Scientific African 30 (2025) e02998

Contents lists available at ScienceDirect

Scientific African

journal homepage: www.elsevier.com/locate/sciaf

Optimization of fermentation conditions for lactic acid production by *Lactiplantibacillus plantarum* OM510300 using plantain peduncles as substrate

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ARTICLE INFO

Editor: DR B Gyampoh

Keywords:
Lactic acid
Optimization
Reducing sugar
Lactiplantibacillus plantarum
Agrowaste

ABSTRACT

Lactic acid (LA) is the most common hydroxycarboxylic acid with optically active chiral carbon atoms, with numerous applications across pharmaceutical, food, textile, chemical, and medical sectors. LA has been used as an antioxidant, decontaminant, acidulant, and cryoprotectant among other functions. This study was aimed at optimizing fermentation conditions for LA production by *Lactiplantibacillus plantarum* (accession number OM510300) using plantain peduncles. The plantain peduncles were subjected to pre-treatment using alkaline and enzymatic hydrolysis. The physical and chemical properties of the pre-treated plantain peduncles were observed using X-ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), and Scanning Electron Microscopy (SEM). Through the design expert, a central composite design was set up and used in designing the experimental runs for fermentation conditions. The results from the runs were analyzed using ANOVA. SEM, XRD, and FTIR analysis revealed changes in the structural arrangement of the untreated and pre-treated plantain peduncles. Results from the ANOVA revealed different levels of interaction by each fermentation condition independently and collaboratively. The reliability of the reduced quadratic model used in the central composite design was observed to be significant with an F-value of 13.25 and a *p-value of* < 0.0001. *This implies that the model can accurately predict the concentration of LA under the investigated experimental settings. Upon conducting a submerged LA production, 483 ml of LA was obtained at the end of the extraction using the phase partitioning technique. Fermentation conditions such as pH and temperature have a significant effect on LA production with optimal conditions observed for the pH to be 9.8 to 10 and for incubation periods of 6 to 7 h* with a temperature range of 40 °C to 42 °C.

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https://doi.org/10.1016/j.sciaf.2025.e02998
Received 21 April 2025; Received in revised form 29 July 2025; Accepted 23 September 2025
Available online 23 September 2025

Introduction

Lactic acid (LA) is the most prevalent hydroxycarboxylic acid with CH3–CH (OH)–COOH as its chemical formula [1,2]. LA isomers are distinguished into d-LA acid and l-LA, with the latter being preferred for application across various industries due to their enantiomeric purity [3]. Enantiomeric purity is essential for the industrial application of LA, such as in the creation of biodegradable plastics and polymers like poly (lactic acid) (PLA). More so, high dosages of the d-lactic acid isomer are thought to be hazardous to humans and can result in acidosis or decalcification. As such, the food and pharmaceutical sectors favour the utilization of l-lactic acid isomer [4,5].

The FOA (U.S. Food and Drug Administration) classified LA as "Generally Recognized as Safe (GRAS)" [6]. Due to this, LA has been adopted for various purposes [7]. In the food sector, LA acid has been utilized as a preservative (for extending the shelf life of products by suppressing microbial growth); it has also been used as a flavouring agent with a well-known functional role, such as pH stabilization [8]. The pharmaceutical industry has numerous applications of LA, including but not limited to mineral preparations, tabletting, dialysis solutions, and surgical sutures [9,10]. More so, LA is used as a cryoprotectant, acidulant, antioxidant, as well as a decontamination agent; LA also possesses prebiotic properties and has been employed to improve the viscosity of some substances [11–13].

Presently, there are two techniques utilized for LA production – chemical synthesis and microbial fermentation [14,15]. A significant drawback to chemical synthesis is that LA made via this route utilizes petrochemical resources, thus, yielding a racemic mixture of LA [16]. On the contrary, LA produced via microbial fermentation is optically pure making it a favorable choice for application across various industries [17]. Moreover, microbial fermentation employs indigenous microorganisms while utilizing cheap substrates for production, a process identified as sustainable with little or no adverse effect on the environment [15].

While microbial fermentation of LA presents numerous advantages over chemical methods, there exist setbacks in terms of the feedstock/substrate used for microbial production. According to de Oliveira et al. [18], microbial fermentation of LA will be heavily challenged by substrate cost and availability, and LAB strains. Moreover, Manandhar and Shah [19] have emphasized the importance of using feedstock that is cheap with a high yield of LA. More so, optimizing the fermentation conditions (i.e., pH, temperature, carbon concentration, incubation time, among others) is paramount for the commercial production of LA [20]. As such, the Central Composite Design (CCD) has been employed by various researchers across the globe for the identification of optimum fermentation conditions for commercial production of LA since it can optimize multiple variables by evaluating their effects on responses [21–24].

To achieve sustainable production of LA using cheap renewable feedstock, researchers and bio-product developers across the globe have sort out different feedstock including but not limited to starch, sugar cane bagasse, molasses, groundnut shells, and plantain peduncle among many others for LA production using varying types of lactic acid bacteria (LAB) such as *Lactiplantibacillus plantarum* a Gram-positive bacteria originally known as *Lactobacillus plantarum* [25]. Plantain peduncles, an agricultural waste, are often found littered all over Nigeria, contributing to post-harvest pollution. Waste management techniques such as landfill, burning, among others, have been proven to be effective in managing wastes generated by plantain peduncle, but with detrimental effects to the environment and the ecosystem at large [26]. Due to this, this study aimed at reducing the environmental pollution caused by plantain peduncles by its utilization as a carbon source for LA production by first identifying the required optimum fermentation conditions.

Materials and methods

Bacterial isolate collection and processing

Lactiplantibacillus plantarum (OM510300), the isolate used in this research, was obtained from our earlier study, Oyewole et al. [26]. *Lb. plantarum* (OM510300), which was isolated from the waste dump of plantain peduncles. Gram staining was used to distinguish the purity of the collected isolate using a 24-hour-old culture, followed by several biochemical (i.e., catalase and oxidase test) and sugar fermentation tests (i.e., glucose, fructose, sucrose, lactose, mannose, and rhamnose) to phenotypically identify the isolate.

Agro-waste collection and alkaline pretreatment

Agro-waste (plantain peduncles) collected from different agro-waste dumpsite locations in Kure Market, Minna metropolis, was used in this study since it was established earlier by Oyewole et al. [26] that among the four agro-wastes (i.e., groundnut shells, sugar cane bagasse, corn cobs and plantain peduncles) screened for reducing sugar concentration, plantain peduncles demonstrated to serve as a reliable source of reducing sugar for LA fermentation. The method outlined by Makut and Ekeleme [27] was used for the alkaline pretreatment of plantain peduncles using NaOH. At the end of the proc,ess, the pH of the substrate was maintained at neutral (i.e., pH 7).

Production of cellulase and the enzymatic hydrolysis of plantain peduncles

According to the procedures described by Omonije et al. [28], *Aspergillus niger* was used for the production of cellulase enzyme locally utilizing sugarcane bagasse in the Microbiology Department, Federal University of Technology Minna, Niger.

The cellulase enzyme produced was then utilized for hydrolyzing plantain peduncles using the methods of Makut and Ekeleme [27]. The hydrolysis process was stopped after 48 h by raising the temperature from 60 °C to 100 °C to block further activities of the enzyme on the substrate. The hydrolysate obtained from filtration using muslin cloth and Whatman No 1 filter paper was used for

determining the reducing sugar concentration [27].

Measuring the reducing sugar concentration in the hydrolysate

The reducing sugar concentration was measured using 3,5-dinitrosalicylic (DNS) methods as described by Oyewole et al. [29].

Physicochemical analysis of agro-wastes used for LA production

According to methods outlined by Oyewole et al. [29], the physicochemical properties of plantain peduncles before and after pretreatment were analyzed using SEM (scanning electron microscopy), FTIR (Fourier-transformed infrared spectroscopy), and XRD (X-ray diffraction).

Inoculum preparation for lactic acid production

Throughout the investigation, the stock culture of *Lb. plantarum* was kept in 250 mL of DeMan's Sharpe and Rogosa (MRS) Broth at 4 °C, with a 10-day interval between subculturing. The growth medium (g/L) was composed of 10.0 g of peptone and meat extract, respectively; 5.0 g of yeast extract and sodium acetate; 2.0 g of tri-ammonium citrate and K_2HPO_4; 0.05 g of $MnSO_4{\cdot}4H_2O$; 0.2 g of MgSO4·7H2O; 20.0 g of d-glucose, and 1.0 mL of Tween 80 [30]. All chemicals used were P.A. grade (Fuga, Darmstadt, Germany). An overnight culture in MRS broth was used to create the inoculum (10 % v/v) with lactose monohydrate (11 g/L). In each experiment, 100 mL of growth media was placed in a 300 mL Erlenmeyer flask, and the carbon content, incubation time, pH, and temperature were all adjusted according to the experimental design.

Experimental design for optimization of fermentation conditions for LA production

Using bacterial isolates and plantain peduncles as the substrate, process parameters were adjusted to provide a high output of LA, as Andler et al. [31] demonstrated. During the optimization process, pH, temperature, carbon source, and incubation time were adjusted accordingly. In a 250 mL Erlenmeyer flask containing 100 mL of fermentation medium, batch cultures with an inoculum (10 % v/v) were experimentally set up. The appropriate number of flasks was used to maximize the pH. Depending on the particular run data, the amount of fermentation media in each flask was adjusted to 100 mL (Supplementary Data, 1).

Each flask was then incubated by the parameters that the design expert had created utilizing the Central Composite Design (CCD) methodologies for surface response. The effect of pH, carbon concentration, incubation time, and temperature on *Lb. plantarum's* lactic acid production was selected to demonstrate the statistical importance of the CCD. A second-degree polynomial of the relationships between the factors and the dependent variable is estimated using the CCD, which also provides information on the interaction between the variables (factors). To ascertain the LA concentration, a spectrophotometric measurement was made at 390 nm after each run period. Design Expert Software Version 11 was used to optimize the production process. Twenty-seven fermentation runs were developed and carried out using varying amounts of inputs. During the lactic acid production phase, the design expert's specific optimized conditions were utilized.

Using submerged fermentation, the isolates' capacity to produce LA in fermentation broth (10 litres) was examined. Each content's growth media was modified to fit the 1000 ml MRS fermentation medium. The composition of the fermentation medium is as follows: MgSO4·7H2O (5 g), ammonium citrate (2 g), K_2HPO_4 (2 g), polysorbate 80 (1 g), sodium acetate (5 g), yeast extract (5 g), beef extract (10 g), animal tissue digest (10 g). The appropriate concentration for plantain peduncle hydrolysate (as a carbon source) was substituted for dextrose [32]. Likewise, the fermentation medium's pH was set to the right value based on the output produced by the software, before incubating for the required temperature and time.

Extraction and purification of lactic acid

At the end of each fermentation run, the medium was centrifuged at 8000 rpm for 15 min at 4 °C to separate the cells from the medium. Subsequently, the supernatant was then passed through the Whatman No 1 filter paper. The filtrate was then used for the extraction and purification of LA using the phase partitioning technique as described by Kumar et al. [33]. A 10 mL of LA-fermented broth was put into a conical flask. Added to it is 5 g of ammonium sulfate, which was allowed to dissolve completely before adding 30 mL of n-butanol, corked, and vortexed to ensure an even solution. The mixture was then allowed to stand for 2 h. Following the separation of the organic and aqueous phases, the mixture was transferred to a separating funnel and allowed to stand. Phase separation was followed by the separation of the upper organic phase, and the final product was evaporated for three hours at 70 °C [33].

Estimation of lactic acid concentration using spectrophotometer

To estimate the LA produced, the spectrophotometric method as described by Borshchevskaya et al. [34] and Uwamahoro et al. [35] was utilized. This method is predicated on the notion of recognizing the coloured substance that is generated when lactate ions and iron (III) chloride react, since these ions absorb at 390 nm. The concentration of LA was known to be 89 % ($\rho = 1.2$ g/mL); a series of LA solutions was then created using two-fold serial dilutions. To make a solution of iron (III) chloride, a volumetric flask containing 100 mL of distilled water was added 0.3 g of iron (III) chloride. All of the diluted LA solutions from the experimental runs were then

subjected to the same procedure, which involved stirring 100 μL of the LA solution with 4 mL of iron (III) chloride. A UV-6100S spectrophotometer was used to measure the mixture's absorbance at 390 nm. The association between absorbance and LA concentration (g/mL) was finally illustrated by a calibration curve (SD 2).

Data analysis

To ascertain the mean differences at the 5 % (0.05) significant level, the data were assessed using Rebbapragada and Kalyanaraman's [36] techniques for the quadratic model analysis of variance (one-way ANOVA).

Result

Lb. plantarum physiological and biochemical properties

Gram's test revealed a positive result for the bacterial isolate. All of the other characteristics of *Lb. plantarum*, including oxidase tests and catalase, were found to be negative **(SD 3).**

Physicochemical analysis of agro-wastes

As shown below, the proper analysis was used to evaluate the plantain peduncle's physical characteristics and elemental components both before and after pre-treatment.

Scanning electron microscopy

The untreated plantain peduncle's SEM image analysis showed that materials of different sizes and shapes were present; Some of the particles had irregular forms, while others were smaller; other shapes were identified to have smooth surfaces with large ovoidal shapes. Some of the particles demonstrated hollow features in their cross-section, and the particles were observed to be widely distributed and densely packed with a variety of forms (Fig. 1A).

The treated plantain peduncle's SEM image analysis showed the presence of materials of different sizes and forms; some of the particles were irregularly shaped, while others appeared to be rods, loosely packed with gaps between the fibres (Fig. 1B). Some of the particles that were seen had microscopic particles strewn all over their surfaces and seemed to be tubular. However, several of the particles had elongated threads with surfaces appearing to be rough.

FTIR analysis of plantain peduncles

The functional groups in the treated and untreated plantain peduncles were identified using FTIR.

The study of the FTIR spectra obtained from the untreated plantain peduncles revealed numerous prominent peaks (12) that correlated to various functional groups, intensities, and wavenumbers (Fig. 2A). The following are the peaks and the functional groupings that are connected with them:

The C—H out-of-plane bending of a peak found at 775.29 cm^{-1} with an intensity of 86.29 suggested the presence of aromatic compounds, most likely substituted benzene derivatives. Alcohols, esters, or ethers may be present, as shown by the peak at 1032.47 cm^{-1} with an intensity of 78.15, which corresponds to C—O stretching. Subsequent investigation revealed that the sample contained ethers, esters, or alcohols as demonstrated by a peak with intensity of 83.38 at 1103.29 cm^{-1} that was also linked to C—O stretching. A

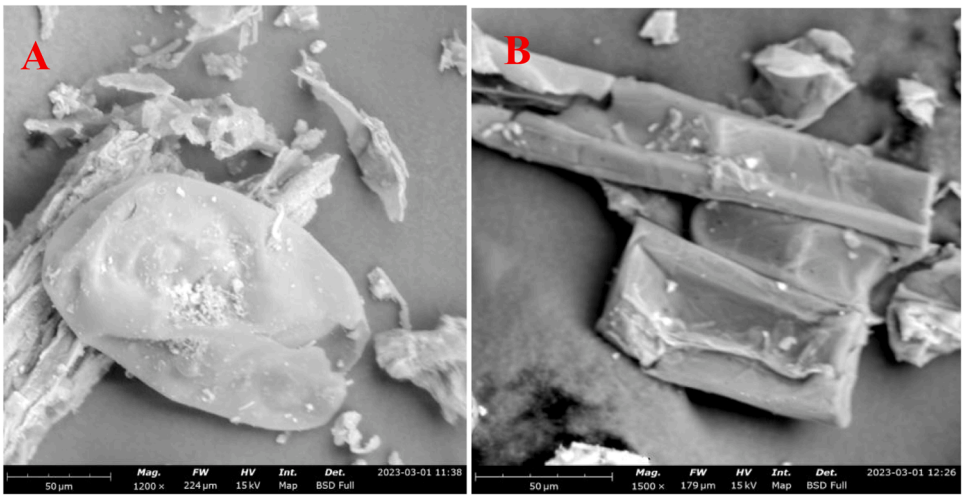

Fig. 1. SEM image of (A) untreated plantain peduncles, and (B) treated plantain peduncles at x1500.

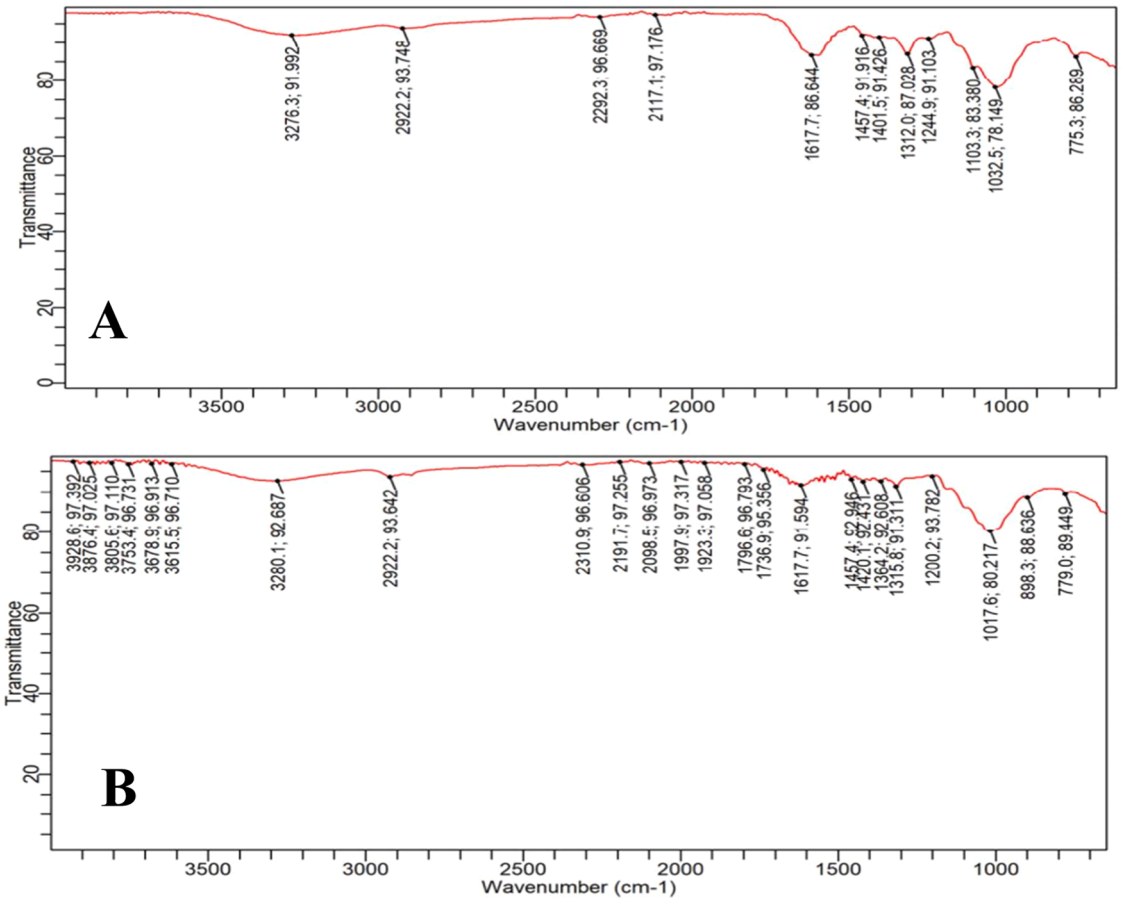

Fig. 2. FTIR Analysis of (A) untreated plantain peduncles, and (B) treated plantain peduncles.

stronger signal with an intensity of 91.10 at 1244.93 cm^{-3} suggested the presence of amines. C—N stretching was associated with this peak. A peak at 1312.02 cm^{-1} (intensity 87.03) was also seen in the spectra, which is indicative of C—H bending, which is typically found in alkanes. O—H bending, which is commonly associated with phenols, was represented by another prominent peak that appeared at 1401.48 cm^{-1} with an intensity of 91.43. The identification of a peak with an intensity of 91.92 at 1457.39 cm^{-1} was linked to C—H bending provided more evidence in favor of alkanes. The peak that emerged at 1617.66 cm^{-1} (intensity 86.64), which was suggestive of $C = C$ stretching, may indicate the presence of alkenes. Two distinct peaks 2292.31 cm^{-3} and 2117.13 cm^{-1}, with intensities of 96.67 and 97.18, respectively, that were associated with C≡C and C≡N stretching, were indicative of alkynes and nitriles. At 2922.23 cm^{-1}, the spectra ultimately displayed a peak with an intensity of 93.75, which was associated with C—H stretching and indicated the existence of alkyl or alkane groups. The last significant peak was linked to O—H stretching at 3276.33 cm^{-1} (intensity 91.99), indicating the presence of alcohols, phenols, or carboxylic acids.

Supplementary Data (SD) 4 presents an in-depth description of the image's 12 FTIR peaks, along with the wavenumber and related functional groups. Several functional groups were linked to peaks in the FTIR analysis of the treated plantain peduncle sample (Fig. 2B). A spectrum of wavenumbers was identified by the investigation, each of which is linked to distinct molecular vibrations that are suggestive of certain sample structures and chemical interactions. The existence of aromatic compounds is indicated by the link between the C—H out-of-plane bending and C—H bending vibrations and peaks seen in the lower wavenumber range, such as 779.01 cm^{-1} and 898.29 cm^{-1}, respectively.

The findings are consistent with the presence of aromatic structures that contain or are attached to benzene rings. C—O and C—O-C stretching vibrations were linked to peaks in the mid-range wavenumbers at 1017.56 cm^{-1} and 1200.20 cm^{-1}, indicating the possible presence of ethers, esters, or alcohols. These compounds are characterized by vibrational modes that reveal the geometry of their molecules. The existence of nitro and amine compounds was indicated by the discovery of additional peaks at 1364.21 cm^{-1} and 1315.75 cm^{-1}, respectively, which were associated with N—O and C—N stretching. Since these functional groups are commonly found in organic compounds, the sample might contain nitrogen-containing substances. Remarkably, the peaks at 1736.94 cm^{-1} and 1617.66 cm^{-1}, respectively, were associated with the stretching vibrations of $C = O$ and $C = C$. Alkenes or aromatic compounds are identified by their $C = C$ stretching, whereas carbonyl groups, like those in ketones, aldehydes, or esters, are identified by their $C = O$ stretching. These peaks show the presence of compounds with unsaturated and carbonyl hydrocarbons. Peaks in the higher wavenumber range

that correlate to C—H and O—H stretching were also found by the analysis; these were located at 3280.06 cm^{-1} and 2922.23 cm^{-1}, respectively. These peaks, which are characteristic of alcohols and alkanes, show the existence of molecules with hydroxyls and saturated hydrocarbons. In Supplementary Data (SD) 5, each of the 24 peaks in the image's FTIR spectrum is described in depth, along with the wavenumber and related functional groups.

It is important to note that the reduction or complete loss of peaks associated with hydroxyl (O–H), carbonyl ($C = O$), and amine (C–N) stretching vibrations implies the partial removal of lignin, hemicellulose, and other extractives from the biomass.

***XRD** analysis of plantain peduncles*

XRD analysis was used to determine the quantitative and qualitative crystallographic phases of both treated and untreated plantain peduncles.

Peaks in the XRD spectrum that corresponded to different crystalline phases found in the plantain peduncles were found by qualitative XRD analysis of untreated plantain peduncles. Davyne, Chaoite, Mellite, Chlorite, Cristobalite, and Refikite were the six

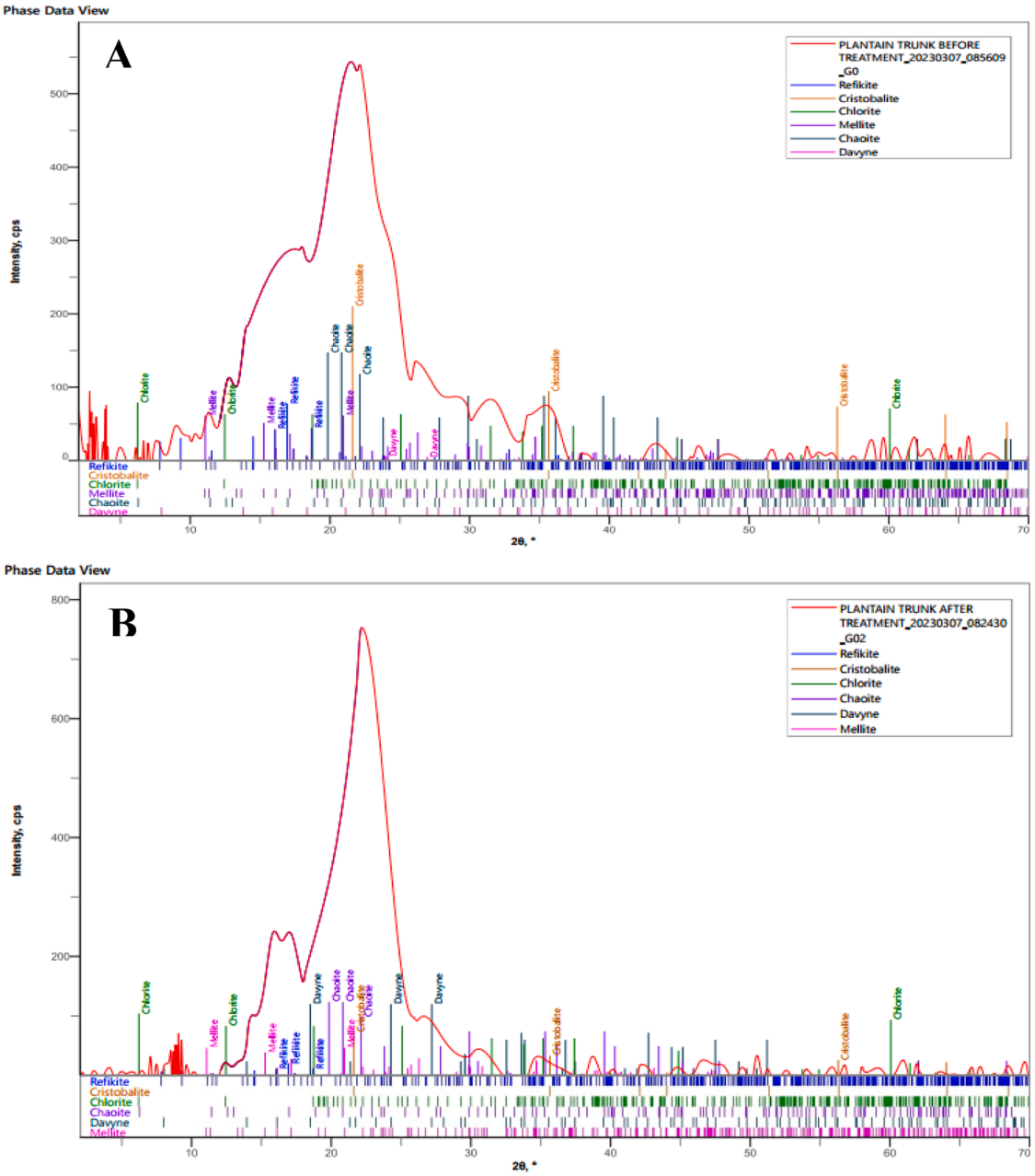

Fig. 3. XRD Analysis of (A) untreated plantain peduncles, and (B) treated plantain peduncles.

crystalline phases that were found (Fig. 3A). A thorough percentage distribution of each phase was quantitatively provided by the XRD spectrum results. Davyne was found to have the lowest distribution of 0.73 %, followed by Chlorite (6.2 %), Cristobalite (6.2 %), and Mellite (6.5 %); Refikite was found to have the largest distribution of the material (72.3 %) followed by Chaoite and Mellite with the distribution of 8.1 % and 6.5 %.

Combining qualitative and quantitative evaluations emphasizes the complexity and variety of the crystalline structure of the material, which is essential for understanding the probable alterations that could follow pre-treatment processes. Therefore, following pre-treatment, XRD analysis showed that Davyne, Chaoite, Mellite, Chlorite, Cristobalite, and Refikite were present (Fig. 3A). As with the crystalline components of the untreated plantain peduncles, Refikite accounted for 72 % of the material, followed by Cristobalite, Chaoite, Chlorite, Davyne, and Mellite with a distribution of 7.5 %, 7.2 %, 5 %, 4.7 %, and 3.4 % respectively (Fig. 3B). The components of the agrowastes persisted following pre-treatment, according to a comparison of the XRD data for the untreated and treated plantain peduncles. The quantitative findings, however, differed somewhat. In contrast to Mellite, which was found to have the lowest percentage (3.4 %) in the treated plantain peduncles, Davyne was found to have the lowest percentage (0.73) in the untreated plantain peduncles. For both the treated and untreated plantain peduncles, refikite retained the greatest percentage.

It is important to note that the XRD result obtained in this study does not significantly change the overall crystalline structure of the plantain peduncles; nevertheless, there are subtle yet meaningful alterations in the phase composition of the treated plantain peduncles, indicating the chemical and enzymatic pretreatments did not destroy or dissolve the main crystalline structure of the treated plantain peduncle.

ANOVA for the quadratic model for lactic acid production using lb. plantarum

The CCD quadratic model for the production of LA using *Lb. plantarum* was examined using the analysis of variance (ANOVA) (Table 1). A *p-value* of <0.0001 and an F-value of 13.25 demonstrated the statistical significance of the model. With a difference of <0.2, the predicted R^2 of 0.6563 and the Adjusted R^2 of 0.8279 are in reasonable agreement. This implies that, within the constraints of the experiment, the model is a reliable predictor of lactic acid generation.

When the main effects of the fermentation parameters were analyzed, the findings indicated that the production of LA was not substantially impacted by temperature, incubation time, or carbon source separately (*p*-values of 0.5522, 0.6689, and 0.7286, respectively). A *p*-value of 0.0003 and an F-value of 20.19, however, showed that pH was a crucial component that had a major impact on LA production. This emphasizes how important it is to keep the pH balance at its ideal level to optimize *Lb. plantarum* fermentation operations. The interaction effects further illustrated the complex interplay among the fermentation factors.

The correlation between carbon supply and incubation time (AB) approached significance (*p*-value = 0.0543), indicating that they may have a combined influence on the production of LA. The findings show that significant relationships between the incubation period and pH (BD, *p*-value = 0.0111) and temperature (BC, *p*-value = 0.0463) were identified. These findings suggest that modifying these factors at the same time could significantly impact LA yield. Despite being only marginally non-significant (*p*-value = 0.0784), the temperature and pH (CD) interaction might nonetheless affect the entire production process. With *p*-values of 0.0170, 0.0075, and 0.0049, respectively, it was also revealed that the quadratic terms for incubation time, temperature and pH were all significant. This implies that the production of LA and these factors have a non-linear relationship, which further makes the optimization process critical. The model accurately and without major errors represents the experimental data, according to the lack of fit test, which revealed no significant lack of fit (*p*-value = 0.1157). SD 6 presents the entire equation in terms of actual factors.

Predictive model's accuracy of CCD

The accuracy of the predictive model in maximizing fermentation conditions utilizing a CCD with LA concentration acting as the response variable was evaluated in this study using a 'Predicted vs. Actual' plot. The model's performance can be diagnostically

Table 1
ANOVA for the experimental results of the CCD quadratic model for Lactic Acid production using *Lb. plantarum*.

Source	Sum of Squares	df	Mean Square	F-value	*p*-value	
Model	336.27	11	30.57	13.25	< 0.0001	Significant
A-Carbon Source	0.4370	1	0.4370	0.1894	0.6689	
B-Incubation Time	0.2871	1	0.2871	0.1244	0.7286	
C-Temperature	0.8485	1	0.8485	0.3678	0.5522	
D-pH	46.58	1	46.58	20.19	0.0003	
AB	9.86	1	9.86	4.27	0.0543	
BC	10.66	1	10.66	4.62	0.0463	
BD	18.75	1	18.75	8.13	0.0111	
CD	8.09	1	8.09	3.51	0.0784	
B^2	16.16	1	16.16	7.00	0.0170	
C^2	21.25	1	21.25	9.21	0.0075	
D^2	24.09	1	24.09	10.44	0.0049	
Residual	39.22	17	2.31			
Lack of Fit	36.09	13	2.78	3.54	0.1157	Not significant
Pure Error	3.13	4	0.7835			
Cor Total	375.49	28				

evaluated by plotting predicted values against actual experimental data. The x-axis of the diagram displays the LA concentrations as observed experimentally, while the y-axis displays the values predicted by the model. Each point on the graph represents a separate experimental run, enabling a visual comparison of the projected and actual values. The data points are seen to cluster along the diagonal line, which indicates a great correlation between the actual and the expected values (Fig. 4). This demonstrates a strong correlation and suggests that the model can precisely forecast the LA yield under the examined experimental conditions. The alignment of most of the data points with the diagonal line indicates that the model used in this experiment is reliable and effective in forecasting lactic acid concentrations. This confirms that the model can be used to improve fermentation conditions in future studies.

Interaction of fermentation conditions and their impact on LA yield

Below, interactions between different fermentation parameters, including pH, incubation duration, carbon source concentration, and fermentation medium temperature, were noted, evaluated, and examined.

Interaction between incubation time and temperature

The response surface analysis (Fig. 5) shows how temperature and incubation time affect Lb. plantarum's ability to produce LA. The findings indicate that, after an incubation period of 6–7 h, the concentration of LA increases marginally with temperature. In particular, the data show that two maximal lactic acid productions at various extreme temperatures can be produced by the combination of temperature and incubation period.

LA yield was observed to be highest at temperatures of 50 °C, with an incubation time of 6 h, after which not much effect was observed until after 23 h of incubation. Optimum production of LA was observed to be within a temperature range of 45 °C to 50 °C with an incubation time of 6 and 22 – 24 h, with which the LA yield was observed to be 42 g/L.

Interaction concentration of carbon and incubation time

Fig. 6 shows below displays the impact of different carbon concentrations and incubation time on the yield of LA. The findings show that when the proportion of the carbon concentration increases from 1 % to 4 %, the concentration of LA yield was understood to increase with longer incubation time.

LA yield was found to be highest at 1 % carbon concentration and decreased with longer incubation times. Additionally, there was a noticeable decrease in the concentration of LA when the carbon concentration rose to 3–4 %, particularly when the incubation period was 11–18 h. When the carbon source was between 1 and 4.5 % and the incubation period was between 6 and 24 h, the LA concentrations were found to be high — approaching 43 g/L. The rise in LA yield, interestingly, levels off after the carbon source concentration reaches 4 %. This suggests that there might be a saturation limit at which more carbon does not increase the yield of LA. This implies that although having a carbon supply is essential, there is a threshold concentration beyond which *Lb. plantarum* may not be able to use more substrate effectively.

Interaction between pH and incubation time

Fig. 7 shows the relationship between incubation time and pH and how their interaction affects the production of LA. The findings indicate that the concentration of LA is significantly influenced by pH, with an ideal range of 8 to 10. This range maximizes the formation of LA, particularly when the incubation time is prolonged to 6–9 h. The highest LA concentrations were obtained at a pH of roughly 9.8 to 10, when incubated for 6–7 h, yielding LA concentrations of 46 g/L. LA production was observed to begin to increase at a

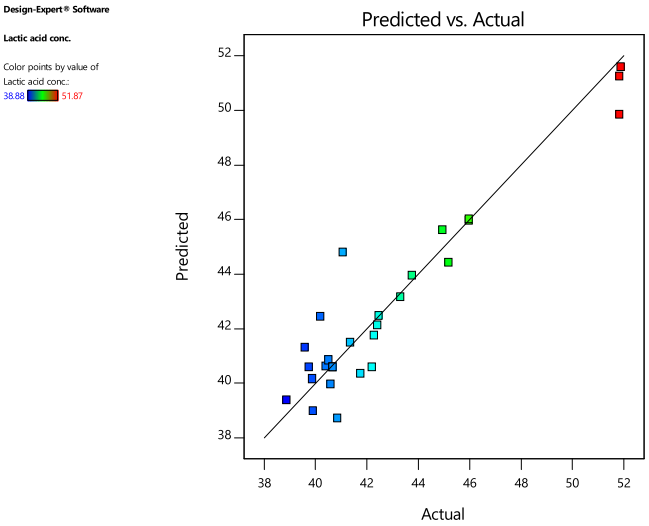

Fig. 4. Correlation between experimental and predicted values of lactic acid production.

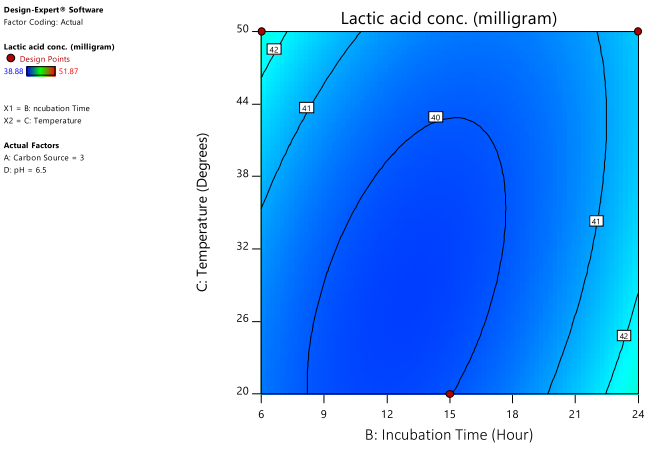

Fig. 5. Effect of incubation time and temperature on LA yield.

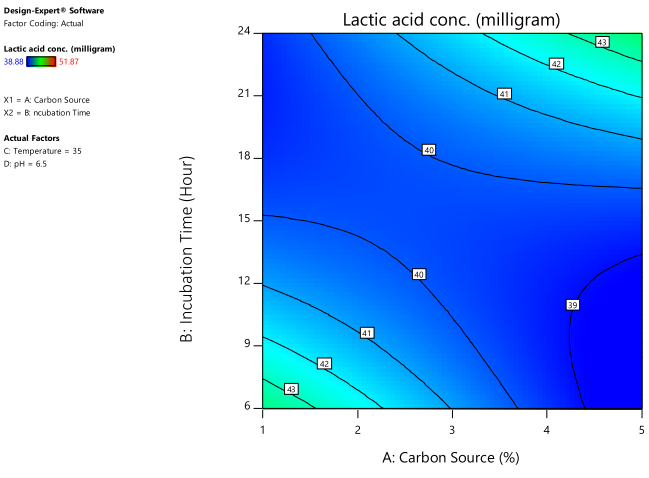

Fig. 6. Effect of incubation time and carbon concentration on the yield of LA.

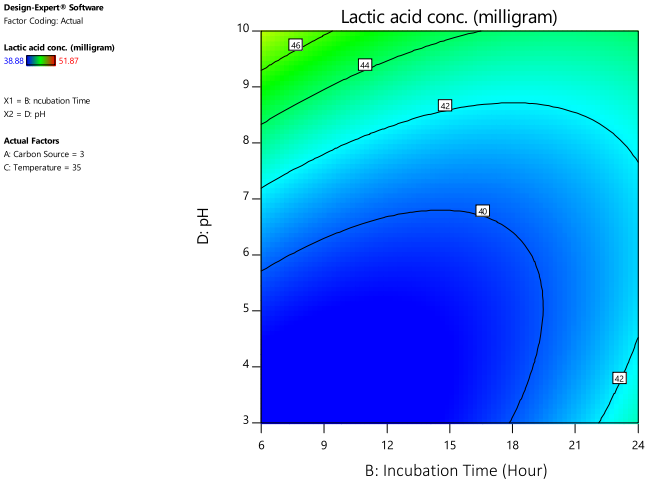

Fig. 7. Effect of incubation time and pH on LA concentration.

pH of 7.2 and the concentration kept rising as the pH approached 8. However, LA production sharply decreases when the pH deviates from this optimal range, becoming more acidic.

This finding emphasizes how sensitive *Lb. plantarum* is to pH variations, which probably have an impact on the activity of important enzymes involved in the production of LA. Therefore, it is essential to keep the pH within the ideal range to maximize production efficiency.

Interaction between temperature and pH

The effect of temperature and pH on the production of LA is shown in Fig. 8. The findings show that in order to obtain high LA production, temperature and pH must be properly regulated. The ideal temperature range for lactic acid synthesis is between 40 °C and 42 °C, while the pH range is limited to 9 to 10.

LA concentrations peak at about 44 g/L within this optimum conditions. Remarkably, neither the temperature nor the amount of LA changed significantly. Even at ideal temperatures, lactic acid production decreases when the pH rises above 8.5. These results demonstrate how temperature and pH interact to regulate the formation of lactic acid. According to the results, even when temperature is maintained or increased, lactic acid yield is discouraged when pH falls.

Optimized conditions desirability response

Several LA concentrations were obtained from the operational runs carried out under optimum conditions (Table 2), with Run 1 being chosen for small-scale production and a confirmatory test.

Production and partial purification of lactic acid

After employing n-butanol as the extraction solvent, 483 mL of crude lactic acid were extracted using the phase partitioning extraction technique (Supplementary Data, 6). After measuring the crude lactic acid's pH, a pH of 6.4 was found following a pH of 9.9. Golden coloration was the observed coloration of the crude lactic acid before it was subjected to vaporization to get the pure lactic acid.

Discussion

Irrespective of the type of pre-treatment technique employed, the biodegradability and digestibility of plant materials (including plantain peduncles) increase due to the effect of pretreatment, which causes the breakdown of complex polymeric lignocellulosic materials into simpler accessible chemical structures with an increased surface area while simultaneously removing the hemicellulose and lignin contents. However, even though agricultural waste provides a cheap carbon source, it presents difficulties owing to the polymeric complex structures it possesses [37]. As such, utilizing pre-treatment, microorganisms, including bacteria, can act on the carbon constituent of the agro-waste with the help of the enzymes they produce.

As this study revealed, various physicochemical analyses were conducted on the untreated and treated plantain peduncle to monitor the effectiveness of pretreatment procedures [38]. The SEM results revealed that the surface architecture of the untreated and treated peduncles differs as anticipated because of the effects of the enzymatic and alkaline treatments, which delignified the material's cell wall and gave the cells a loosely packed appearance. These results are consistent with those of Van et al. [39], who found changes in the surface architecture of agrowastes following pretreatment. More so, the study of Li et al. [40] revealed that pre-treatment of plant biomass causes morphological changes in the material's surface. The FTIR spectrum before pretreatment was dominated by characteristic absorption bands of lignocellulosic biomass observed in this study. The presence of cellulose and hemicellulose was observed owing to a high concentration of hydroxyl groups reported within this study, with a broad O–H stretching vibration reported around 3276 cm^{-1}, similar to the findings of Tsaousis et al. [41]. The FTIR spectrum before pretreatment also

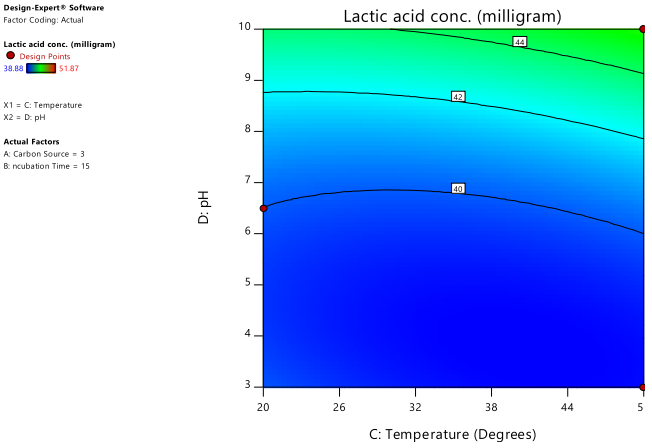

Fig. 8. Effect of temperature and pH on LA concentration.

Table 2
LA Production analyzed data from design expert's desirability response for 25 solutions.

Number	pH	Temperature	Carbon Source	Incubation Time	Lactic acid conc. (g/L)	Desirability	
1	9.999	50.000	5.000	6.000	47.011	0.855	Selected
2	10.000	50.000	4.999	7.976	46.381	0.833	
3	9.999	49.999	4.952	6.000	47.08	0.854	
4	10.000	50.000	5.000	7.775	46.438	0.835	
5	10.000	49.999	5.000	6.145	46.962	0.854	
6	9.863	50.000	5.000	23.702	46.462	0.836	
7	10.000	50.000	4.853	6.000	47.222	0.852	
8	10.000	49.861	4.998	23.315	46.522	0.837	
9	10.000	49.755	4.985	6.000	46.993	0.851	
10	9.919	50.000	5.000	23.588	46.503	0.837	
11	10.000	50.000	4.805	6.000	47.289	0.851	
12	9.994	49.999	5.000	7.412	46.53	0.838	
13	9.957	49.942	4.998	6.000	46.894	0.851	
14	10.000	50.000	4.457	6.000	47.78	0.84	
15	10.000	50.000	4.78	6.000	47.325	0.85	
16	10.000	50.000	4.525	6.001	47.683	0.842	
17	9.974	50.000	5.000	6.414	46.801	0.848	
18	10.000	49.313	4.901	6.000	47.035	0.843	
19	10.000	49.862	5.000	6.538	46.804	0.847	
20	10.000	50.000	5.000	23.711	46.657	0.843	
21	10.000	49.413	4.999	6.000	46.913	0.846	
22	9.880	49.931	5.000	6.000	46.687	0.843	
23	10.000	49.923	5.000	6.738	46.747	0.845	
24	10.000	50.000	5.000	6.94	46.694	0.844	
25	10.000	49.979	5.000	6.849	46.72	0.845	

revealed the presence of lignin substances due to the presence of strong C–H stretching and bending vibrations at 2922, 1457, and 1312 cm^{-1} corresponding to aliphatic chains as reported by Sunkar and Bhukya [42]. More so, the peaks at 1103 and 1032 cm^{-1} correspond to C–O stretching from alcohols, ethers, and esters, indicating the polymeric nature of the plant biomass. Aromatic $C = C$ bonds and out-of-plane C–H bending characteristics of lignin-derived phenolics were observed by peaks observed at 1617 cm^{-1} and 775 cm^{-1} bands, which are similar to the results obtained by Tsaousis et al [41]. After pretreatment, several new absorption bands appeared, while others shifted or decreased in intensity. Notably, peaks at 1736 cm^{-1} and 1796 cm^{-1} (carbonyl $C = O$ stretching) formed, which were not present in the untreated sample. These are usually associated with ketones, aldehydes, or esters created by cleavage of ester and glycosidic bonds during pretreatment, a common feature in recent chemical pretreatment studies [42]. Bands at 2098 and 2194 cm^{-1} ($C{\equiv}N$ stretching) and at 2310 cm^{-1} ($N = C = O$ stretching) indicate the formation or exposure of nitrogen-containing groups, such as protein denaturation or reactions with nitrogenous reagents during pretreatment. Although these high-frequency features are uncommon in untreated biomass, nonetheless study suggests nitrogen-rich materials can produce such features [41]. Additionally, increased N–H stretching vibrations around ≈3786–3928 cm^{-1} point to a higher presence of amine groups after treatment, potentially due to partial protein hydrolysis or deamination that exposes amino groups [41]. While the O–H and C–O bands persisted post-treatment, minor shifts and decreases in their intensity indicate some carbohydrate degradation or solubilization, consistent with partial de-esterification or removal during pretreatment. Aromatic signals at approximately 1617 and 1923 cm^{-1} remained but with possibly reduced intensity, implying lignin structures were only partly removed, a pattern commonly seen in moderate alkaline or oxidative processes as described by Sunkar and Bhukya [42].

The XRD analysis provides important details about the crystallographic phases that existed both before and after the plantain peduncle was pretreated. A comparison analysis of the data revealed that the general crystalline composition remained unchanged, while there were slight quantitative differences between the untreated and the treated samples. The six crystalline phases found in both treated and untreated plantain peduncles were davyne, chaoite, mellite, chlorite, cristobalite, and refikite. Based on the consistency shown in the types of crystalline phases, the pretreatment process may not have significantly altered the core crystalline structure of the plantain peduncles. Refikite, which accounted for about 72 % of the material in both cases and slightly decreased in the treated sample, remained the dominating phase. Given that it kept its structural integrity throughout treatment, this illustrates how resilient refikite is. Both samples also contained additional phases, such as Chaoite, Chlorite, Mellite, Cristobalite, and Davyne, suggesting that these crystalline forms are a normal component of the plantain peduncle's structure and are unaffected by the specific pretreatment that was used.

Although the qualitative investigation revealed no alterations in the crystalline phases, the quantitative research revealed minor yet noteworthy variations in their distribution. Davyne phase in the untreated sample increased from 0.73 % to 4.7 % in the treated sample, indicating the most noticeable alteration. Conversely, Mellite saw a drop from 6.5 % to 3.4 %. These modifications suggest that the pretreatment process affected the crystallization patterns, potentially due to rearrangement or phase transition. As a result, some phases increased in numbers at the expense of others. The ratio of chaoite to cristobalite was changed slightly. After pretreatment, Cristobalite percentage increased to 7.5 % from 6.2 % while Chaoite percentage composition decreased to 7.2 % from 8.1 %. These minor changes imply that the pretreatment procedure changed the proportions of these phases, perhaps by changing the way they crystallized due to heat or chemical effects. Additionally, the chlorite concentration decreased from 6.2 % to 5 %, suggesting a minor

alteration or deterioration during pretreatment. Heat exposure or chemical reactions during pretreatment may have caused this reduction. Although minute, a change in the crystalline structure was nonetheless observed.

ANOVA was used to assess the statistical significance of the predictability of the CCD quadratic model for LA production using Lb. plantarum. The model employed in this study was found to be highly significant; the F-value was reported to be 13.25 with a *p*-value <0.0001, indicating that the experimental design had outstanding predictive power. This finding is similar to the results of Thakur et al. [43] and Tefara et al. [44], where the CCD model for LA production was statistically significant in accurately predicting the optimized conditions to yield a better LA fermentation yield. More so, the ANOVA result revealed there was no significant lack of fit, according to the lack of fit test ($p = 0.1157$), indicating that the model accurately represents the experimental data without significant error, which is similar to the result presented by Thakur et al. [43] and Tefara et al. [44]. The 'anticipated vs the actual' figure also demonstrated a strong agreement between the expected and experimental values for LA yield, with a close clustering of data points along the diagonal line. This suggests that the model has the potential to optimize fermentation systems, as it can accurately predict LA production using the parameters under study.

The fermentation parameters of the carbon source, incubation time, and temperature had non-significant *p*-values of 0.7286, 0.6689, and 0.5522, respectively, demonstrating that these parameters individually had no discernible impact on the production of LA. However, in other studies like that of Thakur et al. [43], all fermentation parameters like carbon (molasse), incubation time, pH, and temperature individually had a significant impact on LA production, which was not the case in our study. The likely reason for this variation could be the difference in the species of microorganisms and the carbon sources utilized by Thakur et al. [43]. Among the fermentation parameters in our study, the impact of pH alone was observed to be statistically significant with a *p*-value of 0.0003. This emphasizes the important role of pH in microbial fermentation. Since it directly influences the bacterial metabolic activities, such as nutrient transport, enzyme function, and integrity of the cell membrane, pH has already been established as a critical component required for bacterial growth and development [45]. This shows why pH in our study had a great role in influencing the extent of LA production by *Lb. plantarum*.

While other factors, such as temperature, incubation time, and carbon concentration assessed in this study, did not individually affect LA fermentation by *Lb. plantarum*, however, their interactions were found to have a varying degree of impact on LA yield. From the ANOVA result, it was identified that a two-factor interaction between incubation time and pH was statistically significant in LA fermentation. According to the results, LA fermentation by *Lb. plantarum* is optimum at pH values between 8 and 10. The production of LA is highly sensitive to pH [45]. It was observed that LA production begins to increase gradually at a pH of about 7.2 and that the concentration continues to rise as the pH approaches 8. This indicates that LA formation is significantly increased within this pH window, particularly if the incubation time is extended to 6–9 h. LA production was observed to be maximum at pH values between 9.8 and 10 with an incubation time of 6 to 7 h. This finding is similar to that of Abdel-Rahman et al. [46], where it was reported that maximum LA production was observed at a pH of 9 using *Enterococcus hirae* BoM 1–2. However, the study of Pau et al. [47] reported otherwise. In their study, lactic acid production was reported to be highest at a pH of 3.11 with an incubation time of 14 days [47]. These findings are different from the results of Omay and Guvenilir [48], who reported a maximum yield of LA at a pH of 6. These varying reports of optimal pH for LA production are an important factor to consider when designing a fermentation system, since varying bacterial strains have different pH functionality and tolerance.

A second interaction evaluated in this study was between temperature and incubation time, which was found to be statistically significant for LA production by *Lb. plantarum*. The findings in this study revealed that these two factors (temperature and incubation time) have an intricate connection and that, generally speaking, LA concentration rises with temperature, particularly during the 6–7 h of incubation. At the opposite temperature extremes of 50 °C and 23 °C, however, the interaction reveals two distinct peaks of LA production. This dual peak shows that LA production can be efficiently carried out by *Lb. plantarum* both at low and high temperatures, albeit under different incubation circumstances as reported by Abdel-Rahman and Sonomoto [49] and Zhou et al. [50], respectively. A longer incubation time appears to be primarily responsible for increasing the yield of LA achieved mostly at a higher temperature. Temperatures of 45 °C and 50 °C were reported to be optimal in this study; however, with extended incubation time ranging between 6 and 20 h, with LA yield of up to 42 g/L. This demonstrates that while LA production is largely influenced by temperature, incubation time can significantly boost yields, particularly in prolonged high-temperature settings.

The interaction between carbon concentration and incubation time was also evaluated. According to our ANOVA result, however, the interaction was marginally significant with a *p*-value of 0.0543. As carbon supply concentration rises from 1 % to 4 %, LA generation gradually decreases. According to Kacaribu and Darwin [51], excessive intake of carbon during fermentation could be the leading cause of a decrease in LA production with an increase in carbon concentration. In their report, they demonstrated that high carbon concentration can cause osmotic stress and cellular lysis, which can result in the decrease of microbial activity, leading to a longer lag phase. This explains why longer incubation times increase the formation of LA, especially at lower carbon concentrations. As witnessed, at 1 % carbon, LA production was still high, but started to decline with longer incubation times. This suggests that poorer production may have resulted from the bacteria eventually using up all of the available substrate at low carbon concentrations. On the other hand, as the carbon concentration increased to 3–4 %, the LA concentration decreased more noticeably, particularly when the incubation time was prolonged to 11–18 h. Inefficiencies in substrate use are indicated by this decline in rising carbon concentrations, which could be brought on by metabolic limitations or the inhibitory effects of excess carbon [51]. Curiously, the highest LA concentrations were achieved with carbon sources at 1 % or 4.5 % with incubation times ranging from 6 to 24 h.

Similarly, temperature and pH were observed to be marginally significant in this study, with a *p*-value of 0.0784. Although the interaction of this factor produces little effect on LA fermentation. Nonetheless, when factoring in this fermentation process, it becomes necessary that both temperature and pH are adjusted correctly for optimal LA production. Specifically, a pH range of 9 to 10 and a temperature range of 40 °C to 42 °C were identified to be optimum conditions. The necessity of maintaining precise fermentation-

related environmental conditions was highlighted by the fact that LA concentrations increased in this optimal window at roughly 44 g/L. Interestingly, when the temperature was adjusted within the 40 °C to 42 °C range under study, there were no appreciable changes in the amount of LA produced. This indicates that pH may be more important in this process, even though temperature is important [47]. Conversely, a pH shift, particularly below 8.5, resulted in a significant reduction in the synthesis of LA even at the optimal temperature. This demonstrates how sensitive Lb. plantarum is to pH changes, which may have an impact on the activity of metabolic pathways and enzymes necessary for the production of LA.

These findings show that temperature and pH are interdependent, with pH being the more crucial factor in controlling the production of LA. Even at the optimum temperature, a drop in pH inhibits the production of LA, emphasizing the need to maintain alkaline conditions (pH 9–10) to maximize fermentation yield. As a result, careful control of both parameters is necessary, with an emphasis on making sure pH stays within the ideal range. Following the phase separation technique and taking the wavelength at 390 nm, 44.5 g/L was obtained after the fermentation conditions were modified based on the desired output produced by the CCD analysis

Conclusion

Lactiplantibacillus plantarum (OM510300) can be isolated from agrowaste dumpsites. Alkaline and enzymatic pre-treatment of plantain peduncles increased the usability of the sugar content of the agrowastes by *Lb. plantarum* for the fermentation of lactic acid. The Central Composite Design (CCD) model has predictive power for accurately predicting the optimum condition required for lactic acid production by *Lb. plantarum.* The fermentation conditions, such as pH and temperature, have a significant effect on lactic acid production, with optimal conditions observed for the pH to be 9.8 to 10 and for incubation periods of 6 to 7 h with a temperature range of 40 °C to 42 °C.

This research revealed that plantain peduncles can be used as a carbon source by *Lb. plantarum* (OM510300) for the production of lactic acid in a controlled environment.

Consent to participate

All authors participated actively in this research and manuscript preparation.

Consent to publish

The content of the article was approved by all authors, and they provided explicit consent to submit.

Ethical approval

Not applicable.

Funding

During this research and manuscript preparation, the authors confirm that no grants or funds were received.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are thankful to the Deanship of Graduate Studies and Scientific Research at University of Bisha for supporting this work through the Fast-Track Research Support Program.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sciaf.2025.e02998.

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