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Research Article

Beauveria Bassiana Amylase-Polygalacturonase Production Using Lignocellulosic Biomass and Application in Juice Processing

Ayodeji Amobonye, Prashant Bhagwat, Suren Singh, Santhosh Pillai*

Department of Biotechnology and Food Science, Faculty of Applied Sciences, Durban University of Technology, P O Box 1334, Durban, 4000, South Africa.

**Corresponding author*: Santhosh Pillai, Department of Biotechnology and Food Science, Faculty of Applied Sciences, Durban University of Technology, P.O. BOX 1334, Durban, 4000, South Africa. Tel/ Fax: +27-313735329, E-mail: santhoshk@dut.ac.za

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Background: The search for sources of industrial biocatalysts, which are non-pathogenic and can utilise cheap nutrient sources, has been a continuous endeavour in the ~ 7 billion USD enzyme industry. *Beauveria bassiana*, an endophytic fungal entomopathogen, is non-pathogenic and possesses the potential to secrete various bioproducts while utilising readily available lignocellulosic biomass.

Objective: This study investigated the optimised production of two glycosyl hydrolases, amylase and polygalacturonase, by *B. bassiana* while utilising readily available agricultural residues. Subsequently, the industrial potential of the enzymes in the clarification of fruit juice was evaluated.

Materials and Methods: Initially, seven agro residues were screened for the concomitant production of amylase and polygalacturonase by *B. bassiana* SAN01. Subsequently, statistical optimisation tools, Plackett Burman Design (PBD) and Central Composite Design (CCD), were employed for the optimisation of enzyme production. The enzyme mixture was partially purified and applied in the clarification of pineapple juice.

Result: The production of *B. bassiana* SAN01 amylase and polygalacturonase was found to be maximal while utilising wheat bran. Subsequent to PBD and CCD optimisation, the optimal conditions for enzyme production were identified to be at 30 °C, pH 6.0 and wheat bran concentration of ~40 $g.L^{-1}$. Under these optimised conditions, heightened production levels of 34.82 and 51.05 $U.mL^{-1}$ were recorded for amylase and polygalacturonase, respectively, which were 179% and 187% of the initial unoptimised levels. In addition, the most effective clarification of the juice (~90%) was observed at 35 °C after an incubation time of 120 min with no significant effect on the pH and total dissolved solids.

Conclusion: *B. bassiana*, a well-known biocontrol agent, was shown to produce amylase and polygalacturonase using readily available agricultural residues for the first time. These enzyme production levels are the highest for these enzymes from any known endophytic fungal entomopathogen. This study further demonstrates the potential applicability of *B. bassiana* in other industrial processes besides its widespread use as a biopesticide.

Keywords: Endophytic fungal entomopathogen, Glycosyl hydrolases, Juice clarification, Statistical optimisation

1. Background

Beauveria bassiana is one of the most prominent entomopathogens due to its wide application in the agriculture industry for pest control; it has also been shown to significantly support plant growth, increasing its potential in integrated pest management. Furthermore,

the level of inquiry into the fungi has increased due to its ability to produce various bioactive metabolites such as beauvericin, bassianolide, oosperin, and pyridovericin, as well hydrolytic enzymes such as amylase, cellulase, chitinase, lipase and xylanase (1). These potentials, along with its established safety for human use, have thus promoted the application of many of its enzymes in chemical biotransformation, as detergent additives, in silk degumming, etc. (2). Interestingly, the fungus can utilise inexpensive plant biomass for its growth and bioproduct secretion. For example, Mishra and Kumar (3) demonstrated the adequacy of jatropha seed cake, pongamia seed cake, rice husk, tea leaf waste, urad bean waste and wheat bran in *B. bassiana* spore production. The fungus has also been described to utilise different agricultural residues to produce other useful metabolites, especially industrially essential enzymes, including cellulase, lipases, proteases, and xylanase (1). Of particular interest are the plant cell wall degrading enzymes (CWDEs), such as amylases, cellulases, xylanases and polygalacturonase, which are very useful in industrial processes.

Amylases (3.2.1.1), which facilitate the depolymerisation of starch to glucose, have been sourced from various microbes including, bacteria, fungi and yeast (4). Amylases are useful in detergents, as desizers in the textile industry, as well as bread softeners and juice clarifying agents in the food industry (5). Pectinases (3.2.1.15), on the other hand, are enzymes that break down pectin into galacturonic acid; the enzyme group comprises mainly pectinesterases, pectin lyase, and polygalacturonase. Furthermore, these enzymes have been found useful in juice processing (clarification and extraction), textile processing (retting and degumming) and plant genetics (cell protoplasts studies) (6). The economic and industrial relevance of these enzymes is further underscored by their significant share in the global food enzyme market (7), hence the need for novel sources of the enzymes. In this regard, a strain of *B. bassiana* isolated in its endophytic state and designated as *B. bassiana* SAN01 was studied for its potential to produce amylase and polygalacturonase.

Initially, different readily available plant biomasses were evaluated as carbon sources for the production of *B. bassiana* SAN01 amylase and polygalacturo-nase, followed by the optimisation of other process parameters via a statistical approach. As against the One Factor at a Time (OFAT) approach, the statistical approach tests and optimises several variables simultaneously, thus saving time and precious laboratory resources (8). Subsequent to the selection of the most productive agricultural biomass, Plackett-Burman Design (PBD) and Central Composite Design (CCD) were utilised for the selection of the most significant enzyme production parameters and the optimisation of the selected factors, respectively. Furthermore, the efficacy of the decolourised amylase-polygalacturonase enzyme mixture in the clarification of pineapple juice was investigated. According to available literature, this is the first study to investigate the amylase and polygalacturonase production ability of *B. bassiana* and the first to optimise the production process using lignocellulosic biomass.

2. Objective

The primary aim of this study was to explore the potential of the endophytic fungal entomopathogen *B. bassiana* as a source for the optimised production of industrial enzymes, specifically amylase and polygalacturonase. Leveraging on the non-pathogenic nature of *B. bassiana* and its ability to utilise cost-effective nutrient sources, we sought to harness its biocatalyst capabilities using readily accessible agricultural residues. Furthermore, the efficacy of these enzymes in the clarification of fruit juice, highlighting a prospective industrial application, was evaluated. Through this research, we aimed to contribute to the enzyme industry's ongoing search for innovative and cost-effective biocatalyst sources.

3. Materials and Methods

3.1. Chemicals

All the chemicals and reagents used in the study were of analytical grade and were purchased from Merck (Germany) and Sigma Aldrich (USA).

3.2. Microorganism

Beauveria bassiana SAN01, the fungal strain under study, was previously isolated in its endophytic state from onion leaves (1). It was grown for five days at 30 °C on potato dextrose agar; subsequently,

fungal inoculum (1x 10^7 spores ml^{-1}) was made by suspending its spores in 0.1% Tween-20 solution.

3.3. Lignocellulosic Biomass Screening

Bambara (*Vigna subterranea*) haulms, corn cob, sugarcane bagasse, spent tea leaves, wheat straw and wheat bran were screened as sole carbon sources

for amylase and polygalacturonase by *B. bassiana* SAN01. Each fermentation flask was made up of 100 mL mineral salt solution, 5 g of the appropriate carbon source and 1 mL of inoculum, while the conditions of fermentation were pH 5.0, 30 °C, 150 rpm and 12 days incubation period. Subsequently, the resulting fermentation broths were clarified using cheesecloth and centrifugation at 10,000 x *g* at 4 °C for 15 min. The supernatants collected served as the enzymes for further experiments.

3.4. Enzyme Assay

The activity of the amylase and the polygalacturonase enzymes were measured using 1% soluble starch and 1% citrus peel pectin, respectively, under standard assay conditions (40 °C, 50 mM phosphate buffer, pH 5.5). The reducing sugars produced in both catalytic reactions were measured using the DNS method (9).

3.5. Statistical Optimisation of Amylase/Polygalacturonase Production

3.5.1. Plackett-Burman Design (PBD)

PBD (Stat-Ease Design Expert**)** was applied in selecting the media composition and fermentation conditions with the most significant effects on the production of the two enzymes. Eleven (11) factors, chosen from previous studies (10, 11) and from our preliminary experiments, were evaluated at both low and high levels to give a set of 12 experimental runs. At the same time, the amylase and polygalacturonase activities were recorded as the responses (**Supplementary Table 1A**).

3.5.2. Central Composite Design (CCD)

Three chosen factors with the most significant effects from PBD were then optimised using CCD at five (5) levels to elucidate their inter-relationship for maximal enzyme production (**Supplementary Table 2A**). The resulting quadratic factorial design led to 14 factorial and 6 replication runs, totalling 20 runs. Subsequently, regression analysis was performed on the resulting data.

3.6. Purification of Crude Enzymes

The crude enzyme was decolourised using activated charcoal to obtain a clarified enzyme solution (12). Finally, the enzyme was vacuum-concentrated and filtered through 0.2 µm sterile syringe filters to eliminate microbes and other suspended particles.

3.7. Juice Processing

Pineapples (Queen variety, ~700 g) were sourced locally and peeled by hand. Afterwards, they were chopped into ~1 cm^3 pieces, and the juice was extracted using a fruit juices and filtered using a stainless-steel sieve (0.5 mm pore size). It was pasteurised at 60 °C for 90 s (13).

3.8. Enzymatic Clarification of Pineapple Juice

For enzyme-assisted juice clarification, the *Beauveria* enzyme solution (~40 U amylase and 60 U polygalacturonase) was added to 50 mL of the pasteurised juice and placed in a shaking water bath at 100 rpm (Memmert WB 22, Germany). The reaction temperatures were set to 30, 35, 40 and 45 °C while clarified samples were taken at an hour interval for 6 h to measure the reducing sugars released (9). The clarification of the samples was then evaluated by measuring the absorbance at 660 nm. In addition, the pH and total soluble solids (TSS) were also recorded.

4. Results

4.1. Biomass Screening for B. Bassiana SAN01 Amylase and Polygalacturonase Production

The production levels of *B. bassiana* SAN01 amylase and polygalacturonase varied significantly across the different lignocellulosic biomass used in the study (**Supplementary Table 3A**). However, the highest secretion levels of the two enzymes were recorded while the fungus utilised wheat bran as its carbon source with amylase and polygalacturonase production of 19.48 $U.mL^{-1}$ and 27.37±0.56 $U.mL^{-1}$, respectively. It is, however, worthy of note that copious amounts of amylase (19.48±1.01 $U.mL^{-1}$) and polygalacturonase (17.85±0.33 $U.mL^{-1}$) were observed with Bambara haulm (an underutilised sub-Saharan crop biomass). In contrast, the production of both enzymes was not detected with sugarcane bagasse and wheat straw.

4.2. Plackett-Burman Design (PBD)

The design matrix of outcomes from the Plackett-Burman experimental design for the screening of significant factors is presented in **Table 1**. It was observed that *B. bassiana* SAN01 amylase and polygalacturonase production were significantly affected by the same parameters, which are incubation temperature, the pH of the fermentation media and the concentration of wheat bran. The significant effects of the evaluated parameters on the production levels of

Table 1. Design matrix of outcomes from Plackett-Burman experimental design for screening significant enzyme production factors

Run	Coded level											Responses (U.mL)$^{-1}$*	
	A	B	C	D	E	F	G	H	I	J	K	Amylase	Polygalacturonase
1	+1	+1	-1	-1	-1	+1	-1	+1	+1	-1	+1	8.86±0.32	19.97±0.08
2	+1	+1	+1	-1	-1	-1	+1	-1	+1	+1	-1	7.05±0.28	14.14±0.44
3	-1	+1	+1	-1	+1	+1	+1	-1	-1	-1	+1	13.66±0.45	22.57±0.92
4	-1	+1	-1	+1	+1	-1	+1	+1	+1	-1	-1	22.85±1.01	29.26±1.33
5	+1	+1	-1	+1	+1	+1	-1	-1	-1	+1	-1	13.85±0.71	22.04±0.46
6	+1	-1	+1	+1	+1	-1	-1	-1	+1	-1	+1	16.47±0.65	26.34±1.21
7	+1	-1	-1	-1	+1	-1	+1	+1	-1	+1	+1	16.8±0.76	20.71±1.02
8	-1	-1	-1	+1	-1	+1	+1	-1	+1	+1	+1	25.67±1.2	36.22±1.57
9	-1	+1	+1	+1	-1	-1	-1	+1	-1	+1	+1	26.49±1.34	28.35±0.94
10	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	25.38±1.18	29.09±1.18
11	-1	-1	+1	-1	+1	+1	-1	+1	+1	+1	-1	19.85±0.88	26.35±1.11
12	+1	-1	+1	+1	-1	+1	+1	+1	-1	-1	-1	16.15±0.75	23.38±1.17

both glycosyl hydrolases are highlighted in the Pareto charts (**Supplementary Fig. 1Aa & 1Ab**).

4.3. Central Composite Design (CCD)

Subsequent to the three-variable-five-level matrix CCD, the yield of *B. bassiana* SAN01 amylase and polygalacturonase was recorded (**Table 2**). The experimental responses for amylase activities were between 10.21 and 36.02 U.mL^{-1}. On the other hand, polygalacturonase production levels were recorded in a range of 19.34 and 52.63 U.mL^{-1}; it should be noted that the highest yields for both enzymes were recorded at the central point (run 2). The accuracy of the results was also further established by the Predicted versus Actual value plots (**Supplementary Fig. 2Aa & 2Ab**), which illustrated the close agreements between actual data and the predicted data.

The analysis of the response data signified that the generated quadratic models fit adequately with the two responses, thus ascertaining their reliability and adequacy. Furthermore, the F-values, 149.21 for amylase and 49.47 for polygalacturonase, as well as their respective low p-values (**Supplementary Table 4A**), all indicate the models' significance, hence, diminishing possibilities that the model F-values could have noise-generated (14). Also, obtained p-values of the two CCD models together with the corresponding values for the terms - A, B, C, AB, AC, A^2, B^2, C^2 - were recorded to be less than 0.05, thus connoting a remarkable level of reliability (**Supplementary Table 4A**). However, the p-value for BC in both models was noted to be insignificant.

Thus, the application of CCD of RSM yielded the following regression equations, which are the derived empirical relationships between the two enzyme production levels and the selected test parameters in coded units:

Amylase activity = $34.63 + 2.26A + 3.40B + 3.18C - 3.14AB + 1.83AC - 0.684BC - 5.34A^2 - 4.642B^2 - 4.48C^2$

Polygalacturonase activity = $50.73 + 1.49A + 4.19B + 4.30C - 4.96AB + 2.40AC - 1.48BC - 6.78A^2 - 6.11B^2 - 6.49C^2$

A is the incubation temperature; B is the pH while, C is the wheat bran concentration.

Furthermore, 3D and 2D plots derived from the quadratic equations of the amylase and polygalacturonase models elucidated the interrelationships between the selected three variables (**Fig. 1 and 2**). These plots denote the interactive effects of varying two factors while keeping the third factor constant at its optimum value. For amylase and polygalacturonase production, the variability of two components, *viz.,* wheat bran concentration vs pH, wheat bran concentration

Table 2. CCD matrix with observed and predicted values for *Beauveria bassiana* SAN01 amylase and polygalacturonase production

	Level			Amylase $(U.mL)^{-1}$*		Polygalacturonase $(U.mL)^{-1}$*	
	Coded (A)	Coded (B)	Coded (C)	Observed value	Predicted value	Observed value	Predicted value
1	-1	+1	+1	25.49±1.16	25.15	40.84±1.54	39.44
2	0	0	0	36.02±1.47	34.67	52.63±2.02	50.73
3	+1	+1	-1	18.33±0.83	18.39	25.26±1.31	26.82
4	-1	-1	-1	10.21±0.44	9.37	19.34±0.84	17.32
5	+1	-1	-1	17.05±0.88	16.51	24.92±0.97	25.42
6	-1	+1	-1	24.74±1.19	23.80	39.12±1.75	38.59
-1	-1	-1	+1	14.39±0.74	13.45	26.55±0.93	24.08
8	0	0	-1.68	15.72±0.67	16.63	25.27±1.03	25.12
9	0	-1.68	0	14.92±0.55	15.84	24.25±0.88	26.40
10	0	0	0	34.25±1.42	34.67	50.29±2.33	50.73
11	+1	-1	+1	27.85±1.27	27.91	42.16±1.67	41.79
12	0	0	0	34.90±1.58	34.67	51.17±2.02	50.73
13	0	0	0	35.04±1.62	34.67	49.13±1.74	50.73
14	0	+1.68	0	26.94±1.13	27.26	41.36±1.89	40.49
15	+1.68	0	0	23.52±1.14	23.36	36.16±1.71	34.05
16	+1	+1	+1	27.09±1.3	27.05	36.17±1.22	37.28
17	0	0	0	33.90±1.24	34.67	49.07±2.28	50.73
18	0	0	0	34.11±1.39	34.67	52.28±2.5	50.73
19	-1.68	0	0	14.36±0.33	15.75	25.67±1.11	29.05
20	0	0	+1.68	27.02±0.81	27.35	38.18±1.48	39.60

* Average of triplicate determinations

vs temperature, and pH vs temperature, all exhibited significant effects on the enzyme production levels (**Fig. 1 and 2**). Furthermore, distinct peaks were observed for both responses, thus demonstrating the fact that the two models highlighted the optimal region for the yield of both enzymes.

A rise in the yield of amylase and polygalacturonase was recorded with elevating pH and incubation temperature until the maximum yield of ~34 $U.mL^{-1}$ and 50 $U.mL^{-1}$ (**Fig. 1A and 2A**) were attained. Maximum production of these enzymes was observed at pH 6 and 30 °C. A steep increase in the production of both enzymes was observed with an increase in substrate concentration until the optimum concentration of ~40 $g.L^{-1}$, after which a decline was observed (**Fig. 1C and 2C**). Increasing the amount of wheat bran beyond the optimum level had less significant effects on the production level of both *B. bassiana* carbohydrases. Furthermore, the interaction between the pH of the media and the incubation temperature (**Fig. 1A and 2A**) had the most significant effect on amylase and polygalacturonase production, while wheat bran- pH interaction (**Fig. 1E and 2E**) had the least effect. The magnitude, as well as the respective positive and negative effects of the parameters on the production of the individual enzymes were also deduced from the coefficient estimate analysis (**Supplementary Tables 5A and 6A**). Positive linear effects were observed for all three optimised parameters with regard to amylase and polygalacturonase production. However, while pH with a coefficient estimates of 3.40, had the highest impact on amylase production, wheat bran concentration, with an estimate of 4.30, had the most significant effect on polygalacturonase production.

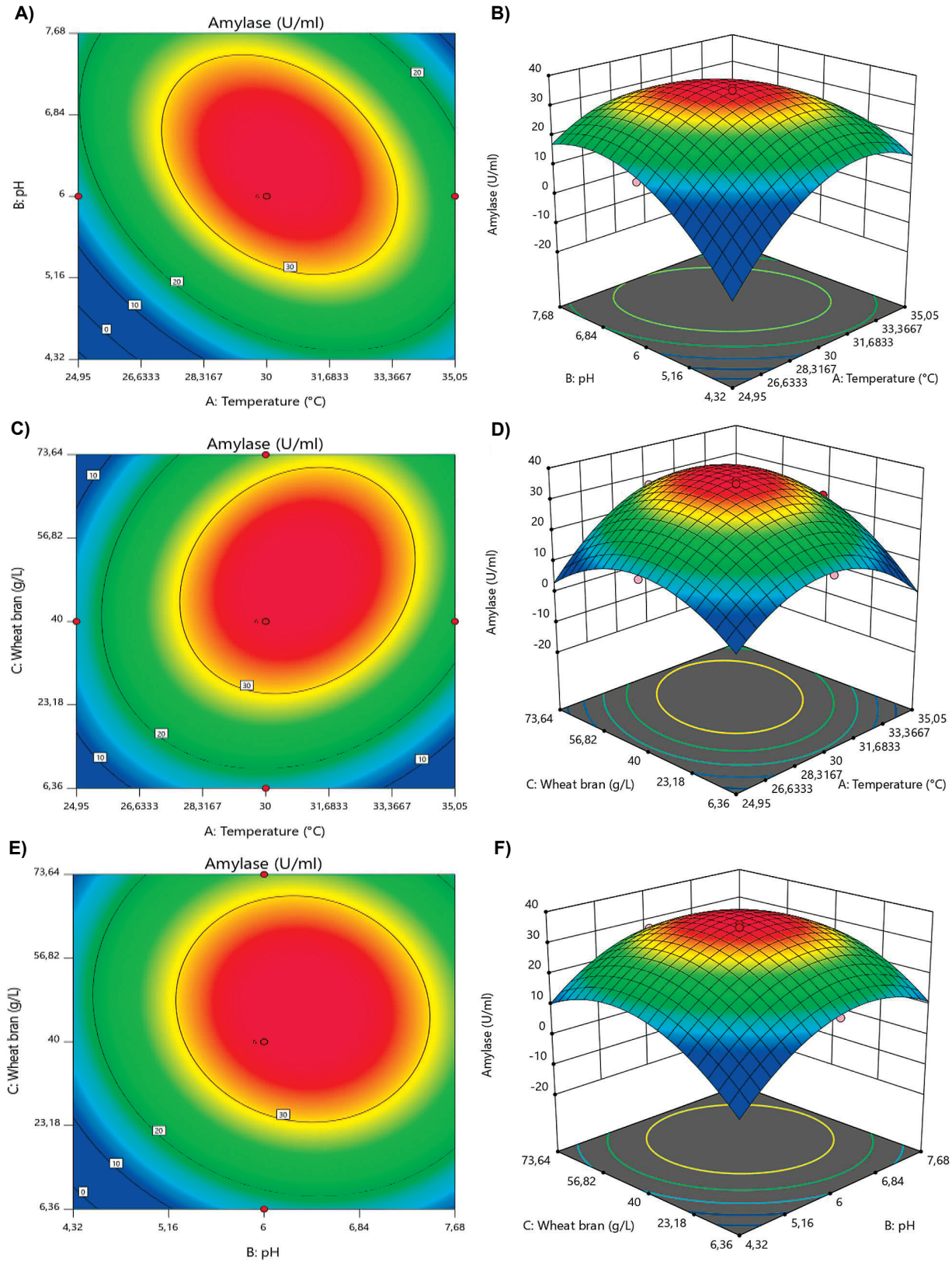

Figure 1. Response surface prediction for Beauveria bassiana SAN01 amylase production: contour **A)** & 3D plots **B)** of combined pH-temperature effect; contour **C)** and 3D plots **D)** of combined wheat bran-temperature effect; contour **E)** and 3D plots **F)** of combined wheat bran-pH interaction.

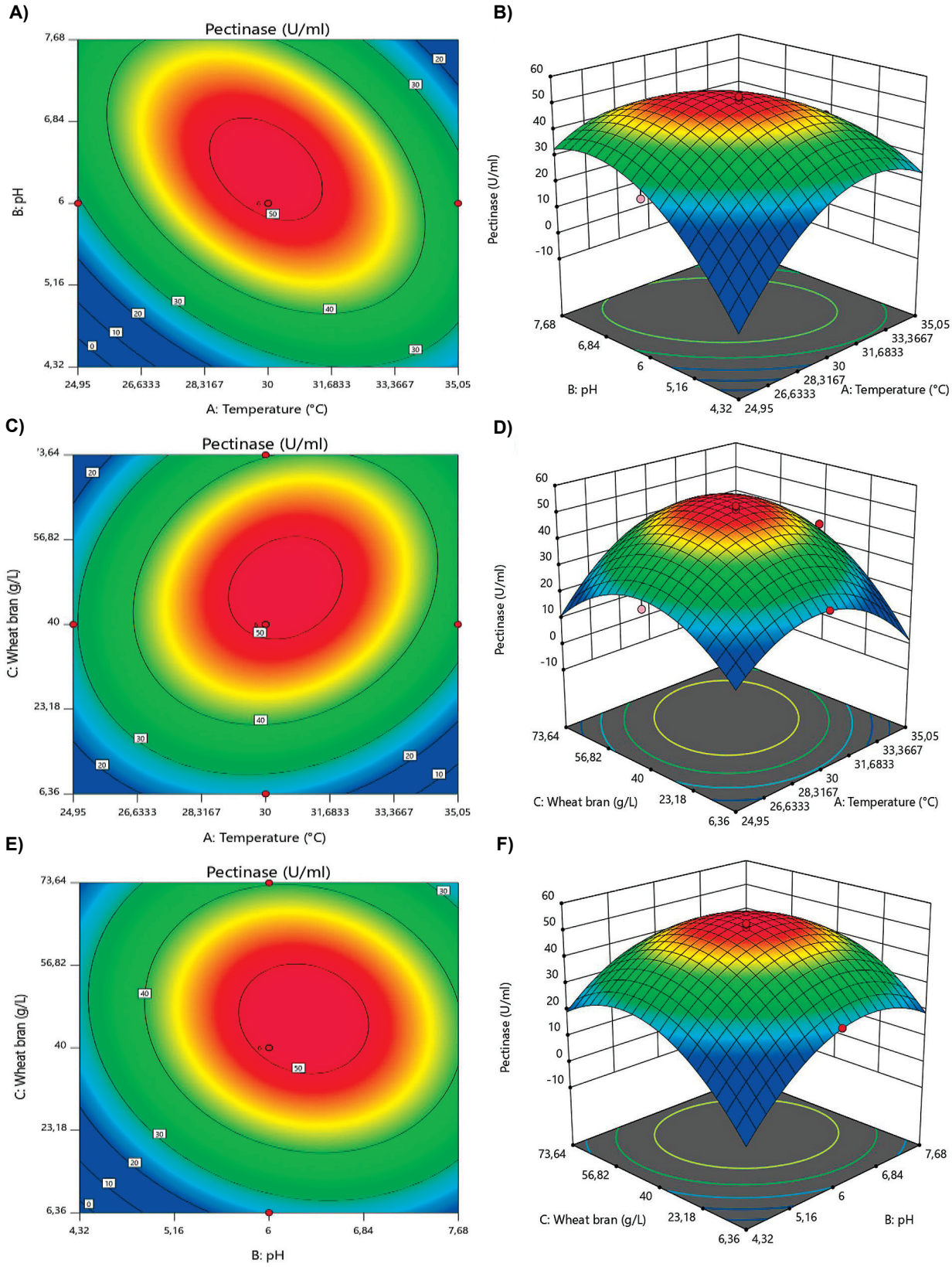

Figure 2. Response surface prediction for Beauveria bassiana SAN01 polygalacturonase production: contour **A)** & 3D plots **B)** of combined pH-temperature effect; contour **C)** and 3D plots **D)** of combined wheat bran- temperature effect; contour **E)** and 3D plots **F)** of combined wheat bran-pH effect.

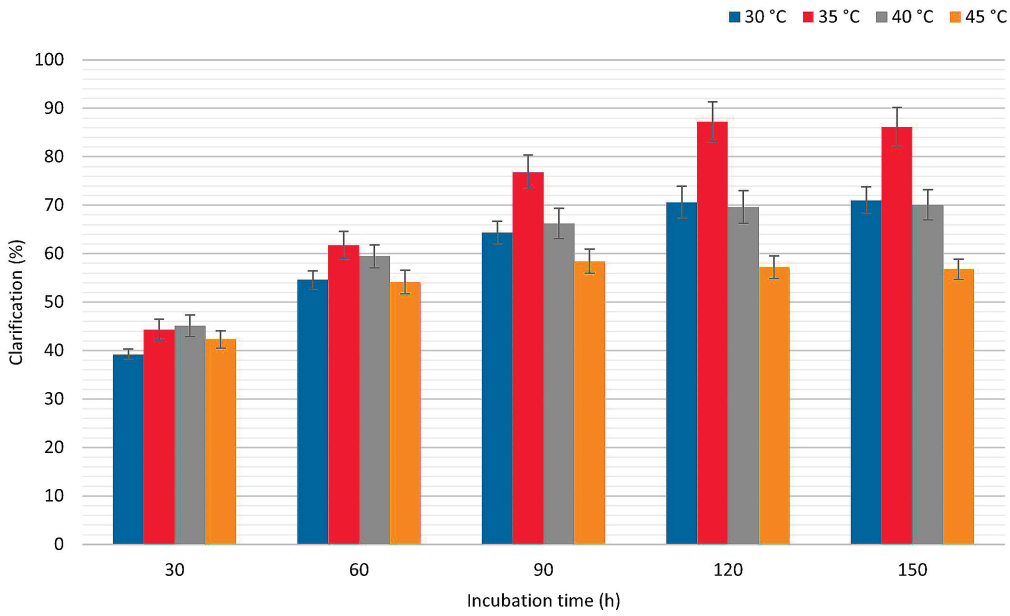

Figure 3. ***Beauveria bassiana*** **SAN01 amylase-polygalacturonase clarification of pineapple juice**

4.4. Model Validation

The optimum parameters predicted for the production of both *B. bassiana* SAN01 amylase and polygalacturonase were pH 6.28, wheat bran concentration of 47.2 $g.L^{-1}$ and incubation temperature of 30.42 °C. The observed experimental values for amylase and polygalacturonase production during the validation experiments were 34.82 ± 1.44 $U.mL^{-1}$ and 51.05 ± 1.92 $U.mL^{-1}$, which are both in close agreement (at 95% PI) with the model-predicted values of 35.87 $U.mL^{-1}$ (amylase) and 51.98 $U.mL^{-1}$ (polygalacturonase). Thus, 1.79- and 1.87-fold increases were finally recorded in amylase and polygalacturonase production relative to unoptimised enzyme activities of 19.48 $U.mL^{-1}$ and 27.37 $U.mL^{-1}$, respectively.

4.5. Clarification of Pineapple Juice

Subsequent to the partial purification of the crude amylase and polygalacturonase, a clear solution with an activity of ~ 40 U amylase and ~ 60 U polygalacturonase was obtained. The *B. bassiana* amylase-polygalacturonase assisted clarification of pineapple juice resulted in significant clarity of the juice sample, with the highest clarification of ~ 90% recorded at 35 °C after 120 min incubation (**Fig. 3**). However, the enzymes were least efficient at 45 °C. Furthermore, prolonged incubation at the optimum temperature had no significant effect on the clarification process, which might have resulted from the diminished thermostability of the enzyme cocktail beyond 2 h. A significant activity of the *Beauveria* cocktail in clarifying pineapple juice was also observed at 30 and 40 °C. An increase in reducing sugar was recorded after enzyme clarification; however, the pH and TSS of the control (untreated juice) and the enzymatically treated juices showed no statistically significant difference (**Supplementary Table 7A**).

5. Discussion

In this study, the concomitant production of amylase and polygalacturonase enzymes was demonstrated for the first time in *B. bassiana* SAN01, using readily available agricultural residues. Previous studies have shown the efficient utilisation of wheat bran by other *Beauveria* strains in the industrial production of various bioproducts, including oosporein (15) and enzymes such as asparaginase (16), endoglucanase and xylanase (1). The notable preference of *B. bassiana* and other fungi for this substrate could be linked to its nutritionally balanced composition, which is sufficient to maintain the growth of microbes and support their other metabolic activities. The proximate analysis of wheat bran has shown the presence of complex sugars including arabinoxylans, cellulose,

pectin, starch and xylan, and the required amount of protein to provide nitrogen to the microbes (17, 18). Recently, significant amylase production of 18. 6 $U.mL^{-1}$ and 36 $U.mL^{-1}$ were recorded with *Aspergillus oryzae* (19) and *Bacillus* sp. DLB, respectively (20). Polygalacturonase levels of 1.12 $U.mL^{-1}$ in *A. oryzae* (21) and 800.0 ± 16.2 $U.g^{-1}$ in *Bacillus subtilis* (22) were also recorded while utilising wheat bran. In contrast, the production of both enzymes was not detected with sugarcane bagasse and wheat straw. The presence of endogenous inhibitors in wheat straw and sugarcane bagasse and their low carbon/nitrogen ratio might be responsible for the failure of these agro residues to support significant production of the enzymes (23-25). Previous studies have overcome these impediments by pretreating the biomass through different methods and by supplementing them with additional nitrogen sources (26, 27). Unlike in this study, where lignocellulosic biomass served as the nutrient source, Ryali and Shankar (10), described *B. bassiana* amylase production using pure starch while pure citrus pectin was utilised by Fernandes and Valério (28), for *B. bassiana* polygalacturonase production. Furthermore, it is remarkable that the production levels of amylase and polygalacturonase observed from *B. bassiana* SAN01 are the highest ever recorded.

Subsequently, PBD showed that the production of both amylase and polygalacturonase from *B. bassiana* SAN01 are significantly influenced by the same parameters, which are the incubation temperature, wheat bran concentration as well as the pH of the fermentation media. Recent optimisation studies on different carbohydrases also identified the same parameters as the most critical in the production of the enzymes (29, 30). Further optimisation of the selected variables using the CCD identified the optimal conditions for the simultaneous production of the *B. bassiana* glycosyl hydrolases. In addition, both the reliability and adequacy of the generated quadratic models were ascertained by fitting them into their respective responses. As observed in the CCD plots, the more the shape of a contour plot tends to that of an ellipse, the higher the interrelationships between the variables. In contrast, the more the shape tends to that of a circle, the lower the interrelationships between the variables (31). The optimal production temperature of 30 °C and pH of 6.0 extrapolated from the CCD plots were observed to be the optimum physiological conditions for *B. bassiana* growth and metabolism (32). However, the effects of these variables on the yield of the *B. bassiana* amylase and polygalacturonase were noticed to diminish beyond these optimum values, which is probably due to the decreased metabolic rate of the fungus at higher pH and temperature as well as the denaturing effects of these conditions on the enzymes. This is in line with the fact that enzymes become denatured at higher temperatures and pH values, leading to their inactivity (33).

It has since been noted that the formation of an unstable cloud, which arises from the presence of different carbohydrate polymers in pineapple juice, reduces the acceptability of the juice to consumers, hence the need for juice clarification in the food industry (34). In this study, the *B. bassiana* amylase-polygalacturonase enzyme mixture was observed to effectively clarify the pineapple juice. The optimum temperature range (30-40 °C) for juice clarification by the *B. bassiana* enzyme mixture is close to the physiological temperature range of *B. bassiana* (32). Previously, the clarification of pineapple juice with some fungal polygalacturonases was most efficient at 40 °C (34, 35). However, many recent studies using fungal depolymerases, including amylase and polygalacturonase have shown 35 °C as the optimum temperature for the clarification of various fruit juices (36, 37). Furthermore, the higher concentrations of reducing sugars observed in the enzyme-clarified juice samples may be due to the depolymerising effect of the enzymes on the carbohydrate polymers, *viz*., pectin and starch, in the pineapple juice. This might be considered an added advantage of the enzyme-assisted clarification of pineapple juice, as the production of the reducing sugars will reduce the need for the addition of artificial sweeteners during further processing. As observed in this research, previous studies have also shown that enzymatic treatment of different fruit juices had no significant effect on the pH as well as on the total dissolved solids (38, 39).

6. Conclusion

The applicability of a widely known endophytic fungal entomopathogen, *B. bassiana*, in industrial biocatalysis was demonstrated in this work, as against its widespread use as a biopesticide. Different readily available agricultural residues were used as substrates for amylase and polygalacturonase production from *B. bassiana* SAN01, with wheat bran being the most promising. RSM was used effectively to establish

the optimum parameters to produce amylase and polygalacturonase from the *B. bassiana* strain. The optimised production levels were 1.79- (amylase) and 1.87- (polygalacturonase) folds higher than the initial set of unoptimised experiments. These data are significant as we recorded the highest secretion levels for both enzymes from *B. bassiana,* judging from the available literature. Furthermore, the potential of the enzyme cocktail in clarifying pineapple juice showed significant results. However, to enhance their applications in industrial processes requiring higher temperatures, the thermostability of the two enzymes can be improved via protein engineering strategies such as rational design and directed evolution.

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