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Development of a Factorial Design To Study the Effect of the Major Hemicellulosic Sugars on the Production of Surface-Active Compounds by *L. pentosus*

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Nowadays, there are no studies about the role of the major hemicellulosic sugars on the production of surface-active compounds by *Lactobacillus pentosus*, although it was demonstrated that the activity of these compounds can be related to the agricultural residue from which they come, as the sugar solutions obtained from different agricultural residues contain different types and ratios of hemicellulosic sugars. Therefore, in this work, an incomplete factorial design was employed to test the relationship between the type and the ratio of hemicellulosic sugars present in hydrolysates from agricultural residues and the activity of surface-active compounds (cell-bond biosurfactants and extracellular bioemulsifiers) produced by *L. pentosus*. This design allowed us to establish models (that include linear, interaction, and quadratic terms) between dependent and independent variables. The independent variables used and their variation limits were as follows: glucose concentration (0−10 g L^{-1}), xylose concentration (5−15 g L^{-1}), and arabinose concentration (0−10 g L^{-1}), whereas the 13 dependent variables studied were based on the measurement of surface tension and emulsifying capability. After the study, it was found that the emulsifier capacity of extracellular bioemulsifiers produced by *L. pentosus* increases at high glucose and xylose concentrations, with glucose concentration as the most influential variable in the range studied. However, the increase of glucose in the absence of xylose produced biosurfactants with low surface activity, with, in this case, the xylose concentration as the most influential variable. Taking into account the xylose/glucose ratio, the best results were obtained with xylose/glucose ratios around 1.5−3.5, which can be found in hemicellulosic hydrolysates from trimming vine shoots or grape marc hydrolysates.

KEYWORDS: Hemicellulosic sugars; *Lactobacillus pentosus*; emulsifiers; biosurfactants; factorial design

INTRODUCTION

In previous works, it was demonstrated that *Lactobacillus pentosus* is able to produce surface-active compounds using the hemicellulosic sugars contained in different agricultural residues (*1*−*3*). In addition, it was observed that when *L. pentosus* ferments hemicellulosic hydrolysates from trimming vine shoots, it produces cell-bond biosurfactants with higher surface activity than when it ferments hemicellulosic hydrolysates from barley bran husks, corn cobs, or *Eucalyptus globulus* chips (*1*). Moreover, it was found that biosurfactants obtained after growing *L. pentosus* cells on grape marc hydrolysates not only have biosurfactant properties but also have emulsifying properties (*3*). In this way, when different agricultural residues were compared, again it was noticed that biosurfactants produced from distilled grape marc hydrolysates gave the highest stabilizing capacity (ES) value to maintain the emulsion, in comparison with biosurfactants from other residues like hazelnut hydrolysates (*3*).

The reason for the differences between the surface activity reduction and the emulsifying activity achieved with biosurfactants from these different agricultural residues is not clear, although some authors have found that the culture medium, the carbon source, and the growth conditions (pH, temperature, limiting nutrients, and trace elements) can influence the types and yields of biosurfactants (*4*). In this way, Batista et al. (*5*) found that glucose was a better carbon source than fructose, sucrose, or kerosene for screening surfactant and/or emulsifier-producing microorganisms. Thus, Amezcua-Vega et al., (*6*) working with *Candida ingens*, observed that the surface tension (ST) reduction of the culture media and the total fatty acid content of the biosurfactant were modified as the media composition changed. The highest biosurfactant production obtained by these authors was reached when higher C/Fe and C/P ratios were combined.

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Moreover, Wei and Chu (*7*) found that the addition of iron-enriched media improved the yield of surfactin by *Bacillus subtilis*.

It is obvious that there is an important effect of nutritional conditions on biosurfactant production. Consequently, on the basis of previous results, the aim of this work was to study the role of the major hemicellulosic sugars, present in lignocellulosic residues, on the production of surface-active compounds by *L. pentosus*. The independent variables studied were glucose, xylose, and arabinose concentrations, whereas the dependent variables were ST reduction produced by cell-bond biosurfactants and the relative emulsion volume (EV), ES, and percentage of emulsified organic phase (EOP) of kerosene/water emulsions stabilized by cell-bond biosurfactants or extracellular bioemulsifiers obtained by fermentation of hemicellulosic sugars with *L. pentosus*.

MATERIALS AND METHODS

Microorganism. *L. pentosus* CECT-4023T (ATCC-8041) was obtained from the Spanish Collection of Type Cultures (Valencia, Spain). The strain was grown on MRS broth at 31 °C for 15 h and at 150 rpm.

Biosurfactant and Bioemulsifier Production. Fermentations were carried out in orbital shakers at 31 °C and 150 rpm for 30 h. The experiments were made in 250 mL Erlenmeyer flasks with a final volume of 100 mL employing different concentrations of glucose, xylose, and arabinose according to the experimental matrix and the codification criteria shown in **Table 1**. In all of the experiments, the fermentation media were supplemented with 10 g L^{-1} corn steep liquor and 10 g L^{-1} yeast extract and minerals ($MnSO_4 \cdot H_2O$, 0.015 g L^{-1}; K_2HPO_4, 5.068 g L^{-1}; $NaOOCCH_3$, 0.045 g L^{-1}; $CaSO_4 \cdot 2H_2O$, 16.260 g L^{-1}; and $MgSO_4 \cdot 7H_2O$, 2.194 g L^{-1}). The mineral concentration was fixed on the basis of a previous work (*8*). Once the fermentation media were sterilized at 100 °C for 60 min, 5 mL of inoculum was added in each Erlenmeyer flask. To keep a constant pH, 5 M NaOH was added to neutralize the lactic acid and keep the pH close to 6. For obtaining cell-bond biosurfactants, *L. pentosus* cells were recovered by centrifugation at 1200*g* for 30 min at 4 °C, washed twice in demineralized water, resuspended in phosphate-buffered saline (PBS; 10 mM KH_2PO_4/K_2HPO_4 and 150 mM NaCl with the pH adjusted to 7.0), left at room temperature up to 2 h with gentle stirring for biosurfactant release, and centrifuged to remove the bacteria, following the methodology proposed by Rodrigues et al. (*9*). The extracellular bioemulsifiers were obtained directly in the fermentation media free of bacteria.

Surface Activity Determination. The ST of PBS in the presence of biosurfactants was determined by measuring the ST of the samples with the Ring method using a KRÜSS Tensiometer equipped with a 1.9 cm De Noüy platinum ring at room temperature (*10*, *11*). Tensiometers determine the ST with the help of an optimally wettable ring suspended from a precision balance. In the Ring method, the liquid is raised until contact with the surface is registered. The sample is then lowered again so that the liquid film produced beneath the ring is stretched. As the film is stretched, a maximum force is experienced, and the force is measured and used to calculate the ST.

Emulsification Studies. Kerosene was mixed in equal volume (2 mL) with 2 mL of fermentation broth containing bioemulsifiers or 2 mL of PBS extract containing biosurfactants, shaken vigorously for 2 min and left to stand for 1 h. After that time (considered the initial time, 0 h) relative EV (%) and ES (%) were measured in intervals up to 72 h using eqs 1 and 2 proposed by Das et al. (*12*).

$$\mathrm{EV}\ (\%) = \frac{\text{emulsion height (mm)} \times \text{cross-section area (mm}^2\text{)}}{\text{total liquid volume (mm}^3\text{)}} \times 100 \quad (1)$$

$$\%\ \mathrm{ES} = \frac{\text{EV at given time}}{\text{EV at 0 h}} \times 100 \quad (2)$$

Additionally, the % of EOP was also calculated using eq 3 as follows:

$$\mathrm{EOP}\ (\%) = \frac{\text{TOP volume (mm}^3\text{)} - [\text{NEOP height (mm)} \times \text{cross-section area (mm}^2\text{)}]}{\text{TOP volume (mm}^3\text{)}} \times 100 \quad (3)$$

Table 1. Nomenclature, Units, and Operational Range of the Independent Variables Assayed

(a) Independent Variables

variable	nomenclature	units	range
glucose	G	g L^{-1}	0−10
xylose	X	g L^{-1}	5−15
arabinose	A	g L^{-1}	0−10

(b) Dimensionless, Coded Independent Variables

variable	nomenclature	definition	range
dimensionless glucose	x_1	(G-5)/5	(−1,1)
dimensionless xylose	x_2	(X-10)/5	(−1,1)
dimensionless arabinose	x_3	(A-5)/5	(−1,1)

Table 2. Nomenclature of the Dependent Variables Considered

(a) Supernatant

variable	nomenclature
% EV_{24h}	y_1
% EV_{72h}	y_2
% ES_{24h}	y_3
% ES_{72h}	y_4
% EOP_{24h}	y_5
% EOP_{72h}	y_6

(b) PBS Extract

variable	nomenclature
ST	y_7
% EV_{24h}	y_8
% EV_{72h}	y_9
% ES_{24h}	y_{10}
% ES_{72h}	y_{11}
% EOP_{24h}	y_{12}
% EOP_{72h}	y_{13}

where TOP is the total volume of organic phase and NEOP is the non-emulsified organic phase.

Experimental Design and Statistical Analysis. An incomplete 3^3 factorial design (*13*) was used to study the influence of glucose, xylose, and arabinose on the ST reduction produced by cell-bound biosurfactants from *L. pentosus* as well as on the relative EV, ES, and percentage of EOP of kerosene/water emulsions stabilized by biosurfactants present in the PBS extracts or extracellular bioemulsifiers present in the cell-free supernatants. **Table 1** shows the nomenclature of the coded and uncoded independent variables assayed. The standardized (coded) adimensional variables employed, having variation limits (−1,1), were defined as x_1 (coded glucose concentration), x_2 (coded xylose concentration), and x_3 (coded arabinose concentration). The correspondence between coded and uncoded variables was established by linear equations deduced from their respective variation limits. Moreover, **Table 2** lists the dependent variables considered. The EV, ES, and EOP in kerosene/water emulsions stabilized by the extracellular bioemulsifiers present in the cell-free supernatants were measured by the variables y_1−y_6 after 24 or 72 h of emulsion formation. In addition, the ST reduction of the PBS extracts containing the cell-bound biosurfactants was measured by the variable y_7, whereas EV, ES, and EOP of the kerosene/water emulsion stabilized by these biosurfactant PBS extracts were measured by the variables y_8−y_{13} after 24 or 72 h of emulsion formation.

The experimental data were analyzed by the response surface method using the software Statistic 5.0. The interrelationship between dependent

Table 3. Coded Experimental Conditions Assayed and the Experimental Results Obtained for All of the Dependent Variables Assayed

				dependent variables												
	independent variables			supernatant						PBS extract						
experiment	x_1	x_2	x_3	y_1	y_2	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}
1	−1	−1	0	18.6	14.6	73.2	57.1	35.3	23.6	57.2	50.5	49.5	96.1	94.1	73.7	73.7
2	1	−1	0	44.2	43.2	93.7	91.5	75.9	73.9	62.9	19.4	19.4	91.1	91.1	26.2	24.3
3	−1	1	0	22.6	19.6	91.9	79.2	40.7	28.9	58.5	37.8	36.9	93.1	90.8	56.5	54.6
4	0	1	0	44.8	36.6	91.7	75.0	83.7	67.4	57.7	38.2	38.2	90.9	90.9	54.1	58.0
5	−1	0	−1	23.2	22.1	85.4	81.3	43.6	37.6	60.4	39.5	37.6	95.2	90.5	56.4	52.5
6	1	0	−1	47.2	46.2	92.2	90.2	79.9	75.9	61.3	20.7	20.7	91.3	91.3	26.9	25.0
7	−1	0	1	18.6	14.7	90.5	71.8	31.5	25.7	59.9	37.2	36.3	93.0	90.7	54.1	54.1
8	1	0	1	46.5	45.5	86.8	84.9	75.7	71.7	58.6	38.4	38.4	90.5	90.5	55.5	55.5
9	0	−1	−1	25.5	24.5	89.9	86.1	40.9	37.0	62.5	19.1	19.1	95.0	95.0	27.5	25.5
10	0	1	−1	32.3	32.3	84.6	84.6	55.0	53.0	56.6	57.0	56.0	96.5	94.7	75.1	79.3
11	0	−1	1	34.4	31.4	87.5	80.0	62.7	52.8	56.6	49.8	47.9	96.3	92.6	73.1	75.1
12	0	1	1	37.6	37.6	86.4	86.4	68.3	64.4	54.5	52.6	52.6	92.9	92.9	77.8	77.8
13	0	0	0	28.9	28.5	85.4	85.4	50.5	47.2	58.2	38.0	38.0	92.1	92.1	54.1	54.1
14	0	0	0	29.5	28.5	86.5	86.5	51.0	47.0	58.2	38.5	38.5	91.8	91.8	51.9	51.9
15	0	0	0	28.3	28.5	87.5	87.5	51.5	47.4	58.2	38.2	38.2	91.5	91.5	53.0	53.0

Table 4. Regression Coefficients and Their Statistical Significance for the Variables y_1-y_{13}

	dependent variables												
	supernatant						PBS extract						
experiment	y_1	y_2	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}	y_{12}	y_{13}
b_0	25.85[c]	19.00[c]	62.55[c]	38.54[c]	49.73[c]	30.56[c]	56.90[c]	52.91[c]	52.00[c]	102.42[c]	101.34[c]	82.62[c]	82.19[c]
b_1	0.17	1.35[c]	4.43[c]	8.61[c]	−0.35	2.10[c]	0.55[c]	−4.63[c]	−4.45[c]	−0.28[a]	0.06	−7.19[c]	−7.26[c]
b_{11}	0.16[c]	0.09[c]	−0.02	−0.30[c]	0.26[c]	0.15[c]	0.05[c]	−0.18[c]	−0.18[c]	−0.04[b]	−0.05[b]	−0.22[b]	−0.27[c]
b_2	−1.63[b]	−0.62[c]	3.07[b]	6.29[c]	−3.71[c]	−1.26[c]	0.85[c]	−3.97[c]	−3.99[c]	−1.41[c]	−1.72[c]	−6.72[c]	−7.53[c]
b_{22}	0.10[b]	0.06[c]	−0.08[a]	−0.21[b]	0.22[c]	0.11[c]	−0.05[c]	0.25[c]	0.24[c]	0.07[c]	0.07[c]	0.39[c]	0.44[c]
b_3	−0.01	−0.66[c]	−0.99[a]	−2.73[b]	0.82[b]	−0.40[b]	−0.75[c]	3.48[c]	3.48[c]	−0.37[a]	−0.23	4.34[c]	5.50[c]
b_{33}	0.04[a]	0.06[c]	0.11[b]	0.13[b]	0.01	0.07[c]	0.02[c]	0.01	−0.02[a]	0.07[c]	0.01	0.03	0.02
b_{12}	0.08[b]	0.03[c]	−0.35[c]	−0.47[c]	0.19[c]	0.09[c]	−0.09[c]	0.45[c]	0.46[c]	0.02[a]	0.05[b]	0.63[c]	0.71[c]
b_{13}	0.04[a]	0.07[c]	−0.10[b]	0.04	0.08[b]	0.08[c]	−0.02[c]	0.20[c]	0.19[c]	0.01	−0.01	0.31[c]	0.29[c]
b_{23}	−0.04[a]	−0.02[c]	0.04	0.08[a]	−0.08[b]	−0.04[b]	0.04[c]	−0.35[c]	−0.32[c]	−0.05[b]	0.01	−0.43[c]	−0.51[c]

[a] Significant coefficients at the 90% confidence level. [b] Significant coefficients at the 95% confidence level. [c] Significant coefficients at the 99% confidence level.

and operational variables was established by a model including linear, interaction, and quadratic terms:

$$y = b_0+b_1x_1+b_2x_2+b_3x_3+b_{12}x_1x_2+b_{13}x_1x_3+b_{23}x_2x_3+b_{11}{x_1}^2+b_{22}{x_2}^2+b_{33}{x_3}^2$$

where y is the dependent variable, b denotes the regression coefficients (calculated from experimental data by multiple regressions using the least-squares method), and x denotes the independent variables.

RESULTS AND DISCUSSION

In a previous work (*8*), it was demonstrated that hemicellulosic sugars from lignocellulosic residues like grape marc can be employed as carbon sources for producing lactic acid with *L. pentosus* rather than commercial sugars. This fact is related with the high concentration of minerals and nitrogen that hydrolysates have. On the other hand, it was observed that when *L. pentosus* ferments hemicellulosic sugars not only produce lactic acid but also biosurfactants (*1*, *2*) that can increase the profits of the biotechnology production of lactic acid by *L. pentosus*. Moreover, some authors (*14*) have pointed out that glucose concentrations higher than 5 g L^{-1} repress the fermentation of xylose by some lactic acid bacteria. This repression does not occur when hemicellulosic hydrolysates from lignocelluosic residues are fermented by *L. pentosus* (*8*). However, it is clear that there are differences between the surface activity reduction and emulsifying activity achieved by the biosurfactants, obtained from *L. pentosus* cells, among the agricultural residue employed as a carbon source. The highest difference between hemicellulosic hydrolysates from agricultural residues is in their sugar composition and in the xylose/glucose ratio. Consequently, to test the influence of sugar composition and sugar ratio on the production of biosurfactants by *L. pentosus*, an incomplete factorial design was carried out using commercial sugars.

The independent variables used in this study and their variations limits were as follows: glucose concentration, 0−10 g L^{-1}; xylose concentration, 5−15 g L^{-1}; and arabinose concentration, 0−10 g L^{-1}. **Table 3** shows the set of experimental conditions assayed (expressed in terms of coded variables) as well as the experimental data obtained for variables y_1-y_{13}. The experimental data allowed the development of empirical models describing the interrelationship between operational and experimental variables by equations including linear, interaction, and quadratic terms. The sequence for the experimental work was randomly established to limit the influence of systematic errors on the interpretation of results. It can be noted that experiments 1−12 allowed the calculation of the regression coefficients, whereas experiments 13−15 were replications in the central point of the design to estimate the influence of the experimental error.

Moreover, **Table 4** lists the regression coefficients and their statistical significance for the variables y_1-y_6 corresponding to the kerosene/water emulsions stabilized by extracellular bioemulsifiers and for the variables y_8-y_{13} corresponding to the kerosene/water emulsions stabilized by *L. pentosus* biosurfactants, whereas variable y_7 corresponds to the surface activity of the PBS extracts.

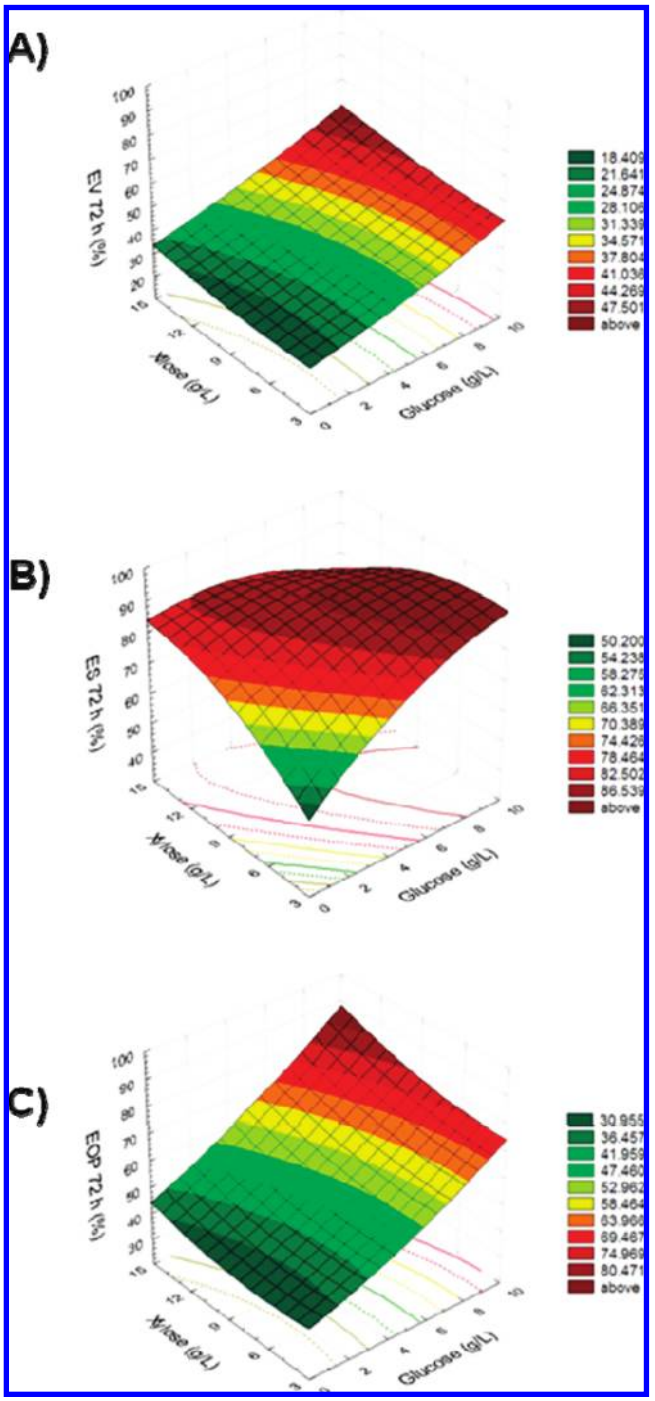

Figure 1. (**A**) Dependence of the relative EV, in emulsions made using cell-free supernatants, on glucose and xylose concentration. (**B**) Dependence of the ES, in emulsions made using cell-free supernatants, on glucose and xylose concentration. (**C**) Dependence of the percentage of EOP, in emulsions made using cell-free supernatants, on glucose and xylose concentration. In all of the cases, the arabinose concentration was fixed at the middle level (5 g L^{-1}), and the cell-free supernatants, containing bioemulsifiers, were obtained after 72 h of fermentation.

The most influential independent variables in the range tested on the EV and ES as well as on the amount of emulsified organic phase (EOP) after 72 h of emulsion formation, by the extracellular bioemulsifiers present in the supernatants, were glucose and xylose, whereas arabinose gave the lowest coefficient values. **Figure 1** shows the dependence of EV, ES, and EOP after 72 h of emulsion formation using cell-free supernatants, with the

Table 5. Statistical Parameters (r^2 and F) and Significance Level of Model for Each Dependent Variable Based on the F Test

variable	r^2	F_{exp}	significance level (based on the F test)
y_1	0.9669	175.07	99.43
y_2	0.9700	194.10	99.49
y_3	0.9696	191.52	99.48
y_4	0.9809	306.90	99.67
y_5	0.9249	73.91	98.66
y_6	0.9408	95.29	98.96
y_7	0.9558	129.74	99.23
y_8	0.967	175.95	99.43
y_9	0.9683	183.27	99.46
y_{10}	0.9835	358.06	99.72
y_{11}	0.9203	69.32	98.57
y_{12}	0.9692	188.64	99.47
y_{13}	0.9798	290.57	99.66

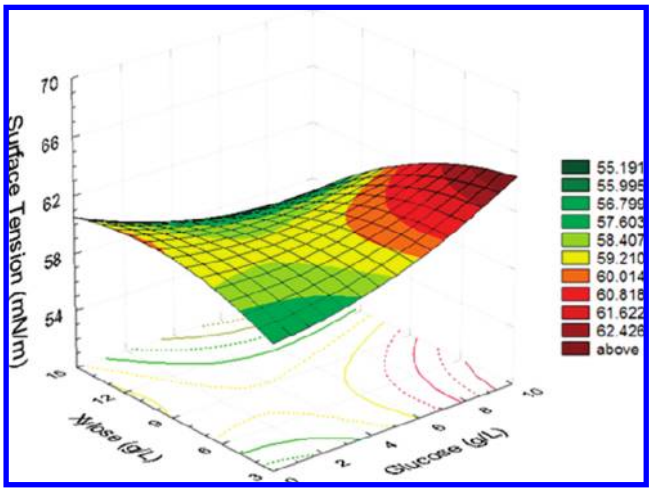

Figure 2. Dependence of the ST for PBS extracts containing biosurfactants from *L. pentosus*, on glucose and xylose concentration, predicted at 5 g L^{-1} of arabinose.

concentration of glucose and xylose in the range assayed, fixing the arabinose concentration at 5 g L^{-1}. The increase of glucose and xylose in the fermentation media enhanced the emulsifying ability of the supernatants, with glucose as the most influential variable. Additionally, in **Figure 1**, it can be observed that those emulsions formed with bioemulsifiers coming from culture media containing mixtures of glucose (5−10 g L^{-1}) and xylose (12−15 g L^{-1}) gave the highest percentage of EV, showing values above the minimum proposed by Willumsen and Karlson (*15*). The ST values of culture broth, containing emulsifiers, are not shown because no reduction of ST was observed.

Table 5 shows the statistical parameters (r^2 and F) to measure the correlation and the statistical significance of the models, respectively. It can be noticed that the models obtained for variables y_1−y_6 showed good statistical parameters for correlation and significance, allowing a close reproduction of experimental data.

On the other hand, in a previous work, we have observed that trimming vine shoot hydrolysates or grape marc hydrolysates gave a higher concentration of biosurfactants, indirectly measured as the dilution needed to reach the critical micelle concentration, than the hydrolysates obtained from other agricultural residues like corn cobs, barley husks, or *Eucalyptus globulus* chips when these hemicellulosic sugars were fermented by *L. pentosus* (*1*). Consequently, to know the reason of the differences about the production of

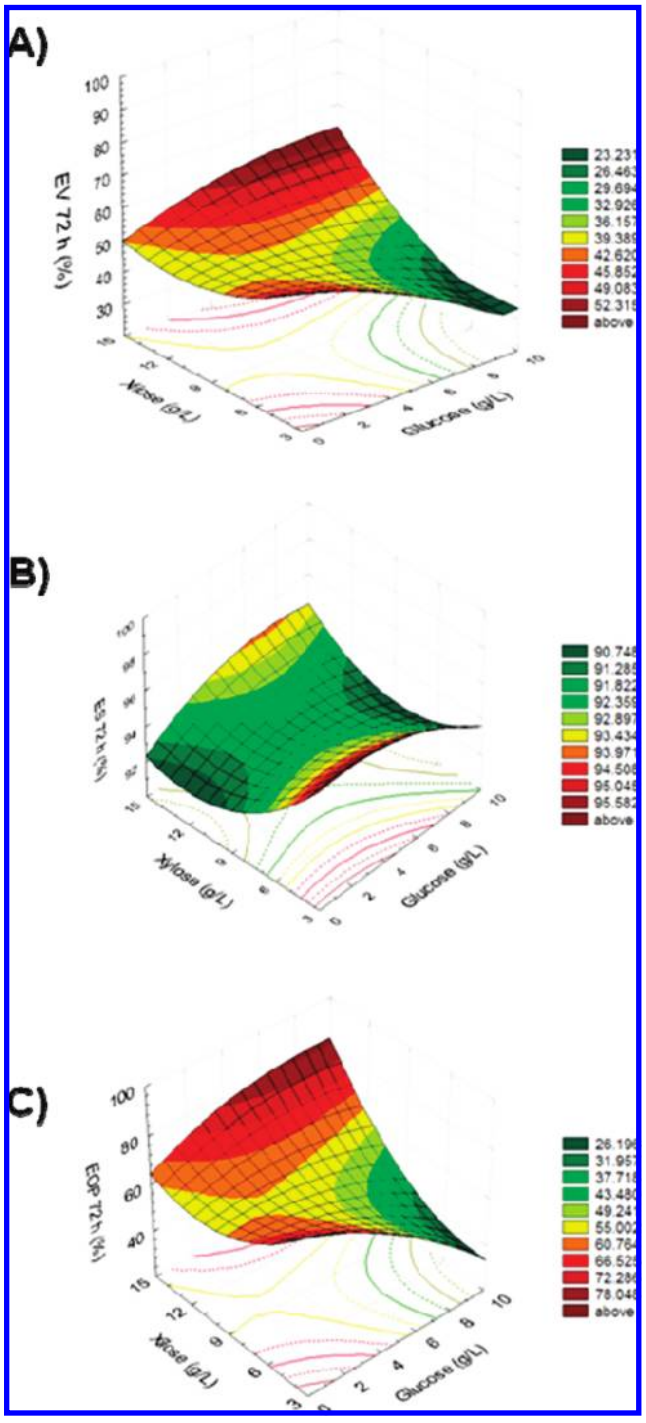

Figure 3. (**A**) Dependence of the relative EV, in emulsions made using PBS extracts, on glucose and xylose concentration. (**B**) Dependence of the ES, in emulsions made using PBS extracts, on glucose and xylose concentration. (**C**) Dependence of the percentage of EOP, in emulsions made using PBS extracts, on glucose and xylose concentration. In all of the cases, the arabinose concentration was fixed at the middle level (5 g L^{-1}) and the extraction of biosurfactants, contained in the PBS, was carried out after 72 h of fermentation.

biosurfactants when *L. pentosus* grows on different sugar concentrations, the ST activity of PBS extracts, containing biosurfactants, was also assayed as the dependent variable. The most influential variables on the ST reduction of *L. pentosus* biosurfactants were xylose and arabinose, showing similar coefficients, whereas glucose gave lower coefficient values (**Table 4**).

Table 6. Maximum and Minimum Values Obtained for the Dependent Variables EV, ES, EOP, and ST When Arabinose Was Fixed at 0, 5, or 10 g L^{-1}, Varying the Glucose and Xylose Concentrations[a]

	supernatants						PBS extracts							
arabinose	EV		ES		EOP		ST		EV		ES		EOP	
(g L^{-1})	min	max	min	max	min	max	min	max	min	max	min	max	min	max
0	20.4	47.4	59.3	93.4	32.3	80.3	56.7	66.0	23.2	51.9	91.3	96.5	25.4	73.6
5	18.4	47.5	50.2	86.5	30.9	80.5	55.2	62.4	23.2	52.3	90.7	95.6	26.2	78.0
10	19.3	50.5	47.4	86.1	33.1	84.3	54.5	59.8	34.7	62.6	90.5	95.2	52.5	95.3

[a] EV, ES, and EOP are given as percentages, while ST is given as mN m^{-1}.

Figure 2 shows that the effect of glucose on the ST value depends on the xylose concentration; the increase of glucose in the fermentation medium (in presence of low xylose concentrations) gave biosurfactants that produced a lower reduction of ST. In the range tested, the highest ST reductions of PBS were obtained using biosurfactants coming from fermentation media containing 15 g L^{-1} xylose and 5−10 g L^{-1} glucose concentration. Consequently, it could be said that the presence of pentoses in the fermentation media enhances the production of surface active substances in the presence of middle to high amounts of glucose in the range studied. These findings could explain the behavior observed in previous works (*1*−*3*), in which the surface activity behavior showed by biosurfactants obtained from trimming vine shoots or grape marc hydrolysates, with a xylose/glucose ratio from 1.9 to 2.6, was similar to that observed in this work for similar xylose/glucose ratios (3−1.5). However, when the xylose/glucose ratio increases to higher values, as happened for other materials as barley bran husks (xylose/glucose ratio = 7), corn cobs (xylose/glucose ratio = 13), and *E. globulus* chips (xylose/glucose ratio = 8.7), Moldes et al. (*1*) observed a lower ST reduction.

Table 4 also shows the regression coefficients and their statistical significance for the variables y_8−y_{13}. In this case, the most influential variables on EV and EOP values were glucose followed by xylose, whereas the most influential variable, in the range tested, on ES values was xylose followed by arabinose. In addition, **Figure 3** shows the EV, ES, and EOP values of the emulsions made with the biosurfactants obtained from *L. pentosus* growing in different concentrations of glucose and xylose. It can be observed that a high glucose concentration in the culture medium in the presence of a low xylose concentration decreases the EV and ES values of the emulsions stabilized by the produced *L. pentosus* biosurfactants, whereas those emulsions stabilized by biosurfactants obtained from fermentation media made with high glucose and xylose concentrations, in the range tested, gave emulsions with favorable EV, ES, and EOP values. The similarity in the behavior shown in **Figure 2** and that observed in **Figure 3** is noticeable, which suggests that when a higher ST reduction is obtained, biosurfactants from *L. pentosus* also show a higher ability to form emulsions and to keep them stable. Additionally, **Figure 3A** shows that biosurfactants from *L. pentosus* gave EV values above 50%, which is the minimum value proposed by Willumsen and Karlson (*15*) for a compound to be considered as a bioemulsifier. The more stable emulsions correspond with those with higher EV and EOP values.

Moreover, **Table 5** shows the statistical parameters (r^2 and F) to measure the correlation and the statistical significance of the models, respectively. It can be noticed that the models obtained for variables y_7−y_{13} showed good statistical parameters for correlation and significance, allowing a close reproduction of experimental data as those obtained for the variables y_1−y_7.

Finally, **Table 6** shows the maximum and minimum values obtained for the dependent variables EV, ES, EOP, and ST when

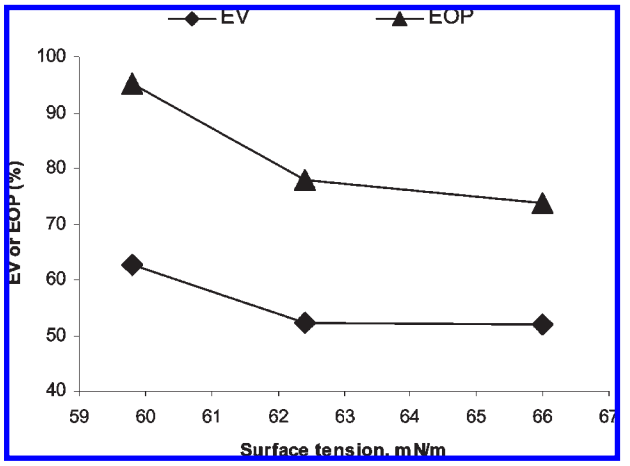

Figure 4. Plot of EV and EOP in emulsions stabilized by biosurfactants vs the ST values achieved in the PBS extract in presence of biosurfactants.

arabinose is fixed at 0, 5, or 10 g L^{-1}, varying the glucose and xylose concentration. No relevant differences were observed for EV, ES, and EOP values of the emulsions coming from supernatants when increasing the arabinose level, but some interesting variations were noticed on the ST, EV, and EOP values of emulsions coming from PBS extracts. In this case, the increase in arabinose concentration produced a remarkable improvement of the surface activity and emulsifying properties of the biosurfactant extracts. According to the coefficients of the models included in **Table 4**, these results indicate that the presence of arabinose has an important positive effect in the case of biosurfactant production, while the effect is negative and of lesser magnitude in the case of bioemulsifiers production. Moreover, when EV and EOP were plotted against ST, as shown in **Figure 4**, a decrease on EV and EOP values of emulsions while increasing the ST values of the PBS extracts was noticeable. This finding is remarkable since it reasserts the behavior observed between **Figures 2** and **3**, which suggest the existence of a correlation between the ability to reduce ST and the ability to form stable emulsions.

All of these findings point to the existence of an effect from the type of sugar on the kind of molecule produced. The production of cell-bound biosurfactants seems to be induced by the presence of pentoses on the fermentation media because higher xylose and arabinose concentrations gave higher EV and EOP values, as well as higher units of ST reduction. Moreover, there is an optimum xylose/glucose ratio (1.5−3.5) for producing biosurfactants, which can be found in hydrolysates coming from trimming vine shoots or grape marc.

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