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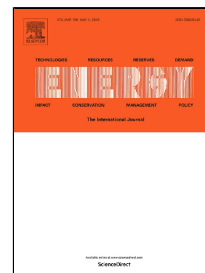
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1 Waste paper and macroalgae co-digestion effect on methane production

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7 8 Abstract

9 The present study investigates the effect on methane production from waste paper when
10 co-digested with macroalgal biomass. Both feedstocks were previously mechanically
11 pretreated to reduce their particle size. The study was planned according two factors: the
12 feedstock to inoculum (F/I) ratio and the waste paper to macroalgae (WP/MA) ratio.

13 The F/I ratios checked were 0.2, 0.3 and 0.4 and the WP/MA ratios were 0:100, 25:75,
14 50:50, 75:25 and 100:0. The highest methane yield ($386 \text{ L kg}^{-1} \text{ VS}_{\text{added}}$) was achieved at
15 an F/I ratio of 0.2 and a WP/MA ratio of 50:50. A biodegradability index of 0.87
16 obtained in this study indicates complete conversion of feedstock at an optimum C/N
17 ratio of 26. Synergistic effect was found for WP/MA 25:75, 50:50 and 75:25 mixing
18 ratios compared with the substrates mono-digestion.

19
20 *Keywords:* Algae, Anaerobic co-digestion, Biomass, Renewable energy, Waste paper

21
22 *Abbreviations:* AD, anaerobic digestion; ANOVA, analysis of variance; F/I,
23 feedstock/inoculum; KDP, potassium dihydrogen phosphate; MC, moisture content;

24 RSM, response surface methodology; TS, total solids; VS, volatile solids; WP/MA,
25 waste paper/macroalgae.

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27

28 1. INTRODUCTION

29 EU and UK Government have tightened their waste disposal regulations, landfill
30 disposal of organic waste will be no longer available after 2020 [1], so alternatives to
31 waste disposal on landfills are required for an efficient and profitable recycling. By the
32 same year of 2020, EU aims to get the 20 % of energy consumption from renewable
33 resources, 10 % coming specifically from biofuels [2,3].

34 Waste management and energy recovery can be effectively combined in the anaerobic
35 digestion process. Anaerobic digestion performed under controlled conditions also
36 allows pollution reduction and emissions control, reducing greenhouse gas emissions
37 compared to fossil fuels by the utilization of local resources [4]. Biogas is obtained from
38 waste materials through the anaerobic digestion process. In the same process, a by-
39 product with fertilizer value is obtained (the digestate) [5–7]. Upgraded biogas, named
40 biomethane, with a concentration greater than 97 % can substitute natural gas in
41 Combined Heat and Power Plants (CHPP) and may be injected into the gas grid or
42 compressed and used as transport fuel [8].

43 Paper and cardboard account for 25-30 % of municipal solids waste (MSW) [9,10]; the
44 biggest source of waste paper is industry and businesses with the 52 % of the total [11].
45 Anaerobic digestion of waste paper is usually studied as part of the anaerobic digestion
46 of MSW. In some cases, the study was carried out on the MSW different fractions that
47 resulted in methane yields for newsprint paper from 58 to 100 L kg⁻¹ VS_{added} [9,12]; for

48 office paper 208-369 L kg⁻¹ VS_{added} [9,12–15] and for cardboard 96 and 217 L kg⁻¹
49 VS_{added} [9,15].

50 The ratio carbon/nitrogen (C/N) is one of the most important factor in anaerobic
51 digestion nutrients balance. Carbon is the source of energy for the process and nitrogen
52 is needed for the formation of enzymes that perform metabolism. A high C/N ratio is an
53 indication of rapid consumption of nitrogen by methanogens and results in lower gas
54 production, while a low C/N ratio causes ammonia accumulation and pH rises
55 excessively. Most authors consider an optimal C/N ratio needs to be in the range 10-30
56 [4,16,17]. Considering other macronutrients, the C:N:P:S ratio in the reactor should be
57 600:15:5:3 [16]. Paper materials have a carbon-to-nitrogen (C/N) ratio ranging from
58 173/1 to greater than 1000/1 [18], these values are very high for anaerobic digestion so a
59 balance of nutrients can be achieved through co-digestion with biomass that contains
60 nitrogen and lower the C/N ratio. Digestion of nitrogenous substrates (C/N ratio less
61 than 15) can lead to problematic digestion caused by excess levels of ammonia,
62 increasing the pH levels in the digester leading to a toxic effect on methanogens
63 population [19,20].

64 Co-digestion is the simultaneous digestion of a mixture of two or more substrates and
65 offers many advantages, including ecological, technological, and economic benefits,
66 compared to digestion of a single substrate [21]. The purpose of co-digestion is usually
67 to balance nutrients (C/N ratio and macro- and micronutrients) and dilute
68 inhibitors/toxic compounds. Moreover, the co-digestion of two or more complementary
69 substrates may induce a synergetic effect on their biodegradability, causing an increase
70 in the methane yield and production rate [22]. Zhong et al achieved maximum methane
71 yield in co-digestion of algae and corn straw at C/N ratios between 20-25 [23]. Co-

72 digestion of waste paper with *Scenedesmus spp.* and *Chlorella spp.* achieved a
73 maximum methane yield at a C/N ratio of 18 [24].

74 Further advantages of co-digestion include the unification of feedstock's management
75 by sharing treatment facilities, reducing investment and operating costs. Successful
76 examples of co-digestion include: cow dung and water hyacinth [25]; algal sludge and
77 waste paper [26]; cattle manure and crude glycerine [27]; grass and sludge and [28];
78 municipal sludge, microalgae and waste paper [4]; algae biomass residue and lipid
79 waste [29] and hay and soybean [21].

80 Co-digestion can result in a positive effect (synergistic effect) on the degradation of
81 each individual substrate in the mixture and/or an increase in the methane yield kinetics
82 [30]. This improvement may arise from the contribution of additional alkalinity,
83 nutrients, enzymes and trace elements that a feedstock by itself may lack and an
84 increased buffering capacity. Evenly allocated nutrients in co-digestion would support
85 microbial growth for efficient digestion, while increased buffering capacity would help
86 maintain the stability of the anaerobic digestion system [31]. Antagonistic effects may
87 result from low C/N ratios resulting in high total ammonia nitrogen (TAN) released and
88 high volatile fatty acids (VFAs) accumulated in the digester leading to a suppression in
89 the cellulase activity and a decrease in the methane yields. Antagonistic effects can
90 come also from other several factors, such as pH inhibition and ammonia toxicity [32].

91 Synergistic effects were found on the co-digestion of primary sludge and paper pulp
92 reject with an improvement of 32 % on methane yield [33] and the co-digestion of
93 Taihu blue algae with corn straw (up to 60 % extra methane) [31].

94 The innovation in this study is that it is the first to assess the optimised conversion of
95 waste paper to biogas through co-digestion with macroalgae (*P. canaliculata*) as a

96 source of nitrogen to balance the C/N ratio in the process. Macroalgae is a great source
97 of biomass in Scotland and its optimization as a feedstock for anaerobic digestion is
98 being addressed. The optimization include both pretreatment and co-digestion for a final
99 improved methane potential. Both feedstock were previously mechanically pretreated in
100 a Hollander beater according to [34,35]. The study was planned to check different levels
101 of feedstock/inoculum ratio (F/I) and waste paper/macroalgal (WP/MA) mixing
102 percentages. A statistical analysis through Response Surface Methodology (RSM) is
103 presented to provide a more comprehensive evaluation of the interaction between the
104 process parameters on the methane production.

105 **2. MATERIALS AND METHODS**

106 **2.1. Feedstock and inoculum**

107 *Pelvetia canaliculata*, a brown macroalgae commonly known as channelled wrack, was
108 collected on-shore (55°55' N 5°09 W) in the Isle of Bute, Scotland in March 2016,
109 refrigerated at 4 °C and used within 4 days. Mature specimens were chosen of minimum
110 length size of tufts of 10 cm. Small contaminants like plastic or stones were removed
111 but the algae was not washed as the algae is considered in this study a waste material to
112 be used as found in the shore. Waste paper was collected from recycle bins at the
113 School of Computing and Engineering at the University of West of Scotland (UWS) in
114 Paisley, Scotland. Feedstock characterization was shown in Table 1. Both feedstocks
115 were previously mechanically pretreated in a Hollander Beater, the optimized time of
116 pretreatment for macroalgae was 50 min and for waste paper was 55 min. During the
117 pretreatment, the biomass is mixed with water and a pulp is produced, this pulp is

118 directly fed the reactor to help to fluidizer the process. Table 1 details the
119 characterization of the macroalgae and the waste paper.
120 The sludge used as inoculum was provided by the Strathendrick Biogas Plant (Balfron,
121 Scotland) which used dairy farm cow slurry, distiller's draff and pot ale syrup from
122 local whisky distilleries and some grass silage as feedstock. The inoculum was
123 refrigerated at 4 °C and used next day of collection (total solids (TS): 7.59 %, volatile
124 solids (VS): 88.63 %, ash content: 11.37 %). Total and volatile solids (TS, VS) of both
125 feedstocks and sludge were calculated in duplicate and were obtained submitting
126 random samples of pretreated biomass at 105 °C (for TS) and 550 °C (for VS) until
127 constant weight. The VS are expressed as percentage of TS.

128

129 **Table 1.** Feedstock characterization.

130

131 **2.2. Biomethane potential test**

132 The biomethane potential test were set according [36,37]. Erlenmeyer flasks of 0.5 L
133 with a working volume of 0.4 L were used as bioreactors; the biogas was collected in
134 airtight Linde PLASTIGAS bags. Nitrogen was flushed into the headspace of each
135 reactor to preserve the anaerobic conditions and clear up any trace of oxygen from the
136 system. The bioreactors were placed in a water-bath to maintain the mesophilic
137 temperature at 37 °C.

138 Reactors were fed with a fixed amount of 200 g of sludge (inoculum) and the quantities
139 of macroalgae and waste paper pulp required to meet the feedstock/inoculum (F/I) ratios
140 (0.2, 0.3 and 0.4) and the waste paper/macroalgae (WP/MA) ratios (0:100, 25:75, 50:50,

141 75:50 and 100:0). The F/I and WP/MA ratios are represent in terms of VS. Control
142 batches were prepared in the same way except for the feedstock addition to assess the
143 inoculum contribution of the methane production. The pH was adjusted to 6.95 ± 0.40
144 with potassium dihydrogen phosphate (KDP) as a buffer solution. To facilitate the
145 contact biomass-inoculum and degasification of the substrate, flasks were daily shaken
146 during the process. The gas volume was measured with an upside-down cylinder
147 connected to a bubbling flask to maintain anaerobic conditions; the methane content
148 was test with a gas analyser (Drager X-Am 7000). Average results were reported in this
149 paper from duplicated tests in terms of mL of methane per g of VS added of feedstock.
150 Methane yields are given for a dry gas in standard conditions of temperature (0 °C) and
151 pressure (1 atm).

152 2.3. Kinetics modelling

153 The methane production is simulated with a first order model as described as follows:

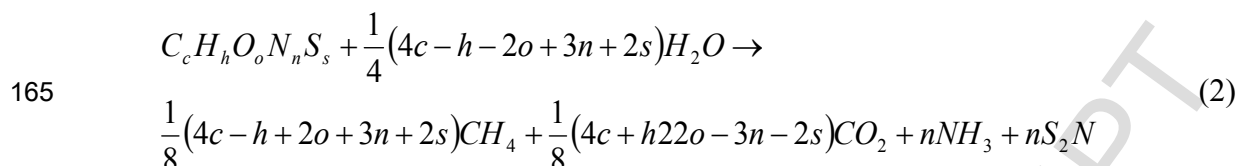
$$154 \quad M(t) = F(1 - e^{-kt}) \quad (1)$$

155 where $M(t)$ is the cumulative methane yield ($L \text{ kg}^{-1} \text{ VS}_{\text{added}}$), F is the maximum methane
156 production ($L \text{ kg}^{-1} \text{ VS}_{\text{added}}$), k is the methane production rate constant (d^{-1}), and t is the
157 time (d). Biodegradability results were compared after a significance statistical analysis
158 by using analysis of variance (ANOVA) for a single factor. Statistical significance was
159 established at $p < 0.05$ level.

160 2.4. Methane production potential

161 Buswell equation provides stoichiometric calculation on the products from the
162 anaerobic breakdown of a generic organic material of chemical composition

163 $C_nH_aO_bN_nS_s$, calculated based on the yield estimates of carbohydrates, lipids, and
 164 proteins [38]:



166 The equation is derived by balancing the total conversion of the organic material mainly
 167 to CH_4 and CO_2 with H_2O as the only external source as under anaerobic conditions.
 168 Note that the methane potentials from (Equation 2) do not consider the nutrients
 169 required for cell maintenance. From this equation, the biodegradability index could be
 170 determined. The biodegradability index (BI) is defined as the ratio of the experimental
 171 methane yield to the theoretical methane yield. Higher biodegradability index
 172 correspond to higher digestion efficiency.

173 2.5. Response surface model

174 A response surface methodology (RSM) with a hexagonal design is used to detect the
 175 interactions between the different factors (WP/MA and F/I ratios) and develop a
 176 predictive model for the response (methane yield). RSM sets an empirical relation
 177 between inputs and outputs variable sets, designing the model that best fit this relation
 178 [39]. In the two factors hexagonal design, one factor has 5 levels and the other factor
 179 has 3 levels. In this study, the 5 levels factor is the WP/MA ratio (0:100, 25:75, 50:50,
 180 75:25 and 100:0) and the 3 levels factor is F/I ratio (0.2, 0.3 and 0.4). The model was
 181 developed with Design Expert v9 software and because of the software configuration,
 182 the WP/MA factor was introduced as waste paper percentage in terms of VS (noted as
 183 WP) and not as a ratio. The adequacy of the model was verified using the determination

184 coefficient R^2 , the adjusted R^2 and the predicted R^2 , all of them close to 1 indicating
185 good regression model. The statistical significance was supported by an F-test and their
186 corresponding P-value at the 5 % significance level. Additionally verification through
187 validation points was carried out experimentally (section 3.5).

188 3. RESULTS AND DISCUSSION

189 3.1. Feedstock elemental composition

190 The feedstock composition was carried out by elemental analysis of carbon, nitrogen
191 and hydrogen components. The oxygen content was calculated by subtracting C, N, H
192 and ash content to the sample total solids. [40]. Carbon content is similar for both
193 feedstock (Table 1), macroalgae contents 52 % more hydrogen and 776 % more
194 nitrogen than waste paper. Nitrogen content in waste paper is at a trace level (0.3 % of
195 TS). As the contribution to methane from inoculum is less than 10 %, the study of C/N
196 ratio is based on the feedstock [23,31,32]. C/N for macroalgae mono-digestion was 15
197 while for waste paper the C/N ratio was 123 (Table 2). Higher methane yields were
198 obtained at WP/MA 50:50 ($386 \text{ L kg}^{-1} \text{ VS}_{\text{added}}$ for F/I 0.2, $369 \text{ L kg}^{-1} \text{ VS}_{\text{added}}$ for F/I 0.3
199 and $357 \text{ L kg}^{-1} \text{ VS}_{\text{added}}$ for F/I 0.4) which correspond to a C/N ratio of 26, these findings
200 corroborate the optimum levels given for anaerobic digestion process. Methane yields
201 from reactors at WP/MA 25/75 and 75/25 (C/N ratios 18 and 42 respectively) are
202 similar with differences less than 13 %. C/N ratio of 18 (correspondent WP/MA 25/75)
203 achieved 15 % and 27 % extra methane than mono-digestion of algae for F/I ratios of
204 0.2 and 0.4 respectively, compared with waste paper digestion these values were around
205 8 %. Smaller increases were found for C/N ratio of 45 (correspondent WP/MA 75/25),
206 where for the lowest F/I ratio, the increase on methane yield compared to macroalgae
207 digestion was 9 % and for the highest F/I ratio was 13 %. Compared with a C/N of 123

208 (waste paper), a C/N of 42 achieved similar methane yields. The salinity in the fed
209 samples was below 1 kg m^{-1} as the unwashed algae was dilute during the pretreatment
210 with 40 L of water. This sodium concentration is far lower than the considered toxic
211 level for anaerobic microflora [41].

212 **3.2. Methane production rate and yield**

213 Experimental conditions and results of methane potentials are shown in Table 2. The
214 inoculum contribution to biogas production was never higher than 10 % and was
215 previously subtracted from final methane yields. Reactor with a WP/MA ratio 50:50
216 produced the highest methane yields for the three F/I ratios studied over a 28-day
217 period, with a maximum value of $386 \text{ L kg}^{-1} \text{ VS}_{\text{added}}$ (F/I 0.2) which represents an
218 increase of 30 % compared with mono-digestion of algae and 22 % with mono-digestion
219 of waste paper. At higher F/I ratios the increase in methane yield of WP/MA 50:50
220 compared with digestion of single substrates is even higher (58 % and 33 % compared
221 with WP/MA 0:100 and 100:0 respectively for an F/I ratio 0.4). For an F/I ratio of 0.3, a
222 50:50 mixing ratio achieved a 48 % and 50 % extra methane compared with the
223 digestion of only macroalgae and only waste paper respectively. At higher F/I ratios,
224 microorganisms population is small and the anaerobic degradation is more influenced
225 by the process parameters and the effect of a specific parameter can be easily noticed.
226 Although the effect of 50:50 co-digestion is more perceptible at higher F/I ratios, the
227 methane yield increased with decreasing F/I ratios regardless the ratio of substrates
228 mixture. An optimum F/I ratio ensures the presence of the microorganisms population
229 required for the complete anaerobic degradation of the substrate. Knowing the optimum
230 F/I ratio allows a better exploitation of the feedstock. Feeding the reactor with high

231 quantities of biomass that the inoculum is not able to process lead to a loss of feedstock,
232 that is not digested [42]. A decrease in methane yield in the range of 4 % (WP/MA
233 50:50) to 22 % (WP/MA 100:0) was found when comparing F/I 0.3 to F/I 0.2. This
234 decrease in methane yield is higher when comparing F/I 0.4 to F/I 0.2, -33 % methane
235 yield for WP/MA 50:50 and -45 % methane yield for WP/MA 0:100.

236

237 **Table 2.** Experimental results obtained at the end of the biodegradability tests.

238

239 Results from kinetic modelling of waste paper and macroalgae co-digestion are shown
240 in Table 2; faster degradation rates, indicated by higher methane production rate (k)
241 were achieved for co-digestion test compared with mono-digestion. WP/MA of 50/50
242 achieved the highest methane production rate for the three different F/I ratios with a
243 maximum k of 0.23 d^{-1} at an F/I ratio of 0.2, which stands for an increment of 43 %
244 compared with only macroalgae and 35 % compared with only waste paper (Figure 1).
245 At higher F/I ratios, similar increments on kinetic constant were found between 50:50
246 co-digestion ratio and mono-digestion systems. Higher methane production rate
247 constants were achieved from WP/MA of 15/75 and 25/75 compared with the mono-
248 digestion test even though the increase in methane yields was not significantly high.
249 Constant rates increased with decreasing F/I ratios for feedstock mono-digestion and co-
250 digestion at 50:50. For WP/MA ratios of 25:75 and 75:25, no evident trend can be
251 noticed on kinetic constants with F/I variation, the values maintain constants around
252 $0.16 \pm 0.2 \text{ s}^{-1}$.

253 **Figure 1.** First order model fitting at various co-digestion and F/I ratios, E:
254 experimental points; KM: first order kinetic model.

255

256 **3.3. Synergistic or antagonistic effect**

257 Synergistic effect is evaluated based on the weighted methane yield from the mixture
258 co-digestion (Equation 3), calculated as the sum of the products of the methane yield of
259 each individual substrate multiplied by its percentage in the mixture in terms of VS.

$$260 \text{ Wiegthed } \text{CH}_4 \text{ yield} = \text{CH}_4 \text{ yield (WP)} * \% \text{WP} + \text{CH}_4 \text{ yield (MA)} * \% \text{MA} \quad (3)$$

261 Table 3 summarizes this analysis for co-digestion mixtures of waste paper with
262 macroalgae *P. canaliculata*, showing the differences between the methane yields from
263 co-digestion samples and the weighted methane yields calculated from Equation 3. A
264 synergistic effect was found for co-digestion ratio of WP/MA 50:50 at the three
265 different F/I ratios, with an improvement of 31 % on methane yield for high F/I ratio
266 while a 21 % on low F/I ratio. Although no evidence was shown in the present study, it
267 was suggested that the presence of waste paper in the digestion might induce cellulase
268 excretion by bacteria such as *Clostridium thermocellum*, facilitating the degradation of
269 cellulosic materials [43]. Further research is required to determine the presence of
270 cellulase-secreting microorganisms in the culture. Smaller increases in methane yield
271 were found on samples WP/MA 25:75 and 75:25 compared with their weighted
272 methane yields. Increasing in methane yield and the synergistic effect increased with
273 increasing F/I ratio for WP/MA 25:75 (11 % increase on methane yield for F/I 0.2 and
274 17 % for F/I 0.4). While for WP/MA 75:25 the synergistic effect was null for F/I ratios
275 of 0.2 and 0.4 and an increase on methane yield of 12 % was achieved for F/I 0.3.

276

277 **Table 1.** Co-digestion effect for waste paper and macroalgae and biodegradability
278 index.

279

280 **3.4. Theoretical methane yield and biodegradability index**

281 Table 3 summarizes the theoretical methane yields obtained from the Buswell equation
282 (Equation 3) the BI for the co-digestion of waste paper and macroalgae.

283 Biodegradability index increases with decreasing F/I ratios, with a maximum percentage
284 of degradation of 87 % at a F/I 0.2 and WP/MA 50:50. Studies have shown that the
285 Buswell formula does not account for around 12-15 % of the organic matter fed to the
286 reactor as this is consumed by the cell protoplasm [32,44], so the 87 % of degradation
287 for a 50 % mixture waste paper and macroalgae means a complete degradation of the
288 substrate. For a F/I of 0.3, the BI of WP/MA 50:50 reactor is still high (0.83), but a big
289 decreased is found for F/I 0.4 (0.58). For mono-digestion of macroalgae, BI range from
290 0.68 for low F/I and 0.37 for high F/I. Similar values were found for mono-digestion of
291 waste paper, with a BI of 0.69 for 0.2 F/I and 0.42 for 0.4 F/I. Reactors with WP/MA of
292 27:75 and 75:25 showed comparable behaviour on their BI, ranging from 0.44 ± 0.3 for
293 high F/I to 0.74 ± 0.02 for low F/I.

294 It must be noted that the theoretical methane yield from Buswell equation is subject to
295 some uncertainty due to sample heterogeneity. Heterogeneity in the sample may have
296 resulted in a difference between the sample characterized and in turn the calculated
297 theoretical methane yield and the tested substrate.

298 **3.5. Process Modelling**

299 The mathematical model associated with the response in terms of actual factors is
 300 shown in Equation 4 and the response surface is showed in Figure 2 (right).

$$301 \quad CH_4 \text{ yield} = -239 + 4.98 \cdot WP + 3955 \cdot F/I - 0.61 \cdot W \cdot F/I - 0.05 \cdot WP^2 - 7683 \cdot F/I^2 \quad (4)$$

302 By considering the coefficients of the model, it was possible to see the extent of impact
 303 of each term on methane yield, the highest impact correspondent to F/I and quadratic
 304 F/I, while the waste paper percentage in the co-digestion had a relative minor impact on
 305 methane yield.

306

307 **Figure 2.** Scatter (left) and response surface (right) plot for methane yield model.

308

309 The adequacy of the model was verified using the determination coefficient R^2 , the
 310 adjusted R^2 and the predicted R^2 , all of them close to 1 indicating good regression
 311 model. The statistical significance was supported by an F-test and their corresponding
 312 P-value at the 5 % significance level (Table 4).

313

314 **Table 2.** ANOVA test for anaerobic process modelling.

315

316 The scatter plot (Figure 2 (left)) shows that the predicted and actual values are distribute
 317 near to a straight line and a satisfactory correlation between them is observed. This
 318 demonstrates that the model on Equation 4 can be effectively applied. Surface plot
 319 (Figure 2 (right)) showed that higher methane yields were obtained where the F/I ratio
 320 was below 0.3 and the waste paper percentage was around 50 %. A strong decrease in

321 methane yield is observed for F/I ratios above 0.3, also showed by line B in the
322 perturbation plot (Figure 3 (left)). Perturbation plot also shows that both factors have a
323 quadratic behaviour, factor A (waste paper percentage) followed a symmetric curve
324 with its maximum at 50 %, this effect of the waste paper percentage on the methane
325 yield is similar for low and high F/I (Figure 3 (right)). The maximum methane yield for
326 factor B (F/I ratio) is achieved at around 0.25, decreasing abruptly after that point.
327 Based on the response surface model showed in Equation 4, an optimization study was
328 conducted using Design-expertV9 software. The optimization criterion was to maximize
329 the methane yield within the design space. A maximum methane yield of 387 L kg^{-1}
330 VS_{added} was found at waste paper percentage of 48 % and an F/I ratio of 0.26. At this
331 optimum point allowed 30 % extra methane compared with the maximum macroalgae
332 mono-digestion and 22 % more methane that the maximum correspondent to mono-
333 digestion of paper.

334

335 **Figure 3.** Perturbation (left) and interaction (right) plots for methane yield model.

336

337 To check the validity of the proposed model, two validation experiments were carried
338 out in duplicate using different input parameters from the design matrix within the
339 experimental range. The validation experiments were performed under the same
340 experimental conditions that the points used to build the model. These results were
341 compared with the predicted results from the model and found to be in good agreement
342 (Table 5).

343

344 **Table 3.** Validation points for methane yield model.

345

346 **4. CONCLUSIONS**

347 A maximum methane yield of 386 L kg⁻¹ VS_{added} was found for a mixing ratio of 50:50
348 achieving an improvement of 30 % and 22 % compared with the mono-digestion of
349 macroalgae and waste paper respectively. Synergistic effect was found for macroalgae
350 and waste paper co-digestion compared with the mono-digestion due to a balance in the
351 C/N ratio. A maximum biodegradability index of 0.87 indicates a complete
352 biodegradation of the feedstock during co-digestion at C/N of 26. F/I ratio had an
353 enormous influence on the methane yield with maximum values achieved at F/I of 0.2.
354 Overall the results showed that co-digestion of waste paper with macroalgae at low F/I
355 ratios is an efficient option for methane production and waste management.

356

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363

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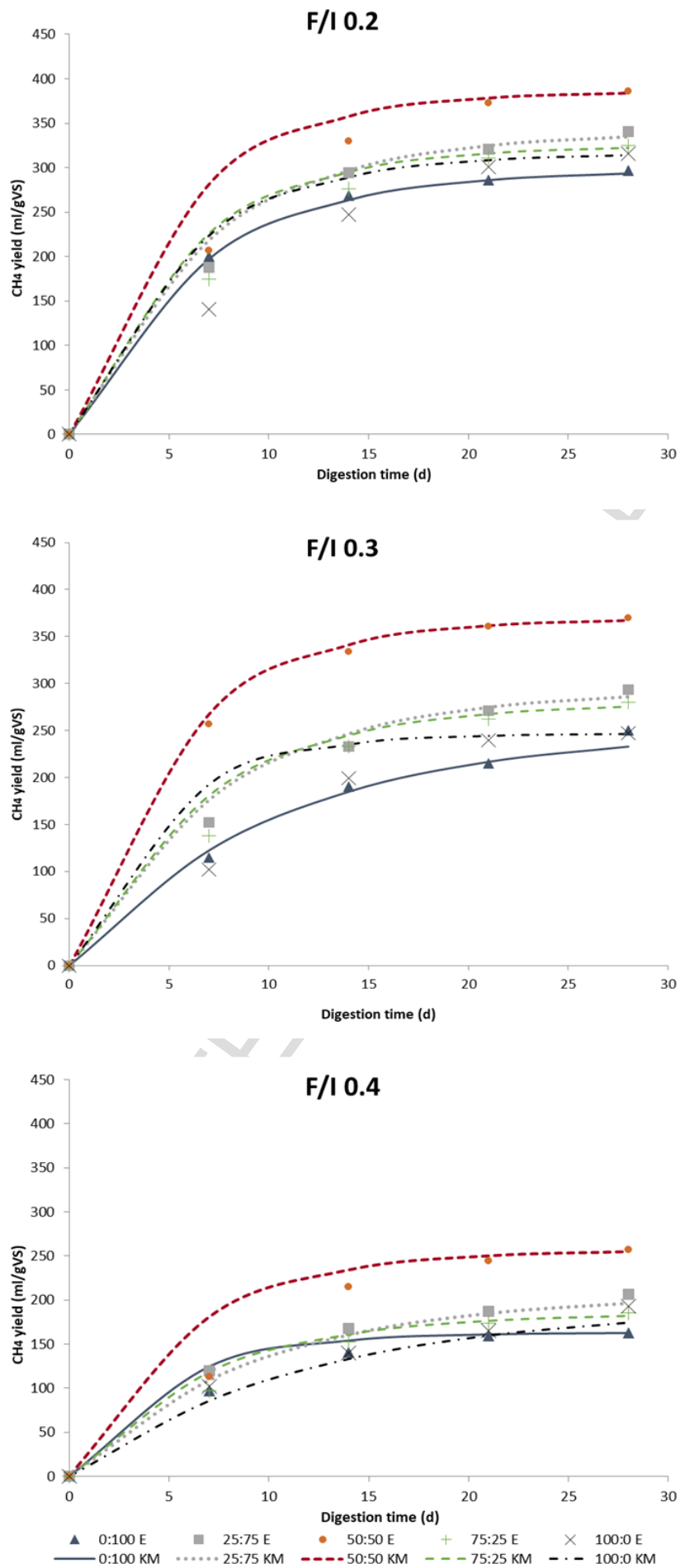


Figure 1. First order model fitting at various co-digestion and F/I ratios, E: experimental points; KM: first order kinetic model.

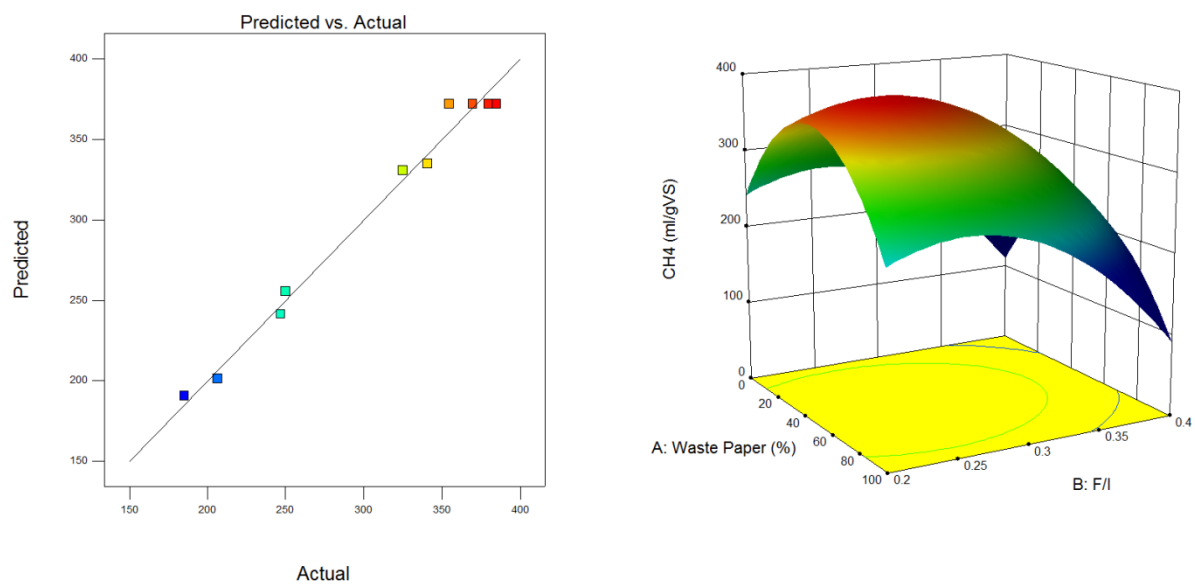


Figure 2. Scatter (left) and response surface (right) plot for methane yield model.

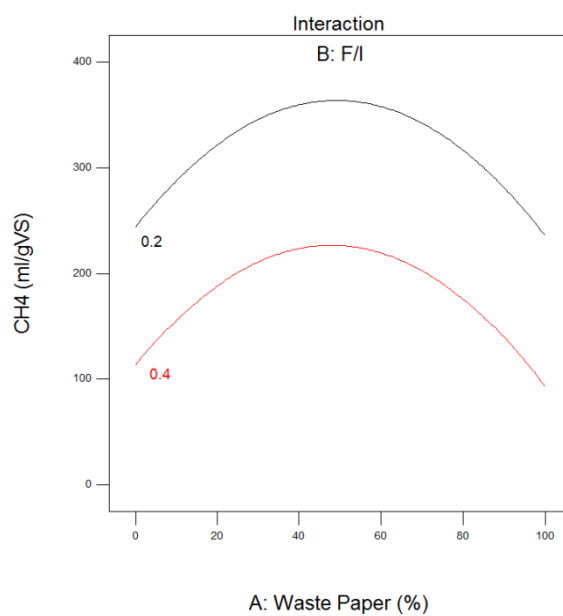
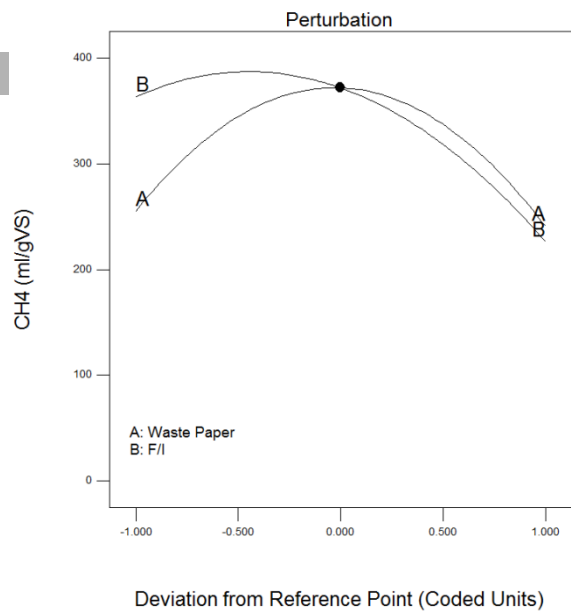


Figure 3. Perturbation (left) and interaction (right) plots for methane yield model.

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Waste paper and macroalgae co-digestion effect on methane production

Highlights

- *Pelvetia canaliculata* was used as a feedstock for anaerobic co-digestion with waste paper.
- Methane yield during co-digestion was 30 % higher compared with mono-digestion.
- Maximum biodegradability index of 0.87 achieved at C/N ratio of 26.
- Synergistic effect was present for waste paper and macroalgae co-digestion.

Table 1. Feedstock characterization

Parameters	Macroalgae	Waste paper
Total Solids (%)	6.17 ± 0.13	2.55 ± 0.02
Volatile Solids (% of TS)	80.18 ± 0.05	97.30 ± 0.07
Ash content (%)	19.82 ± 0.05	2.70 ± 0.03
Carbon (% of TS)	38.15 ±	36.87 ±
Hydrogen (% of TS)	5.48 ±	3.61 ±
Nitrogen (% of TS)	2.63 ±	0.30 ±
Oxygen (% of TS)	34.32 ±	56.52 ±

Table 2. Experimental results obtained at the end of the biodegradability tests

F/I	WP/MA	C/N	CH ₄ yield (ml/gVS)	k (s ⁻¹)
0.2	0:100	15	297 ± 14	0.16 ± 0.01
	25:75	18	341 ± 20	0.18 ± 0.01
	50:50	26	386 ± 25	0.23 ± 0.01
	75:25	42	325 ± 19	0.13 ± 0.01
	100:0	123	316 ± 14	0.17 ± 0.01
0.3	0:100	15	250 ± 12	0.10 ± 0.01
	25:75	18	294 ± 5	0.15 ± 0.01
	50:50	26	370 ± 13	0.18 ± 0.01
	75:25	42	280 ± 25	0.15 ± 0.01
	100:0	123	247 ± 23	0.14 ± 0.01
0.4	0:100	15	163 ± 19	0.11 ± 0.01
	25:75	18	207 ± 15	0.16 ± 0.01
	50:50	26	257 ± 22	0.16 ± 0.01
	75:25	42	185 ± 11	0.15 ± 0.01
	100:0	123	193 ± 16	0.08 ± 0.01

Table 1. Co-digestion effect for waste paper and macroalgae and biodegradability index

F/I	WP/MA	Theoretical CH ₄ yield	BI	Weighted CH ₄ yield	Increasing on CH ₄ yield (%)	Effect
0.2	0:100	436	0.68	297	0	n/a
	25:75	441	0.77	302	11	Synergistic
	50:50	446	0.87	307	21	Synergistic
	75:25	450	0.72	311	4	Synergistic
	100:0	455	0.69	316	0	n/a
0.3	0:100	436	0.57	250	0	n/a
	25:75	441	0.67	249	15	Synergistic
	50:50	446	0.83	249	33	Synergistic
	75:25	450	0.62	248	12	Synergistic
	100:0	455	0.54	247	0	n/a
0.4	0:100	436	0.37	163	0	n/a
	25:75	441	0.47	171	17	Synergistic
	50:50	446	0.58	178	31	Synergistic
	75:25	450	0.41	186	0	n/a
	100:0	455	0.42	193	0	n/a

Table 2. ANOVA test for anaerobic process modelling

Source	Sum of Squares	df	Mean Square	F Value	Prob > F	
Model	49919.85	5	9983.97	55.46	0.0009	significant
A-Waste Paper	155.63	1	155.63	0.86	0.4051	
B-F/I	18773.25	1	18773.25	104.28	0.0005	
AB	9.28	1	9.28	0.052	0.8315	
A ²	20356.10	1	20356.10	113.07	0.0004	
B ²	13993.71	1	13993.71	77.73	0.0009	
Residual	720.12	4	180.03			
Lack of Fit	195.12	1	195.12	1.11	0.3685	not significant
Pure Error	525.00	3	175.00			
Cor Total	50639.97	9				

R² =0.9858; Adj. R² =0.9680; Pred. R²=0.8429; Adeq. Precision=17.45

Table 3. Validation points for methane yield model

Experiment	F/I	WP/MA	Methane yield (ml/gVS)	
1	0.4	50	Experimental	257 ± 0.10
			Model	226
			Error (%)	12
2	0.2	50	Experimental	386 ± 0.15
			Model	363
			Error	6