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Comparison of *Trichoderma longibrachiatum* Xyloglucanase Production Using Tamarind (*Tamarindus indica*) and Jatoba (*Hymenaea courbaril*) Seeds: Factorial Design and Immobilization on Ionic Supports

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Abstract: Xyloglucan (XG) is the predominant hemicellulose in the primary cell wall of superior plants. It has a fundamental role in controlling the stretching and expansion of the plant cell wall. There are five types of enzymes known to cleave the linear chain of xyloglucan, and the most well-known is xyloglucanase (XEG). The immobilization process can be used to solve problems related to stability, besides the economic benefits brought by the possibility of its repeated use and recovery. Therefore, this study aims at the optimization of the xyloglucanase production of *Trichoderma longibrachiatum* using a central composite rotatable design (CCRD) with tamarind and jatoba seeds as carbon sources, as well as XEG immobilization on ionic supports, such as MANAE (monoamine-*N*-aminoethyl), DEAE (diethylaminoethyl)-cellulose, CM (carboxymethyl)-cellulose, and PEI (polyethyleneimine). High concentrations of carbon sources (1.705%), at a temperature of 30 °C and under agitation for 72 h, were the most favorable conditions for the XEG activity from *T. longibrachiatum* with respect to both carbon sources. However, the tamarind seeds showed 23.5% higher activity compared to the jatoba seeds. Therefore, this carbon source was chosen to continue the experiments. The scaling up from Erlenmeyer flasks to the bioreactor increased the XEG activity 1.27-fold (1.040 ± 0.088 U/mL). Regarding the biochemical characterization of the crude extract, the optimal temperature range was 50–55 °C, and the optimal pH was 5.0. Regarding the stabilities with respect to pH and temperature, XEG was not stable for prolonged periods, which was crucial to immobilizing it on ionic resins. XEG showed the best immobilization efficiency on CM-cellulose and DEAE-cellulose, with activities of 1.16 and 0.89 U/g of the derivative (enzyme plus support), respectively. This study describes, for the first time in the literature, the immobilization of a fungal xyloglucanase using these supports.

Keywords: xyloglucanase; *Trichoderma longibrachiatum*; *Hymenaea courbaril*; *Tamarindus indica*; enzyme immobilization

1. Introduction

Xyloglucan (XG) is the predominant hemicellulose in the primary cell wall of superior plants. This includes all the dicotyledonous and non-gramineous monocotyledonous plants [1]. XG is usually strongly associated with cellulose through hydrogen bonds, forming a tridimensional net of cellulose and xyloglucan [2]. It is probably the second most abundant polymer in nature, after cellulose [3]. It is highly soluble in water, which prevents it from forming crystalline microfibrils such as cellulose [4].

XG is composed of a linear chain of glucose residues linked by β-1,4 bonds, which contain up to 75% of their residues joined to α-D-xylopyranose at the position O-6. Although, some structures of xyloglucan may present a β-D-galactopyranose or an α-L-arabinofuranose linked to the residues of xylose, or even an α-L-fucopyranose connected to residues of galactose [5]. This structural diversity is decurrent from the varied species of plants [6].

Xyloglucan plays a fundamental role during the growth and cellular differentiation of plants, which is related to the control of the stretching and expansion of the cell wall. In some terrestrial plants, XG is the main reserve polysaccharide in the seeds [7]. However, the extraction of XG is not an uncomplicated process, mainly due to the strong hydrogen bonds between cellulose and xyloglucan. In addition, the covalent bonds formed with pectins and xylans make the process even more challenging. Usually, the extraction is performed through an alkaline solution combined with chaotropic agents [8].

The enzymes that cleave XG present great utilities in the degradation and conversion of lignocellulosic biomass, mainly due to its synergistic potential with cellulases at the degradation of the plant cell wall [9–11]. Furthermore, the enzymes that degrade and/or modify xyloglucans can be used in the production of new surfactants of oligoxyloglucans [12] in the pharmaceutical [13], textile, and paper industries [14]. Furthermore, the aqueous solutions of XG have a high viscosity, exhibiting Newtonian fluidity, unlike most polysaccharides [15]. That is why they are often used as food additives for increasing viscosity or as stabilizers [16].

Five identified types of enzymes can cleave the linear chain of xyloglucan: endo-β-D-1,4-glucanase, which is specific for xyloglucan, also known as endoxyloglucanase or simply xyloglucanase (XEG) (EC 3.2.1.151); exoxyloglucanase (EC 3.2.1.155); oligoxyloglucan β-glucosidase (EC 3.2.1.120); cellobiohydrolase, which is specific for oligoxyloglucans from the reducing extremities (EC 3.2.1.150); and xyloglucan endotransglucosylase (EC 2.4.1.207) [17].

The immobilization of xyloglucanases can solve problems caused by losses in the stability of free enzymes, which limit their use in large-scale applications. Besides the economic benefits of immobilization, the possibility of its repeated use decreases the general costs of production. This fact is the main reason why, over the last years, science has seen many attempts to obtain immobilized enzymes with high operational and storage stability [18–20].

Due to their constitution, tamarind (*Tamarindus indica* Linn.) and jatoba (*Hymenaea courbaril*) seeds have been utilized for the cultivation of microorganisms or as substrates to produce microbial enzymes [7,21]. In addition, these seeds are rich in xyloglucan, which corresponds to about 40% of their dry mass [22,23].

The ionic adsorption method of enzymatic immobilization is considered simple, cheap, efficient, and reversible [24,25]. In this context, elucidating the enzymatic properties can suggest the vast potential of xyloglucanases for biotechnological applications. Therefore, this study reports the first ever optimization of the production of xyloglucanase from *Trichoderma longibrachiatum* and its immobilization on ionic supports.

2. Material and Methods

2.1. Maintenance of the Fungus and Culture Medium

Trichoderma longibrachiatum LMBC 172 was maintained through the inoculation of its spores in potato dextrose agar medium (PDA) (Sigma-Aldrich, Saint Louis, MO, USA), keeping it through successive transfers in glass tubes containing the same medium, and incubating it at a temperature of 30 °C. Afterward, the test tubes were refrigerated for up to 30 days.

2.2. Submerged Cultivation of the Fungus

The acquirement of xyloglucanases was performed according to Contato et al. [21]. A solution with 10^6–10^7 spores/mL from the fungus was created. The fungus was grown in test tubes and suspended in sterile distilled water, and its spores were counted in a microscope through a Neubauer chamber. The suspension was inoculated into 125 mL Erlenmeyer flasks with 25 mL of Khanna medium (Khanna's salt solution [20x]: NH_4NO_3 (2.0 g), KH_2PO_4 (1.3 g), $MgSO_4.7H_2O$ (0.362 g), KCl (0.098 g), $ZnSO_4.H_2O$ (0.007 g), $MnSO_4.H_2O$ (0.0138 g), $Fe_2(SO_4)_3.6H_2O$ (0.0066 g), $CuSO_4.5H_2O$ (0.0062), distilled water (100 mL) (5.0 mL), yeast extract (0.1 g), carbon source (1.0 g), and distilled water 100 mL) [26]. The media were supplemented with 1% (*w*/*v*) of two different carbon sources: tamarind (*Tamarindus indica*, Fabaceae) or jatoba (*Hymenaea courbaril* L., Caesalpinioideae) seeds, which were previously pretreated (boiled in water, dried, and ground to 20 mesh) to secure the sanitary quality of the seeds and avoid the growth of other associated fungi. The Erlenmeyer flasks were incubated at 30 °C under static conditions or were shaken (at 120 rpm), both up to 96 h, with sampling every 24 h. The samples were filtered with a vacuum pump, and the filtrates were used as enzymatic extracts to determine extracellular enzymatic activities, performed in triplicate.

2.3. Optimization of Production through Factorial Design

The central composite rotatable design (CCRD) type "star" was used to evaluate the influence of different variables on the production of xyloglucanases and obtain the best conditions. The design consisted of assays with two independent variables (temperature and concentration of carbon source) in 4 levels (−1.41; −1; +1; +1.41). The effect of the independent variables was evaluated with respect to the variable response of xyloglucanase activity (mU/mL). The results were adjusted for a second-order polynomial equation. For the construction of the experimental design, the points shown in Table 1 were used, consisting of assays ranging from 24 to 96 h, with samplings every 24 h. The α axial points were chosen due to $\alpha = \sqrt{k}$, where k represents the number of evaluated factors. For $k = 2$, we have the points $\alpha = \pm 1.41$ [27].

Table 1. Values used to construct the factorial design for *T. longibrachiatum* LMBC 172.

	−1.41	**−1**	**0**	**1**	**1.41**
Temperature (°C)	23.95	25.0	30.0	35.0	37.05
Carbon source (%)	0.3	0.5	1.0	1.5	1.7

2.4. Enzymatic Determination

The xyloglucanase activity was measured with xyloglucan (Megazyme®) as substrate [28]. The activity was determined by quantifying the number of reducing sugars using 3,5-dinitrosalicylic acid (DNS), according to the Miller method [29]. The assay mixture consisted of 25 µL of 1% (*w*/*v*) substrate solution in distilled water, 10 µL of 50 mM sodium acetate buffer, pH 5.0, and 15 µL of enzyme extract. A blank was carried out for each enzymatic assay by adding the enzyme extract after the assay time had elapsed with 50 µL of DNS. The absorbance was measured at 540 nm, and reducing sugars were quantified using a standard glucose curve (0–1 mg/mL). The detection limit was 3 µg of reducing

sugar. The activity unit was defined as the amount of enzyme that releases one μmol of reducing sugar per minute per mL, and it was expressed as milliunit per mL (mU/mL).

2.5. Scaling for Bioreactor

The best cultivation condition was verified through a factorial design. Cultivation was carried out in a 5 L BioFlo 310-New Brunswick® bioreactor (Eppendorf, Hamburg, Germany), containing 3.0 L of workload, and under batch fermentation method to increase enzyme production. The same culture medium previously used for cultivation in Erlenmeyer flasks was sterilized in an autoclave at 121 °C for 30 min and aseptically placed in the reactor. The aeration of 1.0 vvm was performed by continuous injection of filtered, compressed air from a sterile filter. The dissolved oxygen concentration (DO) was controlled, employing a DO probe ranging from 0 to 100%. A volume of 3 mL of antifoam 204 (Sigma® A 6426) was added to the culture medium at the beginning of the process. The fermentation was carried out at 30 °C and for 72 h for *Trichoderma longibrachiatum*, using tamarind seeds as carbon source. Protein concentration and DO were monitored every 24 h through an appropriate collector for the bioreactor, allowing the samples to be taken safely.

The following Equation (1) was used to scale and determine that the agitation speed would be 280 rpm:

$$Ni.tm = 1.54\,V/Di^3 \quad (1)$$

where:
Ni = stirring speed (1/s);
tm = mixing time constant;
V = volume of medium;
Di = impeller diameter.

2.6. Protein Quantification

The proteins obtained in extracellular solutions were quantified by Bradford's method [30], whereby 40 μL of Bradford's reagent was added to 160 μL of the enzymatic extracts and incubated for 5 min at room temperature. Finally, the absorbance was read on a spectrophotometer (Shimadzu, Kyoto, Japan) at 595 nm, using bovine albumin as standard. The results were expressed in μg of protein/mL.

2.7. Effects of Temperature and pH on the Enzymatic Activity

The effects of temperature and pH on the enzymatic activity were determined for the enzymes produced in the bioreactor. In order to determine the optimal temperature, different temperatures were applied (40 to 80 °C, with intervals of 5 °C between each). To determine thermostability, the enzymes were incubated for up to 24 h at 30 to 70 °C, with intervals of 10 °C between each. In addition, the influence of pH on the enzymatic activity was verified through the solubilization of the substrate in 100 mM of citrate-phosphate buffer (range of pH 3.0–7.0), glycine (range of pH 7.5–9.0), and borate (range of pH 9.5–10.0). All the assays were performed in triplicate with at least three independent experiments.

2.8. Pretreatment of the Crude Extract

Before the immobilization, the crude extracts were pretreated for the removal of the pigments by adsorption/desorption in activated charcoal. For each mL of crude extract, 5 mg of activated charcoal was added under agitation in an ice bath. After 10 min, the mixture was filtered with filter paper and centrifuged at 11.952 g for 10 min, thereby producing the clarified extract.

2.9. Enzymatic Immobilization through Ionic Adsorption

The supports CM (carboxymethyl)-cellulose, DEAE (diethylaminoethyl)-cellulose, MANAE (monoamine-*N*-aminoethyl), and polyethyleneimine (PEI) were used in the immobilization process. The supports were activated in Tris-HCl 10 mM pH 7.0 buffer. The same buffer was added to the extract. The immobilization was performed according to Monteiro et al. [31]. A volume of 10 mL of extract was added to 1 g of the support, which was incubated in a cold chamber under agitation for 48 h. After this timespan, the derivative (support + immobilized enzyme) was filtered and washed with buffer. The immobilization efficiency (*IE*) was defined as the ratio of the amount of enzyme bound to the support over the total amount of enzyme used, according to Equation (2):

$$IE\ (\%) = \frac{enzyme\ immobilized}{enzyme\ loaded} \times 100 \quad (2)$$

2.10. Statistical Analyses

The experimental results were obtained through the average ± standard deviation of three independent extractions. The programs used were GraphPadPrism® (GraphPad Software, San Diego, CA, USA) and Statistica® (StatSoft, Tulsa, OK, USA).

3. Results and Discussion

3.1. Optimization of Cultivation with Tamarind Seeds

The results of the use of these seeds as a carbon source to produce XEG can be seen in Table 2. From the data, it was possible to verify that the best enzymatic activity was obtained at the periods of 48 and 72 h with the usage of 1.705% tamarind seed extract at 30 °C (545.56 and 545.92 mU/mL, respectively) or the zero points (1% tamarind seed at 30 °C) for 96 h in the stationary condition. Regarding the cultivation under agitation, XEG was produced with a more considerable enzymatic activity at 72 h at 30 °C with 1.705% tamarind seed extract, obtaining a value of 817.28 mU/mL.

Table 2. XEG activity in the tests used for the construction of the experimental design for *T. longibrachiatum* cultivated with tamarind seeds.

	Temperature	Tamarind Seeds	Stationary				Under Agitation			
	(°C)	(%)	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
1	−1 (25)	−1 (0.5)	87.60	66.41	31.34	6.41	19.94	27.12	25.64	7.12
2	1 (35)	−1 (0.5)	27.78	102.82	114.67	138.88	7.48	12.86	135.68	168.80
3	−1 (25)	1 (1.5)	66.24	82.32	120.37	62.32	42.73	214.38	74.78	62.32
4	1 (35)	1(1.5)	55.20	280.97	289.28	415.58	31.52	326.33	451.19	526.69
5	−1.41 (22.95)	0 (1)	71.04	75.85	83.33	48.08	74.78	518.14	430.54	399.56
6	1.41 (37.05)	0 (1)	30.98	61.25	59.47	105.05	56.98	333.68	431.96	632.99
7	0 (30)	−1.41 (0.295)	nd	nd	190.52	411.66	nd	110.04	92.59	145.29
8	0 (30)	1.41 (1.705)	153.13	**545.56**	**545.92**	433.74	99.0	227.91	**817.28**	658.09
9	0 (30)	0 (1)	56.89	312.31	522.42	**537.02**	38.31	148.49	525.04	375.70
10	0 (30)	0 (1)	59.11	314.62	585.38	**628.40**	30.32	142.44	517.80	414.76
11	0 (30)	0 (1)	56.62	335.24	602.90	**633.17**	33.47	215.44	517.81	444.78

Values are expressed in mU/mL. nd = not detected. The maximum values found for each time are in bold.

The best timespan for enabling xyloglucanase activity was 72 h. The ANOVA and the F test (ratio of two variances) were, therefore, calculated only for this interval, where it was verified that the calculated F was 7.93-fold greater than the tabled F when the XEG was produced with tamarind seeds at stationary cultivation and 5.10-fold when produced in cultivation under agitation. Therefore, the null hypothesis was rejected, and there was a significant difference between the groups. According to the graphical representation of

the Pareto diagrams and contour graphs (Figure 1), the temperature and the carbon source strongly influence the xyloglucanase activity.

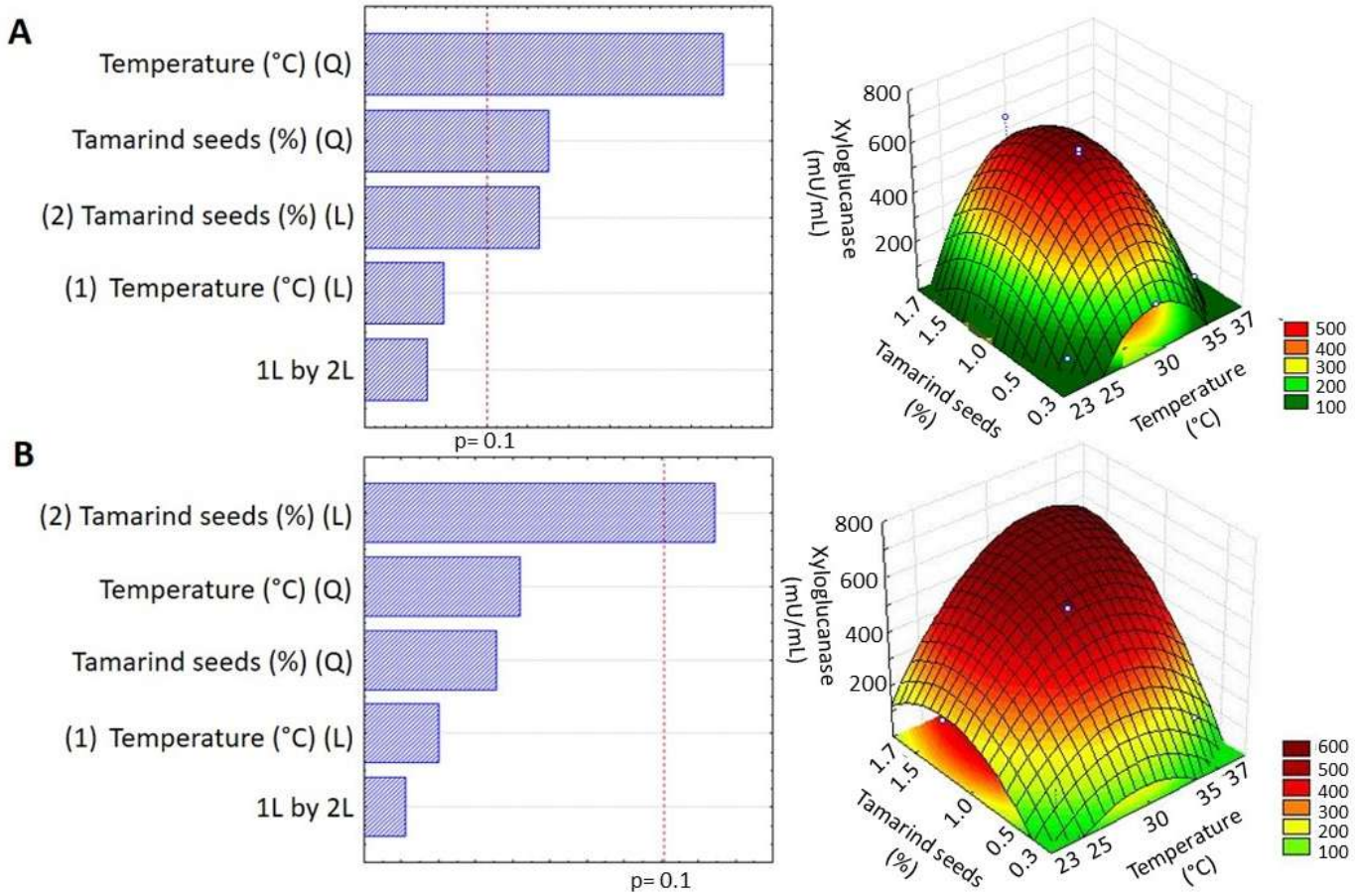

Figure 1. Pareto diagrams and contour graphs of production of *T. longibrachiatum* xyloglucanase with tamarind seeds after 72 h. (**A**)—static cultivation; (**B**)—cultivation under agitation.

3.2. Optimization of Cultivation with Jatoba Seeds

The results of the use of jatoba seeds as a carbon source to produce XEG can be seen in Table 3. It is possible to verify that the most notable enzymatic activity occurred starting from 48 h at the zero points (1% jatoba seed concentration at 30 °C). Still, it doubled 2.3-fold with a 1.705% carbon source and at 30 °C at the 72 h mark for the stationary condition. XEG had a more significant enzymatic activity at 72 h at 30 °C with a 1.705% carbon source for the cultivation under agitation. The approximated values were observed at 48 h (zero points) or 96 h at the same conditions of maximum enzymatic activity (30 °C and 1.705% concentration of the jatoba seed extract).

Via the calculation of the ANOVA and the F test with the time of 72 h (which is also the best timespan for xyloglucanase activity when the jatoba seeds were used as a carbon source), it was verified that the calculated F was 5.25-fold more significant than the tabled F when XEG was produced with jatoba seeds through stationary cultivation, and 2.38-fold greater when it was produced through cultivation under agitation; therefore, the null hypothesis was rejected, with a significant difference between the groups. According to the graphical representation of the Pareto diagrams and contour graphs (Figure 2), the temperature and carbon source strongly influence the xyloglucanase activity when using jatoba seeds as a carbon source.

When comparing the xyloglucanase levels produced with tamarind seeds or jatoba seeds as carbon sources, the best cultivation condition was 1.705% of tamarind seed extract, at 30 °C, under agitation, and at the time of 72 h.

Table 3. XEG activity in the tests used for the construction of the experimental design for *T. longibrachiatum* cultivated with jatoba seeds.

	Temperature (°C)	Jatoba Seeds (%)	Stationary				Under Agitation			
			24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
1	**−1 (25)**	−1 (0.5)	27.06	14.60	11.40	4.11	4.99	26.71	25.28	8.25
2	1 (35)	−1 (0.5)	21.72	113.89	127.48	117.52	12.82	143.16	305.54	337.59
3	−1 (25)	1 (1.5)	26.35	68.02	87.24	78.24	91.52	100.07	82.62	62.80
4	1 (35)	1(1.5)	70.51	254.26	406.68	138.88	69.09	254.26	305.19	475.41
5	−1.41 (22.95)	0 (1)	89.74	90.81	90.81	49.50	25.64	155.98	205.65	54.49
6	1.41 (37.05)	0 (1)	66.59	55.55	55.55	26.24	72.65	264.23	590.43	559.45
7	0 (30)	−1.41 (0.295)	2.0	113.96	340.44	430.18	7.47	75.5	336.53	450.84
8	0 (30)	1.41 (1.705)	96.5	115.5	**642.07**	319.43	96.15	419.5	**652.40**	540.58
9	0 (30)	0 (1)	67.31	312.31	394.0	449.23	101.49	566.79	621.12	508.82
10	0 (30)	0 (1)	62.41	254.55	398.19	379.26	180.62	593.99	622.0	521.88
11	0 (30)	0 (1)	68.02	256.04	398.87	400.09	137.1	491.79	622.10	501.58

Values are expressed in mU/mL. nd = not detected. Bold values indicate the maximum value found for each time.

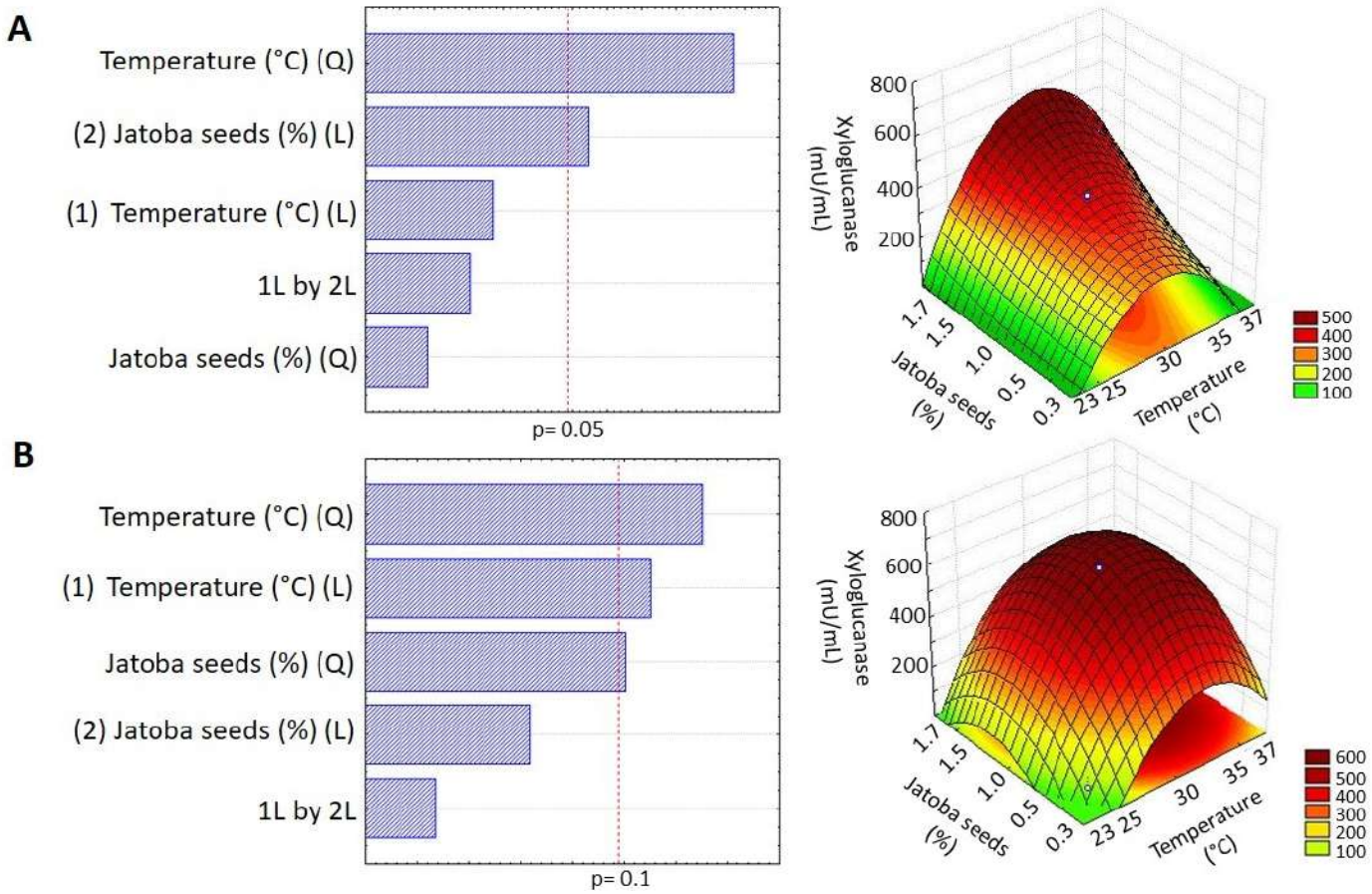

Figure 2. Pareto diagrams and contour graphs detailing the production of *T. longibrachiatum* xyloglucanase with jatoba seeds after 72 h. (**A**)—static cultivation; (**B**)—cultivation under agitation.

The experimental design is a statistically convenient technique for planning experiments in the bioprocessing field, including the optimization of enzyme production for biomass hydrolysis [27]. In recent years, several studies have been using optimization strategies to increase the productivity of enzymes [32–34], especially enzymes of the lignocellulolytic complex [35–37]. For example, Tai et al. [38] increased CMCase and xylanase production in *Aspergillus niger* by 124.5 and 78.5%, respectively, using oil palm frond leaves as carbon sources. In comparison, Ezeilo et al. [39] optimized the extracellular cellulases and xylanases produced by *Rhizopus oryzae* using the same carbon source. Another interesting study was conducted by Naidu et al. [40], who optimized the production of laccase, xylanase, and amylase from *Trametes lactinea* and *Pycnoporus sanguineus* using different substrates and evaluated the interactions between them. More recently, Singhal et al. [41] aimed to optimize the cellulase (CMCase) production in *Aspergillus flavus* using wheat straw, an abundantly available lignocellulosic waste, as a substrate. Three parameters, i.e., the nitrogen content (0.25 to 1%), fungal inoculum (0.25 to 1%), and duration (3 to 12 days), were optimized for maximum CMCase production using the Response Surface Methodology. The maximum output of CMCase of 13.89 U/gds was achieved with 0.25% yeast extract, 0.625% fungal inoculum, and a duration of 12 days. There was an almost threefold increase in CMCase production after optimization compared to the screening experiments (4.7 U/gds). All these works demonstrate the importance of a factorial design to optimizing enzyme production.

3.3. Scaling for the Bioreactor to Increase Enzyme Production

Once the best cultivation conditions were verified regarding the temperature, concentration of the carbon source, and scaling of agitation, *T. longibrachiatum* was cultivated in a bioreactor 5 L BioFlo 310-New Brunswick® (Hamburg, Germany) at 30 °C and with 1.705% of tamarind seed extract as a carbon source. A 1.27-fold increase was verified in the XEG activity since a 0.817 ± 0.028 U/mL level of activity was found in the Erlenmeyer flasks (see Table 2), while the activity was 1.040 ± 0.088 U/mL when the bioreactor was used. This result has shown the advantages of up scaling, as it improved the concentration of proteins. Consequently, this fact might have generated a more significant number of enzymes, subsequently favoring immobilization on ionic supports.

3.4. Biochemical Characterization

The crude extract produced using the bioreactor was characterized concerning the optimal temperature and pH, thermal stability, and pH stability. First, the thermostability was determined at 30 to 70 °C, with intervals of 10 °C, while the pH stability was determined at 3.0 to 8.0. Then, the optimal temperature of the crude extract of xyloglucanase was 50 °C, maintaining an excellent activity at 55 °C (Figure 3A). The optimal pH was 5.0 (Figure 3B). Still, a low thermostability (Figure 3C) and low pH stability (Figure 3D) were detected. Finally, the calculation of the T_{50} was performed, which is the temperature at which there was 50% residual activity (Table 4). XEG had a T_{50} of 185 min at the temperature of 50 °C.

Table 4. T_{50} of XEG of *T. longibrachiatum*.

Temperature (°C)	T_{50} (min)
40	138
50	185
60	67
70	66

The values found in this study are consistent with the ones found in the literature, as many studies [1,10,42–45] have performed a biochemical characterization of these parameters for xyloglucanase activity, and it was verified that the best range varies between the

temperatures of 30–60 °C. However, there is a minor variation in the literature regarding the optimal pH, with the pH range of 5.0 and 5.5 being those most commonly found.

The low thermostability was a determining factor for the enzymatic immobilization, given that there is proof in the literature that immobilization increases the thermostability and allows for the reuse of the enzyme [46–48].

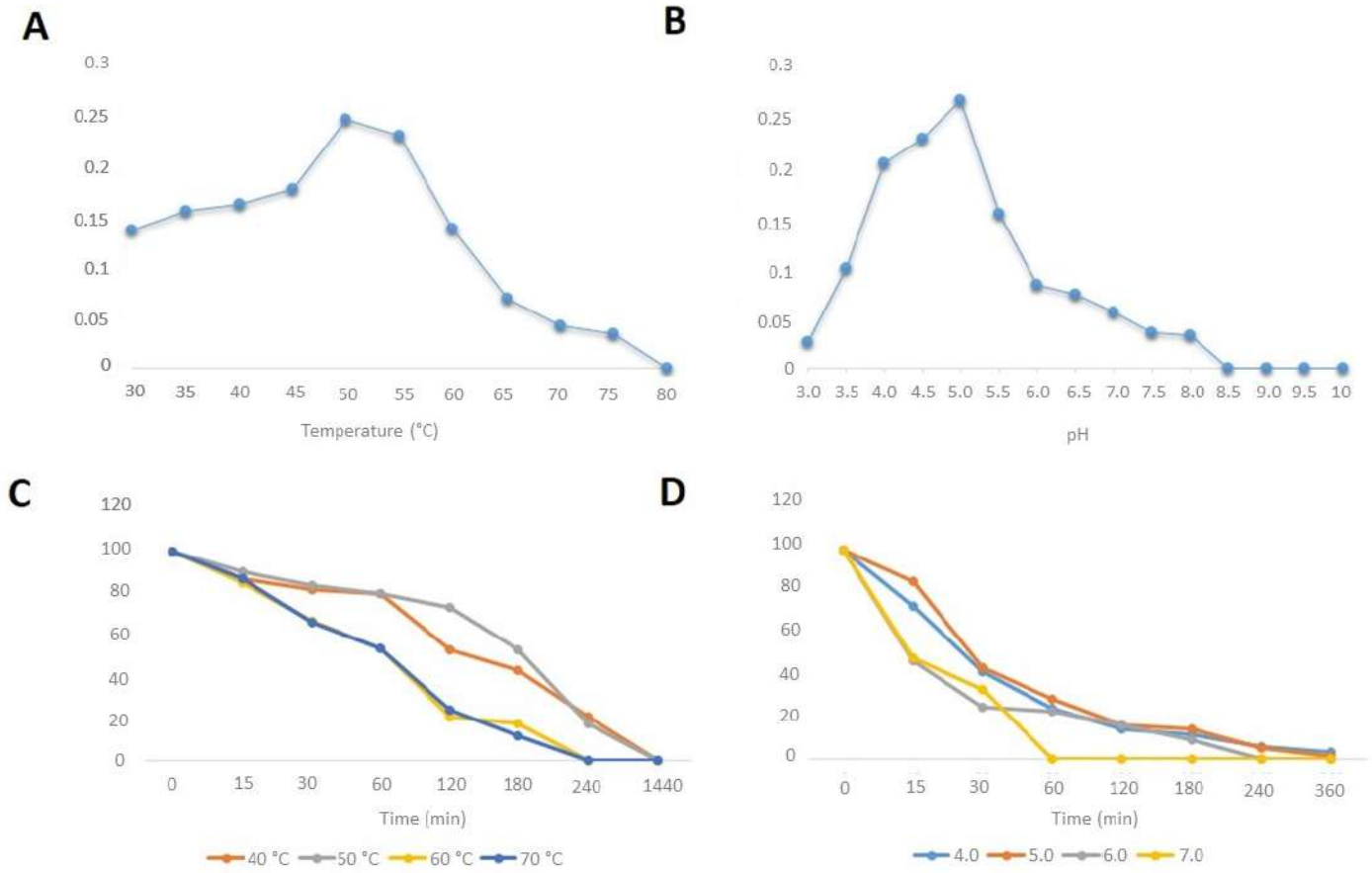

Figure 3. Biochemical characterization of *T. longibrachiatum* xyloglucanase. (**A**)—optimal temperature; (**B**)—optimal pH; (**C**)—thermostability; (**D**)—pH stability.

3.5. Enzymatic Immobilization

The crude enzymatic extract presented a brown-red color, which was a problem for its immobilization. To overcome this problem, the tegument of the tamarind seeds was removed, and it was verified that there was no decrease in the enzymatic activity. Furthermore, the extract was clarified with activated charcoal.

Ionic exchange supports CM-cellulose, DEAE-cellulose, MANAE, and PEI, which efficiently immobilized the xyloglucanase. This is the first time that the immobilization of a xyloglucanase on these supports has been described in the literature. The results, visualized in Table 5, show that the XEG's complete immobilization was obtained in CM-cellulose and DEAE-cellulose, with activities of 1.16 and 0.89 U/g of the derivative (enzyme plus support), respectively, and a hyper-activation of 1.81-fold and 1.39-fold, respectively, which demonstrates an excellent improvement in the XEG activity.

Table 5. Immobilization of XEG of *T. longibrachiatum* on different ionic supports at pH 7.0 after 48 h of immobilization.

Derivative	Total Immobilized Proteins	Derivative Activity	Immobilization Efficiency	Hyperactivation

	(%)	(U/g)	(%)	
CM-cellulose	18.61	1.16	100	1.81
DEAE-cellulose	15.69	0.89	100	1.39
MANAE	27.63	0.32	49	nd
PEI	28.73	0.01	21	nd

nd = not detected.

The primary means of immobilizing enzymes are to boost the enzyme productivity and operational stability, alongside facilitating the reuse of the enzymes. Notwithstanding the aforementioned benefits, enzyme immobilization promotes high catalytic activity and stability and the convenient handling of enzymes, in addition to their facile separation from reaction mixtures without contaminating the products [49]. Among the few studies in the literature on the immobilization of xyloglucanase, Soares et al. [50] immobilized a recombinant xyloglucanase (XegA) from *Aspergillus niveus* on chitosan-coated ferromagnetic iron oxide nanoparticles functionalized with glutaraldehyde, retained 76.9% of the activity compared to the free enzyme. In comparison, Menon et al. [51] chose to immobilize *Debaromyces hansenii* cells on Ca-alginate beads instead of immobilizing xyloglucanase. The immobilized yeast cells were reused six times at 40 °C with a 100% fermentation efficiency.

4. Conclusions

This study allowed us to conclude that, with the aid of a factorial design, high concentrations of the carbon sources in the culture medium, especially of tamarind seeds, were the most favorable conditions for the highest activity of *T. longibrachiatum* xyloglucanase. Furthermore, the up scaling of the Erlenmeyer flasks for the bioreactor was demonstrated to be an essential strategy for increasing the content of the enzymes secreted. Regarding the biochemical characterization of the crude extract, the optimal temperature range was 50–55 °C, and the optimal pH was 5.0. The pH and temperature stabilities were not stable for prolonged periods, which was crucial to choosing the immobilization process on the ion exchange resins (CM-cellulose, DEAE-cellulose, MANAE, and PEI). This was the first time the immobilization of a xyloglucanase on these chemical supports has been described in the literature.

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