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Abstract: *This study investigated factors influencing the production of high nitrogen (N) biofertilizer from mushroom waste, examining five factors: mushroom waste (MW) content (30% to 70%), Bokashi fermentation time (5 to 10 days), agitation (Yes or No), mushroom waste size (Cut or Uncut), and drying temperature (60°C to 105°C). Through Two-Level Factorial Analysis (TLFA), the most impactful factors on N content were identified. Using a HACH Spectrophotometer as measurements devices, optimal conditions were found: 104°C drying temperature, 70% MW content, uncut mushroom waste, 10 days of Bokashi fermentation with agitation, resulting in 1624 mg/L N content. Future research is to assess the long-term stability and efficacy of the biofertilizer in various soil types and climates and conduct scale-up studies to evaluate industrial production feasibility and efficiency. Ongoing research could transform these alternative materials into valuable natural products, promoting sustainable practices and reducing overall waste.*

Keywords: Biofertilizer, Nitrogen content, Factorial analysis, Bokashi fermentation, mushroom waste

1. INTRODUCTION

Due to rising demand brought on by agricultural transformation programs, the mushroom industry has seen tremendous expansion [20]. For every kilogram of mushrooms harvested, the cultivation of mushrooms results in the production of three kilograms of expired mushroom block (EMB) soil-like material made up of peat, straw, and manure. For the cultivation of mushrooms, agricultural wastes, especially lignocellulosic residues, offer a sustainable resource. The biomass left over after commercial mushroom harvesting is referred to as EMB, and it has the potential to yield useful items like animal feed and fertilizers. According to [17], certain mushroom species, such Pleurotus sp., are suitable for use as fertilizer and ruminant feeding. Using mushroom waste, especially mushroom stems, as a biofertilizer is one way to handle this problem. Living microorganisms in biofertilizers increase soil productivity and nutrient availability.

Composting can be a suitable technology that is a cost effective and environmentally friendly option for disposing of mushroom waste. It is a biological process (agricultural waste disinfectant turned into a corresponding and usable flora matter) that occurs in the presence of sufficient oxygen, humidity, and temperature [15]. One of the household composting techniques is Bokashi composting. Bokashi is a product from an anaerobic process using organic matter, effective microorganisms (EM), molasses, and water. Professor Teruo Higa developed Bokashi composting using EM at the University of Ryukyus in Okinawa in 1982 [15]. The anaerobic process of bokashi fermentation, which uses lactic acid fermentation (LAF), is used to create biofertilizers [2]. By removing oxygen from the surrounding environment, this method encourages bacterial growth while maintaining the quality of the nutrients.

By utilizing microbes for functions of N fixation, biofertilizers contribute reduce the demand for chemical fertilizers, potentially reducing climate change and enhancing soil health. Microbial consortia in fertilizers may also have an impact on the carbon cycle, leading to the development of more efficient and long-lasting fertilizers [18]. The advantages of employing soil microorganisms as biofertilizers during the past few decades include enhanced crop output, disease control, increased quality, and promotion of plant growth.

The overall goal of the current study was to investigate the independent factors that affect development of biofertilizer to increase total N content for appropriate biofertilization techniques.

2. MATERIAL AND METHOD

2.1 Collection and Preparation of mushroom waste

Expired mushroom blocks (EMB) and effective microbes (EM) were collected from ADA Farm Fresh, Batu Pahat, Johor Bharu, Malaysia. The sample was deposited in the FTKKP laboratory.

2.2 Process of Bokashi fermentation method

The Bokashi fermentation uses anaerobic conditions, during the Bokashi fermentation process, the condition of the sample was subjected to several parameters. Initially, the mushroom waste was divided into two categories which are uncut and cut into 5 mm thick pieces. After that, the mushroom waste was dried at a certain temperature. ADA Starter Bokashi Bran obtained from Ada Fresh Farm was then mixed with mushroom waste in a tightly closed container, according to the appropriate weight composition. The Bokashi pre-compost, which was the organic waste that had been exposed to ADA Starter Bokashi Bran undergo acidic anaerobic fermentation for a period, about 5 to 10 days, the result of the fermentation process, were then used as a fertilizer by mixing it into the soil the [1].

(a) Mushroom waste

(b) Bokashi bran

Fig. 1. Biofertilizer material

Fig. 2. Weighting dried

Fig. 3. Mixture of mushroom waste and Mushroom and Bokashi bran Bokashi bran tightly sealed for fermentation.

2.3 Experimental set-up for factorial analysis

Five factors were selected for the study and classified into categorical and numerical categories (Table 1.). Among them, three factors, namely drying temperature, mushroom waste content, and reaction time for Bokashi fermentation, were considered numerical factors. Additionally, the size of the mushroom waste (cut or uncut) and agitation of the waste were categorized as categorical factors. The N content was measured using the HACH Spectrophotometer (DR1900) and analyzed with analysis of variance (ANOVA) in Design Expert Software Version 7.0. To conduct the experiment, a 25-1 fractional factorial design was employed, resulting in a total of 16 runs using Design Expert Software Version 7.0. Furthermore, Two-Level Factorial Analysis (TLFA) was utilized to identify the factors that exerted a significant impact on the N content in this study.

Table 1. Factors and actual values of coded levels

No	Variables	Coded	Type of Factor	Actual values of coded levels	
				-1	+1
1	Drying temperature, ˚C	A	Numeric	60	105
2	Size of mushroom waste	B	Categoric	Cut	Uncut
3	Mushroom waste content, %	C	Numeric	30	70
4	Reaction time, Day	D	Numeric	5	10
5	Agitation of mushroom waste	E	Categoric	Yes	No

3. RESULTS AND DISCUSSION

3.1 Factorial analysis of total nitrogen (N) content

Table 2. shows the amount of N obtained within the range of 308 to 1551 mg/L. The highest content of N was 1551 mg/L and was obtained when the Bokashi fermentation was performed by follow their own respective.

The ANOVA-derived R^2 was utilized to assess the proximity of the data to the regression line [6]. A well-fitting model is characterized by an R^2 exceeding 80% [10]. The analysis yielded a highly satisfactory R^2 value of 0.9998, signifying that the model aligns well with both the experimental and predicted values. The conclusive equations in relation to actual factors were established as outlined in equation (1) below.

$$\begin{aligned} N\ (mg/L) = {} & 947.75 + 189.13A \\ & + 71.75B + 49C \\ & + 10.88D - 117E \\ & - 15.87AB - 63.87AC \\ & + 249.25AD - 20.12AE \\ & + 26BC + 30.25BE \\ & - 52.5CE - 31.37DE \end{aligned} \quad (1)$$

Where A is drying temperature, B is size of mushroom waste, C is mushroom waste content, D is reaction time and D is agitation of mushroom waste. Factors of A, B, C, D, and E are referred to as main effects while AB, AC, AD, AE, BC, BE, CE, and DE are the interaction effects. The significance of the model in terms of nitrogen (N) content was assessed through an analysis of variance (ANOVA), as presented in Table 3. To verify the statistical significance of the regression equation, F-values were utilized, and the significance of individual coefficients was examined using p-values [10]. In this model, the F-value was 659.74, and the associated p-value was extremely low ($p < 0.0015$). This low p-value suggests a mere 0.15% likelihood that the model F-value this large could have occurred due to noise. According to [10], a good model is characterized by a calculated F-value substantially greater than the tabulated value. Smaller p-values indicate increased significance of the corresponding variable [10]. In this context, the model term effects of A, B, C, E, AC, AD, AE, BC, BE, CE, and DE were statistically significant in influencing the N content. However, the model term D was not significant, as its p-value exceeded 0.05.

Table 2. The result of the 25 fractional factorial experiments

Standard Order	Coded values of variables					N (mg/L)
	A	B	C	D	E	
1	-1	-1	-1	-1	1	787
2	1	-1	-1	-1	-1	953
3	-1	1	-1	-1	-1	928
4	1	1	-1	-1	1	898
5	-1	-1	1	-1	-1	1145
6	1	-1	1	-1	1	592
7	-1	1	1	-1	1	1128
8	1	1	1	-1	-1	1064
9	-1	-1	-1	1	-1	444
10	1	-1	-1	1	1	1228
11	-1	1	-1	1	1	424
12	1	1	-1	1	-1	1528
13	-1	-1	1	1	1	308
14	1	-1	1	1	-1	1551
15	-1	1	1	1	-1	905
16	1	1	1	1	1	1281

Table 3. Significance of regression coefficient for N content analysis

Source	Coefficient Estimate	F values	p-value Prob > F
Model	947.75	659.74	0.0015
A-Drying Temperature	189.13	2372.20	0.0004
B-Size of MW	71.75	341.43	0.0029
C-MW Content	49.00	159.24	0.0062
D-Reaction time	10.88	7.84	*0.1074
E-Agitation of MW	-117.00	907.87	0.0011
AB	-15.87	16.71	0.0549
BC	26.00	44.83	0.0216
BE	30.25	60.69	0.0161
CE	-52.50	182.80	0.0054
DE	-31.38	65.29	0.0150

R^2 = 0. 9998. *P-values exceeding 0.05 suggest that the model terms lack significance.

3.2 Main and interaction effects of the factors

The Pareto chart, which shows the primary and interaction influences of the process factors, is shown in Figure 4. With the bar heights representing each of the factors, this chart was helpful in examining the most significant factors. The square root of the F-values obtained from the analysis of variance (ANOVA) was represented by the t-values of the bars. The Bonferroni limit line and the t-value limit line, with values of 17.277 and 4.303, respectively, are the two limit lines displayed on the chart. A Pareto chart analysis clearly shows that factors A, B, C, E, AC, AD, CE, DE, BE, BC, and AE have a considerable impact on nitrogen (N) content in biofertilizer production, exceeding the t-value limit of 4.303.

Of the variables, the drying temperature primary factor greatly above the Bonferroni limit, which sets a stricter limit of 0.025 by halving the standard p-value of 0.05. According to [14], the drying procedure is important for minimizing unanticipated mushroom growth when applying biofertilizer made from mushroom waste to gardens. It is notable that the relationship between drying temperature and the length of the Bokashi fermentation process is above the Bonferroni limit, indicating its increased importance. The organic matter in the mushroom waste broke down and decomposed more easily at higher drying temperatures, the amount of N in the biofertilizer increased. According to [10] observations, significant coefficients are indicated by coefficients having t-values greater than the Bonferroni line. Furthermore, if a coefficient's t-value lies between the t-value limit line and the Bonferroni line, it is considered likely.

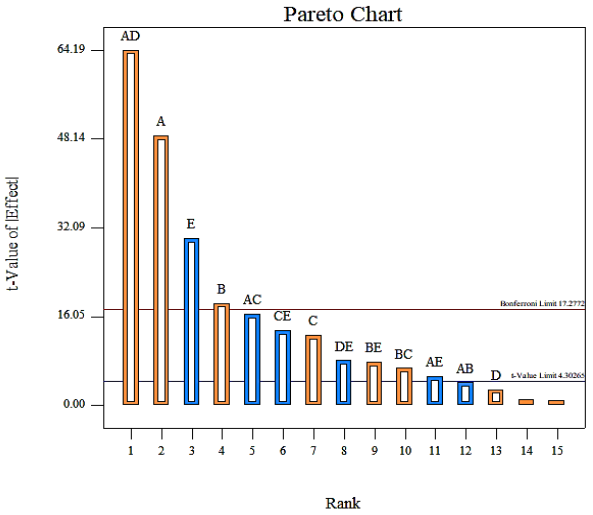
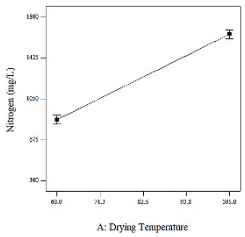


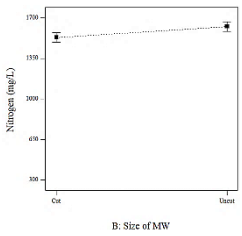
Fig. 4. The effect of factors on N content analysis.

Figures 5. (a), (b), (c), (d) and (e) show the impact of main factors on N contents in the production of biofertilizer using mushroom waste through the Bokashi fermentation method. Figure 5. (a) illustrates that N contents increased with higher drying temperatures. This was because the drying temperature observed in this method was moisture content. By reducing the moisture content through drying, the weight of the material decreased, potentially leading to an increase in the concentration of N and other nutrients [14]. However, it is important to note that the total N content did not change during the drying process, but the concentration of N may increase due to water loss. Figure 5. (b) illustrates that using larger, uncut mushroom waste positively influenced N contents in the biofertilizer. The greater size of the mushroom waste, compared to smaller or cut pieces, led to higher N content. This is due to the larger amount of organic material presented in uncut pieces, provided more substrate for microbial decomposition and the released of N content. The increased surface area and volume of the larger mushroom waste promoted greater microbial activity and nutrient conversion, ultimately resulting in elevated N content in the biofertilizer [3]. Figure 5. (c) shows that as the mushroom waste content increased, there was an increase in microbial

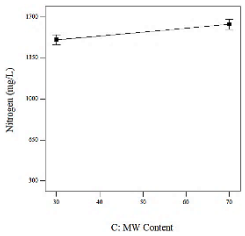
decomposition of the substrate content, resulting in higher contents of N in the biofertilizer. Meanwhile, Figure 5. (d) shows that the N content increased when reaction time increased, allowing for more effective microbial decomposition of the mushroom waste [7]. Figure 5. (d) demonstrates the beneficial effect of agitation in breaking down organic matter, resulting in increased N contents. These findings underscored the significance of analyzing these factors to enhance N content in the biofertilizer derived from the Bokashi fermentation of mushroom waste.



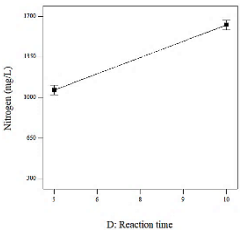
(a) Effect of drying temperature on N content



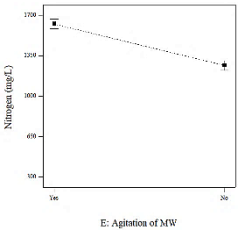
(b) Effect of size of MW on N content



(c) Effect of MW content on N content



(d) Effect of reaction time for Bokashi fermentation on N content



(e) Effect of agitation of MW on N content

Fig. 5. Impact of main factors on N content.

In Figure 5. (a), the interaction effect between drying temperature and reaction time was examined under specific conditions of 66 % mushroom waste content, uncut mushroom size, and agitation during Bokashi fermentation. This figure proves that a higher N content was achieved with 10 days reaction time compared to 5 days reaction time at a higher drying temperature of 105 ℃. This can be attributed to the increased breakdown and decomposition of organic matter at higher temperatures [13]. Meanwhile, at a lower drying temperature of 60 ℃, the N content for 5 days reaction time was higher than temperature at 105 ℃. This difference could be influenced by microbial activity and specific fermentation conditions at lower temperatures, which may favor a shorter reaction time for high N released and conversion.

Figure 6. (a) shows the interaction effect between drying temperature and mushroom waste content. The best conditions for factors was set at uncut mushroom waste, have agitation, and 10 days reaction time for Bokashi fermentation. The figure also shows that N content was observed to increase at high drying temperature (105 ℃) for both mushroom waste contents, but it was higher at 70 % mushroom waste content than 30%. This can be attributed to an increase in microbial activity and the subsequent release of N in the biofertilizer process [16].

Figure 5. (b) illustrates the interaction effect between drying temperature and agitation on N contents. The results showed that as the drying temperature increased and agitation was presented during the Bokashi fermentation process, N contents increased. Agitation played a role in mixing the N content, which is affected by the reduction of moisture content during the drying process. The combination of higher drying temperatures and agitation promoted more efficient mixing, improved N distribution and results in high N contents in biofertilizers [3].

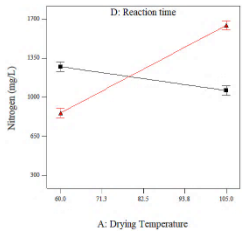
Figure 6. (d) depicts the interaction effect between the size of mushroom waste and mushroom waste content on N contents. The results indicated that a higher mushroom waste content (70 %) and a larger size of mushroom waste (uncut) led to elevated N contents in the biofertilizer. This can be attributed to the increased availability of organic material for microbial activity at higher mushroom waste content, allowed for enhanced nutrient conversion. The larger size of the mushroom waste provided a greater surface area for microbial colonization, promoted efficient nutrient processed and resulted in higher N contents [7]. Conversely, at lower mushroom waste content (30 %), the overall organic material is limited, diminishing the impact of waste size on N contents.

Figure 6. (f) shows the interaction effect between mushroom waste content and agitation. The result showed an increasing in N content as the mushroom waste content increased when agitation occurred. However, without agitation, the N content decreased. According to [8], agitation provided a more homogeneous environment, ensuring equal exposure to microbial activity and facilitated the breakdown of organic matter. So, when there was no agitation during Bokashi fermentation process, microbial activity may decrease and produced low content of N.

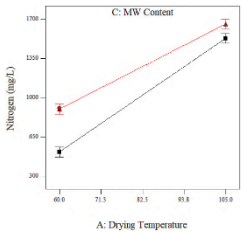
In Figure 6. (f), the interaction effects between mushroom waste content and agitation on N contents are observed. The results demonstrated that higher mushroom waste content, coupled with the presence of agitation during the Bokashi fermentation process, led to increased N contents in the biofertilizer. The higher mushroom waste content provided a greater amount of organic material for microbial decomposition, while agitation promoted better mixing and distribution of nutrients, enhancing microbial activity and nutrient conversion [3]. The combined effect of increased waste content and agitation created favorable conditions for nutrient released and utilization, resulted in higher N contents.

In Figure 6. (g), the interaction effects between reaction time for Bokashi fermentation and agitation on N contents are examined. The findings indicated that higher reaction time, along with agitation, leads to increased N contents. A longer fermentation period allowed for more extensive microbial activity and nutrient conversion, resulting in enhanced N released [22]. The presence of agitation further promoted the distribution and mixing of nutrients, facilitated microbial access to organic material and improved nutrient utilization. The synergistic effect

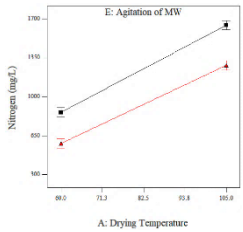
of longer fermentation time and agitation contributed to higher N contents in the biofertilizer.



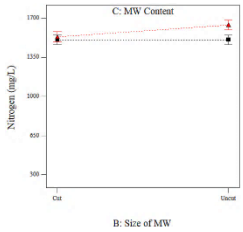
(a) Interaction effect of drying temperature and reaction time on N content



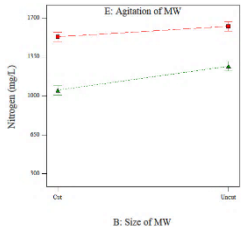
(b) Interaction effect of drying temperature and MW content on N content



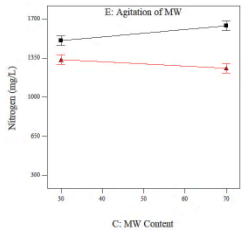
(c) Interaction effect of drying temperature and agitation on N content



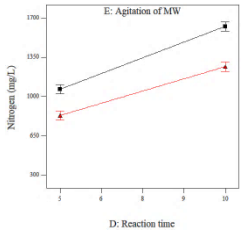
(d) Interaction effect of size of MW and MW content on N content



(e) Interaction effect of size of MW and agitation on N content



(f) Interaction effect of MW content and agitation on N content



(g) Interaction effect of reaction time for Bokashi fermentation and agitation on N content

Figure 6. Impact of interaction effect of factors on N content.

3.3 Validation experiment

The criteria set up to select the best conditions is given in Table 4.

Table 4. Solution obtained for maximum N content

Criteria	Goal	Value
Drying temperature	In range	60 °C – 105 °C
Size of mushroom waste	In range	Cut or uncut
Mushroom waste content	In range	30 % – 70 %
Reaction time	In range	5 days – 7 days
Agitation of mushroom waste	In range	Yes or no
N (N)	Maximize	-
P (P)	Maximize	

Table 5. presents the suggested best processing conditions and predicted N content as determined by Design Expert V.7. Subsequently, experiments were conducted to validate these suggested conditions, and the result was 1624 mg/L exhibiting an error of 2 %, which falls within an acceptable range as it does not exceed 10 %.

Table 5. Solution for 5 combinations of factor levels

Processing condition		Value
Factor 1	A: Drying method (°C)	104
Factor 2	B: Size of MW	Uncut
Factor 3	C:MW Content (%)	66
Factor 4	D: Reaction time (day)	10
Factor 5	E: Agitation of MW	Yes
Exp N content (mg/L)		1624
Predicted N (mg/L)		1591.82

4. CONCLUSION

In summary, the utilization of Design Expert software Version 7.0 confirmed that biofertilizer produced from mushroom waste through Bokashi fermentation contains high N contents. The key factors influencing this high N content include drying temperature, mushroom waste content, size of waste, and agitation. The optimal processing conditions for achieving maximum N (1624 mg/L) content were determined to be 104°C, uncut MW, 66% MW content, and a 10-day reaction time with agitation. The predicted results closely matched the experimental outcomes, with an error rate of only 2%. This suggests that both Expired Mushroom Blocks (EMBs) and mushroom waste (MW) can be effectively utilized to produce biofertilizer with high N content for sustainable agriculture and industrial applications. Future research and development are to assess the long-term stability and efficacy of the produced biofertilizer in various soil types and climates and to conduct scale-up studies to evaluate the feasibility and efficiency of producing biofertilizer at an industrial scale. With

continuous research studies, these alternative materials could be value added natural products, contributing to sustainable practices and reduce overall waste.

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