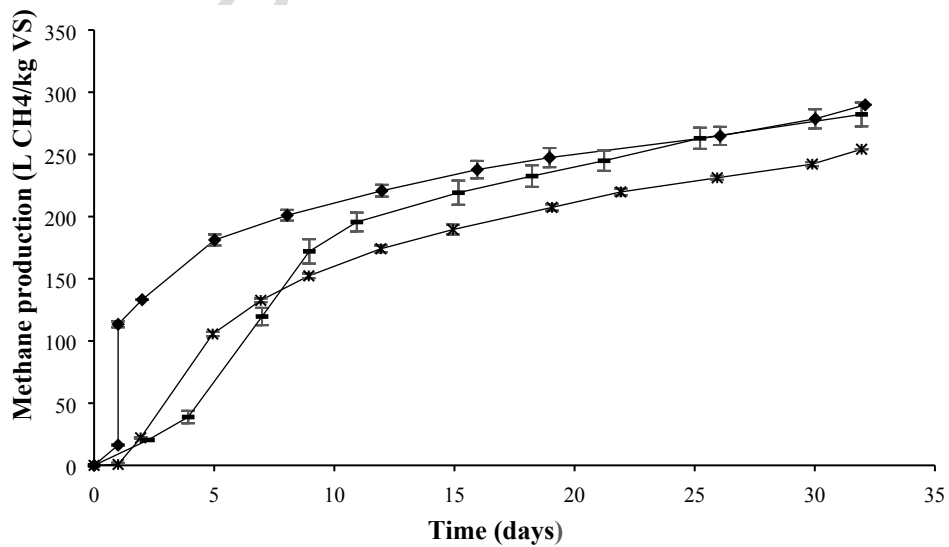
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Figure 3