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Research article

Investigating chemical pre-treatment methods: Valorization of wheat straw to enhance polyhydroxyalkanoate (PHA) production with novel isolate *Bacillus paranthracis* RSKS-3

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ABSTRACT

Wheat is a crucial food crop worldwide, generating straw upon post-harvest. The straw is often burned to enhance soil fertility, leading to massive air pollution. In this study, wheat straw was investigated for the production of Polyhydroxyalkanoate (PHA) using the novel isolate *Bacillus paranthracis* RSKS-3. The wheat straw was pulverized and valorized with different acids (2 % and 4 % H_2SO_4, acetic acid, and hydrochloric acid) and alkalis (2 % and 4 % NaOH, calcium carbonate, and potassium hydroxide). The validation of carbohydrates was done using the Molisch test by analyzing purple-ring production and the DNS test which concluded 4 % H_2SO_4 as an effective treatment with a maximal sugar yield of 5.04 mg/mL at $P < 0.05$. The bioconversion efficiency of the extract to PHA resulted in 0.87 g/L by *Bacillus paranthracis* RSKS-3, later characterized by Ultraviolet (UV)-spectroscopy and FT-IR assessment. The findings of the research offer a potential strategy to mitigate airborne pollutants that result from smouldering wheat straw, thereby contributing significant improvements to sustainable development.

1. Introduction

The accumulation of wheat straw, an agricultural residue remaining after wheat harvest, is a substantial environmental challenge due to its major contribution to soil and water contamination [1]. Wheat straw breakdown emits methane, a highly potent greenhouse gas, which contributes to the phenomenon of climate change [2]. To tackle this problem, different methods for resolving it have been investigated. One strategy entails implementing cutting-edge agricultural techniques, such as reintegrating wheat straw into the soil as an organic fertilizer or utilizing it for bioenergy generation, hence diminishing the reliance on conventional disposal methods [3].

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Furthermore, progress in biotechnology has resulted in the creation of microbial remedies that expedite the breakdown of wheat straw, thereby reducing its ecological footprint [4]. Additionally, investigations into alternate applications of wheat straw, such as its use in the manufacturing of environmentally friendly materials or as a raw material for sustainable biomaterial, present encouraging opportunities for reducing the pollution linked to this agricultural residue [5]. The integration of various methodologies can establish a more sustainable and cyclical framework, effectively tackling the issue of wheat straw pollution while concurrently fostering environmental preservation [6].

Although biological remedies for wheat straw treatment have their advantages, there is an increasing acknowledgment of the necessity for chemical treatments, especially when it comes to developing economically feasible bioproducts like polyhydroxyalkanoates (PHA) [7]. Chemical treatments can provide benefits in terms of the effectiveness of the process, the potential to increase production, and the amount of product obtained. The chemical method may be faster and more effective than some biological methods at breaking down the complex lignocellulosic composition of wheat straw [8]. This offers a faster route to liberate fermentable sugars, which can then be used for future PHA generation. In addition, the use of chemical treatments can improve the purity of extracted cellulose, which is an essential precursor for PHA production. This has the potential to produce bioproducts of superior quality. Although biological treatments are environmentally friendly, incorporating chemical methods into the process could enhance the production of economically valuable bioproducts from wheat straw [9]. This would help in developing a sustainable and economically viable solution to tackle wheat straw pollution.

The objective of the research was to screen nine different chemicals (2 % H_2SO_4, 4 % H_2SO_4, acetic acid, hydrochloric acid, 2 % NaOH, 4 % NaOH, calcium carbonate, potassium hydroxide, and distilled water) for the pretreatment of wheat straw. The screened chemicals were subjected for validation of carbohydrate through Molisch's test and best treatment was selected based on quantitative DNS assay. The selected chemical-based pretreatment was analyzed for growth proliferation of novel isolate *Bacillus paranthracis* RSKS-3 isolated from sewage sample for Polyhydroxyalkanoate (PHA) production. The confirmation of PHA was conferred using UV spectroscopy ranging from 800 to 200 nm, and functional group analysis by FT-IR.

2. Methodology

2.1. Wheat straw collection and subsequent chemical pretreatment

The wheat straw (WS) was acquired from fields near Maheru village (31.248610 latitude, 75.709992 longitude), situated in Punjab, India. To eliminate any impurities, the WS was purified using tap water and subsequently underwent a drying-out procedure in a high-temperature oven at 60 °C for two weeks [10]. Afterward, the dried WS was pulverized using a high-speed multi-functional crusher, a milling device retrieved at Lovely Professional University, Punjab, India, and then filtered through a 1 mm mesh screen. The treatment procedure was carried out using 2 and 4 % sulfuric acid, acetic acid, hydrochloric acid, 2 and 4 % sodium hydroxide (NaOH), calcium carbonate, potassium hydroxide, and distilled water [11]. In 100 mL Erlenmeyer flasks, 50 mL of each chemical treatment solvent with 10 g of WS was added. The flasks were subjected to autoclaving at a temperature of 121 °C for 1 h, following the procedure described [12]. The flask solutions were cooled to the ambient temperature and were filtered sequentially using muslin cloth and Whatman filter paper following autoclaving. The pH of the filtrates obtained after chemical pretreatment was neutralized to 7.0 by the addition of 1 M NaOH and HCl. The remaining remnants were rinsed with distilled water to remove any residual acid or alkali.

2.2. Analytical approach for sugar analysis: Molisch's test and DNS test

The treated WS extracts were assessed to ascertain the sugars that were liberated through the pretreatment operation. The Molisch assay was utilized to identify carbohydrates in various WS extracts after pretreatment [13]. The qualitative analysis of the extracts involved the examination of the carbohydrates, while the quantification of the absolute sugar concentration was carried out employing a standard DNS test [14].

2.3. Compositional analysis of pretreated WS extract

2.3.1. FT-IR analysis

Functional group identification of raw and processed WS was conducted using FT-IR assessment. FT-IR bands were obtained using the PerkinElmer Spectrum 2 instrument. Data peaks were measured and examined within the range of 400–4,000 cm^{-1} using a sensor that had a resolution of 4 cm-1. The study recorded an average of 128 scans per sample [15].

2.3.2. XRD analysis

An X-ray diffraction (XRD) device, namely the Bruker D8 Advance, was used to analyze the crystallite phase of both untreated and chemically altered WS. The diffractograms were acquired by collecting data within the angular range of 10° to 40°.

2.3.3. SEM analysis

The examination of structural structures in untreated and pretreated WS was conducted using scanning electron microscopy with a JEOL JSM-7610F PlusEDS equipped with an Oxford EDS LN2 free system [16]. This analysis was performed at Lovely Professional University in Punjab, India.

2.4. Eco-friendly biopolymer development: PHA production, extraction, and identification using 4 % H_2SO_4 pretreatment

The Bacillus paranthracis RSKS-3 bacterial stock culture was isolated from a sewage sample (31.252194401132233 latitudes, 75.681202985042 longitudes), purified, and subcultured periodically, and stored at 4°C using nutritional agar until needed. The 16s rRNA of the Bacillus paranthracis RSKS-3 bacterium, and the phylogeny relationship, further characterize its genomic features and evolutionary lineage. *The PHA was produced using minimum media: glucose (*10 g/L*), yeast extract (*2.5 g/L*), magnesium chloride (*0.2 g/L*), sodium chloride (*0.5 g/L*), and peptone (*2.5 g/L*)* [17]. *The production tests were performed using* 100-mL *flasks filled with* 50 mL *of the working medium. In an alternative method, the carbon source used was pretreatment wheat straw, which was added in the form of* 2 mL *of* 10 g/L *WS extract of the best treatment based on the DNS test. This was done to substitute glucose in the previously indicated media to conduct comparative research. The flask underwent autoclaving at a temperature of 121 °C and a pressure of 15 psi for a duration of 20 min. Afterward, it was allowed to cool to the surrounding temperature. An inoculum of Bacillus paranthrancis RSKS-3, consisting of a 2 % culture with a cell concentration of* 10^8 *cells per mL, was used. The mixture was thereafter incubated for 48 h at a temperature of 37 °C in an orbital shaker set to 120 revolutions per minute. The liquid component was centrifuged at a force of 8000 RPM for 10 min to collect the cell biomass. The cellular biomass was solubilized by adding* 1 mL *of distilled water to preweighed Eppendorf tubes. The tubes underwent a second round of centrifugation at a force of 10,000 RPM, after which the liquid portion was discarded. The solid residue was thereafter subjected to drying at a temperature of 60 °C until it achieved a stable weight* [18]. *The pellets underwent treatment with a 6 % sodium hypochlorite (NaClO) solution to process the cellular debris* [19]. *Subsequently, positioned in an orbital shaker incubator at a temperature of 30 °C for a duration of 2 h. Following incubation, the tubes underwent centrifugation at a force of 8000 RPM for a duration of 20 min.*

After separating the liquid component, the solid particles were rinsed with water from distillation. The liquid was transferred to Eppendorf tubes and centrifuged at a force of 10000 g for 20 min. Afterward, the liquid part above the sediment was discarded. The remaining cellular waste was removed by washing the solid mass with acetone. In order to ensure a constant weight, the pellets underwent a drying procedure at a temperature of 60 °C. Prior to being immersed in concentrated sulfuric acid (2 mL), the dehydrated PHA granules (1 mg) underwent a 10-min heating procedure. The UV spectra were measured within the wavelength range of 800 to 200 nm [20], utilizing a calibration baseline established using sulfuric acid and a standard crotonic acid purchased from Central Drug House Laboratory Reagent in New Delhi, India.

2.5. FT-IR characterization of polyhydroxyalkanoate (PHA) granules

An analysis was conducted to characterize the functional groups of the PHA granules generated by Bacillus paranthrancis RSKS-3. The FT-IR technique, specifically employing the PerkinElmer Spectrum 2 instrument, was utilized to capture spectra. The detector resolution was set at 4 cm^{-1}, and the spectral range spanned from 500 to 3500 cm^{-1}. The samples underwent around 128 scans, and the resulting data was documented as peaks [20].

3. Result and discussion

3.1. Wheat straw procurement and subsequent chemical pretreatment

The selection of acid or alkali for pre-treatment is indicative of the accessibility of these chemicals, but the specific pre-treatment solutions differed among the investigations. The pre-treatment using 2 % H_2SO_4, 4 % H_2SO_4, acetic acid, hydrochloric acid, 2 % NaOH, 4 % NaOH, calcium carbonate, and potassium hydroxide resulted in the partial breakdown of lignin as compared to distilled water.

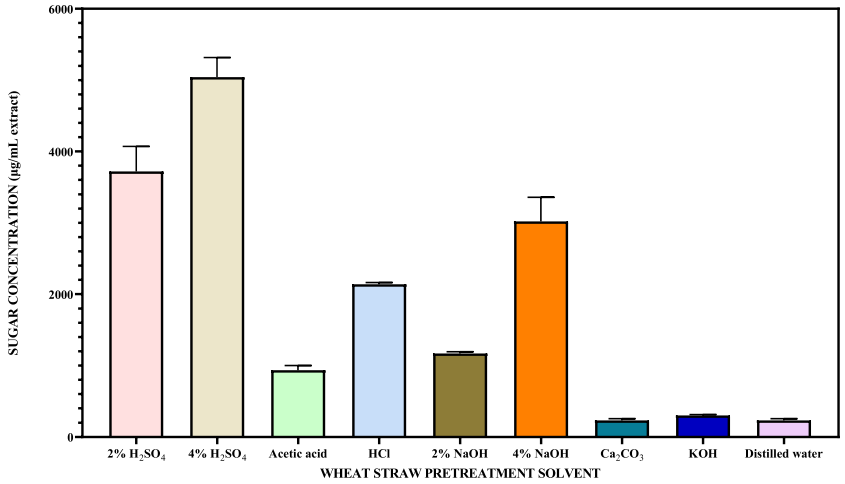

Fig. 1. Analysis of sugar from wheat straw using different acids and alkalis, and distilled water treatment.

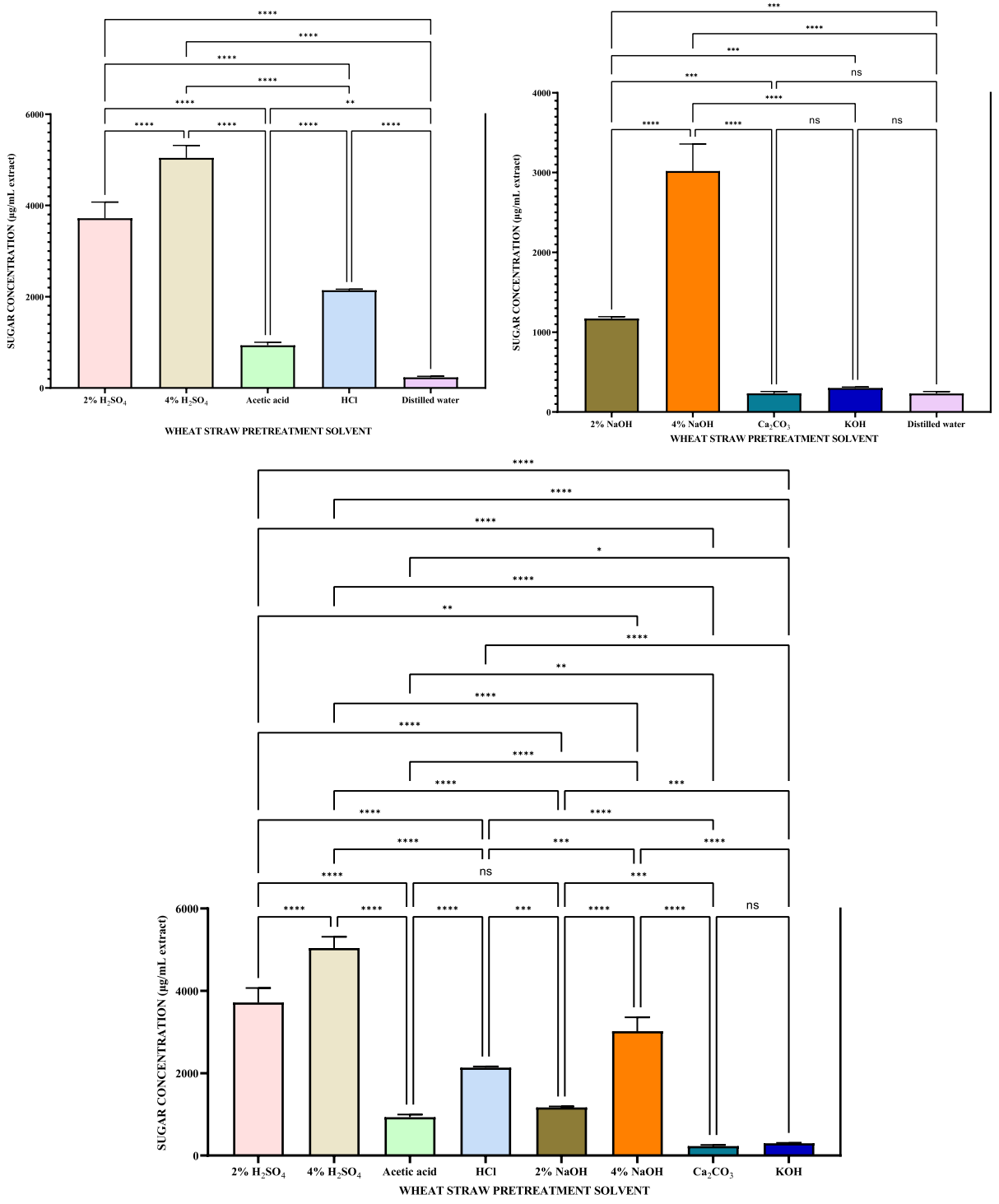

Fig. 2. (a): Tukey's comparison model as a statistical tool for different acid and distilled water treatments of wheat straw, where ** represents $P < 0.01$, *** represents $P < 0.001$, and **** represents $P < 0.0001$.**(b):** Tukey's comparison model as a statistical tool for different alkali and distilled water treatments of wheat straw, where ** represents $P < 0.01$, *** represents $P < 0.001$, and **** represents $P < 0.0001$. **(c):** Tukey's comparison model as a statistical tool for different acid and alkali treatments of wheat straw, where ** represents $P < 0.01$, *** represents $P < 0.001$, and **** represents $P < 0.0001$.

which exposed the intricate cellulose and hemicellulose present in the wheat straw. Significantly, exposed cellulose and hemicellulose are recognized as promising carbon sources for essential industrial substances. This highlights the significance of the pre-treatment method in effectively utilizing wheat straw, which is a copious, cost-efficient, and renewable resource for industrial use. The effectiveness of using 4 % sulfuric acid as a pre-treatment approach is validated across multiple criteria like sugar analysis, FT-IR, XRD, and SEM techniques. This concentration showcases the most effective breakdown of lignin inside the intricate structure of wheat straw, achieving a harmonious combination of efficiency and controlled degradation. The exposure of cellulose and hemicellulose that occurs, as a result, is of utmost importance for industrial applications. Furthermore, the economic viability of utilizing sulfuric acid, which is readily available and cost-effective, boosts the practicality of using wheat straw on a wide scale in industrial applications.

3.2. Analytical approach for sugar analysis: Molisch's test and DNS test

The pretreatment using nine chemicals was screened for the presence of the carbohydrate using the Molisch test, a qualitative approach by the formation of a purple ring. All nine pretreatment processes were positive for Molisch's test, which indicates partial degradation of lignocellulosic material to cellulose and hemicellulose occurred. However, all nine pretreatments were further screened for the quantity of sugar liberated by the DNS assay. The higher amount of reducing sugars in the WS extract indicates a greater capacity to be utilized as a substrate for bioproduct synthesis. Fig. 1 and S1 (Supplementary Material section) display the computed aggregate reducing sugars following the pre-treatment of WS using a 2 % H_2SO_4 solution, 4 % H_2SO_4, acetic acid, hydrochloric acid, 2 % NaOH, 4 % NaOH, calcium carbonate, potassium hydroxide, and distilled water. The results were computed utilizing the formula derived from a standard glucose curve in our prior investigation [12].

The treatment procedures with 2 % H_2SO_4, 4 % H_2SO_4, acetic acid, hydrochloric acid, 2 % NaOH, 4 % NaOH, calcium carbonate, potassium hydroxide, and distilled water gave 3.72, 5.04, 0.93, 2.13, 1.16, 3.02, 0.23, 0.30, and 0.23 mg sugar/mL of extract, respectively. Through Tukey's comparison studies, post-ANOVA analysis was carried out as depicted in Fig. 2 (a), (b), and (c) and S2, S3 (Supplementary Material section). There was no significant difference between acetic acid and 2 % NaOH; distilled water and calcium carbonate; calcium carbonate and potassium hydroxide, and distilled water and potassium hydroxide treatments. Nevertheless, a statistically significant variation of $P < 0.01$ was seen among the treatments including 2 % H_2SO_4 and 4 % NaOH; acetic acid and distilled water; acetic acid and calcium carbonate; acetic acid and potassium hydroxide. A statistically significant difference ($P < 0.001$) was seen when comparing the effects of hydrochloric acid and 4 % NaOH treatments; distilled water and 2 % NaOH treatments; 2 % NaOH and calcium carbonate treatments; and 2 % NaOH and potassium hydroxide treatments. A statistically significant difference

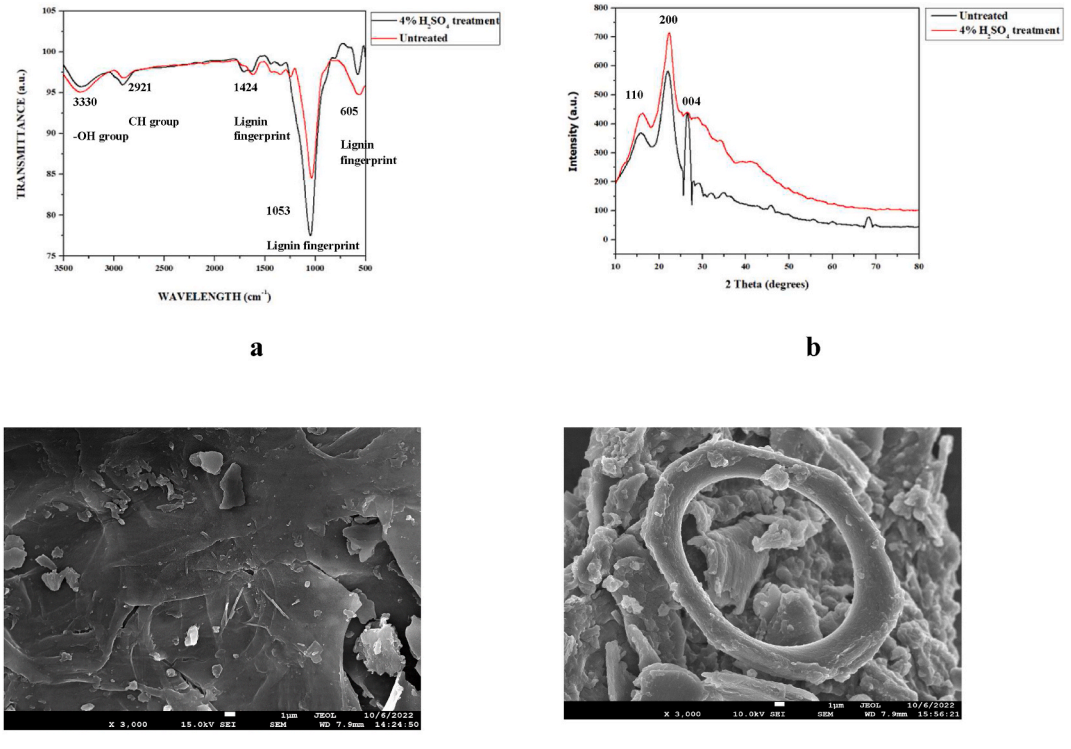

Fig. 3. (a) Fourier transform infrared (FT-IR) spectra pattern, (b) X-ray diffraction (XRD) patterns of untreated wheat straw and after treatment with 4 % sulfuric acid (H_2SO_4), and (c) Scanning electron microscope (SEM) images of untreated and 4 % H_2SO_4 treated wheat straw, respectively. The scale length of 1 μm at 3000× magnification is indicated in the lower right-hand corner.

($P < 0.0001$) was seen when comparing the effects of a 2 % H_2SO_4 solution to a 4 % H_2SO_4 solution, acetic acid, hydrochloric acid, distilled water, 2 % NaOH, calcium carbonate, and potassium hydroxide treatments, respectively. 4 % H_2SO_4 comparison with acetic acid, hydrochloric acid, distilled water, 2 % NaOH, 4 % NaOH, calcium carbonate, and potassium hydroxide showed a statistically significant difference ($P < 0.0001$). Acetic acid exhibited a highly significant distinction ($P < 0.0001$) in comparison to hydrochloric acid and 4 % NaOH, respectively. Hydrochloric acid also showed a statistically significant difference ($P < 0.0001$) when compared with distilled water, 2 % NaOH, calcium carbonate, and potassium hydroxide treatments, respectively. A statistically significant difference ($P < 0.0001$) was analyzed when 2 % NaOH was compared with 4 % NaOH treatment. At last, 4 % NaOH treatment showed a statistically significant difference ($P < 0.0001$) when compared with calcium carbonate, potassium hydroxide, and distilled water treatments, respectively. In the investigation conducted by Deshavath et al. (2020), lignocellulose subjected to 1 % sulfuric acid treatment yielded an estimated mixed sugar concentration of 0.452 mg/mL through DNS assay [21]. In an alternative study, lignocellulose underwent treatment by thermophilic lignolytic bacteria, resulting in a sugar yield of 4.38 mg/mL [22]. In a separate investigation, extractable lignocellulose, containing lignin, demonstrated a sugar concentration of 3.15 mg/mL following pretreatment with KOH [23]. In a subsequent study, researchers reported that a two-step pretreatment involving 1 % NaOH and *Aspergillus* on lignocellulose resulted in a sugar yield of 0.52 mg/mL [24].

Based on the aforementioned observations and analysis, it can be inferred that implementing a 4 % concentration of H_2SO_4 as a pretreatment resulted in the greatest production of reducing sugars, with a significant difference ($P < 0.05$). The findings indicate that a sulfuric acid concentration of 4 % is the optimal choice for breaking down the complex structure of lignocellulose-hemicellulose in WS. This treatment enhances the suitability of WS as a substrate for the production of bioproducts. Collectively, these analyses highlight the significance of pre-treatment and its contribution to enhancing the production of reducing sugars from WS.

3.3. Compositional analysis of pretreated WS extract

The FT-IR method is used to identify the functional groups that are eliminated as a result of the chemical treatment of WS without causing any damage. This technique facilitates the analysis of the physiochemical characteristics of lignocellulosic materials. The FT-IR graph provides a more comprehensive insight into the identification of changes that occurred in the lignocellulosicWS extract following processing. The graph generated through the detection is documented as bands, displaying peaks of varying intensities. The FT-IR technique has demonstrated its efficacy as a effective instrument for elucidating the crystalline structure of cellulose in WS. Fig. 3 (a) shows the changes in structure in WS after the best treatment based on sugar analysis, compared to untreated WS. The 4 % H_2SO_4 treatment resulted in the partial breakdown of cellulose, the total or partial elimination of lignin and hemicellulose, and the formation of a graph due to the breaking and formation of hydrogen bonds between molecules. The modifications are documented on FT-IR spectra, and the investigation is conducted according to the assigned wave number for each functional group. The unprocessed water sample exhibits a spectral band with a wavelength range of 3000–3500 cm^{-1} and a stretching effect, as observed after treatment with 4 % H_2SO_4. This shows that there is an O–H stretching vibration, and the amount of cellulose and hemicellulose in lignocellulosic waste (WS) can be seen by how strong the absorption peaks are across the wavelength spectrum. Another band in the wave number range of 2850–2950 cm^{-1} indicates the stretching of C–H bonds, which reveals the presence of a long aliphatic chain structure in lignin and cellulose after being treated with 4 % H_2SO_4, as opposed to untreated WS. The FT-IR spectral band in the range of 1739, 1618, 1458, 1252, 1071, and 602 cm^{-1} is considered to be the characteristic identification of lignin in untreated WS. The absence or reduction of these groups in any processing procedure suggests the elimination of lignin [25,26]. Fig. 2 (a) exhibits a band at around 1420 and 603 cm^{-1}, indicating the occurrence of C–H bending vibrations, which is a distinctive characteristic of lignin. Finally, a band at 1051 cm^{-1} also demonstrates the C–O stretching vibrations, indicating the presence of cellulose and hemicellulose in the free extract following treatment with 4 % H_2SO_4.

The X-ray diffraction (XRD) peak heights are obtained from the diffraction pattern of cellulose crystallites. Fig. 3 (b) displays the X-ray diffraction (XRD) patterns of both the untreated WS and the best treatment of WS. Both the untreated WS and the wheat straw treated with 4 % H_2SO_4 exhibited light reflection at the (110), (200), and (004) planes, indicating the presence of cellulose crystallinity [27,28]. The plane (200) exhibited a higher peak intensity, suggesting a favored orientation. The crystallite size was calculated with the Scherrer equation ($L = K\lambda/\beta.\cos\theta$). The untreated wheat straw had a crystallite size of 2.092 nm, while the 4 % H_2SO_4 treatment reduced the crystallite size to 0.39 nm. The reduction in crystallite size can be attributed to the effective elimination of amorphous constituents, namely lignin, through acid treatment, resulting in alterations to the structural configuration of the WS. Therefore, the treatment with 4 % H_2SO_4 proved to be the most effective in enhancing the accessibility of cellulose and extracting sugar, as confirmed by sugar analysis. Smaller crystallite size implies reduced cellulose accessibility, facilitating more efficient sugar extraction [29]. The magnitude of the peak is also influenced by the extent of polymerization, which results in the liberation of crystalline cellulose from the lignin-hemicellulose-cellulose complex. A greater degree of polymerization means increased elimination of lignin and hemicellulose, followed by their solubilization [30,31].

The SEM was used to investigate the surface structures of both untreated WS and the most effective treatment for WS. The untreated WS exhibited a regular, stiff, compact, and smooth surface structure, as well as visible fibre bundles when observed at a magnification of 3000× (Fig. 2c). After undergoing pre-treatment, the surface of WS exhibited abrasion, scale development, and layering when observed at a magnification of 3000×. The most likely reason is that the surface structures of untreated WS are made up of a network of cellulose, hemicellulose, and lignin, which makes WS stiff and compact [32,33]. After the 4 % H_2SO_4 treatment, the outside network was partially broken down by water, revealing the inside structures. This resulted in the development of porosity on the fibre surface, revealing cellulose and hemicellulose, as well as the depolymerization of lignin [34]. The modifications enhanced the ease of access to the bioconversion procedures for the generation of bioproducts [35].

3.4. Eco-friendly biopolymer development: PHA production, extraction, and identification using 4 % H_2SO_4 pretreatment

The 16s rRNA of the *Bacillus paranthracis* RSKS-3 bacterium, which was isolated and characterized from sewage water, was sequenced as part of the whole genome sequencing protocol and the phylogeny relationship tree is examined in Fig. 4. Understanding *B. paranthracis* RSKS-3's evolutionary lineage and genomic properties greatly depends on its phylogenetic relationships. One can learn more about the genetic composition of the bacteria and identify conserved areas and putative virulence factors by examining the 16s rRNA. This knowledge advances our understanding of its biological niche, flexibility, and its uses in industrial or environmental processes, among other contexts. Furthermore, phylogenetic analysis aids in the classification and comparison of *B. paranthracis* RSKS-3 with related strains of bacteria, laying the groundwork for future investigations into the organism's ecological function, evolutionary history, and possible biotechnological uses. The study evaluated PHA granules of 0.87 g/L was accomplished using a hypochlorite test which was a 2.55-fold increase as compared to minimum media that produced 0.34 g/L PHA. Fig. 5 of the UV absorption spectra displays a distinct absorption peak within the wavelength range of 230–235 nm. The sample containing PHA was broken down with the use of strong sulfuric acid. The ultraviolet (UV) analysis of crotonic acid, which is produced by digesting PHA with sulfuric acid, showed the presence of a solitary peak with maximum absorption at 235 nm, which corresponds to the standard crotonic acid. Therefore, it illustrates that the substance converted to crotonic acid under the influence of concentrated sulfuric acid was PHA [36, 37].

3.5. FT-IR characterization of polyhydroxyalkanoate granules

The FT-IR tool is an advanced method used to analyze PHA by identifying the stretches in the functional groups and chemical linkages inside the biopolymer. Furthermore, FT-IR analysis offers valuable information regarding the types and configurations of monomers in PHA, which aids in determining the composition of PHA. Fig. 6 illustrates the many functional groups found in PHA granules through the use of FT-IR spectroscopy. The spectroscopy also examined the chemical composition of the polymer, which is indicative of its constituent monomers. The FT-IR graph in Fig. 6 shows the stretch out of various functional groups, such as the aliphatic CH group, C═O (carbonyl), CH_2 (aliphatic), CO (ether), and C–O–C (ester). These are represented by 2923, 1571, 1457, 1155, and 779 cm^{-1} peaks in Fig. 5 and have been confirmed by the studies [12,38–40].

4. Conclusion

The research analyzed nine different chemicals- 2 % H_2SO_4, 4 % H_2SO_4, acetic acid, hydrochloric acid, 2 % NaOH, 4 % NaOH, calcium carbonate, potassium hydroxide, and distilled water for the pretreatment of wheat straw, as its valorization has a significant impact for the development of a sustainable environment. The process was performed at 121 °C with 15 psi for 1 h in an autoclave. The

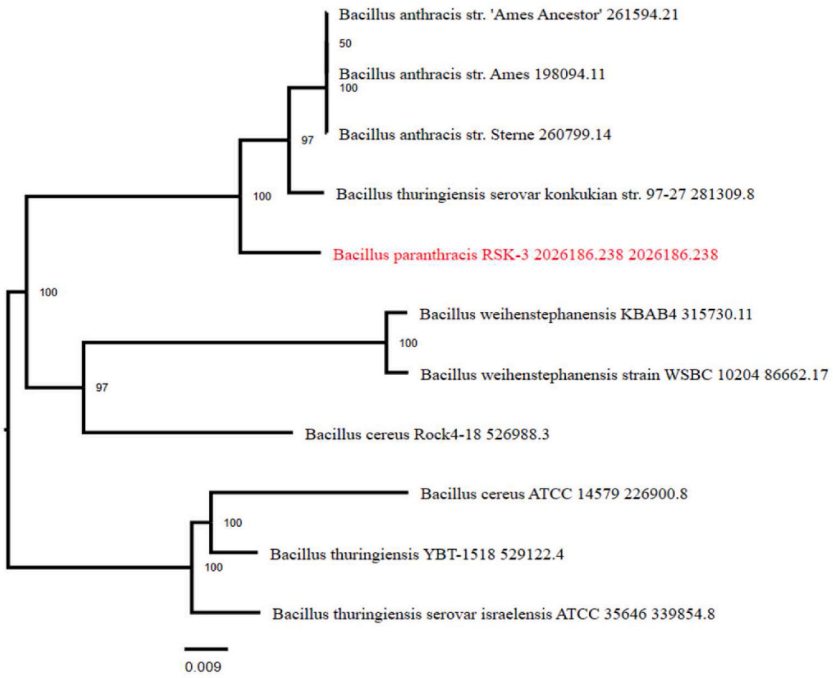

Fig. 4. A tree of phylogenetic relations was produced using the neighbor-joining algorithm in Mega-X software, utilizing Bacillus nucleotide sequences. The tree depicts the positioning of *Bacillus paranthracis* RSKS3. The nodes display 1000 iterations of book strap values.

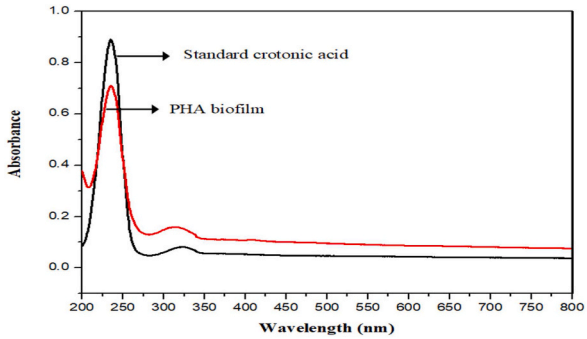

Fig. 5. The UV absorbance spectra illustrate the maxima absorbance peaks of the standard crotonic acid (indicated by the black line) and PHA granules (indicated by the red line) at a wavelength of 235 nm.

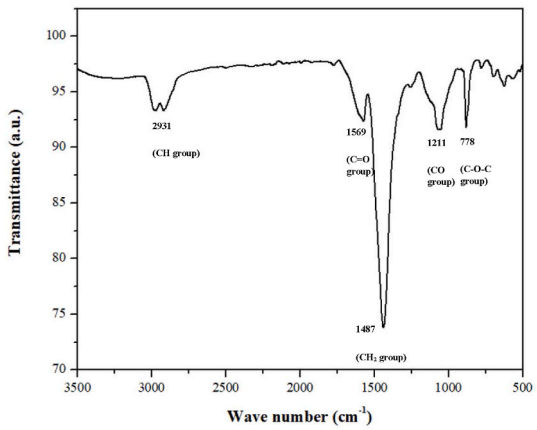

Fig. 6. Fourier transform infrared (FTIR) analysis was conducted on the polyhydroxyalkanoate (PHA) generated by *Bacillus paranthracis* RSKS-3, using an extract of wheat straw treated with 4 % sulfuric acid (H_2SO_4).

efficacy of the pretreatment was validated using Molisch's test which confirmed the presence of carbohydrates in all the nine pretreatment processes and quantitative DNS assay screened 4 % H_2SO_4 as the best solvent for the pretreatment of wheat straw. The treatment yielded 5.04 mg sugar/mL of extract which was chosen as a carbon source to actively promote novel isolate *Bacillus paranthracis* RSKS-3 growth. The growth of the bacterium using 4 % H2SO4 was utilized for PHA production (0.87 g/L) after 72 h of incubation. The finding was characterized by UV spectroscopy which confirmed a sharp peak at 235 nm confirming the PHA molecules after digestion with concentrated sulfuric acid. However, in the future, there is a need to optimize the conditions for the chemical pretreatment and optimize production parameters utilizing 4 % H_2SO_4 treated extract to maximize PHA yield.

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Data availability

The manuscript contains all the accessible data.

CRediT authorship contribution statement

Rohan Samir Kumar Sachan: Writing – original draft, Software, Methodology, Investigation, Data curation. **Inderpal Devgon:** Writing – original draft, Validation, Resources, Methodology, Formal analysis. **Vikas Sharma:** Visualization, Resources, Formal analysis. **Kahkashan Perveen:** Visualization, Validation, Resources. **Najat A. Bukhari:** Software, Resources, Formal analysis, Data curation. **Jamilah A. Alsulami:** Visualization, Resources, Data curation. **Vikash Singh Jadon:** Writing – review & editing, Visualization, Validation, Software. **Deep Chandra Suyal:** Writing – review & editing, Visualization, Validation. **Arun Karnwal:** Writing – review & editing, Writing – original draft, Supervision, Resources, Formal analysis, Conceptualization.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e31572.

Research methodology throughout the manuscript	Important findings
Acid-based and alkali-based pretreatment of wheat straw	Wheat straw was pretreated with 2 % H_2SO_4, 4 % H_2SO_4, acetic acid, hydrochloric acid, 2 % NaOH, 4 % NaOH, calcium carbonate, potassium hydroxide, and distilled water at 121 °C for 1 h.
Qualitative and quantitative sugar analysis	All nine pretreatment processes showed the presence of reducing carbohydrates indicating lignocellulose was broken down to its simpler form.
Qualitative analysis (Molisch's test)	
Quantitative analysis (DNS test)	DNS test quantified sugar liberated from all four pretreatments. 2 % H_2SO_4 had 3.72 mg sugar/mL extract. 4 % H_2SO_4 had 5.04 mg sugar/mL extract. Acetic acid had 0.93 mg sugar/mL extract. Hydrochloric acid had 2.13 mg sugar/mL extract. 2 % NaOH had 1.16 mg sugar/mL extract. 4 % NaOH had 3.02 mg sugar/mL extract. Calcium carbonate had 0.23 mg sugar/mL extract. Potassium hydroxide had 0.30 mg sugar/mL extract. Distilled water had 0.23 mg sugar/mL extract. So, pretreatment with 4 % H_2SO_4 had 5.04 mg sugar/mL extract which was chosen to produce PHA bioproduct using *Bacillus paranthrancis* RSKS-3
FT-IR analysis	FT-IR analysis of all the treatments and raw wheat straw showed different peaks intensities at: 605 cm^{-1}: lignin fingerprint 1053 cm^{-1}: lignin fingerprint 1424 cm^{-1}: lignin fingerprint 2921 cm^{-1}: C–H stretching 3334 cm^{-1}: OH stretching
XRD analysis	XRD analysis of all treatments and wheat straw reflected light at (110), (200), and (004) planes. Peak intensity was higher for the plane (002). The crystallite size was calculated as follows: Untreated wheat straw: 2.455 nm 4 % H_2SO_4 treatment: 0.42 nm. So, 4 % H_2SO_4 was a highly favorable treatment method based on a smaller crystallite size that indicated lesser and easy access to cellulose.
SEM analysis	SEM (at a scale length of 10 μm) provided insight into chemical pretreatment on wheat straw along with morphological changes. The analysis showed that an aggressive breakdown was seen in the case of 4 % H_2SO_4 treatment as compared to untreated wheat straw.
Identification of PHA granules by UV spectrophotometer	4 % H_2SO_4 treated wheat straw extract was used to produce PHA using *Bacillus paranthrancis* RSKS-3. Approx. 0.87 gL^{-1} of the extract was produced as compared to minimal media which extracted 0.34 gL^{-1} PHA. Identification of PHA granules was achieved by converting PHA granules into crotonic acid by reacting with concentrated sulfuric acid. The peak of PHA was compared with standard crotonic acid. The maximum absorbance was observed at 235 nm for both PHA and standard crotonic acid.
FT-IR analysis of PHA granules	The functional groups identified based on stretching were: 2931 cm^{-1}: aliphatic CH group 1569 cm^{-1}: carbonyl group 1487 cm^{-1}: aliphatic CH_2 group 1211 cm^{-1}: CO bond of ether group 778 cm^{-1}: C–O–C bond of ester group

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