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Valorization of sugar extracted from wheat straw for eco-friendly polyhydroxyalkanoate (PHA) production by *Bacillus megaterium* MTCC 453

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ABSTRACT

The valorization of wheat straw can be applied to produce sustainable bioproducts. The objective of this study is to produce a sustainable bioplastic; Polyhydroxyalkanoate (PHA) using wheat straw as a carbon source after pretreatment, using mild acids and alkalis. The qualitative Molisch test validated the presence of carbohydrates by observance of purple-ring formation at the interface, whereas the quantitative DNS test showed a maximum sugar yield of 4.9 μg/mL in the 4 % H_2SO_4-treated wheat straw extract at $p < 0.05$. This extract was efficiently utilized by *Bacillus megaterium* MTCC 453 to produce PHA (30 mg/L), as confirmed by UV-spectroscopy and FT-IR analysis. These results confirm the applicability of producing sustainable PHA excluding the usage of industrial enzymes and any additional carbon/nitrogen sources to produce PHAs. The findings offer a promising alternative to mitigate the effect of global warming caused by the combustion of agricultural biomass and contribute to achieving sustainable development goals.

1. Introduction

Wheat straw, the top five most-produced species of cereals is an obvious choice to be used as a residual low-cost feedstock for the bio-based economy, especially in South- and Southeast Asian countries that are the main producers of wheat (Abdel-Shafy and Mansour, 2018; Adejumo et al., 2020). Every year, approximately 529 tons of wheat straw is produced worldwide, and a portion of wheat straw is collected for cooking, animal feed, roof covering, or mushroom production. However, with improved living conditions of farmers, usage has become less common, and annually approximately 8.5 million tons are burnt across the globe (Nagy et al., 2023; Tufail et al., 2021). Wheat straw burning is projected to release 0.11, 2.306, 0.002, and 0.084 metric tons of cumulative CO, CO_2, N_2O, and NO_x, respectively. Valorization of wheat straw would thus offset such emissions. Studies claim that 35–40 % of the straw may be available for biofuels and other products after leaving that is needed to conserve soil quality and for competitive uses (Bhuvaneshwari et al., 2019; Kathi et al., 2023; Mahmoud et al., 2023).

Studies on the use of wheat straw to produce second-generation biofuels or bioplastics have been reported. Bioplastics presents pragmatic resolutions for a viable and enduring future, supported by empirical scientific substantiation (Donkor et al., 2022; Jędrzejczyk et al., 2019; Kustov et al., 2022). Bioplastics which are obtained from renewable biological resources, make a substantial contribution towards the attainment of Sustainable Development Goals (SDGs) (Olabi et al., 2023). Their alignment with SDG 7 (Affordable and Clean Energy) contributes to the reduction of emissions. Furthermore, scientific evidence supports the connection between biofuels and SDG 9 (Industry, Innovation, and Infrastructure), as they substitute innovation in the fields of construction and manufacturing (Umar et al., 2021; Mahmoud, 2020b; Mahmoud and Mahmoud, 2023). Additionally, bioplastics are shown to align with SDG 12 (Sustainable Consumption and Production) by effectively mitigating environmental impacts. Furthermore, they substantially support the achievement of SDG 13, which pertains to Climate Action, by effectively mitigating greenhouse gas emissions (Dotaniya et al., 2023). Additionally, they demonstrate their commitment to SDG 15 and 14, which focus on Life on Land and Life Below Water, respectively, by actively supporting biodiversity conservation efforts and implementing measures to decrease pollution in marine and terrestrial ecosystems (Ferreira et al., 2021; Khan et al., 2022). Biodegradable bioplastics are microbial-based polyesters commonly known as polyhydroxyalkanoates (PHAs) that possess higher biodegradability

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rates (Cheng and Whang, 2022). A major production cost is related to the choice of carbon source and wheat straw, itself contains over 70 % carbohydrates in the form of cellulose and hemicellulose. Access to these requires the pretreatment of wheat straw to remove lignin from the lignocellulosic complex. Chemical pretreatment methods are commonly used to disrupt the wheat straw components. Alkali-based and acid-based pretreatment enables the modification of the structural makeup of cellulose fibers, making them more thermodynamically stable than the control fiber. Ester bonds found in lignin and hemicelluloses are more strongly affected by chemical pretreatment on cleavage than other ester bond types (Ji et al., 2022; Sarwar et al., 2022). The pretreatment process increases the surface area by swelling the particles of the wheat straw biomass, thus increasing the carbohydrate accessibility with the reduction in cellulose polymerization degree and crystallinity.

The present study investigated sugar concentration and analysis based on the effect of varying percentages of acid (H_2SO_4) and alkali (NaOH) in the chemical treatment of wheat straw and the usage of sugar from the pretreated wheat straw for PHA production using *Bacillus megaterium* MTCC 453. In addition, the feasibility of using raw and chemically processed wheat straw as the medium for the growth of *Bacillus megaterium* MTCC 453, and its ability to use the sugar for PHA production. The study also emphasizes the quantification of sugar release during pretreatment. The utilization of Scanning Electron Microscopy, Fourier Transform Infra-Red, and X-ray diffraction techniques for analysis facilitates the acquisition of a holistic comprehension regarding the potential of wheat straw in the production of PHA. This holds significant implications for the advancement of sustainable bioplastic manufacturing and efficient waste management practices.

2. Materials and methods

2.1. Wheat straw procurement

Wheat straw (WS) was obtained from farms near Lovely Professional University, Punjab, India. To eliminate impurities, grinding, and storing (Fig. S1), we followed the procedure of Mahmoud et al. (2016) and Mahmoud et al. (2021).

2.2. Acid- and alkali-based pretreatment

The treatment process utilized two distinct approaches: sulfuric acid (at concentrations of 2 % and 4 %) and sodium hydroxide (at concentrations of 2 % and 4 %). In summary, 10 g of WS was added to separate Erlenmeyer flasks containing 100 mL of either sulfuric acid or sodium hydroxide. The flasks were appropriately sealed and subjected to autoclave at 121 °C for 1 h, as described by Zheng et al. (2018). Following autoclaving, the contents of each flask were cooled to room temperature and filtered first with muslin cloth and then with Whatman filter paper. The pH of the resulting chemically pretreated filtrates was adjusted to 7.0 using NaOH and HCl. The remaining residues were washed with distilled water to eliminate any residual acid or alkali.

2.3. Sugar analysis

The WS extracts that had been pretreated were examined to identify the carbohydrates released during the pretreatment process. The presence or absence of carbohydrates was performed using Molisch's test, as described in Jain et al. (2021), on various pretreated WS extracts. Following the detection of carbohydrates in the extracts, the total sugar content was quantified using a standard DNS assay (Miller, 1959), as presented in Table S1 (Supplementary Infomation).

2.4. Characterization of pretreated wheat straw

The functional groups of raw and pretreated WS were identified by FTIR analysis. Spectra were acquired using FTIR (Perkin Elmer Spectrum 2). Peak data were recorded and analyzed from 400 to 4000 cm^{-1} using a detector with a resolution of 4 cm^{-1}. Approximately 128 scans per sample were recorded (Zheng et al., 2018). The crystallite phase of raw (untreated) and chemically pretreated WS was detailed using an XRD (Bruker D8 Advance). Diffractograms were recorded from 10° to 40°. Morphological structures in raw and pretreated WS were examined using SEM (FE-SEM: JEOLJSM-7610F PlusEDS: OXFORD EDS LN2 free).

2.5. PHA production, extraction, and identification

2.5.1. PHA production using the 4 % H_2SO_4 pretreated wheat straw extract

Nutrient agar was used to store the bacterial stock culture at 4 °C until needed, while PHA was produced using the liquid media. This media comprised $MgSO_4$ (0.2 g/L), NaCl (0.5 g/L), yeast extract (2.5 g/L), and peptone (2.5 g/L), as reported by De Souza et al. (2020). The production studies were conducted in 100-mL flasks using 50 mL working solution. The carbon source was provided by 2 mL of wheat straw extract (10 g/L) treated with 4 % sulfuric acid, as determined by DNS assay. This carbon source replaced glucose in the aforementioned media. After autoclaving the flask (121 °C, 15 psi for 20 min), it was allowed to cool to room temperature. A 2 % freshly prepared 24-h broth culture of *Bacillus megaterium* MTCC 453 (10^8 CFU/mL) was inoculated, and the mixture was incubated for 48 h at 37 °C in an orbital shaker (120 rpm). For treatment control, media with glucose source was used for the PHA production.

2.5.2. Extraction and identification of PHA granules using UV-spectrophotometer

50 mL of cell culture with the biomass was centrifuged for 10 min at 8000g to collect the cells. The pellets were mixed with 1 mL of distilled water in preweighed Eppendorf tubes. The tubes were centrifuged at 10,000 xg again, and the supernatant was discarded; the pellets were then dried at 60 °C until they reached a constant weight. After that, the cell pellet was processed through a PHA extraction step. The pellets were treated with 15 mL sodium hypochlorite (6 %) solution to break down the cellular debris, and the tubes were placed in an orbital shaker incubator at 30 °C for *2 h*. After incubation, the tubes were centrifuged for 20 min.

After removing the supernatant, the pellets were washed in distilled water. The liquid was transferred to Eppendorf tubes and centrifuged at 10000 g for 20 min; the supernatant was discarded. The remaining cell debris was removed by washing the pellet with acetone. The pellets were dried at 60 °C to ensure a constant weight (De Souza et al., 2020). The dried PHA granules (I mg) were heated for 10 min in concentrated sulfuric acid (2 mL). UV spectra were recorded from 800 to 200 nm, with a calibration baseline established using sulfuric acid and standard crotonic acid.

2.5.3. FTIR characterization of PHA granules

The PHA granules produced by *Bacillus megaterium* MTCC 453 were characterized by analyzing their functional groups. FTIR (Perkin Elmer Spectrum 2) was used to record the spectra, with a detector resolution of 4 cm^{-1} and a range of 500–3500 cm^{-1}. Each sample was scanned about 128 times, and the resulting data were recorded as peaks, as reported by Zheng et al., 2018.

2.6. Statistical analysis

A detailed statistical analysis was conducted to study the impact of different pre-treatment processes of wheat straw on sugar concentrations. The mean values, standard deviations, and standard error were calculated to provide a detailed outline of the central tendency and dispersion within the sugar concentration by different pretreatment processes. Furthermore, ANOVA was utilized to evaluate the overall significance of the difference among the various sugar concentrations by

different pretreatment processes. To identify specific differences, Tukey's multiple comparison test was applied, ensuring comparison while maintaining a 95 % confidence interval.

3. Results and discussion

3.1. Acid- and alkali-based pretreatment

Alkaline and acidic pretreatment of the dried wheat straw was tested with 2 % and 4 % NaOH and 2 % and 4 % H_2SO_4, respectively, at a solid: liquid ratio1:10. The treatment was processed using an autoclave at 121 °C and 15 psi for one hour. This resulted in swelling of the fibers and degradation of lignin to remove cellulose and hemicellulose from the lignin-cellulose-hemicellulose complex (Mittal and Sinha, 2016). Various pre-treatment techniques have been applied to wheat straw (WS) to expose the complex cellulose and hemicellulose in the lignin-cellulose-hemicellulose complex, resulting in the partial degradation of lignin. Different percentages of NaOH and H_2SO_4 were used for pre-treatment because these chemicals were readily available. However, the pretreatment media varied among different studies. Zabihi et al. (2010) utilized acetic acid and ethanol at a temperature of 190 °C with a residence time of 10 min to pre-treat WS. On the other hand, Gaudino et al. (2022) used low-frequency to high-power ultrasound to pre-treat WS mixed with water. Pagano et al. (2023) used wash solutions with different acetic acid and ammonia concentrations to pre-treat WS at 50 °C for two hours. Meanwhile, Tang et al. (2022) used humic acid and ferric chloride to treat WS. Qiu et al. (2018) used phosphoric acid and H_2O_2 to treat WS. Rahmani et al. (2023) used a hydrothermal and thermal alkaline approach to pre-treatment WS. The pretreatment technique resulted in the partial degradation of lignin, which exposed cellulose and hemicellulose complexes. The exposed cellulose or hemicellulose can be a carbon source for several critical industrial compounds. Therefore, the pre-treatment technique used for WS can play a vital role in efficiently using this abundant, low-cost, and renewable resource. The study aimed to examine the alkali and acid pretreatment effect on the structural and compositional modifications of agricultural wastes. These modifications were expected to influence the hydrolysis process, leading to an increment of reducing sugars. The goal was to establish an efficient method to produce alternative energy.

3.2. Qualitative and quantitative DNS sugar analysis

A qualitative Molisch test was used to detect carbohydrates released during pretreatment. The purple ring formation confirms the carbohydrate's presence and suggests that the pre-treatment successfully removed lignin and released cellulose and hemicellulose. In a study by Bekiaris et al. (2015), the sugar released from wheat straw was analyzed using glucose peroxidase and xylose dehydrogenase assays. Zheng et al. (2018) also conducted a qualitative carbohydrate analysis content from the enzymatic treatment of wheat straw using HPLC. Estimation of the sample's total content of reducing sugars, the DNS assay is commonly used (Tishler et al., 2015). A higher amount of reducing sugars in the WS extract corresponds to maximum potential as a substrate for bioproduct production. Fig. 1 and Table S3 provide the calculated total reducing sugars after pretreatment of WS with different NaOH and sulfuric acid concentrations. The results were calculated using the formula X (conc) = (Y-0.0004)/0.0003, derived from a standard glucose curve generated with a 1 mg/mL glucose stock solution (see Supplementary Information S2).

The treatment procedures with 2 % H_2SO_4, 4 % H_2SO_4, 2 % NaOH, and 4 % NaOH gave 3.72 μg sugar/mL of extract, 4.92 μg sugar/mL of extract, 1,05 μg sugar/mL of extract, and 3.02 μg sugar/mL of extract, respectively (see Supplementary Material S3). Comparison studies using Tukey's multiple comparisons were done as post-ANOVA analysis. There was no significant difference between the 2 % H_2SO_4-treated W·S. and 4 % H_2SO_4-treated W.S. as well as between 2 % H_2SO_4 and 4 % NaOH.

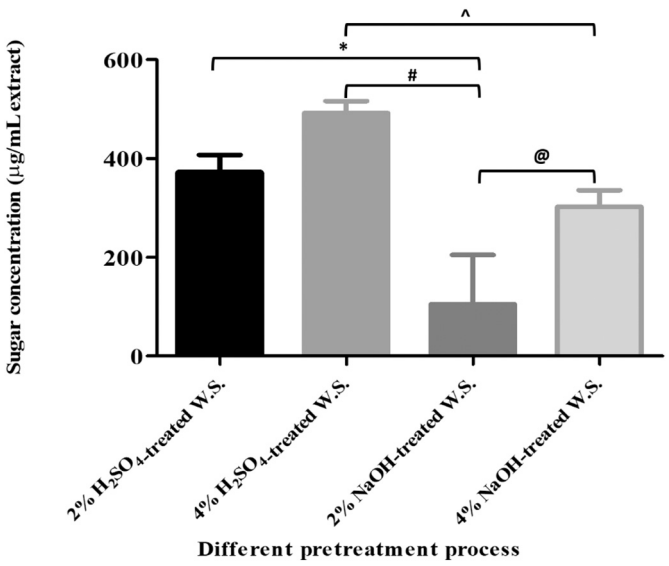

Fig. 1. Total Carbohydrate and Sugar Analysis in the pretreated wheat straw extract. From the graph, 4 % H_2SO_4 shows a higher sugar concentration followed by 2 % H_2SO_4, 4 % NaOH, and 2 % NaOH pretreatment process, where * is significance level ($P < 0.01$), # is significance level ($P < 0.0001$), ^ is significance level ($P < 0.05$), and @ is significance level ($P < 0.05$).

However, a significant difference of $P < 0.01$ was observed between 2 % H2SO4-treated W.S and 2 % NaOH-treated W.S. The comparison between 4 % H_2SO_4 with both 2 % NaOH-treated W.S. and 4 % NaOH-treated W.S. showed significant difference at $P < 0.0001$ and $P < 0.05$, respectively. Additionally, the comparison between 2 % NaOH-treated W.S. and 4 % NaOH-treated W.S. also yielded significance at $p < 0.05$ as shown in Fig. 1 and Table S4 (for table, see Supplementary Material S4). These results indicate that using 4 % H_2SO_4 as a pre-treatment gave the highest reducing sugar yield at $P < 0.05$ significance level (see Supplementary Material S4 and S5). This finding suggests that sulfuric acid concentration is the most effective in breaking down the complex structure of WS, making it a more favorable substrate for byproduct production. Overall, these studies demonstrate the importance of pretreatment and its role in improving reducing sugar yield from WS.

3.3. FTIR analysis of raw and chemically treated wheat straw

A non-destructive method, FTIR, determines the functional groups removed during the chemical treatment of WS. The technique assists in analyzing the physio-chemical properties of lignocellulosic materials (Mahmoud, 2020a). Through the FTIR graph, a better understanding of the detection of changes occurred after pretreatment in the lignocellulosic extract. The graph formed during the analysis is recorded as spectra with various intensities of peaks. The FTIR technique has proved to be a valuable tool for determining the cellulose crystalline structure in lignocellulose. Structural changes occur during the pre-treatment of WS using acid or alkali, such as partial degradation of cellulose, complete or partial lignin, and hemicellulose removal. The FTIR spectra are studied to analyze majorly lignin-cellulose-hemicellulose component reactions with the pretreatment medium (acid or alkali) and breaking and synthesis of another hydrogen linkages (inter and intra-molecular) as seen in supplementary Fig. S2a. These changes are recorded on FTIR spectra, and the study is done as per the wave number allocated to each functional group. For instance, raw WS shows 3000–3500 cm^{-1} wavelength number is allocated to -OH stretching vibration, and the strength of the absorption peaks covering the wavelength number indicates the cellulose content in lignocellulosic waste (WS). β (1➔4) glycosidic linkage between cellulose and hemicellulose also appears in the FTIR spectra as

a specific wave number at 896 cm^{-1}. A wavenumber ranging from 2850 to 2950 cm^{-1} indicated a C—H stretching that showed lignin's long aliphatic chain structure, and the peak band of 1381 cm^{-1} represented C-CH_3 group. The FTIR spectrum range of 1735, 1620, 1460, 1257, 1070, and 604 cm^{-1} is regarded as the lignin fingerprint of raw WS (Tsegaye et al., 2019) and decreased or disappearance of these bands from any treatment process indicates lignin removal (Asghar et al., 2015).

Moreover, other functional groups that build up lignin substance, i.e., uronic esters and acetyl groups of feluric and p-coumaric acid monomers, showed peak bands at 1733 cm^{-1}. The spectra of WS pre-treatment using alkali show noticeable results. The acid-based treatment efficiently removed hemicellulose; however, poorly removed lignin substances and partial degradation of the cellulose. Thus, acid proved to be more potent in the pre-treatment of lignocellulose than alkali. Acid uniformly reacts with lignocellulosic components and their subsequent removal or extraction into the medium.

Compared with acid, which can react only with the hemicellulose part of lignocellulose (WS) removal. Moreover, pretreatment with NaOH increased the wave number of O—H groups as new O—H groups were introduced from the demethylation of O-CH_3 linkages. Interestingly, released cellulose confirmed new bands in FTIR spectrum for all NaOH-based pre-treatment. The new bands appeared because of C—O bond and β (1➔3) polysaccharides' vibrational stretch. A study by authors concluded intensity of O—H group extension and band decreased to 3315.26 cm^{-1} pretreated using 2.5 % NaOH of WS compared to the raw WS with band number 3336 cm^{-1}.

Furthermore, the authors analyzed a decrease or disappearance of the lignin band compared with the raw WS with a lignin band at 1316 cm^{-1} (Asghar et al., 2015; Kumar Trivedi et al., 2022). A study by the authors analyzed spectrum of FTIR for raw WS and chemically treated (3 % potassium hydroxide and 2 % potassium hydroxide +1 % calcium hydroxide) WS that recorded decreased wave number of lignin due to stretching as compared with raw WS that had wave number 1535, 1488, 1425 cm^{-1} for lignin substances in the lignocellulose materials. There was also a decrease in the wave number of the -C-O-C- functional group compared to the raw WS with wave number 158 cm^{-1} (Shen et al., 2019).

From the FTIR analysis, H_2SO_4 is a potent acid that can precisely target and extract cellulose without aggressively breaking down lignocellulose, whereas NaOH is a potent alkaline reagent that can more aggressively damage the lignocellulosic structure (Lee et al., 2014). Therefore, conclusively, the use of 2 % H_2SO_4 concentration during the pre-treatment caused a excessive release of lignin and cellulose than 4 % H_2SO_4 concentration, and NaOH (2 % and 4 %) treated wheat straw.

3.4. XRD analysis of the raw and treated wheat straw

Cellulosic crystallite diffraction pattern results in XRD peak heights. The XRD of raw and untreated wheat straw and the various treatment methods shown in supplementary Fig. S2b. Raw and untreated WS, and chemically treated wheat straw both reflected light at the (101) and (002) planes. The peak intensity was higher for a plane (002), indicating a preferred orientation (Barman et al., 2012; Lavarda et al., 2019; Naz et al., 2020; Irfan et al., 2016). The complete width at half maximum of peaks (β) in radians found at any two points in the pattern were used to compute the crystallite size using the Scherrer eq. ($L = K\lambda/\beta.\cos\theta$). K ranges from 0.62 to 2.08 but is typically estimated to be around 0.89. The crystallite size of the untreated wheat straw was 2.455 nm. The crystallite size for different treatments was 1.69 nm for 2 % H2SO4, and 0.42 nm for 4 % H_2SO_4 treated, 1.84 nm for 2 % NaOH, 1.77 nm for 4 % NaOH treated wheat straw. Hence, 4 % H_2SO_4 treatment is more likely to be an efficient treatment for the higher accessibility of cellulose and subsequent sugar extraction. The fact is that smaller crystallite size indicates that lesser and easier access to cellulose is available for better extraction of sugars (Momayez et al., 2019). Lignocellulose comprises lignin, cellulose, hemicellulose, and, to some extent, pectin. Although lignocellulosic biomass has other crystallinity indices depending on the lignin, cellulose, and hemicellulose matrix. The compactness of the matrix also affects CrI. raw WS has a reflection at 101 and 002 planes, which is determined as the peak height in the XRD graph and corresponds to the cellulose crystals. However, treated WS showed increased or enhanced intensity in peak height at the exact plane reflection, suggesting that pre-treatment was a success that increased disruption of the matrix linkages and augmented area of the WS fibers. The strength of peak at the 002 planes was more vigorous, indicating that the 002 plane was the preferred orientation for diffraction.

The XRD of pre-treatment of WS using an alkali (NaOH) measures cellulose crystallinity with estimation of qualitative and semi-quantitative of crystalline cellulose, amorphous lignin, and hemicellulose (Asghar et al., 2015) CrI is calculated using XRD peaks of both crystalline and amorphous constituents of the WS, which depends on the biomass's crystalline component (cellulose). Therefore, the lignocellulosic biomass, wheat straw's crystallinity is a critical factor influencing cellulose removal from the matrix and reflecting it on XRD as peaks. The similar XRD diffractogram of both pre-treatments suggested identical structural changes, i.e., relative removal of similar amorphous substances was achieved.

3.5. SEM of analysis of raw vs. chemically treated wheat straw

Surface structures of untreated or raw untreated WS and pretreated WS were observed under SEM to study the effect of acidic and alkali-based pretreatment. A regular, rigid, compact, and smooth surface structure and fiber bundles of raw WS were visualized under 500× magnification, as shown in Fig. S3. Post pretreatment, surface structures of WS showed abrasion, scale formation, and layering under 500× magnification, as shown in supplementary Fig. S3. A plausible explanation is that raw WS surface structures are made of the cellulose –hemicellulose–lignin network, which gives WS rigid and compact structures. Treatment with alkali or acid destroys the network that makes up the external structures, exposing internal structures—this results in fiber porosity on the surface, which is not observed in raw WS. Barman et al. (2012) documented a report on NaOH-based (0.5–2 %) and hot water treatment of WS. The report concluded that raw WS, 0.5 % and 1 % NaOH-based pretreatment, and hot water-based pretreatment showed rigid and well-organized surface structures under SEM with 1000× magnification. 1.5 % and 2 % NaOH-based WS pretreatment destroyed these rigid and ordered structures. The rigidity structures were due to the lignin and hemicellulose linkage, which was demolished after the treatment exposing the cellulose surface area (Barman et al., 2012).

The WS samples that were pre-treated with NaOH exhibited a distinct surface morphology characterized by cylindrical forms, filaments, cells, and pores. This morphology facilitated adhesion between the WS and a polymer matrix, as demonstrated by the results obtained from the composite film. The application of acid/base pre-treatments resulted in a reduced water-soaking (WS) resistance, hence improving the digestibility of cellulose (Dixit and Yadav, 2020).

3.6. Identification of PHA granules using a UV spectrophotometer

30 mg/L of PHA granules extracted using a hypochlorite test were analyzed for the study. A prominent absorption peak between 230 and 235 nm (Fig. 2) of the UV absorption spectra is observed. The PHA-containing sample was digested using concentrated sulfuric acid. The presence of a single peak with a maximum absorption with that of standard crotonic acid was confirmed by UV analysis of crotonic acid, a byproduct of PHA digestion in sulfuric acid. Hence, the product formed after digestion exhibited a UV spectrum that matched standard crotonic acid (Central Drug House, New Delhi) (Duvigneau et al., 2021; Mojaveryazdi et al., 2014; Panda et al., 2008; Singh et al., 2019).

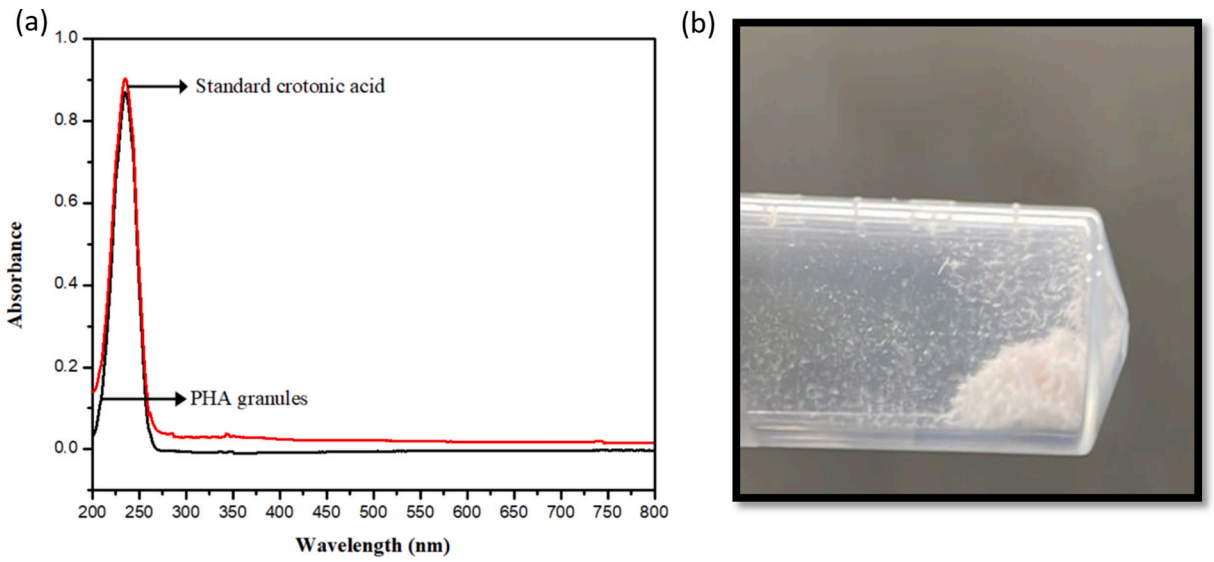

Fig. 2. (a) UV spectra showing standard crotonic acid (red line) and PHA granules (black line), (b) Sample of PHA granules produced by *B. megaterium* MTCC 453. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.7. FTIR of PHA granules

The FTIR tool is a sophisticated instrument for characterizing PHA based on identifying functional groups and their bonds in the polymer. In addition, FTIR provides insight into monomer types and arrangements in PHA for its determination of PHA composition. FTIR spectroscopy depicted different functional groups present in PHA granules, as indicated in Supplementary Fig. S4. Spectroscopy also analyzed the features of the chemical characteristics of the polymer that reflects its monomers. In Fig. S4, stretching of functional groups such as aliphatic -CH group, carbonyl group, aliphatic CH_2 group, CO bond of ether group, and C-O-C bond of ester group represented as 2923, 1571, 1457, 1155, and 779 cm^{-1} bands. These peaks coincide with the previous studies on polyhydroxyalkanoate production (Asad-ur-Rehman et al., 2016; Hong et al., 1999; Trakunjae et al., 2021).

4. Conclusion and recommendations

The findings of this work have significant ramifications for sustainable development and cost-effective methods for producing bioplastic from agricultural waste. The present work involved analyzing the efficacy of the pretreatment procedure utilized in the extraction of sugar from wheat straw (WS), as well as an evaluation of its viability as a growth medium for the synthesis of Polyhydroxyalkanoate (PHA) through the involvement of *Bacillus megaterium* MTCC 453. A positive Molisch test confirmed the presence of fermentable sugars in pretreated WS. The experimental conditions for the pretreatment used were at 121 °C with 15 psi for 1 h, as evidenced by the DNS test. Using chemically treated wheat straw extract as a carbon source can enhance the growth proliferation of industrially essential microorganisms and produce bioplastics. When *Bacillus megaterium* MTCC 453 was grown on 4 % H_2SO_4 pretreated wheat straw, it produced Polyhydroxyalkanoate (PHA), as confirmed by UV spectra at 235 nm after digestion with concentrated sulfuric acid. Further studies are required to optimize the pretreatment conditions of wheat straw extract for maximum PHA production.

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, 2 % NaOH, and 4 % NaOH at 121 °C for 1 h.
Qualitative and quantitative sugar analysis	All four pretreatment processes showed the presence of reducing carbohydrates indicating lignocellulose was broken down to its simpler form.
Qualitative analysis (Molisch's test)	DNS test quantified sugar liberated from all four pretreatments.
Quantitative analysis (DNS test)	2 % H_2SO_4 had 3.72 µg sugar/mL extract. 4 % H_2SO_4 had 4.92 µg sugar/mL extract. 2 % NaOH had 1.05 µg sugar/mL extract. 4 % NaOH had 3.02 µg sugar/mL extract. So, pretreatment with 4 % H_2SO_4 had 4.92 µg sugar/mL extract which was chosen to produce PHA bioproduct using *Bacillus megaterium* MTCC 453
FTIR analysis	FTIR analysis of all the treatments and raw wheat straw showed different peaks intensities at: 603 cm^{-1}: lignin fingerprint 1051 cm^{-1}: lignin fingerprint 1420 cm^{-1}: lignin fingerprint 2920 cm^{-1}: C—H stretching 3400 cm^{-1}: OH stretching
XRD analysis	XRD analysis of all treatments and wheat straw reflected light at (101) and (002) planes. Peak intensity was higher for the plane (002). The crystallite size was calculated as follows: Raw wheat straw: 2.455 nm 2 % H_2SO_4: 1.69 nm. 4 % H_2SO_4: 0.42 nm. 2 % NaOH: 1.82 nm. 4 % NaOH: 1.77 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

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Research methodology throughout the manuscript	Important findings
	breakdown was seen in the case of NaOH as compared to H_2SO_4. 4 % H_2SO_4 treated wheat straw extract was used to produce PHA using *Bacillus megaterium* MTCC 453. Approx. 30 mg PHA/L of the extract was produced.
Identification of PHA granules by UV spectrophotometer	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.
FTIR analysis of PHA granules	The functional groups identified based on stretching were: 2923 cm^{-1}: aliphatic CH group 1571 cm^{-1}: carbonyl group 1457 cm^{-1}: aliphatic CH_2 group 1155 cm^{-1}: CO bond of ether group 799 cm^{-1}: C-O-C bond of ester group

CRediT authorship contribution statement

Rohan Samir Kumar Sachan: Writing – original draft, Methodology, Conceptualization. **Inderpal Devgon:** Writing – original draft. **Arun Karnwal:** Writing – review & editing, Supervision. **Alaa El Din Mahmoud:** Writing – review & editing, Writing – original draft, Visualization, 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.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biteb.2024.101770.

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