Research article

Impact of composting factors on the biodegradation of lignin in *Eichhornia crassipes* (water hyacinth): A response surface methodological (RSM) investigation

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ABSTRACT

Water hyacinth (*Eichhornia crassipes*) is a hydrophyte weed that causes havoc in the aquatic ecosystem as an invasive plant that can obstruct waterways and bring about nutrient imbalance. This study aims to address how this invasive hydrophyte can be physically harvested and biochemically transformed into a bioproduct that can enhance the restoration of damaged soil. Biocomposting, a low-cost biotechnological technique, was designed to degrade the lignocellulosic *Eichhornia crassipes* biomass and transform it into a valuable bioproduct. The process used response surface methodology (RSM) to investigate the aggregate effect of moisture content, turning frequency, and microbial isolate (*Chitinophaga terrae*) inoculum size on the breakdown of lignin over 21 days. The moisture content (A), (45, 55, 65) % v/w, inoculum size (B), (5, 7.5, 10)% v/v, and turning frequency (C), (1, 3, 5) days were considered independent variables, while percentage lignin degradation was considered a response variable. The optimal conditions for lignin breakdown were 65.7 percent (v/w) moisture, 7.5 percent (v/v) inoculum concentration, and 5-day interval turning. The R^2 score of 0.9733 demonstrates the model's integrity and reliability. Thus, the RSM approach resulted in a fine grain dark brown Nutri-compost that proved effective in enhancing soil fertility. This procedure is recommended for a scale-up process where large quantities of the hydrophyte could be treated for conversion into Nutri compost.

1. Introduction

The hydrophyte weed *Eichhornia crassipes* (Water Hyacinth) grows in freshwater bodies as a nuisance (Quilliam et al., 2015; Udume et al., 2021). It is considered an invasive species because of its characteristic prolific growth. It resurfaces despite huge sums of money allocated each year for its excavation. Biocomposting of Water Hyacinth offers a low-cost biotechnological solution that can tackle its menace by transforming it into biofertilizers (Udume et al., 2021).

The biocomposting process is likened to the formulation or design of new molecules. The product satisfies ecological theories/laws such as Liebig's law of the minimum, Shelford's law of tolerance, and Odums' combined law. These laws deal with the nutritional and physicochemical requirements of organisms in the ecosystem. All the requirements are summarized as limiting nutrients and conditions. Are the permutations in

the RSM for the formulation of compost from *Eichhornia crassipes* deemed adequate to satisfy the nutritional and physicochemical requirements of the organism? The product, which is compost, is the answer.

Composting, a process that combines and influences biological events, is meant to speed up the breakdown of complex, heterogeneous molecules found in biowaste in a wide range of conditions (Duran al., 2022; Meghvansi and Varma, 2020). Soil improvers from Carbon-rich phytobiomass, lignocellulosic agricultural waste products, and nitrogen-rich domestic solid waste are examples of products of this process. Compost biofertilizers can be used in agricultural and environmental management (Xiao et al., 2019; Li et al., 2019) by offering nutrients to indigenous soil microbes, and improving their ability to eliminate pollutants such as hydrocarbons, and augmenting microbial population (Semple et al., 2001; Reid et al., 2002; Sayara et al., 2010a,b; Wu et al., 2013; Taiwo et al., 2016; Yan et al., 2016). When compost is

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agitated, it exposes new material to microbial colonization and releases ammonia from the internal vacuum area (Kalamdhad and Kazmi, 2009; Jiang-Ming, 2017). The turning rate of compost material prevents the establishment of anoxic conditions and ensures appropriate airflow (Khalil et al., 2011). However, too much aeration and agitation may cause moisture and NH_3 loss (Jiang-Ming, 2017). Optimizing turning frequencies or developing a suitable turning regime is essential for enhanced compost output. The optimum moisture content of the compost mixture is essential for the microbial decomposition of the hydrophyte organic waste (Ali et al., 2014). However, the combined effects of turning frequencies with moisture content and the microbial inoculum size are the priming factors that can initiate biodegradation of the organic matter in the water hyacinth biomass.

From the literature, various statistical methods in composting factor optimization studies of different lignocellulosic waste products have been conducted to improve the overall performance of the compost in terms of quality and stability (Table 1). However, no known work has evaluated the compost performance in terms of lignin degradation using RSM. Previous studies focused on waste from agriculture, kitchens, food, municipal waste, industry (e.g., paper mill effluent), et cetera, but none

of the works employed a functional gene-specific organism such as *Chitinophaga terrae* to handle the menace–causing Water Hyacinth. This is part of the novelty of the study.

Several researchers have lately carried out optimization experiments in compost by regulating various essential factors, such as optimizing variable factors for improved nutritional yield (Chowdhury et al., 2014; Eklind et al., 2007). It is also critical to maintaining appropriate levels of moisture and oxygen for the best microbial activity (Bernal et al., 2009), maximize nutrient turnover (Nigussie et al., 2016), and minimize N losses and CO_2 emissions during composting (Bernal, 2017; Chowdhury et al., 2014; Nigussie et al., 2016). Some scientists have argued that compost feedstock contains a high concentration of microbes; hence, bacterial inoculants may be of limited benefit because of the already existing bacteria. The optimization of lignocellulose degrading microbial inoculants are believed to (i) shorten compost maturity time, (ii) accelerate lignocellulose degradation, and (iii) improve fertilizing value of the compost (Alikhani et al., 2016; Busato et al., 2017; Estrada-Bonilla et al., 2017; Chen et al., 2019). This has resulted in varied opinions across research groups about the degree of inoculum effects (Alikhani et al., 2017; Busato et al., 2017; Estrada-Bonilla et al., 2017).

Table 1. Some waste optimization methods.

Optimization method	Compost feedstock (waste source)	Variable Factor(s)	Response factors	Findings/correlation coefficient	Reference
One-Variable-at-a-time	Municipal solid	Moisture, temperature, aeration rate, free air space	Performance of biological treatment plants	Improvement from 53% to 107%.	Baptista et al., 2011
One-Variable-at-a-time	Palm (Oil mill effluent and empty fruit branches)	Particulate size, pH, initial carbon dosage, mix ratio of substrates	Electrical conductivity, (EC) protein content, organic matter content and C/N ratio),	Increase in all response factors	Mohammad et al., 2015
One-Variable-at-a-time	Medlar pruning, cattle manure	Cattle manure addition	EC, total N, and organic-matter stability	Increase in all response factors	Paredes et al., 2015
One-Variable-at-a-time	Solid waste (Biodegradable)	pH, moisture content, composting method	Thermophilic phase	Optimal thermophilic phase achieved with 60% moisture level	Sarkar et al., 2016
One-Variable-at-a-time	Food	Natural zeolite, and biochar	N & heavy metals	Decrease in Conc. of heavy metals due to Zeolite affinity to cations	Waqas et al., 2018
RSM (Central Composite Design- CCD)	Municipal solid	Pyrene conc.), soil/compost mixing ratio, compost stability expressed as respiration index	Pyrene biodegradation.	$R^2 > 0.69$	Sayara et al. (2010a),b
RSM (BBD)	Kitchen	1. Fly ash and bulking agent), 2. (Lime, temperature, and inoculum size)	Moisture, C/N	Moisture $R^2 = 0.9975$, and C/N $R^2 = 0.9947$.	Iqbal et al. (2015)
3-Factorial BBD	Municipal solid	Aeration, moisture, C/N ratio, time	compost stability (organic matter, chemical O_2 demand, nitrate concentration and biodegradability)	$R^2 > 0.8$	Cabeza et al. (2013)
Full Factorial experimental design (ANOVA- test)	Pulp and paper mill sludge	Composting time, moisture, addition of hazelnut kernels	NH_4^+ Removal	60% removal of ammonium at 50% moisture,25% Kernel in 5 weeks	Aycan et al. (2014)
Full factorial design (statistical)	Papermill (sludge), corn	Process duration, Cornhusk, corn cob, moisture, material ratio	NH_4^+ Removal	91.84% removal of ammonia	Aycan and Turan (2016)
Factorial optimization	cattle dung with maize/silage and peach-juice pulp	pH, C/N ratio, and moisture content	Optimal composting conditions. by Dewar autothermal assay	$R^2 = 0.8$.	Torres-Clement et al., 2015
Radial basis functional (RBF) neural network model	Agricultural	Volatile solids, BOD (soluble) and CO_2 evolution	ideal agro-waste composting mix proportion	($R^2 = 0.99$) Achieved with Vegetable waste, cow manure, and sawdust. (62.0 kg, 17.0 kg, and 9.0 kg respectively)	Varma et al. (2017)

Biodegradation of lignocellulosic materials is crucial throughout an unrestricted or regulated composting process (Feng et al., 2011). Due to its highly difficult-to-degrade character and variability, lignin is the most recalcitrant, abundant, and sustainable material in the biosphere (Wang et al., 2021). It is hard to degrade lignin under the conventional oxygen-requiring (aerobic) process (De Gonzalo et al., 2016; Wei et al., 2019). The structure of lignin inhibits the breakdown of related cellulosic and hemicellulose components of biological materials and the isolation and growth of novel microbes capable of degrading lignin may be difficult (Wang et al., 2021). Numerous microbial and physical parameters affect biomass bioconversion and microbial deterioration during the composting process (Bhatia et al., 2012; Zhang et al., 2020). Research on lignin biodegradation has advanced significantly within the last two decades because of the increased lignin transformations to valuable products, mediated by isolated and characterized biotechnologically important microbes for lignocellulosic biomass degradation (Shuai et al., 2016). Some researchers, before now, have reported that lignin is resistant to breakdown and is unchanged after composting (Carrasco et al., 2020; Jurak et al., 2015; Zhang et al., 2019b). However, it is also reported that microbial biomass could interfere with gravimetric and spectrophotometric methods, leading to an overvaluation of lignin in compost and an undervaluation of the lignin biodegradability potential (Carrasco et al., 2020; Zhang et al., 2019b). Researchers must consider these possibilities of biomass interference when evaluating lignin biodegradability prospects in compost (Zhang et al., 2019a).

Nevertheless, the alteration of lignin can be achievable during composting, leading to structural modifications such as reducing monomeric unit ratios (Albrecht et al., 2008; Chen et al., 1989). Tuomela et al. (2000) stated that lignin was not inert, but it was not easy to measure precisely (. *Pseudomonas* sp. and *Sphingobium* sp. are known to decompose and mineralize lignin; these have been discovered in mushroom compost (Siyoum et al., 2016). A work by Duran et al. (2022) found that lignin levels dropped and changed structurally during phase 2 of composting mushroom stem.

Temperature, pH levels, moisture content, and other variables like particle size, nutrient content, and oxygen availability can influence the composting process efficiency. All of these factors and their relationships must be considered to get the most out of composting. Statistical optimization can aid in this endeavor. Over time, techniques and mathematical models have been established. To guarantee that the eventual compost output is of excellent quality, it is critical to conduct the composting operation in a way that is best suited to the unique conditions occurring. Setting the proper moisture content and airflow rate is critical for optimizing the compost degradation rate because these conditions affect the bacterial population, which regulates organic material breakdown and consequently controls compost maturity (Echarrafi et al., 2018; Onwosi et al., 2017). Studies have outlined different methods for improving a system's performance. Single-variable optimization has been used to determine the best processing conditions (Yang et al., 2021; Shimizu, 2017). However, this method might not be very accurate and could lead to misinterpretation of data; because it does not show how variables interact and does not support the choices of the best processing conditions. In statistical experimentation, all variables and associated interactions can be studied simultaneously (Iqbal et al., 2015; Hema et al., 2016; Asadu et al., 2019; Pottipati et al., 2022; Gao et al., 2021; Dümenci et al., 2021).

The Box-Behnken design (BBD) is a popular form of RSM (Asadu et al., 2019). RSM is a non-parametric statistical approach commonly employed in R&D initiatives. The experimental design evaluates process drivers (factors) and determines optimal yield and productivity. In other words, process optimization maximizes results within restrictions (Datta, 2011; Maravi and Kumar, 2021). The technique uses regression equations and analysis to describe the relationships between variables and responses (Adeyanju et al., 2016). RSM allows evaluating and analyzing the impact of independent variables on the response(s) and their correlations (Datta, 2011; Maravi & Kumar, 2021). The degradation of

lignin-containing biomass has long been a global issue, but little research has been done on the conditions that promote degradation. In addition, the outcome of the interaction between lignin-consuming bacteria and other physical characteristics in a compost pile is unknown. As a result, an immediate demand exists for unique or extraordinary lignin-digesting bacterial isolates or consortia from various biological environments, including the rumen chamber. Though the lignocellulosic biomass in a compost system is degraded collaboratively by diverse microorganisms within the compost and other physical factors such as moisture and turning frequency (aeration), the application of RSM techniques aids in the understanding of the effects of these interactions.

Literature has shown state-of-the-art designs in the effects of single factors on composting like turning frequency, moisture, and microbial inoculum and the need for optimization for better compost yield in terms of nutrient, stability, organic material degradation, gaseous emissions, et cetera. However, there is a dearth of information on the effect of combined-optimized variables on lignin breakdown; this study aims to demonstrate this. Thus, these were the key goals of this research. (a) isolate, identify and characterize lignocellulose-degrading bacteria and (b) determine the optimum values of the factors that would give the most lignin degradation within the ranges studied.

2. Materials and method

2.1. Reagents and composting

Analytical grade chemicals reagents and standards were purchased from a commercial distributor, Joechem Ventures Limited, in Choba, Rivers State. Fresh Water Hyacinth plants were manually harvested into canoes from the Orashi River located within Ahoada-East LGA, Rivers State, Nigeria. The plants were air-dried and fragmented with a shredder to improve ease of handling during composting.

2.2. Isolation of lignin degrading bacteria

A lignocellulose-degrading organism was isolated from the rumen content of cows slaughtered at an abattoir located at Choba Community Market, within Obio-Akpor LGA, Rivers State, Nigeria. Lignin degradation screening was done according to El-Hanafy et al. (2007). One gram of sterile powder of the Water hyacinth biomass, acting as the source of carbon, was aseptically transferred into a 100 ml capacity Erlenmeyer flask containing 98 ml of MSM (*Mineral Salt Medium*) made of 3 g per liter $NaNO_3$, 0.5 g per liter KCl, 1g per liter of KH_2PO_4; 10 and 0.5 g per liter of 7M anhydrous $FeSO_4$ and $MgSO_4$ respectively, adjusted to pH 6.3. The MSM was sterilized for 15 min at 120 °C, cooled, and seeded with rumen juice. For 20 days, the MSM broth seeded with rumen juice was agitated on a bench top shaker incubator at 120 rpm and 37 °C. The re-activation of the rumen bacteria was conducted in nutrient broth incubated at 37 °C for 24 hrs. After that, the isolates were sub-cultured into nutrient broth in a sterile Erlenmeyer flask for 24hrs and inoculated into the composting setup for the Water Hyacinth lignin degradation optimization study.

2.2.1. DNA extraction, amplification, sequencing

Extraction was performed using an Inqaba South Africa-supplied ZR bacteria DNA micro prep extraction kit. Fifty microliters of a vigorously growing (24hrs) culture of the rumen bacterial isolates were suspended in 200 μL of isotonic buffer in a ZR Bashing Bead Lysis tubes, followed by the addition of 750 μL of lysis solution. The tubes were placed in a bead beater equipped with a 2ml tube holder assembly and treated for 5 min at maximum speed. The ZR Bashing Bead Lysis tubes were centrifuged for 1 minute at 10,000xg. A collection tube was used to obtain 400 μL of supernatant and centrifuged at 7000xg for one minute using a Zymo-Spin IV spin Filter (orange top). The collecting tubes' filtrate was increased in volume to 1600 μL with a 1,200 μL DNA binding solution introduced. The filtrate was passed to a Zymo-Spin IIC column in a collecting tube and swirled at 10,000xg for 1 minute and discarded from the collection

tube. The remainder of the volume was spun on the same Zymo-spinner. In a new collection tube, 200 μL of the DNA Pre-Wash buffer were added to the Zymo-spin IIC and spun at 10,000xg for 1 minute before adding 500 μL of fungal DNA. After buffer washing, 1-min centrifugation was done at 10,000xg. Using a clean 1,500 μL centrifuge tube, the Zymo-spin IIC column was loaded with 100 μL of DNA, eluted and swirled at 10,000xg for 30 seconds. After purification, the ultra-pure DNA was kept at -20 degrees Celsius for use in subsequent reactions. Amplifying the Internal Transcribed Spacer: 35 cycles of amplification of the ITS segment of the rRNA genes of the organisms were performed utilizing primers. Two sequences from ITS1F and ITS4 are 5′-TTGGTCATTTA-GAGGAAGTAA-3′, and 5′TCCTCCGCTTATTGATATGC-3′ respectively. The PCR mixture contained the Inqaba X2 Dream Taq Master mix ((Taq enzyme, dNTPs, MgCl), 0.4M primers, and DNA (extracted) template. For the amplification of the 16S rRNA gene, the PCR protocol was followed under the conditions of preliminary denaturation at 94 °C for 5 mins, 36 denaturation cycles at 94 °C for 30 sec, 56 °C annealings for 30 sec, plus 72 °C elongation for 45 sec, and final 72 °C for 7 min elongation, then 10 °C forever. The result was resolved at 120V for 15 min on a 1% agarose gel and visualized using a UV transilluminator. Inqaba Biotechnological, Pretoria, South Africa, performed ITS and 16SrRNA sequencing using the Big Dye Terminator kit on an ABI 3510 sequencer.

The sequencing was carried out in a final volume of 10ul, and the following components were used: 0.25ul Big Dye® terminator v1.1/v3.1, 2.25ul 5x Big Dye sequencing buffer, 10uM Primer PCR primer, and 2–10ng PCR template per 100bp. The following conditions were applied to the sequencing: 32 cycles of 10 seconds at 96 °C, 5 seconds at 55 °C, and 4 min at 60 °C. The obtained sequences were altered using the bioinformatics method and Trace edit to construct the phylogenetic tree. Similar sequences were downloaded using the BLASTN algorithm from the National Center for Biotechnology Information (NCBI) data source. ClustalX was used to align these sequences. MEGA 6.0's Neighbor-Joining approach was used to infer the evolutionary history (Saitou and Nei, 1987). The evolutionary history of the taxa investigated is assumed to be represented by the bootstrap consensus tree estimated from 500 replicates (Felsenstein, 1985). The Jukes-Cantor technique was used to calculate the evolutionary distances (Jukes and Cantor, 1969).

2.3. Composting method

The compost optimization study was carried out using seventeen 10L capacity reactors kept in an open space. The reactors were supported on a raised platform. An equal amount of Water Hyacinth biomass was added to the reactors. Compost operating parameters such as percentage moisture content, frequency of aeration by turning, and microbial seeding were maintained according to experimental design. One gram (1g) of the compost was drawn from each reactor after 21 days for lab investigation to determine the percentage of lignin degradation, and the microbial abundance using molecular next-generation sequencing.

2.4. Experimental design and data analysis

Experimental Design (Box-Behnken) was applied for a process optimization study. Here, three variables, namely turning frequency, moisture content, and inoculum size were recognized as critical parameters. The effect of interaction between these significant parameters [moisture (A), inoculum size (B), and turning frequency (C)] and their optimum level was studied by response surface statistical analysis utilizing Box and Behnken design. The experimental range was set up using Design expert software version 12 (State- Ease Inc., Minneapolis, Minnesota, USA), and levels of the three independent variables were used in Box–Behnken Design (−1, 0, and +1) as shown in Table 2a. These levels (−1, 0, and +1) represent the maximum and minimum ranges of composting factors as reported in the literature. The changeable factors optimized were moisture content, which ranged between upper and lower limits of 65% and 45% v/w; inoculum size, 5 and 10% v/v; and turning frequency, 1 and 5

Table 2a. Factor and rates (levels/range) considered in BBD.

Factor/Symbol	Rates		
	-1 (low)	0 (centre)	1 (high)
%Moisture Content v/w	45	55	65
Turning frequency (day)	1	3	5
Inoculum size v/w	5	7.5	10

days, respectively. The experimental responses were the changes in lignin concentration (i.e., the percentage of lignin reduction). The three coded factor levels (β_1, β_2, and β_3) were −1 low, 0 centers, and +1 high, respectively. Eq. (1) below was used to calculate the cumulative number of experiments (N) required for the construction of a Box–Behnken design.

$$N = 2k\,(k-1) + C0 \tag{1}$$

N denotes the set of experiments, the total range of different factors, and C0 is the overall set of central points. Using the abovementioned formula, a numerical correlation between these three factors on *Eichhornia crassipes* lignin breakdown was established by doing 18 experimental runs comprising four recurrence points at a centralized location. Employing the quadratic Eq. (2), the response data was subjected to the second-order polynomial.

$$Y = \beta0 + \Sigma\,\beta i\,Xi + \Sigma\,\beta ii\,Xi2 + \Sigma\,\beta ij\,Xij \tag{2}$$

Y represents the anticipated response in this scenario.

Xi symbolizes independent variables, β0 denotes the coefficient of the modeled response, βii & βi is quadratic and linear coefficients, respectively, and βij is the interaction coefficient; Xij indicates factors interacting with one another. Table 2b displays the experimental design constructed using BBD for the four separate variables with coded levels. A temperature of 37 °C was employed for all 18 test runs. After 48 hours of incubation, the medium was evaluated for *Eichhornia crassipes* lignin breakdown, as specified by Box–Behnken Design

2.5. Determination of lignin content in compost

Three grams of air-dried powdered samples were extracted with acetone using the Soxhlet apparatus for 6 hours (Dence, 1992). Next was the solvent vaporization, followed by the drying of the residue at a temperature of 103 °C and weighing after it had cooled in a desiccator. The acid-insoluble lignin content was determined using the Klason method, where 300 mg of the extracts were treated for an hour under vacuum with 3 ml of 72% H_2SO_4 before being thinned with 82 ml of H_2O and autoclaved for an hour at 125 °C. The "precipitates" were extracted using the suction filtration technique, collected with a glass fiber filter, and then flooded with water. Acid-insoluble lignin, also known as "Klason lignin," is filtered out, dried at 103 °C, and weighed after cooling. To determine the quantity of lignin solubilized by acid, 250 ml H_2O was added to the filtrate and read using a spectrophotometer. Absorbance equals the quantity of lignin solubilized in acid solution as measured by the spectrophotometer reading at 203 nm. Total lignin content, which is acid-soluble plus acid-insoluble, was determined from Eq. (3).

$$Lignin\ content = y\,(100\ x)/c \tag{3}$$

where.

y = Amount of precipitation (in grams)
x = Extraction solvent (percentage)
c = The extracted sample's constant weight (in grams).

The 128 L/g.cm was used as a standard for lignin absorbance to calculate the acid-soluble lignin content as reported by KCL (1982). Measurements were done in duplicate, and the mean was taken as the total lignin content.

Table 2b. BBD coding for three independent factors/actual and predicted lignin value.

Standard	Runs	Moisture content%		Inoculum size%		Turning Frequency		Actual Lignin Value (%)	Predicted Lignin Value (%)
		code	value	code	value	code	value		
11	1	0	55	-1	5	+1	5	8.64	8.39
2	2	+1	65	-1	5	0	3	8.85	8.86
7	3	-1	45	0	7.5	+1	5	7.20	7.02
15	4	0	55	0	7.5	0	3	14.64	14.25
9	5	0	55	-1	5	-1	1	8.73	8.54
1	6	-1	45	-1	5	0	3	8.02	8.45
6	7	+1	65	0	7.5	-1	1	7.19	7.37
3	8	-1	45	+1	10	0	3	8.49	8.48
5	9	-1	45	0	7.5	-1	1	7.57	7.33
13	10	0	55	0	7.5	0	3	13.62	14.25
10	11	0	55	+1	10	-1	1	8.13	8.38
12	12	0	55	+1	10	+1	5	7.93	8.12
4	13	+1	65	+1	10	0	3	8.81	8.38
8	14	+1	65	0	7.5	+1	5	7.04	7.28
17	15	0	55	0	7.5	0	3	14.06	14.25
14	16	0	55	0	7.5	0	3	14.70	14.25
16	17	0	55	0	7.5	0	3	14.22	14.25
11	control	-	-		-		-		

Variance predictions over the whole design are indicated by the center points occurring thrice.
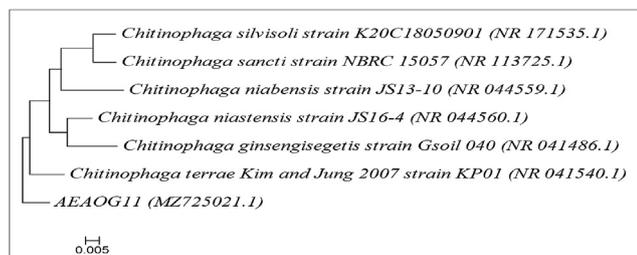
Figure 1. A phylogenetic tree based on the 16S rRNA sequence of *Chitinophaga terrae*.

3. Result and discussion

3.1. Bacterial identification

The molecular approach to microbial identification is more authentic and reliable than the culture-based. This is because it aligns, compares, and relates gene sequences of isolates within or across microbial taxa based on 16S rRNA gene sequences which are highly conserved sequences. The results generated in terms of the similarities in their 16S rRNA gene sequences are revealed by the BLAST analysis, identifying their nearest homology. Figure 1 identified the lignocellulose bacteria used as a factor in the experiment as *Chitinophaga terrae* (KP076216.1) with 96% homology closeness. Genbank MZ725021 was assigned to submission SUB10163725 SeqAEAOG11.

Composting was performed by applying the BBD design of RSM, which generated seventeen experimental runs and a control. The responses to the experimental variable inputs were statistically analyzed concerning the coded levels. The polynomial regression equation demonstrates that the rate of breakdown of the Water Hyacinth lignin constituent was an experiment function of variable factors in which coded elements were applied. Figure 2a shows that lignin content was reduced in all the compost setups, with runs 3, 7, and 14 recordings of approximately 52.2%, 51.25%, and 52.27% degradation, respectively, when compared with the control setup; while Figure 2b presents the graph showing actual and predicted values.

3.2. The second-order polynomial regression model

The polynomial regression equation of the second-order consists of triplicate linear, quadratic, and interaction terms (Maravi & Kumar, 2021), as presented in Eq. (4). Using the Design-Expert tool, the equation for lignin degradation in Water Hyacinth compost was developed.

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11}A^2 + \beta_{22}B^2 + \beta_{33}C^2 + \beta_{12}AB + \beta_{13}AC + \beta_{23}BC, \tag{4}$$

Where:

β_0 = center point of the design (a fixed value showing the response)
β_1, β_2, and β_3 = linear coefficients.
β_{11}, β_{22}, and β_{33} = quadratic coefficients.
β_{12}, β_{13}, and β_{23} = interaction outcome coefficient regression terms.
A, B, and C = Independent variable factors at three levels.

The F test and P values define the degree of significance of the individual coefficients that make up the polynomial equation. The F-test demonstrates that all variable factors and mutual interactions between variables evaluated in the experimental design are statistically significant (P 0.05) at the 95 percent confidence level. As a result, lignin degradation is a product of the various input variables: moisture content, inoculum concentration, and turning frequency. Equations 5 and Equation 6 summarize all terms in the experiment, regardless of their significance.

$$\text{Lignin} = +14.25 + 0.0762A - 0.1100B - 0.1013C - 0.1275\,AB + 0.0550AC - 0.0275BC - 3.41A^2 - 2.30\,B^2 - 3.59C^2 \tag{5}$$

$$Y = +14.25 + 0.0762A - 0.1100B - 0.1013C - 3.41A^2 - 2.30B^2 - 3.59C^2 - 0.1275AB + 0.0550AC - 0.0275BC \tag{6}$$

Where:

A = Moisture.
B denotes the inoculum size
and C = turning frequency.

Table 3 presents the response surface model ANOVA which indicates the fitness of the model produced, considering the results of the
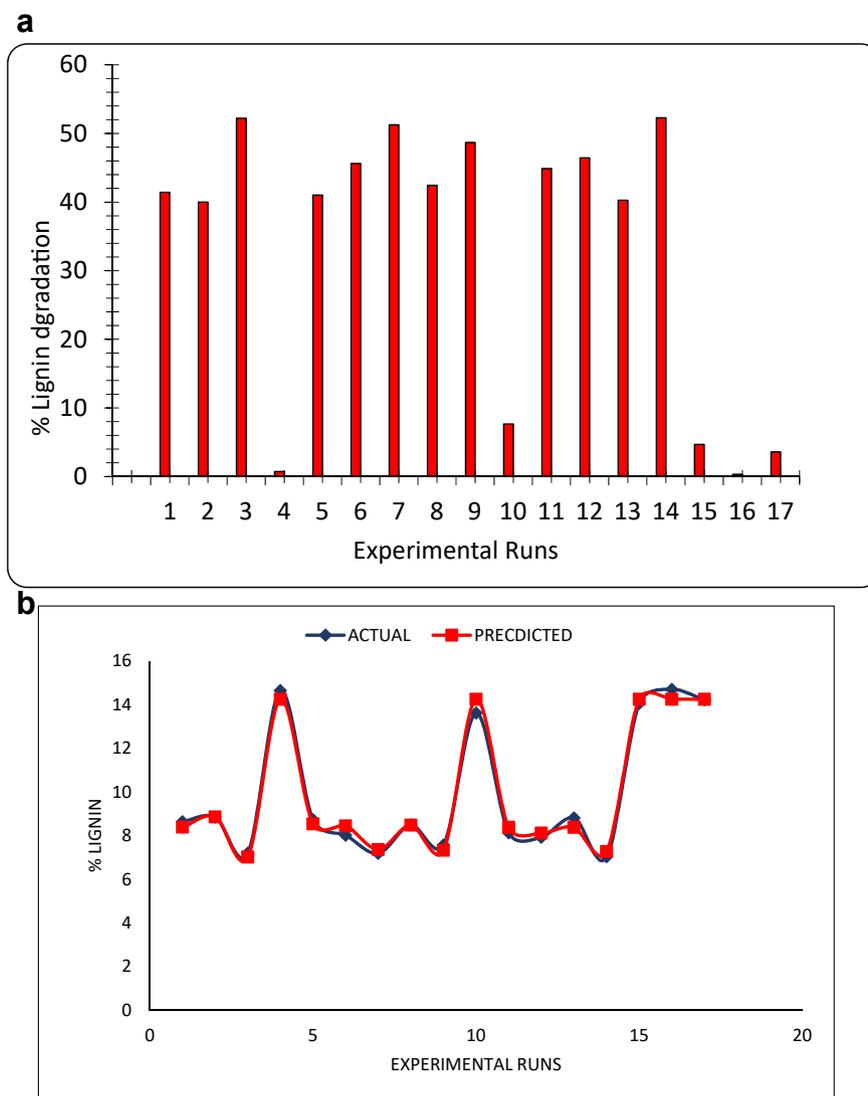
a

b

Figure 2. a: Changes in the lignin content over the experiment period. b: Actual and Predicted Values of lignin content in the Experimental runs.

determination coefficient (R^2) alongside the regression. The F-test score of 71.04 indicates that the model is significant for lignin degradation. The model is evaluated using a variety of indices and statistics. For example, if the probability value for the lignin degradation being studied is less than 0.0001, it indicates that the model is significant. The R^2 value is another metric that assesses the "goodness of fit." As a result, the determination coefficient (R^2) of 0.9892 indicates a strong connection with the values of "the observed" and "the expected." The explanation for an R^2 of 0.9892 for lignin degradation is that the variable components contributed over 98.92 percent of the variations that make the model highly significant for lignin degradation, but the model for lignin only

explains 1.08 percent of the entire variance. The analysis of variance result in Table 3 shows a nominal value for the lack-of-fit test. This is produced through a comparative analysis of the actual residual model's inconsistencies with those observed across the factors included in the experiment. The "lack of fit" in the experiment plays a role in determining whether the model used could adequately define the observed data or if a more sophisticated model should be sought.

The predicted and adjusted R^2 values of 0.9073 and 0.9752 are both considered appropriate. "Signal to noise" with a high value (of 4) indicates that the model has good prediction, efficacy, and attractiveness (Taran and Aghaie, 2015). The ratio of 20.161 achieved in this study is

Table 3. Response surface model ANOVA.

Source	Sum of squares	Degree of freedom (Df)	Mean square	F-value	Probability greater than an F	P-value
% Lignin						
Residual model	1.53	7	0.1971			
Pure error	0.7885	4	0.1971			
Lack of fit	0.7421	3	0.2474	1.25	0.4015	Not significant
Total correlation	141.33	16				

R^2 - 0.9892, Adjusted R^2- 0.9752, Predicted R^2- 0.9073, Adequate precision 20.1612.

Table 4. Model Coefficient for lignin degradation.

Factor*	Coefficient estimate	Standard error	F-value	Probability greater than F(P-value)	Degree of freedom§	Comment
Lignin						
β 0	14.25	0.2091	71.04	<0.0001	9	significant
β1 (A)	0.0762	0.1653	0.2127	0.6586	1	
β2 (B)	-0.1100	0.1653	0.4427	0.5271	1	
β 3 (C)	-0.1013	0.1653	0.3751	0.5596	1	
β 11 (A^2)	-3.41	0.2279	223.46	<0.0001	1	significant
β22 (B^2)	-2.30	0.2279	101.78	<0.0001	1	Significant
β33 (C^2)	-3.59	0.2279	248.39	<0.0001	1	Significant
β12 (AB)	-0.1275	0.2338	0.2974	0.6025	1	
β13 (AC)	0.0550	0.2338	0.0553	0.8208	1	
β23 (BC)	-0.0275	0.2338	0.0138	0.9097	1	

A, B, and C are the linear coefficients.
AB, AC, and BC are quadratic coefficients. A2, B2, and C2 are the regression terms for interaction outcome coefficients.
§ Df = free-to-vary values.
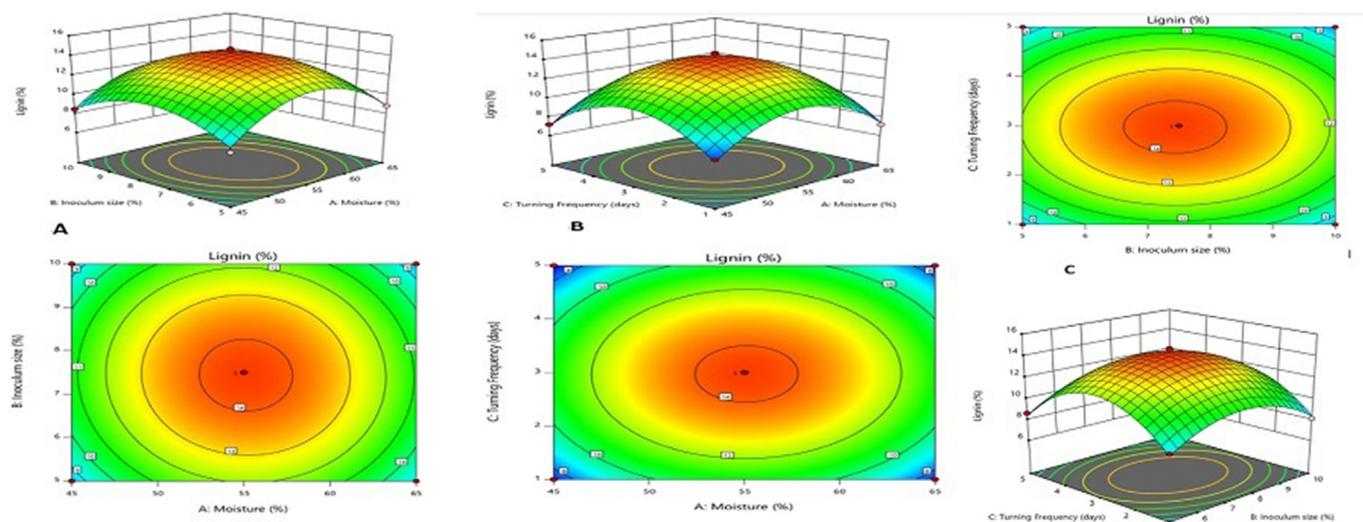* A is Moisture, B is Inoculum size, and C is Turning frequency.

promising. As a result, the model may be used to navigate design areas and when necessary, a model could be copied or duplicated. This reproducibility is determined by the coefficient of variation (CV) (Chen et al., 2012). When the CV exceeds 10%, the model is deemed non-reproducible and non-repeatable (Chen et al., 2012). The determined CV value of 4.74 percent is a strong indication of the model's repeatability potential. The expected values are the results obtained after imputing the independent parameter values into the quadratic model and comparing them to the observed experimental values. The model coefficient is shown in Table 4.

3.3. Plot analysis from the lignin degradation study

The interactions of the parameters considered for the lignin degradation study are presented in 3D contour plots. Figures 3a - c show the racetrack of the combined effect of inoculum size (%) v/v, moisture content (%)v/w, and turning frequencies (days). The plots show the highest degradation of lignin at the center points of the design. The two variables were inextricably linked to the yield parameters. The 3-D plots showed that the percentage of lignin decreased with increased microbe concentration and moisture.

3.4. Optimization and authentication

A process such as composting would usually operate with a range of critical factors that are dynamically influential (Asadu et al., 2019). It is essential to confirm the ideal numerical arrangement. The workability of the optimum conditions for lignin degradation was determined using an arithmetical optimization technique based on the "desirability function." To produce a model case for water hyacinth compost lignin degradation, variable parameters were set in range (minimum and maximum) based on the change reported in the literature to favor the degradation of compost organic matter. The predicted optimum (uncoded) values of moisture, inoculum size and turning frequency were correspondingly found to be 65%, 7.5%, and 5.0 days respectively, to achieve 52.27% optimal lignin degradation. However, an authentication test was carried out to establish the optimal lignin degradation if the adjusted variables were fixed at the good optimal points set using the Box Behnken Design. An authentication test was also done for both the percent error and the standard deviation, which were studied for validation. Eq. (7) shows the formula for calculating errors between expected (predicted) and actual values. An error of 3.336 was made when applying the formula below. The results are indicative of no significant variance.

Figure 3. (a) 3D and contour plots demonstrate the interaction of inoculum size and moisture; (b) 3D and contour plots demonstrate interactions between turning frequency and moisture; 3(c) 3D and contour plots demonstrate the interaction of turning frequency and inoculum size.

$$\text{Error} = \frac{\text{Actual value} - \text{Predicted value}}{\text{Acutal value}} \times 100 \qquad (7)$$

One of the experimental setups used in this investigation resulted in a lignin breakdown rate of 74 percent. Similarly, Tomati et al. (1995) reported a 70 percent lignin degradation from olive-mill effluent and wheat straw composted in a pile for 140 days in a thermophilic environment. The high temperature, which frequently encouraged lignin degradation even in an unregulated process, could be ascribed to the high percentage of degradation attained. Horwath and Elliott (1996) investigated lignin breakdown in a ryegrass straw over 45 days at 25 degrees Celsius. Within the study's time frame, the initial lignin concentration was 12.25 percent, and a 7 percent lignin breakdown was obtained within the time frame of the study. Jiang et al. (2020) investigated the effects of red mud and microbial additions on the breakdown of lignin in aqueous solutions. According to their findings, the final lignin degradation in the setup with red mud addition was 18.67 percent higher than the final lignin degradation in the setup without red mud. According to the findings, the laccase-producing bacteria *Pseudomonas, Phenylobacterium*, and *Caulobacter* were substantially associated with lignin breakdown.

Chen et al. (2021) have found that inoculation with lignocellulosic microorganisms is a practical approach for increasing the breakdown of lignocellulosic materials. *According to their research, Acetobacter orientalis* XJC-C (a novel cellulolytic bacterium isolated from a soft marine coral) was capable of degrading a variety of lignocellulosic biomass derived from agricultural leftovers, including banana straw. The inclusion of the bacteria *Acetobacter orientalis* XJC-C strain during composting at 40 days resulted in a 47.08 percent and a 21.85 percent increase in degradation rate in the negative and positive controls respectively. Aside from the addition of bacteria, ligninolytic enzymes added to composting material have demonstrated the capacity to stimulate the metabolic activities of the compost microbes. The addition of ligninolytic enzymes resulted in a 5.25 percent increase in lignin breakdown, according to Feng et al. (2011). Based on the studies of Zhong et al. (2021), bioaugmentation resulted in a decrease in the lignocellulose content of compost (rice straw, pig dung, and biogas residue) by 27.14–66.30 percent, which was accomplished through an increase in the activity of the xylanase enzyme by 98.5 percent throughout the compost operation's lifespan. When microbe inoculation is performed on compost, the stage of the compost at which inoculation is done can exert a significant influence on the rate of compost breakdown and humification. Inoculating a compost with *Phanerochaete chrysosporium* during the cooling period of a maize straw and canola residue co-compost resulted in 40.00% and 64.30% increases in cellulose and lignin degradation, respectively, and a 55.40% and 75.20% increase in humus content (Chen et al., 2018). Mei et al. (2020) employed *Bacillus amyloliquefaciens* SL-7, a lignin-degrading bacterium, to achieve 28.55 percent degradation of tobacco straw lignin within 15 days of composting and 50.81 percent degradation by day 41.

The impact of lignin-degrading bacteria strains on compost efficiency when compared to fungi has also been studied. Yu et al. (2018) investigated the practicality of applying QROD (quadratic regression orthogonal design) to optimize a consortium of microbes and establish an inoculum cocktail to stimulate the lignocellulose breakdown of green waste throughout composting. They found that QROD was effective for optimization with a p-value of less than 0.05, i.e. (0.0108). This demonstrates the model's usefulness for capturing the interaction between microbes, physical conditions (such as moisture and turning frequency), and the degradation of lignocellulosic materials. Temperature, pH, and the size of the material are all elements that can influence deterioration (Trinh and Kang, 2010). Examples of waste composting optimization strategies, factors optimized, and the results are presented in Table 1.

In the past 15 years, a variety of experimental designs, including BBD, CCD, and factorial designs, have been used to examine the biodegradability of waste materials. Sokac et al. (2021) utilized BBD (43) and RSM to enhance the biodegradability of organic wastes in adiabatic reactors with and without microbial inoculants. In addition, in a composting

experiment conducted by Zhang et al. (2019a,b), the effects of turning frequency and microbial inoculum were evaluated using dairy dung as the composting material. It was discovered that rotating frequency (4 days), straw, and microbial inoculum (1.3 liters) affected the composting efficiency of dairy manure in outdoor compost. Similarly, an ANOVA coefficient was employed to assess the impact of every process variable. In their experiment, they investigated the impacts of compost temperature, moisture, and pH on lignin breakdown as response factors. In a separate study, Sharma et al. (2018) used RSM and CCD to maximize the composting of flower debris. Inoculum (cow manure) was added to the stirred pile method, and the pH, TOC (total organic carbon), EC (electrical conductivity), and NH4–N of the mixture were measured to determine its stability and maturity. The results indicated that quadratic models could appropriately characterize experimental data dependent on input factors. Calabi-Floody et al. (2019) investigated the effects of the size of particulate matter, N-addition, and *T. harzianum* inoculum concentration on wheat bran biodegradation using a three-level factorial design. According to the ANOVA data, their findings indicated that all three input components and their interactions significantly affected the composting process. When composting efficiency was utilized to establish the optimal way for changing wheat straw, it was discovered that particle size reduction has a favorable effect on water retention capacity. Ajmal et al. (2020, 2021) adjusted the C/N ratio in agro-waste decomposition using a Taguchi experimental design dependent on startup inoculum volume, temperature, and time. Roman et al. (2021) investigated the effects of hydro-treatment duration, initial compost mass, and hydro-treatment temperature on municipal composting material using 15 independent experimental trials (the Doehlert matrix). Numerous investigations have demonstrated that compost volume is the most important aspect of the composting process. Gao et al. (2021) employed simplex centroid design plus response surface simulation coupled with an analysis of variance with pig dung, human faces, rice husks, and kitchen garbage to optimize the composition of composting mixtures.

Figure 4 shows the microbial phyla levels of the optimized compost (OC), Their abundance consist of members of Proteobacteria (3.48%), Firmicutes (8.22%), Gemmatimonadetes (14.78%), Actinobacteria (5.03%) Planctomycetes (12.50%), Bacteroidetes (4.49%), Chloroflexi (4.21%) Verrucomicrobia (3.05%), Fibrobacteres (1.59%), Cyanobacteria (1.45%) and Acidobacteria (<1%)

3.5. Practical implications of this study

Improvements in compost technology have occurred through the years; for example, in the 1970s, in the United States, an aeration system was created for the introduction of air into a pile (aeration) of compost stems. Rather than relying on mechanical turning, a fan was employed to either force air into or suck out air from the pile. The innovation was to
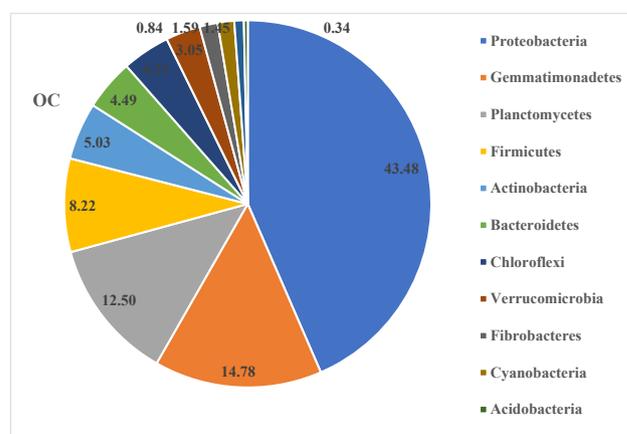

Figure 4. Microbial abundance in optimized compost (OC) at Phylum Level.

solve the problem of temperature fluctuations caused by un-optimized aeration. The outcome of this study will improve waste management systems, reduce organic waste in landfills, and restore poor soil. In agriculture and oil industries, this compost process model can be replicated on large scale as a sustainable system for bio-waste management, which can be used for the production of Nutri-compost. This compost is a soil conditioner to improve soil quality for crop production and reclaim soil impacted by anthropogenic pollutants. While mathematical simulation is critical for composting, there are significant limitations such as not having a generalized model for all substrate types, the design of experiments, and the complexity of the influences of critical factors in the process (Deaconu and Coleman, 2000).

With the advancement in environmental and human sustainability, there is a need to phase out the widespread poor waste treatment practiced in landfills in favor of more eco-friendly alternatives, like composting. Composting has a critical role in a nature-based solution (NBS) to replace synthetic fertilizer, which is commonly used, There are different organisms capable of digesting complicated, bulky-recalcitrant molecules which can be mined and kept in culture collection facilities, and be made available as inoculum to accelerate the composting process. This could be utilized in commercial and public waste management organizations to accelerate material degradation. The end product of the composting process can be utilized as a nutrient supplement for contaminated soil cleanup as well as a biofertilizer for agricultural purposes.

4. Conclusions

This study establishes the viability of using the response surface methodology to investigate the combined effects of moisture, inoculum size, and turning frequency on the degradation of water hyacinth lignin. The use of RSM with Box-Behnken design to model and optimize lignin degradation in lignocellulosic waste material has proven to be a predictable and robust process. As optimizing process variables can considerably accelerate lignocellulosic material degradation. This model could be utilized on a large industrial scale to manage massive amounts of the invasive hydrophyte (Water Hyacinth) that has become an uncontrollable scourge in water bodies. Compost derived from water hyacinth would serve as a nutrient supplement for the cleanup and rehabilitation of soil harmed by anthropogenic contaminants, as well as a biofertilizer for the enhancement of agricultural crop output. Thus, the outcome of this molecular geo-environmental biotechnology is a Nutri-compost.

Declarations

Author contribution statement

Udume Ogochukwu Ann & Abu Gideon: Conceived and designed the experiments; performed the experiment, Analysed and interpreted the data; wrote the paper.

Abu Gideon & Ijeoma Vincent-Akpu & Stanley Herbert: Performed the experiment; Analyse and interpreted the data; wrote the paper.

Yusuf Momoh: Analysed and interpreted the data.

Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

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