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RESEARCH ARTICLE

THE EFFECT OF HYDROGEN PEROXIDE TREATMENT ON FUNCTIONAL PROPERTIES OF PADDY HUSK BIOCHAR AND ITS APPLICATION FOR WASTEWATER TREATMENT

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Abstract

Paddy husk is one of the biomass raw materials and byproducts of the rice production and processing industry. It is very much abundant in Sri Lanka, which is readily available as an energy source. Paddy husk contains silica as a special characteristic component. Application of paddy husk is novel in Sri Lanka. Though it has perceived implications, the process may impose limitations in its application. Appropriate knowledge of the functional properties of paddy husk is required for applying them in thermochemical conversion processes like pyrolysis. The ultimate objective of this study is to identify the effectiveness of wastewater treatment using biochar, derived from H_2O_2-treated paddy husk. The changes in functional properties: moisture content, proximate analysis, true density, bulk density, and porosity of paddy husks, before and after H_2O_2 treatment were evaluated in this study. Two main variables: concentration of H_2O_2 and treatment temperature, were considered. The H_2O_2 treatment method was investigated to remove the lignin distributed in the lignocellulosic matrix of paddy husk. The results obtained from this experimental study showed that there was a significant difference in the functional properties of the paddy husk after H_2O_2 treatment. The biosorbent adsorption process is the finest treatment method, for the removal of methylene blue dye. The use of H_2O_2-treated paddy husk bio sorbent as an alternative cost-effective adsorbent in the removal of methylene blue has been extensively studied and compiled. Biochar as a firm carbonaceous material shows reasonable potential to oversee wastewater contaminants, due to induced pore spaces that are responsible for the removal of contaminants.

Keywords: Biochar characterization, Functional properties, Delignification, H_2O_2 treatment, Pyrolysis, Wastewater treatment

INTRODUCTION

Sri Lanka is an agriculture based developing country where paddy cultivation occupies a major share, since rice is considered as their staple food. Therefore, there is a heavy demand for paddy in Sri Lanka. Sri Lankan economy is agriculture based (Arachchige and Sakuna 2019), and it contributes to 11.38% of the GDP (Neshankine and Kannan 2021). In the process of milling paddy for consumption, paddy husk is produced as a byproduct from the milling of paddy, which provides an abundant agricultural residue that is considered to be a waste product (Murtey and Seeni 2020). Paddy comprises 20 - 25% of paddy husk, which is a cellulose-based hard protective leafy outer covering of rice (Risfaheri *et al*. 2018; Labaran *et al*. 2019).

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Pathak *et al*. (2016) reported that paddy husk consists of 32% cellulose, 21% hemicellulose, 21% lignin, and 20% inorganic silica. The percentage of this composition could change according to the variety of rice, climate, place of cultivation, and other environmental factors. After pretreatment of paddy husk, it has a very high potential use as an energy source. However, a large amount of paddy husk is still being dumped or burned as waste due to low bulk density, smaller particle size, high C : N ratio, low content of total digestible nutrients, lack of knowledge, and the general complexity of using it as a raw material for pyrolysis (Wang *et al*. 2016). In rice-producing countries like Sri Lanka, it is a burning problem how to profitably utilize this low-value paddy husk.

Over recent decades, there has been a development of numerous pretreatments to degrade lignin through oxidizing agents like hydrogen peroxide (H_2O_2). In H_2O_2 treatment of paddy husk, it decomposes into hydroxyl radical and superoxide anion that utilize oxidative delignification to solubilize lignin and lose the lignocellulose matrix (Ho *et al*. 2019). Since H_2O_2 is expensive, in order to overcome economic drawbacks, a lower concentration treatment can be selected. H_2O_2 treatment heavily depends upon the concentration of H_2O_2, temperature, reaction time, pH, and liquid to solid ratio (Díaz *et al*. 2014).

The use of H_2O_2 leads to formation of oxygenated functional groups in biochar and results in activated carbon with high porosity and surface area (Jain *et al*. 2015). The functional properties of paddy husk are considerably modified by H_2O_2 treatment, which solubilizes part of the lignin and reduces cellulose crystallinity by breaking the hydrogen bonds within and between the chains, resulting in a more open internal structure (Galdeano and Grossmann 2005). Paddy husk can be made into an attractive biosorbent material through H_2O_2 treatment. It is used as an absorbent in removing heavy metals, chemicals, and dyes (Noor and Rohasliney 2012).

Methylene blue ($C_{16}H_{18}N_3SCl.3H_2O$) is an organic cationic compound, an intensely toxic dye which can be adsorbed on to adsorbents like H_2O_2-treated paddy husk (Bhattacharya and Sharma 2005). Although Methylene blue (MB) is seen in some medical uses in enormous quantities, it also has wide applications that include dyeing wools, cotton, paper, and hair colorant. MB is preferred as a model compound to assess the capacity of biosorbents like paddy husk for the removal of methylene blue dye from aqueous solutions (Han *et al.* 2007). The amount of dye adsorbed was found to vary with adsorbent dosage, temperature, initial solution pH, and contact time (Kumar and Kumaran 2005).

Biochar is a black, highly porous, and carbonaceous material generated by thermochemical pyrolysis of organic material in an oxygen-limited environment (Koyama and Hayashi 2017). Production of biochar and its functional properties strongly depend upon the conditions during rapid pyrolysis. Pyrolysis favors the elimination of hydrogen and oxygen over carbon leading to a solid residue (biochar) progressively with high carbon content (Crombie *et al.* 2013). Biochar is viable as a sustainable material that has gained growing attention for environmental remediation as it has a proven potential for wastewater treatment. H_2O_2 treatment leads to the formation of oxygenated functional groups with the associated high porosity and surface area which helps delignification that can affect the properties of biochar (Ho *et al*. 2019).

Over the last few centuries, wastewater production from several industries has been significantly increasing (Rafatullah *et al.* 2010). Presently, vast conventional technologies are available for wastewater treatment. But essentially, these technologies do have their drawbacks including high power and chemical consumption, complexity, high maintenance and operational costs (Enaime *et al.* 2020). This work deals with an evaluation of H_2O_2 treatment for improving functional properties of paddy husk and wastewater treatment using pyrolyzed biochar from H_2O_2-treated paddy husk. In the past, several research have been undertaken with H_2O_2-treated paddy husk, but the production of biochar using that H_2O_2-treated paddy husk is unknown. This study is to discover the use of hydrogen peroxide as a potential oxidizing agent for delignification of paddy husk and to check the potential of H_2O_2-treated biochar as a compound for wastewater treatment.

MATERIALS AND METHODS

Materials

Hydrogen peroxide (6% v/v) was purchased at

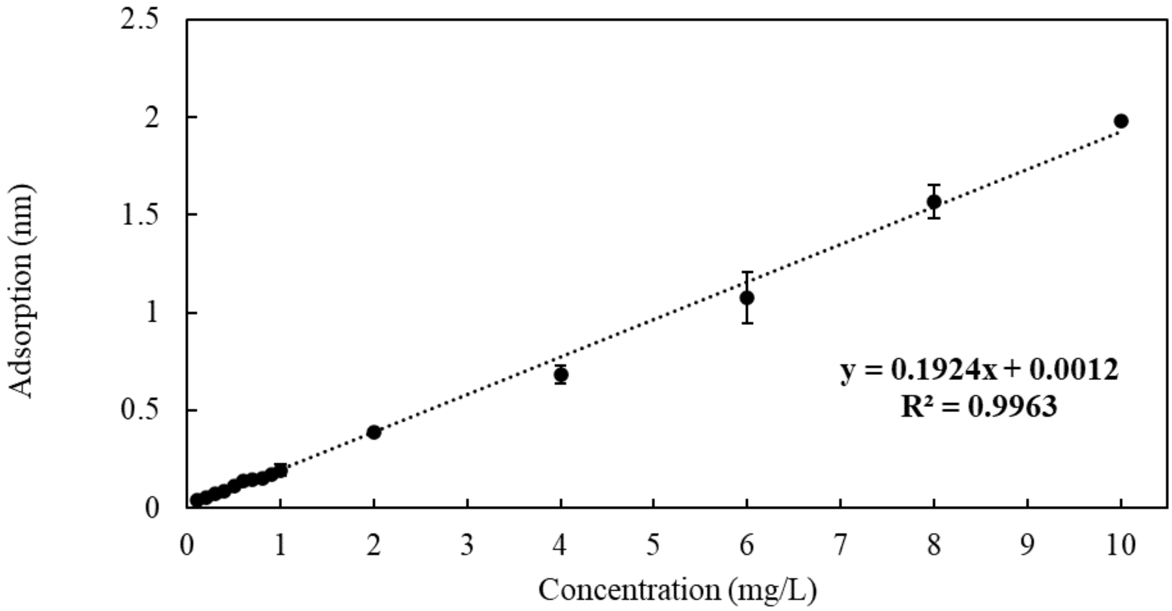

Figure 1: Calibration curve of methylene blue

a commercial-level pharmacy located in Jaffna, Sri Lanka. Methylene blue (MB) dye was purchased from Win Lab Ltd. company in the form of fine powder. Paddy husk was obtained from the Durga mill located at Karugampanai, Sri Lanka. Paddy husk samples above 2 mm in size were separated by sieving. Different concentrations of H_2O_2 solutions: 2%, 4%, and 6% (v/v) were prepared using commercial H_2O_2 solution (6% assay). 100 mg/L concentration of methylene blue stock solution was prepared by dissolving 100 mg of MB in 1 L of distilled water. The calibration curve for MB is given in the Fig. 1.

H_2O_2 Treatment

H_2O_2 concentrations of 2%, 4%, and 6% (v/v) were used to pretreat 10 g of paddy husk samples at a weight/volume (g/mL) ratio of 1:10 (Wang *et al*. 2016). Treatments were performed at different temperatures of 50 °C, 70 °C, and 90 °C in 3 replicates. H_2O_2-treated paddy husk samples were washed with distilled water, followed by mild acid (0.01 M HCl), and solar-dried. Well-dried samples were sealed and stored in a storage bottle for investigating the adsorption properties. The best concentration of treatment was selected based on the adsorption experiment.

Adsorptive Experiment

Batch adsorption experiments were conducted for H_2O_2-treated paddy husk along with raw paddy husk. In each trial, 30 mg of raw paddy husk and H_2O_2-treated paddy husk samples were placed into 15 mL glass bottles, and each bottle was then filled with 10 mL of a methylene blue solution with a known concentration of 100 mg/L. Then pH of methylene blue was adjusted to the desired level (pH 6) with 0.01 M HCl and 0.01 M NaOH. Samples were incubated in a shaking incubator at 25 °C and 150 rpm for 24 hours. After this incubation period, the absorbance of the appropriately diluted solution was measured using a UV-vis spectrophotometer (Model: UH5300 spectrophotometer) at a wavelength of 664.5 nm (Labaran *et al*. 2019). This was repeated three times for an average value of absorbance.

The removal percentage and adsorptive capacity were measured using equations (1) and (2), respectively (Kannan and Sundaram 2001).

$$\text{Removal (\%)} = \frac{(C_i - C)}{C} \times 100 \text{ ....Eqn 01}$$

$$\text{Adsorptive capacity } (q_e) = \frac{(C_i - C)\,V}{W} \text{ ...Eqn 02}$$

Where: c_i is the initial adsorbate concentration (mg/L), c is the adsorbate concentration (mg/L) in solution at time t (h), W is the weight of biochar (g), and V is the volume of solution (L). The suitable concentration of H_2O_2 treatment was selected through adsorptive capacity.

Determination of Functional Properties before and after Treatment

The functional properties of raw paddy husk and best selected H_2O_2-treated paddy husk were measured. The considered parameters and their measurement procedures are tabulated (Table 1). Changes in functional properties before and after treatment were recorded and effectiveness was compared by t-test.

Biochar Production and characterization

The selected best H_2O_2-treated paddy husk was used for biochar preparation. H_2O_2-treated paddy husk was filled into a ceramic crucible, and it was heated at 300 °C, 400 °C, and 500 °C, at a rate of 72.73 °C min^{-1} for 2 hours in a muffle furnace. The resulting biochar produced at 300 °C, 400 °C, and 500 °C was designated as PHBC 300, PHBC 400, and PHBC 500 respectively, with "PHBC" indicating Paddy Husk Biochar.

In the characterization of H_2O_2-treated biochar, biochar yield, point zero charge (pzc), and X-ray diffraction spectroscopy (XRD) were considered. The biochar yield was ascertained by evaluating the ratio of the weight of the H_2O_2-treated biochar to the weight of biomass (Manoharan *et al.* 2022). pzc of H_2O_2-treated biochar was determined by the pH drift method using 3 replicates. In here, accurately weighed 0.1 g of biochar pyrolyzed at different temperatures were mixed with 100 mL of NaCl solution at different pH values separately (3, 4, 5, 6, 7, 8, 9, 10, and 11). Organized solutions were placed in a shaking incubator for 24 h at 30 °C and 150 rpm. The final pH was determined after 24 h using a multimeter. The pzc was determined at the point where the final pH and initial pH values are equal. Raw paddy husk, selected best H_2O_2-treated paddy husk, and biochar pyrolyzed at different temperatures (PHBC 300,400 and 500) were powdered for determination of crystallographic arrangements using XRD spectroscopy (Manoharan *et al.* 2022).

Wastewater Treatment (WWT)

Wastewater (WW) from a canteen was used to check the performance of H_2O_2-treated, pyrolyzed biochar. Initial wastewater parameters: pH, Turbidity, Colour, Total Solids (TS), Total Dissolved Solids (TDS), Total Suspended Solids (TSS), Total Volatile Solids (TVS), and Electrical Conductivity (EC) were measured. For wastewater treatment of biochar, 20 mL of wastewater sample was poured into 100 mg biochar and the mixture was placed in a shaking incubator for 24 h at 30 °C and 150 rpm with 3 replicates. Then final parameters after treatment were measured and their effectiveness in wastewater treatment was compared by statistical analysis (paired t-test).

RESULTS AND DISCUSSION

Adsorption Experiment for Selection of Best H_2O_2 Treatment

The removal percentage and adsorptive capacity (q_e) of raw paddy husk and differently treated paddy husk are illustrated in the Fig. 2. The removal percentage and adsorptive ca-

Table 1: Functional properties considered in the study and procedure of measurement

Parameter	Method or Instrument used	Reference
Moisture content	Oven dry method	Naqvi *et al*. 2015
Length breadth ratio (lbr)	Vernier caliper	Neshankine and Kannan 2021
Proximate analysis	Chesson-Data method	Ma'Ruf *et al*. 2017
True density	Toluene displacement method	Varnamkhasti *et al*. 2008
Bulk density	Mass/ Volume ratio	Neshankine and Kannan 2021
Porosity	$\frac{\text{True density} - \text{Bulk density}}{\text{True density}} \times 100$	Hu *et al*. 2008

pacities varied with varying sorbent treatments as shown in the Fig.2 due to the deviations in surface functional properties like constricted carbon structure and functional groups.

Raw paddy husk expressed the lowest removal percentage of 38.2%, while 6% H_2O_2 treatment at 90 °C exhibited the highest removal percentage of 77.7%. Raw paddy husk showed the lowest adsorptive performance of 12.7 mg/g, whereas 6% H_2O_2 treatment at 90 °C exhibited the highest adsorptive performance of 25.9 mg/g. Both removal percentage and q_e values increased with the increase in H_2O_2 concentration and treatment temperature, because they dissolved lignin and enhanced the surface area, and carbon network. Moreover, they induced the hydroxyl, carboxyl, and oxygen containing functional groups to their active stage with the increased concentration and temperature (Nadarajah *et al.* 2021).

Selection process

The highest equilibrium quantity (q_e) was achieved for 6% concentration of H_2O_2 treatment at 90 °C, which showed the best adsorption. Therefore 6% concentration of H_2O_2 treatment at 90 °C was selected as the best one, and functional properties after the treatment were determined for this particular group, so on to decide the preparation of targeted biochar product.

Effect of H_2O_2 Treatment on Functional Properties

Tabulated changes in functional properties (physical characteristic) of paddy husk due to H_2O_2 treatment are presented in the Table 2. It shows the functional properties of paddy husk before and after treatment and the p-values of the paired t-test for comparison. As per the results, there is a significant increment in moisture content, true density, porosity, cellulose content, and hemicellulose content while there were small increases in length breadth ratio (lbr) and bulk density after H_2O_2 treatment of paddy husk.

There is a significant decline in lignin content after treatment due to delignification by H_2O_2 treatment. H_2O_2 acts as an oxidizing agent and utilizes oxidative delignification to solubilize

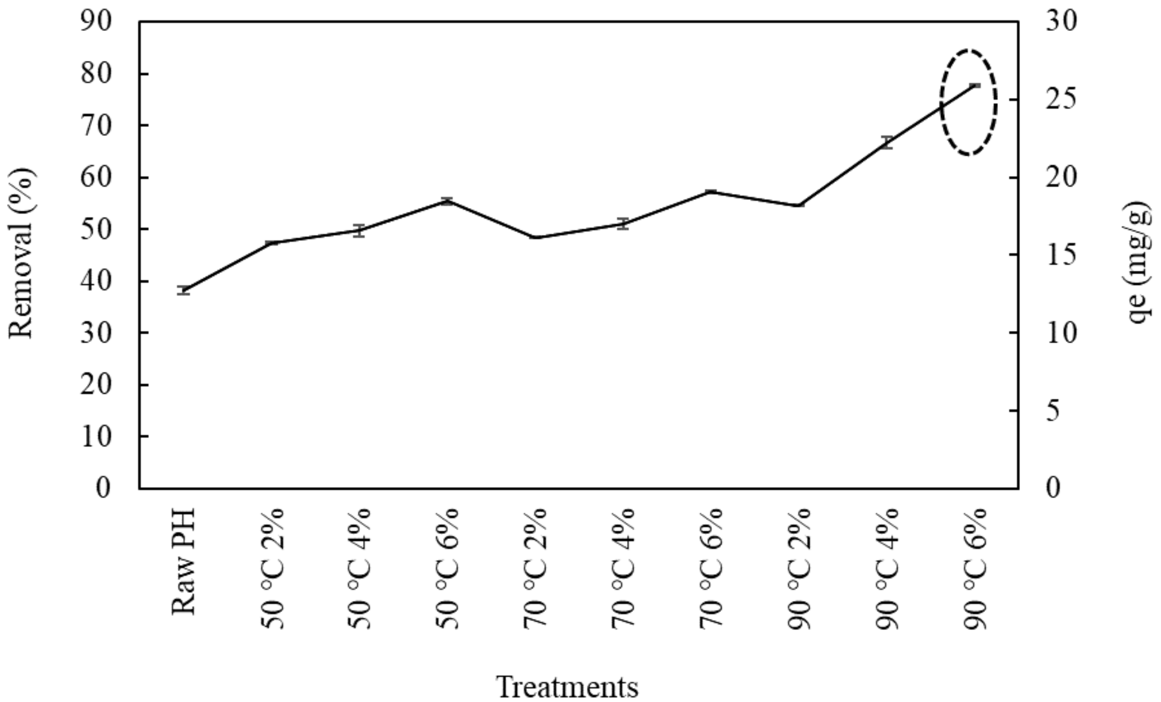

Figure 2: Removal percentage and adsorptive capacity of raw paddy husk and paddy husk with different H_2O_2 treatments

Table 2: Changes in physical characteristics due to H_2O_2 treatment

Physical character	Before treatment	After treatment	p-value
Moisture content (%)	7.03	8.51	0.037
Lbr	3.39	3.61	0.508
Bulk density (gcm^{-3})	0.1033	0.1047	0.367
True density (gcm^{-3})	0.47	0.78	0.003
Porosity (%)	78.35	88.93	0.047
Cellulose (%)	32.81	38.64	0.007
Hemicellulose (%)	15.87	22.12	0.004
Lignin (%)	27.93	20. 98	0.017

lignin, which leads to lose the lignocellulose matrix (Wang *et al*. 2016). The true effect of H_2O_2 depends on the concentration of H_2O_2 and treatment temperature. The ability of H_2O_2 to act as an oxidizing agent increased with the increase of H_2O_2 concentration and treatment temperature that resulted in modified activated paddy husk to be more porous with increased surface area and less lignin content (Manoharan *et al*. 2022).

Biochar Characterization: The effect of temperature on biochar

The biochar yield at 300 °C, 400 °C, and 500 °C were 46.62%, 33.78%, and 17.57%, respectively (Fig. 3). The biochar yield reduced with the increase in the pyrolysis temperature from 300 °C to 500 °C, while with a further increase in temperature, it became paddy husk ash. The highest yield of 46.62% specified the occurrence of partial pyrolysis at 300 °C. The major constituents, cellulose and hemicellulose play a key role in the determination of the biochar characterization through the pyrolysis process (Wang *et al*. 2016).

Previous studies reported that the moisture liberation had occurred at 220 °C, while degradation of hemicellulose at temperature ranged from 220 to 300 °C, whereas degradation of cellulose occurred at 340 °C and lignin degraded at elevated temperatures above 400 °C (Al-Wabel *et al*. 2013). There was an extreme yield reduction from 400 °C to 500 °C. This reduction in yield was due to the decomposition of primary and secondary compounds of biochar, diffusion of some organic matter, and destruction of components like cellulose and hemicellulose at elevated temperatures (Manoharan *et al*. 2022).

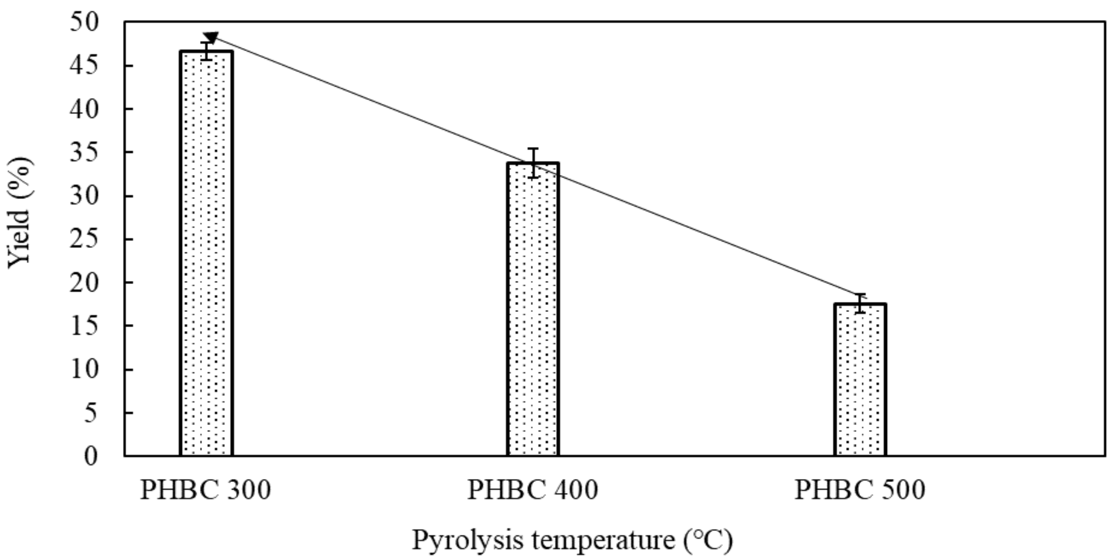

Figure 3: Effect of pyrolysis temperature on biochar yield

Biochar Characterization: Surface charge distribution

The point zero charges (pzc) of H_2O_2-treated biochar pyrolyzed at different temperatures (300 °C, 400 °C, and 500 °C) achieved using the pH drift method (Tran *et al*. 2016) as illustrated in the Fig. 4. The point zero charge is a rudimentary description of the point, which is equivalent to zero with the net charge of the biochar (Banik *et al*. 2018). With the increasing pyrolysis temperature, the point zero charge increased due to high concentrations of oxonium groups and low concentrations of carboxylate groups at elevated temperatures (Manoharan *et al*. 2022). The pzc values of PHBC 300, 400, and 500 are 3.38, 4.22, and 5.41, respectively. Since the experimental setup involved the use of NaCl, NaOH, and HCl, negatively charged sites were occupied by H^+ and Na^+ ions, while positively charged sites were filled by Cl^- and OH^- ions.

When the pH of the solution is lower than the pzc value, the biochar's surface charge becomes positive, facilitating the adsorption of anions. Conversely, when the pH of the solution exceeds the pzc value, the biochar's surface charge turns negative, enabling the adsorption of cations (Tran *et al*. 2016). The pzc is a key factor influencing electrical conductivity in wastewater treatment and the determination of optimum pH in adsorptive experimental studies.

Biochar Characterization: Carbon Structure

The selected biosorbent H_2O_2-treated paddy husk was further characterized in X-ray diffraction (XRD) to study their crystalline phases along with raw paddy husk. The XRD of H_2O_2-treated paddy husk exposes information relevant to the chemical composition of biomass. The XRD spectra obtained for selected paddy husk before and after H_2O_2 treatment are illustrated in the Fig. 5. The blue line in the Fig.5 expressed the XRD pattern of the powdered raw paddy husk before H_2O_2 treatment, while the red line denoted the XRD pattern of the powdered paddy husk after surface modification with H_2O_2.

Both XRD patterns showed that the paddy husk consisted of both amorphous and crystalline silica (Manoharan *et al*. 2022). The differences visible in the XRD spectra of each biosorbent represented the differences among their crystallographic features. The peak at 25.47° was observed in the spectrum of raw paddy husk, while the peak at 25.68° was ob-

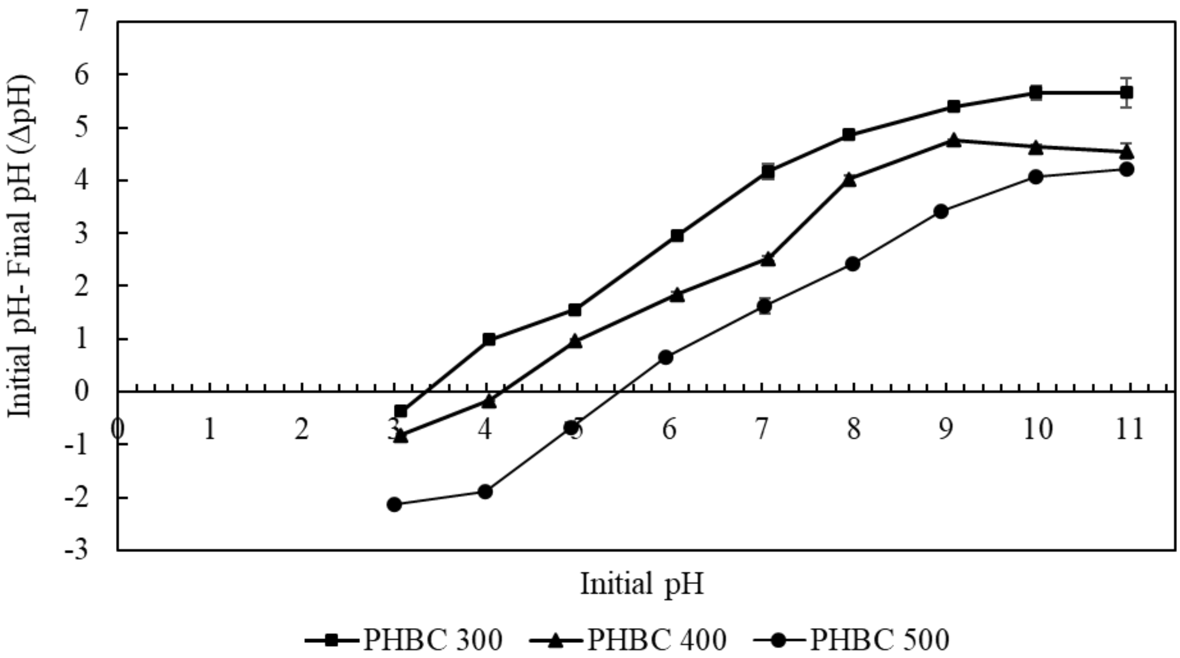

Figure 4: pzc of PHBC 300, 400, and 500

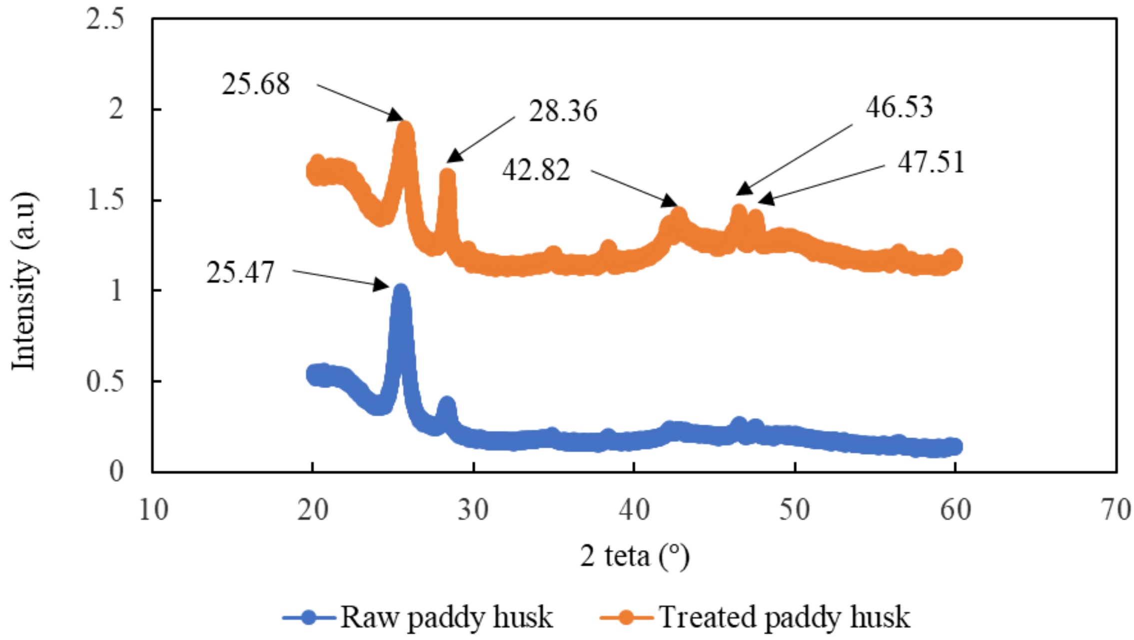

Figure 5: XRD graph before and after H_2O_2 treatment

served in the spectrum of H_2O_2-treated paddy husk. Remarkable peaks also obtained in H_2O_2-treated paddy husk at 28.36°, 42.82°, 46.53°, and 47.51°. These differences could be correlated to the variations in the microcrystalline structure of cellulose in the selected biosorbent and cellulosic compounds with active -OH functional groups (Nadarajah *et al*. 2021). The peaks have decreased in intensity caused by the amorphous nature of silica because of the carbon present in the sample. It is evident from the XRD results that the crystalline structures of the raw paddy husk showed significant differences after the H_2O_2 treatment (Wang *et al*. 2016).

The XRD pattern of paddy husk biochar pyrolyzed at the temperature of 500 °C (PHBC 500) before and after wastewater treatment (WWT) is illustrated in the Fig. 6. The blue line in the Fig.6 expressed the XRD pattern of the powdered PHBC 500 before WWT, while the red line denoted the XRD pattern of the powdered PHBC 500 after WWT. XRD was employed to identify crystalline components in the samples by analyzing the prominent peaks that described prospective minerals that are present in the resulting biochar (Zhang *et al*. 2017).

The XRD spectrum displayed numerous peaks, affirming the formation of a wide array of mineral crystals and other inorganic components throughout pyrolysis. The XRD patterns of the major peaks were found at 25.57°, 28.70°, 46.86°, and 47.83° of biochar PHBC 500 before WWT while the XRD patterns of the major peaks were found at 25.54°, 28.61°, 46.57°, and 47.50° of biochar PHBC 500 after WWT. The most prominent peak emerged at 25.54° for PHBC 500 after WWT. Several new peaks formed at PHBC 500 before WWT, which indicated the creation of new compounds as a result of degradation at elevated temperatures (Manoharan *et al*. 2022).

Effectiveness of different Biochar on the wastewater treatment

Biochar, highly porous, biostable, widely accessible substance, has gained growing attention as a substitute for adsorbents such as zeolite and activated carbon. The biochar sorption capacity toward different contaminants and effectiveness of modified biochar in WWT have been widely reported in many works of literature (Kamali *et al*. 2021).

PHBC 500 has given exceptionally good effectiveness for all these parameters compared to other PHBC samples, which could be due

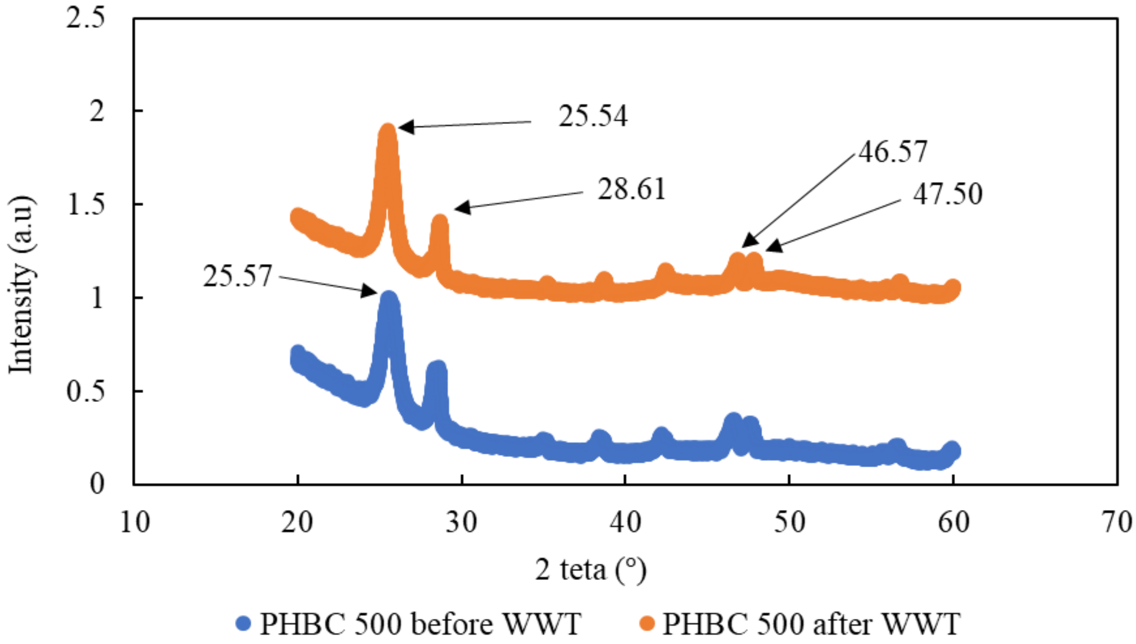

Figure 6: XRD graph before and after WWT

to the development of high surface area and porosity. The treatment removes lignin material from the pore space of biochar, thus making the pore spaces available for mass diffusion of pollutants. Soluble substances migrate into the pore spaces, and adsorption of these into the biochar is responsible for a significant reduction (Nadarajah *et al*. 2021). Therefore, H_2O_2 treatment is highly productive in removing all these substances (TS, TDS, TVS, and TSS) from wastewater. The Fig. 7 displays the effectiveness of H_2O_2-treated biochar produced at different temperatures in the removal of WW parameters: TS, TDS, TSS, and TVS.

Turbidity and colour were removed by different biochar materials (PHBC 300, 400 and 500), and PHBC 500 showed an effective reduction compared to other PHBC because of the development of high porosity and surface area (Manoharan *et al*. 2022).

Electrical conductivity (EC) is the ability to transfer charges. EC increases after wastewater treatment, and PHBC 500 showed the highest EC value probably due to the well-organized development of carbon rings at an elevated temperature of 500 °C (Qambrani *et al*. 2017). pH indicates the hydrogen ion concentration. pH was increased after wastewater treatment and PHBC 500 showed the highest pH value because of its ability to efficiently adsorb substances responsible for the acidic condition of wastewater. Therefore, PHBC 500 is highly effective for the removal of all these parameters considered for the selected wastewater. The following Fig. 8 displays the effectiveness of H_2O_2-treated biochar produced at different temperatures in the removal of WW parameters: pH, EC, Turbidity, and Color.

Effectiveness of selected biochar for wastewater treatment

Statistical analysis was done before and after wastewater treatment only for the best treatment PHBC 500 through Microsoft Excel Software. The Table 3 shows the results of wastewater treatment with a significant p-value of t-test for wastewater treatment with PHBC 500. According to the results there is a significant reduction in TS, TDS, TVS, TSS, color, and turbidity values while there is a significant increment in pH and EC values after wastewater treatment with PHBC 500, due to the above-mentioned reasons.

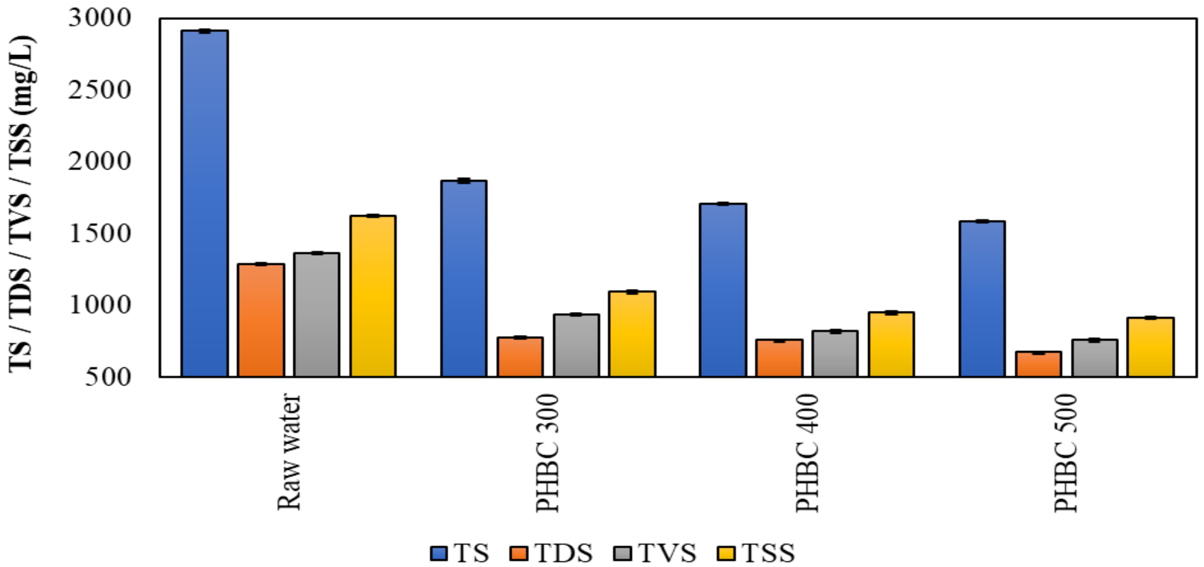

Figure 7: Effectiveness of H_2O_2-treated biochar produced at different temperatures in the removal of WW parameters: TS, TDS, TSS, and TVS

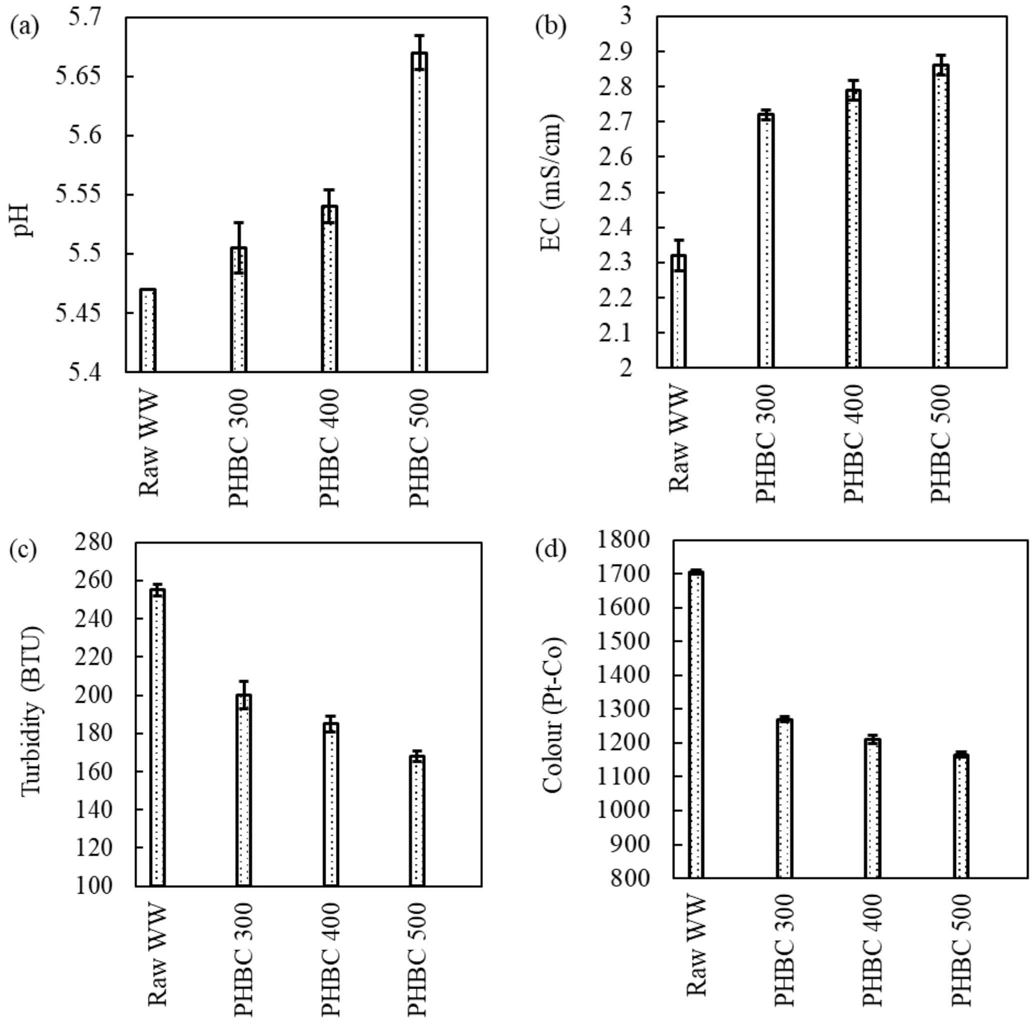

Figure 8: Effectiveness of H_2O_2-treated biochar produced at different temperatures in the removal of WW parameters: (a) pH; (b) EC; (c) Turbidity; (d) Colour

Table 3: Outcome of the wastewater treatment with PHBC 500

Wastewater Parameters	Before treatment (Raw WW)	After treatment (PHBC 500)	Paired t-test p-value
TS (mg/L)	2910	1586	< 0.0001
TDS (mg/L)	1288	672.5	< 0.0001
TVS (mg/L)	1364	757	< 0.0001
TSS (mg/L)	1622	913.5	0.0003
Colour (Pt-Co)	1707	1165	0.0001
Turbidity (BTU)	255	168	0.0007
pH	5.47	5.67	0.0008
EC (mS/cm)	2.32	2.86	0.0040

CONCLUSION

The functional properties of paddy husk were improved after H_2O_2 treatment. Therefore, the raw paddy husk can be upgraded using H_2O_2 treatment. Both H_2O_2 concentration and treatment temperature influenced the functional properties of paddy husk. The treatment with 6% H_2O_2 at 90 ℃ showed the highest removal percentage and adsorptive performance due to better development of surface functional groups at higher concentrations and elevated temperature. The capacity of H_2O_2-treated paddy husk can be further improved by biochar production at different pyrolysis temperatures. It is possible to pyrolyze the upgraded H_2O_2-treated paddy husk to produce highly porous biochar. PHBC 500 exhibited significantly superior pollutant removal capabilities compared to other forms of PHBC. Therefore, H_2O_2-treated PHBC 500 is a viable option for wastewater treatment, although it may not achieve recommended portable water standards. Future research endeavors should explore the potential for even greater effectiveness by investigating higher H_2O_2 concentrations.

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AUTHOR CONTRIBUTION

K. N. and R. C. P. devised the ideas. A. M., K. N. and M. T. collected samples, analyzed, and interpreted the data, and A. M. and K. N wrote the article with input from all the authors.

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