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Optimization of the operating conditions for the removal of alcoholic insoluble compounds contained in sugar beet vinasse

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ABSTRACT

The purification of sugar beet distillery vinasse, a by-product of bioethanol manufacturing, was studied with a view to removing the insoluble compounds by precipitation in ethanol. Two influential operating parameters, i.e. temperature and ethanol:vinasse (w/w) ratio of the precipitation, were optimized by a simplex method. An optimum was reached for a weight ratio of 4.6 at 21 °C, that allowed 52.4% of the vinasse dry matter to be separated. Additional experiments were then carried out to establish a second-order polynomial model from a Doehlert design and measure the weight of solid removed as a function of temperature and ethanol:vinasse (w/w) ratio used. In the experimental domain studied, results showed the very weak positive influence of temperature and the weak negative influence of ethanol:vinasse (w/w) ratio on weight of precipitate obtained. In addition, the statistical model enables to determine the response surface in order to predict the variation in response when the values of variables are modified. Lastly, the sugar beet precipitation was successfully scaled up, enabling a mass balance of the process to be established. Results showed that a large proportion of proteins and pectins were separated during the process.

1. Introduction

In recent decades, many sectors of industrial production have devoted increasing attention to ecological–environmental problems by [1]: (i) evaluating the inputs and outputs of the traditional production process, (ii) identifying the weak points in the process which could be involved in environmental problems, (iii) searching for solutions which could allow by-products or waste products to be reused as secondary raw materials, (iv) optimizing the consumption of energy and water, (v) controlling gaseous emissions and solid/liquid wastes, and (vi) studying alternative technologies that are more eco-friendly.

In sugar beet processing, environmental problems are mainly related to the production of pulp, the consumption of large amounts of lime, the production of vinasse (residue obtained after rectification of ethanol), and the consumption of energy and water [1]. To reduce production costs and set up an environmental policy, French industrialists have therefore to find beneficial uses for their by-products. Besides, environmental restrictions have limited the disposal of vinasse as a waste and have consequently increased the supply of vinasse as an additive for animal feed and as a fertilizer. As all the water-soluble compounds biosynthesized by beets are concentrated in vinasse, there is an increased interest in recovering some valuable compounds from it. These valuable compounds are betaine, polyphenolic compounds, polysaccharides, succinic acid, citric acid, etc., depending on the raw materials and fermentation process [2]. Because of the high betaine concentration in sugar beet vinasse (more than 15% of the dry matter) and its economic potential, we have focused on betaine recovery. A multi-stage process for betaine recovery was thus developed in our laboratory [3] and has shown that the betaine was conveniently isolated after proceeding in several steps: (i) the removal of most insoluble compounds from the ethanolic medium, (ii) the separation of most polyphenolic and other colored compounds contained in ethanolic medium, and (iii) the purification of the betaine by ion exchange.

Since betaine is soluble in hydroethanolic medium, this study concerns the first step of the process, which is the primary purification of vinasse by removing ethanolic insoluble compounds. The aim of the study was to optimize the operating conditions for the removal of insoluble compounds (proteins and pectins) from vinasse by precipitating them in ethanol while keeping betaine in solution. To carry out this optimization, a simplex method was first used to locate the optimum area by varying the level of variables

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Nomenclature

ANOVA	analysis of variance
b_j	coefficients of the polynomial model
BSA	bovine serum albumin
HPLC	high-pressure liquid chromatography
k	number of control variables in the simplex optimization
ratio	ethanol:vinasse weight ratio
rpm	rotation per minute
R^2	coefficient of determination
T	temperature
$x_{i,j}$	real coordinate of control variable in the simplex optimization and Doehlert design
$x_{0,j}$	real coordinate of control variable at the center of the experimental domain in the simplex optimization and Doehlert design
Δx_j	step size of the control variable in the simplex optimization and Doehlert design
$X_{i,j}$	reduce coordinate of the control variable in the simplex optimization and Doehlert design
Y_D	experimental response in Doehlert design
Y_S	experimental response in simplex optimization

(temperature and ethanol:vinasse, w/w ratio) on the experimental response (precipitate:vinasse, w/w ratio).

While the simplex methods [4–6] enable to encircle the optimal area, the influence of every variable can also be determined by a response surface methodology. A large number of experimental designs adapted to various types of problems are available such as factorial designs [7], centroid composite matrices [8] or Doehlert shells [9,10]. In this study, vinasse precipitation was optimized by a simplex method. Furthermore, a Doehlert matrix was built in order to determine the response surface in the optimum area by using the previous simplex experiments and adding several complementary experiments. Finally, the process was extrapolated with a vessel homothetic to an industrial reactor in order to establish a mass balance and calculate characteristic parameters.

2. Experimental

2.1. Materials

The vinasse used was sugar beet vinasse obtained from the ethanol production line of a sugar refinery (La Vermandoise, Toury, France). Table 1 reports the chemical composition of depotassified vinasse used. The protein content was determined with the Bradford method [11] by using a UV–vis spectrophotometer (Thermo Labsystems Multiscan Spectrum, Thermo Fisher Scientific, France) and a commercial bovine serum albumin (BSA) standard. The determination of the pectin content was carried out by Laboratoire des Biopolymères Interactions Assemblages (INRA Nantes, France). In this study, pectins were characterized by their content in uronide material [12] and neutral sugars [13]. The betaine content was determined by high-pressure liquid chromatography (HPLC) [14,15]. The Folin–Ciocalteu method [16] was used to estimate the amount of polyphenolic compounds within the sample. A calibration curve, obtained from caffeic acid with a spectrophotometer (Jasco V-530, Jasco France, France), gave the content of polyphenolic compounds in mg L^{-1} of equivalent caffeic acid. The analysis of mineral salts and other compounds was carried out by UNGDA (Paris, France). These different analytical methods were also used, after the sugar beet precipitation, for the characterization of the precipitate and the filtrate. Ethyl alcohol was 96.2% (v) purchased from Carlo Erba (Val de Reuil, France).

Table 1
Chemical composition of the concentrated depotassified vinasse used for the process

Components	Content
pH	5
Dry matter (%)	65
Betaine (g kg^{-1})	116.76
Polyphenolic compounds (g equiv. kg^{-1})	17.99
Proteins (g kg^{-1})	1.99
Pectins	
Uronic acids (g kg^{-1})	6.64
Neutral sugars (g kg^{-1})	76.93
Inorganic matter (%)	2.7
Total nitrogen (%)	6.2
Phosphorus (%)	0.5
Potassium (%)	0.5
Sodium (%)	0.7
Calcium (%)	Inf. 0.1
Total sulfur (%)	1.2
Reducing sugars (%)	Inf. 0.1
Total sugars (%)	1.5

2.2. Experimental set-up

2.2.1. Optimization of the process

The results of preliminary experiments [3] showed that ethanol separated a greater part of the vinasse dry matter than methanol and that it is less toxic. Ethanol was therefore chosen as solvent precipitation in order to carry out the optimization experiments.

In this study, all the optimization experiments were performed at different temperatures by using appropriate weight of vinasse (generally 30 g) and different weights of ethanol. The precipitation was carried out in a 250-mL three-neck round-bottom flask equipped with a condenser to avoid ethanol evaporation, a dropping funnel, a thermometric probe and a magnetic stirrer. The temperature was regulated with a thermostated bath and a thermoregulator (Huber Ministat, Fisher Scientific Bioblock, France). The appropriate weight of ethanol was transferred into the round-bottom flask by the dropping funnel and the media was set at the studied temperature. When the appropriate temperature was reached, a weight of 30 g of vinasse was added by the dropping funnel and the medium was stirred at the studied temperature.[1] After 15 min stirring, the precipitate was filtered through a 0.45-μm membrane filter and dried in a dessiccator at ambient temperature until the weight of solid was constant.

2.2.2. Scale-up of sugar beet precipitation

The scale-up of sugar beet precipitation was performed in a 5-L 316-L stainless double-jacketed reactor (1, Fig. 1, purchased by Pignat, Genas, France) equipped with a marine impeller and a reflux condenser. The experimental set-up is shown in Fig. 1. The temperature was kept constant with a thermometric probe (7, Fig. 1) and a Vulcanic 10,803 thermo-regulator (Pignat, Genas, France, 6, Fig. 1). The experiments were carried out under the optimized conditions obtained from simplex optimization of the precipitation process (T = 21 °C and ratio = 4.58). To use all the efficient volume of the reactor (5 L), it was necessary to carry out the experiment by adding 636 g of vinasse to 2913 g of ethanol. The weight of ethanol was first introduced in the reactor and set at 21 °C. The

[1] For a better precipitation of insoluble compounds, it is more advisable to add vinasse to ethanol rather than in the reverse order.

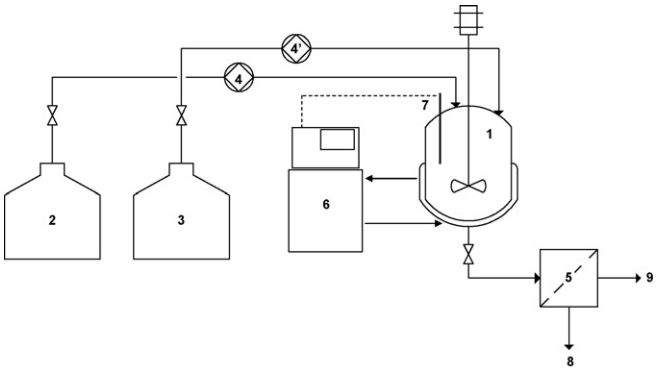

Fig. 1. Experimental set-up of the removing of ethanolic insoluble compounds contained in sugar beet vinasse (1: 5 L double-jacketed reactor; 2: sugar beet vinasse tank; 3: ethanol tank; 4, 4′: peristaltic pumps; 5: filter; 6: thermo-regulator; 7: thermometric probe; 8: filtrate; 9: precipitate).

medium was then mixed and the weight of vinasse was transferred into the reactor by using a peristaltic pump (Watson–Marlow 503U, Watson–Marlow pumps SA, France). After an extrapolation procedure (not described in the paper), the impeller speed was adjusted to 400 rpm and the introduction flow-rate of solvent was set at $2\,g\,s^{-1}$. After 15 min, the precipitate was filtered as in the optimization set-up and dried at ambient temperature under vacuum.

2.3. Simplex method

Simplex methods are evolutionary operation techniques in which a series of experiments is set up in such a way that the operating conditions, for a given experiment, are dictated by the results of the preceding experiments in that series [4–6]. They are based on an initial design of $k+1$ trials, where k is the number of control variables. The simplex in two dimensions has three vertices, represented by an equilateral triangle. After the initial experiments, which compose the initial simplex, its evolution occurs in a sequential order, with the addition of a new experiment, performed on the direction opposite from the worst result [4–6]. Fig. 2 shows an example of the simplex reflection for a two-dimensional factor space, drawn according to the Czech method [17]. The three vertices of the initial simplex are labeled W for the vertex giving the worst response, B for the vertex giving the best response, and N for the vertex giving the next-to-worst response. Vertices G and R are, respectively the centroid and reflection points of the simplex.

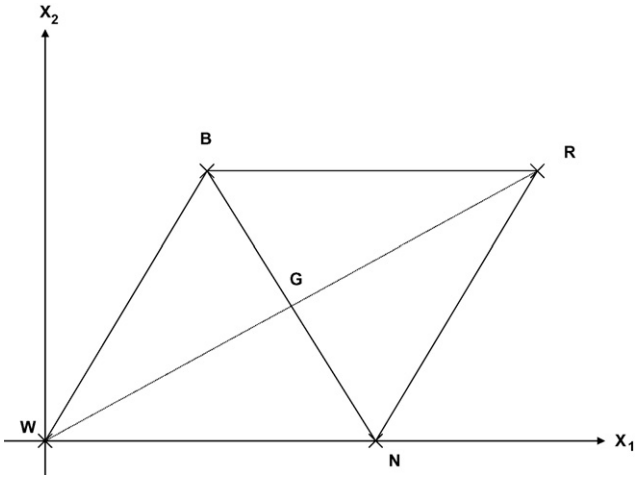

Fig. 2. Building of first vertices of a two-variable simplex according to the Czech method [17] and simplex reflection move (B, N and W are, respectively the vertex giving the best, the next-to-worst and the worst response. G and R are, respectively the centroid and the reflex of the simplex).

The optimization process ends when the optimization objective is reached or when the responses cannot be improved further [4,5]. The simplex algorithm consists of few rules:

- The evolution is performed by the rejection of the worst point, which is replaced by its symmetrical point with respect to the hyperface containing the other simplex vertices.
- If the symmetrical point is the worst vertex of the $(i+1)$ simplex, the (i) simplex is considered again and the next-to-worst point of this simplex is eliminated.
- The vertex retained in the simplex for $k+1$ steps has to be reevaluated. The reevaluation rule avoids the simplex being stuck around a false favorable response.

The simplex optimization was applied to the first step of our process. The choice of the solvent (ethanol in this study) is an important factor. For a chosen solvent, the solubility of compounds depends on the temperature and the weight ratio of added solvent per initial vinasse. Therefore, these two parameters (temperature (T) and ethanol:vinasse, w/w ratio (ratio)) were chosen as parameters of simplex. The experimental response selected is the (w/w) ratio between the precipitate and the vinasse used (Y_S) and the initial simplex was built in accordance with the method of Czech [17].

It is necessary to use adimensional variables to obtain a regular figure (reduced variables). The real coordinate of each parameter is obtained from the adimensional variables (or coded variables) according to the following relation:

$$x_{i,j} = x_{0,j} + X_{i,j}\,\Delta x_j \tag{1}$$

where i corresponds to simplex point from 0 to k, j corresponds to the variable from 1 to k, $x_{0,j}$ is the real coordinate for the variable j at the center of the studied domain, corresponding to the reduced coordinate equal to 0, $X_{i,j}$ corresponds to the reduced coordinate to the variable j at the point i, Δx_j is the step size for the variable j corresponding to a variation of +1 in reduced coordinate.

2.4. Doehlert design

When a two-variable simplex optimization surrounds the optimum, a hexagon is formed and the results can be used in a Doehlert design in order to calculate a response surface. The geometry of the Doehlert experimental design is represented in Figs. 3 and 4, where the matrix can be represented in normalized variables (X_i) by apexes and the center of a hexagon. Several results of the simplex optimization were thus used again (Figs. 3 and 4, experiments E, G, I, J and K) and only two complementary experiments were added (Figs. 3 and 4, experiments L and M). The experimental results obtained when using a Doehlert matrix lead to the estimation of 6 coefficients for a second-order polynomial model, according to the following equation:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2 \tag{2}$$

The model consists of first-order terms (b_i), square terms (b_{ii}) and first-order interactions (b_{ij}).

Moreover, the levels of the independent variables are normalized, usually as reduced and centered variables, according to Eq. (1). The estimation of the coefficients of the polynomial model (b_0, b_i, b_{ii} and b_{ij} in Eq. (2)) were calculated using the least-square method of

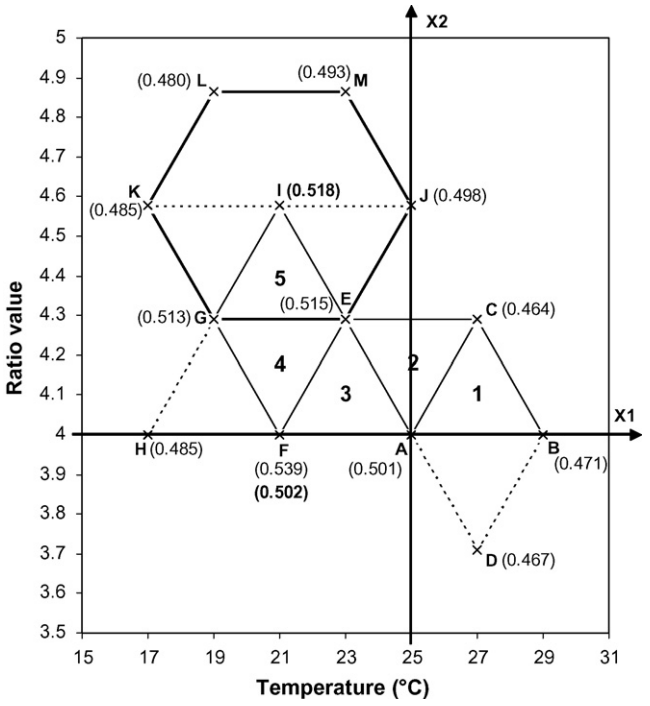

Fig. 3. Movement of the fixed size simplex for the distillery vinasse precipitation (L and M are the experiments added for the establishment of the Doehlert experimental design; the values shown in brackets are the results of Y_S).

Statgraphics 5.1 Software (Sigma-Plus, Paris, France). The resulting model equation was used for drawing the response surface. The significance of the effects was checked by analysis of variance (ANOVA) and using *p*-value significance levels.

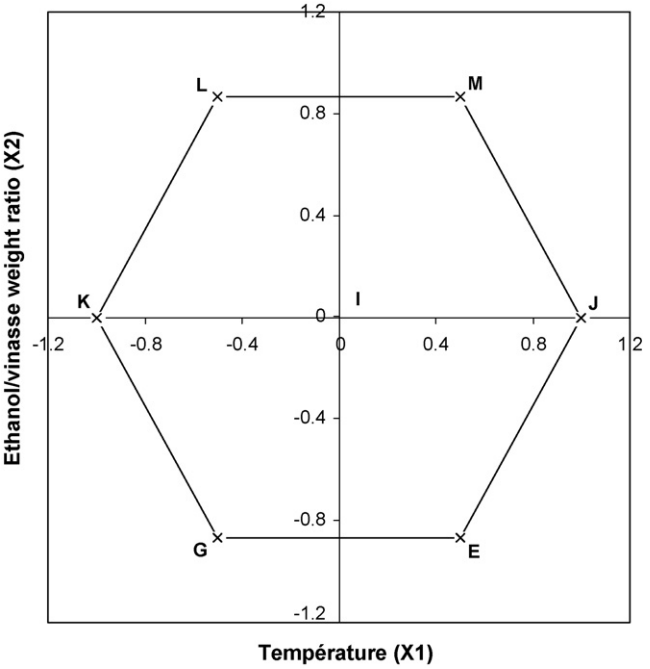

Fig. 4. Building of a classical two-variable Doehlert matrix (*T* and ratio) centered on optimal point of simplex (experiment I). X_1 is the normalized temperature (*T*) and X_2 is the normalized ethanol:vinasse weight ratio (ratio).

3. Results and discussion

3.1. Optimization by the simplex method

3.1.1. Initial simplex

The step size values of the initial simplex must be suitably selected in order to (i) obtain equivalent effects on the experimental response and (ii) generate significant variation on the response without exceeding the optimum (steps over-estimated) and increasing the number of experiments (steps under-estimated). In this study, the initial values ($x_{0,j}$) were 25 °C and 4.00, and the step size values (Δx_j) were 4 °C and 0.33 for, respectively *T* and ratio control variables. These values were chosen from preliminary experiments and industrial constraints. The experimental matrix is shown in Table 2.

3.1.2. Evolution of simplex

The parameters and results of experiments of each simplex are given in Table 2 and the projections of the simplex in space are shown in Fig. 3. Table 2 shows the values of control variables (*T* and

Table 2
Evolution of simplex

	Experiment number	Control variables***		Response function (Y_S)	
		T (°C)	Ratio		
Simplex 1	A	25	4.00	0.501	B*
	B	29	4.00	0.471	N*
	C	27	4.29	0.464	W*
	D (sym/W)	27	3.71	0.470	Ignored
	E (sym/N)	23	4.29	0.515	Retained
Simplex 2	A	25	4.00	0.501	N
	E	23	4.29	0.515	B
	C	27	4.29	0.464	W
	F (sym/W)	21	4.00	0.539	Retained
Simplex 3	A	25	4.00	0.501	W
	E	23	4.29	0.515	N
	F	21	4.00	0.539	B
	G (sym/W)	19	4.29	0.513	Retained
Simplex 4	G	19	4.29	0.513	W
	E	23	4.29	0.515	N
	F	21	4.00	0.539	B
	H (sym/N)	17	4.00	0.485	Ignored
	F	21	4.00	0.502	
Simplex 4′	G	19	4.29	0.513	N
	E	23	4.29	0.515	B
	F	21	4.00	0.502	W
	I (sym/W)	21	4.58	0.518	–
Simplex 5	G	19	4.29	0.513	W
	E	23	4.29	0.515	N
	I	21	4.58	0.518	B
	J (sym/W)	25	4.58	0.498	Ignored
	K (sym/N)	23	4.58	0.485	Ignored
	I (a)	**21**	**4.58**	**0.518****	
	I (b)	**21**	**4.58**	**0.522****	
	I (c)	**21**	**4.58**	**0.516****	
	I (d)	**21**	**4.58**	**0.523****	
	I (e)	**21**	**4.58**	**0.525****	

* W corresponds to the worst point of the simplex, B to the best point of the simplex and N to the next-to-worst point of the simplex.

** Average value is 0.521 ± 0.011 (p = 0.95).

*** The step size values are 4 °C for temperature and 0.33 for ratio.

Table 3
Doehlert experimental matrix*

w/w ratio (ratio)	Temperature (T)		Ethanol:vinasse (w/w) ratio (ratio)		Experimental response ($Y_{D,exp}$)	Predicted response ($Y_{D,cal}$)	$Y_{D,exp} - Y_{D,cal}$
	X_1	x_1 (°C)	X_2	x_2			
J	1	25	0	4.58	0.498	0.498	0
M	0.5	23	0.866	4.87	0.493	0.490	0.003
L	−0.5	19	0.866	4.87	0.480	0.483	−0.003
K	−1	17	0	4.58	0.485	0.485	0
G	−0.5	19	−0.866	4.29	0.513	0.511	0.002
E	0.5	23	−0.866	4.29	0.515	0.517	−0.002
I (a)	0	21	0	4.58	0.518**	0.521	
I (b)	0	21	0	4.58	0.522**	0.521	
I (c)	0	21	0	4.58	0.516**	0.521	
I (d)	0	21	0	4.58	0.523**	0.521	
I (e)	0	21	0	4.58	0.525**	0.521	

Variables equation: $x_1 = 21 + 4X_1$ and $x_2 = 4.58 + 0.33X_2$.

* Effective (x_i) and normalized (X_i) variables and corresponding experimental ($Y_{D,exp}$) and predicted responses ($Y_{D,cal}$) for the experiments contained in the Doehlert matrix used in this work.

** Average value is 0.521 ± 0.011 ($p = 0.95$).

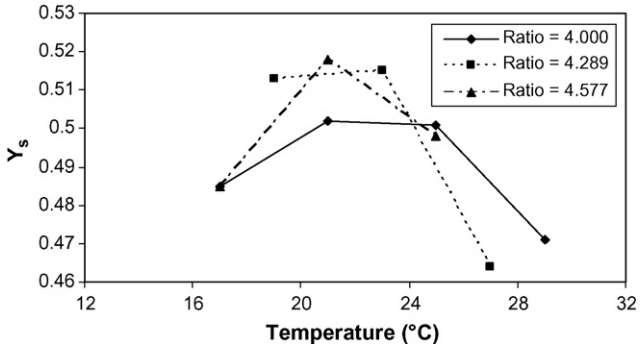

Fig. 5. Influence of temperature in the precipitation of sugar beet vinasse.

ratio), the experimental response (Y_S) and simplex number with the best point (B), the worst point (W) and the next-to-worst point (N). In the case of the initial simplex experiments (simplex 1), the best point is experiment A and the worst one (experiment C) is excluded. Experiment D is a reflection of the worst trial (experiment C) in the initial simplex. Its response value was also the worst in simplex (ABD) and, in accordance with the second rule of the simplex method, experiment E, the reflection of experiment B, was performed (simplex 2). The simplex is then carried on until point F ($Y_S = 0.539$) is the best in 3 successive simplex (simplex 3, 4 and (FGH)). Following the reevaluation rule of the simplex, experiment F was repeated and a response of 0.502 was obtained. Consequently, the simplex evolution was continued from simplex 4′. Finally, the simplex led to an optimum represented by point I (simplex 5, $Y_S = 0.518$, the best point in simplex 5 (EIJ) and (GIK)). This simplex optimum ($T = 21$ °C and ratio = 4.58) was verified by carrying out five experiments in order to evaluate reproducibility. Experiment I in bold characters in Table 2. Their mean was 0.521 with a relative standard deviation of 0.004.

Therefore, from the initial domain (experiments A, B and C) the evolution of simplex leads to a weaker temperature (21 °C instead of 25 °C) and a higher ratio value (4.58 instead of 4.00), corresponding to an ethanol weight increase of 14.4%. Moreover, it is possible to remove more than 4% of solid (0.521 instead of 0.501) in these conditions. The evolution of the response (Y_S) is shown as a function of temperature for several ratio values (Fig. 5). Whatever the ratio value, the results showed an optimum area at about 21–22 °C.

3.2. Doehlert experimental design

3.2.1. Determination of the regression model

The Doehlert matrix was built from the points surrounding the optimal experiment (I) of the simplex and points L and M were then added in order to draw the Doehlert shell (Table 3 and Fig. 4). The responses of points L and M are worse than in experiment I and confirm that the boundaries of the optimal area are defined from simplex. The effective and normalized variables (x_i and X_i) as well as the experimental response ($Y_{D,exp}$) and calculated response ($Y_{D,cal}$), obtained from Eq. (3), without the synergic term ($b_{12}X_1X_2$), are presented in Table 3. All the five duplicates for experiment I (Table 3), the center of the experimental domain, was taken into account for the establishment of the Doehlert experimental design.

By applying multiple regression analysis on the experimental data, a second-order polynomial equation was found which expresses the precipitate per vinasse weight ratio (Y_D) versus the variables in coded units (X_1 and X_2). The coefficients of the regression model (Eq. (2), calculated by the least-square method, are listed in Table 4, which contains two linear (b_1 and b_2) and quadratic terms (b_{11} and b_{22}), and one interaction (b_{12}) and block term (b_0).

Table 4
Analysis of variance (ANOVA) for the quadratic polynomial model and coefficients estimated by multiple linear regression

Term	Coefficient	Coefficient value	Sum of squares (SS)	Degree of freedom (DF)	Mean square (MS)	F-value	p-Value	Standard error of coefficient
Constant	b_0	0.5208	–	–	–	–	–	0.0017
Temperature (T)	b_1	0.0068	0.000140	1	0.000140	12.63	0.0163*	0.0022
Ratio	b_2	−0.0159	0.000756	1	0.000756	68.17	0.0004*	0.0019
$T \times T$	b_{11}	−0.0293	0.001226	1	0.001226	110.55	0.0001*	0.0032
Ratio × ratio	b_{22}	−0.0176	0.000444	1	0.000444	40.04	0.0015*	0.0024
$T \times$ ratio	b_{12}	–	0.000030	1	0.000030	2.73	0.1596*	–
Total error			0.000055	5	0.000011	–	–	–

$R^2 = 0.967$; R^2-adjusted = 0.945; standard error of estimate = 0.0038.

* Significant at $p < 0.05$

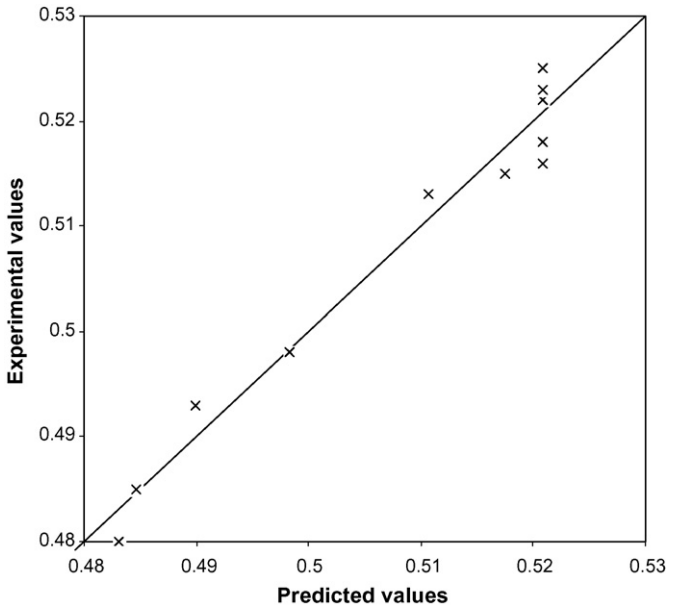

Fig. 6. Parity plot showing the distribution of experimental vs. predicted values of precipitate per vinasse weight ratio (Y_D).

The significance of each coefficient was determined by ANOVA and p values are also listed in Table 4. As shown in this table, the first-order and second-order main effects of temperature and ethanol:vinasse (w/w) ratio (ratio) are significant, seeing that their respective p values are below 0.05 (by convention). On the contrary, the interaction effect of temperature and ratio was found to be insignificant (p-value = 0.1596). Consequently, it was decided to exclude the interaction term and express the response by the following equation:

$$Y = \underset{\pm 0.0017}{0.5208} + \underset{\pm 0.0022}{0.0068}X_1 - \underset{\pm 0.0019}{0.0159}X_2 - \underset{\pm 0.0032}{0.0293}X_1^2 - \underset{\pm 0.0024}{0.0176}X_2^2 \quad (3)$$

Standard errors of coefficients are given in Table 4 and under each coefficient of Eq. (3). Eq. (3) shows the very weak positive influence of temperature (X_1) and the weak negative influence of ethanol:vinasse (w/w) ratio (X_2) on the precipitate:vinasse (w/w) ratio (Y_D) in the optimal area. Correction by the quadratic term is weak and negative on the experimental response for each parameter.

The fit of the model (Eq. (3)) was checked by the coefficient of determination (R^2). In this case, the value of the determination of coefficient (R^2) is 0.967. The R^2 value also indicates that only 3.3% of the variation is not explained by the model. The value of adjusted R^2 (adjusted R^2 = 0.945) is high enough to assert the high significance of the model. Furthermore, the parity plot shows a satisfactory correlation between the experimental and predicted values of precipitate:vinasse (w/w) ratio (Fig. 6). The F-ratio between the lack of fit and the pure error is 1.13, corresponding to a p-value of 41%.

3.2.2. Validation of the regression model

The final step of the statistical modeling is checking the predictive power in the experimental domain. Several experiments were therefore carried out in the experimental area of the Doehlert design and the experimental response was compared with the predicted one. These experiments (N, O, P and Q, Table 5) were chosen randomly in the experimental domain and experiments N and P were duplicated. Table 5 shows the validation results of the model, with these experimental points. In the experimental domain studied, the variation between experimental and calculated Y_D values was found to be less than the statistical model error. Therefore, the second-order statistical model (Eq. (3)) was totally validated.

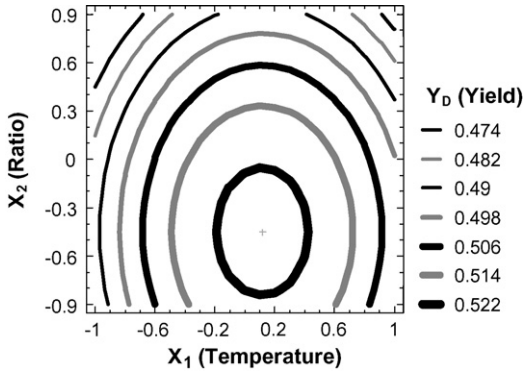

Fig. 7. 2D contour plot of temperature vs. ethanol:vinasse (w/w) ratio on the response (precipitate:vinasse, w/w ratio).

3.2.3. Interpretation of the model

The 2D contour plot of the model is presented in Fig. 7. The results show an optimum point for $X_1 = 0.117$ and $X_2 = -0.450$ in normalized variables, corresponding to effective values of 21.5 °C for the temperature and 4.43 for the ratio value. In these operating conditions, the value of the response (Y_D) is equal to 0.525. Indeed, for the low ratio values, the precipitating compounds were not entirely converted into the solid form. On the contrary, for higher ratio values, it was assumed that the less insoluble part of the precipitate was redissolved. In order to confirm these assumptions, experiments were carried out at 21 °C (optimal temperature for the simplex optimization), with different ratio values ranging from 3 to 7.5. The results, presented in Fig. 8, show the evolution of the Y_D value as a function of ratio value. The results show that the shape of the curve was also parabolic. It confirms the results obtained with the predicted values in the 2D contour plot (Fig. 7). In fact, the solvent of sugar beet vinasse is mainly water (35% in weight of initial vinasse), enabling pectins and proteins to solubilize. The addition of ethanol modifies the polarity of the solvent and reduces the solubility of some species, which precipitate. Thus, in the first part of the curves, the weight of precipitates increases with the addition of ethanol by reducing the solubility up to an amount of ethanol. Above this value, the solubility of compounds increases even for a constant temperature. Moreover, the maximum Y_D value (0.525) was reached for a ratio value of 4.9.

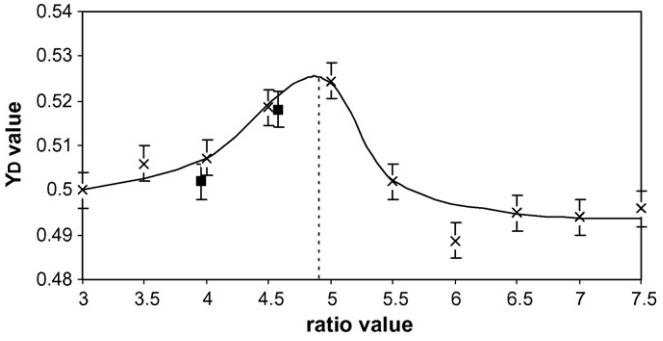

Fig. 8. Evolution of the experimental response (Y_D) as a function of ratio value, at 21 °C. (■) corresponds to the experiments of the simplex optimization: experiments F and I.

Table 5
Values of effective (x_i) and normalized (X_i) variables and corresponding experimental and predicted response for statistical model validation experiments

Points	Temperature		Ethanol:vinasse (w/w) ratio		Experimental response ($Y_{D,exp}$)	Predicted response ($Y_{D,cal}$)	$Y_{exp} - Y_{cal}$
	x_1 (°C)	X_1	x_2	X_2			
N	23.3	0.575	4.66	0.241	0.508*	0.510 ± 0.010	0.002
N′	23.3	0.575	4.66	0.238	0.513*	0.510 ± 0.010	−0.003
O	18.7	−0.575	4.30	−0.832	0.515*	0.509 ± 0.012	−0.006
P	23.3	0.575	4.37	−0.613	0.513*	0.518 ± 0.011	0.005
P′	23.3	0.575	4.36	−0.655	0.517*	0.518 ± 0.011	−0.001
Q	18.7	−0.575	4.56	−0.066	0.508*	0.508 ± 0.010	0.000

* Standard deviation of the experimental response is 0.004.

Table 6
Mass balance of sugar beet vinasse precipitation (T = 21 °C and ratio = 4.58)

Components	Input				Output			
	Vinasse[a] (636 g)		Ethanol (2913 g)		Precipitate[a] (349 g)		Filtrate[a] (3175 g)	
	C[b]	W[b]	C[b]	W[b]	C[b]	W[b]	C[b]	W[b]
Betaine (g kg^{-1})	117.6	74.79	0	0	40.0	13.96	19.8	62.87
PPC[c] (g equiv. kg^{-1})	17.99	11.44	0	0	19.77	6.90	1.31	4.16
Proteins (g kg^{-1})	1.99	1.27	0	0	3.56	1.24	0.05	0.16
Uronic acids (g kg^{-1})	6.64	4.22	0	0	7.87	2.75	0.34	1.08
Neutral sugars (g kg^{-1})	76.93	48.93	0	0	108.46	37.85	2.04	6.48

[a] Dry matter: 65.0% (vinasse), 98.5% (precipitate) and 2.9% (filtrate).
[b] C = concentration (unit shown with compound) and W = weight (g).
[c] Polyphenolic compounds.

3.3. Scale-up of sugar beet precipitation

In order to scale-up the process to an industrial scale, the precipitation was carried out in a 5-L reactor homothetic of an industrial vessel. In this experiment, 636 g of vinasse (65.0% dry matter) was stirred with 2913 g of ethanol (ratio = 4.58) at 21 °C. After precipitation, the weight of precipitate and filtrate was, respectively 349 g (98.5% in dry matter) and 3175 g (2.9% in dry matter) and 54.1% of the weight of solid was removed from vinasse (83.2% of the dry matter). The mass balance in weight and in dry matter was verified with an error respective of 0.7% (weight) and 5.4% (dry matter). The small increase in removing (54.1% as compared with 52.5% during optimization) is certainly due to a better stirring system, which enhances matter and thermic transfers. The weight balance of input and output analyzed compounds was established and is presented in Table 6.

The results shown in Table 6 indicate that the mass balance is verified for betaine, seeing that the weight of betaine in the input and output of the precipitation process was, respectively 74.79 and 76.83 g (62.87 + 13.96), representing a variation of 2.7%. Moreover, 84.1% of betaine is recovered into the filtrate. For the other sugar beet components, the variations were 3.3%, 10.2%, 9.2% and 9.4%, respectively for polyphenolic compounds, proteins, uronic acids and neutral sugars. The material balance is worst for the last three components because of the unreliability of biochemical analysis. It should be noted that the major part of proteins and pectins (uronic acids and neutral sugars) was separated during the process (respectively 97.6%, 65.2% and 77.3%). It is also shown that about 18.7% of betaine is retained by the gummed solid.

4. Conclusion

In order to separate ethanol insoluble compounds from sugar beet vinasse, precipitation in ethanol was carried out. This paper shows the optimization of the precipitation process that allows eliminating more than 52% of the matter of vinasse (55% in the scale-up precipitation) by studying the two main parameters: temperature and ratio. This optimization was firstly performed by applying the simplex method that allows us to reach an optimum and therefore, to define a domain with its optimum. The experimental results showed that the maximum Y_S response value was reached for 21 °C and a ratio value of 4.58. In these experimental conditions, 52.1% of the matter of sugar beet vinasse was separated (80.2% of the dry matter).

This study was completed by a Doehlert shell in the optimal area by adding 2 new experiments. With this design, a second-order model can be obtained by measuring the weight of solid as a function of temperature and ratio values. The predictive model obtained was checked with several validation experiments. In the experimental domain studied, the response surface appeared to be stable near the center of the domain, which is the optimum of the simplex method. In addition, the optimal operating conditions, calculated by the second-order model, were reached for 21.5 °C and a ratio value of 4.43. In this case, 52.5% of the matter of the sugar beet vinasse could be separated (80.8% of the dry matter), which is comparable with the results obtained by the simplex method (52.1%). Moreover, the validity of the method was confirmed by statistical analysis.

Finally, the precipitation process was verified on a larger scale with a view to industrialization and a better yield is obtained by using an appropriate stirring system (54.1% of the matter of vinasse removed, corresponding to 83.2% of the dry matter). The results confirmed the methods and showed that sugar beet vinasse precipitation in ethanol is a good alternative to remove protein and pectin substances. Indeed, such an operation separates about 54% of the matter contained in the crude vinasse and leads to an enrichment of betaine in the filtrate.

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References

[1] G. Vaccari, E. Tamburini, G. Sgualdino, K. Urbaniek, J. Klemeš, Overview of the environmental problems in beet sugar processing: possible solutions, J. Clean. Prod. 13 (2005) 499–507.
[2] H. Heikkila, G. Hyoky, J. Kuisma, H. Paananen, Method for fractionating a solution, US Patent US 248,089 (1999).
[3] V. Caqueret, Conception d'un procédé multi-étapes d'obtention de la bétaïne à partir de la filière bioéthanol, Ph.D. Thesis, Université d'Orléans, France, 2006.
[4] W. Spendley, G.R. Hext, F.R. Himsworth, Sequential application of simplex designs in optimization and evolutionary operation, Technometrics 4 (1962) 441–461.
[5] C. Porte, Analyse et caractérisation: méthodes directes d'optimisation, méthodes à une variable et Simplex, Techniques de l'Ingénieur P228 (2002) 1–14.
[6] T. Matsumoto, H. Du, J.S. Lindsey, A parallel simplex search method for use with an automated chemistry workstation, Chemometrics Intell. Lab. Syst. 62 (2002) 129–147.
[7] G.E.P. Box, W.G. Hunter, J.S. Hunter, Statistic for experimenters: an introduction to design, in: Data Analysis and Model Buildings, Wiley, New York, 1978.
[8] A.C. Atkinson, Optimum Experimental Designs, Clarendon, Oxford, 1992.
[9] A.L. Khuri, J.A. Cornell, Response Surfaces: Designs and Analysis, ASQC Quality Press, New York, 1987.
[10] D.A. Doehlert, Uniform shell design, Appl. Stat. 19 (1970) 231.
[11] M.M. Bradford, A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein–dye binding, Anal. Biochem. 72 (1976) 248–254.
[12] J.F. Thibault, Automatisation du dosage des substances pectiques par la méthode au métahydroxy-diphényl, Lebensm. Wiss. Technol. 12 (1979) 247–251.
[13] M.T. Tollier, J.P. Robin, Adaptation de la méthode à l'orcinol sulfurique au dosage automatique des glucides neutres totaux: conditions d'application aux extraits végétaux, Ann. Technol. Agric. 28 (1979) 1–15.
[14] J. Gorham, Separation of plant betaines and their sulphur analogues by cation-exchange high-performance liquid chromatography, J. Chromatogr. 287 (1984) 345–351.
[15] A. Zamarreno, R.G. Cantera, J.M. Garcia-Mina, Extraction and determination of glycinebétaïne in liquid fertilizers, J. Agric. Food Chem. 45 (1997) 774–776.
[16] V.L. Singleton, J.A. Rossi Jr., Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents, Am. J. Enol. Vitic. 16 (1965) 144–158.
[17] F.P. Czech, Simplex optimized J-acid method for the determination of formaldehyde, J. Assoc. Off. Anal. Chem. 56 (6) (1973) 1489–1495.