

Article

Optimization of Xanthan Gum Production by Demerara Sugar Using Response Surface Methodology

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Abstract: Xanthan gum (XG) production using three *Xanthomonas* sp. strains (290, 472, and S6) was evaluated by applying a 2³ full factorial central composite design response to study the interactive effects of the fermentation medium component concentrations as parameters to determine the efficiency of the gum production in batch experiments. The experimental variables were the carbon source (demerara sugar or sucrose), potassium phosphate dibasic, and magnesium sulfate. Experimental results showed the K₂HPO₄ concentration as the important parameter for XG production by using *Xanthomonas axonopodis* pv. *manihotis* IBSBF 290 and *X. campestris* pv. *campestris* IBSBF 472, while for the *Xanthomonas* sp. S6 strain, the MgSO₄·7H₂O concentration was the determining factor in XG production using demerara sugar or sucrose as a carbon source. The strains of *Xanthomonas* 472 and S6, using demerara sugar and higher concentrations of salts, exhibited a higher yield of XG (36 and 32%) than when using sucrose and the same concentration of salts. The experimental outcomes highlighted demerara sugar as a suitable and efficient alternative carbon and micronutrient source for XG production. Despite the bacterial strain influence, the medium composition is crucial for this fermentation process. Therefore, the evaluated salts are important factors for XG production, and the demerara sugar can partially replace this mineral salt requirement as indicated by the face-centered composite experimental design due to its chemical composition. Overall, demerara sugar provides promising properties for XG production.

Keywords: xanthan gum; demerara sugar; mineral salts; response surface methodology; fermentation



Citation: Ramos, L.C.; Jesus, M.S.; Pires, P.; Fontes-Junior, A.S.; Nunes, E.S.; Santos, K.S.; Teixeira, J.A.; Padilha, F.F.; Ruzene, D.S.; Silva, D.P. Optimization of Xanthan Gum Production by Demerara Sugar Using Response Surface Methodology. *Sustainability* **2023**, *15*, 5080. <https://doi.org/10.3390/su15065080>

Academic Editor: Idiano D'Adamo

Received: 14 February 2023

Revised: 4 March 2023

Accepted: 9 March 2023

Published: 13 March 2023



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1. Introduction

Xanthan gum (XG) is a water-soluble microbial exopolysaccharide (EPS) produced by bacteria of the genus *Xanthomonas* [1]. The monomeric structure of the XG is formed by two units of glucose (cellobiose) bonded in the main chain (the backbone) with a branch formed by two units of mannose and one unit of glucuronic acid (Figure 1) [2,3]. The XG is an EPS, and a biopolymer, chemically characterized by a structural chain with a molar mass ranging between 2×10^6 and 20×10^6 Da [4,5]. Furthermore, its molecular weight is influenced both by bacterium strains and fermentation conditions, producing a variety of gums of industrial interest [6]. These aspects are important since the XG molecular structure and conformational state are closely related to its rheology, stability, and function [7,8]. These properties allow its application as a thickening, dispersant, emulsifier, and viscous aqueous solution at low concentrations (0.05–1%), and are stable over a wide range of pH values

and temperatures. Moreover, XG is a dry, tasteless, white-to-cream, and biodegradable powder, and is less expensive compared to synthetic polymers [9].

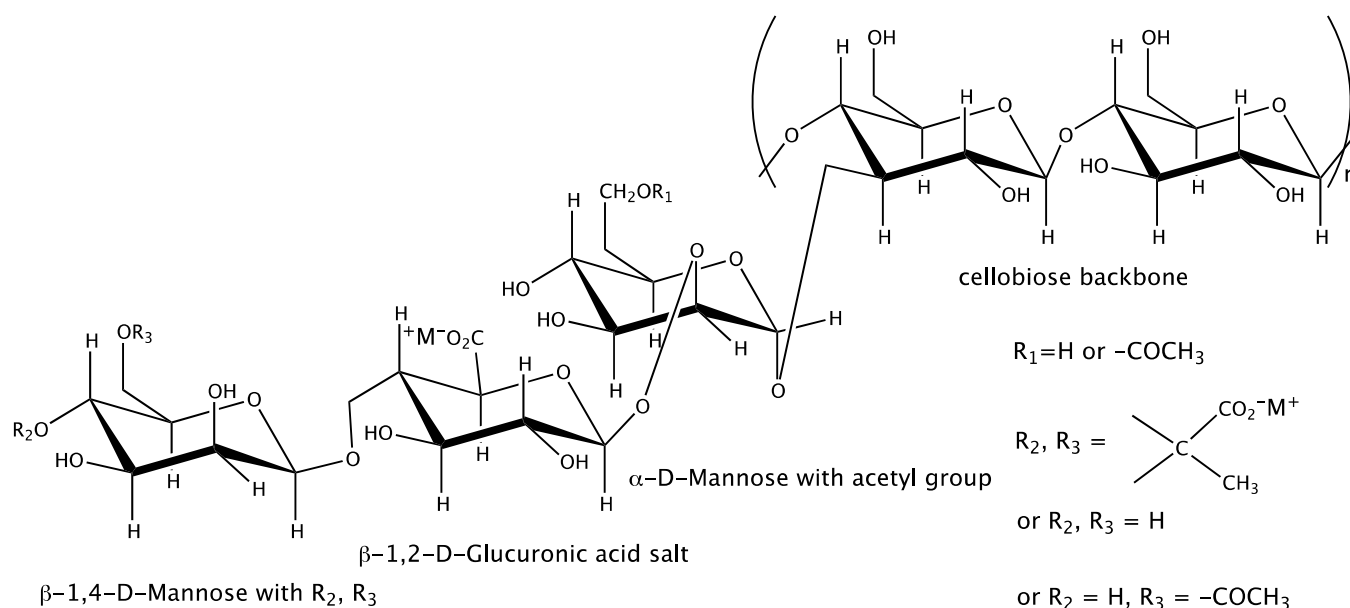


Figure 1. The structure of the xanthan gum molecule.

These properties add industrial value and explain the wide commercial acceptance of this polysaccharide, whereby the xanthan market is constantly expanding to meet its global demand [7,10]. The global XG market grows at a significant rate of 5.6% since 2019, predicting that its market value will reach USD 1.2 billion by 2030 [11]. Its demand increase is due to the growth in processed food consumption, and applications in the agrochemical, cosmetics, driller fluid, and foam stabilizer segments [7]. This commercial demand is a key factor that stimulates studies to increase xanthan production on an industrial scale by sustainable processes to exploit the micro-organism potential [12,13].

Therefore, studies have been carried out analyzing the variables involved in XG production to obtain efficient results, since this productivity can be affected by biotic and abiotic factors [4,7]. Fermentation depends on many parameters and variables, including micronutrients (e.g., potassium, iron, and calcium salts), macronutrients such as carbon and nitrogen sources, oxygen transfers, agitation rate, pH, temperature, and fermentation duration beyond the *Xanthomonas* strain production efficiency [14,15]. These conditions represent a pivotal point in XG production by affecting the fermentative medium cost, further promoting changes in the gum characteristics, downstream processes, and, consequently, in its productivity [7,16]. In addition, the adjustment of the strains with the conditions applied in the fermentation are important as they directly influence the characteristics, techniques, yields, compositions, and structures [7,10] of the XG produced. The different strains present different productivity levels depending on the factors implemented during the fermentation [17]. The *Xanthomonas* strains used to produce XG that are most reported in the literature are *X. campestris* pv. *campestris* 629, *X. campestris* pv. *campestris* 1078, *X. campestris* pv. *campestris* S6, *X. campestris* pv. *campestris* 254, *X. sp.* 1537, *X. campestris* pv. *campestris* 729, *X. campestris* pv. *campestris* 607, *X. campestris* pv. *campestris* 1167, *X. campestris* pv. *mangiferaeindicae* 1230, *X. campestris* pv. *arracia* 1198, *X. axonopodis* pv. *manihotis* 1182, and *X. melonis* 68 [18,19].

On an industrial scale, glucose and sucrose are the usual carbon sources for XG production. Considering that the raw material cost is a factor which burdens its production, alternative carbon sources have been evaluated to obtain this polysaccharide [20–22]. Regarding Brazil, sucrose as a carbon source is a favorable point for its production since its soil and climate allow an intense development of sugarcane cultivation [23]. Therefore,

the binomial XG–sugarcane exhibits a positive effect on profitable income for the agro-industrial market.

In this regard, sugarcane juice is a raw material option for this gum production due to the high content of sucrose, minerals, and vitamins [24]. This broth is used to produce demerara sugar, which, after a brief refinement process, still maintains its rich chemical composition compared to refined sugar [25]. Considering its cost of production in Brazil [26], demerara sugar is an alternative carbon source for XG production due to its nutritional profile and little processing [27]. In addition, as it is an abundant, low-cost, and little-processed product, it can be considered an economical solution and a possible low-cost approach to the production of XG, which can contribute to a sustainable bioeconomy [28].

Nonetheless, several variables affect xanthan production, including technical, biochemical, and microbiological aspects, whose evaluation is crucial for potential industrial implementation [12]. Hence, experimental designs and related statistical methods have been applied systematically to investigate different problems correlating to development and production [15,29,30]. These designs include blocking and factorial experiments to determine the steepest climb ascent path to identify the individual factor effect and approach the neighborhood of the optimal response [17,31,32].

Screening research on alternative or conventional fermentation media for this polymer synthesis has previously been reported [7,33], for example, agricultural residues and biomass with lignocellulosic content [20] (such as tapioca pulp [34], sugarcane bagasse [35], orange peel [36], kitchen waste [37], rice bran [38], chicken feathers [39], coconut shell, cocoa husk [20], potato crop [40], cellar wastewater [41], and corncob [19]); however, these studies found some limitations in the pre-treatments and production yields. Furthermore, there are no studies reporting and describing the use of demerara sugar in XG production. This raw sugar appears to be a promising source since its chemical composition displays adequate nutritional requirements for a fermentative medium, allowing it to be an excellent substrate alternative for the industrial production of XG.

Therefore, the objective of the research was to design and compare XG production processes and thus maximize its yield through a full central factorial composite design (2^3). Furthermore, the interactive effects of MgSO_4 , K_2HPO_4 , and the carbon source (camera sugar or sucrose) as variable parameters of the fermentation medium using three strains of *Xanthomonas* will be evaluated, thus finding ideal conditions for the biosynthesis of XG, with a greater yield and using an alternative carbon source to obtain the most effective operating conditions, and being able to contribute to a bioeconomy sustainable.

2. Materials and Methods

2.1. Strains

Xanthomonas axonopodis pv. *manihotis* IBSBF 290, *X. campestris* pv. *campestris* IBSBF 472, and *Xanthomonas* sp. S6 from the Cultures Collection of the Biomaterials Laboratory (Institute of Technology and Research, Aracaju, SE, Brazil) were used as xanthan-producing micro-organisms. Bacteria were grown on MY-agar medium with the following composition ($\text{g}\cdot\text{L}^{-1}$)—yeast extract, 3.0; malt extract, 3.0; peptone, 5.0; glucose, 10.0; and agar, 20.0—and subcultured every three weeks. After incubation ($25\text{ }^\circ\text{C}/48\text{ h}$), cultures were stored at $4\text{ }^\circ\text{C}$ [42]. Inoculum cultures were prepared in MY liquid medium in 125 mL Erlenmeyer flasks containing 14 mL of culture medium at $28\text{ }^\circ\text{C}$, at 100 rpm for 24 h until reaching a log growth of 10^{11} CFU/mL [43].

2.2. Xanthan Gum Production

The xanthan production was performed in 250 mL Erlenmeyer flasks containing 85 mL of the culture medium under experimental conditions of $28\text{ }^\circ\text{C}$, 96 h, and 180 rpm. The fermentation medium was constituted by the carbon source (demerara sugar or sucrose) ranging from 30 to $70\text{ g}\cdot\text{L}^{-1}$, supplemented with $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ (ranging from 0.2 to $1.0\text{ g}\cdot\text{L}^{-1}$), K_2HPO_4 (ranging from 0.01 to $1.0\text{ g}\cdot\text{L}^{-1}$), $(\text{NH}_4)_2\text{SO}_4$ ($2.2\text{ g}\cdot\text{L}^{-1}$), H_3BO_3 ($0.0066\text{ g}\cdot\text{L}^{-1}$), FeCl_3 ($0.0026\text{ g}\cdot\text{L}^{-1}$), CaCl_2 ($0.0022\text{ g}\cdot\text{L}^{-1}$), and ZnSO_4 ($0.0022\text{ g}\cdot\text{L}^{-1}$). Fer-

mentation started with an inoculum concentration of 15% (v/v), according to medium composition conditions established by experimental design.

2.3. Xanthan Gum Recovery

After fermentation, the samples were subjected to centrifugation ($9626 \times g/5^\circ\text{C}/60\text{ min}$) to remove cells from the fermented broth. Then, the cell-free supernatant was precipitated with 98% ethanol at a 3:1 (v/v) ratio for biopolymer recovery. After recovery, the precipitated gum was collected and dried at $30 \pm 2^\circ\text{C}$ for 72 h. Its production was expressed as $\text{g}\cdot\text{L}^{-1}$ (grams of XG per liter of fermentation broth) [42].

2.4. Experimental Design and Data Analysis

For each *Xanthomonas* strain used experimentally, a 2^3 full factorial central composite design was used to optimize XG production, with variable concentrations of $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ ($0.2\text{--}1\text{ g}\cdot\text{L}^{-1}$), K_2HPO_4 ($0.01\text{--}1\text{ g}\cdot\text{L}^{-1}$), and demerara sugar or sucrose ($30\text{--}70\text{ g}\cdot\text{L}^{-1}$). Table 1 shows the conditions of the performed experiments. All experiments were conducted in triplicate. For statistical calculation, factor levels (in coded values) were -1 , 0 , and $+1$, where 0 corresponded to the central point. The range and levels of the variables investigated in this study are depicted in Table 1. The variables were coded using Equation (1):

$$xi = (X_i - X_0) / \Delta X_i \quad (1)$$

where xi is the independent variable coded value, X_i is the independent variable real value, X_0 is the independent variable real value on the center point, and ΔX_i is the step change value.

Table 1. Real and coded values of independent variables according to each 2^3 full factorial design, for all strains of *Xanthomonas* (*X. axonopodis* pv. *manihotis* IBSBF 290, *X. campestris* pv. *campestris* IBSBF 472, and *Xanthomonas* sp. S6).

Independent Variable (Concentration on $\text{g}\cdot\text{L}^{-1}$)	Symbol	Range and Levels		
		-1	0	$+1$
Sucrose or demerara sugar	X_1	30	50	70
$\text{MgSO}_4\cdot 7\text{H}_2\text{O}$	X_2	0.2	0.6	1.0
K_2HPO_4	X_3	0.01	0.50	1.00

The individual and interactive effects of carbon source (demerara sugar or sucrose), and $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ and K_2HPO_4 concentrations on the xanthan production (Y) as response variables were studied. Thereby, a second-order polynomial model using a least-squares method was fitted to evaluate the production (Y), resulting in Equation (2):

$$Y = b_0 + b_{1 \times 1} + b_{2 \times 2} + b_{3 \times 3} + b_{11 \times 1^2} + b_{22 \times 2^2} + b_{33 \times 3^2} + b_{12 \times 1 \times 2} + b_{13 \times 1 \times 3} + b_{23 \times 2 \times 3} \quad (2)$$

where (Y) is the calculated response function; X_1 , X_2 , and X_3 represent the coded variables (carbon source, $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$, and K_2HPO_4 , respectively); and b_0 , b_i , and b_{ij} ($i, j = 1, 2, 3$) are the coefficient estimates.

The software “Statistic” (version 8.0) was applied for regression and graphical analysis of experimental data. The statistical significance of the regression coefficients was determined by the Student *t*-test, while second-order model equations were determined by the *F* test, and the proportions of variance explained by the obtained models were given by the multiple coefficients of determination, R^2 .

3. Results and Discussion

The xanthan production by the three selected strains (*X. axonopodis* pv. *manihotis* IBSBF 290, *X. campestris* pv. *campestris* IBSBF 472, and *Xanthomonas* sp. S6) was developed using six experimental designs (two for each strain). Thus, the 2^3 full factorial central composite

design with five replicates at the center point comprised a set of 78 experiments. The design matrix of the variables in coded units is depicted in Table 2, as well as the predicted and experimental values of the response factor (xanthan production).

Table 2. 2^3 full factorial central composite design with five replicates at the center point along with the experimental values of xanthan gum production.

Runs	X_1	X_2	X_3	Production of Xanthan Gum ($\text{g}\cdot\text{L}^{-1}$)					
				Strain 290		Strain 472		Strain S6	
				Sucrose	Demerara Sugar	Sucrose	Demerara Sugar	Sucrose	Demerara Sugar
1	−1	−1	−1	0.2993	0.3192	0.2857	0.3931	0.1528	0.4533
2	−1	−1	+1	0.5166	0.2465	0.7171	1.2739	0.0200	0.0600
3	−1	+1	+1	0.5992	0.2070	0.8308	1.3075	0.9382	1.3839
4	−1	+1	−1	0.2847	0.2317	0.2585	0.3619	0.5968	0.4644
5	+1	+1	+1	0.2784	0.3924	0.5254	0.3750	0.0712	0.3219
6	+1	−1	+1	0.6840	0.2741	1.0034	0.6109	0.0920	0.3996
7	+1	−1	−1	0.1127	0.2263	0.3713	0.1373	0.0619	0.4166
8	+1	+1	−1	0.1454	0.1811	0.4683	0.8674	0.9255	0.3644
9	0	0	0	0.4926	0.3761	0.3480	0.7854	0.4634	0.7212
10	0	0	0	0.2859	0.4738	0.3436	0.6200	0.5775	0.7488
11	0	0	0	0.4182	0.4273	0.2495	0.4562	0.6876	0.6121
12	0	0	0	0.2750	0.3885	0.2326	0.6213	0.4677	0.6193
13	0	0	0	0.3010	0.4708	0.2875	0.6315	0.7182	0.6533

The concentration of demerara or sucrose sugar, and the concentration of $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ and K_2HPO_4 are important factors that influence the efficiency of the fermentation process for XG production. The conditions of these experiments and the results obtained are listed in Table 2.

As seen in Table 1, the lowest XG production was achieved with the *Xanthomonas* S6 strains in experiment 2 ($0.0600 \text{ g}\cdot\text{L}^{-1}$), where lower concentrations of demerara sugar and $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ were used, and higher concentrations of K_2HPO_4 . Silva et al. [16] also did not find significant effects in relation to the concentration of $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$; in addition, the presence of K_2HPO_4 improved the yield of XG production. The improvement in extraction was observed when the $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ concentration increased up to $1 \text{ g}\cdot\text{L}^{-1}$ in experiment 3, where the highest XG production in this study was obtained for strains 472 and S6 (1.3075 and $1.3839 \text{ g}\cdot\text{L}^{-1}$, respectively). These results agree with Jesus et al. [19], which achieved higher yields of XG production using the S6 strain supplemented with salts and a hemicellulose fraction obtained by the alkaline hydrolysis of corncob. Furthermore, $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ at lower concentrations $0.2 \text{ g}\cdot\text{L}^{-1}$ and $30 \text{ g}\cdot\text{L}^{-1}$ of demerara sugar showed similar results for strain 472. It was verified that the highest productions of XG for strains *Xanthomonas* 290 and 472, when using sucrose as the main source of carbon, were obtained in the tests containing higher levels of K_2HPO_4 and sucrose (experiment 6, 0.6840 and $1.0034 \text{ g}\cdot\text{L}^{-1}$, respectively). However, for strain 290, the best results were obtained in the conditions applied in the central points (50 , 0.6 , and $0.50 \text{ g}\cdot\text{L}^{-1}$ of demerara sugar, $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$, and K_2HPO_4 , respectively). In addition, the lowest concentration of XG produced by this strain using demerara sugar was obtained in experiment 8 or more; there were no significant differences between the other experiments. Other authors [44] state in the literature that the most promising culture medium for producing XG is sucrose supplemented with salts. On the other hand, in this study, it is observed that demerara sugar has a higher XG production compared to the experiments with the same conditions using sucrose as a carbon source, making it a very important factor for producing XG.

The quadratic regression models in terms of a coded factor expressed by Equations (3)–(5) represent the XG productions (Y) as a function of the sucrose (X_1),

MgSO₄·7H₂O (X₂), and K₂HPO₄ (X₃) concentrations reached by each of the *Xanthomonas* strains (290, 472, and S6), respectively.

$$Y = 0.0105 X_1^2 - 0.0599 X_1 - 0.0381 X_2 + 0.1545 X_3 - 0.0551 X_1 X_2 + 0.0215 X_1 X_3 - 0.0426 X_2 X_3 + 0.3545 \quad (3)$$

$$Y = 0.2653 X_1^2 + 0.0345 X_1 - 0.0368 X_2 + 0.2116 X_3 - 0.0584 X_1 X_2 - 0.0393 X_1 X_3 - 0.0543 X_2 X_3 + 0.2922 \quad (4)$$

$$Y = -0.2256 X_1^2 - 0.0696 X_1 + 0.2756 X_2 - 0.0769 X_3 - 0.0649 X_1 X_2 - 0.1291 X_1 X_3 - 0.0513 X_2 X_3 + 0.5823 \quad (5)$$

Regarding the carbon source alteration in fermentation, the quadratic regression models in terms of a coded factor indicating the gum production (Y) as a function of demerara sugar (X₁), MgSO₄·7H₂O (X₂), and K₂HPO₄ (X₃) concentrations are expressed by Equations (6)–(8), also achieved with each studied strain (290, 472, and S6), respectively.

$$Y = -0.1675 X_1^2 + 0.0087 X_1 - 0.0067 X_2 + 0.0202 X_3 + 0.0250 X_1 X_2 + 0.0446 X_1 X_3 + 0.2064 X_2 X_3 + 0.4273 \quad (6)$$

$$Y = 0.0430 X_1^2 - 0.1682 X_1 + 0.0621 X_2 + 0.2260 X_3 + 0.0615 X_1 X_2 - 0.2306 X_1 X_3 - 0.1126 X_2 X_3 + 0.6229 \quad (7)$$

$$Y = -0.1879 X_1^2 - 0.1074 X_1 + 0.1506 X_2 + 0.0583 X_3 - 0.1831 X_1 X_2 - 0.0732 X_1 X_3 + 0.1609 X_2 X_3 + 0.6709 \quad (8)$$

3.1. Analysis of Variance (ANOVA)

The statistical significance of the main interaction effects of the factors on the dependent variable was assessed by the *F* test of the analysis of variance (ANOVA). Tables 3 and 4 depict the experiments with sucrose and demerara sugar, respectively, showing significant results at a 95% confidence level.

Table 3. Analysis of variance (ANOVA) for the model regression representing xanthan gum production (Y) using sucrose by *X. axonopodis* pv. *manihotis* 290, *X. campestris* pv. *campestris* 472, and *Xanthomonas* sp. S6, respectively.

	SS	dF	MS	F	p-Value
Strain 290					
(1) sucrose (L)	0.028716	1	0.028716	1.97056	0.219346
sucrose (Q)	0.000339	1	0.000339	0.02327	0.884727
(2) MgSO ₄ ·7H ₂ O (L)	0.011621	1	0.011621	0.79742	0.412776
(3) K ₂ HPO ₄ (L)	0.190993	1	0.190993	13.10634	0.015215
1L by 2L	0.024299	1	0.024299	1.66746	0.253072
1L by 3L	0.003720	1	0.003720	0.25524	0.634884
2L by 3L	0.014544	1	0.014544	0.99802	0.363654
Error	0.072863	5	0.014573		
Total SS	0.347094	12			
Strain 472					
(1) sucrose (L)	0.009543	1	0.009543	0.63434	0.461891
sucrose (Q)	0.216603	1	0.216603	14.39851	0.012699
(2) MgSO ₄ ·7H ₂ O (L)	0.010841	1	0.010841	0.72066	0.434676
(3) K ₂ HPO ₄ (L)	0.358239	1	0.358239	23.81361	0.004553
1L by 2L	0.027320	1	0.027320	1.81604	0.235625
1L by 3L	0.012364	1	0.012364	0.82187	0.406201
2L by 3L	0.023555	1	0.023555	1.56582	0.266171
Error	0.075217	5	0.015043		
Total SS	0.733682	12			
Strain S6					
(1) sucrose (L)	0.038809	1	0.038809	0.67477	0.448775
sucrose (Q)	0.156573	1	0.156573	2.72232	0.159867
(2) MgSO ₄ ·7H ₂ O (L)	0.607753	1	0.607753	10.56694	0.022679
(3) K ₂ HPO ₄ (L)	0.047370	1	0.047370	0.82362	0.405737
1L by 2L	0.033722	1	0.033722	0.58632	0.478413
1L by 3L	0.133334	1	0.133334	2.31827	0.188357
2L by 3L	0.021033	1	0.021033	0.36570	0.571750
Error	0.287573	5	0.057515		
Total SS	1.326168	12			

SS: sum of squares; DF: degrees of freedom; MS: mean square.

Table 4. Analysis of variance (ANOVA) for the model regression representing xanthan gum production (Y) using demerara sugar by *X. axonopodis* pv. *manihotis* 290, *X. campestris* pv. *campestris* 472, and *Xanthomonas* sp. S6, respectively.

	SS	dF	MS	F	p-Value
Strain 290					
(1) Demerara sugar (L)	0.000604	1	0.000604	0.30652	0.604
Demerara sugar (Q)	0.086340	1	0.086340	43.83216	0.001
(2) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (L)	0.000363	1	0.000363	0.18436	0.685
(3) K_2HPO_4 (L)	0.003268	1	0.003268	1.65925	0.254
1L by 2L	0.005005	1	0.005005	2.54089	0.172
1L by 3L	0.015887	1	0.015887	8.06512	0.036
2L by 3L	0.005592	1	0.005592	2.83865	0.153
Error	0.009849	5	0.001970		
Total SS	0.126907	12			
Strain 472					
(1) Demerara sugar (L)	0.226397	1	0.226397	6.05021	0.057
Demerara sugar (Q)	0.005688	1	0.005688	0.15200	0.713
(2) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (L)	0.030826	1	0.030826	0.82380	0.406
(3) K_2HPO_4 (L)	0.408427	1	0.408427	10.91476	0.021
1L by 2L	0.030233	1	0.030233	0.80795	0.410
1L by 3L	0.425595	1	0.425595	11.37356	0.020
2L by 3L	0.101520	1	0.101520	2.71301	0.160
Error	0.187099	5	0.037420		
Total SS	1.415786	12			
Strain S6					
(1) Demerara sugar (L)	0.092257	1	0.092257	1.930789	0.223
Demerara sugar (Q)	0.108667	1	0.108667	2.274231	0.192
(2) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (L)	0.181533	1	0.181533	3.799212	0.109
(3) K_2HPO_4 (L)	0.027226	1	0.027226	0.569801	0.484
1L by 2L	0.268242	1	0.268242	5.613882	0.064
1L by 3L	0.042881	1	0.042881	0.897424	0.387
2L by 3L	0.207143	1	0.207143	4.335177	0.092
Error	0.238909	5	0.047782		
Total SS	1.166857	12			

SS: sum of squares; DF: degrees of freedom; MS: mean square.

The *Xanthomonas* growth time can vary according to the fermentation conditions. Xanthan production is closely related to the carbon source consumption up to its maximum concentration. Afterward, the enzymatic polysaccharide hydrolysis starts to restore the carbon source in the culture medium as an attempt to maintain bacterial viability [45]. Therefore, the carbon source is a critical factor in any bioproduct formation, including XG [46]. Thereby, it is noteworthy to evaluate each carbon source interaction with respect to the other variables in the medium composition independently, to establish the carbon-source–micronutrient interactions, such as $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and K_2HPO_4 [14].

The statistical study using an ANOVA-linked Fisher (*F*) test was applied to determine the significant variables, whose significance degree was ranked based on the *F*-ratio value. In fact, the greater the magnitude of the *F* value and the lower the “Prob < *F*” value, the more significantly displayed the corresponding model and individual coefficient. The *p*-value serves as a tool to check the significance of each of the coefficients, also indicating the interaction strength between the parameters. Low *p*-values (<0.05) denote a greater correlation of the significance between corresponding coefficients [32,47].

Hence, in the study using sucrose as a carbon source (Table 3), a significant linear effect (*p* < 0.05) of K_2HPO_4 was observed with strain 290 (*p* = 0.015) and strain 472 (*p* = 0.004), and of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ with strain S6 (*p* = 0.023). Silva et al. [16] and Niknezhad et al. [47] also reported potassium as a significant factor in fermentations using cheese whey, K_2HPO_4 , and MgSO_4 for xanthan production. Notwithstanding, Umashankar et al. [48] observed the nutrient influence on *X. campestris* growth under a high phosphate concentration, which

inhibited gum production. Nonetheless, the sucrose quadratic term coefficients ($p = 0.013$) were significant for strain 472, indicating that both the concentration and carbon source type are important for the cells during the fermentation process for xanthan production. This result is consistent with that reported by Demirci et al. [21], verifying the significant glucose effect on polymer production.

The demerara sugar concentration evaluation depicted in Table 4 shows the ANOVA statistical analysis concerning the influence of this sugar concentration, K_2HPO_4 , and $MgSO_4 \cdot 7H_2O$ on xanthan production. The effects of $MgSO_4 \cdot 7H_2O$ associated with the demerara sugar rate displayed no statistical significance for the strains tested. However, the significant effect ($p < 0.05$) on the response for strain 290 was the demerara sugar concentration quadratic term ($p = 0.001$), while the linear term of the K_2HPO_4 concentration ($p = 0.021$) was significant for strain 472. Furthermore, the interaction between demerara sugar and the K_2HPO_4 concentration exhibited a significant effect ($p = 0.036$ and $p = 0.020$) for the 290 and 472 strains, respectively.

The measures of the models' goodness-of-fit were confirmed by the determination coefficients (R^2), with R^2 values of 0.79008, 0.89748, and 0.78316 by the 290, 472, and S6 strains, respectively, for the sucrose experiments, while for the demerara sugar experiments, the R^2 values for the 290, 472, and S6 strains were 0.92239, 0.86785, and 0.79525, respectively. The R^2 value provides a variability measure in the observed response values, which can be explained by the experimental parameters and their interactions. R^2 values close to 1 indicate the best correlation between the experimental and predicted values and the better predictive response model [32]. Therefore, the R^2 predicted values > 0.78 for all strains pointed to a reasonable agreement between the experimental and predicted values for xanthan production.

3.2. Response Surface Methodology (RSM)

The response surface methodology (RSM) is often applied in order to provide effective tools for optimization since it involves many critical factors for determining high accuracy [32,49]. Figures 2 and 3 represent the response surfaces of the regression study using sucrose.

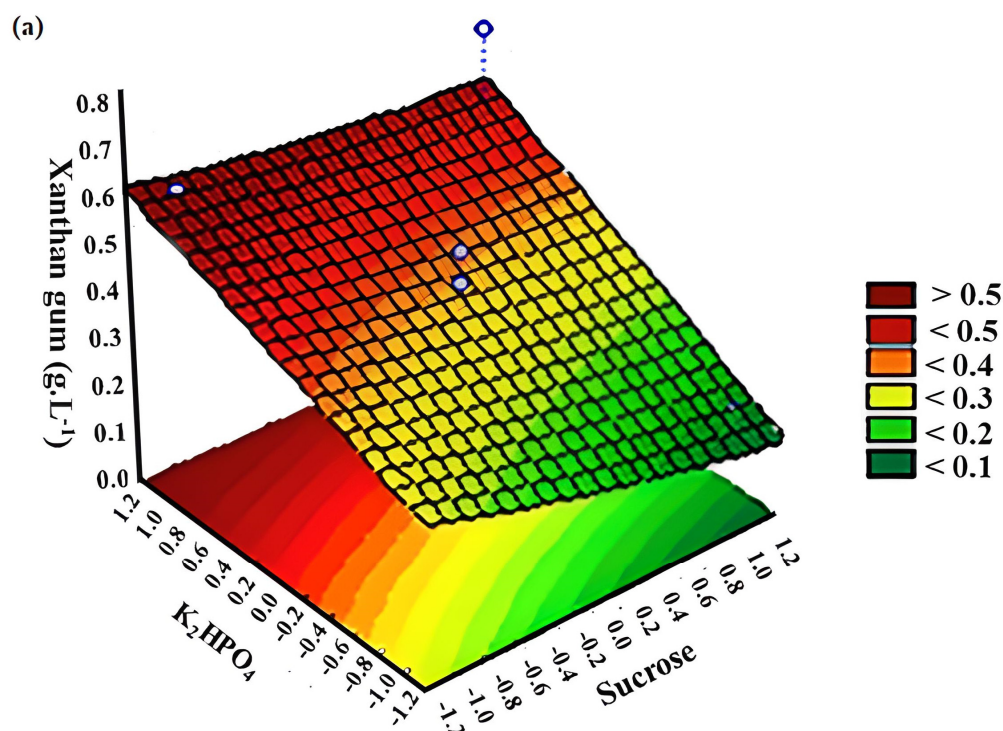


Figure 2. Cont.

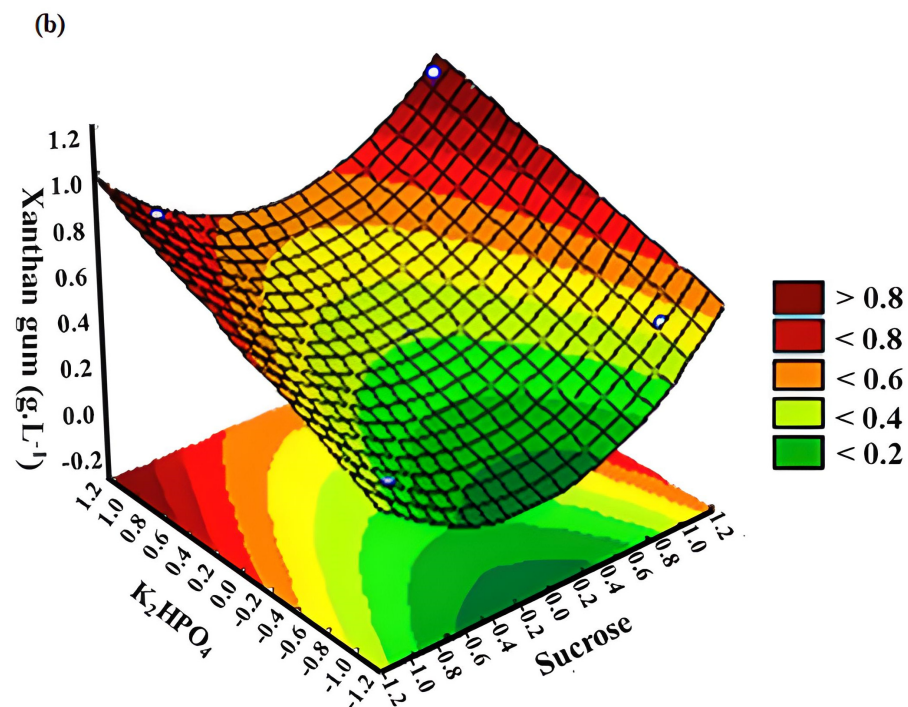


Figure 2. Response surface plots depicting the sucrose and K_2HPO_4 concentration effect and their mutual effects on xanthan production by (a) *X. axonopodis* pv. *manihotis* 290 and (b) *X. campestris* pv. *campestris* 472.

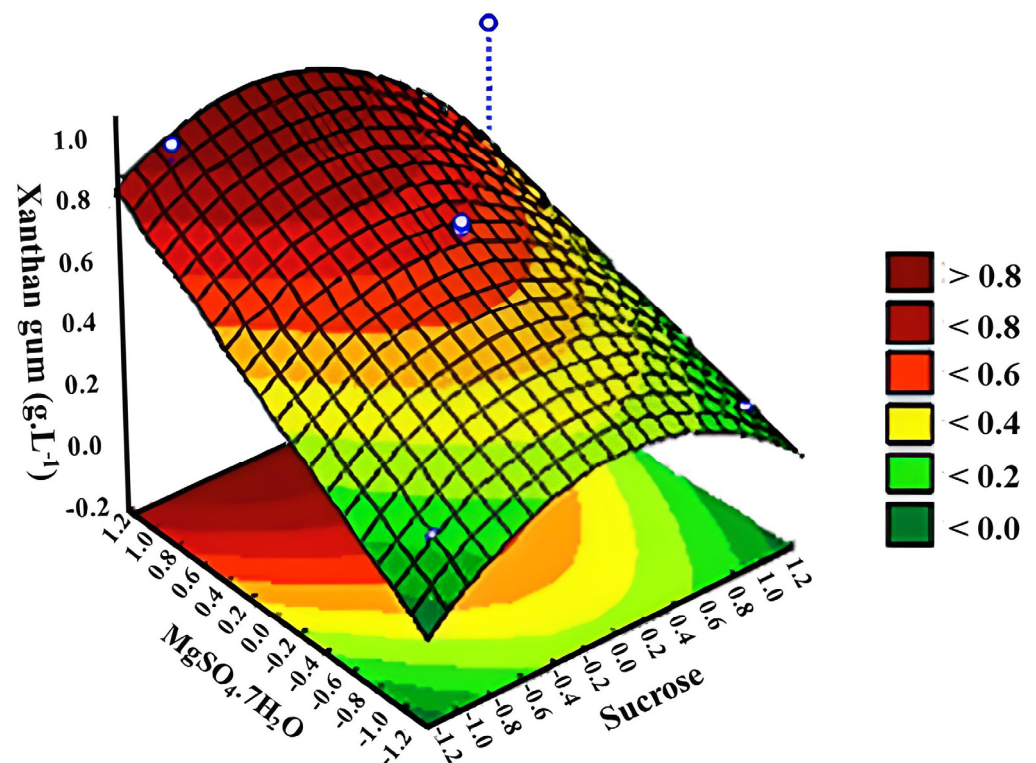


Figure 3. Response surface plot representing the sucrose and $MgSO_4 \cdot 7H_2O$ concentration effect and their mutual effects on xanthan production by *Xanthomonas* sp. S6.

Figure 2a,b show the response surface plots for xanthan production at different concentrations of sucrose and K_2HPO_4 for the *Xanthomonas* 290 and 472 strains, respectively. Likewise, Figure 2 depicts the relationship between the $MgSO_4 \cdot 7H_2O$ concentrations with

sucrose for the S6 strain. According to the response surface plots, the xanthan production yield rises with the increased K_2HPO_4 concentration relative to the 290 and 472 strains, while an increased $MgSO_4 \cdot 7H_2O$ concentration promoted a higher yield by the S6 strain. In both cases, the independent variables $MgSO_4 \cdot 7H_2O$ and K_2HPO_4 significantly affected xanthan production in each *Xanthomonas* strain.

The results show the importance of screening the *Xanthomonas* strains in association with a fermentation variable study since the culture medium composition can promote different behaviors on the strains used for production. Therefore, the response surface methodology aimed to detect the experimental parameters responsible for signals superior to those caused by noise. The phosphate addition to the medium can increase xanthan gum production by acting as a buffering agent, minimizing pH fluctuations [50].

Concerning Figure 4a,b, the response surface displays the relationship between the K_2HPO_4 concentrations and demerara sugar for the *Xanthomonas* 290 and 472 strains, respectively. High K_2HPO_4 concentrations provided a significant xanthan production increase, previously observed in fermentations using sucrose. Moreover, it was observed that the *Xanthomonas* strain 290 grown in demerara sugar, even with a low K_2HPO_4 content, kept gum production close to its highest level when grown in demerara sugar at the central point level. The data suggest that the demerara sugar and K_2HPO_4 use provided a high potassium content, affecting gum production. The experiment performed with maximum values of demerara sugar and $MgSO_4 \cdot 7H_2O$ and a minimum value of K_2HPO_4 displayed the third-largest gum production by the *Xanthomonas* 472 strain. Therefore, demerara sugar could supply the potassium amount required for gum production.

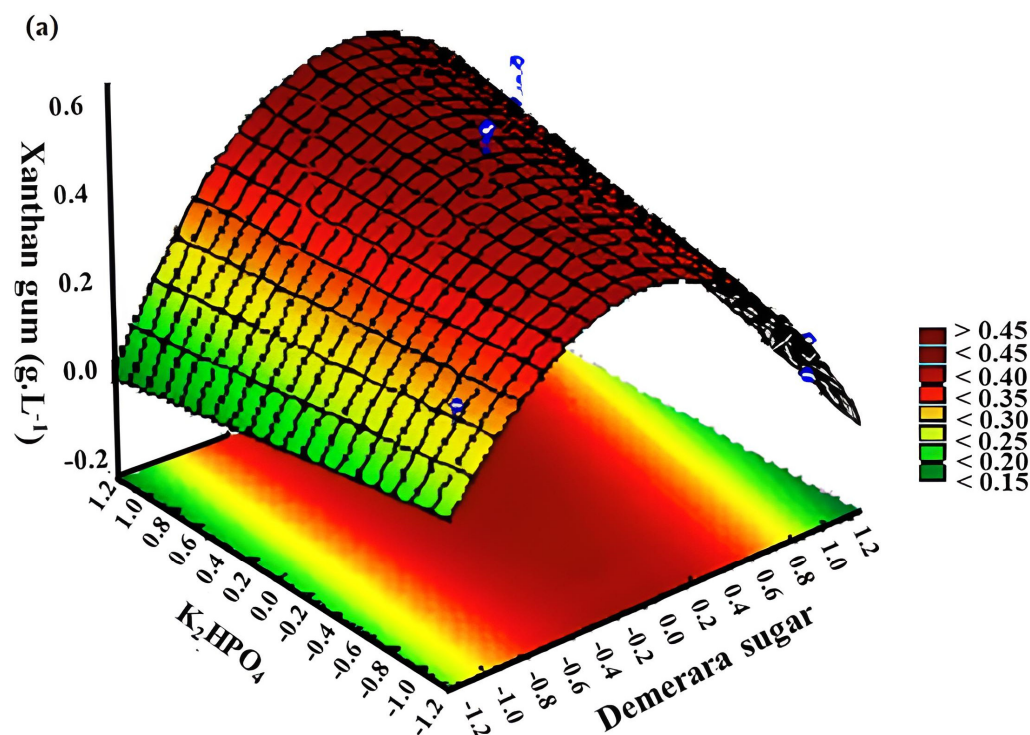


Figure 4. Cont.

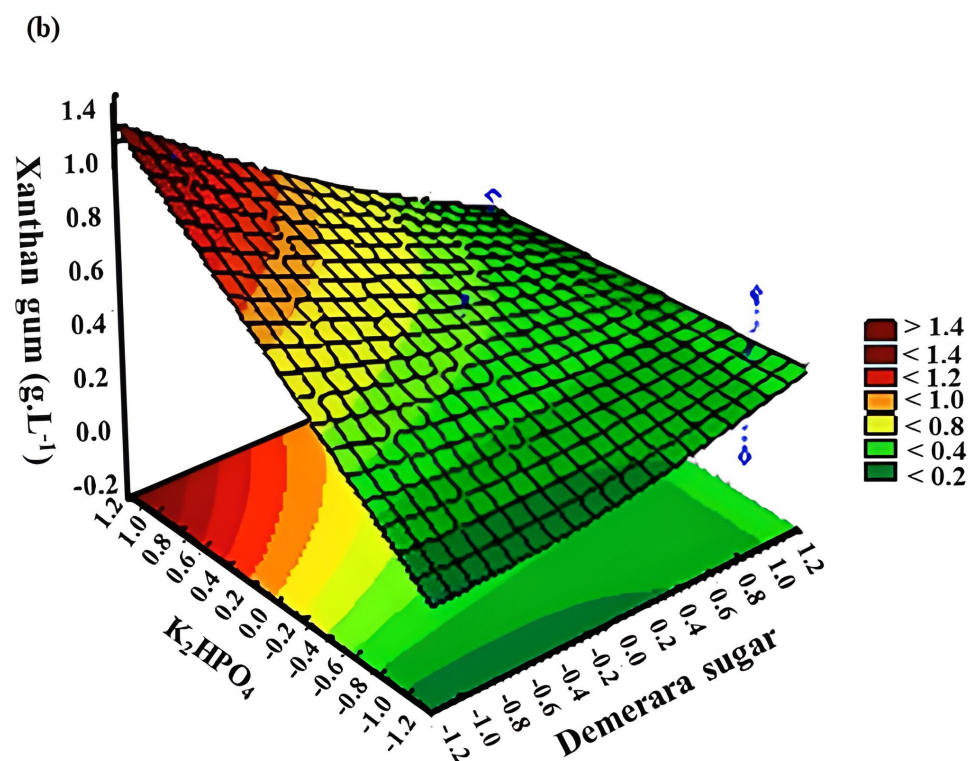


Figure 4. Response surface plots depicting the demerara sugar and K_2HPO_4 concentration effect and their mutual effects on xanthan production by (a) *X. axonopodis* pv. *manihotis* 290 and (b) *X. campestris* pv. *campestris* 472.

Overall, these results are supported by Faria et al. [51] on the influence of the initial sucrose concentration on xanthan production, and by García-Ochoa et al. [4] on the effects of nitrogen, phosphorus, and magnesium on bacterial growth and polymer production. Micronutrients may be required for pyruvilation, although the literature reports several results regarding its effect on the pyruvilation degree in the xanthan molecular structure [14]. Pyruvate influences the polymer–polymer interaction increase related to the affinity with the neighboring xanthan molecules, promoting more viscous aqueous solutions [52].

The significant effects of K_2HPO_4 were similar to those described by Silva et al. [16] and Mesomo et al. [53]. These authors evaluated the combined effects of K_2HPO_4 and $MgSO_4 \cdot 7H_2O$ with the cheese whey addition, observing the higher xanthan production with the maximum K_2HPO_4 supplementation by *X. campestris* pv. *mangiferaeindicae* 1230 and *X. campestris* pv. *manihotis* 1182. Kalogiannis et al. [50] also reported the maximal production using pretreated beet molasses (carbon source), supplemented with K_2HPO_4 , yeast extract, and Triton 80. Moreover, potassium can significantly increase the xanthan viscosity [54] which, in the form of K_2HPO_4 and KH_2PO_4 , are significant medium components, acting as buffering agents as well as nutrients for *Xanthomonas* growth [55].

Despite evidence showing the influence of the microbial strain, time, and fermentation medium composition on XG production, no alternative medium has even replaced the use of sucrose or glucose with a significant effect on the polysaccharide quality and productivity. Nevertheless, the results with demerara sugar showed its efficiency as a carbon source in gum production due to its nutritional richness, which can partially replace the evaluated salt addition. Even so, further studies with demerara sugar or other potential substrates are required, whose nutrient content can provide operational and economic advantages for a xanthan production process since the analyzed variables are significant for the polymer-producing strains.

4. Conclusions

This study evaluated the possibility of supplementing the fermentation medium with mineral salts, using demerara sugar or sucrose as substrates, in XG production. Thereby, the sucrose or demerara sugar, K_2HPO_4 , and $MgSO_4 \cdot 7H_2O$ concentration effects on its production were explored by an experimental design and a response surface analysis. The proposed model studies pointed out a reasonable experimental agreement for xanthan production, indicating specific nutritional requirements for each *Xanthomonas* strain. The three selected strains displayed a different influence from the fermented conditions for xanthan production. Statistical significance values suggested that the sugar concentration and K_2HPO_4 played an important role for this production by the *Xanthomonas* 472 strain, using sucrose or demerara sugar as a carbon source. Regarding the S6 strain, $MgSO_4 \cdot 7H_2O$ played a significant role in gum synthesis with sucrose but not with demerara as a carbon source. With strain 290, the demerara sugar concentration increases the XG but with sucrose as the carbon source, the production is higher with K_2HPO_4 . The evaluated salts are important factors for XG production, and the demerara sugar can partially replace this mineral salt requirement. Economically, the experimental data show the relevance of demerara sugar as an efficient and low-cost alternative source of carbon in the production of XG compared to a carbon source used industrially. These results contribute to the production of XG through sustainable biotechnology, efficient in the context of the circular bioeconomy.

Author Contributions: Conceptualization, D.P.S., D.S.R. and L.C.R.; methodology, L.C.R., A.S.F.-J., E.S.N. and K.S.S.; formal analysis, D.P.S., D.S.R. and L.C.R.; investigation, L.C.R., A.S.F.-J., E.S.N., and K.S.S.; resources, D.P.S., P.P., and D.S.R.; data curation, D.P.S., D.S.R., L.C.R., J.A.T., P.P. and M.S.J.; writing—original draft preparation, L.C.R. and F.F.P.; writing—review and editing, D.P.S., D.S.R., P.P. and M.S.J.; supervision, D.P.S. and D.S.R.; project administration, D.P.S. and D.S.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data generated or analyzed during this study are included in this published article.

Acknowledgments: The authors acknowledge the financial assistance from the Brazilian research funding agencies including the Co-ordination for the Improvement of Higher Education Personnel (CAPES) under Finance Code 001, a Brazilian foundation within the Ministry of Education (MEC); the National Council for Scientific and Technological Development (CNPq), a Brazilian foundation associated to the Ministry of Science, Technology, and Innovations (MCTI); the Foundation of Support to Research and Technological Innovation of the State of Sergipe (FAPITEC/SE); and the Regional Co-operative of Agrarian Reform Settlers of Sergipe's Semi-Arid. The authors acknowledge the Foundation for Science and Technology (FCT, Portugal) for the financial support to the CISAS UIDB/05937/2020 and UIDP/05937/2020, including the postdoc grant and the contract of the two authors.

Conflicts of Interest: The authors declare no conflict of interest.

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