

1 **Comparison of several mathematical models for describing the joint effect of**
2 **temperature and pH on glucanex activity**

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15 **ABSTRACT**

16 The aim of the present work was to evaluate with different statistical criteria the suitability of
17 nine equations for describing and optimizing the simultaneous effect of temperature and pH on
18 glucanex activity using two characteristic polysaccharides (curdlan and laminarin) as substrates.
19 The most satisfactory solutions were found with an empirical equation constituted with
20 parameters of practical interest (Rosso model), and a hybrid model between the Arrhenius
21 equation and the mathematical expression generated by the protonation-hydroxylation
22 mechanism (Tijskens model). The joint optimal values of pH and temperature calculated with the
23 Rosso model were obtained at 4.64 and 50°C with curdlan and 4.64 and 48°C using laminarin as
24 substrate.

25
26 **Keywords:** glucanex, enzymatic activity, curdlan, laminarin, mathematical modelling, pH and
27 temperature effects.

28
29 **1. INTRODUCTION**

30 Describing the combined effect of temperature and pH on the enzymatic reaction rate is a
31 frequent problem that is not always solved satisfactorily. When they are studied independently,
32 the two variables produce well-known rate profiles that increase to a maximum point which is
33 then followed by a drop in activity.^{1,2,3} The results from experiments repeatedly demonstrate that
34 the profiles obtained for the independent variables are linked to each other, generally in a non-
35 additive interaction.^{1,2,3} The correct modeling of these experimental profiles is especially
36 important when a rigorous and predictive quantification of the maximum enzymatic activity, and
37 minimum, optimum and maximum pH and temperature values are necessary, for example, in the
38 case of an enzyme reactor that is controlled by software.

39

40 The Arrhenius model provides a possible resource for modelling temperature when it is applied
41 to the three rates involved in the process: the substrate transformation rate and the enzyme
42 denaturation rate at high and low temperatures. This approach was used to describe the
43 metabolism of poikilotherm organisms in which the restrictive factor of the rate is defined by
44 only one enzyme.^{4,5} The equation obtained was later reformed by Schoolfield et al.⁶ who inserted
45 a reference rate at a pre-established temperature and redefined the parameters (six in both
46 mathematical expressions) in order to reduce the correlation among them. In spite of the formal
47 basis of this last approach, the empirical equation proposed by different authors^{7,8,9} and modified
48 by Zwietering et al.¹⁰ generated the best fits (with only four parameters) when several
49 mathematical models for assessing the effect of temperature on the bacterial growth of
50 *Lactobacillus plantarum* on a conventional culture medium MRS were compared.¹⁰ The same
51 model led to satisfactory fits for describing *Salmonella* growth on soudjouk-style fermented
52 semi-dry sausage.¹¹ In enzyme kinetics, the Arrhenius equation has been used extensively to
53 model the effect of temperature on the increase in reaction rate,^{12,13,14} on the deactivation
54 constant^{15,16} and on the thermal stability of enzymes.^{17,18}

55
56 The requirements for modelling pH are similar or larger than those defined by the Arrhenius
57 equation for temperature. The most formal description is based on an acid-base dissociation
58 mechanism.^{1,2,3} The use of this resource provides good fit results as well as parameters with a
59 clear physical meaning and in some cases for defining the optimal intervals of enzyme activity.

60
61 Glucanex is a multicomponent enzyme preparation consisting of several isoenzymes that contain
62 β -1,3 glucanase activity,¹⁹ that is used commonly for hydrolyzing the oligosaccharides from
63 yeast cell walls in order to obtain β -glucans,²⁰ to control wine spoilage yeasts, protoplast
64 preparation and as a biocontrol agent against plant pathogenic fungi.^{21,22,23} Nevertheless, there
65 are no studies describing the kinetic characteristics of this enzyme, such as the combined effect

66 of temperature and pH on glucanex activity, and there are no data on the optimum temperature
67 and pH in the literature.

68
69 The aim of the present work was to evaluate and compare the suitability of nine equations for
70 describing the combined effect of pH and temperature on the catalytic activity of a commercial
71 glucanolytic preparation (glucanex) in the hydrolysis of two gluco-polysaccharides substrates:
72 curdlan ($\beta(1\rightarrow3)$) and laminarin ($\beta(1\rightarrow3):\beta(1\rightarrow6)$ ratio of 3:1).

73

74 **2. MATERIALS AND METHODS**

75 *2.1. Chemicals*

76 Both substrates, laminarin from *Laminaria digitata* and curdlan from *Alcaligenes faecalis* were
77 provided by Sigma (St. Louis, MO, USA). Glucanex[®] 200G was obtained from Novozymes
78 Corp (Copenhagen, Denmark). Other reagents used in enzymatic assays were of analytical grade
79 and purchased from Sigma.

80

81 *2.2. Methodology for measuring the enzyme activity of Glucanex[®] 200G*

82 Two different substrates (curdlan and laminarin) were dissolved in 0.02M citric/phosphate buffer
83 at various pHs. The enzyme activity was tested by placing 0.4 mL of the substrate solution, at the
84 required pH for the analysis, into a 30 mL tube (in duplicate) with a teflon cap. The tubes were
85 then put into a controlled thermostatic water bath with continuous mild agitation. After reaching
86 the bath temperature, 0.1 mL of a fresh enzyme solution (0.005 M citric/phosphate buffer for
87 each pH assay) of Glucanex[®] 200G (the concentration varied) was added, and thus 2.5 mg/L of
88 substrate was obtained in the final reaction solution. The reaction was ended when the analytical
89 time (varied) finished by adding 0.5 mL of 3,5-dinitrosalicylic acid (DNS). The enzyme activity
90 was measured by determining the sugars released by the reaction with DNS using glucose as the
91 substrate.²³

92

93 *2.3. Preliminary assays for establishing the initial conditions*

94 In order to determine the proper conditions for evaluating the joint effect of temperature and pH
95 on the enzymatic activity, initial experiments were carried out to establish: a) an appropriate
96 range for the temperature and pH; b) a suitable ratio between the substrate and enzyme
97 (Glucanex[®] 200G); and c) an analytical time in which the product formation rate would continue
98 to show a linear profile.

99

100 *2.3.1. The temperature and pH range*

101 The appropriate pH and temperature ranges were obtained by studying the two variables
102 separately (with a constant pH of 4.5 for the temperature assays, and at 45°C for the pH assays)
103 with curdlan as the substrate, an analytical time of 20 min and an enzyme-substrate ratio of 3:10
104 (750 µg/L). The maximum enzyme activity was obtained at 45 °C and pH 4.5 (Figure 1a). The
105 final range selection for pH (3.5 to 6) and temperature (32 to 60 °C) were chosen around the
106 individual optima where the product conversion reaches 50% (see experimental points included
107 into the selected range box, Figure 1a). The experimental data were expressed as the percentage
108 of the maximum concentration of the product formed (RS).

109

110 *2.3.2. The substrate and enzyme ratio*

111 The suitable substrate/enzyme ratio was selected by carrying out a kinetic assay, measuring the
112 enzyme activity with different enzyme concentrations (from 0 to 1000 µg/L) at pH 4.5 and 45°C
113 with a constant curdlan concentration of 2.5 mg/L in the final solution. The experimental results
114 are shown in Figure 1b together with the profiles obtained by fitting the data to a first-order
115 kinetic model:

116

117
$$A_{Ez} = K(1 - e^{-at}) \quad [1]$$

118
119 where A_{Ez} as the enzymatic activity of glucanex (mg/L of RS released), t is the time of hydrolysis
120 (min), K is the asymptotic product formation (mg/L) obtained and α is the specific RS
121 production rate (min^{-1}). In the case of Glucanex, due to the absence of saturation, the value of K
122 it is equal to the maximum possible product conversion (2.5 mg/L) for all enzymatic
123 concentrations tested except for the case when no enzyme is present. The parameter α increases
124 as the enzyme concentration increases.

125
126 Finally, Figure 1c shows the nonlinear relation between the specific rate α , and the enzyme
127 concentration along with the fit to a similar mathematical expression [1], allowing us to analyse
128 its derivative and to obtain the optimum value for the enzyme-substrate ratio. The ideal
129 concentration was found to be approximately 250 μg of glucanex/L (a ratio of 1:10) and this
130 value was maintained for all subsequent experiments.

131
132 *2.3.3. The optimum analytical time*
133 The results obtained for the initial times (<1 h) are plotted in Figure 1d. A linear correlation
134 between the product formed and reaction time was observed for all enzyme concentrations over
135 the initial 30 minutes. An analytical time of 15 min was chosen in order to ensure the linearity of
136 product formation (mg/L) throughout the experiment. This time choice also avoids enzyme
137 denaturation, shortens the time of the assay, and produces enough reducing sugars for accurate
138 quantification.

139
140 *2.4. Combined effect of pH and temperature on glucanex activity*
141 The combined effect on glucanex activity (GA) was measured at several pHs (from 3.5 to 6 in
142 steps of 0.5) and at different incubation temperatures (from 32 to 60°C with different interval
143 steps for each substrate) with curdlan and laminarin as substrates. The enzyme activity (with

144 0.02M citric/phosphate buffer) was tested following the procedure described above using the
145 conditions previously selected: an analytical time of 15 min and enzyme/substrate ratio of 1:10
146 (250 µg/L) in the final solution. The experimental data were expressed as a percentage of the
147 maximum concentration obtained for each substrate.

148

149 *2.5. Fitting procedure and common statistical values*

150 Fitting procedures and parametric estimates from the experimental results were performed by
151 minimizing the sum of quadratic differences between the observed and model-predicted values,
152 using the nonlinear least-squares (quasi-Newton) method provided by the macro ‘*Solver*’ of the
153 *Microsoft Excel XP* spreadsheet. The confidence intervals from the parametric estimates
154 (Student’s *t* test) and the goodness of fit and consistency of the mathematical models (Fisher’s *F*
155 test) were determined using *DataFit 9.0.59* (Oakdale Engineering, Oakdale, PA, USA).

156

157 *2.6. Criteria used to assess the selection of the best model*

158 *2.6.1. Criteria based on model selection criteria (MSC)*

159 In the present work, the AICc, BIC, RIC, Cp, R^2_{adj} , FPE and MSC criteria (Table 1) were
160 directly obtained using an *Excel* spreadsheet. The leave one out cross-validation (LOO-CV)
161 procedure and Monte Carlo cross-validation (MCCV) were obtained with an *Excel* spreadsheet
162 using the *Excel* add-in *Solverstat* macro. This selected group is a combination of different criteria
163 that can discriminate between the models based on their goodness of fit, complexity, overfitting
164 and generalizability.

165 *2.6.2. Additional statistical criteria*

166 Additional criteria based on the following features were used to evaluate the mathematical
167 models: a) the residual distribution; b) the number of non-significant (NS) parameters ($\alpha = 0.05$);
168 c) the number of parameters with biological or physical meaning.

169

170 3. RESULTS AND DISCUSSION

171 3.1. Mathematical models describing the combined effect of temperature and pH on GA

172 We reviewed and studied the appropriateness of nine models from the literature for predicting
173 GA under different pH and temperature conditions. Those equations have different origins and
174 mathematical structure and can be classified as: a) regular models with empirical forms
175 (polynomials) whose parameters do not have any physical meaning; b) models useful and widely
176 in other fields of knowledge (i.e., microbial growth) for similar purpose; c) structured models
177 developed to study the combined effect of temperature and pH on enzymatic reactions.

178

179 3.1.1. Regular models

180 Model 1: The simplest approach for describing the joint effect of temperature and pH is defined
181 by a quadratic polynomial with a multiplicative term that combines the action of the two
182 independent variables on the response (enzymatic activity). This resource has been applied, for
183 example, to study amylase,¹ chitinase³³ and 1,3-glucanase³⁴ activity. When r is the enzymatic
184 activity, the mathematical function is as follows:

185

$$186 \quad r = b_0 + b_1T + b_2pH + b_{12}TpH + b_{11}T^2 + b_{22}pH^2 \quad [2]$$

187

188 Model 2: The previous equation [2] defines a parabolic surface that can acceptably approach the
189 response in a nearby environment to its maximum value. Thus, the following equation [3] could
190 represent a useful resource for determining the temperature and pH values that maximize the
191 activity. However, the response is generally asymmetric, and thus better fits are obtained by
192 adding two more terms to [2]:

193

$$194 \quad r = b_0 + b_1T + b_2pH + b_{12}TpH + b_{11}T^2 + b_{22}pH^2 + b_{112}T^2pH + b_{122}TpH^2 \quad [3]$$

195

196 3.1.2. Models used in other fields of knowledge

197 Model 3: If the objective is to describe a profile with an asymmetric dome form, a function with
198 bias towards the right side (*i.e.*, with an abrupt drop when $X \rightarrow 0$ and gentle drop when $X \rightarrow \infty$)
199 is:

200
201
$$r = aX^n \exp(-bX) \quad [4]$$

202
203 When $n = 1$, the equation represents a classic model of the population dynamics that describes
204 with basic principles the effect of intraspecific competition on reproductive success.³⁵ The
205 arbitrary resource of allowing n values that are different from 1 makes the profile of the
206 mathematical function more versatile.

207
208 Equation [4] was used by Murado et al.³⁶ to describe the production of amylases in solid-state
209 cultures by *Aspergillus oryzae* as a function of the saturation of the support in the liquid phase.
210 The same authors found that the results obtained by Lindenfelser and Ciegler,³⁷ relative to the
211 effect of the humidity percentage in ochratoxin A production with *Aspergillus ochraceus* using
212 solid-state fermentation on wheat grains, were satisfactorily fitted to equation [4].

213
214 The bivariate model applied to the activity is the multiplication of two equations [4]:

215
216
$$r = aT^{n_1} pH^{n_2} \exp(-b_3T - b_4pH) \quad [5]$$

217
218 However, since equation [4] defines a curve with an initial null ordinate, to use equation [5] in
219 our context we need to modify the origin by introducing two parameters (T_0 and pH_0) that
220 represent the T and pH values that make the activity null:

221

222 $r = a(T - T_0)^{n_1} (pH - pH_0)^{n_2} \exp[-b_3(T - T_0) - b_4(pH - pH_0)]$ [6]

223

224 Model 4: The effect of temperature on the rate of nucleotide decomposition (r) in cold-stored
 225 carp muscle was described by Ohta and Hirabara³⁸ with the empirical relation: $r^{1/2} = 0.065T +$
 226 0.518 , which⁸ is generalized as:

227

228 $r^{1/2} = c(T - T_0)$ [7]

229

230 It was applied to bacterial growth in a range of temperatures (in K) that covers a range from the
 231 minimum temperature (T_0) when the growth rate is null to the maximum temperature of growth.

232

233 Later on, the equation was reformed⁷ to expand its descriptive capacity to any temperature, and
 234 took on the following mathematical form:

235

236 $r^{1/2} = c(T - T_{\min}) \{1 - \exp[a_1(T - T_{\max})]\}$ [8]

237

238 where T_{\min} (with the same meaning as T_0 in equation [7]) and T_{\max} represent the limits of the
 239 temperature range beyond which the growth rate is null. Indeed, the exponential term becomes
 240 nil when $T_{\max} \gg T$ (so that equation [8] can be simplified to equation [7]) and increases when T
 241 is close to T_{\max} , so that r decreases from a certain T value and tends to zero when $T = T_{\max}$.

242

243 In a subsequent modification, Pronk et al.¹⁰ proposed the equation:

244

245 $r = c(T - T_{\min})^2 \{1 - \exp[a_1(T - T_{\max})]\}$ [9]

246

247 This equation differs from [6] because the decrease in r from a maximum is due to an
 248 exponential function instead of its square. In both cases, equations [8] and [9] produce more
 249 versatile profiles than [4], with the possibility of obtaining biases to the left or right.

250
 251 The inclusion of the pH in this model can be done by multiplying [9] by a polynomial equation
 252 formulated with this variable:

$$254 \quad r = c(T - T_{\min})^2 \left\{ 1 - \exp \left[a_1 (T - T_{\max}) \right] \right\} (c_0 + c_1 pH + c_2 pH^2) \quad [10]$$

255
 256 Model 5: Another option would be to accept that the relationship between the pH and the
 257 enzymatic activity leads to a function with the same structure as that used for the temperature.
 258 Thus, the combined response could be described by multiplying the two effects:

$$260 \quad r = c(T - T_{\min})^2 \left\{ 1 - \exp \left[a_1 (T - T_{\max}) \right] \right\} (pH - pH_{\min})^2 \left\{ 1 - \exp \left[a_2 (pH - pH_{\max}) \right] \right\} \quad [11]$$

261
 262 Model 6: Initially, the Rosso equation was used to describe the joint effect of temperature and
 263 pH on microbial growth,³⁹ but it can also be used in other fields, such as enzyme kinetics. It
 264 establishes the enzymatic activity (r) as a dependent variable:

$$266 \quad f(T) = \frac{(T - T_{\min})^2 (T - T_{\max})}{(T_{\text{opt}} - T_{\min}) \left[(T_{\text{opt}} - T_{\min})(T - T_{\text{opt}}) - (T_{\text{opt}} - T_{\max})(T_{\text{opt}} + T_{\min} - 2T) \right]}$$

$$268 \quad f(pH) = \frac{(pH - pH_{\min})(pH - pH_{\max})}{\left[(pH - pH_{\min})(pH - pH_{\max}) - (pH - pH_{\text{opt}})^2 \right]}$$

270 $r = r_m f(T) f(pH)$ [12]

271
 272 where r is the enzymatic activity, r_m is the maximum enzymatic activity, T is the temperature
 273 ($^{\circ}\text{C}$), T_{min} is the temperature below which no activity occurs, T_{max} is the temperature above which
 274 no enzymatic activity occurs, T_{opt} is the temperature at which the enzyme activity is optimal,
 275 pH_{min} is the pH below which no catalytic activity occurs, pH_{max} is the pH above which no
 276 activity occurs, and pH_{opt} is the pH at which the enzyme activity is optimal.

277
 278 Model 7: The accumulated function of the Weibull distribution is a very versatile resource when
 279 a symmetric sigmoid or parabolic profiles do not need to be simulated.⁴⁰ It has been successfully
 280 used in diverse experimental fields such as the study of biotoxins⁴¹ and the antioxidant capacity
 281 of different compounds.⁴² Its mathematical expression, in the case of defining the combined
 282 effect of temperature and pH on GA, can be written as:

283
 284
$$r = k \left(\frac{\alpha T^{\alpha-1}}{q^{\alpha}} \right) \exp \left[- \left(\frac{T}{q} \right)^{\alpha} \right] \left(\frac{\beta pH^{\beta-1}}{p^{\beta}} \right) \exp \left[- \left(\frac{pH}{p} \right)^{\beta} \right]$$
 [13]

285
 286 where r is the enzymatic activity, k , α , q , β and p are empirically determined parameters, and T is
 287 the temperature ($^{\circ}\text{C}$).

288
 289 *3.1.3. Structured models*

290 Model 8: The model proposed by Sharpe et al.⁴ is based on a combination of three Arrhenius
 291 equations:

292

293
$$r = \frac{k_r \exp\left(\frac{-E_r}{RT}\right)}{1 + k_a \exp\left(\frac{-E_a}{RT}\right) + k_b \exp\left(\frac{-E_b}{RT}\right)}$$
 [14]

294
 295 where T is the temperature (K), R is the ideal gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$), and the meaning
 296 of the parameters is defined by the Arrhenius model for each reaction considered: 1) substrate
 297 transformation (represented by subindex r), and 2) the reversible enzyme deactivations at high
 298 (subindex a) and low (subindex b) temperatures. Thus, k_i is the pre-exponential terms and E_i the
 299 activation energies (J mol^{-1}).

300
 301 The reformulation used by Schoolfield et al.⁶ is:

302
 303
$$r = \frac{r_r \frac{T}{T_r} \exp\left[\frac{H_r}{R} \left(\frac{1}{T_r} - \frac{1}{T}\right)\right]}{1 + \exp\left[\frac{H_a}{R} \left(\frac{1}{T_a} - \frac{1}{T}\right)\right] + \exp\left[\frac{H_b}{R} \left(\frac{1}{T_b} - \frac{1}{T}\right)\right]}$$
 [15]

304
 305 involving the enzymatic activity r_r to a reference temperature T_r (319 K), the substitution of
 306 activation energies by the enthalpies H_i and the introduction of temperatures T_a and T_b that
 307 determine, for excess and defect, respectively, the 50% drop in enzymatic activity. As in
 308 equation [9], the profile can be biased to the left or right.

309
 310 The effect of pH could also be included by multiplying equation [15] and a quadratic polynomial
 311 as follows:

312

$$r = \frac{r_r \frac{T}{T_r} \exp\left[\frac{H_r}{R} \left(\frac{1}{T_r} - \frac{1}{T}\right)\right]}{1 + \exp\left[\frac{H_a}{R} \left(\frac{1}{T_a} - \frac{1}{T}\right)\right] + \exp\left[\frac{H_b}{R} \left(\frac{1}{T_b} - \frac{1}{T}\right)\right]} (c_0 + c_1 pH + c_2 pH^2) \quad [16]$$

314
 315 Model 9: The only theoretical approach developed for fitting the joint effect of temperature and
 316 pH on enzymatic activity was proposed by Tijssens et al.³ in order to study this effect in phytase,
 317 peroxidase and lipase catalysis.⁴³ This equation combines the Arrhenius model that explains the
 318 temperature effect with an acid-base dissociation reaction for the pH effect:

$$r = \frac{r_m k_{sr} \exp\left[\frac{E_s}{R} \left(\frac{1}{T_r} - \frac{1}{T}\right)\right] \exp\left[-k_{dr} t \exp\left[\frac{E_d}{R} \left(\frac{1}{T_r} - \frac{1}{T}\right)\right]\right]}{1 + \frac{H^+}{K_{EH}} + \frac{K_w}{K_{EOH}} \frac{1}{H^+}} \quad [17]$$

321
 322 where r is the enzymatic activity, r_m is the maximum enzymatic activity, which was maintained
 323 constant at a value of 100, t is the reaction time (10 min), T is the temperature (K), T_r is the
 324 reference temperature (313 K), k_{sr} is the specific reference rate for the enzymatic process (min^{-1}),
 325 k_{dr} is the specific reference rate for the deactivation enzymatic process (min^{-1}), E_d is the
 326 activation energy for the catalytic process (J mol^{-1}), E_s is the deactivation energy for the catalytic
 327 process (J mol^{-1}), R is the ideal gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$), H^+ is the pH value with the
 328 expression $H^+ = 10^{-\text{pH}}$, K_w is the water dissociation constant, and K_{EH} and K_{EOH} are the equilibrium
 329 constants of the protonation and hydroxylation reactions respectively.

330
 331 The combined effects of pH and temperature on GA using laminarin and curdlan as substrates
 332 are displayed in Figure 2 and Figure A (Supplemental Material), respectively. In both cases, the
 333 experimental domains ranged from 32 to 60°C and from 3.5 to 6.0 for pH. In these figures the
 334 experimental data were fitted to the equations specified on the top of the graphs. The parametric

335 estimates and corresponding confidence intervals of the proposed equations are summarized in
336 Table 2. In all cases for both substrates, the fitting of experimental data to equations ($F_{\text{ratio}} > 45$,
337 $p < 0.005$) was statistically satisfactory and consistent (data not shown).

338

339 *3.2. Model selection by statistical criteria*

340 Since there are many models able to fit the combined effects of T and pH reasonably well for the
341 data presented, a selection process was carried out to determine the model that best predicts the
342 joint effect of the two variables in the interval studied. In order to assist us with selecting the best
343 model, we used different statistical criteria to evaluate the multivariable fit and explanatory
344 appropriateness of the equations.

345

346 *3.2.1. Model selection criteria (MSC)*

347 The usefulness of MSC to compare a group of possible models is well-documented.⁴⁴ A model
348 should be complex enough to extract the regularities in data, but simple enough not to overfit it
349 and thereby reduce predictiveness. MSC adjust the goodness of fit in order to penalize model
350 complexity, overfitting and lack of generalizability. Currently, there are a variety of MSC
351 available,^{26,45} but there is no one criterion that can lead to a perfect choice.⁴⁶ A summary of the
352 MSC used to evaluate the results obtained for the nine models with curdlan and laminarin as
353 substrates is shown in Table 1.

354

355 Table 3 shows the model rank (Rk) obtained for each MSC and the final ranking (Rk_F) based on
356 the ranking sum of each MSC ($\sum Rk$) for the two substrates. With curdlan, equation [3] was the
357 best model with respect to the sum of all MSC, followed by equations [12], [13], [11] and [17].
358 In the case of laminarin, equation [16] was the best model with respect to the sum of all MSC,
359 followed by equations [17], [12], [11] and [10]. When the sum of the model rank (Rk) for the

360 two substrates is applied, equation [12] followed by [17] and [16] are the models most likely to
361 be correct.

362

363 3.2.2. Additional statistical criteria

364 The residuals should be randomly scattered around zero to avoid autocorrelation.⁴⁷ These
365 residuals should not be grouped and should not increase or decrease as a function of the
366 independent variable. In general terms all the models used showed a relatively good distribution
367 of the residuals, and autocorrelation was not observed with the Durbin-Watson test (data not
368 shown).

369

370 The confidence intervals at a level of 95% for each parameter are reported in Table 2. The
371 parametric estimates in many cases led to large confidence intervals, and therefore these
372 parameters were considered not significant. For example, in equation [6] only one parameter (b_3)
373 was significant. In equations [10] and [11] the most important coefficients with physical
374 meaning (T_{max} , T_{min} , pH_{max}) were significant. Equation [16] has good fitting levels in both cases
375 (the best fit was when laminarin was used); however, just three out of the nine parameters were
376 significant.

377

378 Only in equations [12] and [13] were all the parameters statistically significant. In equation [16],
379 the parameters K_{dr} and K_{sr} showed confidence levels below 95% in both cases (laminarin and
380 curdlan). As explained by Tijssens et al.³, fitting should be carried out in two steps: first, obtain
381 the parameters K_{dr} , E_d , K_{sr} and E_s ; and second, adjust the other two parameters (K_{EH} and K_{EOH})
382 separately. When these indications are followed all the parameters are significant ($\alpha = 0.05$).
383 Conversely, when the fitting procedure is applied in one single step, as we reported here, the
384 parameters K_{dr} and K_{sr} are not statistically significant.

385

386 Finally, many parameters from equations [2], [3], [6] and [13] do not have a biological or simple
387 physical meaning, and therefore these equations are not very appropriate for describing
388 enzymatic activity. Equations [10] and [11] have a small number of interpretable parameters.
389 Only in equations [12] and [17] do all the parameters have physical meaning. In equation [17],
390 additional values for pH_{opt} , pH_{min} and pH_{max} can be calculated from the parameters obtained.
391 However, equation [12] seems to be more convenient (from an industrial point of view) because
392 its parameters that describe the limits and optimal conditions of pH and T as well as the
393 maximum amount of product released are easily interpreted. Many authors have also emphasized
394 that the applied models should have a clear meaning and use the minimum number of parameters
395 that can be successfully employed for biological optimizations and descriptions.^{39,48}

396

397 *3.3. Model selection and application*

398 The combination of the above mentioned criteria indicated that equations [12] and [17] are
399 suitable for predicting the joint effect of temperature and pH on GA with both substrates. In the
400 case of curdlan, equation [12] seems to be more accurate than [17]; however, with laminarin,
401 equation [17] was the best model for predicting the experimental data. These models can be used
402 to determine a set of parameters with geometric and physical meaning that describe completely
403 the joint effect of pH and T on the enzyme activity.

404

405 *3.3.1. Application when curdlan is used as substrate*

406 Figure 3 shows the interactive effects of pH and temperature on GA using curdlan as a substrate.
407 Experimental data were fitted to equation [12], which showed that a joint maximum is achieved
408 from 46 to 53°C and in the pH range 4.5 to 5.0.

409

410 Practical and operative descriptions of the limits and optimal glucanex activity can be established
411 using the numerical values of the biologically meaningful parameters from equation [12] (Table

412 2). Thus, the joint optimal pH (pH_{opt}) and temperature (T_{opt}) for GA was 4.64 ± 0.22 and
413 $50.48\pm 1.38^\circ\text{C}$, respectively, using curdlan as substrate. Both values were in agreement with the
414 results obtained by⁴⁹ using 1,3-glucanase from *Trichoderma harziaum*, but studying one factor at
415 a time. Other interesting parameters obtained from equation [16] were the maximum temperature
416 (T_{max}) and pH (pH_{max}) and minimum temperature (T_{min}) and pH (pH_{min}) for enzymatic activity.
417 The values for glucanex with curdlan were $65.55\pm 2.29^\circ\text{C}$, 6.83 ± 0.51 , $22.96\pm 4.63^\circ\text{C}$ and
418 2.88 ± 0.42 respectively.

419

420 3.3.2. Application when laminarin is used as substrate

421 The experimental data and the simulated profiles fitted to equation [17] are shown in Figure B
422 (Supplementary Material). The optimal activity was found at $47.58\pm 0.70^\circ\text{C}$ for T and 4.64 ± 0.11
423 for pH. Using a conventional study of one factor at a time with laminarin, other authors
424 established the optimal pH and T conditions for an exo- β -1,3-glucanase from *Trichoderma*
425 *asperellum* as 5.1 and 55°C , respectively [35].

426

427 The large number of runs in the residual plot indicates that there is no clustering in the
428 distribution in certain zones, which suggests that this distribution is random and that the equation
429 estimated all datasets perfectly (avoiding under or overestimations) (see Figure B, Supplemental
430 Material).

431

432 4. CONCLUSIONS

433 The results of the comparison of several mathematical models for describing the experimental
434 profiles of the combined effect of pH and temperature on glucanex activity highlighted the fitting
435 and description capacities of the Rosso [12] and Tijskens [17] equations. The two models were
436 used separately to obtain a set of parameters, based on first principles or with clear geometric
437 and physical meaning, which described the GA characteristics completely.

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441 Project: 200930I183) and Xunta de Galicia (Programa de consolidación de unidades de
442 investigación 2008-2010, IN845B-2010/004) for financial support.

443

444 **FIGURE CAPTIONS**

445
446 **Figure 1:** Determination of the optimal conditions for enzyme assay in order to evaluate the
447 combined effect of temperature and pH on GA: a) selection of pH and T intervals; b) kinetics
448 data obtained at different enzyme concentrations (0 ■, 15 □, 30 ●, 75 ○, 150 ▲, 250 △, 500
449 ◆, 1000 ◇ µg/L) and adjusted to the equation (1); c) values of specific rate of RS produced (α)
450 for each glucanex concentration; d) selection of the analytical time in the linear section of the
451 initial rates.

452
453 **Figure 2:** Response surfaces of the combined effect of temperature and pH on GA (%) with
454 laminarin as substrate. Fit of the experimental results (●) according to the equations defined in
455 the text.

456
457 **Figure 3:** Combined effect of temperature and pH on GA (%) with curdlan as substrate. **A:** 2D
458 representation of pH and T effects. Fit of the experimental results (●) according to the equation
459 (12) (continuous line). **B:** correlation between expected and observed data and plots of residuals
460 (%) in relation with pH, T and GA. **C:** 3D representation of pH and T effects on GA. Fit of the
461 experimental results (●) according to the equation (12) (response surface).

462
463 **Supplemental Figure A:** Response surfaces of the combined effect of temperature and pH on
464 GA (%) with curdlan as substrate. Fit of the experimental results (●) according to the equations
465 defined.

466
467 **Supplemental Figure B:** Combined effect of temperature and pH on GA (%) with laminarin as
468 substrate. **A:** 2D representation of pH and T effects. Fit of the experimental results (●)
469 according to the equation (17) (continuous line). **B:** correlation between expected and observed
470 data and plots of residuals (%) in relation with pH, T and GA. **C:** 3D representation of pH and T
471 effects on GA. Fit of the experimental results (●) according to the equation (17) (response
472 surface).

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TABLE CAPTIONS

Table 1: Different model selection criteria (MSC) used to compare the nine models reviewed from the bibliography to predict the joint effect of pH and T. *n*: number of independent measurements considered in the fit. *k*: number of fitted parameters. *RSS*: residual sum of squares. *ESS*: explained sum of squares.

Table 2: Parametric estimates and confidence intervals obtained from the equations used in the evaluation of the joint effect of pH and temperature on the glucanex activity with curdlan (A) and laminarin (B) as substrate. CI: confidence intervals were evaluated by t-Student test ($\alpha=0.05$). NS: non significant. ** Further interesting values calculated from the parameters of equation (17).

Table 3: Model ranking (*R_k*) obtained for each MSC and the final ranking (*R_{kF}*) based on the total ranking average ($\sum R_k$) for the two substrates.

- 493
494 **REFERENCES**
495
496 1. Murado MA, Siso MIG, Gonzalez MP, Montemayor MI, Pastrana L, Pintado J.
497 Characterization of microbial biomasses and amyolytic preparations obtained from mussel
498 processing waste treatment. *Bioresour Technol.* 1993;43:117-125.
499
500 2. Lacki K and Duvnjak Z. Enzymatic transformation of sinapine using polyphenol oxidase from
501 *Trametes versicolor*. Effect of pH and temperature and model development. *Chem Eng J.*
502 1997;65:27-36.
503
504 3. Tijsskens LMM, Greiner R, Biekman ESA, Konietzny U. Modeling the effect of temperature
505 and pH on activity of enzymes: The case of phytases. *Biotechnol Bioeng.* 2001;72:323-330.
506
507 4. Sharpe PJH, Curry GL, DeMichele DW, Cole CL. Distribution model of organism
508 development times. *J Theor Biol.* 1977;66:21-38.
509
510 5. Sharpe PJH and DeMichele DW. Reaction kinetics of poikilotherm development. *J Theor*
511 *Biol.* 1977;64:649-670.
512
513 6. Schoolfield RM, Sharpe PJH, Magnuson CE. Non-linear regression of biological temperature-
514 dependent rate models based on absolute reaction-rate theory. *J Theor Biol.* 1981;88:719-731.
515
516 7. Ratkowsky DA, Lowry RK, McMeekin TA, Stokes AN, Chandler RE. Model for bacterial
517 culture growth rate throughout the entire biokinetic temperature range. *J Bacteriol.*
518 1983;154:1222-1226.
519
520 8. Ratkowsky DA, Olley J, McMeekin TA, Ball A. Relationship between temperature and
521 growth rate of bacterial cultures. *J Bacteriol.* 1982;149:1-5.
522
523 9. Pronk W, Boswinkel G, van't Riet K. Parameters influencing hydrolysis kinetics of lipase in a
524 hydrophilic membrane bioreactor. *Enzyme Microb Technol.* 1992;14: 214-220.
525
526 10. Zwietering MH, De Koos JT, Hasenack BE, De Wit JC, van't Riet K. Modeling of bacterial
527 growth as a function of temperature. *Appl Environ Microbiol.* 1991;57:1094-1101.
528
529 11. Juneja VK, Melendres MV, Huang L, Subbiah J, Thippareddi H. Mathematical modeling of
530 growth of *Salmonella* in raw ground beef under isothermal conditions from 10 to 45 °C. *Int J*
531 *Food Microbiol.* 2009;131:106-111.
532
533 12. Wang R, Godoy LC, Shaarani SM, Melikoglu M, Koutinas A, Webb C. Improving wheat
534 flour hydrolysis by an enzyme mixture from solid state fungal fermentation. *Enzyme Microb*
535 *Technol.* 2009;44:223-228.
536
537 13. Marx JC, Collins T, D'Amico S, Feller G, Gerday C. Cold-adapted enzymes from marine
538 Antarctic microorganisms. *Mar Biotechnol.* 2007;9:293-304.
539
540 14. Jurado E, Camacho F, Luzón G, Vicaria JM. Kinetic models of activity for β -galactosidases:
541 influence of pH, ionic concentration and temperature. *Enzyme Microb Technol.* 2004;34:33-40.
542

- 543 15. Gonçalves EM, Pinheiro J, Abreu M, Brandão TRS, Silva CLM. Carrot (*Daucus carota L.*)
544 peroxidase inactivation, phenolic content and physical changes kinetics due to blanching. *J Food*
545 *Eng.* 2010;97:574-581.
546
- 547 16. Ricca E, Calabrò V, Curcio S, Iorio G. Optimization of inulin hydrolysis by inulinase
548 accounting for enzyme time- and temperature-dependent deactivation. *Biochem Eng J.*
549 2009;48:81-86.
550
- 551 17. Serrano-Martínez A, Fortea MI, del Amor FM, Núñez-Delicado E. Kinetic characterisation
552 and thermal inactivation study of partially purified red pepper (*Capsicum annuum L.*)
553 peroxidase. *Food Chem.* 2008;107:193-199.
554
- 555 18. Chang MY and Juang RS. Activities, stabilities, and reaction kinetics of three free and
556 chitosan-clay composite immobilized enzymes. *Enzyme Microb Technol.* 2005;36:75-82.
557
- 558 19 Elvig SG and Pedersen PB. Safety evaluation of a glucanase preparation intended for use
559 in food including a subchronic study in rats and mutagenicity studies. *Regul Toxicol Pharm.*
560 2003;37:11-19
561
- 562 20. Kim KS and Yun HS. Production of soluble β -glucan from the cell wall of *Saccharomyces*
563 *cerevisiae*. *Enzyme Microb Technol.* 2006;39:496-500.
564
- 565 21. Lomolino G and Curioni A. Protein haze formation in white wines: Effect of *Saccharomyces*
566 *cerevisiae* cell wall components prepared with different procedures. *J Agric Food Chem.*
567 2007;55:8737-8744.
568
- 569 22. Enrique M, Ibáñez A, Marcos JF, Yuste M, Martínez M, Vallés S, Manzanares P. β -
570 Glucanases as a tool for the control of wine spoilage yeasts. *J Food Sci.* 2010;75:1253-1269.
571
- 572 23. Kim KS, Chang JE and Yun HS. Estimation of soluble β -glucan content of yeast cell wall by
573 the sensitivity to Glucanex[®] 200G treatment. *Enzyme Microb Tech.* 2004;35:672-677.
574
- 575 24. Bernfeld P. Enzymes of starch degradation and synthesis. *Advances in Enzymology.*
576 1951;12:379-427.
577
- 578 25. Yi G and Judge G. Statistical model selection criteria. *Economics Lett.* 1988;28:47-51.
579
- 580 26. Shi P and Tsai C. Regression Model Selection: A Residual Likelihood Approach. *J Royal*
581 *Statistical Soc.* 2002;64:237-252.
582
- 583 27. Schwarz G. Estimating the dimension of a model. *The Annals of Statistics.* 1978;6:461-464.
584
- 585 28. Myung JI and Pitt MA. Model comparison methods, In: Ludwig Brand and Michael L.
586 Johnson (Ed) . *Methods in Enzymology*, Academic Press. 2004;383:351-366.
587
- 588 29. Browne MW. Cross-validation methods. *J Math Psychol.* 2000;44:108-132.
589
- 590 30. Homburg C. Cross-validation and information criteria in causal modeling. *J Market Res.*
591 1991;28:137-144.
592
- 593 31. Boulesteix A. Wilcox CV: an R package for fast variable selection in cross-validation.
594 *Bioinformatics.* 2007;23:1702-1704.

- 595
596 32. Xu Q and Liang Y. Monte Carlo cross validation. *Chemometrics Intellig Lab Syst.*
597 2001;56:1-11.
598
- 599 33. Kapat A and Panda T. pH and thermal stability studies of chitinase from *Trichoderma*
600 *harzianum*: A thermodynamic consideration: A thermodynamic consideration. *Bioprocess Eng.*
601 1997;16:269-272.
602
- 603 34. Rana DS, Thèodore K, Naidu GSN, Panda T. Stability and kinetics of β -1,3-glucanase from
604 *Trichoderma harzianum*. *Proc Biochem.* 2003;39:149-155.
605
- 606 35. Ricker WE. Stock and recruitment. *J Fish Res Board Canada.* 1954;11:559-623.
607
- 608 36. Murado MA, González MP, Pastrana L. Production of microfungus metabolites on inert solid
609 supports. *Recent Research Developments in Biotechnology and Bioengineering.* Pandalai SG
610 (Ed). Trivandrum, India: Research Signpost Publisher. 1998;1:405-432.
611
- 612 37. Lindenfelser LA and Ciegler A. Solid substrate fermentor for ochratoxin A production. *Appl*
613 *Microbiol.* 1975;29:323-327.
614
- 615 38. Ohta F and T Hirabara T. Rate of degradation of nucleotides in cool-stored carp muscle.
616 *Memo Fac Fish Kagoshima Univ.* 1977;26:97-102.
617
- 618 39. Rosso L, Lobry JR, Bajard S, Flandrois JP. Convenient model to describe the combined
619 effects of temperature and pH on microbial growth. *Appl Environ Microbiol.* 1995;61:610-616.
620
- 621 40. Murado MA, González MP, Vázquez JA. Dose-response relationships: An overview, a
622 generative model and its application to the verification of descriptive models. *Enzyme Microb*
623 *Technol.* 2002;31:439-455.
624
- 625 41. Riobó P, Paz B, Franco JM, Vázquez JA, Murado MA. Proposal for a simple and sensitive
626 haemolytic assay for palytoxin: Toxicological dynamics, kinetics, ouabain inhibition and thermal
627 stability. *Harmful Algae* 2008;7:415-429.
628
- 629 42. Murado MA and Vázquez JA. Mathematical model for the characterization and objective
630 comparison of antioxidant activities. *J Agric Food Chem.* 2010;58:1622-1629.
631
- 632 43. Seyhan F, Tijssens LMM, Evranuz O. Modelling temperature and pH dependence of lipase
633 and peroxidase activity in Turkish hazelnuts. *J Food Eng.* 2002;52:387-395.
634
- 635 44. Rivers D and Vuong Q. Model selection tests for nonlinear dynamic models. *Econometrics J*
636 2002;5:1-39.
637
- 638 45. Forster MR. Key concepts in model selection: Performance and generalizability. *J Math*
639 *Psychol.* 2000;44:205-231.
640
- 641 46. Rust RT, Simester D, Brodie RJ, Nilikant V. Model selection criteria: An investigation of
642 relative accuracy, posterior probabilities, and combinations of criteria. *Management Sci.*
643 1995;41:322-333.
644
- 645 47. Zwietering MH, Wiltjes T, Rombouts FM, van't Riet K. A decision support system for
646 prediction of microbial spoilage in foods. *J Ind Microbiol.* 1993;12:324-329.

- 647
648 48. Noronha EF and Ulhoa CJ. Characterization of a 29-kDa β -1,3-glucanase from *Trichoderma*
649 *harzianum*. *FEMS Microbiol Lett.* 2000;183:119-123.
650
651 49. Neter J, Wasserman W, Kutner MH. *Applied linear statistical models*. 2nd edition. Illinois:
652 Irwin; 1985.
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FIGURES

Figure 1

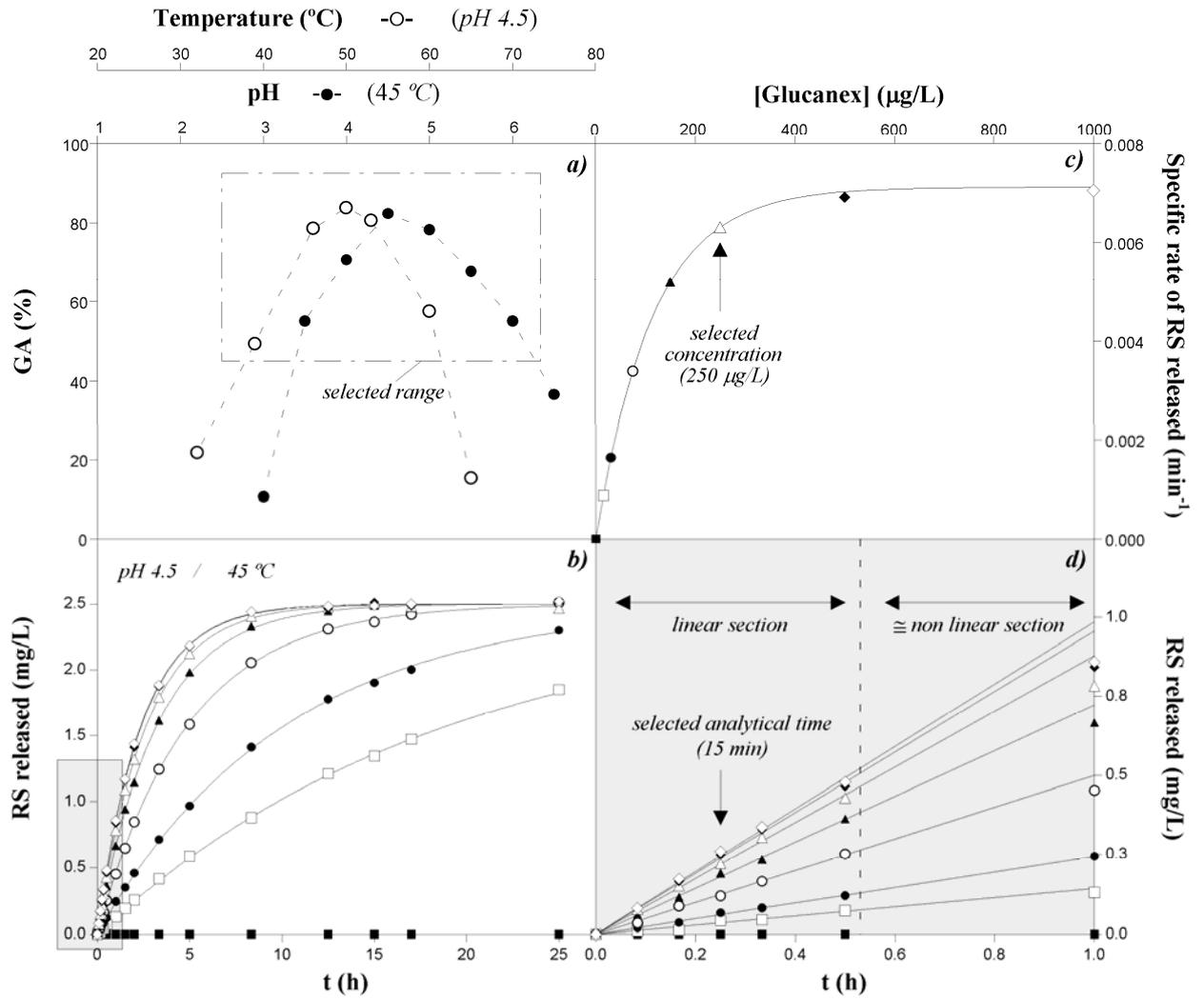


Figure 2

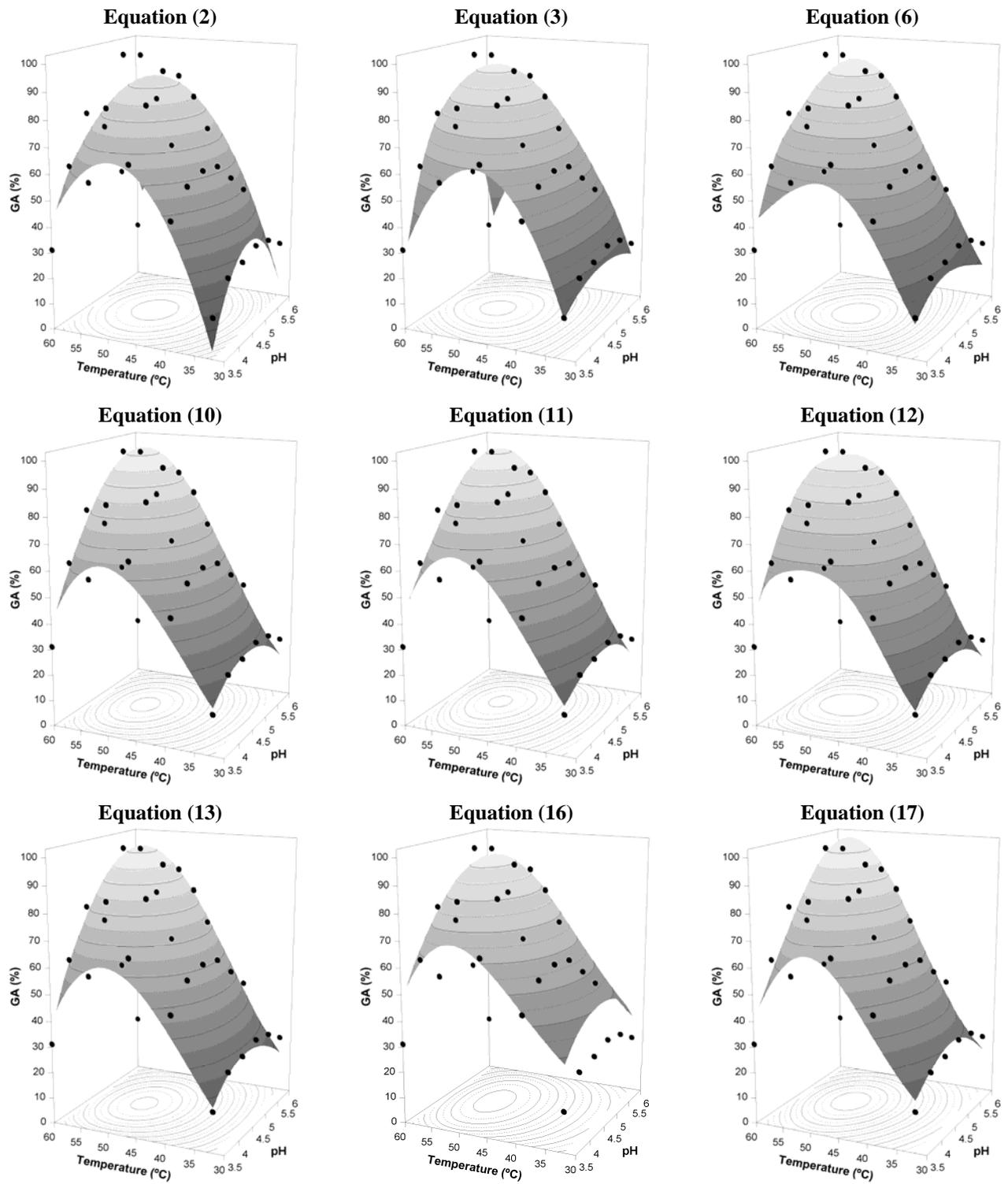


Figure 3

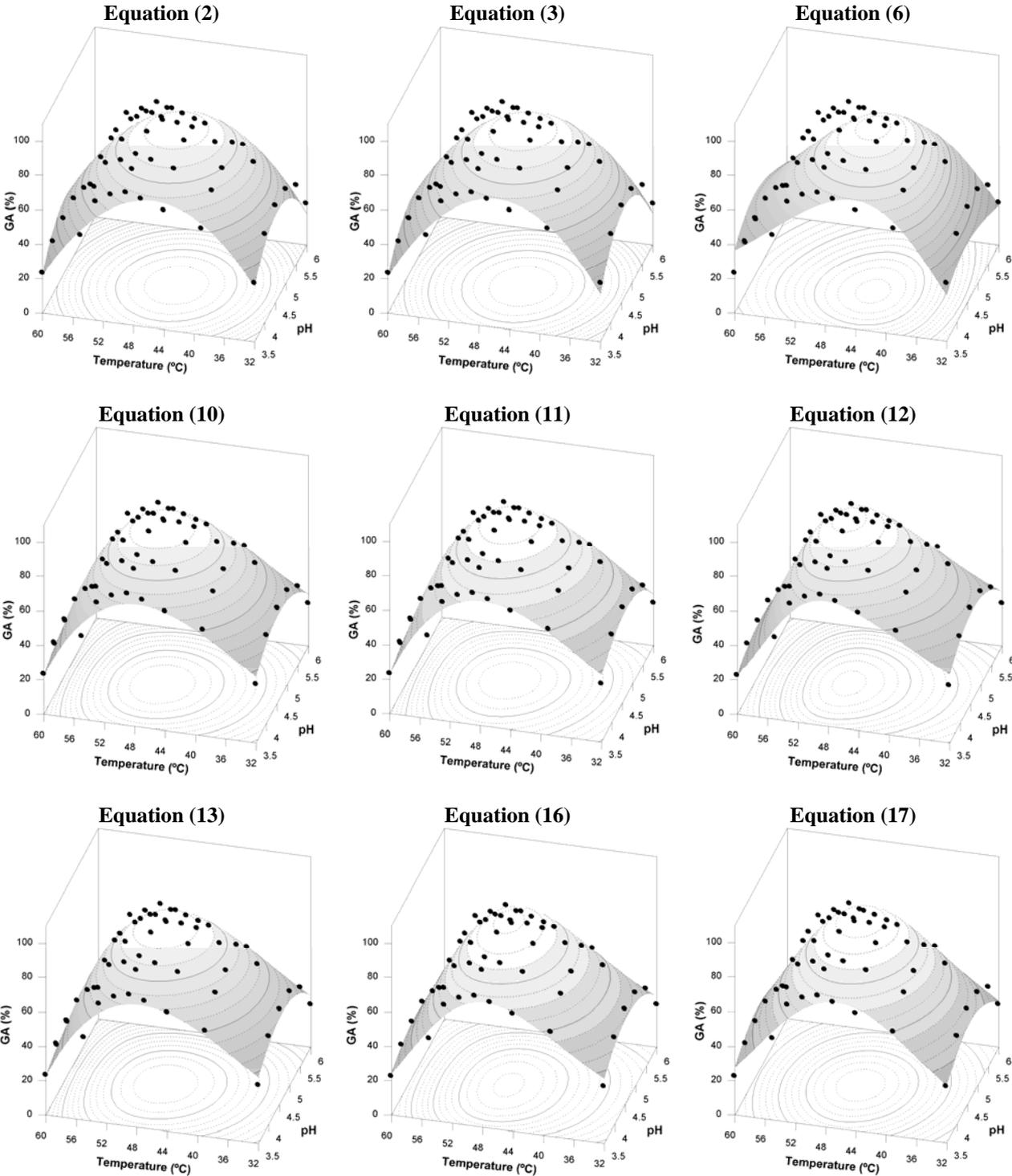


Figure 4

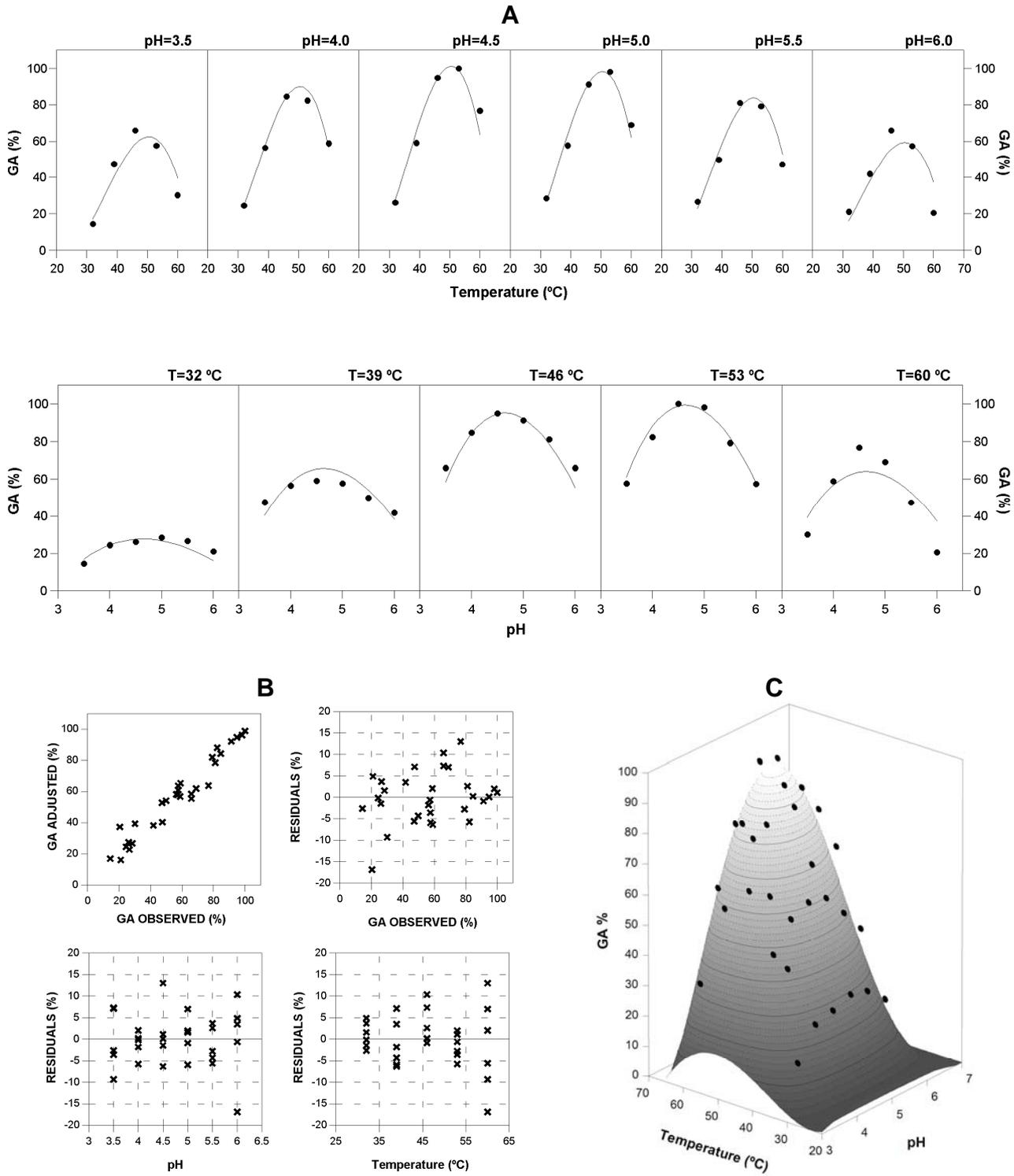
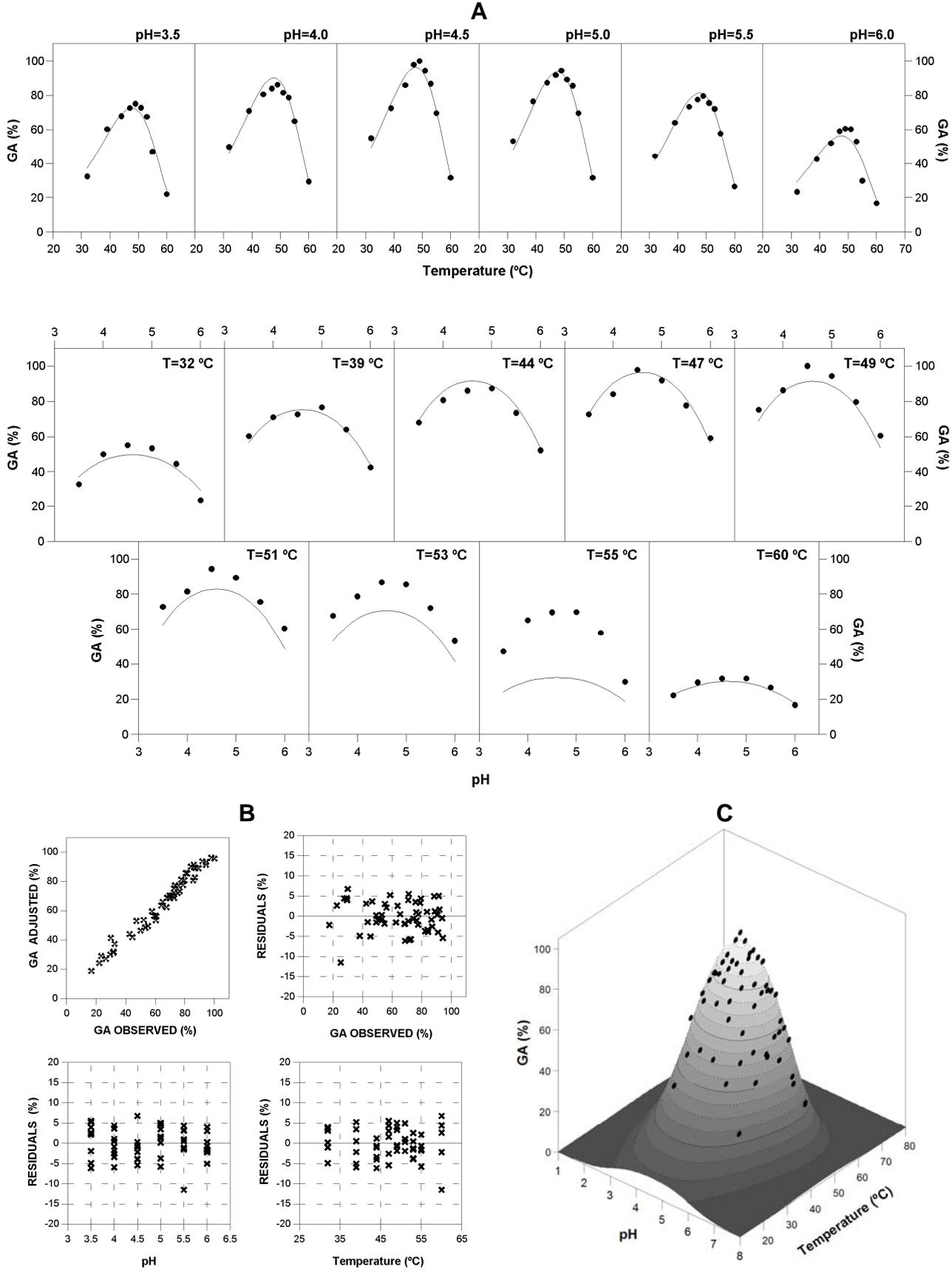


Figure 5



TABLES

Table 1: Different model selection criteria (MSC) used to compare the nine models reviewed from the bibliography to predict the joint effect of pH and T. n: number of independent measurements considered in the fit. k: number of fitted parameters. RSS: residual sum of squares. ESS: explained sum of squares.

Criterion	Key	Claim	Formula
Akaike Information Criterion Corrected ^{23,24}	AICc	complexity (efficient)	$AIC_c = n \ln \left(\frac{RSS}{n} \right) + \left(\frac{2(k+1)}{n-k-2} \right)$
Bayesian Information Criterion ²⁵	BIC	complexity (consistent)	$BIC = n \ln (RSS) + \ln (n) k$
Akaike's Final Prediction Error ²⁴	FPE	complexity	$FPE = n \frac{RSS(n+k)}{(n-k)}$
Mallows' Cp ^{23,24}	Cp	goodness of fit / overfitting	$C_p = n \left[\frac{RSS}{(ESS/n-1)} \right] - n + 2k$
Adjusted Coefficient of Determination ²⁴	R ² _{adj}	goodness of fit / complexity	$R_{adj}^2 = \frac{(n-1)R^2 - k}{n-1-k}$
Residual Information Criterion ²⁴	RIC	goodness of fit / overfitting	$RIC = (n-k) \ln (RSS) + k [\ln (n) - 1] + \frac{4}{n-k-2}$
Model Selection Criterion ²⁶	MSC	goodness of fit	$MSC = \ln \left(\frac{ESS}{RSS} \right) - \frac{2k}{n}$
Leave One Out Cross-Validation ^{26,27,28,29}	LOO-CV	generalizability	--
Monte Carlo Cross-Validation ^{27,28,30}	MCCV	generalizability / overfitting	--

Table 2: Parametric estimates and confidence intervals obtained from the equations used in the evaluation of the joint effect of pH and temperature on GA with curdlan (A) and laminarin (B) as substrate. CI: confidence intervals were evaluated by t-Student test ($\alpha=0.05$). NS: non significant.

A) PARAMETERS OBTAINED WITH CURDLAN AS SUBSTRATE									
Equations	b_{00}	b_{12}	b_1	b_{11}	b_2	b_{22}	b_{221}	b_{112}	--
(2)	-856±164	-0.186 (NS)	181±53	-18.37±5.22	21.42±4.10	-0.21±0.04	--	--	--
(3)	116.2 (NS)	9.01±4.42	-240±170	25.90±16.64	0.146 (NS)	-0.21±0.15	-0.0006 (NS)	-0.96±0.35	--
(6)	a	T_0	n_1	pH_0	n_2	b_3	b_4	--	--
	6x10 ⁻⁶ (NS)	17.17 (NS)	6.54 (NS)	17.17 (NS)	6.31 (NS)	0.210±0.207	2.06 (NS)	--	--
(10)	T_{max}	T_{min}	c	a_1	c_0	c_1	c_2	--	--
	65.12±2.25	21.97±4.98	22.20 (NS)	0.0003 (NS)	-5.92 (NS)	3.05 (NS)	-0.32 (NS)	--	--
(11)	T_{max}	T_{min}	pH_{max}	pH_{min}	c	a_1	a_2	--	--
	65.11±2.45	21.98±5.42	6.52±0.36	1.26 (NS)	40.68 (NS)	0.0001 (NS)	0.083 (NS)	--	--
(12)	T_{max}	T_{min}	pH_{max}	pH_{min}	r_m	T_{opt}	pH_{opt}	--	--
	65.55±2.29	22.96±4.63	6.83±0.51	2.88±0.42	101.76±6.55	50.48±1.38	4.64±0.22	--	--
(13)	k	α	q	β	p	--	--	--	--
	8445±758	5.32±0.40	52.35±0.70	4.33±0.45	5.04±0.09	--	--	--	--
(16)	c_0	c_1	c_2	r_r	H_r	T_a	H_a	T_b	H_b
	-5.71 (NS)	2.950 (NS)	-0.313 (NS)	1.189 (NS)	-17.37 (NS)	334.0±46.6	687.7 (NS)	311.9±41	-177 (NS)
(17)	E_s	E_d	pH_{max}^{**}	pH_{min}^{**}	K_{EH}	K_{EOH}	pH_{opt}^{**}	k_{sr}	k_{dr}
	65.5±42.6	104.28±92.59	7,96**	3,38**	0.0004±0.0002	1.1x10 ⁻⁸ ±10 ⁻¹⁰	4,70**	7.5x10 ¹⁰ (NS)	3.83x10 ¹⁵ (NS)
B) PARAMETERS OBTAINED WITH LAMINARIN AS SUBSTRATE									
Equations	b_{00}	b_{12}	b_1	b_{11}	b_2	b_{22}	b_{221}	b_{112}	--
(2)	-747.8±94.4	0.032 (NS)	153.1±28.2	-16.77±2.69	21.58±2.42	-0.24±0.02	--	--	--
(3)	-1029±447	-2.59 (NS)	245.3±164.4	-23.5±16.3	30.72±14.11	-0.31±0.13	0.01 (NS)	0.14 (NS)	--
(6)	a	T_0	n_1	pH_0	n_2	b_3	b_4	--	--
	0.0001 (NS)	15.22 (NS)	6.26 (NS)	1.13 (NS)	6.08 (NS)	0.21 (NS)	1.82 (NS)	--	--
(10)	T_{max}	T_{min}	c	a_1	c_0	c_1	c_2	--	--
	62.17±0.52	12.50±5.51	0.38 (NS)	0.03 (NS)	-2.29 (NS)	1.28 (NS)	-0.14 (NS)	--	--
(11)	T_{max}	T_{min}	pH_{max}	pH_{min}	c	a_1	a_2	--	--
	62.11±0.52	12.51±5.60	6.60±0.18	0.75 (NS)	0.026 (NS)	0.047 (NS)	0.18 (NS)	--	--
(12)	T_{max}	T_{min}	pH_{max}	pH_{min}	r_m	T_{opt}	pH_{opt}	--	--
	62.18±0.54	13.10±4.58	6.69±0.22	2.26±0.45	94.82±2.44	47.58±0.70	4.64±0.11	--	--
(13)	k	α	q	β	p	--	--	--	--
	9019±280	4.87±0.24	48.81±0.42	3.83±0.26	5.00±0.06	--	--	--	--
(16)	c_0	c_1	c_2	r_r	H_r	T_a	H_a	T_b	H_b
	-318.6 (NS)	178.8 (NS)	-19.43 (NS)	1.14 (NS)	-52.33 (NS)	327.4±25.6	226.4 (NS)	329.2±27.6	-98.35±46
(17)	E_s	E_d	pH_{max}^{**}	pH_{min}^{**}	K_{EH}	K_{EOH}	pH_{opt}^{**}	k_{sr}	k_{dr}
	59.09±14.3	109.1±24.9	7,91**	3,11**	8x10 ⁻⁴ ±2x10 ⁻⁴	1x10 ⁻⁸ ±2x10 ⁻⁹	4,60**	7.8x10 ⁹ (NS)	3.1x10 ¹⁶ (NS)

** Further interesting values calculated from the parameters of equation (17).

Table 3: Model ranking (Rk) obtained for each MSC and the final ranking (Rk_F) based on the total ranking average ($\sum Rk$) for the two substrates.

CRITERIA	AICc		BIC		RIC		Cp		R ² _{adj}		FPE		MSC		LOO-CV (MEP)		MCCV (MEP)		AVERAGE	
Equations	Value	Rk	Value	Rk	Value	Rk	Value	Rk	Value	Rk	Value	Rk	Value	Rk	Value	Rk	Value	Rk	$\sum Rk$	Rk _F
A) MODEL RANK USING CURDLAN AS SUBSTRATE																				
(2)	123.30	(9)	245.11	(8)	194.35	(9)	65.1	(9)	0.8798	(9)	80551.8	(9)	1.95	(9)	114.24	(9)	124.57	(6)	77	(9)
(3)	96.70	(1)	225.04	(1)	164.49	(1)	19.9	(1)	0.9448	(1)	37878.6	(1)	2.71	(1)	37.87	(2)	47.35	(3)	12	(1)
(6)	115.63	(8)	240.72	(7)	183.30	(6)	48.1	(8)	0.9005	(8)	66629.4	(7)	2.14	(7)	43.06	(3)	43.02	(2)	56	(7)
(10)	113.96	(6)	239.04	(6)	182.01	(5)	44.6	(5)	0.9213	(4)	63014.2	(5)	2.20	(5)	58.94	(7)	164.41	(8)	51	(6)
(11)	108.80	(4)	233.88	(4)	178.05	(4)	35.1	(4)	0.9074	(7)	53050.8	(4)	2.37	(4)	54.07	(5)	135.91	(7)	43	(4)
(12)	107.76	(2)	232.84	(3)	177.26	(3)	33.3	(3)	0.9241	(3)	51243.9	(3)	2.40	(3)	36.43	(1)	40.69	(1)	22	(2)
(13)	108.31	(3)	226.83	(2)	187.03	(8)	30.6	(2)	0.9288	(2)	45786.7	(2)	2.51	(2)	56.99	(6)	60.87	(5)	32	(3)
(16)	113.85	(5)	245.44	(9)	172.20	(2)	47.8	(7)	0.9122	(6)	71771.1	(8)	2.08	(8)	95.81	(8)	234.96	(9)	62	(8)
(17)	114.89	(7)	238.70	(5)	186.22	(7)	47.1	(6)	0.9160	(5)	65061.9	(6)	2.16	(6)	53.87	(4)	54.87	(4)	50	(5)
B) MODEL RANK USING LAMINARIN AS SUBSTRATE																				
(2)	189.74	(8)	428.77	(7)	377.88	(8)	163.4	(8)	0.9191	(7)	121683.6	(7)	2.41	(7)	41.42	(7)	42.61	(7)	66	(7)
(3)	187.79	(7)	434.70	(8)	367.12	(6)	159.7	(7)	0.9186	(8)	126311.3	(8)	2.38	(8)	47.25	(8)	50.27	(8)	68	(8)
(6)	237.01	(9)	479.99	(9)	414.48	(9)	452.3	(9)	0.8018	(9)	302935.9	(9)	1.50	(9)	74.33	(9)	74.05	(9)	81	(9)
(10)	152.21	(5)	395.18	(5)	340.66	(5)	62.4	(5)	0.9610	(4)	62992.5	(5)	3.07	(5)	19.86	(3)	16.78	(3)	40	(5)
(11)	150.23	(4)	393.20	(4)	338.94	(4)	58.7	(4)	0.9603	(5)	60727.3	(4)	3.11	(4)	20.24	(4)	21.02	(4)	37	(4)
(12)	148.64	(3)	391.61	(3)	337.55	(3)	55.8	(3)	0.9614	(3)	58962.6	(3)	3.14	(3)	20.45	(5)	21.56	(5)	31	(3)
(13)	176.93	(6)	412.03	(6)	370.81	(7)	118.1	(6)	0.9374	(6)	92548.7	(6)	2.69	(6)	32.57	(6)	33.39	(6)	55	(6)
(16)	129.43	(1)	380.27	(1)	313.96	(1)	31.0	(1)	0.9719	(1)	44470.9	(1)	3.42	(1)	13.80	(2)	11.84	(1)	10	(1)
(17)	141.31	(2)	380.35	(2)	334.83	(2)	41.8	(2)	0.9670	(2)	49632.8	(2)	3.31	(2)	13.62	(1)	13.50	(2)	17	(2)