

1 **Optimisation of the production of fermentable monosaccharides from algal biomass**
2 **grown in photobioreactors treating wastewater**

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17 ABSTRACT

18 Biomass grown in wastewater treatment photobioreactors is a cheap raw material
19 with high contents of carbohydrates, proteins and lipids. This work studies the production of
20 fermentable monosaccharides from three biomasses grown in piggery wastewater (P),
21 domestic wastewater (W) and synthetic medium (S) by applying chemical pretreatment and
22 enzymatic hydrolysis, using a Taguchi design.

23 ANOVA identified temperature, chemical reagent type and chemical reagent
24 concentration as significant operational parameters. However, the biomass concentration,
25 pretreatment time, enzyme dosage and enzymatic hydrolysis time had no remarkable effect.
26 The bacterial content of the biomass had no relevant impact on carbohydrate and protein
27 solubilisation but had a remarkable effect on the degradation of the released carbohydrates
28 (57, 60 and 37% for P, W and S), while also affecting lipid solubilisation. Pretreatment with
29 HCl 2M at 120°C resulted the optimal conditions, achieving a monosaccharide recovery of
30 53, 59 and 80% for P, W and S biomasses, respectively.

31

32 **Highlights**

- 33 • Temperature was the most influential factor on sugar production from algal biomass.
- 34 • HCl resulted in higher monosaccharide recovery than NaOH.
- 35 • No effect of enzymatic hydrolysis operational factors on sugar production was found
- 36 • High carbohydrate solubilisations were achieved from biomasses grown in
37 wastewater.
- 38 • Biomass grown in synthetic medium achieved the highest monosaccharide recovery.

39

40 Keywords: Enzymatic hydrolysis; Lipids; Pig manure; Pretreatment; Proteins; Taguchi

41 method

42 **1. Introduction**

43 Microalgae are considered a promising bio-based feedstock and a great source of
44 carbohydrates, proteins and lipids, which has increased their use in the recent years.
45 Microalgae photosynthetically consume CO₂ as a carbon source, use sunlight as an energy
46 source, can treat different types of wastewaters and exhibit high areal productivities in non-
47 arable land (Jankowska et al., 2017; Su et al., 2017). Nowadays, the cultivation of axenic
48 microalgae is costly (Zhuang et al., 2018), but the integration of microalgae cultivation and
49 wastewater treatment significantly reduces the production costs of microalgae biomass. By
50 contrast, complex mixtures of different microalgae species and bacteria grow symbiotically
51 in these treatment photobioreactors hinder the valorisation of the biomass (Kadir et al.,
52 2018; Chen et al., 2013).

53 At an industrial scale, microalgae are currently used to produce extracts of specific
54 high added value products, such as astaxanthin or pigments, but the rest of components are
55 typically not valorised, which jeopardises the economic sustainability of these processes
56 (Koutra et al., 2018). Thereby, one of the main challenges of microalgae cultivation is the
57 valorisation of every fraction of the microalgae biomass. Among the different components,
58 the carbohydrate fraction could be used as a carbon source for fermentation processes for the
59 production of biofuels like bioethanol, biohydrogen, biobutanol (Sankaran et al., 2018) and
60 even for the production of polyhydroxyalkanoates (Rahman and Miller, 2017).

61 Cell wall disruption is typically the main bottleneck to valorise the components of
62 algal biomass. This step becomes even more critical for algal-bacterial biomass grown in
63 wastewater treatment photobioreactors, due to the resistant and recalcitrant cell wall of
64 microalgae species able to growth in these media (Onumaegbu et al., 2018). Among the
65 possible alternatives, chemical pretreatments have been successfully tested to support
66 microalgae cell wall disruption, resulting in a fast and relatively inexpensive cell breakdown

67 while providing high carbohydrate solubilisation. As examples of effective chemical
68 pretreatments, Shokrkar et al., (2017) achieved a monosaccharide recovery of 94% from a
69 mixture of pure microalgae species using 2M HCl at 120°C for 30 min. Markou et al.,
70 (2013) obtained a carbohydrate solubilisation of 90% from *Spirulina platensis* using 0.5N
71 HNO₃ at 100°C for 3h. Likewise, Harun et al., (2011) pretreated *Chlorococcum infusionum*
72 biomass with alkali, achieving a maximum yield of 0.350 g_{glucose} g_{dw}⁻¹ at 0.75% (w/v) NaOH,
73 120°C for 30 min. In addition, the potential sterilisation effect of chemical pretreatment is of
74 great interest when pretreating microalgae-bacteria consortia, due to the prevention of the
75 microbial degradation of the released components by microorganisms present in the
76 cultivation broth (Fuentes et al., 2016).

77 The high variability and the bacterial content of the biomass grown in wastewater
78 treatment photobioreactors are also major challenges to be considered (Oh et al., 2018).
79 Biomass grown in open photobioreactors is strongly dependent on uncontrollable factors,
80 such as climatic and environmental conditions (Kumar et al., 2019), as well as on the
81 characteristics of the wastewater (García et al., 2017; Iasimone et al., 2018; Lv et al., 2018;
82 Ganeshkumar et al., 2018). A robust optimisation of the process that would be able to
83 provide high extraction yields independently of the intrinsic variability of biomass grown in
84 wastewater treatment photobioreactors is a requirement to successfully implement the
85 process at both pilot and industrial scales (El-Dalatony et al., 2019).

86 This work aims at optimising the production of fermentable monosaccharides from
87 the carbohydrate fraction of algal-bacterial biomass grown in photobioreactors. Based on
88 previous results (Martín Juárez et al., 2018), a two-step process with a chemical
89 pretreatment followed by an enzymatic hydrolysis was selected. A Taguchi L₂₇(3¹³) design
90 was used to evaluate the influence of the main experimental parameters and their interaction
91 effects on carbohydrate solubilisation and monosaccharide recovery, and to analyse the loss

92 of released sugars via chemical or metabolic degradation. The effect of the pretreatment and
93 the enzymatic hydrolysis on proteins and lipids was also evaluated by applying the concept
94 of bio-refinery. In order to achieve a robust optimisation, independent of the substrate
95 characteristics, the complete experimental design was applied to three types of biomass
96 grown in piggery wastewater, domestic wastewater and a synthetic medium. These
97 particular wastewater streams were selected in order to obtain a wide variation of bacterial
98 content in the microalgae biomass, which is a main objective of this study. The microalgae
99 grown in synthetic medium, without bacteria, is an extreme condition and is comparable to
100 most of the previously published research in this field which worked with pure microalgae.

101

102 **2. Materials and methods**

103 **2.1. Raw materials**

104 The biomass used in this work was cultivated in a 1.2 m³ outdoor thin-layer
105 photobioreactor operating under steady-state at the facilities of the Cajamar Foundation
106 (Almería, Spain) (Morales-Amaral et al., 2015). Three experiments were performed feeding
107 the photobioreactor with different media: piggery wastewater (P), domestic wastewater (W)
108 and synthetic culture medium (S). The different types of biomass cultivated were
109 concentrated through centrifugation up to a concentration of 20% (P), 24% (W) and 18%
110 (S). The biomass was refrigerated at 4 °C prior to use for a maximum of 48 h. The chemical
111 composition of these fresh biomasses was as follows: 22.3% of carbohydrates (including
112 1.7% of starch), 51.7% of proteins and 13.4% of lipids for P grown biomass; 24.2% of
113 carbohydrates (including 1.4% of starch), 45.4% of proteins and 14.0% of lipids for W
114 grown biomass; and 21.9% (including 1.9% of starch) of carbohydrates, 58.0% of proteins
115 and 13.7% of lipids for S grown biomass (percentages refer to dry mass).

116 The main microalgae species present in the three biomasses were as follows:
117 *Scenedesmus acutus* (32%), *Chlorella kessieri* (23%), *Scenedesmus obliquus* (17%),
118 *Scenedesmus* sp. (12%) and *Aphanothece saxicola* (12%) in biomass P; *Scenedesmus acutus*
119 (65%), *Scenedesmus acuminatus* (27%) and *Chlorella kessieri* (7%) in biomass W; and
120 *Scenedesmus acutus* (98%) in biomass S.

121 The identification and quantification measurements of the microalgae species were
122 performed by microscopic examination (OLYMPUS IX70) using at least three different
123 samples using a counting chamber according to Sournia, (1978). Biomass samples were
124 fixed with lugol acid at 5% and stored at 4 °C prior to analysis.

125

126 **2.2. Pretreatments**

127 Weighted amounts of biomass and the corresponding volumes of 5 M HCl or NaOH
128 and distilled water – to achieve a total volume of 300 mL of suspension – were introduced in
129 1 L borosilicate bottles. The bottles were introduced in a thermostatic bath or in an autoclave
130 at the pre-established temperature during the time selected for each experiment. The
131 pretreated suspensions were stored at 4 °C for a maximum period of 24 h for further
132 enzymatic hydrolysis experiments. Additional aliquots were centrifuged at 10,000 rpm for 6
133 min to separate the solid and liquid fractions, which were then weighted. The content of
134 carbohydrates, proteins and lipids was analysed in the solid fractions and the
135 monosaccharide concentration was measured in the liquid fractions. In order to check the
136 mass balances, total and volatile solids were determined in the solid and liquid fractions, as
137 well as in the whole suspensions.

138

139 **2.3. Enzymatic hydrolysis**

140 Assays to study the enzymatic hydrolysis conditions in the pretreated biomass were
 141 carried out at a biomass concentration of 5 % w/w and adjusting the final concentration with
 142 distilled water when necessary. The pH was adjusted to 4.9 ± 0.1 . The tests were performed
 143 in 100 mL Erlenmeyer flasks with a working volume of 25 mL by adding the required
 144 enzyme dosage (Celluclast 1.5L - Cellulase from *Trichoderma reesei*) and a 1 M citrate
 145 buffer (Travaini et al., 2016). The assays were carried out in a rotatory shaker at 50 °C and
 146 300 rpm at the tested incubation times. The experiments were performed in duplicate.

147 The solid and liquid fractions were separated by centrifugation (10 min, 10,000 rpm)
 148 and weighted after the enzymatic hydrolysis. The carbohydrate, protein and lipid
 149 concentrations were determined in the solid fractions and the monosaccharide concentration
 150 was determined in the liquid fractions (Martín Juárez et al., 2016). Total and volatile solids
 151 were determined in the solid and liquid fractions as well as in the whole suspensions to
 152 check the mass balances. All analyses were carried out in duplicate.

153

154 **2.4. Calculation of yields**

155 The following parameters were defined to understand the process and to determine
 156 the solubilisation of carbohydrates, proteins and lipids, the loss of carbohydrates via
 157 degradation and the recovery of monosaccharides in the liquid fractions during the
 158 pretreatment step and the global process (pretreatment + enzymatic hydrolysis):

$$159 \text{ Component solubilisation yield} = \left(1 - \frac{\text{g component in solid fraction}}{\text{g component in PR}}\right) \cdot 100 \quad \text{Eq. (1)}$$

$$160 \text{ Monosaccharide recovery yield} = \frac{\text{g monosaccharides in liquid fraction}}{\text{g carbohydrates in PR}} \cdot 100 \quad \text{Eq. (2)}$$

$$161 \text{ Carbohydrate degradation factor} = \left(1 - \frac{\text{g monosaccharides in liquid fraction}}{\text{g carbohydrates in PR} - \text{g carbohydrates in solid fraction}}\right) \cdot 100 \quad \text{Eq. (3)}$$

162 where “components” are carbohydrates, proteins and lipids and “PR” is the initial biomass.

163 The solid and liquid fractions were from the pretreatment for the pretreatment step yields
 164 and from the enzymatic hydrolysis for the global yields.

165

166 **2.5. Optimisation of operational conditions by Taguchi's robust parameter design**

167 Seven operational parameters (control factors) were selected in this study based on
168 previous works on monosaccharide production from solid wastes by applying chemical
169 pretreatments and enzymatic hydrolysis: biomass concentration (C_A), chemical reagent (H),
170 chemical reagent concentration (C_Q), temperature (T) and pretreatment time (t) on the
171 pretreatment step and enzyme dosage (E) and time (t_H) for the enzymatic hydrolysis.

172 Interaction effect of some control factors ($C_Q \times T$, $C_Q \times t$ and $T \times t$) were also considered. The
173 optimisation was carried out using the Taguchi's orthogonal arrays (OA) $L_{27}(3^{13})$ design.
174 This experimental design, with 27 freedom degrees, permits three levels for each control
175 factor in order to detect quadratic or non-linear effects of the parameters and to obtain
176 information over a wide range of the factors. Additionally, this design provides information
177 about the interaction effect of 3 combinations of control factors (Taguchi et al., 2007).

178 The range as well as the specific values of each operational parameter were selected
179 based on previous results and unpublished research (Table 1). Individual control factors and
180 interactions of control factors were assigned to the columns of the OA according to the
181 adequate triangular table and linear graph (Taguchi and Konishi, 1987). The chemical
182 reagent type (H) was tested at only two levels, using HCl and NaOH solutions. The dummy
183 treatment allowed for the accommodation of the factor H at only two levels into a column
184 with three levels while orthogonality was maintained by repeating one of the two levels
185 (Ross, 1995). The experimental design matrix is shown in Table 2. The execution order of
186 each set of 27 experiments was randomised.

187 The variability of the microalgae biomass, inherent and uncontrollable in a real
188 wastewater treatment process, was introduced in the experimental design as a noise factor by
189 using three microalgae biomass grown in rather different media to achieve a robust

190 response. Each of the 27 combinations of factor levels defined by the OA were run at the
191 three levels of the noise factor.

192 The effect of the individual control factors and the interactions of control factors on
193 the different target responses was studied by analysis of variance (ANOVA). No replicate of
194 experiments was performed, and hence residual error was estimated from the results of the
195 unassigned degree of freedom of the design (dummy error in factor H, e_H). Sums of squares
196 and degrees of freedom of dummy error and of its interaction with the noise factor, $e_H \times N$,
197 were pooled for a first estimation of the residual variance. Non-significant
198 factors/interactions were then iteratively pooled into the residual error until only significant
199 effects arose. To estimate the experimental conditions less affected by the variability of
200 microalgal biomass, the ANOVA of the signal-to-noise ratio (S/N) of the 27 combinations
201 was analysed (Taguchi et al., 2007).

202 For those factors that contributed considerably to the target responses, the Duncan
203 multiple range test was used. This test allowed for the evaluation of the statistically
204 significant differences between the tested factor values for the identification of the factor
205 level that yielded the optimum response (Ross, 1988). A significance level $p=0.05$ was used
206 in all statistical calculations.

207

208 **2.6. Analytical methods**

209 The total and volatile solid contents were measured according to the NREL protocols
210 in the raw material, solid and liquid fractions, and whole suspensions to check the mass
211 balance in all the experiments (Van Wychen and Laurens, 2015a). The lipid content was
212 determined using a modified protocol based on a chloroform-methanol 2:1 extraction by
213 applying the Kochert method (Kochert, 1978) and the protein content was calculated by
214 multiplying the Kjeldahl Total Nitrogen by a factor of 5.95 (González Lopez et al., 2010).

215 The carbohydrate content was determined as total monosaccharides in the raw
216 materials and solid fractions by using an NREL procedure (Van Wycken and Laurens,
217 2015b). The biomass samples (300 mg dry biomass) were subjected to a concentrated acid
218 hydrolysis for 1 h by adding 3 mL of 72% w/w H₂SO₄ at 30 °C. Then, 84 mL of deionised
219 water was added to dilute the acid concentration to 4% w/w and the samples were
220 autoclaved at 121 °C for 1 h. Then, solid and liquid fractions were separated by filtration and
221 the resulting liquid fraction was stored at 4 °C for in order to determine the total
222 carbohydrate content by HPLC-RI.

223 A Bio-Rad HPX-87H ion-exclusion column installed in a Waters e2695 separation
224 module was used for the quantification of the monosaccharide content. A refractive index
225 detector (Waters 2414) was used to quantify the monosaccharide concentration obtained in
226 the liquid fractions. An aqueous solution of 0.025 M H₂SO₄ was eluted at a flow rate of 0.6
227 mL/min and 50°C (Martín-Juárez et al., 2016). The external calibration method was used for
228 quantification. Multi-standard calibration solutions were prepared by adequate dilution of
229 individual standards commercially available with a purity >95% (Sigma Aldrich, Spain).
230 The starch content was determined using the polarimetric methodology using an internal
231 procedure of the Laboratory of Animal Nutrition (Serida, Spain).

232

233 **3. Results and discussion**

234 *3.1. Effect of the experimental parameters on the performance of the pretreatment step*

235 High solubilisation yields of the different macromolecular components of biomass
236 were achieved in the pretreatment step for some of the combinations of the operational
237 parameters (Table 2). Specifically, an average carbohydrate solubilisation yield of 64% was
238 obtained, with similar values ranging from 25% to 94% for biomasses grown in piggery and
239 domestic wastewaters and slightly lower (from 13% to 85%) for microalgae grown in

240 synthetic medium. A high protein solubilisation yield was also achieved, with average yields
241 of 53% (identical for the three biomass) and experimental values ranging from 13% to 96%.
242 These similar carbohydrate and protein solubilisation yields concurred with the analogous
243 composition and predominant microalgae species determined in the three biomasses used in
244 this study. Therefore, these results could indicate the insignificant effect of the bacteria
245 present in the biomass in the release of these components during acid or basic diluted
246 pretreatment. Lipid solubilisation resulted in the largest differences with average yields of
247 only 18% for biomass grown in piggery wastewater, while 48% and 52% of the lipid
248 fraction was solubilised from biomass W and S, respectively.

249 The experimental design applied allowed for the elucidation of the individual effects
250 that each operational parameter, interaction of selected factors and noise factor had on
251 carbohydrate, protein and lipid solubilisation, as well as on the monosaccharide recovery.

252

253 *3.1.1. Carbohydrate solubilisation and monosaccharide recovery*

254 The effect of each factor level on the mean values of carbohydrate solubilisation
255 yields during the pretreatment step is shown in Figure 1a. The mean results at the different
256 noise factor levels have been represented separately to highlight the variability of the type of
257 biomass.

258 The ANOVA analysis revealed that temperature, chemical reagent concentration and
259 chemical reagent type were the most influential parameters with the respective percentages
260 of contributions of 38, 13 and 12%, being higher than the residual error (8%). Similarly, the
261 ANOVA S/N disclosed the most influential factors in the robustness of the carbohydrate
262 solubilisation during the pretreatment step against the variability of microalgae biomass
263 used as a substrate. The main parameters identified by ANOVA were confirmed by the
264 ANOVA S/N, with a contribution of 48% for temperature and 15% for the chemical reagent

265 concentration and a residual contribution of 9%. It was also determined that the effect of the
266 reagent type depended on the biomass.

267 The effect of temperature was very similar for the three types of biomass, with a
268 rapid increase in the yields between 80 and 100°C and slight differences between 100 and
269 120 °C. For instance, the carbohydrate solubilisation yield in experiments with microalgae
270 grown in synthetic medium pretreated with HCl 0.5 M increased from 13% at 80°C to 69%
271 at 100°C and to 75% at 120°C. HCl provided higher carbohydrate solubilisation yields than
272 NaOH, increasing the significance of the type of chemical reagent with the concentration of
273 chemical reagent (Figure 1a). The biomass type exhibited a significant influence on the
274 effect of the chemical reagent factor, with significant differences for algal-bacteria biomass
275 grown in wastewater, but minor variances for microalgae grown in synthetic medium.

276 Despite the insignificant effect of the pretreatment time in the mean responses of the
277 three biomasses, this control factor had a significant impact on the results from microalgae
278 grown in synthetic medium. Indeed, carbohydrate solubilisation yields increased remarkably
279 from Level 1(10 minutes) to Level 2 (20 minutes) in the S biomass. The bacteria present in
280 the biomasses grown in wastewater jeopardised the effect of pretreatment time.

281 Monosaccharide recovery yields varied from 3% to 76% for biomass grown in
282 piggery wastewater, from 8% to 62% for biomass grown in domestic wastewater and from
283 4% to 80% for microalgae grown in synthetic medium (Table 2). These values were low
284 compared with the high monosaccharide recovery yields reported by Shokrkar et al., (2017),
285 who achieved a maximum yield of 94% from mixed microalgae grown in synthetic medium
286 by applying acid pretreatment with 2M HCl at 121°C for 30 min. This difference could be
287 attributed to the previous drying and grinding applied to the biomass or to the microalgae
288 species composition (data not provided).

289 Despite the fact that comparable average carbohydrate solubilisation yields were
290 obtained for the three types of biomass, the average monosaccharide recovery yields were
291 significantly higher for the S microalgae (41%) than for biomasses grown in wastewaters
292 (31% for P and 28% for W). These differences revealed average carbohydrate degradation
293 factors of 37% for the S microalgae and ~ 60% for the P and W biomasses. The presence of
294 bacteria in the biomass exerted a relevant and negative influence on monosaccharide
295 recovery by increasing the microbial degradation of the monosaccharides released (Fuentes
296 et al., 2016).

297 The impact of the control factor levels on the mean monosaccharide recovery yields
298 during the pretreatment step is shown in Figure 2a. According to the ANOVA analysis, the
299 effects of temperature (33% of the share) and the reagent concentration (9% of the share) in
300 the monosaccharide recovery were very similar to those obtained for carbohydrate
301 solubilisation. However, a higher contribution of the chemical reagent type was calculated
302 for monosaccharide recovery (20% of the share) than for carbohydrate solubilisation.
303 Chemical degradation of the solubilised carbohydrates could also increase with the severity
304 of the pretreatment conditions, resulting in lower recovery yields (Anburajan et al., 2018).
305 No significant contributions were found for the rest of individual and combined operational
306 parameters in the pretreatment step. Some authors have reported the significant influence of
307 the microalgae concentration (Shokrkar et al., 2017) and the pretreatment time
308 (Sivaramakrishnan and Incharoensakdi, 2018) on monosaccharide recovery, but these
309 studies only used microalgae species grown in synthetic media and conducted non-statistical
310 analysis.

311 The ANOVA S/N confirmed that temperature was the most influential factor (with a
312 share of 42%). The effect of the other factors was rather variable dependent on the different
313 biomass and, hence, common conclusions cannot be drawn (23% of residual). Higher impact

314 of temperature on monosaccharide recovery was recorded from Level 1 (80°C) to 2 (100°C)
315 than from Level 2 to 3 (120°C). Sivaramakrishnan and Incharoensakdi, (2018) observed a
316 similar effect of temperature during the chemical pretreatment of *Scenedesmus* sp. with
317 0.3M NaOH, with an increase in the monosaccharide recovery yield, from 45% at 60°C to
318 78% at 100°C, but with no further improvement at 120°C.

319 Despite the differences among biomasses, the mean values of monosaccharide
320 recovery were higher using HCl instead of NaOH (Figure 2a). Therefore, a monosaccharide
321 recovery of 80% was achieved with HCl, while the maximum monosaccharide recovery
322 using NaOH was only 40%. The superior performance of acid reagents was also reported by
323 Shokrkar et al., (2017) when comparing the hydrolysis of microalgae mixtures with different
324 acid reagents (H₂SO₄, HCl, H₃PO₃) and NaOH. However, Sivaramakrishnan and
325 Incharoensakdi, (2018) achieved higher monosaccharide recovery yields with NaOH (45%)
326 instead of HCl (28%) under mild pretreatment conditions (0.2M, 80°C).

327 Monosaccharide recovery increased with the chemical reagent concentration in the
328 three types of biomass tested in this study. Only a slight difference was observed in
329 monosaccharide recovery from the W biomass, where the recovery yield increased slightly
330 when the reagent concentration increased from 1M to 2M. In this context, the carbohydrate
331 solubilisation from the W biomass using acid pretreatment at 80°C increased from 28% at
332 HCl 0.5M to 84% at HCl 2M. Similarly, Sivaramakrishnan and Incharoensakdi, (2018) also
333 reported an increment on the monosaccharide recovery yields with a chemical reagent
334 concentration from 35% at 0.1M NaOH to 60% at 0.3M NaOH.

335 According with the carbohydrate solubilisation results, the contribution of
336 pretreatment time on monosaccharide recovery was particularly relevant in microalgae
337 grown in synthetic medium, but it was not significant for the mean values of the three
338 biomasses.

339
340 *3.1.2. Protein and lipid solubilisation*

341 The application of chemical pretreatments resulted in the solubilisation of other
342 macromolecular components of the biomass (proteins and lipids) (Lorenzo Hernando et al.,
343 2018). Thus, similar protein solubilisation yields were obtained for the three types of
344 biomass, ranging from 13% to 96% (Table 2). Figure 3a displays the effect of the control
345 factors on the mean protein solubilisation yields for the three noise levels. No divergence on
346 protein solubilisation for the three microalgae was detected and, hence, a great robustness of
347 this result against the variations of microalgae biomass in the process was determined.

348 The ANOVA analysis provided the contributions of the most influential parameters
349 to protein solubilisation: temperature (39%), chemical reagent type (21%), and the chemical
350 reagent concentration (11%), with residual of 8%. These results, analogous to those obtained
351 for the carbohydrate solubilisation yields, were confirmed by ANOVA of S/N.

352 Protein solubilisation increased with temperature and chemical reagent
353 concentration, reaching the maximum at 2M and 120°C, which confirmed the simultaneous
354 solubilisation of carbohydrates and proteins. However, the best chemical reagent for protein
355 solubilisation was NaOH. It is well known that alkaline pHs promote protein solubilisation,
356 whereas carbohydrates are better solubilised under acidic conditions (Phong et al., 2018).
357 The highest protein solubilisation yield was obtained for the S microalgae with NaOH 2M
358 and 120°C (96%), while only a maximum yield of 75 % was achieved for this biomass with
359 HCl 2M at 120°C.

360 The noise effect exerted a significant impact on lipid solubilisation yields along with
361 chemical reagent type used according to the ANOVA. The impact of the type of biomass is
362 shown in Figure 4a. The lipid solubilisation yields from the P biomass were remarkably
363 lower than those obtained from the W and S biomasses. HCl solubilised lower amounts of
364 lipids than NaOH under all experimental conditions tested. This effect was especially

365 notable for the S microalgae. The chemical reagent was also the only significant factor in
366 ANOVA signal to noise, with 55% of the share (residual: 45%). Therefore, the use of acid
367 reagents was selected as the best option to minimise lipid release.

368

369 *3.2. Effect of the operational parameters on the global process yields*

370 The application of enzymatic hydrolysis after chemical pretreatment was also
371 evaluated using the same experimental design. Two additional factors of the enzymatic
372 process were also included (enzyme dosage, E, and time, t_H). Considering the low
373 concentration of starch in the microalgae biomasses used in this work, a commercial cocktail
374 containing cellulases and β -glucosidases was selected for the enzymatic hydrolysis in order
375 to obtain fermentable monosaccharides, as previously reported by other authors (González-
376 Fernández et al., 2012; Hernández et al., 2015; Passos et al., 2014; Yin et al., 2010). The
377 assessment of global yields (pretreatment followed by enzymatic hydrolysis) was
378 investigated in this section in order to determine the feasibility of an additional enzymatic
379 hydrolysis step compared to a single chemical pretreatment stage. Despite the use of specific
380 enzymes for carbohydrates, enzymatic hydrolysis increased the average global solubilisation
381 values of all the macromolecular components to 83% for carbohydrates, 77% for proteins
382 and 59% for lipids. This simultaneous solubilisation of intracellular content (carbohydrates,
383 proteins and lipids) could be attributed to the cell wall breakthrough by the enzymatic
384 hydrolysis. The multilayer cell wall of microalgae present in these biomasses contain
385 structural polysaccharides (cellulose and hemicellulose) which were degraded by the
386 enzymes actions (Cordova et al., 2018). Proteins are also an integral cell wall constituent,
387 covalently linked to algaenan or carbohydrates (Zhang et al., 2018). Thus, it could be
388 expected that these proteins release in the media after polysaccharides hydrolysis.

389 The effect of enzymatic hydrolysis was different depending on the type of biomass.
390 Therefore, enzymatic hydrolysis resulted in a lower impact on the global carbohydrate
391 solubilisation of the P biomass (average of 78%) than in the W biomass (average of 89%)
392 and the S microalgae (average of 81%). The opposite effect was found in the global protein
393 solubilisation, with the highest yields recorded in the P biomass (average of 83%) compared
394 to the W and S biomass (76% and 70%, respectively).

395 The enzymatic hydrolysis also boosted the global monosaccharide recovery yields,
396 but to a lower extent than the global carbohydrate solubilisation yields, with average yields
397 of 39% in the P biomass, 44% in the W biomass and 53% in the S microalgae. The
398 maximum global monosaccharide recovery yields were 86% for the P biomass, 72% for the
399 W biomass and 91% for the S biomass. The biomass cultivated in the synthetic medium also
400 provided the highest global monosaccharide recoveries. Differences between the global
401 carbohydrate solubilisation yields and the global monosaccharide recovery yields allowed
402 for an estimation of the global carbohydrate degradation factors – 57% for the P biomass,
403 60% for the W biomass and 37% for the S microalgae. These factors, very similar to those
404 previously estimated for the chemical pretreatment step highlighted the metabolic
405 degradation of solubilised carbohydrates by the bacteria present in biomasses grown in
406 wastewater.

407

408 *3.2.1. Global carbohydrate solubilisation and monosaccharide recovery*

409 The effect of the operational parameters on the global carbohydrate solubilisation
410 yields is shown in Figure 1b. The ANOVA showed that temperature was the only factor
411 with an important contribution on the global yields (37%). The enzymatic hydrolysis stage
412 counteracted the differences found in the pretreatment step for the rest of the operational
413 parameters. No influence of the analysed operational factors of the enzymatic hydrolysis

414 was identified. Rehman and Anal, (2019) also detected no impact of enzyme concentration
415 on sugar yields from *Chlorococcum* sp. using cellulase enzyme at 45°C, 72h.

416 Regarding the noise effect, the W biomass provided higher global carbohydrate
417 solubilisation yields than the P and S biomass. The ANOVA S/N confirmed that temperature
418 was the most influential factor with a 58% of the share, where an increase in the
419 carbohydrate solubilisations yields was observed at increasing temperatures.

420 Temperature was also the most influential parameter on the mean values of global
421 monosaccharide recovery (Figure 2b), with a 41% of the share. The ANOVA S/N of the
422 global monosaccharide recovery yields confirmed this major contribution of temperature
423 (51%, with a residual of 30%).

424 Regarding the results for each biomass, temperature, chemical reagent type and
425 chemical reagent concentration exhibited a noteworthy impact on the global monosaccharide
426 recovery yields in the P biomass. Average global monosaccharide recoveries of 45% were
427 obtained using HCl, whereas a recovery of 26% was reached with NaOH. Moreover, an
428 increase in chemical reagent concentrations greatly improved the yields (24% at 0.5M and
429 58% at 2M). However, only temperature and chemical reagent type exerted a significant
430 effect on global monosaccharide recovery yields in the W biomass. In this case, the average
431 values were 49% using HCl and 34% using NaOH. Finally, only temperature exhibited a
432 relevant impact on the global monosaccharide recovery yields in the S biomass. Therefore,
433 the effect of the chemical reagent type and concentration on monosaccharide recovery yields
434 seems to be related to the sterilising effect of the pretreatment, and with the metabolic
435 degradation of solubilised carbohydrates by the viable bacteria remaining after pretreatment.
436
437 *3.2.2. Global protein and lipid solubilisation*

438 Figure 3b shows the effect of the control factors on the mean values of global protein
439 solubilisation yields. The trend was similar to the results obtained in the protein
440 solubilisation tests conducted with a single pretreatment step. However, the significant
441 operational parameters had a lower influence on these yields. Temperature and chemical
442 reagent type were the most influential factors with 29% and 18% of the share, respectively
443 (residual 13%). Unlike of the results obtained in the chemical pretreatment step, the noise
444 factor exerted a significant impact on this global yield, with remarkably different results
445 among the three types of biomass tested. The enzymatic hydrolysis step increased the
446 average protein solubilisation yield by 30% in the P biomass, 31% in the W biomass and
447 17% in the S biomass. The bacteria present in the biomass could contribute to the proteins
448 release during the enzymatic hydrolysis step. It could be corroborated with the fact that
449 Maffei et al., (2018) obtained constant protein content after the application of cellulase on
450 pure *Nannochloropsis* at 53°C and pH 4.4.

451 The ANOVA S/N confirmed the key role of temperature (39% of the share) and the
452 chemical reagent type (23% of the share) on the global protein solubilisation, but to a lesser
453 extent than the ANOVA analysis, because of the differences between the biomasses (38% of
454 residual). The global protein solubilisation yields increased with temperature and NaOH as
455 the chemical reagent. These results were consistent with those previously recorded for the
456 pretreatment step.

457 On the other hand, the effect of the individual parameters on the global lipid
458 solubilisation yields was identical to that found in the chemical pretreatment tests (Figure
459 4b). The only difference was the increase in the yields after enzymatic hydrolysis in all the
460 experiments. The chemical reagent and biomass type were identified as the only influential
461 control factors on the global lipid solubilisation yields. The highest global lipid
462 solubilisation yields were recorded in microalgae grown in the synthetic medium and the

463 lowest yields were recorded in microalgae grown in piggery wastewater. The ANOVA
464 established the global lipid solubilisation dependence of only these two parameters, with
465 contributions of 40% for the type of biomass and 13% for the chemical reagent type
466 (residual of 24%). In this regard, Zhang et al., (2018) identified temperature, enzyme dosage
467 and enzymatic hydrolysis time as the key variables in the optimisation of lipid solubilisation
468 in *Scenedesmus* sp. using enzymatic hydrolysis, although these tests were conducted with an
469 initial chemical pretreatment step.

470 Finally, the ANOVA S/N demonstrated that the chemical reagent type was
471 significant in every biomass, with a 61% of the share. HCl was the chemical reagent that
472 caused minimal global lipid solubilisation and was less sensitive to noise.

473

474 *3.3. Process optimisation*

475 In order to optimise a robust process capable of coping with a variable biomass
476 composition, the typical effects of the main significant control factors should be used. A
477 Duncan multiple range test of the most influential parameters was performed to elucidate the
478 factor levels providing the highest improvement of the target variables. The analysis of the
479 protein solubilisation yields showed an inevitable co-solubilisation of carbohydrates and
480 proteins. Most of the operational conditions mediating a carbohydrate release also caused a
481 solubilisation of proteins. Therefore, the protein solubilisation yields cannot be used as a
482 target response and process optimisation should target maximising carbohydrate
483 solubilisation and/or monosaccharide recovery and minimising lipid solubilisation. Thus, a
484 fractional valorisation of macromolecular components of microalgae-based biomass using
485 HCl or NaOH pretreatment would require a further step to separate monosaccharides and
486 proteins (Suarez Garcia et al., 2018).

487 The temperature of the pretreatment was identified as the most important factor, with
488 higher temperature increasing carbohydrate and protein solubilisation and monosaccharide
489 recoveries in both the chemical pretreatment tests and the global process. Interestingly, no
490 significant influence of temperature on lipid solubilisation yields was recorded. Differences
491 between temperature levels were all significant for carbohydrate solubilisation and
492 monosaccharide recovery, with Level T3 (120°C) being selected as the optimal temperature.
493 The reagent type exerted a higher influence on the pretreatment step than on the global
494 process. The use of HCl favored carbohydrate solubilisation and monosaccharide recovery,
495 mainly in the pretreatment step, while the NaOH pretreatment favored protein and lipid
496 solubilisation. Therefore, HCl was selected as the optimal chemical reagent. The increase in
497 the chemical reagent concentration induced higher carbohydrate and protein solubilisation
498 and monosaccharide recovery in both the chemical pretreatment tests and the global process
499 but exhibited no impact on lipid solubilisation. The Duncan Test conducted revealed that the
500 only significant difference was between Level 1 (0.5M) and Level 3 (2M), and between
501 Level 2 (1M) and Level 3 (2M) during carbohydrate solubilisation and monosaccharide
502 recovery. Therefore, Level 3 was selected as the optimal concentration.

503 Carbohydrate solubilisation increased with the pretreatment time from Level 1 (10
504 minutes) to Level 2 (30 minutes), but no significant differences were found from Level 2 to
505 Level 3 (60 minutes). Nevertheless, the effect of the pretreatment time on the
506 monosaccharide recovery was highly dependent on the type of biomass, with the
507 degradation factor increasing remarkably in biomass grown in wastewater. An optimal
508 pretreatment time of 10 minutes was selected based on economic considerations. Finally,
509 economic or technical criteria should be applied for the values selection of the rest of the
510 operational parameters since no significant impact was recorded (Lam et al., 2017).

511 The results obtained in experiment number 25, which involved all the selected levels
512 of the influential parameters, provided carbohydrate solubilisations of 85%, 85% and 84% in
513 the pretreatment step, and monosaccharide recoveries of 53%, 59% and 80% in the P, W and
514 S biomasses, respectively. Likewise, protein solubilisation yields of 85%, 85% and 84% and
515 lipid solubilisation yield of 16%, 1% and 59% were obtained in the chemical pretreatment
516 tests in the P, W and S biomasses, respectively, under optimal operational conditions.

517 In the particular case of the P biomass, experimental conditions numbers 8 and 15
518 provided high monosaccharide recovery yields (76 and 73%, respectively). Carbohydrate
519 solubilisation was similar or lower in these experiments than in experiment number 25. The
520 high monosaccharide recovery recorded in experiments 8 and 15 was likely due to the low
521 degradation of the solubilised carbohydrates under these particular combinations of
522 operational parameters.

523 The enzymatic hydrolysis of pretreated samples obtained under the selected optimal
524 conditions supported global carbohydrate solubilisation values of 97%, 98% and 95% and,
525 therefore, global monosaccharide yields of 64%, 68% and 91% in the P, W and S biomasses,
526 respectively. This slight improvement in the yield was not likely sufficient to counterbalance
527 the additional cost of the enzymatic step. The economic viability of applying an enzymatic
528 hydrolysis step could be considered only in the case that a relevant enhancement of the
529 monosaccharide recovery is achieved. Interestingly, enzymatic hydrolysis did not solubilise
530 additional proteins under these conditions, but lipid solubilisation yields increased up to
531 49%, 46% and 66% in the P, W and S biomass, respectively.

532

533 **4. Conclusions**

534 This study optimised the operational conditions of the chemical pretreatment and the
535 enzymatic hydrolysis for the fermentable monosaccharide production from microalgae

536 biomass. The experimental design provided the optimal conditions for the significant control
537 factors (120°C, 2M HCl) independently of the kind of microalgae biomass. The other
538 parameters (10 min, 75g/L) were selected applying economic considerations. At these
539 conditions, the carbohydrate solubilisations were 84% for all biomasses with a degradation
540 of 37, 31 and 5% for biomass grown in piggery wastewater, domestic wastewater and
541 synthetic medium, respectively. The global process improved the solubilisation up to 97%
542 while the degradation remained constant.

543

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550

551 **Appendix A. Supplementary data**

552 Supplementary data associated with this article can be found in the online version.

553

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696

697

698 **Figure Captions**

699 **Figure 1.** Main effect plots on the carbohydrate solubilisation yields (in %) for **(a)** the
700 chemical pretreatment step, **(b)** the global process (pretreatment followed by an enzymatic
701 hydrolysis). Plotted values represent the mean yields for each factor level considering
702 individual noise levels P (\diamond), W (\circ) and S (\square) and the mean response of the three noise
703 levels (*).

704

705 **Figure 2.** Main effect plots on the monosaccharide recovery yields (in %) for **(a)** the
706 pretreatment step, **(b)** the global process (pretreatment followed by an enzymatic
707 hydrolysis). Plotted values represent the mean yields for each factor level considering
708 individual noise levels P (\diamond), W (\circ) and S (\square) and the mean response of the three noise
709 levels (*).

710

711 **Figure 3.** Main effect plots on the protein solubilisation yields (in %) for **(a)** the
712 pretreatment step, **(b)** the global process (pretreatment followed by an enzymatic
713 hydrolysis). Plotted values represent the mean yields for each factor level considering
714 individual noise levels P (\diamond), W (\circ) and S (\square) and the mean response of the three noise
715 levels (*).

716

717 **Figure 4.** Main effect plots on the lipid solubilisation yields (in %) for **(a)** the pretreatment
718 step, **(b)** the global process (pretreatment followed by an enzymatic hydrolysis). Plotted
719 values represent the mean yields for each factor level considering individual noise levels P
720 (\diamond), W (\circ) and S (\square) and the mean response of the three noise levels (*).

721 **Tables**

Table 1: Levels of the studied control factors^a for the optimisation by Taguchi design compared with other published works.

| References | Statistical design | Microalgae biomass | C _Q | T | T min | C _A g/L | H | E FPU/g | t _H hours | Remarks |
|---|--|---|---------------------------|------------------------------------|-------------------------|--------------------------------------|---|-------------------------|-----------------------|--|
| This study | Taguchi design, three levels (1, 2, 3) | P, W: Mixed microalgae-bacteria S: <i>Scenedesmus Almeriense</i> | 1: 0.5M 2: 1M 3: 2M | 1: 80 °C 2: 100 °C 3: 120 °C | 1: 10 2: 30 3: 60 | 1: 50 g/L 2: 75 g/L 3: 100 g/L | 1: HCl 2: NaOH 3 ^b : HCl | 1: 10 2: 30 3: 60 | 1: 3 2: 6 3: 12 | |
| Asyraf Kassim and Bhattacharya, (2016) | Response surface method | <i>Chlorella sp.</i> | 0.1 to 0.5 M | 60 to 120°C | 30 to 120 | | NaOH | - | - | Sugar yield: 88mg/g at 120°C, 2% NaOH, 30 min |
| Harun et al., (2011) | Central composite design | <i>Chlorococcum humicola</i> | 0.2 to 0.75 M | 60 to 140°C | 15 to 60 | | NaOH | | | Glucose yield: 350 mg/g at 0.75%, 120°C, 30 min |
| Hernández et al., (2015) | | <i>C.sorokiniana</i> <i>N.gaditana</i> <i>S. almeriensis</i> | 0 to 2.5M | 121°C | 30 | | H ₂ SO ₄ | 15 Celluclast 1.5L | | Maximum sugar release <i>C.sorokiniana</i> : 100mg/g <i>N.gaditana</i> : 125mg/g <i>S. almeriensis</i> : 50mg/g |
| Pancha et al., (2016) | | <i>Scenedesmus sp.</i> CCNM 1077 | 0.1 to 3M | 121°C | 15 to 60 | 20 to 100 | HCl, H ₂ SO ₄ , NaOH, KOH | Cellulase | 6, 24, 48, 72 | HCl, 60 min, 0.5M, 6% of biomass, 72h. |
| Shokrkar et al., (2017) | | Mixed microalgae-bacteria biomass | 0.5, 1 and 2M | 121°C | 10 to 40 | | HCl, H ₂ SO ₄ , NaOH | | | Sugar yield: 95% at HCl, 2M, 30 min |
| Sivaramakrishnan and Incharoensakdi, (2018) | | <i>Scenedesmus sp.</i> | 0.1, 0.2 and 0.3N | 60 to 120°C | 10 to 40 | | HCl, H ₂ SO ₄ , NaOH, KOH | | | Sugar yield: 80% at 0.3N, 120°C, 20min, NaOH |

^aC_Q: Concentration of chemical reagent; T: Temperature; t: time; C_A: concentration of microalgae biomass; H: reagent; E: dosage of enzyme; t_H: time during the enzymatic hydrolysis.

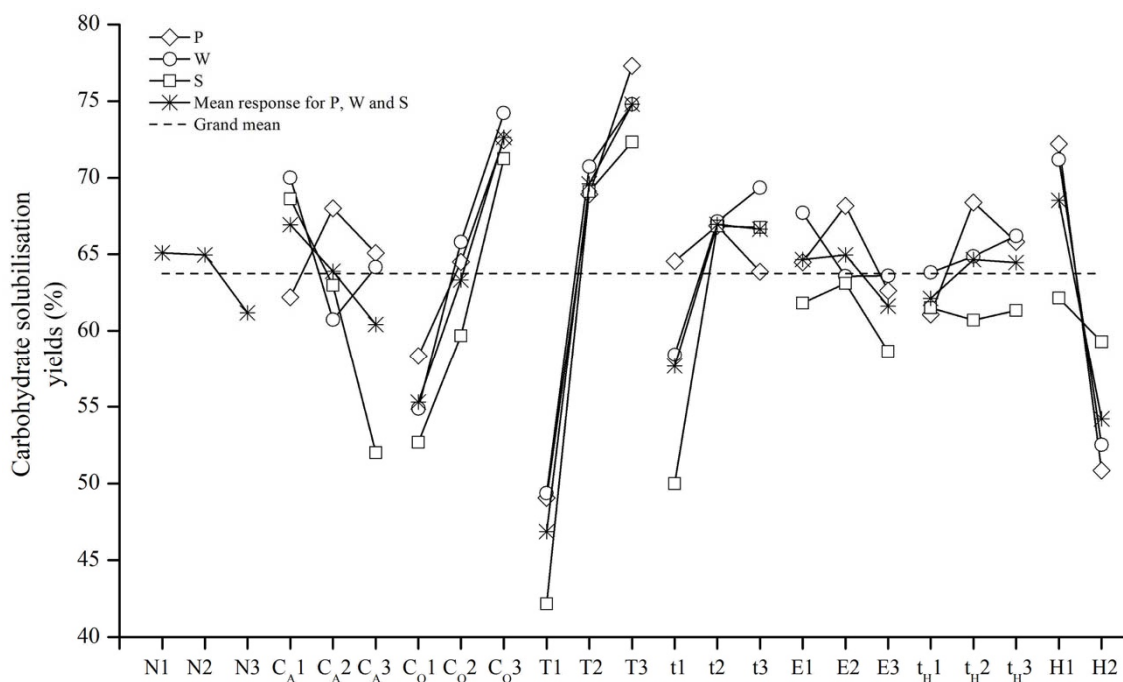
^bLevel 3 for chemical reagent corresponds again to HCl (dummy effect).

Table 2: Taguchi's $L_{27}(3)^{13}$ orthogonal array and experimental results of carbohydrate, protein and lipid solubilisation yields, and monosaccharide recovery yields during the pretreatment step.

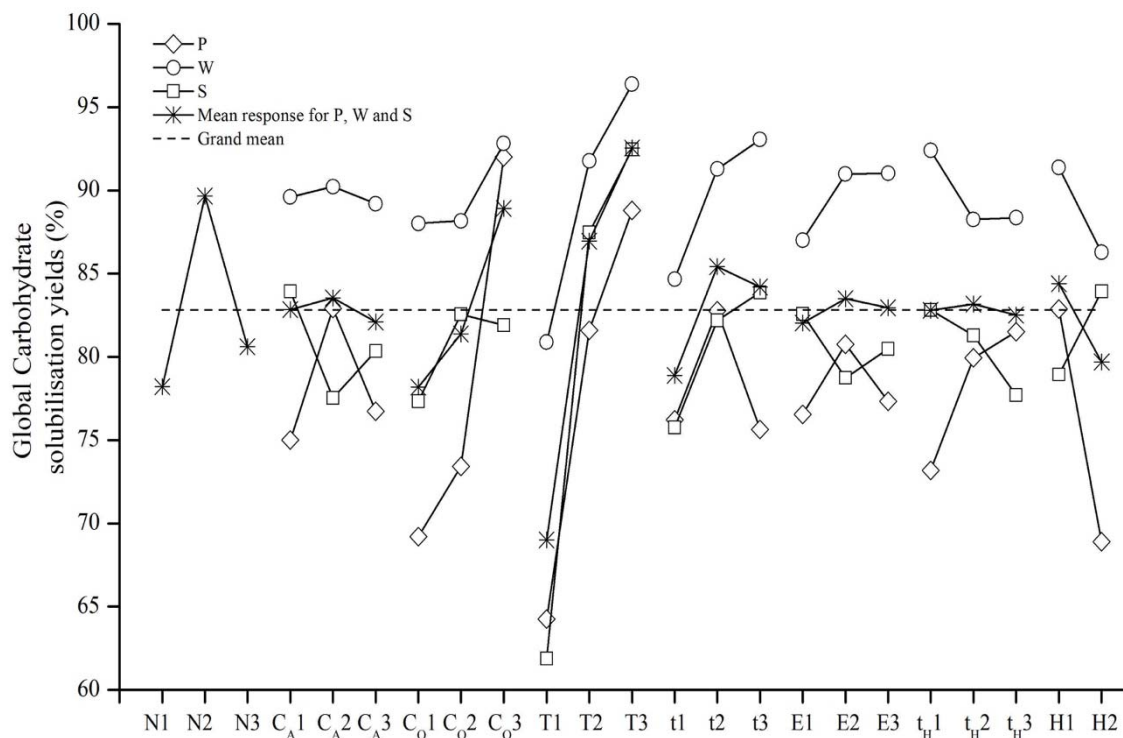
| Orthogonal array matrix | | | | | | | | | | | | | | Experimental results, in % | | | | | | | | | | | |
|-------------------------|---------|-------|----------------|----------------|-------|----------------|----------------|--------------|---------|-------|--------------|---------|-------|----------------------------|-------|-------|-----------------|-------|-------|----------|-------|-------|--------|-------|-------|
| Exp. No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | Carbohydrates | | | Monosaccharides | | | Proteins | | | Lipids | | |
| | C_Q^a | T^b | $C_Q \times T$ | $C_Q \times T$ | t^c | $C_Q \times t$ | $C_Q \times t$ | $T \times t$ | C_A^d | E^e | $T \times t$ | t_H^f | H^g | P^h | W^i | S^j | p^h | W^i | S^j | p^h | W^i | S^j | p^h | W^i | S^j |
| 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 28 | 37 | 13 | 4 | 10 | 4 | 18 | 13 | 18 | 1 | 62 | 44 |
| 2 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 45 | 33 | 48 | 9 | 8 | 17 | 37 | 34 | 48 | 2 | 40 | 78 |
| 3 | 1 | 1 | 1 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 40 | 54 | 20 | 5 | 9 | 7 | 26 | 23 | 33 | 11 | 67 | 26 |
| 4 | 1 | 2 | 2 | 2 | 1 | 1 | 1 | 2 | 2 | 2 | 3 | 3 | 1' | 75 | 44 | 57 | 10 | 15 | 31 | 34 | 46 | 29 | 30 | 69 | 45 |
| 5 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 1 | 1 | 1 | 73 | 67 | 69 | 16 | 15 | 30 | 45 | 26 | 38 | 20 | 29 | 14 |
| 6 | 1 | 2 | 2 | 2 | 3 | 3 | 3 | 1 | 1 | 1 | 2 | 2 | 2 | 40 | 45 | 76 | 12 | 17 | 32 | 67 | 73 | 88 | 63 | 71 | 88 |
| 7 | 1 | 3 | 3 | 3 | 1 | 1 | 1 | 3 | 3 | 3 | 2 | 2 | 2 | 55 | 54 | 40 | 4 | 12 | 28 | 62 | 56 | 51 | 59 | 65 | 77 |
| 8 | 1 | 3 | 3 | 3 | 2 | 2 | 2 | 1 | 1 | 1 | 3 | 3 | 1' | 85 | 85 | 78 | 76 | 56 | 70 | 67 | 57 | 68 | 12 | 32 | 16 |
| 9 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 1 | 1 | 1 | 85 | 75 | 75 | 56 | 52 | 51 | 58 | 49 | 35 | 7 | 19 | 23 |
| 10 | 2 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 1' | 52 | 34 | 22 | 3 | 9 | 8 | 13 | 17 | 20 | 7 | 66 | 46 |
| 11 | 2 | 1 | 2 | 3 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 57 | 64 | 51 | 4 | 8 | 16 | 13 | 21 | 22 | 13 | 44 | 44 |
| 12 | 2 | 1 | 2 | 3 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 25 | 53 | 67 | 14 | 10 | 27 | 67 | 53 | 81 | 5 | 50 | 89 |
| 13 | 2 | 2 | 3 | 1 | 1 | 2 | 3 | 2 | 3 | 1 | 3 | 1 | 2 | 61 | 61 | 45 | 19 | 9 | 30 | 64 | 54 | 64 | 9 | 7 | 77 |
| 14 | 2 | 2 | 3 | 1 | 2 | 3 | 1 | 3 | 1 | 2 | 1 | 2 | 1' | 82 | 84 | 67 | 44 | 47 | 57 | 56 | 58 | 58 | 16 | 44 | 49 |
| 15 | 2 | 2 | 3 | 1 | 3 | 1 | 2 | 1 | 2 | 3 | 2 | 3 | 1 | 74 | 80 | 74 | 73 | 51 | 64 | 54 | 61 | 55 | 2 | 39 | 28 |
| 16 | 2 | 3 | 1 | 2 | 1 | 2 | 3 | 3 | 1 | 2 | 2 | 3 | 1 | 87 | 88 | 78 | 54 | 62 | 72 | 52 | 63 | 62 | 12 | 34 | 41 |
| 17 | 2 | 3 | 1 | 2 | 2 | 3 | 1 | 1 | 2 | 3 | 3 | 1 | 2 | 58 | 45 | 65 | 22 | 15 | 37 | 86 | 75 | 86 | 14 | 64 | 78 |
| 18 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 2 | 3 | 1 | 1 | 2 | 1' | 85 | 82 | 67 | 55 | 58 | 52 | 71 | 63 | 43 | 22 | 30 | 22 |
| 19 | 3 | 1 | 3 | 2 | 1 | 3 | 2 | 1 | 3 | 2 | 1 | 3 | 2 | 52 | 28 | 28 | 8 | 8 | 21 | 56 | 61 | 34 | 3 | 53 | 92 |
| 20 | 3 | 1 | 3 | 2 | 2 | 1 | 3 | 2 | 1 | 3 | 2 | 1 | 1' | 60 | 67 | 67 | 24 | 15 | 31 | 28 | 50 | 35 | 10 | 20 | 51 |
| 21 | 3 | 1 | 3 | 2 | 3 | 2 | 1 | 3 | 2 | 1 | 3 | 2 | 1 | 84 | 74 | 64 | 60 | 30 | 55 | 54 | 24 | 41 | 17 | 41 | 43 |
| 22 | 3 | 2 | 1 | 3 | 1 | 3 | 2 | 2 | 1 | 3 | 3 | 2 | 1 | 86 | 94 | 85 | 49 | 44 | 77 | 51 | 92 | 75 | 10 | 78 | 59 |
| 23 | 3 | 2 | 1 | 3 | 2 | 1 | 3 | 3 | 2 | 1 | 1 | 3 | 2 | 55 | 76 | 79 | 13 | 14 | 32 | 82 | 67 | 89 | 37 | 93 | 96 |
| 24 | 3 | 2 | 1 | 3 | 3 | 2 | 1 | 1 | 3 | 2 | 2 | 1 | 1' | 75 | 84 | 71 | 68 | 52 | 59 | 42 | 71 | 51 | 5 | 48 | 18 |
| 25 | 3 | 3 | 2 | 1 | 1 | 3 | 2 | 3 | 2 | 1 | 2 | 1 | 1' | 85 | 85 | 84 | 53 | 59 | 80 | 60 | 67 | 75 | 16 | 1 | 59 |
| 26 | 3 | 3 | 2 | 1 | 2 | 1 | 3 | 1 | 3 | 2 | 3 | 2 | 1 | 88 | 83 | 78 | 48 | 50 | 67 | 67 | 72 | 51 | 26 | 40 | 33 |
| 27 | 3 | 3 | 2 | 1 | 3 | 2 | 1 | 2 | 1 | 3 | 1 | 3 | 2 | 67 | 77 | 87 | 33 | 21 | 40 | 86 | 78 | 96 | 44 | 93 | 96 |

^aConcentration of chemical reagent (mol/L). 1=0.5, 2=1, 3=2.^bTemperature (°C). 1=80, 2=100, 3=120^ctime (min). 1=10, 2=30, 3=60.^dConcentration of microalgae biomass (g/L). 1=50, 2=75, 3=100.^eDosage of enzyme (FPU/g). 1=10, 2=30, 3=60.^fTime during the enzymatic hydrolysis (h). 1=3, 2=6, 3=12.^gChemical reagent. 1=HCl, 2=NaOH, 1'=HCl.^hP: microalgae biomass grown in pig manure wastewater.ⁱW: microalgae biomass grown in domestic wastewater.^jS: microalgae biomass grown in synthetic media.

725 **Figure 1**
726 **(a)**



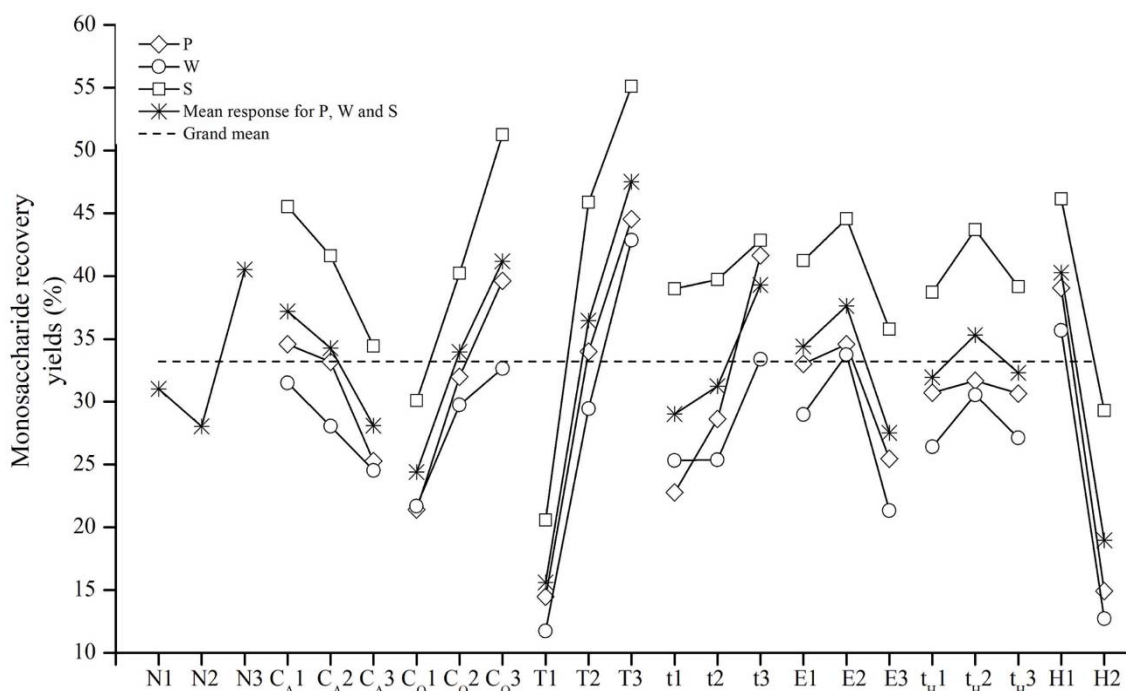
727 **(b)**
728
729



730

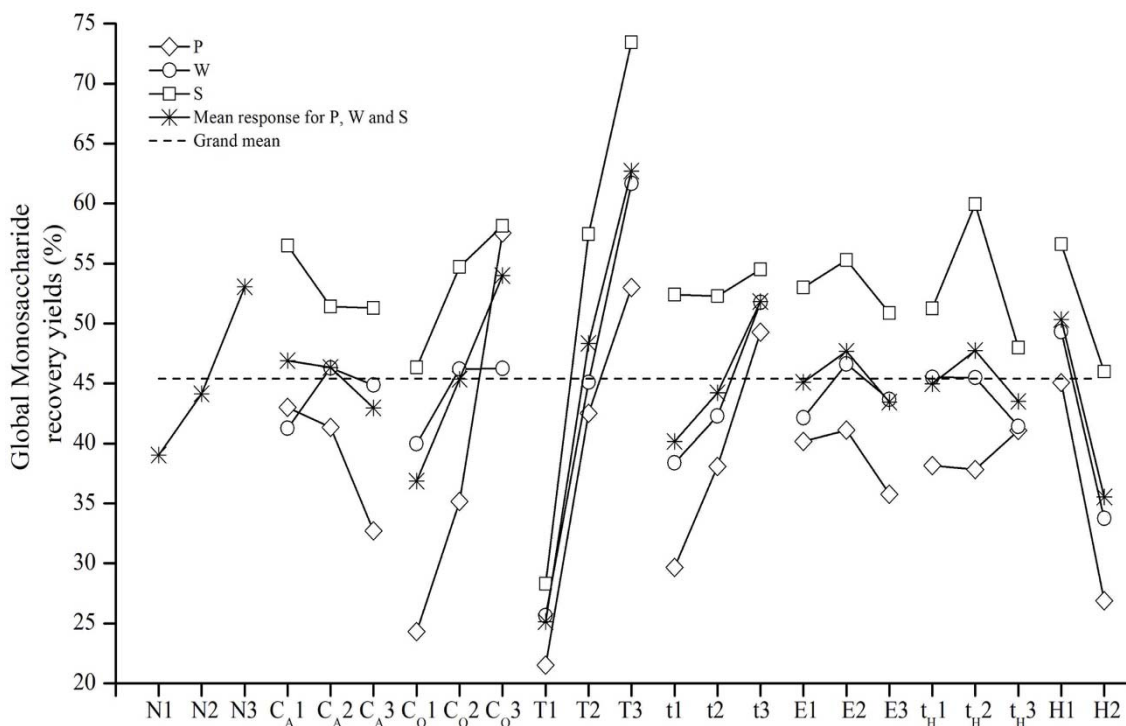
731
732
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734

Figure 2
(a)



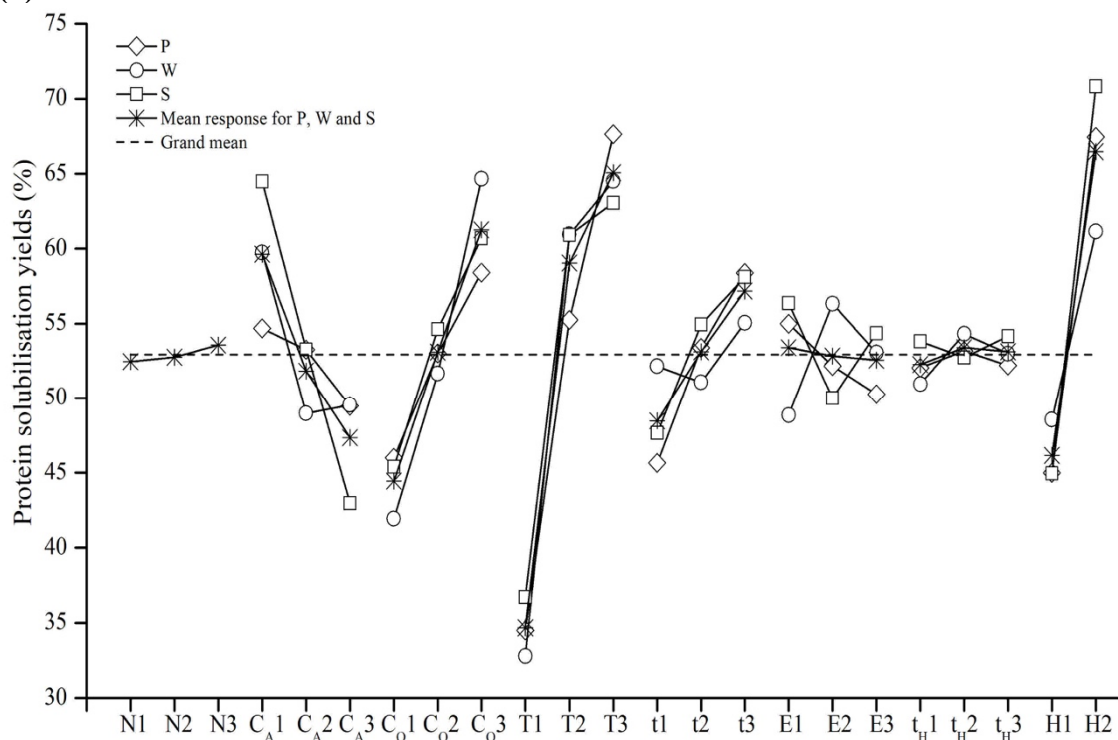
735
736
737

(b)

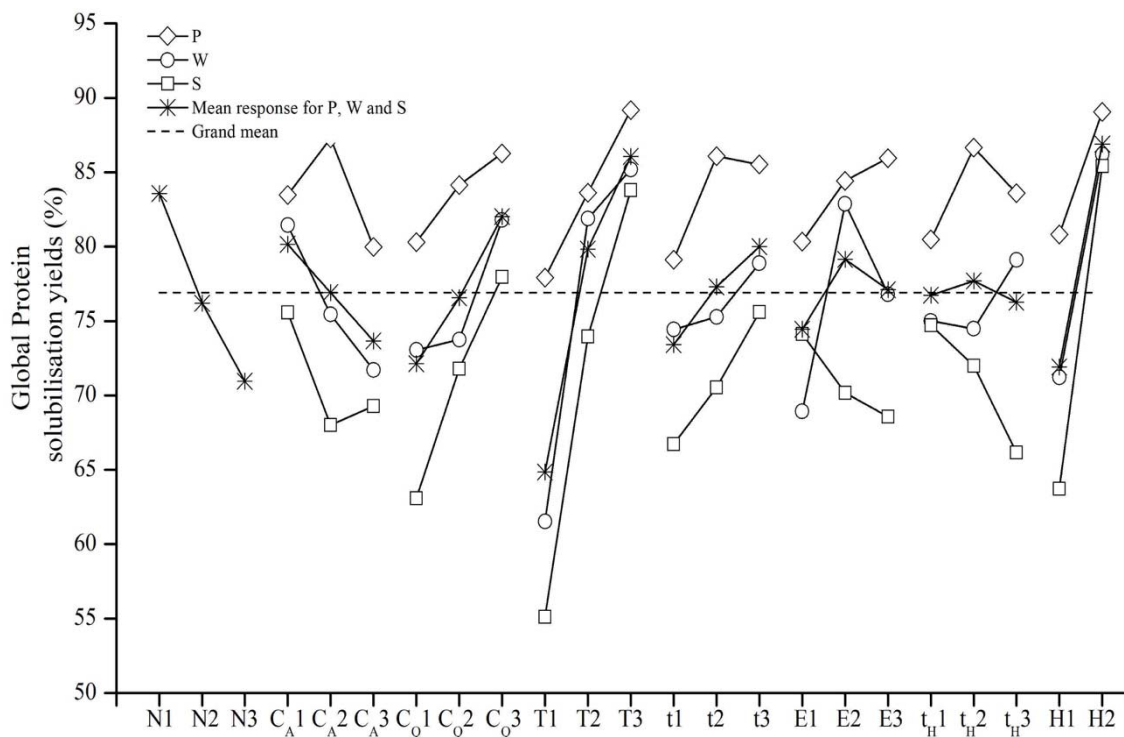


738

739 **Figure 3**
740 **(a)**

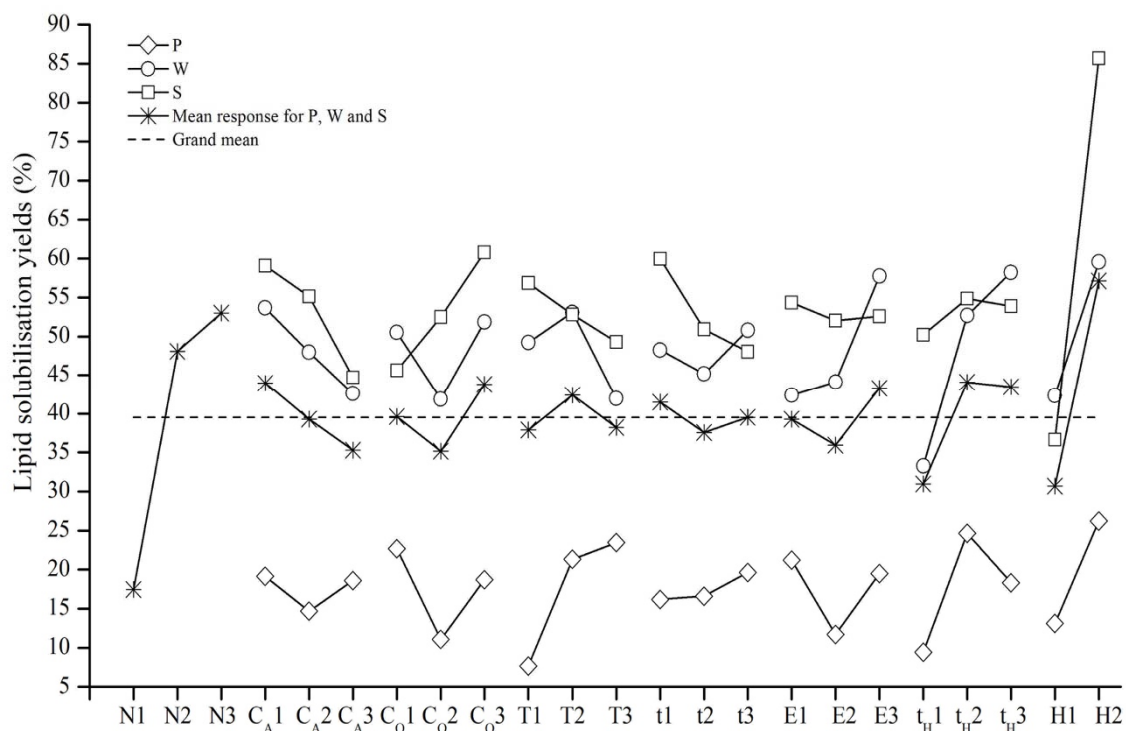


741 **(b)**
742
743

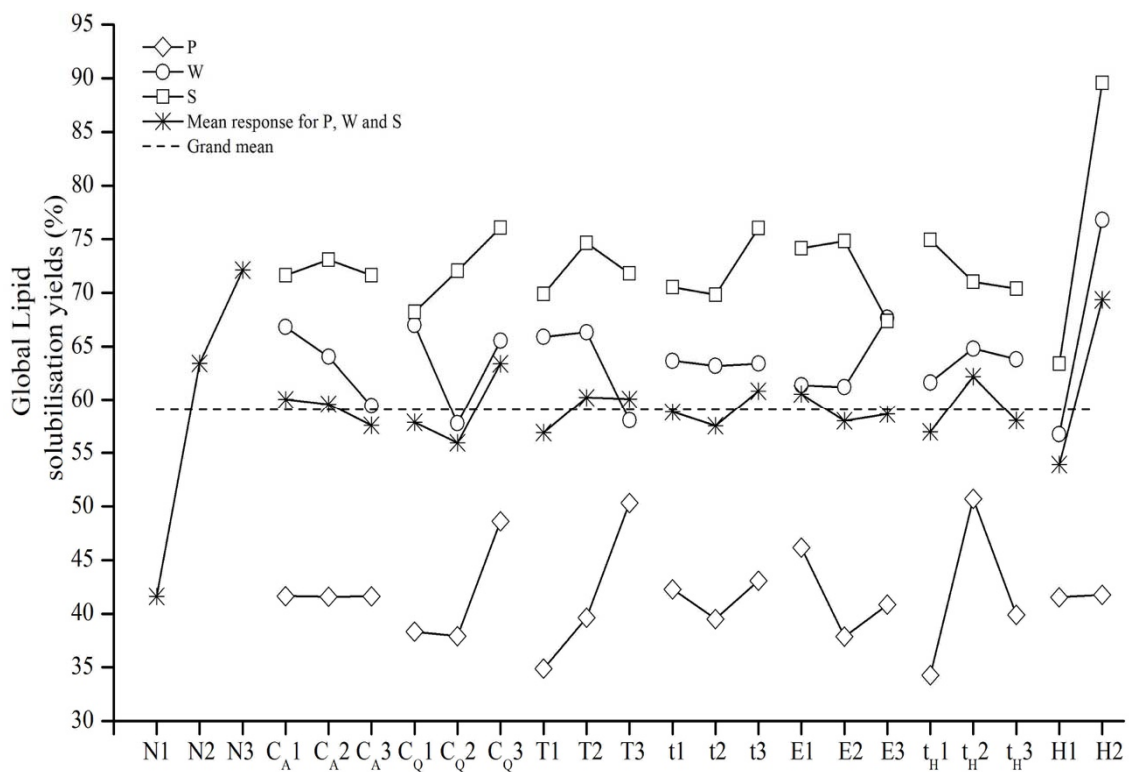


744

745 **Figure 4**
746 **(a)**



747 **(b)**
748



749

SUPPLEMENTARY MATERIALS

Optimisation of the production of fermentable monosaccharides from algal biomass grown in photobioreactors treating wastewater

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CONTENT

- Table S1
- Table S2
- Table S3
- Table S4
- Table S5
- Table S6

| Orthogonal array matrix | | | | | | | | | | | | | | Experimental results, in % | | | | | |
|-------------------------|-----------------------------|----------------|-------------------|-------------------|----------------|-------------------|-------------------|-----|-----------------------------|----------------|-----|-----------------------------|----------------|----------------------------|--------|----------------|--------|----------------|--------|
| Exp. No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | P ^h | Global | W ⁱ | Global | S ^j | Global |
| | C ₀ ^a | T ^b | C ₀ ×T | C ₀ ×T | t ^c | C ₀ ×t | C ₀ ×t | T×t | C _A ^d | E ^c | Txt | t _H ^f | H ^g | PR | Global | PR | Global | PR | Global |
| 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 22 | 47 | 7 | 51 | 8 | 28 |
| 2 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 24 | 67 | 10 | 80 | 39 | 64 |
| 3 | 1 | 1 | 1 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 1' | 16 | 30 | 17 | 29 | 18 | 44 |
| 4 | 1 | 2 | 2 | 2 | 1 | 1 | 1 | 2 | 2 | 2 | 3 | 3 | 1' | 26 | 51 | 26 | 69 | 32 | 54 |
| 5 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 1 | 1 | 1 | 30 | 55 | 24 | 49 | 26 | 55 |
| 6 | 1 | 2 | 2 | 2 | 3 | 3 | 3 | 1 | 1 | 1 | 2 | 2 | 2 | 62 | 85 | 32 | 69 | 77 | 94 |
| 7 | 1 | 3 | 3 | 3 | 1 | 1 | 1 | 3 | 3 | 3 | 2 | 2 | 2 | 46 | 71 | 34 | 62 | 41 | 79 |
| 8 | 1 | 3 | 3 | 3 | 2 | 2 | 2 | 1 | 1 | 1 | 3 | 3 | 1' | 51 | 83 | 50 | 80 | 48 | 86 |
| 9 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 1 | 1 | 1 | 53 | 86 | 45 | 69 | 28 | 85 |
| 10 | 2 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 1' | 15 | 38 | 18 | 38 | 12 | 22 |
| 11 | 2 | 1 | 2 | 3 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 15 | 43 | 12 | 38 | 19 | 51 |
| 12 | 2 | 1 | 2 | 3 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 54 | 65 | 33 | 87 | 68 | 87 |
| 13 | 2 | 2 | 3 | 1 | 1 | 2 | 3 | 2 | 3 | 1 | 3 | 1 | 2 | 25 | 52 | 40 | 65 | 44 | 82 |
| 14 | 2 | 2 | 3 | 1 | 2 | 3 | 1 | 3 | 1 | 2 | 1 | 2 | 1' | 49 | 76 | 52 | 79 | 38 | 75 |
| 15 | 2 | 2 | 3 | 1 | 3 | 1 | 2 | 1 | 2 | 3 | 2 | 3 | 1 | 44 | 73 | 50 | 74 | 43 | 73 |
| 16 | 2 | 3 | 1 | 2 | 1 | 2 | 3 | 3 | 1 | 2 | 2 | 3 | 1 | 43 | 74 | 53 | 79 | 58 | 83 |
| 17 | 2 | 3 | 1 | 2 | 2 | 3 | 1 | 1 | 2 | 3 | 3 | 1 | 2 | 51 | 77 | 38 | 78 | 66 | 92 |
| 18 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 2 | 3 | 1 | 1 | 2 | 1' | 53 | 77 | 47 | 75 | 28 | 85 |
| 19 | 3 | 1 | 3 | 2 | 1 | 3 | 2 | 1 | 3 | 2 | 1 | 3 | 2 | 16 | 35 | 41 | 61 | 13 | 35 |
| 20 | 3 | 1 | 3 | 2 | 2 | 1 | 3 | 2 | 1 | 3 | 2 | 1 | 1' | 27 | 64 | 19 | 58 | 26 | 62 |
| 21 | 3 | 1 | 3 | 2 | 3 | 2 | 1 | 3 | 2 | 1 | 3 | 2 | 1 | 51 | 82 | 26 | 53 | 35 | 73 |
| 22 | 3 | 2 | 1 | 3 | 1 | 3 | 2 | 2 | 1 | 3 | 3 | 2 | 1 | 38 | 77 | 54 | 78 | 70 | 85 |
| 23 | 3 | 2 | 1 | 3 | 2 | 1 | 3 | 3 | 2 | 1 | 1 | 3 | 2 | 55 | 79 | 75 | 92 | 72 | 92 |
| 24 | 3 | 2 | 1 | 3 | 3 | 2 | 1 | 1 | 3 | 2 | 2 | 1 | 1' | 39 | 79 | 51 | 81 | 38 | 84 |
| 25 | 3 | 3 | 2 | 1 | 1 | 3 | 2 | 3 | 2 | 1 | 2 | 1 | 1' | 54 | 87 | 52 | 83 | 66 | 86 |
| 26 | 3 | 3 | 2 | 1 | 2 | 1 | 3 | 1 | 3 | 2 | 3 | 2 | 1 | 66 | 94 | 56 | 85 | 53 | 79 |
| 27 | 3 | 3 | 2 | 1 | 3 | 2 | 1 | 2 | 1 | 3 | 1 | 3 | 2 | 58 | 83 | 80 | 94 | 91 | 97 |

^aConcentration of chemical reagent (mol/L). 1=0.5, 2=1, 3=2.

^bTemperature (°C). 1=80, 2=100, 3=120

^ctime (min). 1=10, 2=30, 3=60.

^dConcentration of microalgae biomass (g/L). 1=50, 2=75, 3=100.

^eDosage of enzyme (FPU/g). 1=10, 2=30, 3=60.

^fTime during the enzymatic hydrolysis (h). 1=3, 2=6, 3=12.

^gChemical reagent. 1=HCl, 2=NaOH, 1'=HCl.

^hP: microalgae biomass grown in pig manure wastewater.

ⁱW: microalgae biomass grown in domestic wastewater.

^jS: microalgae biomass grown in synthetic media.

Table S2: ANOVA tables of the results from the pretreatment step showing degrees of freedom (DF), sum of squares (SS), p-value (p) and percentages of contributions (C) of factors and interactions for the experimental design at three noise levels. In italics, non-significant factors/interactions pooled to estimate the residual variance.

| Source of variation ^a | Carbohydrates | | | | Monosaccharides | | | | Proteins | | | | Lipids | | | |
|----------------------------------|---------------|------------|-------|----|-----------------|--------|-------|----|----------|-------|-------|----|--------|-------------|-------|----|
| | DF | SS | p | C | DF | SS | p | C | DF | SS | p | C | DF | SS | p | C |
| C _Q | 2 | 4063 | 0.000 | 13 | 2 | 3829 | 0.000 | 9 | 2 | 3819 | 0.000 | 11 | 2 | <i>1009</i> | | |
| T | 2 | 11913 | 0.000 | 38 | 2 | 141809 | 0.000 | 34 | 2 | 13985 | 0.000 | 39 | 2 | <i>342</i> | | |
| C _Q x T | 4 | <i>349</i> | | | 4 | 2022 | 0.000 | 5 | 4 | 92 | | | 4 | <i>1826</i> | | |
| t | 2 | 1508 | 0.000 | 5 | 2 | 1579 | 0.000 | 4 | 2 | 1023 | 0.001 | 3 | 2 | <i>206</i> | | |
| C _Q x t | 4 | 831 | 0.010 | 3 | 4 | 2787 | 0.000 | 7 | 4 | 1034 | 0.004 | 3 | 4 | <i>2796</i> | | |
| T x t | 4 | 1523 | 0.000 | 5 | 4 | 1306 | 0.004 | 3 | 4 | 886 | 0.008 | 2 | 4 | <i>301</i> | | |
| C _A | 2 | 576 | 0.009 | 2 | 2 | 1173 | 0.001 | 3 | 2 | 2096 | 0.000 | 6 | 2 | <i>1015</i> | | |
| H | 1 | 3685 | 0.000 | 12 | 1 | 8179 | 0.000 | 20 | 1 | 7430 | 0.000 | 21 | 1 | 12607 | 0.000 | 21 |
| N | 2 | 265 | | | 2 | 2303 | 0.000 | 6 | 2 | 18 | | | 2 | 19993 | 0.000 | 33 |
| C _Q xN | 4 | <i>109</i> | | | 4 | 277 | | | 4 | 289 | | | 4 | <i>1183</i> | | |
| TxN | 4 | <i>164</i> | | | 4 | 156 | | | 4 | 343 | | | 4 | <i>1820</i> | | |
| (C _Q xT)xN | 8 | 1265 | 0.011 | 4 | 8 | 430 | | | 8 | 681 | | | 8 | 2365 | | |
| txN | 4 | 834 | 0.010 | 3 | 4 | 568 | | | 4 | 313 | | | 4 | 696 | | |
| (C _Q xT)xN | 8 | 270 | | | 8 | 355 | | | 8 | 390 | | | 8 | 919 | | |
| (TxT)xN | 8 | 418 | | | 8 | 474 | | | 8 | 1370 | 0.008 | 4 | 8 | 1537 | | |
| C _A xN | 4 | 1254 | 0.001 | 4 | 4 | 70 | | | 4 | 781 | 0.016 | 2 | 4 | 636 | | |
| HxN | 2 | 1197 | 0.000 | 4 | 2 | 183 | | | 2 | 571 | 0.011 | 2 | 2 | 4641 | 0.001 | 8 |
| Residual | 45 | 2477 | | 8 | 57 | 4365 | | 10 | 49 | 2818 | | 8 | 75 | 22698 | | 38 |
| Total | 80 | 31126 | | | 80 | 41722 | | | 80 | 35814 | | | 80 | 59939 | | |

^aC_Q: Concentration of chemical reagent, T: Temperature, t: time, C_A: Concentration of microalgae biomass, H: Chemical reagent, and N: microalgae biomass harvested from different wastewater treatments (noise).

Table S3: ANOVA tables for the signal to noise values of the 27 experiments for pretreatment results, showing degrees of freedom (DF), sum of squares (SS), p-value (p) and percentages of contributions (C) of factors and factor interactions for the experimental design at three noise levels.

| Source of variation ^a | Carbohydrates | | | | Monosaccharides | | | | Proteins | | | | Lipids | | | |
|----------------------------------|---------------|-----|-------|----|-----------------|------|-------|----|----------|-----|-------|----|--------|-----|-------|----|
| | DF | SS | p | C | DF | SS | p | C | DF | SS | p | C | DF | SS | p | C |
| C _Q | 2 | 47 | 0.001 | 15 | 2 | 208 | 0.014 | 13 | 2 | 41 | 0.023 | 9 | 2 | 12 | | |
| T | 2 | 153 | 0.000 | 48 | 2 | 676 | 0.000 | 42 | 2 | 231 | 0.000 | 50 | 2 | 16 | | |
| C _Q x T | 4 | 13 | | | 4 | 77 | | | 4 | 7 | | | 4 | 25 | | |
| t | 2 | 28 | 0.007 | 9 | 2 | 142 | 0.045 | 9 | 2 | 17 | | | 2 | 20 | | |
| C _Q x t | 4 | 10 | | | 4 | 105 | | | 4 | 23 | | | 4 | 32 | | |
| T x t | 4 | 32 | 0.020 | 10 | 4 | 31 | | | 4 | 21 | | | 4 | 3 | | |
| C _A | 2 | 2 | | | 2 | 76 | | | 2 | 19 | | | 2 | 10 | | |
| H | 1 | 26 | 0.003 | 8 | 1 | 212 | 0.004 | 13 | 1 | 94 | 0.000 | 21 | 1 | 185 | 0.000 | 55 |
| Residual | 15 | 30 | | 10 | 19 | 368 | | 23 | 21 | 93 | | 20 | 25 | 150 | | 45 |
| Total | 26 | 315 | | | 26 | 1606 | | | 26 | 459 | | | 26 | 335 | | |

^aC_Q: Concentration of chemical reagent, T: Temperature, t: time, C_A: Concentration of microalgae biomass, H: Chemical reagent, and N: noise.

Table S4: Taguchi's L₂₇(3)¹³ orthogonal array and experimental results for carbohydrates, proteins and lipids solubilisation, and monosaccharides recovery in the global process (pretreatment followed by enzymatic hydrolysis).

| Orthogonal array matrix | | | | | | | | | | | | | | Experimental results, in % | | | | | | | | | | | |
|-------------------------|-----------------------------|----------------|-------------------|-------------------|----------------|-------------------|-------------------|-----|-----------------------------|----------------|-----|-----------------------------|----------------|----------------------------|----------------|----------------|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Exp. No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | Carbohydrates | | | Monosaccharides | | | Proteins | | | Lipids | | |
| | C _Q ^a | T ^b | C _Q xT | C _Q xT | t ^c | C _Q xt | C _Q xt | Txt | C _A ^d | E ^e | Txt | t _H ^f | H ^g | P ^h | W ⁱ | S ^j | P ^h | W ⁱ | S ^j | P ^h | W ⁱ | S ^j | P ^h | W ⁱ | S ^j |
| 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 38 | 75 | 39 | 5 | 14 | 9 | 54 | 41 | 40 | 11 | 77 | 65 |
| 2 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 67 | 87 | 58 | 11 | 20 | 24 | 89 | 78 | 68 | 14 | 87 | 83 |
| 3 | 1 | 1 | 1 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 1' | 47 | 99 | 54 | 6 | 53 | 15 | 67 | 66 | 50 | 26 | 68 | 46 |
| 4 | 1 | 2 | 2 | 2 | 1 | 1 | 1 | 2 | 2 | 2 | 3 | 3 | 1' | 86 | 87 | 69 | 12 | 25 | 34 | 77 | 72 | 35 | 40 | 71 | 60 |
| 5 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 1 | 1 | 1 | 82 | 98 | 91 | 19 | 46 | 47 | 85 | 61 | 50 | 31 | 57 | 53 |
| 6 | 1 | 2 | 2 | 2 | 3 | 3 | 3 | 1 | 1 | 1 | 2 | 2 | 2 | 50 | 69 | 100 | 15 | 34 | 55 | 86 | 85 | 91 | 68 | 79 | 89 |
| 7 | 1 | 3 | 3 | 3 | 1 | 1 | 1 | 3 | 3 | 3 | 2 | 2 | 2 | 69 | 87 | 97 | 15 | 35 | 83 | 86 | 77 | 79 | 60 | 69 | 78 |
| 8 | 1 | 3 | 3 | 3 | 2 | 2 | 2 | 1 | 1 | 1 | 3 | 3 | 1' | 91 | 92 | 97 | 78 | 61 | 87 | 88 | 91 | 81 | 37 | 53 | 58 |
| 9 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 1 | 1 | 1 | 94 | 97 | 92 | 57 | 72 | 64 | 91 | 87 | 74 | 57 | 41 | 82 |
| 10 | 2 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 1' | 65 | 61 | 49 | 7 | 23 | 18 | 80 | 45 | 42 | 56 | 76 | 61 |
| 11 | 2 | 1 | 2 | 3 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 72 | 70 | 67 | 7 | 11 | 25 | 71 | 32 | 38 | 47 | 53 | 61 |
| 12 | 2 | 1 | 2 | 3 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 34 | 89 | 84 | 16 | 18 | 38 | 91 | 84 | 87 | 23 | 80 | 89 |
| 13 | 2 | 2 | 3 | 1 | 1 | 2 | 3 | 2 | 3 | 1 | 3 | 1 | 2 | 66 | 91 | 94 | 20 | 38 | 41 | 77 | 79 | 95 | 40 | 55 | 94 |
| 14 | 2 | 2 | 3 | 1 | 2 | 3 | 1 | 3 | 1 | 2 | 1 | 2 | 1' | 89 | 98 | 86 | 48 | 59 | 76 | 83 | 82 | 72 | 36 | 48 | 71 |
| 15 | 2 | 2 | 3 | 1 | 3 | 1 | 2 | 1 | 2 | 3 | 2 | 3 | 1 | 81 | 91 | 86 | 76 | 57 | 75 | 91 | 84 | 64 | 16 | 41 | 65 |
| 16 | 2 | 3 | 1 | 2 | 1 | 2 | 3 | 3 | 1 | 2 | 2 | 3 | 1 | 93 | 100 | 93 | 56 | 74 | 84 | 81 | 88 | 70 | 48 | 42 | 51 |
| 17 | 2 | 3 | 1 | 2 | 2 | 3 | 1 | 1 | 2 | 3 | 3 | 1 | 2 | 71 | 98 | 90 | 30 | 67 | 61 | 97 | 95 | 93 | 27 | 76 | 82 |
| 18 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 2 | 3 | 1 | 1 | 2 | 1' | 91 | 96 | 94 | 57 | 70 | 74 | 87 | 75 | 85 | 48 | 51 | 75 |
| 19 | 3 | 1 | 3 | 2 | 1 | 3 | 2 | 1 | 3 | 2 | 1 | 3 | 2 | 76 | 63 | 57 | 27 | 19 | 32 | 85 | 91 | 64 | 30 | 58 | 98 |
| 20 | 3 | 1 | 3 | 2 | 2 | 1 | 3 | 2 | 1 | 3 | 2 | 1 | 1' | 86 | 85 | 77 | 46 | 21 | 38 | 78 | 68 | 58 | 44 | 50 | 64 |
| 21 | 3 | 1 | 3 | 2 | 3 | 2 | 1 | 3 | 2 | 1 | 3 | 2 | 1 | 95 | 98 | 71 | 69 | 53 | 56 | 86 | 50 | 49 | 62 | 44 | 62 |
| 22 | 3 | 2 | 1 | 3 | 1 | 3 | 2 | 2 | 1 | 3 | 3 | 2 | 1 | 98 | 99 | 91 | 61 | 49 | 80 | 91 | 99 | 82 | 46 | 79 | 62 |
| 23 | 3 | 2 | 1 | 3 | 2 | 1 | 3 | 3 | 2 | 1 | 1 | 3 | 2 | 91 | 94 | 87 | 46 | 31 | 39 | 92 | 91 | 94 | 53 | 94 | 98 |
| 24 | 3 | 2 | 1 | 3 | 3 | 2 | 1 | 1 | 3 | 2 | 2 | 1 | 1' | 92 | 99 | 84 | 86 | 67 | 71 | 70 | 84 | 81 | 26 | 73 | 80 |
| 25 | 3 | 3 | 2 | 1 | 1 | 3 | 2 | 3 | 2 | 1 | 2 | 1 | 1' | 97 | 98 | 95 | 64 | 68 | 91 | 82 | 77 | 93 | 49 | 46 | 66 |
| 26 | 3 | 3 | 2 | 1 | 2 | 1 | 3 | 1 | 3 | 2 | 3 | 2 | 1 | 97 | 99 | 86 | 57 | 66 | 74 | 92 | 80 | 80 | 65 | 51 | 59 |
| 27 | 3 | 3 | 2 | 1 | 3 | 2 | 1 | 2 | 1 | 3 | 1 | 3 | 2 | 97 | 99 | 89 | 62 | 42 | 42 | 99 | 96 | 99 | 61 | 94 | 96 |

^aConcentration of chemical reagent (mol/L). 1=0.5, 2=1, 3=2.

^bTemperature (°C). 1=80, 2=100, 3=120

^ctime (min). 1=10, 2=30, 3=60.

^dConcentration of microalgae biomass (g/L). 1=50, 2=75, 3=100.

^eDosage of enzyme (FPU/g). 1=10, 2=30, 3=60.

^fTime during the enzymatic hydrolysis (h). 1=3, 2=6, 3=12.

^gChemical reagent. 1=HCl, 2=NaOH, 1'=HCl.

^hP: microalgae biomass grown in pig manure wastewater.

ⁱW: microalgae biomass grown in domestic wastewater.

^jS: microalgae biomass grown in synthetic media.

Table S5: ANOVA tables for the global process (pretreatment and enzymatic hydrolysis) responses showing degrees of freedom (DF), sum of squares (SS), p-value (p) and percentages of contributions (C) of factors and interactions for the experimental design at three levels of noise. In italics, non-significant factors/interactions pooled to estimate the residual variance.

| Source of variation ^a | Carbohydrates | | | | Monosaccharides | | | | Proteins | | | | Lipids | | | |
|----------------------------------|---------------|-------|-------|----|-----------------|-------|-------|----|----------|-------|-------|----|--------|-------|-------|----|
| | DF | SS | p | C | DF | SS | p | C | DF | SS | p | C | DF | SS | p | C |
| C _Q | 2 | 1639 | 0.000 | 7 | 2 | 3953 | 0.000 | 8 | 2 | 1319 | 0.000 | 6 | 2 | 819 | 0.048 | 2 |
| T | 2 | 8158 | 0.000 | 37 | 2 | 19387 | 0.000 | 41 | 2 | 6409 | 0.000 | 29 | 2 | 190 | | |
| C _Q x T | 4 | 359 | | | 4 | 2874 | 0.001 | 6 | 4 | 317 | | | 4 | 1098 | | |
| t | 2 | 651 | 0.032 | 3 | 2 | 1903 | 0.001 | 4 | 2 | 591 | 0.009 | 3 | 2 | 151 | | |
| C _Q x t | 4 | 349 | | | 4 | 1640 | 0.016 | 3 | 4 | 1442 | 0.000 | 6 | 4 | 549 | | |
| T x t | 4 | 1274 | 0.011 | 6 | 4 | 1710 | 0.013 | 4 | 4 | 181 | | | 4 | 603 | | |
| C _A | 2 | 29 | | | 2 | 249 | | | 2 | 571 | 0.011 | 3 | 2 | 94 | | |
| E | 2 | 29 | | | 2 | 247 | | | 2 | 299 | | | 2 | 98 | | |
| t _H | 2 | 6 | | | 2 | 251 | | | 2 | 29 | | | 2 | 412 | | |
| H | 1 | 395 | 0.040 | 2 | 1 | 3940 | 0.000 | 8 | 1 | 4032 | 0.000 | 18 | 1 | 4311 | 0.000 | 13 |
| e _H | 1 | 16 | | | 1 | 1 | | | 1 | 66 | | | 1 | 47 | | |
| N | 2 | 1973 | 0.000 | 9 | 2 | 2734 | 0.000 | 6 | 2 | 2171 | 0.000 | 10 | 2 | 13341 | 0.000 | 40 |
| C _Q xN | 4 | 1292 | 0.011 | 6 | 4 | 2121 | 0.004 | 5 | 4 | 278 | | | 4 | 567 | | |
| TxN | 4 | 683 | | | 4 | 522 | | | 4 | 938 | 0.006 | 4 | 4 | 1432 | 0.034 | 4 |
| (C _Q xT)xN | 8 | 1045 | | | 8 | 1097 | | | 8 | 736 | | | 8 | 3322 | 0.004 | 10 |
| txN | 4 | 312 | | | 4 | 718 | | | 4 | 137 | | | 4 | 120 | | |
| (C _Q xT)xN | 8 | 358 | | | 8 | 1159 | | | 8 | 175 | | | 8 | 1267 | | |
| (TxT)xN | 8 | 1017 | | | 8 | 1026 | | | 8 | 132 | | | 8 | 578 | | |
| C _A xN | 4 | 472 | | | 4 | 581 | | | 4 | 399 | | | 4 | 170 | | |
| E _x N | 4 | 223 | | | 4 | 81 | | | 4 | 881 | 0.009 | 4 | 4 | 769 | | |
| t _H xN | 4 | 572 | | | 4 | 592 | | | 4 | 603 | 0.046 | 3 | 4 | 1000 | | |
| H _x N | 2 | 1083 | 0.004 | 5 | 2 | 177 | | | 2 | 543 | 0.013 | 2 | 2 | 2203 | 0.001 | 7 |
| e _H xN | 2 | 2 | | | 2 | 5 | | | 2 | 198 | | | 2 | 129 | | |
| Residual | 61 | 5472 | | 25 | 55 | 6704 | | 14 | 51 | 2946 | | 13 | 61 | 7842 | | 24 |
| Total | 80 | 21938 | | | 80 | 46966 | | | 80 | 22447 | | | 80 | 33269 | | |

^aC_Q: Concentration of chemical reagent, T: Temperature, t: time, C_A: Concentration of microalgae biomass, E: dosage of enzyme, t_H: time of enzymatic hydrolysis, H: Chemical reagent, e_H: dummy effect, and N: noise.

Table S6: ANOVA tables for the signal to noise values of the 27 experiments for global (pretreatment and enzymatic hydrolysis) results, showing degrees of freedom (DF), sum of squares (SS), p-value (p) and percentages of contribution (C) of factors and factor interactions for the experimental design at three noise levels.

| Source of variation ^a | Carbohydrates | | | | Monosaccharides | | | | Proteins | | | | Lipids | | | |
|----------------------------------|---------------|----|-------|----|-----------------|------|-------|----|----------|-----|-------|----|--------|----|-------|----|
| | DF | SS | p | C | DF | SS | p | C | DF | SS | p | C | DF | SS | p | C |
| C _Q | 2 | 12 | 0.014 | 13 | 2 | 203 | 0.004 | 20 | 2 | 9 | | | 2 | 4 | | |
| T | 2 | 53 | 0.000 | 58 | 2 | 527 | 0.000 | 51 | 2 | 45 | 0.000 | 39 | 2 | 0 | | |
| C _Q x T | 4 | 5 | | | 4 | 97 | | | 4 | 5 | | | 4 | 6 | | |
| t | 2 | 5 | | | 2 | 55 | | | 2 | 5 | | | 2 | 1 | | |
| C _Q x t | 4 | 3 | | | 4 | 63 | | | 4 | 14 | | | 4 | 2 | | |
| T x t | 4 | 7 | | | 4 | 11 | | | 4 | 1 | | | 4 | 5 | | |
| C _A | 2 | 1 | | | 2 | 18 | | | 2 | 4 | | | 2 | 0 | | |
| E | 2 | 0 | | | 2 | 22 | | | 2 | 3 | | | 2 | 0 | | |
| t _H | 2 | 1 | | | 2 | 6 | | | 2 | 1 | | | 2 | 2 | | |
| H | 1 | 4 | | | 1 | 35 | | | 1 | 27 | 0.001 | 23 | 1 | 34 | 0.000 | 61 |
| e _H | 1 | 0 | | | 1 | 1 | | | 1 | 1 | | | 1 | 0 | | |
| Residual | 22 | 26 | | 29 | 22 | 310 | | 30 | 23 | 43 | | 38 | 25 | 21 | | 39 |
| Total | 26 | 91 | | | 26 | 1040 | | | 26 | 114 | | | 26 | 55 | | |

^aC_Q: Concentration of chemical reagent, T: Temperature, t: time, C_A: Concentration of microalgae biomass, E: dosage of enzyme, t_H: time of enzymatic hydrolysis, H: Chemical reagent, and e_H: dummy effect.