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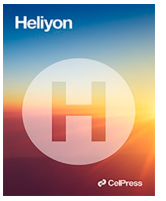

Sustainable use of giant reed to produce industrialized enzymes

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ABSTRACT

The giant reed (*Arundo donax*) is a fast-growing plant adapted to different climatic and soil conditions; although its origin is Asian, the species has spread throughout the world. During its development, it consumes three times more water than typical native vegetation and is responsible for changing the landscape of riparian areas; the high biomass productivity and the annual harvest period make this crop an alternative to produce and/or extract industrial bioproducts. The main objective of this research was to evaluate the feasibility of using giant reed in a bioprocess that produces enzymes by a solid-state fermentation experiment, four fungal species were tested (*Aspergillus niger* GH1, *Aspergillus niger* PSH, *Trichoderma harzianum*, and *Rhizopus oryzae*); enzyme activities were performed using reported methodologies varying only reaction volumes. The *A. niger* GH1 and PSH strains were the best adapted to the plant material, *A. niger* GH1 was capable to produce 4 of the 5 evaluated enzymes (cellulase-endoglucanase (174.39 ± 19.62 U/L), xylanase (1313.31 ± 39.25 U/L), invertase (642.22 ± 23.55 U/L), and polyphenol oxidase (6094.01 ± 306.54) while *A. niger* PSH was able to produce 3 of the 5 evaluated enzymes (cellulase-endoglucanase (147.09 ± 13.88 U/L), xylanase (1307.76 ± 31.40 U/L), and invertase (603.92 ± 3.14 U/L).

1. Introduction

Cuatrociénegas Valley is located within the Chihuahuan desert, in the state of Coahuila; this place has been characterized as a site with a high biological diversity despite its extreme climatic conditions. It has an area of 840 km^2 surrounded by mountains that rise to 3500 m above sea level and is divided into seven main and permanent drainage systems that form hundreds of lagoons and small lakes (locally known as "pozas") with turquoise-blue crystalline waters [1]. The giant reed is an opportunistic plant that inhabits the Cuatrociénegas valley; and can come to unbalance mainly the aquatic ecosystems, competes with the local flora and for this reason, is considered invasive species. During its development, it consumes three times more water than typical native vegetation and is responsible for changing the landscape of riparian areas.

The giant reed is a rhizomatous perennial species, introduced by humans globally as a cultivated ornamental plant. It is a sterile plant without viable seeds, which can be propagated vegetatively from the rhizome or stems [2]. Although its origin is Asian, the species has been dispersed worldwide and is cultivated in many regions and under different climates. The plant tolerates diverse

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ecological and soil conditions and is resistant to most pests [3]. Traditionally, the giant reed has been used to produce walking sticks, baskets, fishing rods, construction materials, and musical instruments; the high biomass productivity and the annual harvest period have made this crop a very attractive lignocellulosic raw material for the production of industrial bioproducts [4]. Giant reed has been used industrially for low-carbon energy production [5]; it has also been used as a wood substitute in industry due to its high cellulose content; additionally, giant reed has shown potential in medicine due to its ability to prevent the growth of biofilms [6]; otherwise, the giant reed fermentation is not reported yet, and the investigation elucidates the potential of the plant.

Solid-state fermentation (SSF) it's an eco-friendly biotechnological process, where a fungal strain growth uses as support-substrate a selected residue (mainly agro-industrial wastes), that takes place in the absence or near absence of free water, however, the substrate used must have sufficient moisture to support the growth and metabolism of the microorganism [7]. SSF is currently used in the production of enzymes, antibiotics, bioethanol, and biodiesel as an alternative energy source, biosurfactants for environmental purposes, and in the production of organic acids and bioactive compounds [8]. In recent decades, there has been a growing trend towards the use of SSF for the production of various enzymes and bulk chemicals because it offers higher yields, higher productivity, lower production cost, products with higher stability (which is especially important if an enzyme is the target product), higher asepsis, lower energy requirement, simple and inexpensive substrates (agro-industrial wastes) [9].

This work aimed to generate a bioprocess (fungal/support) of SSF with giant reed from the wetland areas of Cuatrocinegas, Coahuila, which allows the production of enzymes with potential use in the industry.

2. Materials and methods

2.1. Collection and milling of plant material

The giant reed was collected in Cuatrociénegas, Coahuila. It was dried in a conventional heating oven at 50 °C for 72 h and ground to a particle size of 2 mm. Storage until use in darkness and dry conditions.

2.2. Physicochemical characterization of giant reed

The water absorption index and critical moisture point were carried out used a mix 1:12 of plant: distilled water, mixing 10 min and centrifugated (3200 g at 4 °C) for 30 min [10]. For the determination of packing density, 10 g of plant material was placed in a graduated cylinder; placed in manual vertical agitation for 5 min and the volume of the resulting material was reported.

2.3. Reactivation of fungal strains

Four fungal strains from the strain collection of the Food Research Department (DIA), Faculty of Chemical Sciences of the Autonomous University of Coahuila were evaluated: *Aspergillus niger* GH1, *Aspergillus niger* PSH, *Rhizopus oryzae,* and *Trichoderma harzianum*. The strains were reactivated on potato dextrose agar (PDA-Bioxon) for 5 days at 30 °C, agar explants were used as inoculum in radial growth experiments, while the spores were used for the SSF.

2.4. Kinetics of radial growth and SSF

3 g of plant material was placed in sterile Petri dishes and 7 mL of sterile distilled water was added to obtain a moisture of 70 %. Each of the above-mentioned fungal strains was inoculated in triplicate with agar explants in the center of the Petri dishes and incubated at 30 °C. Mycelial growth was measured with a vernier every 6 h until the complete invasion of the plate.

Acrylic reactors of 125 cm^3 were used for SSF. To each reactor (27 reactors in total), 1.5 g of giant reed (support/substrate) and 3.5 mL of water with inoculum (1×10^7 spores/mL) were added [5], followed by the incubation at 30 °C. Sampling was performed every 6 h obtaining an enzymatic extract with 7 mL of 0.1 M citrate buffer at pH 5.

2.5. Determination of soluble protein and enzyme activities

Soluble protein was quantified using the Bradford technique [11].

Cellulase activity (Endoglucanase) used carboxymethyl cellulose at 500 ppm as the substrate, while dextrose at 200 ppm was used for the standard curve. To the tubes labeled BE (Blank Enzyme) were added 200 μL of the substrate and 50 μL of 0.05 M citrate buffer pH 5, and to the tubes labeled BS (Blank Substrate) were added 200 μL of 0.05 M citrate buffer pH 5 and 50 μL of enzyme extract, finally to the tubes labeled Rx (Reaction tube) were added 200 μL of the substrate and 50 μL of enzyme extract. All tubes were incubated in a thermos bath at 50 °C for 10 min, the reaction was stopped by boiling in a water bath for 5 min and all tubes were placed in an ice bath for 1 min [12], the release of reducing sugars was determined utilizing DNS (3,5-dinitro salicylic acid) reagent [13].

Invertase activity used sucrose at 40 mM as substrate and dextrose at 1000 ppm as the standard curve was prepared. To tubes labeled BE added 500 μL substrate and 500 μL citrate buffer 0.05 M pH 5, to tubes labeled BS were added 500 μL citrate buffer 0.05 M pH 5 and 500 μL enzyme extract, and to tubes labeled Rx were added 500 μL substrate and 500 μL enzyme extract. All tubes were incubated in a thermos bath at 25 °C for 10 min. The reaction was stopped by boiling in a water bath for 5 min and all tubes were placed in an ice bath for 1 min [14], the release of reducing sugars was determined by DNS [13].

Xylanase activity used xylans as substrate at 500 ppm and a standard curve of xylose 500 ppm in ethanol was prepared. To tubes

labeled BE were added 200 μL of the substrate and 50 μL of 0.05 M citrate buffer pH 5, to tubes labeled BS were added 200 μL of 0.05 M citrate buffer pH 5 and 50 μL of enzyme extract, and to tubes labeled Rx were added 200 μL of the substrate and 50 μL of enzyme extract. All tubes were incubated in a thermos bath at 50 °C for 10 min, the reaction was stopped by boiling in a water bath for 5 min and all tubes were placed in an ice bath for 1 min [15], the release of reducing sugars was determined by DNS [13].

Polyphenol oxidase activity used pyrocatechol 0.1 M was used as substrate and a pyrocatechol standard curve was prepared at 500 ppm. To tubes labeled BE were added 500 μL substrate and 500 μL citrate buffer 0.05 M pH 5, to tubes labeled BS were added 500 μL citrate buffer 0.05 M pH 5 and 500 μL enzyme extract, and to tubes labeled Rx were added 500 μL substrate and 500 μL enzyme extract. An additional 1.7 mL of 0.05 M citrate buffer pH 5 was added to all tubes. All tubes were incubated in a thermos bath at 30 °C for 10 min [16].

Tannase activity was performed using the methanolic rhodanine method [17] using methyl gallate 0.01 M as substrate and gallic acid 100 ppm for the standard curve.

2.6. Data treatments

Statistical analysis and graphing were conducted using the Origin Pro version 8.5 statistical software package (Origin Lab, Northampton, Massachusetts, USA). The experimental design was completely randomized, all experiments were performed in triplicate to increase the reliability of the results, and all data obtained in the physicochemical tests and the radial growth test was given a treatment in Excel software calculating averages and standard deviations. The enzyme activities were evaluated by analysis of variance (ANOVA), and the comparison of the means test was performed by Tukey's test using the Origin Pro.

3. Results

The physicochemical characterization is a preliminary determination when a new support/substrate is proposed for an SSF bioprocess, with this information the moisture, particle size, and support/culture media can be decided to predict a successful proposal; regarding the giant reed presented a water absorption capacity of 4.00 ± 0.01, a moisture percentage of 6.00 ± 0.01, and supports a maximum moisture value of 76.50 ± 0.01% (Table 1).

Filamentous fungi are the principal microorganisms used for SSF due to the growth requirements that include low moisture and wide temperature range, because of this the fungal selection was based on radial growth of the principal strains that the research group has significant results in SSF processes. Obtained microbial growth is shown in Fig. 1, *A. niger* strains (GH1 and PSH) were able to adapt to the giant reed and complete the invasion of the Petri dish after 36 h, having a maximum growth of 4.03 ± 0.01 cm and 4.00 ± 0.01 cm; respectively, *R. oryzae* needed 42 h to complete the invasion with a maximum growth of 3.95 ± 0.09 cm and *T. harzianum* did not complete the invasion of the plate after 42 h, reaching a maximum growth of 1.36 ± 0.04 cm.

The *A. niger* strains were the best adapted to giant reed and the complete invasion of the Petri dishes in lower time, for this reason, were selected to continue with the SSF process and subsequent enzymatic production.

As primary determination the quantification of soluble protein production was done kinetically every 6 h, results profile is similar for *A. niger* strains (Fig. 2), GH1 had a decrease at 18 h but then rise again until the final quantification performed at 48 h, achieving 411.48 and 300.28 mg/L *A. niger* GH1 and *A. niger* PSH, respectively.

In the industry the enzymes are useful to complete several processes and/or to obtain products/molecules; for this reason, new biotechnological approaches where the enzymes are obtained from new sources with interesting enzymatic activity titles are sought. In this study, some of them were addressed, such as cellulase, invertase, xylanase, polyphenol oxidase, and tannase.

Giant reed is a lignocellulosic material, because of this the cellulase activity was inferred as present, and the enzyme quantification was proposed. *A. niger* GH1 cellulase production was detected since 6 h fermentation (47.18 ± 9.81 U/L) (Table 2), being 36 h where the highest enzyme activity titer was obtained (174.39 ± 19.62 U/L). The *A. niger* PSH strain showed cellulase activity up to 12 h (8.33 ± 11.77 U/L) (Table 3), with enzyme activity titers at 30, 36, 42, and 48 h (97.14 ± 3.92, 147.09 ± 13.88, 86.04 ± 11.77, and 116.57 ± 15.70 U/L, respectively), while the highest value was at 36 h.

Invertase is an applied enzyme used in the food industry, mainly in confectionery manufacturing, and produced by diverse filamentous fungal species. *A. niger* GH1 invertase activity was detected since 6 h fermentation (Table 2), and showed enzyme titers at 6, 24, 42, and 48 h (493.74 ± 21.59, 339.71 ± 23.55, 642.22 ± 23.55, and 449.33 ± 9.81 U/L, respectively), being 642.22 ± 23.55 U/L, the highest enzyme activity obtained. *A. niger* PSH exhibited invertase activity at 12, 18, 30, 42, and 48 h (603.92 ± 3.14, 102.13 ± 69.08, 81.04 ± 7.85, 220.92 ± 117.75 and 84.37 ± 0.01 U/L, respectively, Table 3), being at 12 h where the highest enzyme activity titer (603.92 ± 3.14 U/L) was obtained.

Table 1
Physicochemical characterization of giant reed (dry basis).

Parameters	Results
Moisture (%)	6.00 ± 0.01
Solids (%)	94.00 ± 0.01
Water absorption capacity (g of gel/g of dry weight)	4.00 ± 0.01
Maximum moisture of the support/substrate (%)	76.50 ± 0.01
Packing density (final resultant volume)	13.33 ± 1.15

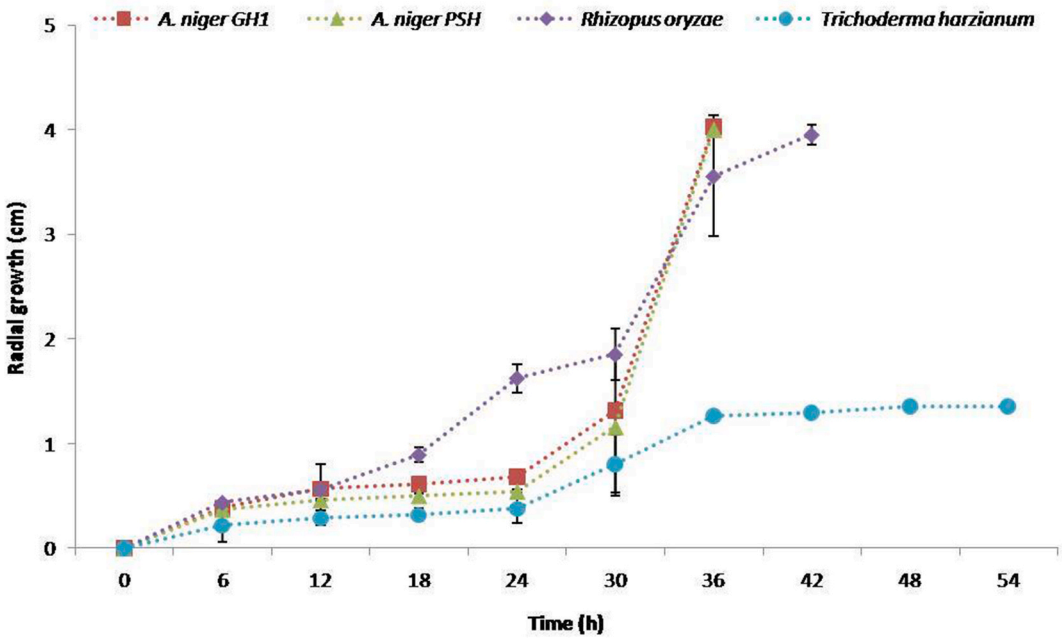

Fig. 1. Radial growth of fungal strains on giant reed.

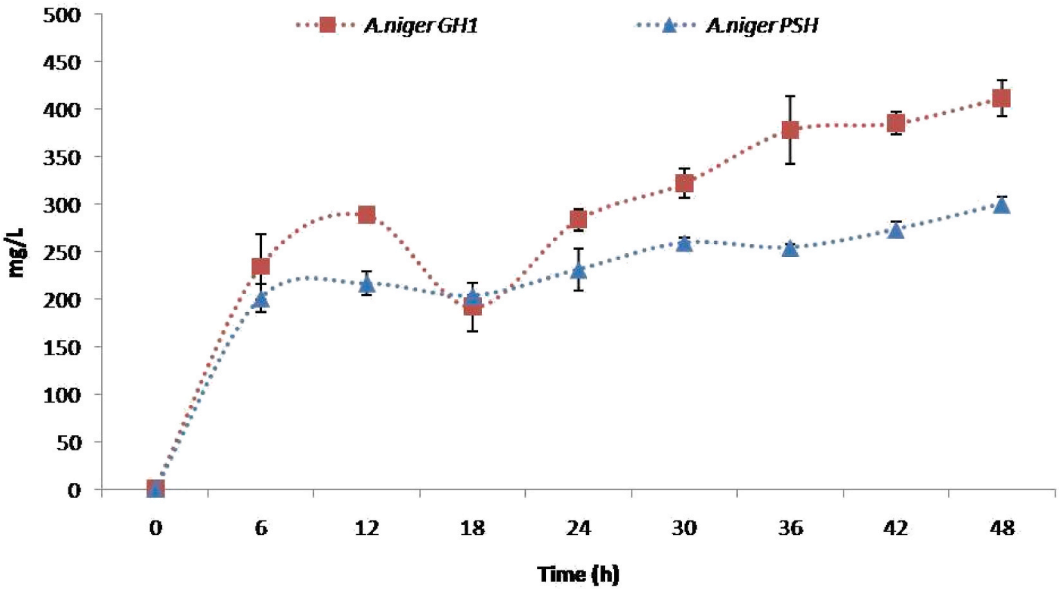

Fig. 2. Comparison of soluble protein production between *A. niger* GH1 and *A. niger* PSH strains.

Xylanases are enzymes capable to metabolize hemicellulose into xylo-saccharides (XOS) of different sizes, with use in the food and paper industries. *A. niger* GH1 began to produce xylanase at 6 h (Table 2), with more quantifications at 18, 24, 30, 36, 42, and 48 h (1096.83 ± 62.80, 1163.44 ± 47.10, 1052.42 ± 47.10, 1302.20 ± 7.85, 1313.31 ± 39.25, and 1302.20 ± 39.25 U/L, respectively). The highest enzyme activity titer was obtained at 42 h; otherwise, *A. niger* PSH produced xylanase at 6, 12, 24, 30, 36, 42, and 48 h (863.70 ± 157.00, 919.20 ± 15.70, 952.51 ± 31.40, 885.90 ± 15.70, 1307.76 ± 31.40, and 919.20 ± 94.20 U/L, respectively), being the 42 h where the highest enzyme activity titer was obtained (Table 3).

Polyphenol oxidase catalyzes the hydroxylation from monophenols to *o*-diphenols and its oxidation to *o*-quinones. In plants, this enzyme is related to the defense mechanism against pathogens and bugs. *A. niger* PSH did not show polyphenol oxidase activity on the support substrate used for the experiment; instead, *A. niger* GH1 had results at 12, 24, 42, and 48 h (6094.01 ± 306.54, 1863.76 ± 735.69, 1679.84 ± 797.00, and 3825.61 ± 490.46 U/L, respectively), being at 12 h the highest titer of enzyme activity.

Table 2
Enzymatic activities of *A. niger* GH1 on giant reed.

Time (h)	Cellulase (U/L)	Invertase (U/L)	Xylanase (U/L)	Polyphenol oxidase (U/L)
0	ND	ND	ND	ND
6	47.18 ± 9.81	493.74 ± 21.59	364.13 ± 235.50	ND
12	168.60 ± 44.16	141.27 ± 52.99	264.22 ± 141.30	6094.01 ± 306.54
18	169.76 ± 0.01	245.34 ± 11.10	1096.83 ± 62.80	1066.76 ± 551.77
24	95.75 ± 0.01	339.71 ± 23.55	1163.44 ± 47.10	1863.76 ± 735.69
30	128.13 ± 13.08	149.59 ± 29.44	1052.42 ± 47.10	453.68 ± 306.54
36	174.39 ± 19.62	275.87 ± 0.01	1302.20 ± 7.85	ND
42	70.31 ± 25.44	642.22 ± 23.55	1313.31 ± 39.25	1679.84 ± 797.00
48	123.50 ± 0.01	449.33 ± 9.81	1302.20 ± 39.25	3825.61 ± 490.46

*ND means Non Detected.

Table 3
Enzymatic activities of *A. niger* PSH on giant reed.

Time (h)	Cellulase (U/L)	Invertase (U/L)	Xylanase (U/L)
0	ND	ND	ND
6	ND	ND	275.32 ± 222.03
12	8.33 ± 11.77	603.92 ± 3.14	863.70 ± 157.00
18	36.08 ± 3.92	102.13 ± 69.08	586.16 ± 47.10
24	22.20 ± 15.70	ND	919.20 ± 15.70
30	97.14 ± 3.92	81.04 ± 7.85	952.51 ± 31.40
36	147.09 ± 13.88	ND	885.90 ± 15.70
42	86.04 ± 11.77	220.92 ± 117.75	1307.76 ± 31.40
48	116.57 ± 15.70	84.37 ± 0.01	919.20 ± 94.20

*ND means Non-Detected.

A. niger GH1 and PSH did not show tannase activity on the support substrate used for the experiment although the same strains are reported as principal tannase producers by the research group, using different supports and culture media.

As a resume, we can say *A. niger* GH1 presented the highest enzyme titers of cellulase at 36 h (174.39 ± 19.62), invertase at 42 h (642 ± 23.55 U/L), xylanase at 42 h (1313.31 ± 39.25 U/L), and polyphenol oxidase at 12 h (6094.01 ± 306.54 U/L) (Table 2). *A. niger* PSH presented the highest enzyme titers of cellulase at 36 h (147.09 ± 13.88 U/L), invertase at 12 h (603.92 ± 3.14 U/L) and xylanase at 42 h (1307.76 ± 31.40 U/L) (Table 3).

Values of specific enzymatic activities (U/mg) obtained with *A. niger* GH1 using giant reed as support/substrate can be observed in Table 4.

A. niger GH1 presented the highest cellulase enzyme titer at 36 h (174.39 ± 19.62 U/L, with a specific activity of 0.46 U/mg, but presented the highest specific activity at 18 h with 0.89 U/mg; the highest invertase enzyme titer was obtained at 42 h (642.22 ± 23.55 U/L, specific activity of 1.67 U/mg), but presented the highest enzymatic specific activity at 6 h with 2.11U/mg; the highest enzymatic titer of xylanase was presented at 42 h (1313.31 ± 39.25 U/L, specific activity of 3.41 U/mg), but presented the highest specific activity at 18 h with 5.72 U/mg; and polyphenol oxidase presented the highest enzymatic activity at 12 h (6094.01 ± 306.54 U/L, specific activity of 19.91 U/mg) being this time where it also presented the highest specific activity.

Values of enzyme-specific activities (U/mg) obtained with *A. niger* PSH using giant reed as support/substrate can be observed in Table 5.

A. niger PSH presented the highest enzyme titers at the same time with the specific activities, cellulase at 36 h (147.09 ± 13.88 U/L, specific activity of 0.58 U/mg); invertase enzyme at 12 h (603.92 ± 3.14 U/L, with a specific activity of 2.79 U/mg); and xylanase was presented at 42 h (1307.76 ± 31.40 U/L, with a specific activity of 4.78 U/mg).

Table 4
Specific enzyme activities from *A. niger* GH1.

Time (h)	Cellulase (U/mg)	Invertase (U/mg)	Xylanase (U/mg)	Polyphenol oxidase (U/mg)
0	0.00	0.00	0.00	0.00
6	0.20	2.11	1.55	0.00
12	0.58	0.49	0.92	19.91
18	0.89	1.28	5.72	5.11
24	0.34	1.20	4.10	5.91
30	0.40	0.46	3.27	0.23
36	0.46	0.73	3.45	0.00
42	0.18	1.67	3.41	4.60
48	0.30	1.09	3.16	10.30

Table 5
Specific enzymatic activities of *A. niger* PSH.

Time (h)	Cellulase (U/mg)	Invertase (U/mg)	Xylanase (U/mg)
0	0.00	0.00	0.00
6	0.00	0.00	1.37
12	0.04	2.79	3.99
18	0.18	0.50	2.88
24	0.10	0.00	3.97
30	0.37	0.31	3.67
36	0.58	0.00	3.48
42	0.31	0.81	4.78
48	0.39	0.28	3.06

4. Discussions

The giant reed can support a maximum moisture value of 76.50 % (Table 1) before free water in the medium appears, which is not suitable in an SSF [18]; the previously mentioned authors obtained similar values for the parameters evaluated when they evaluated Castilla Rose (*Purshia plicata*), which is also a semi-desert plant. They reported a moisture value of (6.8 %), a solids percentage of (93.2 %), a water absorption capacity of (5.3 g gel/g of dry material), and a maximum substrate moisture value of (83 %); the authors also mentioned that if the microorganism used for fermentation is a filamentous fungus, as in the present work, it is not convenient to carry out the fermentation at a high moisture value, since this can interfere with oxygen transfer, in addition to causing particle agglomeration and favoring bacterial growth, so based on the parameters evaluated, it was decided to carry out the SSF with 70 % moisture value.

In 2022, the use of giant reed was proposed as a tool for pathogen control in horticulture, due to the lignocellulosic composition of the plant and the biological activity of lignin and lignin-derived compounds, which have been shown to have antimicrobial and antifungal activity with some fungal species [19], which may explain why the fungal strain *T. harzianum* was not able to adapt and completely invade the giant reed Petri dish (Fig. 1).

The positive trend increase observed in the graph (Fig. 2) may be explained because fungi secrete enzymes to degrade the plant material and have access to the nutrients in it, and use them as a substrate to continue growing; the enzymes, being of protein origin, are detected in the Bradford test; however, it is still not possible to ensure that the fungi produced enzymes because they can be false positives, that is why to infer whether the enzymes sought are produced it is necessary to evaluate the corresponding enzymatic activities.

A. niger can produce cellulase by induction of D-xylose or by a limitation of carbon in the medium leading to a repression of the enzyme and under these conditions, monomeric sugars such as D-xylose can function as inducers. In *A. niger* the cellulase enzyme activator is expressed in the presence of xylan and leads to cellulose utilization; in the absence of xylan, this system could be activated or poorly activated, leading to reduced fungal growth when cellulose is the only carbon source [20]; therefore, based on the above, cellulase in *A. niger* is considered an inducible enzyme. Additionally, was reported cellulase activity as well as isolation, purification, and characterization of the enzyme from *A. niger* [21]; therefore, it can be deduced that *A. niger* strains (GH1 and PSH) can produce endoglucanase to hydrolyze the β 1–4 bonds of cellulose present in giant reed and thus release glucose units to be used as substrate.

A. niger GH1 was capable of producing cellulase at 6 h, presenting the highest enzyme activity titer at 36 h; *A. niger* PSH was not able to produce enzyme until 12 h, presenting lower enzyme activity titers than those obtained by GH1; however, after 30 and 36 h the enzyme activity of *A. niger* PSH behaved similarly to *A. niger* GH1. In general, it was determined that the *A. niger* GH1 strain obtained better cellulase enzyme production compared to the *A. niger* PSH strain.

Invertase is a repressible enzyme since it is synthesized when there are low levels of hexoses in the medium [22], *A. niger* synthesizes and secretes in large quantities only those enzymes of the hydrolase type that it needs to degrade some medium components; the mechanism of invertase induction of *A. niger* is different from that of yeasts in which invertase synthesis is constitutive, even so, the levels of excretion of the enzyme to the culture medium are low compared to the excretion levels of filamentous fungi [23]. Additionally, several species of filamentous fungi (*Trichoderma* spp., *Rhizopus* spp., *Neurosporas* spp., *Chaetomium* spp., *Fusarium* spp., *A. niger LP5*, and *Penicillium* spp) were isolated from longan pollen and upon evaluating the production of invertase from longan pollen determined that only the *Aspergillus* strain could produce invertase [24]. As mentioned above, the production of invertase by both *A. niger* strains (GH1 and PSH) was expressed probably by the low concentration of carbon (hexoses) in the medium.

A. niger GH1 produced invertase at 6 h and had the highest titer of enzyme activity at 42 h; *A. niger* PSH did not produce invertase until 12 h, this being the time at which it presented the highest enzyme activity and was also higher than that obtained with *A. niger* GH1 at the same time. It was determined that the *A. niger* GH1 strain obtained better invertase enzyme production compared to the *A. niger* PSH strain.

The high levels of xylanase activity observed in this study may be because xylanase is an enzyme produced by fungi that degrade plant material. The giant reed, being a hard and fibrous plant material, contains structural polysaccharides such as cellulose, cellobiose, and hemicellulose. Xylan is one of the main structural components of plant cell walls [25]. Therefore, it is likely that the *A. niger* GH1 and PSH strains produced xylanase to hydrolyze the xylans present in giant reed and obtain xylose as a substrate. The production of xylanase by these strains is also consistent with the fact that they were able to produce cellulase. The presence of xylan in the medium promotes the production of cellulase enzymes [20], so it can also be considered that xylanase is an inducible enzyme by xylan

action. To reinforce this idea was founded a report that xylanases are inducible enzymes and that commercial xylan is the substrate per excellence for xylanases, but it is expensive [26]. The high levels of xylanase activity observed in this study suggest that the *A. niger* GH1 and PSH strains were able to degrade the xylans present in the giant reed to obtain xylose as a substrate. Both fungal strains showed similar behavior in the production of xylanase. However, after 18 h, *A. niger* GH1 had higher enzymatic activity titers than *A. niger* PSH. Both strains showed the highest enzymatic activity at 42 h. Based on experimental results, it was determined that the *A. niger* GH1 strain obtained slightly better xylanase production compared to the *A. niger* PSH strain.

A. niger GH1 results showed the production of the polyphenol oxidase enzyme; lignins are one of the main components of the cell walls of plants, it is the second natural polymer of the organism, and obtaining polyphenols derived from lignin was reported [27]. The enzyme activity titers obtained with *A. niger* GH1 can be attributed to the presence of lignin and lignin-derived compounds in the structure of giant reed. Additionally, this enzyme is strongly related to a defense mechanism against pathogenic agents [19]. The high production of the enzyme may be related to factors such as giant reed being rich in lignin, in addition, since it is an oxidation reaction, factors such as exposure to light during the experimental procedure as well as environmental factors such as temperature may have an influence. Although giant reed contains lignin, which can induce the production of polyphenol oxidase, *A. niger* PSH was not capable to produce this enzyme. Even though both strains belong to the *A. niger* genus, it does not necessarily mean that they produce the same enzymes or have the same enzymatic activities. This can be seen in the soluble protein values and enzymatic activity titers, where *A. niger* GH1 performed better. Therefore, it is most likely that *A. niger* PSH cannot produce polyphenol oxidase.

A. niger GH1 and PSH did not exhibit tannase activity. Although authors such as [28,29], to mention a few, have reported that *A. niger* GH1 is capable to produce tannase in SSF, the support, and substrate have a great influence on enzyme production; this is because the enzyme is inducible by the presence of hydrolyzable tannins, so if the support or substrate used is not rich in tannins the enzyme is not produced; everything indicates that the reason why tannase could not be produced is that the giant reed does not contain tannins in its structure. Additionally, there are reports about the tannase production of *A. niger* GH1 and *A. niger* PSH [30].

The specific activity of an enzyme refers to the activity of an enzyme per mg of total soluble protein (Bradford); in other words, it is the number of units of enzyme activity divided by the milligrams of protein in the sample evaluated [31]. The specific activity defines the purity of an enzyme in a protein mixture; a probable reason is that the times of the highest enzyme titers differed with the specific activities of *A. niger* GH1.

Particularly, *A. niger* PSH presented the highest enzymatic titers and specific activities at the same time, which could be due to the purity of the produced enzymes higher than that of the GH1 strain; however, it should be emphasized that *A. niger* GH1 presented higher soluble protein values, which could explain the specific enzymatic activities varied concerning its higher enzymatic titers, since having a large amount of soluble protein, the purity of the enzymes evaluated could be lower.

Finally, it should be clarified that to determine the purity of an enzyme more experiments are necessary, so the above-mentioned are only explanations.

5. Conclusions

The physicochemical analyses showed that the giant reed can support fungal growth on it. *A. niger* GH1 and PSH strains were the best adapted to the plant material. The giant reed proved to be useful as a support/substrate to carry out the SSF, where both strains used the giant reed as a source of carbon and nutrients.

A. niger GH1 produced cellulase, invertase, and xylanase. Cellulase had the highest enzyme titer at 36 h (174.39 $\pm$ 19.62 U/L, specific activity of 0.46 U/mg), invertase had the highest enzyme titer at 42 h (642.22 $\pm$ 23.55 U/L, specific activity of 1.67 U/mg), and xylanase had the highest enzyme titer at 42 h (1313.31 $\pm$ 39.25 U/L, specific activity of 3.41 U/mg).

A. niger PSH produced the highest enzyme titer of cellulase at 36 h (147.09 $\pm$ 13.88 U/L, specific activity of 0.58 U/mg), invertase had the highest enzyme titer at 12 h (603.92 $\pm$ 3.14 U/L, specific activity of 2.79 U/mg), and xylanase had the highest enzyme titer at 42 h (1307.76 $\pm$ 31.40 U/L, specific activity of 4.78 U/mg).

The enzymatic behavior of both strains was similar, although *A. niger* GH1 was able to produce concentrated enzymes that traduce in higher titers of enzymatic activities compared to *A. niger* PSH. These results suggest that *A. niger* GH1 is the recommended fungal to continue with the optimization work.

Author contribution statement

Alonso Ascacio-Valdés: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Jose Carlos León-Medina: Analyzed and interpreted the data.

Elan Iñaky Laredo-Alcalá, Miguel Angel León-Zapata: Contributed reagents, materials, analysis tools or data.

Adriana Carolina Flores-Gallegos: Analyzed and interpreted the data, Contributed reagents, materials, analysis tools or data.

N. Paola Melendez-Rentería: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data associated with this study has been deposited at Special issue latín food conference http://www.biochemtech.uadec.mx/2023/01/09/jbct-no-29-special-edition-latin-food-2022/

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Melendez-Renteria, N.P. reports financial support was provided by Federal Government of Mexico Secretaria of Public Education.

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