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Enhanced bioconversion of potato byproducts into natural blue pigments by *Streptomyces lydicus* PM7: bioprocess optimization and biochemical insights

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Abstract

Background Artificial colorants raise health and environmental concerns, creating demand for sustainable natural alternatives. Blue pigments are particularly scarce due to their structural complexity and instability, with actinorhodin standing out among *Streptomyces* metabolites. A major challenge for actinorhodin production is to improve yields and reduce costs to enhance process feasibility. Discarded potato, an abundant and underutilized agricultural byproduct, is a nutrient-rich, low-cost substrate for microbial processes. Recently, a *Streptomyces lydicus* strain was reported to convert this byproduct into actinorhodin, but with relatively low production compared to traditional media and other *Streptomyces* species. This study aimed to optimize the conversion of discarded potato into actinorhodin-related blue pigments by *S. lydicus* PM7 and to evaluate biochemical responses that influence pigment production.

Results A Plackett–Burman design identified temperature, agitation, pH, and KH_2PO_4 supplementation as significant factors among 11 tested variables. Optimization using a central composite face-centered design (CCD) within the framework of response surface methodology (RSM) increased pigment production up to 8000 mg L^{-1}. Model validation using point prediction identified optimal conditions of 30 °C, 180 rpm, an initial pH of 9, and 0.15 g L^{-1} KH_2PO_4. Growth kinetics under optimized conditions revealed two exponential phases and shifts in α-glucosidase and α-amylase activities, indicating a possible sequential use of carbohydrates. Catalase activity coincided with the onset of exponential growth and pigment production.

Conclusions The optimized process yielded an 8.5-fold increase in pigment production, supporting the use of potato byproducts as an effective and low-cost fermentation substrate. The biochemical responses of *S. lydicus* PM7 provide initial insight into metabolic features associated with pigment formation. Overall, the findings establish a laboratory-scale proof of concept and a basis for future bioreactor-scale and application-oriented studies on microbial blue pigments by *Streptomyces* spp.

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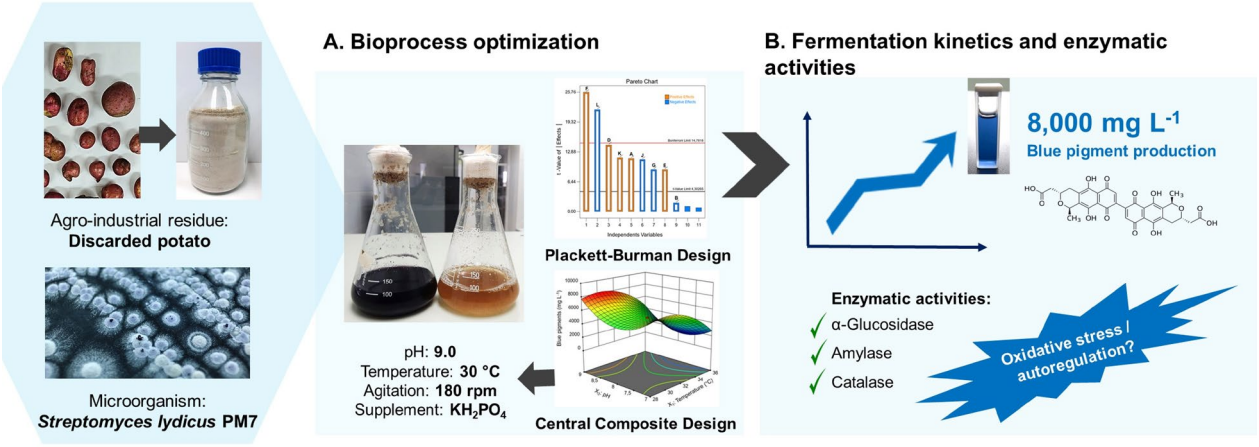
Keywords Actinomycete, Actinorhodin, Agricultural byproduct, Potato waste, *Streptomyces* pigment, Submerged fermentation

Graphical abstract



Background

Agroindustrial waste increases by approximately 1,300 million tons yearly, representing nearly one-third of global agricultural production [1]. Among them, potato waste is particularly significant due to the high global output of this crop and the high proportion of potato tubers discarded during harvesting and storage, estimated at approximately 30% of total production [2]. Globally, potato production was estimated at 383 million tonnes in 2023, with China (93.4 million tonnes) and India (60.1 million tonnes) being the top producers [3]. This volume presents an opportunity to enhance the profitability of local production systems, diversify agricultural outputs, and support regional economic development. Given their high nutrient content [4], discarded potatoes have been used as animal feed byproducts or discarded to prevent rapid spoilage and contamination with plant pathogens in the field. However, the high carbohydrate, protein, and mineral content of potato tubers opens opportunities to convert them into multinutrient substrates for microbial production of pigments and other valuable biocompounds [5]. The high production cost of microbial products is primarily attributed to the culture media, which can account for 38–73% of total production costs [6]. Thus, replacing conventional culture media with discarded potatoes [7] offers a viable alternative to reduce the operational costs of high-value microbial products and valorize this underutilized potato byproduct.

The *Streptomyces* genus consists of Gram-positive, filamentous, spore-forming bacteria with a high guanine-cytosine content (around 70%), classified under the Actinomycetota phylum [8], formerly classified as actinobacteria in earlier taxonomic schemes [9, 10]. This group of bacteria is well known for its versatile metabolism and ability to produce a wide range of secondary metabolites under various culture conditions, including antibiotics, enzymes, biosurfactants, and pigments with diverse biological activities [9, 11]. Several strains have been studied for their capacity to produce natural pigments such as undecylprodigiosin (red), coelimycin P1 (yellow), actinorhodin (blue), and pyomelanin (brown/black), as well as carotenoids like leprotene, β-isorenieratene, and lycopene [12–17]. The demand for natural pigments has risen across industries, as safer, health-promoting alternatives to synthetic pigments [18]. Natural blue pigments are especially sought after by the cosmetic, pharmaceutical, and food sectors, primarily due to concerns over the safety of synthetic blue dyes. For example, chronic exposure to the synthetic blue colorant E133 in animal models has shown potential effects on immune and hematological parameters [19].

The search for new sources of blue pigments is therefore a priority. Phycocyanin extracted from *Spirulina*, for example, reached a global market of $155.3 million in 2020, with projections to grow to $409.8 million by 2030 [20]. According to Transparency Market Research [21], the global market for natural (representing 11% of the total) and synthetic (71%) pigments was valued at $33 billion in 2021 and is estimated to reach $59 billion by 2035. In parallel, regulatory trends are accelerating this demand: in April 2025, the U.S. Food and Drug Administration (FDA) announced the progressive elimination of petroleum-based synthetic dyes from the food supply, including FD&C Blue No. 1 and Blue No. 2, with a complete ban scheduled by the end of 2026 [22]. These

measures will have global repercussions as multinational companies adapt their formulations to comply with U.S. standards. Together, these market and regulatory drivers indicate a strong economic and strategic potential for incorporating a natural, safe, and stable blue pigment into the industry to meet the growing demand for functional, sustainable, and health-oriented ingredients.

Given this context, there is a clear need to explore novel microbial sources of stable blue pigments through optimized fermentation processes and genetic regulation studies. In this way, the economic feasibility of natural pigment extraction relies on using raw materials with a stable commercial supply [23]. Some agroindustrial wastes have been successfully explored as low-cost substrates for pigment production using *Streptomyces* strains. For example, the use of sugarcane waste for melanin synthesis [24] and discarded potato to produce reddish-purple pigment [5] and actinorhodin [5, 25] has been reported. Actinorhodin, a pH-sensitive polyketide with red-to-blue color variation, is typically produced by *Streptomyces coelicolor* [26–29]. However, recent studies have also identified *Streptomyces lydicus* PM7 as a novel and promising producer of actinorhodin [25].

In this context, statistical tools such as Plackett–Burman Design (PBD) and Central Composite Design (CCD) are valuable for screening influential variables and optimizing culture conditions. The PBD is a statistical method for identifying significant factors influencing a response variable with a minimal number of experimental runs [30]. Therefore, PBD enables the simultaneous evaluation of multiple factors, while CCD refines the working ranges of selected variables [31–33]. Moreover, understanding microbial growth kinetics is crucial for enhancing the yield of secondary metabolites and improving process efficiency and economic benefits [34].

Recent studies reported that *S. lydicus* PM7 can produce blue pigments when cultivated with discarded potato under standard *Streptomyces* growth conditions, reaching 0.87 g L^{-1} of actinorhodin-related blue pigments [25]. However, these yields remain relatively low and require further optimization to support future scale-up efforts. Therefore, the aim of this study was to optimize the conversion of discarded potato into actinorhodin-related blue pigments by *S. lydicus* PM7 and to evaluate biochemical responses that influence pigment production.

Methods

Materials

All reagents and chemicals used for extractions and preparation of traditional culture media were purchased from Sigma-Aldrich/Merck (Darmstadt, Germany).

Discarded potato: collection and processing

Discarded potatoes (var. Puyehue) were collected from a local farm in the La Araucanía region, Chile. The samples were processed as described by Schalchli et al. [5]. Briefly, the tubers were washed, manually peeled, and cut into small pieces of approximately 1 cm^3. Then, potato pieces were dried at 60 °C until they reached a constant weight and subsequently ground to obtain a fine powder.

Streptomyces strain and inoculum preparation

The *S. lydicus* strain PM7 was obtained from the strain collection at the Laboratory of Biotechnology, Universidad de La Frontera (Chile). The strain was originally isolated from soil samples and deposited in the Chilean Collection of Microbial Genetic Resources (CChRGM) under the number RGM 3663. The strain was maintained on International *Streptomyces* Project N°2 medium (ISP2) agar slants for assays and stored at 4 °C.

Spore suspensions were prepared from fresh cultures of *S. lydicus* PM7. Spores were harvested from 7-day ISP2 agar plates by flooding each plate with 10 mL of 0.9% NaCl and gently scraping the surface with a sterile loop; the resulting suspension was enumerated using a Neubauer chamber. Appropriate three-fold dilutions were prepared in 0.9% NaCl to obtain countable. Based on these counts, the suspension was adjusted to 1.0×10^4 spores mL^{-1} by dilution with sterile medium or saline. The inoculum preparation was conducted in 250 mL Erlenmeyer flasks containing 100 mL of ISP2, which consists of the following per liter of distilled water: 10 g malt extract, 4 g yeast extract, and 4 g glucose, with the pH adjusted to 7.0. After autoclave sterilization, the ISP2 medium was inoculated with a final spore concentration of 1.0×10^4 spores mL^{-1} and incubated in the dark at 28 °C with agitation at 120 rpm for 72 h.

Experimental design for process optimization

Each experimental unit consisted of a 250 mL Erlenmeyer flask, using a culture medium prepared with the processed discarded potatoes (powder) as the basal nutrient source. The working volume and composition of the medium varied according to the conditions established in the experimental designs described later. The inoculum, prepared in ISP2 broth, was added at different concentrations depending on the experimental setup. When specified by the experimental conditions, the discarded potato medium was supplemented with additional components such as antifoam, soluble starch, yeast extract, or KH_2PO_4, to evaluate their influence on blue pigment production by *S. lydicus* PM7. All assays were conducted in triplicate and incubated in the dark under controlled temperature and agitation conditions. Samples were collected at specific intervals and processed for subsequent analyses under a destructive mode of operation.

Table 1 Independent variables and their levels used in the Plackett–Burman design for blue pigment production by *Streptomyces lydicus* PM7

Variable code	Variables	Levels	
		Low (– 1)	High (+ 1)
A	Residue concentration (g L^{-1})	4	32
B	Inoculum size (%v v^{-1})	4	8
C	Incubation time (days)	6	10
D	pH	6	8
E	Temperature (°C)	20	30
F	Agitation (rpm)	50	150
G	Working volume (mL in 250 mL $flask^{-1}$)	50	100
H	Antifoam (%v v^{-1})	0	1.0
J	Starch (g L^{-1})	0	4.0
K	KH_2PO_4 (g L^{-1})	0	0.1
L	Yeast extract (g L^{-1})	0	4.0

Screening of significant variables using Plackett–Burman design (PBD)

The PBD was used to identify the most influential medium components and process conditions affecting the production of blue pigments by *S. lydicus* PM7. Twelve experimental runs were conducted to evaluate the effects of eleven independent variables based on literature. Each variable was tested at low (– 1) and high (+ 1) levels. The response variable was the blue pigment concentration in mg L^{-1}, estimated from the absorbance at 640 nm using the calibration curve (Eq. 1). The factors evaluated were discarded potato concentration (A), inoculum size (B), incubation time (C), initial pH (D), temperature (E), agitation (F), working volume in 250 mL flasks (G), antifoam addition (H), starch (J), KH_2PO_4 (K), and yeast extract supplementation (L), as summarized in Table 1.

The PBD model was based on the first-order polynomial Eq. (1):

$$Y1 = \beta_0 + \sum \beta_i X_i \quad (1)$$

Where, Y_1 denotes the predicted *Streptomyces* blue pigments in mg L^{-1}, β_0 is the model intercept, β_i are the linear coefficients, and X_i represent the coded levels of the independent variables. Each design point was performed in triplicate ($n = 3$ independent flasks).

Optimization using central composite design (CCD)

Based on the PBD results, a central composite design (face-centered, $\alpha = 1$) was implemented to optimize the most significant variables positively affecting blue pigment production by *S. lydicus* PM7. The four key factors, temperature (X_1), agitation (X_2), pH (X_3), and KH_2PO_4 concentration (X_4) were selected for further evaluation.

Table 2 Independent variables and levels selected for optimization by central composite design (CCD)

Code	Factors	Low level (– 1)	Medium (0)	High level (+ 1)
X_1	Temperature (°C)	28	32	36
X_2	Agitation (rpm)	120	150	180
X_3	pH	7	8	9
X_4	KH_2PO_4 (g L^{-1})	0.10	0.15	0.20

Each variable was tested at three levels (– 1, 0, + 1), as shown in Table 2. The experimental data were analyzed using response surface methodology (RSM) to predict the optimal conditions. Each design point was performed in triplicate ($n = 3$ independent flasks).

Kinetic fermentation assay

The kinetic fermentation assay was conducted under the optimized conditions established by the CCD-RSM. Therefore, each experimental unit consisted of a 250 mL Erlenmeyer flask containing 50 mL of discarded potato medium at a concentration of 16 g L^{-1}. The medium was supplemented with KH_2PO_4 at 0.15 g L^{-1}, adjusted to an initial pH of 9 before sterilization, and inoculated with an 8% v v^{-1} inoculum of *S. lydicus* PM7 prepared in ISP2 medium. Incubation was performed in the dark at 30 °C with orbital agitation at 180 rpm for 8 days. All experiments were conducted in triplicate under a destructive sampling mode. Samples were collected every 12 h to evaluate blue pigment production, bacterial growth, reducing sugar concentration, enzymatic activities (α-glucosidase, amylase, catalase) and pH.

Analytical methods

Estimation of extracellular blue pigment production

The production of extracellular blue pigments by *S. lydicus* PM7 was estimated from the cell-free supernatant (CFS) by spectrophotometry, as described by Bystrykh et al. [26] with modification. After incubation, the cultures were centrifuged at 6,000 x g 10 min at 4 °C to separate the biomass. The resulting supernatant was filtered through a 0.22 µm membrane to obtain the cell-free supernatant (CFS). An aliquot (0.5 mL) of this CFS was adjusted to pH 12 using 0.5 M KCl/NaOH buffer. Absorbance was measured at 640 nm using a spectrophotometer (Orion AquaMate 8000, Thermo Scientific). Samples were diluted as necessary to ensure absorbance values remained within the range of 0.1–0.9. The pigment concentration was determined based on a calibration curve according to Eq. (2):

$$Y2 = 0.0024x + 0.0032 \cdot 0020 \quad (2)$$

Where Y_2 denotes the absorbance at 640 nm and x the pigment concentration in mg L^{-1}. The calibration curve was constructed using a semi-purified fraction of blue pigments produced by *S. lydicus* PM7. For this, CFS was lyophilized and fractionated on a chromatography column (10 × 2.5 cm) packed with LiChroprep RP-18 (5 g; 15–25 µm). An elution was performed with 50 mL of solvents of increasing polarity: EtOH, EtOH/H_2O (1:1), and H_2O; the major fraction eluted with EtOH/H_2O (1:1, v/v). This fraction was concentrated to dryness to obtain a semi-purified extract of blue pigments. The extract (fraction F.2) was dissolved using 0.5 M KCl/NaOH buffer pH 12 at 10–300 ppm (mg L^{-1}) to construct the calibration curve. Known pigment masses were correlated with their corresponding absorbance values at 640 nm, and the resulting curve was used to quantify blue pigment production in CFS.

Bacterial growth by colony-forming units (CFU)

Bacterial growth was estimated as colony-forming units (CFU). One milliliter of the culture was serially diluted (10^{-1} to 10^{-5}) in sterile 0.9% NaCl. From each dilution, 250 µL aliquots were spread on ISP2 agar plates and incubated at 28 °C for 48 h. Colonies were counted on plates with 30–200 colonies and converted to CFU mL^{-1} [35].

Reducing sugar concentration

The reducing sugar was determined using the dinitrosalicylic acid (DNS) method [36], with glucose as standard.

Enzymatic activities

α-Glucosidase activity. An aliquot of 500 µL of CFS was mixed with 500 µL of potassium phosphate buffer (100 mM, pH 7.0) and 500 µL of maltose solution (60 mM) as the substrate. The reaction mixture was incubated at 37 °C for 40 min. Glucose released during the reaction was determined using the DNS method [36]. As a control, a reaction mixture containing 1000 µL of 100 mM potassium phosphate buffer (pH 7.0) and 500 µL of maltose was prepared under the same conditions. One unit of α-glucosidase activity was defined as the amount of enzyme that releases one µmol of glucose per reaction under the assay conditions.

Amylase activity. An aliquot of 500 µL of CFS was mixed with 500 µL of potassium phosphate buffer (100 mM, pH 7.0) and 500 µL of 1% soluble starch solution as substrate. The mixture was incubated at 37 °C for 40 min. The amount of reducing sugars generated was measured using the DNS method [36]. The control consisted of 1000 µL of buffer and 500 µL of 1% starch solution. One unit of amylase activity was defined as the amount of enzyme that releases one µmol of reducing sugars per reaction under the specified conditions.

Catalase activity. Catalase activity was determined as described by Iwase et al. [37], with modifications. A reaction mixture consisting of 100 µL of sample, 100 µL of 1% Triton X-100, and 100 µL of 30% hydrogen peroxide was prepared in a borosilicate glass tube (13 mm diameter × 200 mm height). The reaction proceeded at room temperature, and the height of the O_2-forming foam, representing oxygen release, was measured after 15 min. The catalase activity was expressed as foam height (mm), indicative of oxygen evolution.

pH monitoring

The pH was directly measured in the CFS using a calibrated pH meter.

Statistical analysis

The experimental designs and statistical analyses were conducted using Design-Expert software version 23.1.8 (Stat-Ease Inc., Minneapolis, MN, USA). Model adequacy was assessed from diagnostic plots of externally studentized residuals: normal Q–Q (normality), residuals vs. fitted (homoscedasticity), and residuals vs. run order (independence). Residual normality was also formally evaluated using the Shapiro–Wilk test applied to the externally studentized residuals ($\alpha = 0.05$) for both the PBD and the RSM/CCD models. Statistical significance was set at $\alpha = 0.05$ (two-sided); model terms with $p < 0.05$ were considered significant.

Results

Screening of main factors affecting the production of *Streptomyces* blue pigments

The PBD was used to determine the most significant variables affecting blue pigment production by *S. lydicus* PM7. The experimental design matrix and the corresponding responses are shown in Table 3.

Pigment production ranged widely from 58 to 5,416 mg L^{-1}, reflecting the importance of medium optimization for enhanced biosynthesis. Among the eleven factors tested, as summarized in Table 4, statistical analysis revealed that inoculum size (B), incubation period (C) and antifoam addition (H) had minimal contributions (0.18%, 0.07% and 0.04%, respectively) and were therefore considered statistically insignificant.

Analysis of variance (ANOVA) (Table S1) indicated that the model was statistically significant ($p = 0.0010$). The model exhibited a high coefficient of determination ($R^2 = 0.9971$) and an adjusted R^2 of 0.9895, indicating the model's adequacy. Residual diagnostics on externally studentized residuals supported model assumptions: the normal Q–Q plot was approximately linear and the Shapiro–Wilk test indicated no departure from normality ($W = 0.92$, $p = 0.434$). Residuals vs. fitted showed no

Table 3 Twelve-trial Plackett-Burman design for evaluation of eleven independent variables used for *Streptomyces* blue pigments production

Run	Coded values of the independent factors											Blue pigments (mg L^{-1})		Residual
	A	B	C	D	E	F	G	H	J	K	L	Actual value	Predicted value	
1	− 1	1	− 1	1	1	-1	1	1	1	-1	-1	101	90	11
2	-1	-1	− 1	1	− 1	1	1	− 1	1	1	1	788	713	75
3	1	1	1	− 1	− 1	− 1	1	− 1	1	1	− 1	58	70	− 11
4	− 1	1	1	− 1	1	1	1	− 1	− 1	− 1	1	128	140	− 11
5	1	− 1	1	1	− 1	1	1	1	− 1	− 1	− 1	3,151	3,226	-75
6	1	1	− 1	1	1	1	− 1	− 1	− 1	1	− 1	5,416	5,340	75
7	1	− 1	− 1	− 1	1	− 1	1	1	− 1	1	1	92	81	11
8	− 1	− 1	1	− 1	1	1	− 1	1	1	1	− 1	2,594	2,669	-75
9	1	1	− 1	− 1	− 1	1	− 1	1	1	− 1	1	168	157	11
10	− 1	− 1	− 1	− 1	− 1	− 1	− 1	− 1	− 1	− 1	− 1	73	-2	75
11	1	− 1	1	1	1	− 1	− 1	− 1	1	− 1	1	108	119	− 11
12	− 1	1	1	1	− 1	− 1	− 1	1	− 1	1	1	82	157	− 75
Level	g L^{-1}	%v v^{-1}	days	pH	°C	rpm	mL	%v v^{-1}	g L^{-1}	g L^{-1}	g L^{-1}			
− 1	4	4	6	6	20	50	50	0	0	0	0			
1	32	8	10	8	30	150	10	1	4	0.1	4			

Independent variables: A (Residue concentration, discarded potato powder); B (Inoculum size); C (Incubation time); D (pH); E (Temperature); F (Agitation); G (Working volume (mL 250 mL flask^{-1}); H (Antifoam); J (Starch); K (KH_2PO_4); L (Yeast extract)

funneling and residuals vs. run order showed no trend (Fig. S1).

According to Table 4, positive effects were observed for discarded potato concentration, pH, temperature, agitation, and KH_2PO_4, while inoculum size, incubation time, working volume, antifoam, starch, and yeast extract had a negative influence.

The Pareto chart (Fig. 1) highlights the relative importance of each factor; factors with negative effect and bars below the Bonferroni limit were treated as non-significant and were not carried forward to the RSM (inoculum size, incubation time, and antifoam addition).

Effect of discarded potato concentration

The effect of different concentrations of discarded potato on pigment production was evaluated considering the results from the PBD screening. These results confirmed that potato concentration significantly influences pigment yield. The highest blue pigment production was observed at 16 and 32 g L^{-1}, with 4,563 ± 324 mg L^{-1} and 4,749 ± 607 mg L^{-1}, respectively (Fig. 2). In contrast, the lowest quantity (1,814 ± 99 mg L^{-1}) was obtained at 4 g L^{-1}. In all the tested concentrations (4, 8, 16, and 32 g L^{-1}), the blue pigment production began from day 3 of incubation.

Optimization of *Streptomyces* blue pigments production by Central Composite Design (CCD)

The optimization of blue pigment production by *Streptomyces* was carried out using a CCD, based on four variables previously identified as influential through the PBD. The independent variables temperature (X_1), agitation (X_2), pH (X_3), and KH_2PO_4 concentration (X_4) were selected due to their positive contribution to pigment yield. Variables that showed a negative effect in the PBD were excluded from the optimization or fixed at their minimum levels to reduce their potential inhibitory effect. Each selected variable was evaluated at three levels: low, middle, and high. Table 5 shows the experimental design matrix and the actual and predicted pigment production values for each run.

The highest pigment yields were obtained in runs 9 and 27, reaching 8010 mg L^{-1} and 7,864 mg L^{-1}, respectively. In contrast, the lowest production was recorded in run 22, with 1732 mg L^{-1} of blue pigment. The data were analyzed through analysis of variance (ANOVA) to develop a quadratic response surface model (Table 6).

A second-order polynomial regression equation was generated to describe the relationship between the response (Y) and the independent variables (Eq. 3):

Table 4 Plackett–Burman screening of blue pigment production in *S. lydicus* PM7: main-effect estimates

Source		Coefficient Estimate	Standardized effect	% Contribution	F-value	*p*-value
Model		1063.28	–	–	130.54	0.0010
A-Residue concentration		435.42	870.834	6.87	71.93	0.0034
D-pH		544.24	1088.47	10.73	112.37	0.0018
E-Temperature		343.12	686.249	4.26	44.67	0.0068
F-Agitation		977.5	1955	34.61	362.51	0.0003
G-Working volume in 250 mL flask		– 343.54	– 687.083	4.28	44.78	0.0068
J-Starch		– 427.01	– 854.028	6.61	69.18	0.0036
K-KH2PO4		441.67	883.333	7.07	74.01	0.0033
L-Yeast extract		– 835.56	– 1671.11	25.3	264.87	0.0005
Mean	1,063.28	R^2	0.9971			
Std. Dev.	177.85	Adjusted R^2	0.9895			
C.V. %	16.73	Predicted R^2	0.9542			
PRESS	1.5.E + 09	Adeq Precision	35.605			

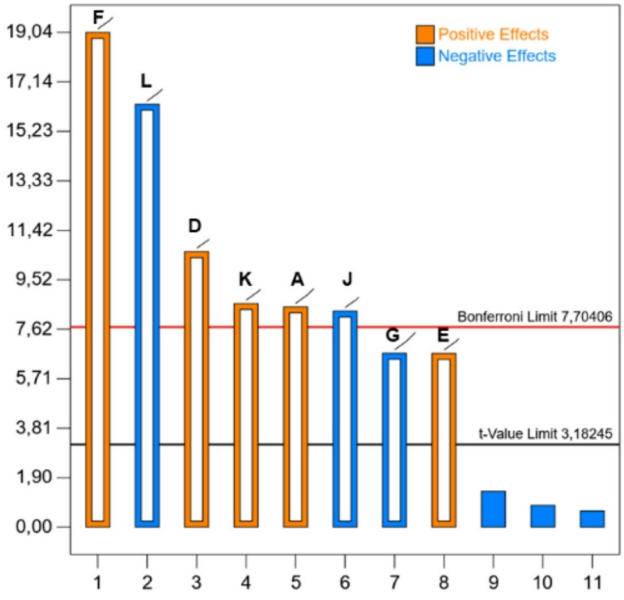


Fig. 1 The Pareto chart shows the amount of influence of each factor on *Streptomyces* blue pigments production. A (Residue concentration); D (pH); E (Temperature); F (Agitation); G (Working volume (mL·250 mL flask^{-1}); J (Starch); K (KH_2PO_4); L (Yeast extract)

$$\begin{aligned} Y_1 = {} & 3985.02 - 685.34X_1 + 428.81X_2 + 822.11X_3 \\ & + 63.88X_4 - 1036.71X_1X_2 + 152.02X_1X_3 + 115.90\ X_1X_4 \\ & - 84.73\ X_2X_3 + 33.52X_2X_4 + 80.28\ X_3X_4 - 1688.18X_1^2 \\ & + 385.18X_2^2 + 2311.82X_3^2 + 237.97X_4^2 \end{aligned} \quad (3)$$

Where Y_1 denotes the predicted *Streptomyces* blue pigments in mg L^{-1} and X_1, X_2, X_3 and X_4 are temperature (X_1), agitation (X_2), pH (X_3) and KH_2PO_4 (X_4); respectively.

The model was statistically significant, with a Model F-value of 52.38, indicating a very low probability (0.01%) that such a result could arise from random variation. Among the model terms, X_1, X_2, X_3, X_1X_2, X_1^2, and X_3^2 were identified as significant contributors to the response. The Lack of Fit F-value was 3.25, which is not statistically significant relative to the pure error, suggesting the model fits the data well. There is a 25.84% probability that such a Lack of Fit F-value could be due to random noise.

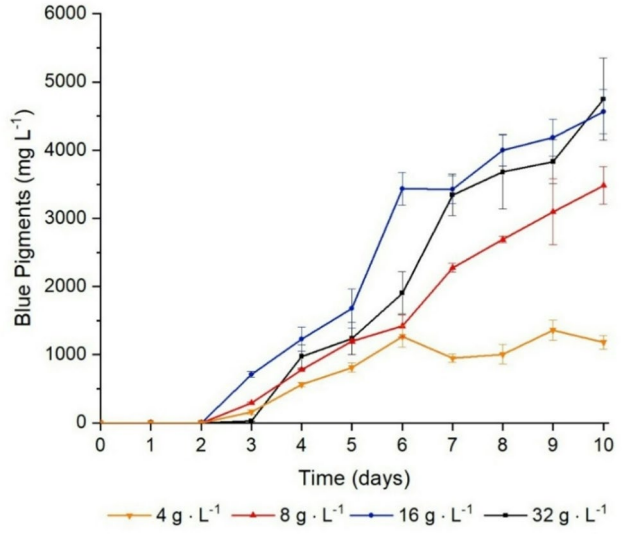


Fig. 2 Influence on the production of blue pigments by *Streptomyces lydicus* PM7 at different concentrations of discarded potato powder under optimal culture conditions by Plackett-Burman design

The coefficient R^2 was 0.9839, suggesting a strong correlation between the experimental and predicted values (Fig. S2) and demonstrating the model's robustness and reproducibility for pigment production. Additionally, the model's significance was further supported by a *P*-value of < 0.0001, while the Lack of Fit *P*-value of 0.2584 reinforced its adequacy. For the quadratic RSM model, residual diagnostics likewise supported model adequacy: the Q–Q plot was near-linear and the Shapiro–Wilk test was non-significant ($W = 0.95$, $p = 0.423$). Residuals vs. fitted showed no heteroscedastic pattern and residuals vs. run order showed no serial structure (Fig. S3).

Table 5 Central composite design CCD of independent variables of blue pigments production

Std	Run	Variables				Blue pigments (mg L^{-1})		Residue
		X_1	X_2	X_3	X_4	Actual	Predictive	
6	1	1	– 1	1	– 1	6,098	5,987	111
21	2	0	0	– 1	0	5,445	5,475	-30
3	3	– 1	1	– 1	– 1	7,131	6,896	235
14	4	1	– 1	1	1	6,219	6,440	-221
10	5	1	– 1	– 1	1	4,103	4,161	-58
2	6	1	– 1	– 1	– 1	4,210	4,030	180
23	7	0	0	0	– 1	4,032	4,159	– 127
8	8	1	1	1	– 1	4,313	4,534	-221
7	9	– 1	1	1	– 1	8,010	7,906	104
22	10	0	0	1	0	6,909	7,119	-210
19	11	0	– 1	0	0	3,676	3,942	-266
27	12	0	0	0	0	4,266	3,985	281
16	13	1	1	1	1	5,437	5,122	315
5	14	– 1	– 1	1	– 1	5,326	5,212	114
24	15	0	0	0	1	4,174	4,287	– 113
1	16	– 1	– 1	– 1	– 1	3,593	3,863	-270
26	17	0	0	0	0	4,376	3,985	391
9	18	– 1	– 1	– 1	1	3,767	3,531	236
20	19	0	1	0	0	4,825	4,799	26
12	20	1	1	– 1	1	3,082	3,182	– 100
4	21	1	1	– 1	– 1	2,789	2,916	– 127
18	22	1	0	0	0	1,732	1,611	121
25	23	0	0	0	0	4,032	3,985	47
17	24	– 1	0	0	0	2,622	2,982	-360
13	25	– 1	– 1	1	1	5,374	5,201	173
11	26	– 1	1	– 1	1	6,633	6,699	-66
15	27	– 1	1	1	1	7,864	8,030	– 166

Level	X_1: Temperature (°C)	X_2: Agitation (rpm)	X_3: pH	X_4: KH_2PO_4 (g L^{-1})
– 1	28	120	7	0.10
0	32	150	8	0.15
1	36	180	9	0.20

The predicted R^2 value of 0.9069 confirmed the model's reliability in capturing the response trends. The standard deviation, mean, and Predicted Residual Sum of Squares (PRESS) were 297.8, 4,816.2, and 6.1×10^6, respectively.

Three-dimensional response surface plots were generated to visualize the interactive effects of key variables. These plots facilitated the interpretation of variable interactions under the following fixed conditions: temperature = 30 °C, agitation = 180 rpm, pH = 9, and KH_2PO_4 concentration = 0.15 g L^{-1}. These conditions correspond to the optimal solution derived from the numerical optimization, which yielded a desirability value of 1.000 for maximum blue pigment production by *S. lydicus* PM7.

The interaction effects of the optimized variables on *Streptomyces* blue pigment production are shown in Fig. 3a–f. A significant interaction ($p < 0.0001$) was observed between temperature (X_1) and agitation (X_2), with higher pigment yields at increased agitation, especially between 30 and 32 °C. However, production declined at temperatures above 34 °C, regardless of agitation intensity.

The interaction between temperature and pH ($X_1 \times _3$), though not statistically significant ($p = 0.0638$), revealed decreased pigment production at 35 °C and increased yields under alkaline conditions, suggesting that pH supports the biosynthetic process. Likewise, increased agitation and pH increased pigment levels at a constant 30 °C.

Model validation using point prediction identified optimal conditions as 30 °C, 180 rpm, pH 9, and 0.15 g L^{-1} KH_2PO_4. Under these settings, experimental pigment production reached 7491 ± 47.8 mg L^{-1}, showing a 91% correlation with predicted values.

Table 6 Analysis of variance (ANOVA) for face central composite design results used for optimizing blue pigments production

Source	Sum of Squares	df	Mean Square	F-value	*p*-value
Model	6.50E + 07	14	4.65E + 06	52.38	< 0.0001*
X_1 - Temperature	8.46E + 06	1	8.46E + 06	95.35	< 0.0001*
X_2 - Agitation	3.31E + 06	1	3.31E + 06	37.32	< 0.0001*
X_3 - pH	1.22E + 07	1	1.22E + 07	137.17	< 0.0001*
X_4 - KH_2PO_4	73,600.06	1	73,600.06	0.83	0.3802
$X_1 \times_2$	1.72E + 07	1	1.72E + 07	193.91	< 0.0001*
$X_1 \times_3$	3.70E + 05	1	3.70E + 05	4.17	0.0637
$X_1 \times_4$	2.15E + 05	1	2.15E + 05	2.42	0.1458
$X_2 \times_3$	1.15E + 05	1	1.15E + 05	1.29	0.2775
$X_2 \times_4$	18,023.06	1	18,023.06	0.2032	0.6602
$X_3 \times_4$	1.03E + 05	1	1.03E + 05	1.16	0.3019
X_1^2	7.33E + 06	1	7.33E + 06	82.64	< 0.0001*
X_2^2	3.82E + 05	1	3.82E + 05	4.31	0.0602
X_3^2	1.37E + 07	1	1.37E + 07	154.98	< 0.0001*
X_4^2	1.46E + 05	1	1.46E + 05	1.64	0.2245
Residual	1.06E + 06	12	88,678.84		
Lack of Fit	1.00E + 06	10	1.00E + 05	3.25	0.2583
Pure Error	61,730.67	2	30,865.33		
Cor Total	6.61E + 07	26			

R^2 = 0.9839; Adj. R^2 = 0.9651; Pred. R^2 = 0.9069; Adeq. Precision = 28.9177; *Statistically significant ($p < 0.05$)

Kinetics of growth and metabolic activities under optimized conditions

To study the fermentation kinetics of *S. lydicus* PM7 under the previously optimized conditions (30 °C, 180 rpm, pH 9, 0.15 g L^{-1} KH_2PO_4), we monitored the bacterial growth (CFU mL^{-1}), blue pigment production, residual reducing sugars, and key enzymatic activities (α-glucosidase, amylase, catalase) in the culture for 192 h (Fig. 4). At 192 h, the culture reached a final titer of 6.97 g L^{-1} of blue pigments. Volumetric productivity (Q_p) was estimated from the most linear production segment (108–168 h) as ΔP/Δt, yielding Q_p = 0.057 g L^{-1} h^{-1} (R^2 = 0.905), the overall 0–192 h productivity was 0.036 g L^{-1} h^{-1}. Reducing sugars were monitored as a proxy for substrate availability. However, because transient accumulation followed by a decline in reducing sugar concentration, a global substrate-to-product yield ($Y_{P/S}$) was not estimated at the shake-flask scale to avoid biased calculations.

The growth kinetics of *S. lydicus* PM7 displayed a biphasic pattern consistent with diauxic growth in the discarded-potato medium. During the first 48 h, cell counts remained relatively stable, indicating a lag and early adaptation phase. Between 48 and 72 h, a clear initial exponential phase occurred, reaching approximately 1×10^7 CFU mL^{-1}. This period coincided with the maximum α-glucosidase activity (0.88 U) and a gradual decrease in reducing sugars, suggesting the consumption of readily available carbohydrates. Interestingly, the onset of blue pigment production aligned with this growth phase. After 72 h, a transitional period was observed (72–144 h), where bacterial growth slowed, α-glucosidase activity dropped sharply -particularly at 92 h- and α-amylase activity began to increase. Reducing sugars remained relatively stable and then started to decline again as the culture progressed toward 144 h. A second exponential phase was observed between 144 and 168 h, during which bacterial cells peaked at 3.8×10^7 CFU mL^{-1}. At the end of this period, α-amylase activity reached its maximum (~ 0.25 U) and a further decrease in reducing sugars. Blue pigment production increased notably during both exponential phases, reaching its highest concentration around 168 h. Then, a decline phase began (168–192 h), characterized by reduced cell viability, decreasing enzymatic activities, diminishing reducing sugars, and a slight decrease in pigment concentration. Catalase activity increased markedly during the first 48 h (reaching ~ 49 U), remained relatively stable until 84 h, and then gradually decreased throughout the incubation time.

The initial pH of the culture medium was 8.2 ± 0.1 after sterilization and the inoculum addition (Fig. 4). After 24 h, the initial pH decreased to 5.9 ± 0.1, likely due to the accumulation of organic acids from glucose metabolism. The pH of the culture medium gradually increased by ~ 0.2 units every 24 h from 48 h of incubation, stabilizing at 8.02 ± 0.1 by 192 h during the kinetic fermentation assay. In contrast, the control medium without inoculum maintained a constant pH of 8.2 ± 0.1.

Discussion

Commonly, the production of secondary metabolites associated with ecological functions is not readily observed under standard laboratory conditions, as their synthesis is often triggered by environmental stressors such as nutrient fluctuations in the growth medium [38]. In the case of the discarded potato used as a basal nutrient source, the peel particles could contribute with non-starch polysaccharides and lignin [39]. These insoluble components may provide physical support for the microparticle-enhanced cultivation of S. lydicus PM7, thereby influencing secondary metabolite production depending on the fermentation approach [40]. For example, Holtmann et al. [41] reported an 85% increase in actinorhodin production by *S. coelicolor* M145 upon addition of SiO_2 particles to the culture medium. Similarly, López-García et al. [42] observed enhanced actinorhodin synthesis when using sodium alginate for cell encapsulation, compared to unencapsulated cultures.

In our previous studies, a potato-based culture medium was used under non-optimized cultivation conditions. The resulting pigment was characterized by chromatographic and mass spectrometry analyses

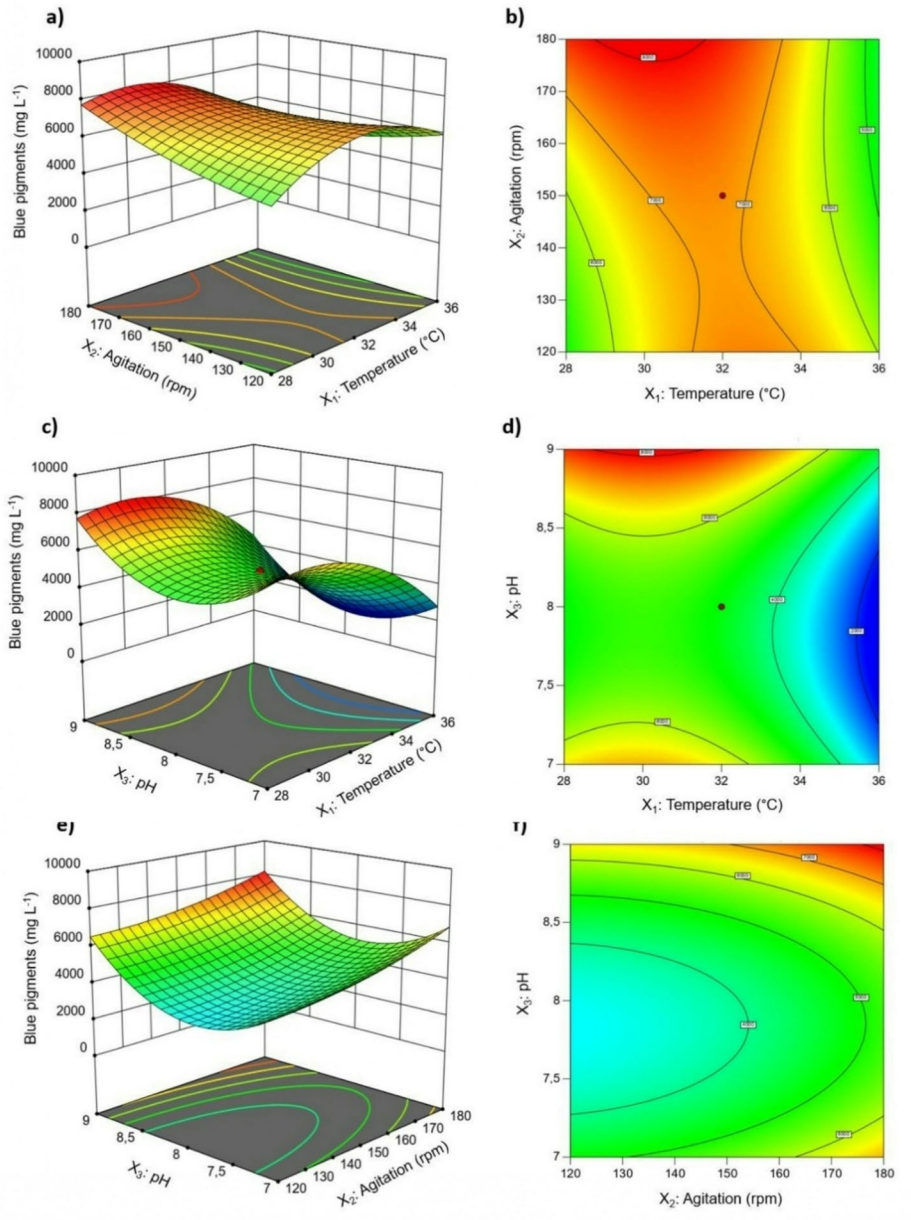


Fig. 3 **a–f** 3D surface and 2D contour plot and plots explaining the individual and interactive effects of factors on blue pigments production by *Streptomyces lydicus* PM7

and was consistent with actinorhodin [25]. In line with these findings, under optimized cultivation conditions, the chemical properties of the blue pigment, particularly its acid/base indicator behavior and maximum absorption wavelength at 640 nm [26], suggest the presence of actinorhodin and/or actinorhodin-related compounds. Actinorhodin-related compounds include α-, β-, ε-actinorhodin and actinorhodinic acid [26].

Beyond a potential microcarrier role, substrate composition may also steer metabolic flux toward pigment biosynthesis, which is consistent with reports highlighting the substrate as a key determinant of microbial pigment production [43]. Considering that discarded potatoes share the nutritional profile of food-grade potatoes [4], starch is expected to predominate (~ 65%), followed by protein (~ 5.7%) and minor lipids (~ 0.7%); increased starch hydrolysis can elevate acetyl-CoA availability and favor pigment pathways [44].

In addition to the composition of the culture substrate, temperature is a key environmental factor influencing the growth and metabolic activity of *Streptomyces* strains in submerged fermentation. Optimal biosynthesis of secondary metabolites typically occurs within a narrow temperature range [45]. For example, maximum

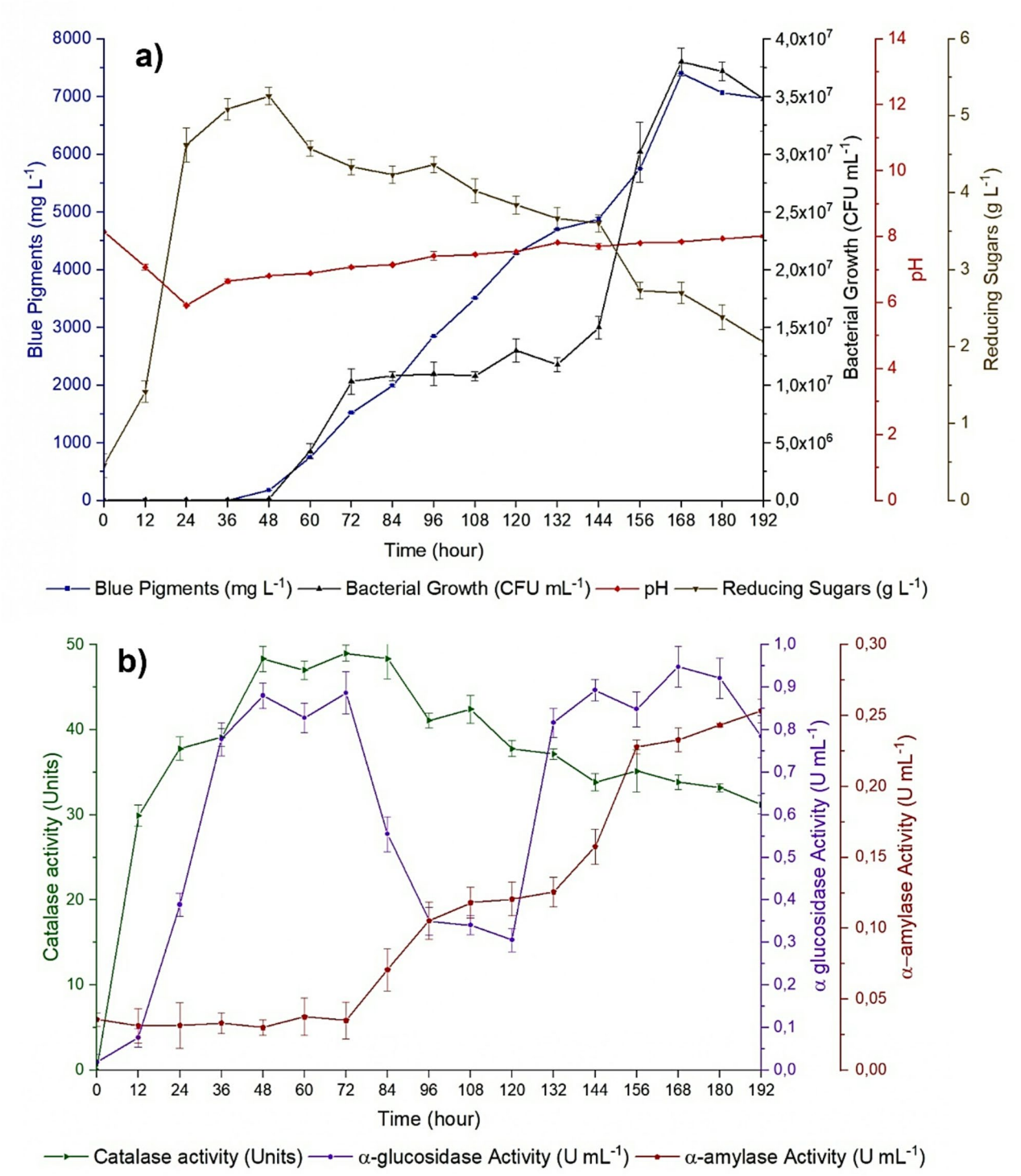


Fig. 4 Kinetic response of *Streptomyces lydicus* PM7 cultivated in discarded potato medium under optimized conditions. **a** Fermentation dynamics showing changes in blue pigment concentration (mg L^{-1}), microbial growth (CFU mL^{-1}), reducing sugar concentration (g L^{-1}), and pH, **b** Enzymatic activity profiles, including α-glucosidase, α-amylase, and catalase activities during the fermentation process

endoglucanase activity in some *Streptomyces* isolates was observed between 26 and 30 °C [46], while tetracycline production declines outside of 35 °C [47]. These findings support the temperature sensitivity observed in the present study, where pigment production decreased at ≥ 35 °C. Although the mechanisms remain to be fully elucidated, temperature likely influences enzyme activity and energy metabolism, with a direct impact on secondary

metabolite biosynthesis. However, more research is needed to confirm and quantify the effect of temperature on the production of secondary metabolites by *Streptomyces*.

Alongside temperature, agitation is equally significant in ensuring homogenous nutrient distribution and facilitating oxygen transfer, which are critical factors in submerged cultures [48]. Agitation speed has been directly linked to oxygen availability and metabolite production [49]. However, excessive agitation may suppress cell growth due to shear stress or oxygen oversaturation, as reported by Umar et al. [50]. Interestingly, such stress conditions may not hinder, but favor metabolite synthesis. For instance, in *Streptomyces clavuligerus*, increased agitation reduced biomass but enhanced clavulanic acid production [51]. Therefore, this parameter must be carefully optimized, as it can indirectly affect secondary metabolite production by modulating growth kinetics and stress responses. Taken together, temperature and agitation act in concert with the substrate to define workable windows for productivity.

Another critical factor influencing secondary metabolism is pH, which plays a crucial role in regulating metabolic pathways in *Streptomyces*. While most strains grow optimally at pH 6.5-8.0, there are also acidophilic and alkaliphilic variants [52]. Our findings of enhanced pigment production at alkaline pH align with previous studies on actinorhodin, where solubility and yield improved at pH 8.5 [27, 53]. Similarly, an alkaline shock (pH 8) increased validamycin-A production by 26% in *Streptomyces hygroscopicus* [54], likely due to improved proton transport and respiratory efficiency. Overall, alkaline pH appears to favor pigment biosynthesis by modulating intracellular pH homeostasis and metabolic flux. Biosynthesis was favored under alkaline conditions, with an optimum around pH 9, consistent with the proposed improvement in respiratory efficiency. In this context, achieving 7,491 mg L^{-1} represents an approximately 8.5-fold improvement over the previously reported non-optimized baseline [25], reinforcing the effectiveness of the selected conditions. Beyond pH, phosphate availability can further modulate secondary metabolism in *Streptomyces*. In our system, phosphate acted as a permissive factor (presence vs. absence), yet within the narrow interval tested it did not produce a detectable dose–response, suggesting that the process was not phosphate-limited under those conditions. A broader survey of concentrations would help delineate the operational phosphate window and its interaction with nitrogen. Consistently with the phosphate-nitrogen balance, nitrogen-rich supplementation showed the opposite trend: the addition of yeast extract negatively impacted pigment production, in agreement with reports where ammonium/nitrate and trace elements suppressed actinorhodin in *S. coelicolor* A3(2) [26, 55].

The biphasic growth profile observed in *S. lydicus* PM7 is consistent with classical diauxic behavior reported in *Streptomyces* species growing on complex substrates containing multiple carbon sources. During the first exponential phase, the high α-glucosidase activity and the decline in reducing sugars strongly suggest preferential utilization of simple carbohydrates (e.g., glucose, sucrose-derived monomers) naturally present in potato tissues. These sugars typically feed into glycolysis and the pentose phosphate pathway, providing rapid precursor and energy supply, as documented in *Streptomyces* spp. and other actinobacteria [56]. As readily assimilable sugars become limited, *Streptomyces* species commonly shift toward the utilization of more complex polysaccharides through the induction of amylolytic systems. In our study, the sharp decline in α-glucosidase activity and the progressive increase in α-amylase activity observed between 72 and 144 h are indicative of the metabolic transition. The second exponential growth phase in our study corresponds well to the onset of maximal α-amylase activity, suggesting that starch-derived compounds became the primary carbon source. Such stepwise substrate utilization mirrors metabolic models proposed for *Streptomyces*, where diauxic shifts reflect adaptive strategies to optimize growth in nutrient-heterogeneous environments [57, 58].

Importantly, pigment formation occurred during both exponential phases and became more pronounced as reducing sugars declined. Although actinorhodin biosynthesis has traditionally been associated with the stationary phase in *S. coelicolor* [59, 60], both previous reports [61] and our results indicate pigment formation during exponential growth, especially as reducing sugar concentrations decline in the medium. This diauxic behavior mirrors observations in *S. coelicolor*, where the organism transitions from glutamate to maltose utilization as nutrient availability changes [57]. Chu and Barnes [62] highlighted that such metabolic flexibility relies on tightly regulated genetic adaptations, enabling *Streptomyces* to dynamically reprogram its enzymatic machinery in response to complex nutrient environments. Thus, diauxic growth is generally interpreted as an adaptive strategy to maximize population growth in multi-nutrient substrates such as discarded potato.

The early peak in catalase activity further suggests that the initial phase of simple-sugar metabolism involves intense aerobic respiration and reactive oxygen species (ROS) generation. High levels of ROS in the culture medium are likely a result of the high dissolved oxygen, which produces superoxide (O_2^-) and hydrogen peroxide (H_2O_2) [63]. Actinorhodin, a redox-active molecule produced by *S. lydicus* PM7, can contribute to oxidative

stress through its quinone/semiquinone transitions [64]. Catalase helps mitigate this by breaking down H_2O_2 into water and oxygen [65], and its elevated activity from 12 to 48 h could be a key defense mechanism. Interestingly, actinorhodin may also modulate oxidative stress in *S. lydicus* PM7. In *S. coelicolor*, actinorhodin has been shown to act as an electron acceptor and suppress oxidative stress response genes, which help protect the cells [66]. This suggestion is supported by our observation that when actinorhodin in cell-free supernatant was exposed to a low concentration of H_2O_2, a color change was observed from blue (λ_{max} = 640) to turquoise (λ_{max} = 580) but maintaining its characteristic of acid/base indicator. This effect suggests its potential antioxidant property. Further studies are needed to confirm the role of actinorhodin in oxidative stress resistance in *S. lydicus* PM7. Altogether, these observations support a context-dependent redox function for actinorhodin that aligns with prior evidence in *S. coelicolor*.

The accumulation of organic acids in *S. coelicolor*, such as pyruvate and α-ketoglutarate during early growth stages leads to a pH decline, which has been associated with antibiotic production [67]. The subsequent re-assimilation of these acids contributes to pH stabilization, feeding into the tricarboxylic acid (TCA) cycle [68] and supporting the biosynthesis of secondary metabolites, including actinorhodin [69]. A similar pattern observed in *S. lydicus* PM7 suggests that the initial pH drop is driven by acid accumulation and re-assimilation, coupled with a metabolic shift toward secondary metabolite production, thereby optimizing intracellular conditions for pigment biosynthesis. These findings underscore the complex interplay between physicochemical parameters, nutrient dynamics, and oxidative stress in modulating actinorhodin production. Integrating substrate characteristics, operational conditions, and metabolic regulation thus provides a coherent framework for understanding the observed behavior and identifies potential strategies for process intensification.

It should be noted that the blue pigment production trials were performed without controlled dissolved oxygen, aeration, mixing, or pH. Consequently, the process performance may differ under bioreactor conditions, where factors such as volumetric oxygen mass transfer coefficient (kLa), agitation speed, and shear stress could significantly influence growth and pigment biosynthesis [70, 71]. Future studies with *S. lydicus* PM7 will focus on evaluating and optimizing culture conditions in a stirred-tank bioreactor, as well as downstream processing, to enhance actinorhodin yield and process scalability.

Conclusions

This study demonstrated that Plackett–Burman and central composite designs are effective tools for identifying and optimizing key variables to enhance the production of actinorhodin-related blue pigments by *S. lydicus* PM7 using discarded potato as a low-cost substrate. The optimized conditions resulted in an 8.5-fold increase in pigment yield compared with non-optimized conditions. Beyond process optimization, the growth kinetic, reducing sugars, and enzyme activities provide preliminary insight into metabolic responses associated with pigment formation. The two exponential growth phases, together with shifts in α-glucosidase and α-amylase activities, suggest a possible sequential use of readily available sugars followed by starch-derived carbohydrates. However, confirming this metabolic pattern will require targeted analyses of specific carbohydrates and regulatory pathways. Overall, this work establishes a laboratory-scale proof of concept for converting potato byproducts into microbial blue pigments and identifies key physicochemical and metabolic parameters that influence pigment biosynthesis in *S. lydicus* PM7. These findings provide a basis for future bioreactor-scale and application-oriented studies aimed at advancing cost-effective and sustainable microbial pigment production.

Abbreviations

PBD	Plackett–Burman design
CCD	Central composite design
CFS	Cell-free supernatant
EtOH	Ethanol
ISP2	International Streptomyces project n°2 medium
CFU	Colony-forming units
DNS	Dinitrosalicylic acid
PRESS	Predicted residual sum of squares
Q_P	Volumetric productivity
Y_p/s	Substrate-to-product yield
ROS	Reactive oxygen species

Supplementary Information

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Supplementary Material 1

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Author contributions

H.S., E.H. and M.C.D. planned experiments; A.A. performed experiments; H.S. and A.A. wrote the original draft; E.S. and H.S. contributed to data analysis and visualization; A.A. and O.R. performed biochemical analyses; S.M.A. and G.B. reviewed and edited the final manuscript version. All authors reviewed the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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