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Multivariate optimization of *Staphylococcus xylosus* AD1 biomass production using sugarcane molasses plus yeast extract and soybean meal

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ABSTRACT. *Staphylococcus xylosus* is a microorganism that has important physiological and technological characteristics that make it suitable for use as a starter culture in fermented meat products. For the development of these products in the food industry, it is necessary to produce biomass by the multiplication of starter cultures using low-cost media. This study developed a culture medium based on sugarcane molasses (SCM) supplemented with yeast extract (YE) and soybean meal (SM) to produce *S. xylosus* AD1 biomass employing a Box Behnken multivariate optimization design, using the best concentrations of the constituents of the culture medium for *S. xylosus* AD1 growth. By combining the mathematical models by the desirability function, it was possible to establish the optimal condition for the maximum production of viable cells and biomass. The optimal experimental condition was found when the fermentative process medium was composed of 10% SCM, 2% YE and 4% SM. In addition, the results of all experiments, except for the medium formulated with only SCM, presented a better performance than the commercial medium Brain Heart Infusion for the growth of *S. xylosus* AD1. The culture medium with agro-industrial byproduct (SCM) supplemented with YE and SM is an excellent alternative for producing *S. xylosus* AD1 biomass.

Keywords: Agro-industrial by products; Microbial production; Starter culture.

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Introduction

Coagulase-negative staphylococci (CNS) play a major role in the food processing industry, notably the species *Staphylococcus carnosus* and *Staphylococcus xylosus*, which are traditionally used in starter cultures for fermented meat products (Sánchez-Mainar, Stavropoulou, & Leroy, 2017). *Staphylococcus xylosus* is a microorganism that has important physiological and technological characteristics that make it suitable for use as a starter culture (Laranjo, Elias, & Fraqueza, 2017). Among these characteristics are the production of catalase, its ability to reduce nitrate, and its lipolytic and proteolytic activity (Chajęcka-Wierzchowska, Zadernowska, Nalepa, Sierpińska, & Łaniewska-Trokenheim, 2015; Cruxen et al., 2017; Fiorentini, Sawitzki, Bertol, & Sant'Anna, 2009). Moreover, these effects are most effective when the microorganisms are isolated from the microbiota of traditional products, such as the bacterium *S. xylosus* AD1, which has been isolated from sausage produced in Southern Brazil (Fiorentini et al., 2009).

For the development of fermented meat products in the food industry, it is necessary to produce biomass by the multiplication of starter cultures. To enable the large-scale industrial production of starter cultures, low-cost culture media that ensure good bacterial growth are essential (Makowski et al., 2017). Obtained commercially, the medium that is currently used extensively for multiplication of *Staphylococcus* is Brain Heart Infusion broth (BHI) (Miranda, Sant'anna, & Porto, 1999).

It has been well established that bacterial cell biomass production is strongly influenced by the composition of media, including carbon, nitrogen, growth factors, and inorganic salts (Li, Bai, Cai, &

Ouyang, 2002). Microorganisms, depending on their nutritional requirements, need access to different sources of constituents, and both a shortage and excess of even one component may inhibit the growth and activity of the cells, and consequently decrease the process efficiency of cell biomass production (Makowski et al., 2017). For this, due to the number of variables, optimization of media composition is a great challenge.

In this way, various byproducts and raw materials from the food industry have been employed for the growth of microorganisms, thereby reducing the costs associated with the culture medium, besides being more economical, affordable and avoiding improper disposal, thus minimizing environmental problems. Wheat bran, oat bran, molasses sugar and fruit flour are examples of agro-industrial byproducts that may be used in a culture medium (Bispo, Andrade, Souza, Teles, & Nascimento, 2018; Dumbrepatil, Adsul, Chaudhari, Khire, & Gokhale, 2007; Li, Han, Ji, Wang, & Tan, 2010; Parra-Matadamas, Mayorga-Reyes, & Pérez-Chabela, 2015).

Of these byproducts, sugarcane molasses (SCM) can be useful for fermentation processes because it contains high levels of fermentable sugars such as fructose, sucrose and glucose, and vitamins and minerals, reasons why it has been considered the most economical carbon source in the fermentation industry (Fernández-López, Torrestiana-Sánchez, Salgado-Cervantes, Mendoza García, & Aguilar-Uscanga, 2012; Machado et al., 2018). It is noteworthy that molasses is an inexpensive renewable carbon source, easily handled, low-cost, and has the potential for many industrial bioprocesses (Oliveira, Sousdaleff, Lima & Lima, 2009). Furthermore, Brazil is the major sugarcane producer worldwide, occupying the first position in the ranking of sugarcane-producing countries, being a potential source of SCM for fermentation industries (Brasil, 2019).

According to Portilla et al. (2017), the growth medium supplemented with sugarcane molasses as carbon sources improved biotechnological process regarding enzyme and biomass production. Machado et al. (2018) observed that the use of SCM as supplement as culture media consist on a viable and promising strategy for reducing costs and enhancing bacterial cellulose production, which the increasing concentration of SCM into culture media, smoother and more flexible bacterial cellulose membranes surfaces were detected. Tyagi and Suresh (2016) verified that the composition of molasses contains nitrogen and vitamins sources, which may play an important role in optimizing the production of bacterial cellulose.

Although SCM is a cheap source of carbon, supplementation of nitrogen source can enhance biomass production. For this, some researchers have supplemented molasses with organic nitrogen compounds, such as yeast extract (YE) and soybean meal (SM) (Dumbrepatil et al., 2007; Oliveira et al., 2009). YE is an excellent source of vitamin B complex, amino acids, peptides, proteins and carbohydrates (Dumbrepatil et al., 2007). SM is a byproduct of oil extraction, has high protein content (Siqueira et al., 2008) and can hydrolyze SM, thereby releasing amino acids from proteins and facilitating the uptake of these compounds by microorganisms. In study performed before, was detected that molasses supplemented with peptone and YE could be used as a good production medium for large scale production of yeast biomass (Sarlin & Philip, 2013). Su, Cheng, Hsiao, Han, and Yu (2018) investigated the optimal parameters for solid-state fermentation of SM by *Lactobacillus* species and *Clostridium butyricum*, and the results showed that two days of fermentation plus exogenous protease supplementation of SM was sufficient to increase the viable count of bacteria and consequently lactic acid levels.

It is noteworthy that the composition of the medium and final quality of molasses vary a great deal among batches, having different titers of nutrients (e.g., minerals, sucrose, glucose, fructose, vitamins, fatty acids, etc.), and for this its efficient utilization by microbial cell should not be taken for granted (Basso, Basso, & Rocha, 2011; Lino, Basso, & Sommer, 2018). Considering that multivariate optimization can be used to evaluate the relative significance of several factors in the presence of complex interactions (Polak-Berecka, Waśko, Kordowska-Wiater, Targoński, & Kubik-Komar, 2011), in this study a Box Behnken multivariate design was employed with the aim to develop a culture medium based on sugarcane molasses (SCM) supplemented with yeast extract (YE) and soybean meal (SM) for the production of *S. xylosus* AD1 biomass.

Material and methods

Bacterial strain

The strain *S. xylosus* AD1 used in this study was isolated from microbiota of handmade fermented sausages from the Southern region of Brazil. It was molecularly characterized (Fiorentini et al., 2009) and stored frozen at -80 °C in BHI broth (Oxoid, UK), containing 20% (v v^{-1}) glycerol (Sigma-Aldrich, UK).

Physicochemical characterization of molasses

The SCM was obtained from Cooper Tereza Company in Campinas das Missões, Rio Grande do Sul, Brazil. The physicochemical characterization of molasses was performed according to the methods of the Association of Official Analytical Chemists (AOAC, 2000) to determine the moisture, ash, nitrogen, and reducing and non-reducing sugar contents. The potassium and sodium contents were determined photometrically (Flame Photometer, Digimed, Brazil). The magnesium and manganese contents were determined by atomic absorption (Atomic Absorption Spectrophotometer in Flames, Gilson, USA). The phosphorus and iron contents were quantified using spectrophotometry at 400 nm and 550 nm, respectively (Spectrophotometer, Hitachi U-2010, Japan). YE (AES Chemunex BioMérieux Company, France) and SM (Camera Agroalimentos SA, Brazil) were assessed to determine their nitrogen contents according to the AOAC method. Determination of the nitrogen content was critical because the YE and SM were added to alternative culture media to address the low amount of nitrogen present in SCM.

Multivariate optimization for cell biomass production

Multivariate optimization was performed using an incomplete 3^3 Box Behnken design (Hauly, Oliveira, & Oliveira, 2003) for the optimization of three variables: concentration of SCM (m v^{-1}), YE (m v^{-1}) and SM (m v^{-1}). Therefore, three levels were investigated for each variable, as shown in Table 1. Multivariate planning was performed in random order and at the central point triplicates were performed to estimate experimental variance, totaling 15 experiments.

Responses investigated were viable cell content, biomass and optical density. The experimental results were analyzed by analysis of variance in order to evaluate the quality of the mathematical models and the significance of the regression coefficients, with 95% confidence. Simultaneous optimization of responses was performed using the desirability function of Derringer and Suich (1980), through the combination of mathematical models, in order to obtain the prediction of the optimal condition. Analyses were performed using Statistica software 7.0 (2004) and Design Expert 6.0.4. (2000) software. The optimal condition predicted by the models was experimentally validated in triplicate, and their results were compared using the confidence interval (95%).

Table 1. Multivariate design experiments for biomass production of *Staphylococcus xylosus* AD1 in culture media based on sugarcane molasses supplemented with yeast extract and soybean meal

Experiments	Coded Levels and Variables		
	X1	X2	X3
1	-1	-1	0
2	+1	-1	0
3	-1	+1	0
4	+1	+1	0
5	-1	0	-1
6	+1	0	-1
7	-1	0	+1
8	+1	0	+1
9	0	-1	-1
10	0	+1	-1
11	0	-1	+1
12	0	+1	+1
13	0	0	0
14	0	0	0
15	0	0	0
Decoded Variables	Decoded Levels		
	-1	0	+1
X1, sugarcane molasses	2%	6%	10%
X2, yeast extract	0%	2%	4%
X3, prepared soybean meal	0%	2%	4%

Cell biomass production process

The growth of *S. xylosus* AD1 was initiated by propagation of inoculum. Firstly, stock culture was inoculated into BHI agar petri dishes with incubation at 35°C for 24 hours. A colony was transferred to 10 mL of BHI broth and incubated at 35 °C for 24 hours, followed by transfer to 10 mL of media made using the

experimental design presented in Table 1 with incubation at 35 °C for 12 hours. After this period, 10 mL of each broth was transferred to flasks containing 20 mL of medium with the same composition and incubated at 35 °C for 12 hours, under stirring of 100 rpm. This process yielded a total of 30 mL inoculum, at a concentration of approximately 9 log CFU mL^{-1}, as determined from the viable cell count in BHI petri dishes.

Biomass production was carried out in a Bio-Tec bioreactor Plus (Tecnal, Brazil), containing 3.0 L of the formulated medium. *Staphylococcus xylosus* AD1 was inoculated 1% (v v^{-1}) and the mixture was fermented under the following conditions: temperature of 35 °C, agitation at 100 rpm and aeration of 0.7 v v m^{-1} (L of air per L of medium per minute) for 12 hours (based on preliminary tests, ensuring that bacterial growth would not exceed the exponential phase).

In addition to the culture media treatments tested, BHI broth was used as the standard commercial medium. Previously, SM plus water (for every gram of SM, 7 mL of water was added) was subjected to heat treatment by autoclaving at 121°C for 15 min. After that, the mixture was sieved to yield SM filtrate containing 3.4% (m v^{-1}) nitrogen.

Analytical determinations

During the biomass production, samples were taken every two hours for determination of the pH, the viable cell count, biomass, optical density (OD), and contents of total and non-reducing sugars expressed as sucrose, with the purpose of monitoring the growth of the microorganism.

The pH was determined in the bioreactor through use of a pH probe. The OD measurements were performed using a spectrophotometer at 630 nm. The viable cell counts of *S. xylosus* AD1 were measured by plating 0.1 mL of medium on BHI petri dishes with incubation at 35 °C for 48 hours. For determination of the biomass, 10 mL of fermentation broth were centrifuged (Cientec, Brazil) at 2,420 g for 30 min. plus three washes with 0.85% (w v^{-1}) saline solution (Synth, Brazil). Subsequently, drying was performed at 60 °C in a drying oven (DeLeo, Brazil) until a constant weight, and the value was expressed in grams of cells per liter of fermentation broth. The determination of total and non-reducing sugar contents expressed as sucrose was performed using the Lane-Eynon method (AOAC, 2000).

Composition characterization of the formulated medium

The culture medium that presented the highest viable cell counts of *S. xylosus* AD1 during the 12 hours fermentation was evaluated to determine the nitrogen content, contents of reducing and non-reducing sugars, and crude fiber content according to AOAC (2000).

Results and discussion

Characterization of the sugarcane molasses (SCM)

The composition of SCM is detailed in Table 2. It can be observed that the total sugar content was 48.12%, with the highest percentage accounted for by non-reducing sugars, such as sucrose. Some studies on the use of SCM as a culture medium for the growth of microorganisms found similar values for the contents of total sugars, reducing sugars and non-reducing sugars, which had values of 48.50, 14.72 and 33.78%, respectively (Miranda et al., 1999).

Table 2. Composition of sugarcane molasses

Composition	Concentration
Moisture	22.43% ± 0.12
Ash	1.25% ± 0.06
Nitrogen	0.04% ± 0.00
Reducing sugars	12.49% ± 0.35
Non-reducing sugars	35.63% ± 1.00
Sodium	46.12 mg per 100g ± 0.00
Potassium	292.46 mg per 100g ± 0.01
Manganese	8.36 mg per 100g ± 0.00
Magnesium	118.38 mg per 100g ± .01
Iron	0.83 mg per 100g ± 0.01
Phosphorus	65.5 mg per 100g ± 0.01

Feltrin, Sant'Anna, Porto, and Torres (2000) analyzed the composition of SCM and found higher values for the ash content than those obtained in this study. However, when the phosphorus and potassium contents are compared, only the potassium values are higher. Variations in the levels of SCM constituents are expected because the composition is dependent on the variety and ripeness of sugarcane, climate and growing conditions as well as industrial factors. Determination of the phosphorus and potassium contents is important because both participate in the formation of nucleic acids, phospholipids and energy substances from the ATP series, thereby allowing accumulation and distribution of the cells' energy; furthermore, potassium and magnesium are elements that microorganisms often require as cofactors for enzymatic reactions (Madigan, Martinko, Bender, Buckley, & Stahl, 2014).

For the growth of microorganisms, the culture medium should meet the nutritional and energy needs by providing sources of carbon, nitrogen (amino acids and peptides), vitamins, nitrogenous bases and nucleic acids (Arumugam & Kumar, 2017; Madigan et al., 2014). Thus, the SCM was supplemented with YE and SM. Vitamins and other organic factors are supplied by the YE, in addition to organic nitrogen and carbon compounds. Soybean meal is also a rich source of B vitamins and proteins, and contains calcium and iron. The YE exhibited a nitrogen content of 12.48%, whereas the filtered SM nitrogen content was 7.53%.

Monitoring the growth of *S. xylosus* AD1

The fermentative process was monitored for 12 hours. The behavior of the microorganisms in BHI culture medium was also analyzed for comparison with culture medium formulated using the experimental design. The sugar consumption varied from 11.43% (T1) to 100% (BHI). The variation in sugar consumption was due to the composition of the medium, which influenced the growth of the microorganism during the fermentative process. The lowest consumption of sugar was observed in the formulated broth from T1, which had a low sugar concentration and low supplementation, only 2% of SM. This occurred because the initial sugar concentration of T1 is lower than those of most of the media formulated using the treatments described in the experimental design (Table 1), as well as the type of sugar that comprised the BHI broth, glucose.

The mean sugar consumption values corresponding to treatments T3, T5 and T7 were 55.23, 43.97 and 42.96%, respectively. The range of sugar consumption percentage values is in agreement with the results obtained by Miranda et al. (1999), in a study that found that the consumption of sugar reached 50.53% during fermentative growth of *Micrococcus varians* for 24 hours in a medium based on sugarcane molasses.

The final pH of the fermentation broth ranged from 3.73 (T3) to 6.97 (T9) (Table 3). The low pH reduction obtained in T9 was due to the absence of supplementation, which restricted the growth of the microorganism and consequently the production of metabolites.

Table 3. Results obtained from the Box Behnken design for the biomass production of *Staphylococcus xylosus* AD1

Experiments	Molasses of sugarcane (X1) (%)	Yeast extract (X2) (%)	Soybean meal (X3) (%)	Consumption of total sugar	pH	Count viable cells (log CFU mL^{-1})	Biomass (g L^{-1})	Optical density
1	2	0	2	11.43	4.65	9.48	6.80	0.50
2	10	0	2	21.78	5.25	9.00	4.90	1.88
3	2	4	2	55.23	3.73	10.43	8.20	1.46
4	10	4	2	21.38	4.07	10.76	7.30	1.78
5	2	2	0	43.97	4.01	10.11	5.60	1.59
6	10	2	0	22.84	4.38	8.80	4.91	1.83
7	2	2	4	42.96	4.04	10.00	7.95	0.78
8	10	2	4	31.30	3.96	11.92	8.35	0.85
9	6	0	0	20.39	6.97	5.30	2.85	0.66
10	6	4	0	18.95	4.91	9.60	4.31	1.98
11	6	0	4	23.36	4.94	8.72	7.96	1.68
12	6	4	4	23.21	4.44	9.43	9.50	1.34
13	6	2	2	17.75	4.07	9.23	4.36	2.04
14	6	2	2	17.26	3.93	9.30	5.30	2.29
15	6	2	2	17.46	4.15	9.48	5.42	1.63
BHI	NA*	NA	NA	100	6.62	9.11	2.53	1.96

*Not applicable.

Microorganisms use an organic source and produce metabolites such as organic acids, which are formed during the exponential growth phase, while new cells are produced (Madigan et al., 2014). The nutritional components of the growth medium depend on the microorganism being cultivated. Each organism has

specific metabolic pathways for obtaining energy and production of metabolites, and this should be considered. The great variety of species and microbial strains can also lead to specific nutritional needs (Vogel & Todaro, 2014).

Regarding viable cell count, T8 showed the best experimental outcome with 11.92 log CFU mL^{-1} followed by T4 (10.76 log CFU mL^{-1}), T3 (10.43 log CFU mL^{-1}), and T5 (10.11 log CFU mL^{-1}) (Table 1). Miranda et al. (1999) found an approximate value of viable cells of *M. varians* after 12 hours of cultivation, obtaining 14 log CFU mL^{-1} of *M. varians* using a medium based on SCM, reaching the value of 19.43 log CFU mL^{-1} after 24 hours.

The best experimental results for biomass production were obtained using T12, with a value of 9.5 g L^{-1}, and the second-best result was obtained using T8, with a value of 8.35 g L^{-1}. The least favorable condition was T9, with a biomass of 2.85 g L^{-1}, which was constituted by only 6% (m v^{-1}) SCM, without protein supplementation (Table 1). These results are higher than those found by Miranda et al. (1999), with *M. varians* production in a culture medium composed of SCM.

The OD values obtained for experiments T13 and T10 were higher than those obtained using the BHI medium. The best experimental results for the OD analysis were obtained using T13, which corresponds to the central point – 6% (m v^{-1}) SCM, 2% (m v^{-1}) YE and 2% (m v^{-1}) SM, with an OD value of 2.04, 2.29 and 1.63, respectively. However, none of the variables had a statistically significant effect at the levels studied.

Multivariate optimization

Table 4 presents the validation data of the multivariate planning mathematical models, the desirability conditions employed for simultaneous optimization of the responses, the optimal condition predicted by the models and the experimental validation of the optimal condition.

Table 4. Validation of multivariate planning mathematical models, desirability conditions employed, predicted optimal condition and experimental validation

Validation of mathematical models													
Responses	Indicated Model	F Lack of Fit[a]	F Regression Significance[b]	Significant coefficients (error)									
				Intercept	A	B	C	A^2	B^2	C^2	AB	AC	BC
Viable cells	Quadratic	14.84	19.54	9.34 (0.23)	-	0.96 (0.14)	0.78 (0.14)	1.26 (0.20)	-0.68 (0.20)	-	-	0.81 (0.20)	-0.90 (0.20)
Biomass	Linear	3.47	12.89	6.25 (0.26)	-	0.85 (0.36)	2.01 (0.36)	-	-	-	-	-	-
Optical density	Quadratic	2.56	1.65	-	-	-	-	-	-	-	-	-	-

Desirability conditions, predicted optimal condition and experimental validation					
Variable/Response	Goal	Importance	Optimum conditions	Predicted Responses	Experimental responses observed (Mean ± Standard deviation)
A	Is in range: -1 a 1	3	1		
B	Is in range: -1 a 1	3	0.01		
C	Is in range: -1 a 1	3	1		
Viable cells	Maximize	5		11.86	11.73 ± 0.21
Biomass	Maximize	3		7.88	8.05 ± 0.27

A: Sugarcane molasses; B: Yeast extract; C: Soybean meal. [a]Model showed no significant lack of fit (95% confidence) when F value was below critical $F_{(9,2)}$=19.38 for linear model; [b] Model showed significant regression (95% confidence) when F value was higher than critical $F_{(9,5)}$ =4.77 for quadratic model and critical $F_{(3,11)}$= 3.59 for linear model; -: Non-significant coefficient.

For the viable cell content, the quadratic model was more appropriate to describe the behavior of the variables and showed no evidence of lack of fit, with 95% confidence, since the experimental F obtained was lower than the critical $F_{(3.2)}$ of 19.16. The regression was significant, and the coefficients that affected the response behavior were B and C (linear), A and B (quadratic). The AC interaction was significant and the simultaneous increase of the two variables improved the responses. Simultaneous increase in BC evidenced a decrease in response. Regarding biomass, parameter, the linear model adequately described the behavior of the variables, without evidence of lack of adjustment, with 95% confidence, since the experimental F obtained was lower than the critical $F_{(9.2)}$ of 19.38. Regression was significant. The variable that most affected the response was C. Variable B also had a significant effect and the increase of both improved the amount of biomass. For optical density, the variables did not affect the response, indicating that any of the levels studied can be used.

Considering the quality of the mathematical models for viable cells and biomass, they were used to predict the optimal conditions. The models were combined through the desirability algorithms, establishing

viable cell content (5) as the most important criterion over biomass (3). This decision was made because of the importance of cells remaining viable during the biomass harvesting process. Through the simultaneous optimization of the models, 28 possible combinations of the variables that met between 0.90 and 0.92 of the desired desirability were obtained. In all these combinations, the responses predicted by the models did not differ from each other. One of the suggested combinations was similar to one of the conditions already evaluated in the experimental design (T8), which is why it was chosen as the optimal point.

The optimal point condition was validated through experimental trials, where statistically similar responses (95% similarity) to the predicted model responses were obtained.

Figure 1 shows the response surface obtained for viable cell counts, while Figure 2 shows the biomass of *S. xylosus* AD1. Through the response surfaces, it can be observed that the best condition was obtained when variable A is used at level +1.0, B at level 0.0 and C at level +1.0. In decoded conditions, the optimum point was when the medium used in the fermentation process was composed of 10% SCM, 2% YE and 4% SM.

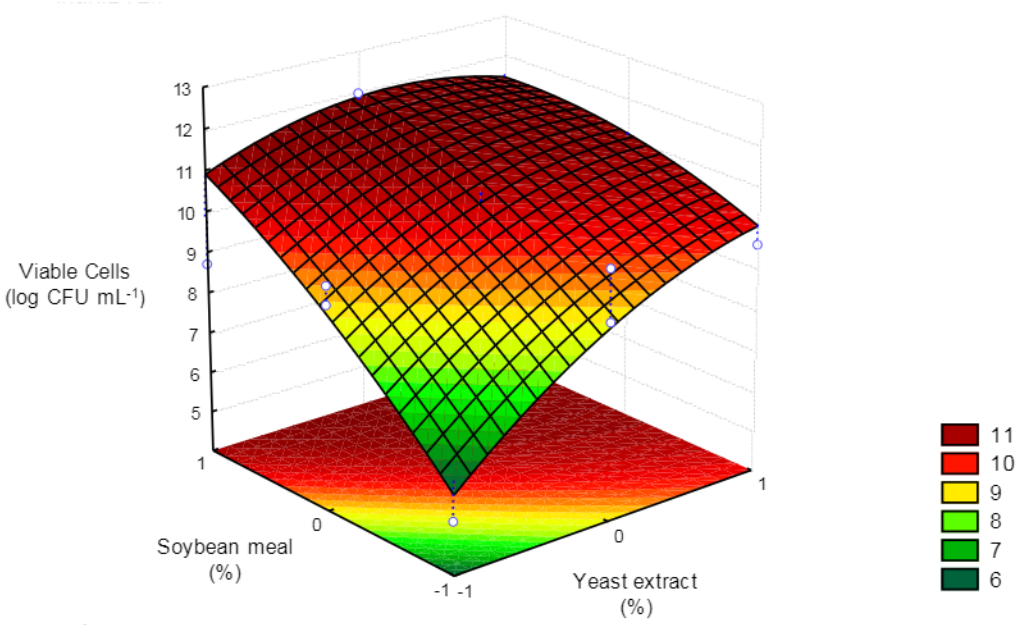

Figure 1. Response surface obtained for viable cell counts of *Staphylococcus xylosus* AD1. (The sugarcane molasses variable was set at level +1.0.).

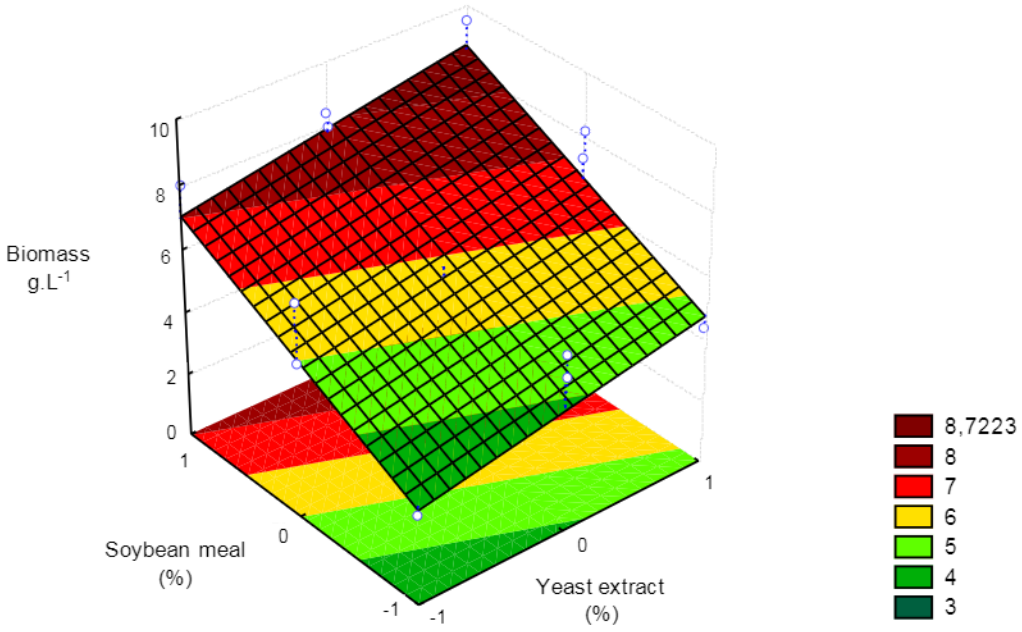

Figure 2. Response surface obtained for biomass production of *Staphylococcus xylosus* AD1. (The sugarcane molasses variable was set at level +1.0.).

Considering the mathematical model presented on the biomass response surface (Figure 2), it was observed that a higher biomass content would be obtained if the multivariate planning were shifted to higher levels of the SCM and SM variables. However, the curvature observed for viable cell response has already reached the optimal condition. This corroborates the simultaneous optimization of the models performed by the desirability function (Table 4). From a practical point of view, the viable cell count is the most important parameter when the aim is to use the cells as a starter culture, because when the microorganisms are added they should be viable in the product, to ensure that the fermentation proceeds in a satisfactory manner. Table 5 presents the results for the composition of T8 medium and its contents of nitrogen, reducing, non-reducing, and total sugars, as well as crude fiber.

Table 5. Composition of the culture medium in treatment eight (T8) for *Staphylococcus xylosus* AD1 growth

Composition	Concentration (g%)
Nitrogen	4.71 ± 0.09
Reducing sugars	1.30 ± 0.27
Total sugar	7.07 ± 1.23
Non-reducing sugars	5.52 ± 0.91
Crude fiber	0.005 ± 0.01

Moreover, all experimental conditions – with the exception of T9, which consisted of only SCM, without supplementation – have the potential for use as culture media for growth of *S. xylosus* AD1, especially considering the viable cell counts obtained. The values obtained for 12 of the 13 formulated culture media were similar to that obtained using the BHI culture medium. These results demonstrate the importance of using a medium supplemented with a nitrogen source, because when using the T9 treatment (without supplementation), the viable cell counts were lower than in the other treatments.

Conclusion

Agro-industrial byproducts, such as sugarcane molasses (SCM), supplemented with yeast extract (YE) and soybean meal (SM), provide an excellent alternative for the biomass production of *S. xylosus* AD1. The results of this study demonstrate the best combination by Box Behnken multivariate optimization design and, due to the desirability function of Deringer and Suich, it was possible to establish the optimal condition for the maximum production of biomass. The optimal decoded point was obtained using the medium composed of 10% SCM, 2% YE and 4% SM, which obtained 8.05 g L^{-1} biomass of *S. xylosus* AD1 after 12h of fermentation.

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