Original Article

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Water hyacinth biorefinery: Improved biofuel production using *Trichoderma atroviride* pretreatment

Obianuju P. Ilo, Mulala D. Simatele, School of Geography, Archaeology and Environmental Studies, University of the Witwatersrand, Johannesburg, South Africa

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Abstract: The pyrolysis of water hyacinth is gaining attention and acceptance as a resource recovery technique due to its availability and economic viability. However, the presence of lignin, one of the three major biomass fractions, presents significant challenges for profitable water hyacinth processing for biofuel production. Fungal pretreatment of water hyacinth for lignin breakdown has been explored but the application of *Trichoderma atroviride* as a pretreatment for pyrolysis is relatively novel. The efficacy of *T. atroviride* pretreatment in improving water hyacinth's pyrolytic products using a fixed-bed reactor was therefore investigated in this study. The optimization process was studied using a central composite design in response surface methodology with Design Expert 13. Delignification of the biomass was established because the elemental analysis showed a 25.42% increase in cellulose content and a 23.40% and 3.37% decrease in lignin and hemicellulose content, respectively. The biomass pretreatment applied influenced the physical and chemical characteristics of the pyrolytic products. The highest pyrolysis oil yield increased by 25.81% at 575 °C and particle size 2290 μm, and the highest char yield decreased by 4.23% at 273 °C and particle size 1500 μm. This research is crucial for policy and research conversations as it offers a scientific basis for the application of *T. atroviride* pretreatment in biomass pyrolysis technology and emphasizes the optimal utilization of water hyacinths to obtain socio-environmental benefits. © 2024 The Author(s). *Biofuels, Bioproducts and Biorefining* published by Society of Industrial Chemistry and John Wiley & Sons Ltd.

Supporting information may be found in the online version of this article.

Key words: biomass; decarbonization; invasive alien plants; pyrolysis; resource recovery

Introduction

The depletion of fossil resources, escalating environmental concerns, and the imperative to foster regional and rural development have resulted in an increased focus on transitioning towards a circular economy. A circular economy is an economic system that aims to restore and regenerate products and resources, ensuring that they always remain at their highest level of functionality and value.[1] This approach emphasizes the systematic use of

Correspondence to: Obianuju P. Ilo, School of Geography, Archaeology and Environmental Studies, University of the Witwatersrand, Johannesburg, South Africa. E-mail: obianuju.ilo@wits.ac.za

biomass as a means of achieving sustainable growth, allowing for the efficient utilization of waste and byproducts to generate valuable outputs.[2]

The biorefinery is a key approach in the circular economy, enabling the recycling and reuse of biomass materials to minimize waste effectively.[1] The biorefinery of lignocellulosic biomass in a circular economy is receiving attention and acceptance because of its environmental and economic sustainability. It utilizes technological processes to convert biomass into valuable products such as fuels, biofertilizers, and energy, in a similar manner to the way in which an oil refinery refines crude oil to produce usable products like gasoline, kerosene, and diesel using separation, conversion, and treatment techniques.[3]

Pyrolysis, one of the various conversion routes of lignocellulose biomass, involves the thermal depolymerization of the biomass in an inert atmosphere to produce residual char and volatile products, condensable liquid, and noncondensable gas.[4,5] The lignocellulosic composition of the biomass and process parameters affect the pyrolytic product yield – for example, char texture and structure[6] and polymeric elements in the liquid product.[7] This is because lignocellulosic biomass comprises three major fractions – cellulose, hemicellulose, and aromatic polymer lignin – which are entwined to form a complex plant cell-wall structure. Cellulose and hemicellulose can be altered easily during pyrolysis but lignin is not susceptible.[8] Lignin is therefore a significant challenge to the profitable processing of biomass, as it is considered to be responsible for the high viscosity and low stability of the pyrolysis oil.[9] The pyrolysis oil is also corrosive and has high water and oxygen content. These factors have reduced its commercialization as a fossil fuel replacement. However, pretreatment of the biomass feedstock before the pyrolytic conversion has an enriching outcome on the biochar and liquid fraction's quality and yield compared to post-treatment.[10,11] Most commonly used pretreatment methods, such as dilute acid and liquid hot water pretreatment, necessitate an extra detoxification stage due to the harsh conditions that lead to the production of pollutants such as furans.

Researchers have recently used fungi to pretreat lignocellulosic biomass and have studied their effects on the kinetic behavior of biomass pyrolysis, highlighting their sustainability and cost-efficiency.[11–13] The present study aims to (i) evaluate the efficiency of *Trichoderma atroviride* as a pretreatment agent on water hyacinth's pyrolytic products and (ii) enhance the process parameters for scale-up potential. *Trichoderma atroviride* is a producer of a complex array of cellulose-degrading enzymes such as β-glucosidase,[14] and it is readily available. This research is innovative as there are no studies on the use of *T. atroviride* for the pretreatment of water hyacinth for pyrolysis. Water hyacinth, also called *Eichhornia crassipes*, is an invasive alien species, originally from the Amazon basin in South America. It poses a significant threat to the pillars of sustainability by reducing aquatic biodiversity through dissolved oxygen depletion, impacting local economies by obstructing recreational spaces and fishing areas, and fostering the breeding of disease vectors such as mosquitoes.[15] Efforts to control it have been largely unsuccessful. Nonetheless, *E. crassipes* presents a promising biomass resource for energy recovery and the production of valuable chemicals. Utilizing it as feedstock does not adversely affect food security, water resources, or land reforms.[16] The invasive plant has enormous carbon and hydrogen matter, high hemicellulose and cellulose content and low lignin content.[17] Its valorization for biorefinery production reduces its invasiveness, its adverse effects on the environment, and the cost of mitigating them.

As much as fungal pretreatment is sustainable and cost-effective for optimal pyrolytic yield, it is considered time consuming and may not be practical for large-scale processes. Rahman[18] opined that the composition and yield of pyrolytic products on a large scale depend heavily on operational factors such as temperature, particle size, heating rate, inert gas flow rate, and the type of feedstock. Insights have been derived using the one-variable-at-a-time (OVAT) experimental method; however, evaluating multiple factors simultaneously has proven to be more effective.[19] The study noted that it reduces the number of experiments, validates the regression model, and optimizes process conditions. To enhance process parameters for scale-up, the influence of temperature and particle size on the pyrolysis process of pretreated water hyacinths was explored, and a response surface methodology (RSM) with central composite design (CCD) of Design-Expert 13 Software (Stat Ease, Inc, Minneapolis, MN, USA) was fitted. According to Arrhenius' law, temperature significantly affects the rate of a chemical reaction and it is necessary to achieve the isoreactive temperature for deoxygenation to occur.[20] The pyrolytic products were further characterized for elemental and volatile organic compound compositions to understand the effect of the pretreatment on the quality of the products. The structural changes in the biomass and char were also examined. This study is important for policy and research conversations as it provides a scientific basis for the use of *T. atroviride* pretreatment in biomass pyrolysis technology and

underscores the optimal utilization of water hyacinths to obtain socio-environmental benefits.

Materials and methods

Collection and preparation of substrate

A permit was obtained from the Department of Environmental Affairs, South Africa, to carry out this research as water hyacinth is controlled through the Alien and Invasive Species (AIS) Regulations, 2014. Permit numbers 50922210601101319 and 50922210525113851 were issued for the possession and study of the invasive plant, and for conveying it. The aquatic weed was collected from Ixopo Dam in KwaZulu-Natal Province, South Africa (30° 8′ 53″ S, 30° 4′ 35″ E) in closed containers and conveyed to the laboratory. They were rinsed carefully with distilled water to remove dirt; the roots were cut off and autoclaved with the wastewater before discarding. The leaves and stems were oven-dried at 105 °C for 24 h to a constant weight. After this, they were pulverized in a slow-speed granulator (Zerma, Shanghai, China) and further, with a grinder (IKA, Staufen, Germany) to infiltrate a vibratory sieve shaker (Fritsch, Idar-Oberstein, Germany) with 380–2629 µm openings. The water hyacinth particles were bagged and stored at ambient temperature for further use.

Inoculum preparation

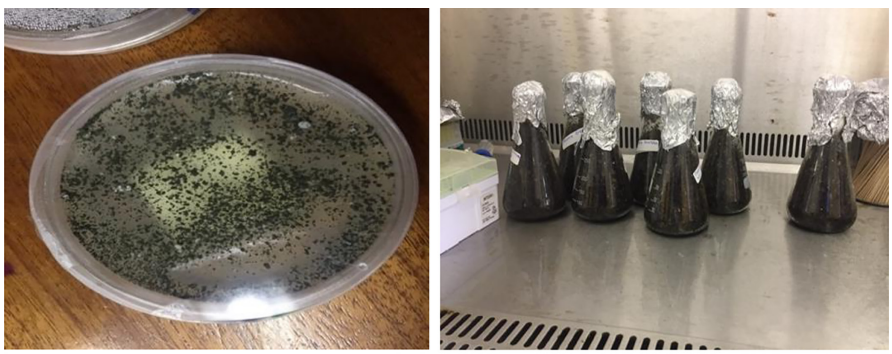
Trichoderma atroviride (PPRI 4705), maintained on potato dextrose agar (PDA), was purchased from the Agricultural Research Council (ARC), Pretoria, South Africa. The *T. atroviride* was activated by inoculating at the center of the plates containing 5 g PDA (Sigma-Aldrich Darmstadt, Germany), 5 g bacteriological agar (Biolab, Budapest, Hungary), and 500 mL distilled water. They were left to grow for 7 days at 25 °C. The spores were prepared according to Kumar *et al.*[14] Sporogenesis was performed by microscopic enumeration with a hematocytometer.

Fungal pretreatment

Pretreatment was achieved as expressed by García-Torreiro *et al.*[21] Water hyacinth samples weighing 16 g in different particle sizes: 380, 710, 1500, 2290, and 2620 µm were mixed with 48 mL of distilled water in each 250 mL Erlenmeyer flask, which was closed with a cotton stopper and aluminium foil to reduce moisture loss, and autoclaved (Equitron, Mumbai, India) at 121 °C for 15 min. The flasks were left to cool, after which 16 mL of the inoculum was added to each flask aseptically and maintained statically in an incubator (Labotec, Durban, South Africa) at 30 °C for 10 days (Fig. 1). The pretreated biomass was dried in an oven at 60 °C for 3 days. The physicochemical compositions of the pretreated water hyacinth, presented in Table 1, were performed to ascertain the key components and elemental compositions and were compared with that of the untreated water hyacinth reported by Ilo *et al.*[22]

Experimental design and setup

Temperature and particle-size ranges were chosen in accordance with the conventional ranges mentioned in the literature to enable comparison with previous studies.[18,22–24] Inert gas flow and heating rates were kept constant due to the constraints of the fixed-bed batch reactor, which limited the concurrent investigation of other factors. Furthermore, keeping the inert gas flow constant ensured a controlled environment and prevented counter airflow during experiments. Design-Expert 13 Software (Stat-Ease, Inc.) was used with response surface methodology (RSM) and a central



(a) (b)

Figure 1. Pictorial representation of (a) the *Trichoderm atroviride* strain and (b) inoculated samples for pretreatment of water hyacinth.

Table 1. Physicochemical composition of pretreated and untreated water hyacinth biomass

Proximate analysis	Pretreated water hyacinth (wt%)	Untreated water hyacinth (wt%) Ilo *et al.*[22]
Ash	11.8 ± 0.22	12.66 ± 0.30
Crude fiber	23.85 ± 0.08	21.18 ± 0.65
Hemicellulose	27.24 ± 0.32	28.19 ± 0.33
Cellulose	24.82 ± 0.07	19.79 ± 0.76
Lignin	5.27 ± 0.05	6.88 ± 0.12
Elemental analysis (wt%)		
Carbon	54.24	48.06
Hydrogen	4.03	3.78
Nitrogen	3.31	3.19
Oxygen[a]	38.42	40.65
Sulfur	1.14	–
Phosphorus	0.81	–
Sodium	–	0.57
Magnesium	2.95	0.67
Aluminium	–	0.38
Chlorine	9.04	4.03
Potassium	14.71	4.22
Calcium	5.01	0.43
Silicon	–	0.70
Iron	–	0.30
Calorific value ($MJ.Kg^{-1}$)		
HHV	17.51	14.76

[a]By difference.

composite design (CCD) in a two-level factorial design with replicates to optimize temperature and particle-size variables. Temperatures ranging from 300–600 °C and particle sizes between 500–2500 µm were treated as independent variables, while the liquid fraction, char, and gaseous fraction were the expected responses. Design-Expert processed the low and high values, center point, and lower and upper axial points for each variable range. A correlation between the independent variables and the responses was established. The samples were coded with unique identification numbers such as T273.22-PS1500, meaning a temperature of 273.22 °C and particle size of 1500 µm.

The experimental setup, which consisted of a fixed-bed reactor, as presented in Fig. 2, was fed with 16 g of dried water hyacinth, with particle sizes ranging from 380–2620 µm. To create an inert atmosphere, and to carry away the volatiles that were produced, nitrogen (N_2) gas (99.9% purity, Afrox, Durban, South Africa) was used as a carrier gas. The reactor was set at temperatures of 273–626.78 °C for each experiment and heated externally with 1.8 kW of electric power. The pyrolysis reactor was linked to a shell and tube condenser, where the hot volatiles from the reactor were cooled using circulating water at 5 °C, facilitated by a pump within a water bath. Condensed liquid fractions were collected in three flasks. Noncondensable gases were directed through a vent to an extractor, and the char remained in the reactor. The experiment was conducted in triplicate and the results were reported as mean values with standard deviations.

Analytical methods

The moisture content of the pyrolysis oil was determined using a Karl Fischer moisture titrator (KEM, Tokyo, Japan). The elemental composition of char and liquid fractions was determined using an organic elemental analyzer (Thermo Scientific, California, USA).

Analysis of variance (ANOVA), available in the Design-Expert 13 software package, was used to analyze the significance of the CCD. The coefficient determination (R^2) established the polynomial model equation for its quality. The F-test analyzed the statistical significance, and the desirability rate for optimized process factors was analyzed. The elemental composition of char was also explored using scanning electron microscopy–energy dispersive X-ray (SEM–EDX) to ascertain other chemical components such as Mg, Cl, and Ca. The pyrolysis oil obtained was analyzed using gas chromatography–mass spectrometry (GC–MS) (Shimadzu, Johannesburg, South Africa). The temperature program was regulated to increase the extraction of chemical components from the liquid fraction. Precisely 1 µL of the pyrolysis oil with dichloromethane solvent (Sigma–Aldrich) was injected in a 30 m × 0.25 mm ID × 0.25 µm capillary column (Shimadzu), with 5% diphenyl/95% dimethyl polysiloxane packing. Helium gas (99.9% purity, Air Products, Pinetown, South Africa) was used as carrier gas at a constant flow rate of 1.2 $mL.min^{-1}$. The National Institute of Standards and Technology mass spectral library was used in the qualitative exploration of the chromatograph to classify the compounds. The functional groups were further characterized using Fourier transform infrared (FTIR) spectroscopy (IRSpirit, Shimadzu) with spectra recorded between 400 and 4000 cm^{-1} over 64 scans. The structural characteristics of the biomass before and after pretreatment were examined using scanning electron microscopy (SEM) (Zeiss,

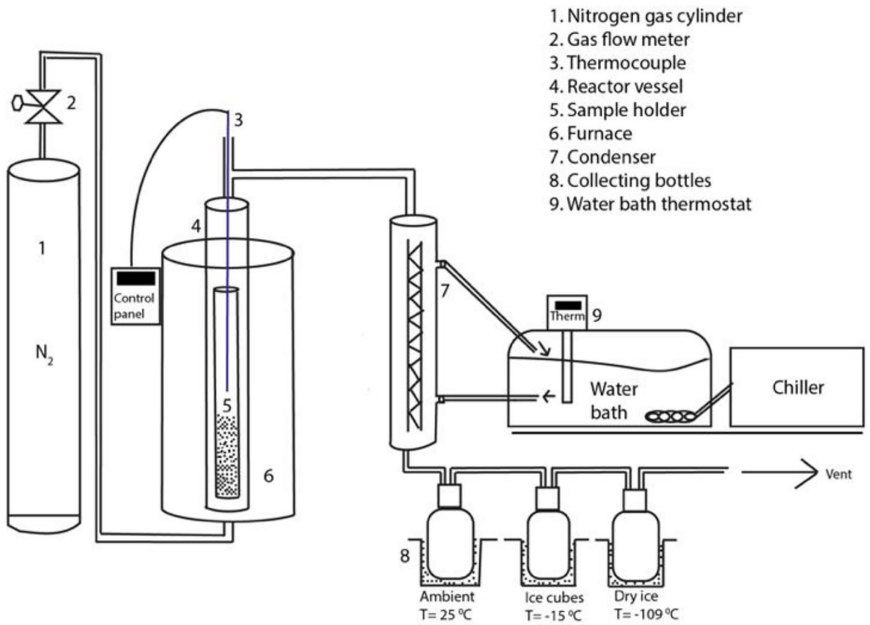


Figure 2. A fixed bed reactor.

Johannesburg, South Africa) to determine the impact of the fungal pretreatment. The surface structure of the char products was also observed.

Results and discussion

Characterization of *T. atroviride* pretreated water hyacinth

The physicochemical components of the water hyacinth play a substantial role in the effectual conversion of biomass into valuable products. These components were altered significantly by the fungal pretreatment (Table 1). In the proximate analysis, the lignin and hemicellulose content in the pretreated feedstock decreased from 6.88 wt% to 5.27 wt% and 28.19 wt% to 27.24 wt%, respectively, compared with the untreated water hyacinth biomass, whereas cellulose increased from 19.79 wt% to 24.82 wt%.,[12] after fungal pretreatment of wheat straw for pyrolysis, reported a 49.49% increase in cellulosic content and 19.31% and 38.08% decrease in hemicellulose and lignin content, respectively. The significant increment in the cellulose content is therefore based on the comparative reduction in the lignin and hemicellulose constituent, as hemicellulose and lignin function as actual obstacles that keep cellulose from degradation. Yang *et al.*[25] opined that the alteration of the lignin component is considered to cause the biomass to be more suitable for thermal devolatilization and faster. In the ultimate analysis, carbon (48.06–54.24 wt%), hydrogen (3.78–4.03 wt%), and nitrogen (3.19–3.31 wt%) were present in higher amounts, while oxygen (38.42–40.65 wt%) was lower in comparison with the untreated feedstock. Yu *et al.*[26] found a similar outcome: more carbon and hydrogen content and reduced oxygen content after fungal pretreatment of corn stover. A higher HHV of 17.51 ($MJ.Kg^{-1}$ was recorded for pretreated water hyacinth than for untreated water hyacinth (14.47 $MJ.Kg^{-1}$).

Effect of pretreatment on product distribution

Heating rates are shown in Table 2. The fraction yield of the pyrolytic products was estimated following the method described by Mohamed *et al.*[27] An increase in temperature resulted in increased liquid and gas fractions and a decrease in the char yield, which corresponds to results reported in the literature.[12,24,28] Among the different temperatures and particle sizes, the highest pyrolysis oil yield of 60.98 wt% was noted at temperature 575 °C and particle size 2290 µm, which is higher than the yield of 48.47 wt% at the same temperature and particle size from untreated water hyacinth in the study reported by Ilo *et al.*[22] This suggests that the pretreatment deconstructed the lignin content and improved the cellulose because, according to Tomczyk *et al.*,[29] high lignin content results in a high char yield. The results also confirmed that the pyrolysis oil yield is dependent on cellulose, whereas the char yield is dependent on the lignin.[12]

Table 2. The pyrolysis of *Trichoderm atroviride* pretreated water hyacinth

Std	Run	Factor 1 temperature (°C)	Factor 2 particle size (μm)	Heating rate (°C. min^{-1})	Nitrogen gas flow rate (mL. min^{-1})	Response 1 pyrolysis oil (wt%)	Response 2 char fraction (wt%)	Response 3 gas fraction (wt%)
3	1	325	2290	30	25	45.29 ± 0.24	39.52 ± 1.03	15.20 ± 0.79
10	2	450	1500			45.56 ± 1.10	35.47 ± 0.15	18.96 ± 0.95
6	3	626.78	1500			38.84 ± 1.13	20.18 ± 0.09	40.98 ± 1.04
4	4	575	2290			60.98 ± 1.39	26.72 ± 1.39	12.30 ± 2.48
5	5	273.22	1500			28.18 ± 0.19	55.75 ± 2.09	16.07 ± 2.28
2	6	575	710			50.75 ± 1.10	30.09 ± 1.35	19.16 ± 0.25
11	7	450	1500			45.56 ± 1.10	35.47 ± 0.15	18.96 ± 0.95
9	8	450	1500			45.56 ± 1.10	35.47 ± 0.15	18.96 ± 0.95
7	9	450	380			36.92 ± 0.71	41.85 ± 0.18	21.23 ± 0.53
13	10	450	1500			45.56 ± 1.10	35.47 ± 0.15	18.96 ± 0.95
1	11	325	710			41.34 ± 0.09	39.51 ± 0.17	19.15 ± 0.35
8	12	450	2620			58.26 ± 1.10	30.93 ± 1.14	10.81 ± 0.05
12	13	450	1500			45.56 ± 1.10	35.47 ± 0.15	18.96 ± 0.95

Optimization of pyrolytic product distribution using RSM

Table 3 presents the experimental runs proposed by Design-Expert version 13, based on the specified process factors (temperature and particle size). Surface and contour graphs were created to ascertain the optimal pyrolytic product yields from *T. atroviride* pretreated water hyacinth. These graphs are the predictions of the response surface; a surface plot specifies a three-dimensional outlook for a more precise response surface, and a contour indicates a two-dimensional view where all the points with similar responses are linked to form contour lines of the responses.[30] Figures 3–5 show the 3D surface and contour graphs of the pyrolytic products of pretreated water hyacinth at different temperatures and particle sizes.

The surface plot of the pyrolysis oil (Fig. 3) shows a sharp drop in the value of the wt% yield at low levels of both temperature and particle size. At a temperature of 273 °C, 28.18 wt% of pyrolysis oil was recorded because the reaction was not complete but, as the temperature increased to 575 °C, a high yield of 60.98 wt% was noted. As temperature increases, the pyrolysis oil yield increases. The increase in pyrolysis oil yield is a result of an increased supply of heat, which enhances primary devolatilization. However, as the temperature increased further to 626 °C, a decrease of 38.84 wt% was recorded due to the activation of secondary reactions in the condensable hot volatiles, leading to increased noncondensable gas.[24] It signifies that the thermal cracking of pretreated water hyacinth ended at 575 °C. The increase in particle size also produced a higher yield of liquid fraction; a particle size of 2620 μm gave rise to 58.25 wt% of liquid content, while the smallest particle size of 380 μm resulted in 36.92 wt% of the liquid fraction. This is a result of the reaction rate of large particle sizes increasing more quickly as temperature rises and yielding more volatiles than small particle sizes. According to Niu and Liu,[31] particle size has a perspicuous influence on the mass loss rate; increased particle size causes a subsequent rise in mass loss.

The surface plot of the char (Fig. 4) indicates a sharp decline in the wt% yield as temperature and particle size increase. At a low temperature of 273 °C, 55.75 wt% of char was generated, whereas at a high temperature of 626 °C, 20.18 wt% of char was generated. As the temperature increased, the char fraction yield decreased. This decrease in char yield at high temperatures is a result of the inner particle temperature of water hyacinth being slow to get to the pyrolysis temperature. Considering the effect of particle size, at a small particle size of 380 μm, 41.85 wt% of char was generated, while 55.75 wt% of char yield was generated at a particle size of 1500 μm. Figure 5 shows that the wt% yield of the gas fraction increased as temperature increased but decreased as particle size increased. At a temperature of 273 °C, a gas yield of 16.07 wt% was recorded, and at an increased temperature of 626 °C, a high yield of 40.98 wt% was noted. This is attributed to the breakdown of the longer chained condensable volatiles and the loss of char to noncondensable gases. At a particle size of 2620 μm, a 10.81 wt% yield of the gas fraction was generated, whereas at the smallest size of 380 μm, a 21.03 wt% yield was produced. Small particle sizes

Table 3. Analysis of variance

Source	Liquid fraction					Char					Gaseous fraction				
	Sum of squares	Df	Mean squares	*F*-value	*P*-value	Sum of squares	Df	Mean squares	*F*-value	*P*-value	Sum of squares	Df	Mean squares	*F*-value	*P*-value
Model	436.25	2	218.12	5.24	0.0278	686.79	2	343.40	25.03	0.0001	369.40	5	73.88	2.21	0.1647
A-temperature	181.85	1	181.85	4.37	0.0632	638.99	1	638.99	46.57	<0.0001	119.67	1	119.67	3.58	0.1002
B-particle size	254.39	1	254.39	6.11	0.0330	47.81	1	47.81	3.48	0.0915	88.15	1	88.15	2.64	0.1482
AB	–	–	–	–	–	–	–	–	–	–	2.25	1	2.25	0.0674	0.8026
A^2	–	–	–	–	–	–	–	–	–	–	91.41	1	91.41	2.74	0.1419
B^2	–	–	–	–	–	–	–	–	–	–	47.93	1	47.93	1.44	0.2698
Residual	416.52	10	41.65	–	–	137.20	10	13.72	–	–	233.68	7	33.38	–	–
Lack of fit	416.52	6	69.42	–	–	137.21	6	22.87	–	–	233.68	3	77.89	–	–
Pure error	0.0000	4	0.0000	–	–	0.0000	4	0.0000	–	–	0.0000	4	0.0000	–	–
Corrected Total Sum of Squares (Cor. Total)	852.77	12	–	–	–	824.00	12	–	–	–	603.08	12	–	–	–

causing high gaseous yields during pyrolysis are similar to the outcome described by Hu *et al.*[32] This is a result of the greater heat transfer resistance from the surface of the large particle sizes to the center. Small particle sizes have higher surface area than large particle sizes, resulting in high gaseous yield from pyrolysis reactions.

Statistical parameters

An ANOVA was conducted to understand the developed response surface methodology's reliability, reproducibility and efficiency. As Table 3 shows, the *F*-values for the liquid, char, and gaseous models are 5.24, 25.03, and 2.21, respectively, and their corresponding *P*-values are 0.0278, 0.0001, and 0.1647, respectively. A *P*-value lower than 0.05 indicates that the model is statistically significant. The chances that the *F*-value occurred due to noise are 2.28%, 0.01%, and 16.47% for liquid, char, and gas, respectively. The models for liquid and char fractions are therefore significant, confirming the applicability of the predicted model, whereas that of gas is not significant. Aside from the *P*-value, other statistical factors such as R^2, adjusted R^2, predicted R^2 and coefficient of variation were also used to evaluate the model's fitness. As seen in Table 4, the estimated R^2 for liquid, char and gas fractions are 0.5116, 0.8335, and 0.6125, respectively. Adequate precision quantifies the signal-to-noise ratio; a ratio above four is preferred. This study attained good precision ratios of 6.7134, 14.2059, and 5.7740 for liquid, char, and gas, respectively. This indicates that the model has a suitable signal-to-noise ratio to navigate the design space.

Desirability function

One substantial factor considered for optimization is the desirability function.[30] All three responses are evaluated concurrently in the optimization but the optimal outcome in one response may have a conflicting effect on another response. The desirability function approach was therefore applied to determine favourable conditions that satisfy all responses. As a result, the composite desirability of the product yields, as seen in Fig. 6, was found to be 1, which signifies a positive effect on the optimization of the response variables.

Elemental composition

Chemical analyses measuring the concentrations of carbon, hydrogen, nitrogen, and oxygen were conducted on two pyrolytic product fractions – liquid and char – to evaluate their elemental composition and product quality. Table 5 presents the percentage (wt/wt) of the elements. It is apparent

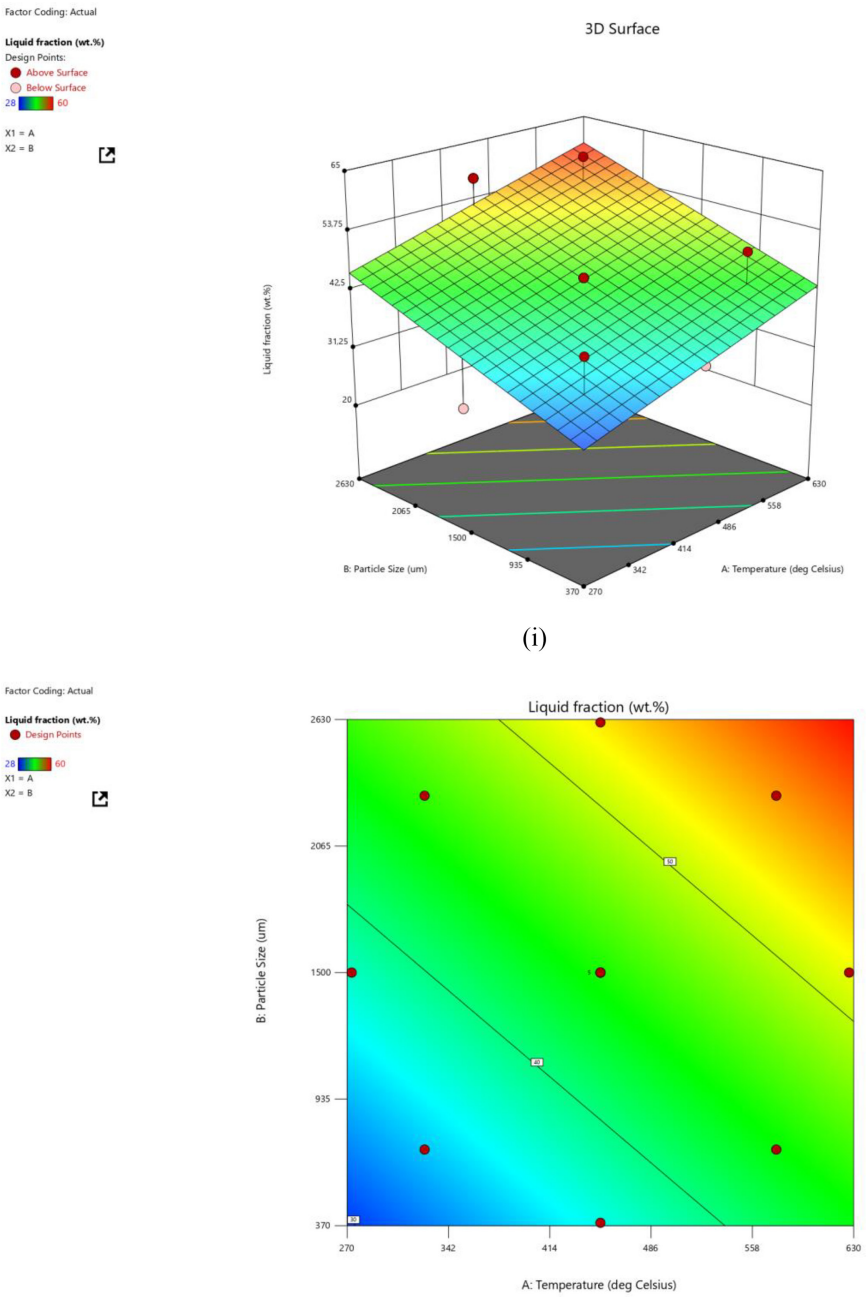


Figure 3. Response surface methodology of the pyrolysis oil from pyrolysis of *Trichoderm atroviride*-pretreated water hyacinth. (i) Surface plot. (ii) Contour graph.

that the liquid faction has quite low nitrogen (0.13–0.57 wt%) and no sulfur, which signifies that there is a negligible chance of NOx and SOx emission while using it as fuel. A gradual increment in the carbon content (56.05–59.21 wt%) was observed until the highest temperature, when it was reduced to 55.75 wt%. A decrease in the oxygen content (30.84–34.40 wt%) and an increase to 34.75 wt% at T626-PS1500 was also noted, which is slightly lower than the pyrolysis oil from untreated water hyacinth in literature; 31.07–35.43 wt% and 31–33 wt% by Ilo *et al.*[22] and Rahman,[18] respectively.

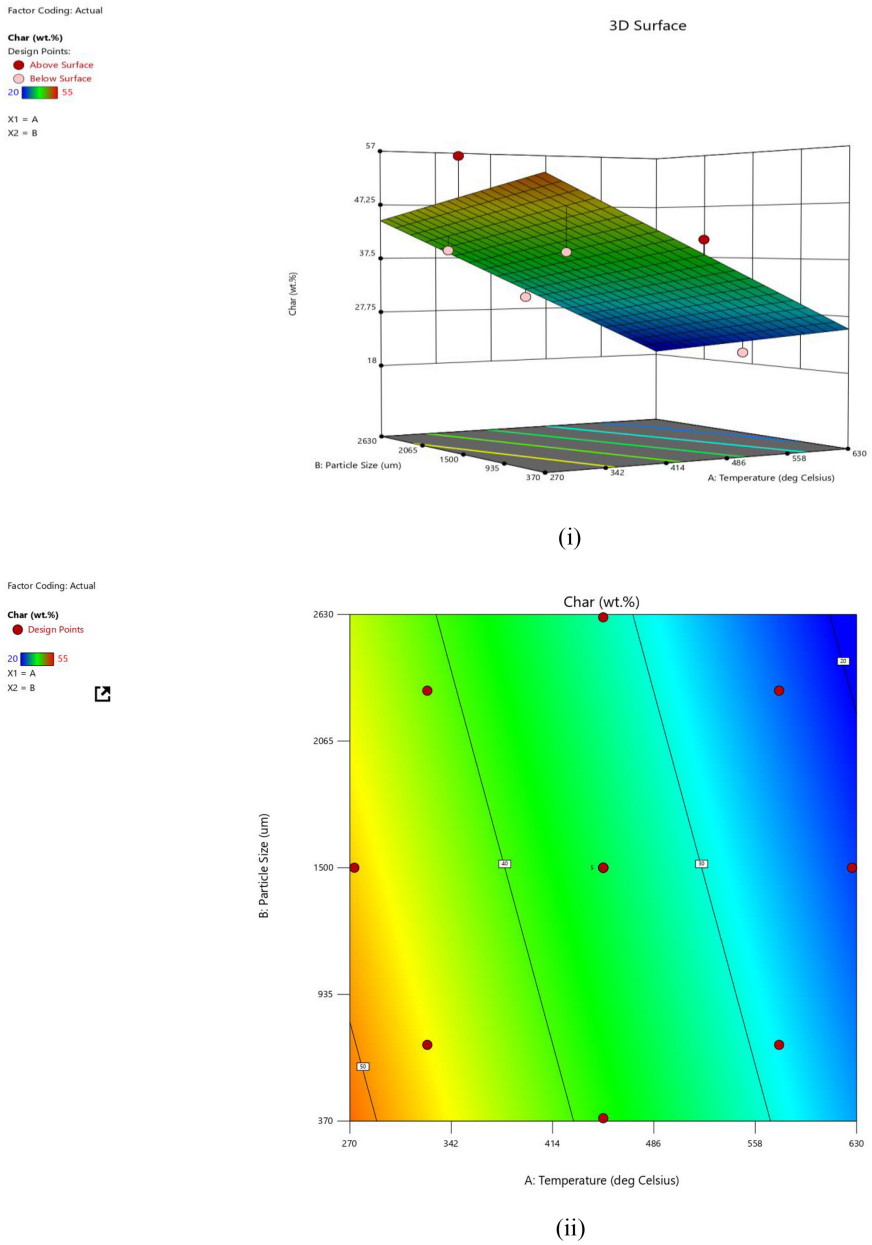


Figure 4. Response surface methodology of the char fraction from pyrolysis of *Trichoderm atroviride*-pretreated water hyacinth. (i) Surface plot. (ii) Contour graph.

The gradual increase and decrease of carbon and oxygen, respectively, are similar to the results of Ghosh *et al.*[10] They described the fungal decarboxylation of the biomass during pretreatment to be accountable for reducing the oxygen content. The HHV, which is closely linked to CHNO content, also increased gradually (26.71–28.96 wt%) until it reached T626-PS1500, where it reduced to 25.95 wt%. As Table 5 also indicates, carbon of the char fraction decreased gradually (59.36–54.37 wt%) as temperature increased while oxygen increased (34. 41–42.75 wt%).

Gas chromatography–mass spectrometry

The emergence of biorefineries is prompting a reawakening in carbohydrate chemistry, resulting in the production of novel sustainable polymers that can tackle global problems in energy, healthcare, and agriculture. The liquid fractions obtained at different temperatures and particle sizes were therefore comprehensively analyzed with GC–MS to identify the compounds. They are presented in Supporting Information, Table S1. The results were compared to the

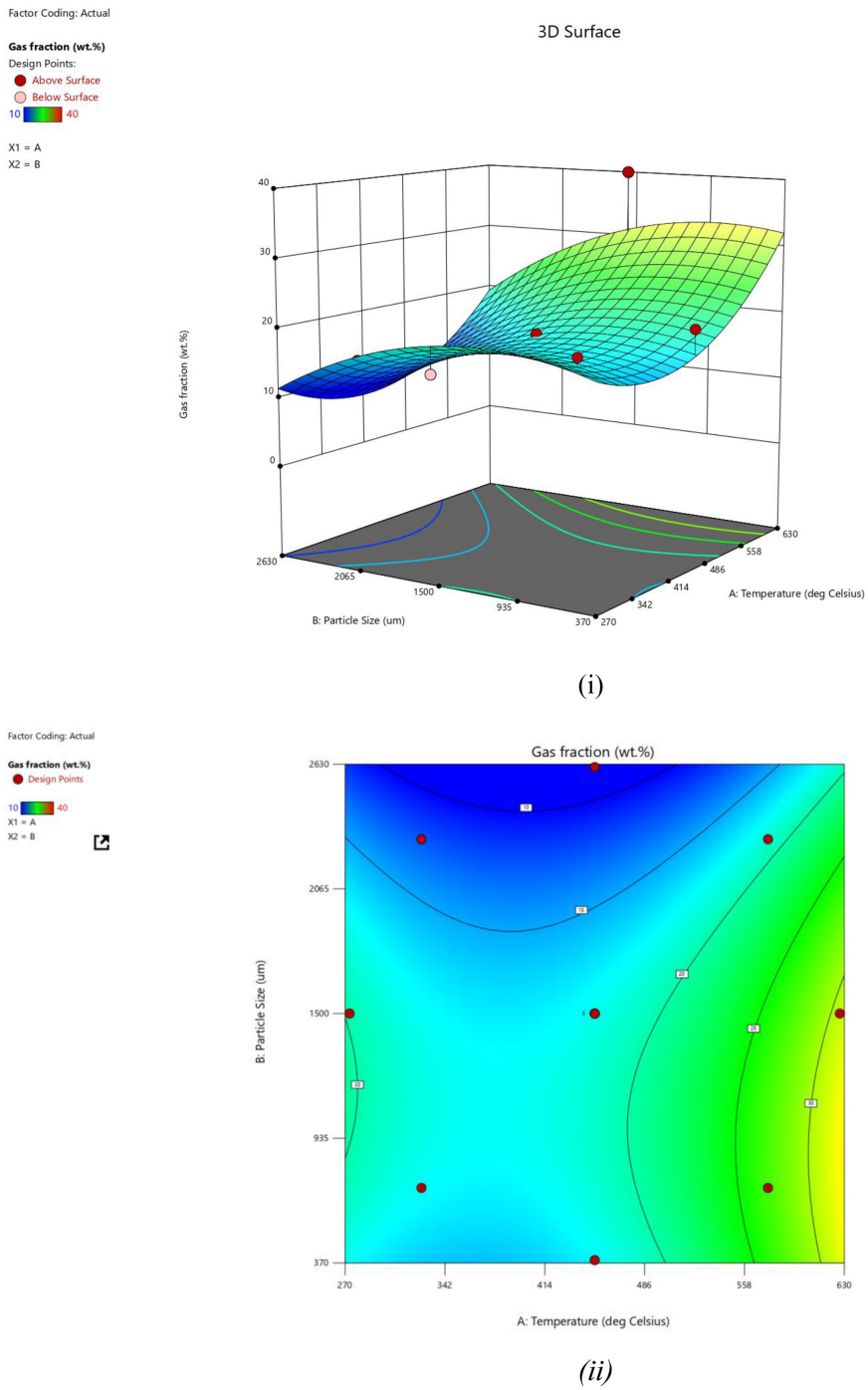


Figure 5. Response surface methodology of the gas fraction from pyrolysis of *Trichoderm atroviride*-pretreated water hyacinth. (i) Surface plot. (ii) Contour graph.

pyrolytic products from the untreated water hyacinth reported by Ilo *et al.*[22] As anticipated, the relative peak areas of pyrolysates from *Eichhornia crassipes* altered considerably following treatment with *Trichoderma atroviride*. Significant quantities of phenol and its derivatives from lignin were detected, which is similar to results reported by You *et al.*[33] According to Fu *et al.*,[34] the reduction in phenolic compounds with three carbon atoms and the increase in phenolic compounds with one or two carbon atoms corroborates the fungal degradation of the lignin. For example, the relative area of 2-methoxy-4-vinylphenol decreased from 0.46% to 0.22% in T525-PS2290.

The breakdown of hemicellulose was observed to occur alongside lignin removal during the fungal treatment of the lignocellulosic biomass. Research indicates that acetic acid and furfural are the primary compounds produced during the pyrolysis of hemicellulose.[35,36] It was noted that, at the highest temperature (T626-PS1500), acetic acid increased slightly from 31.11 to 31.58%. Typically, the cellulose left after fungal treatment becomes more accessible to enzymes than before treatment. Wang *et al.*[37] stated that the products of cellulose pyrolysis primarily include levoglucosan, glycolaldehyde, and 5-hydroxy furfural, among others. Oxazolidine,2,2-diethyl-3-methyl- was absent in the pyrolysis oil from the untreated water hyacinth at T250-PS1500; however, a relative area of 0.27% was noted at the same temperature for the pyrolysis oil of the pretreated water hyacinth. Likewise, there was an absence of maltol at T2273-PS1500 for the pyrolysis oil from untreated water hyacinth, but a relative area of 0.25% was noted in the same condition for the pyrolysis oil of pretreated water hyacinth.

Table 4. Statistical parameters

Parameter	Liquid fraction	Char	Gas
Standard deviation	6.45	3.70	5.78
Mean	44.69	35.00	18.62
R^2	0.5116	0.8335	0.6125
Adjusted R^2	0.4139	0.8002	0.3358
Predicted R^2	0.0776	0.5342	1.6504
Adequate precision	6.7134	14.2059	5.7740

Furthermore, fungal pretreatment improved water hyacinth's structural characterization, resulting in increased aromatic yield. It can therefore be inferred from the above outcomes that *T. atroviride* pretreatment can substantially enhance the thermochemical conversion of water hyacinth; however, there was a slight influence on enhancing the quality of the pyrolysis oil as there was still a presence of oxygenated compounds in the liquid fractions. According to Fateh *et al.*,[38] the water, acids, and aldehydes in the pyrolysis oil cause the product's poor quality, resulting in low heating value, instability, and high corrosiveness.

Fourier transform infrared spectra

Figure 7 shows the FTIR spectra identifying numerous characteristic functional groups existing in the pyrolysis oil as a result of *T. atroviride* pretreatment. The FTIR graphs are plotted with percentage transmittance (%T) on the *y*-axis and wavelength (cm^1) on the *x*-axis. Deep broadbands were noted for five samples (T325-PS710, T325-PS2290, T450-PS380, T450-PS2620, and T575-PS2290), while shallow bands were recorded for four samples (T273-PS1500, T450-PS1500, T575-PS710, and T676-PS15000). Across all samples, the band at ~3470/cm is attributed to O-H stretching vibrations in hydroxyl groups. The location and shape of this band imply that the groups are associated with hydrogen bonding and water impurities. Additional small peaks at 2993/cm for T273-PS1500, T450-PS1500, T575-PS710, and T676-PS1500 show C-H vibrations attributable to the presence of alkanes. Strongly cumulative concentrations at the 1731/cm band were recorded across all samples, indicating the C=O stretching of carbonyl groups. The occurrence of numerous tiny peaks

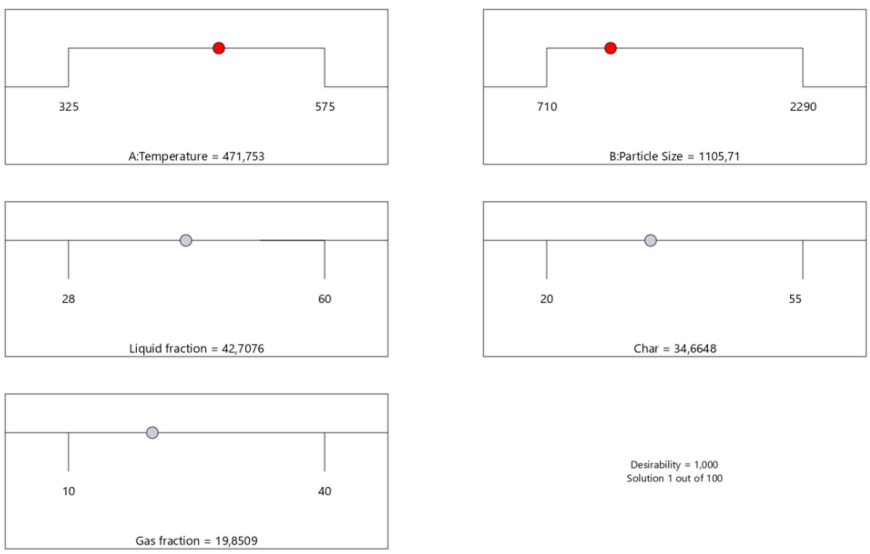


Figure 6. Numerical optimization ramps for desirability analysis.

Table 5. Elemental composition of the pyrolytic products

Elemental composition	T273.22-PS1500	T325-PS710	T325-PS2290	T450-PS380	T450-PS1500	T450-PS2620	T575-PS710	T575-PS2290	T626.78-PS1500
Pyrolysis oil (wt%)									
C	56.049	57.189	57.022	57.724	57.605	57.831	58.497	59.213	55.754
H	9.322	8.542	9.584	9.036	9.397	9.707	9.898	9.766	8.926
N	0.231	0.252	0.129	0.379	0.344	0.132	0.547	0.184	0.569
O[a]	34.398	34.017	33.265	32.861	32.654	32.330	31.058	30.837	34.751
HHV ($MJ.Kg^{-1}$)	26.711	26.038	27.599	27.080	27.591	28.189	28.819	28.958	25.945
Char (wt%)									
C	59.360	55.760	55.607	55.895	55.524	54.489	54.678	54.386	59.781
H	3.682	2.970	3.129	2.138	2.460	2.561	1.528	1.369	1.768
N	2.551	2.151	1.997	2.029	2.025	2.069	1.653	1.499	2.003
O[a]	34.407	39.119	39.267	39.938	39.991	40.881	42.141	42.746	36.448
HVV ($MJ.Kg^{-1}$)	19.457	16.570	16.744	15.323	15.649	15.302	13.762	13.367	16.639

[a]By difference.

in the fingerprint region of T273-PS1500, T450-PS1500, T575-PS710, and T676-PS1500 with a frequency of 500–700/cm indicates the existence of several alkyl halides.

Structural characteristics

Figure 8 illustrates SEM images obtained from the pretreated and untreated water hyacinth biomass. The image of the untreated biomass appears shrunk and compact with noticeable folds and ravines, signifying that the fibrillates were unbroken, whereas the pretreated biomass displays a layer with fibrillary structures and distorted cellular arrangements, suggesting the removal of lignin. The pretreatment process enhanced porosity and increased surface area, facilitating greater cellulosic exposure for effective pyrolysis.

Figure 9 shows the morphological features of char at different temperatures and particle sizes after pyrolysis of *T. atroviride* pretreated water hyacinth. These chars showed notably diverse surface structures, losing their firmness through the thermal degradation process. However, in the case of T272-PS1500, the char had a slightly intact structure with small fibrils, while increased coarseness of the char was visibly seen from T325-PS710. Macropores were observed from T575-PS710 to T626-PS1500 due to the extensive devolatilization of the char, which caused the collapsing of pores. Char is made up of a series of plant nutrients, which are valued as a soil amendment. The surface structure of char produced during pyrolysis gives rise to potential for filtration and adsorption of organic and inorganic matter. In contrast, char produced at a high pyrolysis temperature is efficient in the sorption of organic contaminants, whereas char produced at a low pyrolysis temperature is more appropriate for eradicating inorganic contaminants.[39]

The economic and environmental implications of pyrolysis technology

Pyrolysis of *T. atroviride*-pretreated water hyacinth has the potential to reduce reliance on fossil resources and create a sustainable cycle; however, its economic feasibility is crucial for practical application. The use of pyrolysis products holds considerable promise for lowering greenhouse gas (GHG) emissions in comparison with relying on fossil fuels and traditional chemicals but the profitability of pyrolysis also hinges on the costs of the feedstock, the yields of the products, and the capacity to generate higher value bioproducts. The yearly removal of water hyacinth places a substantial and avoidable strain on the economies of developing nations. Hence, its utilization for value-added bioproducts helps to

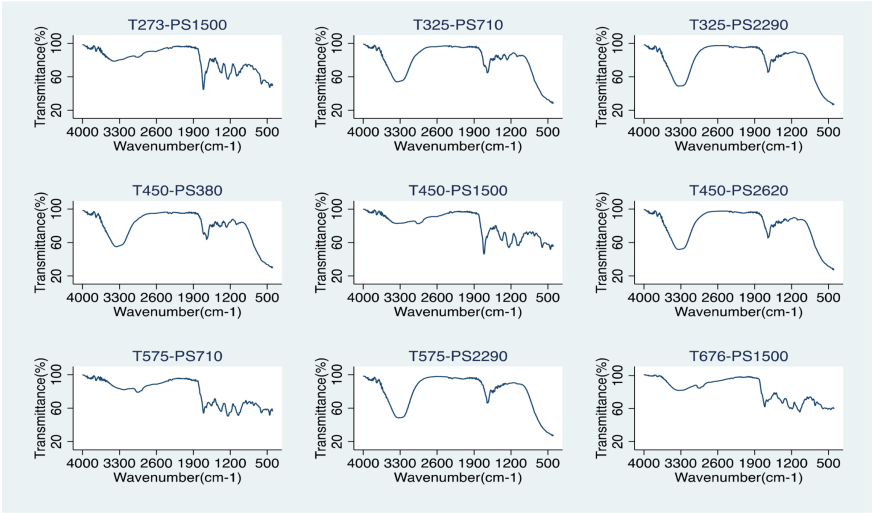


Figure 7. Fourier transform infrared spectra of pyrolysis oil from the pyrolysis of *Trichoderm atroviride*-pretreated water hyacinth.

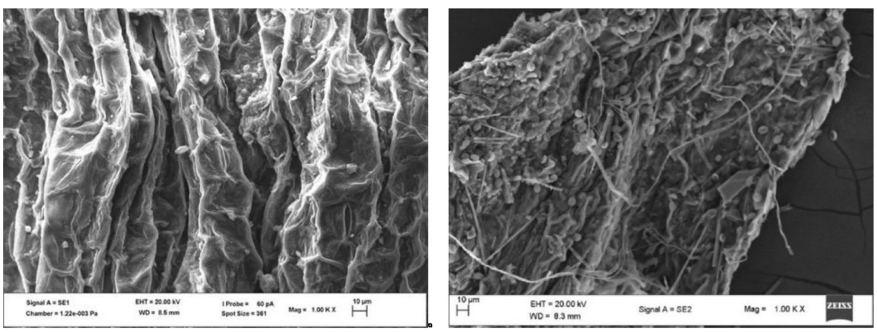


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Figure 8. Scanning electron microscopy images of (i) untreated water hyacinth, (ii) *Trichoderm atroviride*-pretreated water hyacinth.

reduce the negative ecological and socio-economic impacts. *Eichhornia crassipes* has a rapid growth rate and contains large proportions of cellulose and hemicellulose, making it a prime candidate for sustainable feedstock across multiple industries. The logistics of managing biomass present a significant obstacle to enhancing pyrolysis efficiencies in the extensive bioenergy systems.[40] Reducing the expenses associated with handling and transporting feedstock is therefore crucial for enhancing cost efficiency.

When a sustainable biomass supply is abundant, enhanced pyrolysis oil emerges as a cost-effective and environmentally friendly option. Fonseca *et al.*[41] opined that producing bio-oil in a lab-scale plant is more costly than the average cost of conventional diesel fuel due to higher power and electricity usage and the limited capacity of lab-scale reactors. However, in a scaled-up plant, the bio-oil production cost is estimated to be 63% lower than diesel. This underscores the commercial viability and economic feasibility of the process in larger industrial operations. Although pyrolysis oil has been criticized for possessing a significant moisture content, which poses challenges for direct combustion,[40,42] it also carries substantial oxygen content and can be enhanced to mimic crude oil. Upgrading the pyrolysis oil or co-pyrolysis of feedstocks to improve pyrolysis oil is an effective method to make the product commercially viable.

Apart from generating bio-oil, the process can produce pyrolytic gases and biochar as additional potentially valuable products. The pyrolysis unit constitutes a significant expense but revenue from co-products like district heat sales and biochar has the potential to reduce costs by a factor of three.[20] Biochar holds promise as a valuable material for agricultural purposes, serving as a soil amendment and aiding in carbon sequestration. Valuable chemical recovery can also be considered to improve the techno-economic feasibility of water hyacinth pyrolysis. For example, acetic acid, the main component of vinegar, finds applications in the food industry as a preservative and additive. It is also utilized in various

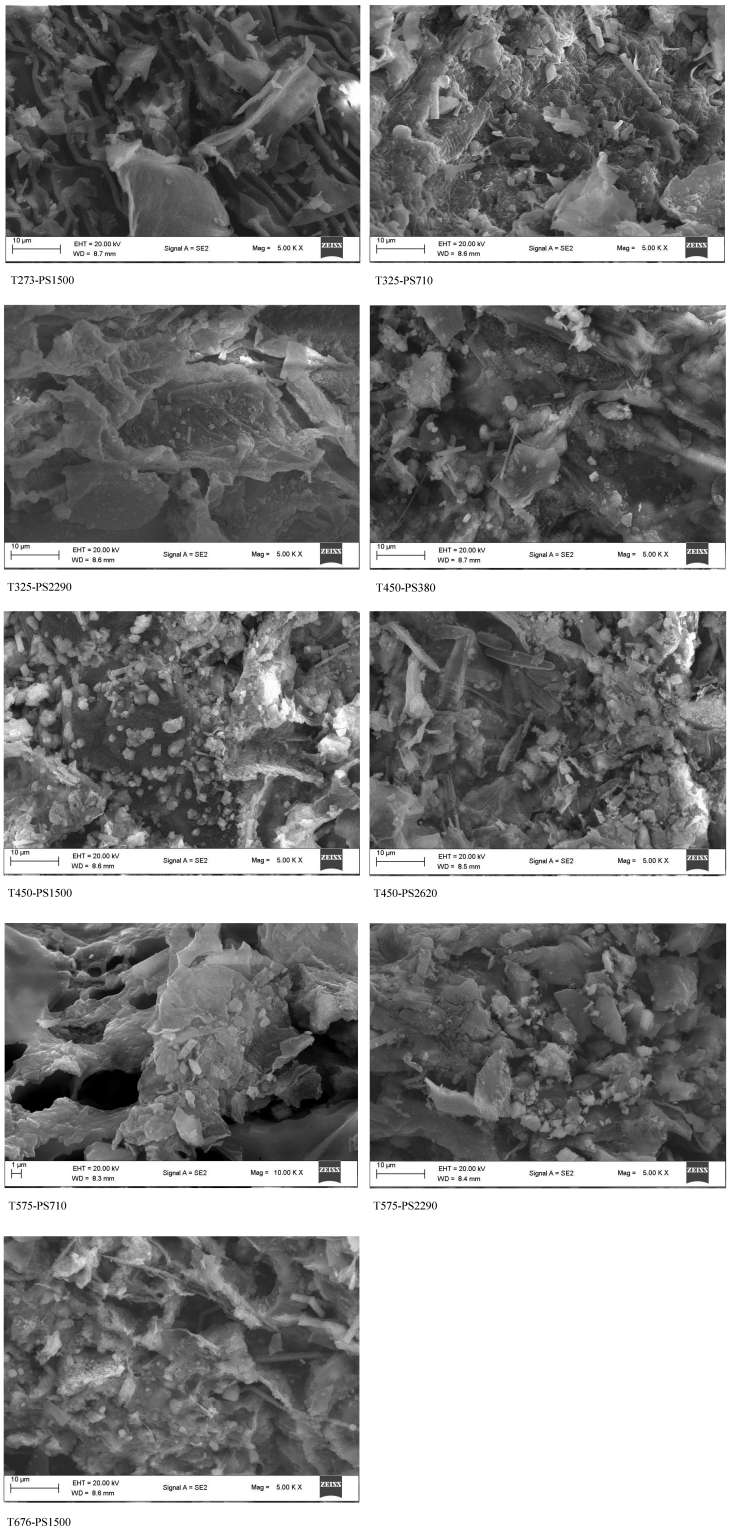

Figure 9. Scanning electron microscopy images of the char.

other industries, for example for the fabrication of printing inks, pesticides, pharmaceuticals, and household cleaning products.[22]

Introducing pyrolysis bioproducts into a country's energy sector will help to broaden the range of energy sources for electricity generation, offering an alternative to the

persistent reliance on imported crude oil for transportation and enhancing crop productivity to bolster food security. Efforts are being made to enhance pyrolysis technologies but issues such as production costs and a lack of established markets for pyrolysis bioproducts restrict their potential as standalone technologies.[41] Policy and financial support are therefore crucial to drive the adoption of this technology on a commercial scale, including the provision of incentives and the establishment of markets for pyrolysis bioproducts.

Conclusions

This study aimed to investigate the efficiency of *Trichoderma atroviride* pretreatment on water hyacinth's pyrolytic products. Pretreatment increased the cellulosic content and decreased the lignin and hemicellulosic content of water hyacinth, which enhanced pyrolytic polygeneration. Analysis of variance confirmed the effects to be significant. The product's elemental analysis outcome showed an increase of carbon and a decrease of oxygen in the pyrolysis oil and the opposite in the char fraction. Gas chromatography–mass spectrometry revealed a decline in hemicellulose and lignin derivatives, such as acetic acid and phenol, respectively, and increased cellulose derivatives, such as furfural. It can therefore be concluded that the fungal pretreatment made the thermal devolatilization of water hyacinth easier and enhanced the yield of the pyrolytic products; however, the quality of the pyrolysis oil was insignificantly affected. The study presents promising solutions for improving pyrolytic yield, enhancing substrate efficiency, and establishing processes in biorefineries. Efficiently reducing lignin content in water hyacinth improves the overall yield and quality of biofuels and makes the process economically competitive. The results highlight the potential value of utilizing water hyacinth as a valuable resource material, offering benefits for a circular economy. This aspect of our study provides valuable insights for industries looking to adopt sustainable biorefining methods.

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Conflict of interest statement

The authors declare no conflict of interest.

Data availability statement

The data that support the findings of this study are available in the supporting information for this article.

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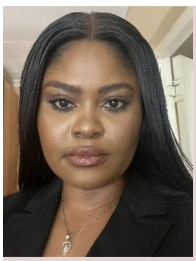

Obianuju P. Ilo

Obianuju P. Ilo is a postdoctoral research fellow at the School of Geography, Archaeology and Environmental Studies, University of the Witwatersrand, South Africa. Dr Ilo has more than 12 years of significant industry experience, which includes climate change mitigation and adaptation, sustainability, energy security, biodiversity conservation, solid waste management, and environmental resource management. She has won several prestigious awards and scholarships.

Mulala D. Simatele

Mulala D. Simatele is a professor of environmental management and sustainability at the School of Geography, Archaeology and Environmental Studies, University of the Witwatersrand, South Africa. Professor Simatele's research involves human-environment interactions, particularly mining, climate change adaptation, community-based natural resource management, education for sustainability, environmental rehabilitation and sustainability, and solid-/mine-waste management. He has served as a member of the advisory and steering committees for different international organizations and governments.