Multiple Objective Optimization of High Nitrogen content Biofertilizer Production using Mushroom waste

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Abstract
The study aimed to optimize nitrogen content in biofertilizer production using mushroom waste. Five factors were analyzed using two-level full factorial design (TLFD) including mushroom waste content, reaction time for Bokashi fermentation, agitation of mushroom waste, size of mushroom waste and drying method. The nitrogen content was measured using a HACH Spectrophotometer. The factors were further optimized using a central composite design (CCD) through response surface methodology. The parameters studied included drying temperature and mushroom waste content.

Analysis of variance (ANOVA) showed that the best conditions for producing high nitrogen biofertilizer were a drying temperature of 104 ℃, 70% mushroom waste content, uncut mushroom waste size, a Bokashi fermentation reaction time of 10 days with agitation and nitrogen content of up to 1624 mg/L. The suggested optimum condition for producing high nitrogen biofertilizer was a temperature of 80°C and 70% mushroom waste content, yielding the highest nitrogen content of 1305mg/L. This research suggests mushroom waste as a new alternative for biofertilizer production, adding value-added natural products.

Keywords: Biofertilizer, Central composite design, Full factorial design, Optimization, Response surface methodology.

Introduction

The growing demand for agricultural products encourages the use of unsustainable fertilizing techniques. While the agriculture industry uses a lot of synthetic fertilizers, organic waste from the food and crop processing sectors is often regarded as waste and its nutritional value is frequently overlooked. Mushroom waste (MW) is one of the organic wastes beneficial for the development of organic fertilizer or biofertilizer. It is estimated that for every 1 kg of fresh mushroom harvested, approximately 5 kg of MW is generated[2,21]. After the mushroom harvest, MW still contains adequate levels of organic matter including nitrogen (N), phosphorus (P), potassium (K) and other mineral nutrients that are essential for crop growth[2,21]. Chemical fertilizers can have a harmful impact on the environment, for example soil degradation, groundwater pollution and greenhouse gas emission. Rather than relying on chemical or synthetic fertilizers, biofertilizers offer a sustainable alternative to agriculture by enhancing soil fertility through natural processes. MW is a rich source of nutrient that can enhance soil fertility and can promote plant growth. One way to avoid any waste leftover from mushroom production is by converting the waste to biofertilizer. MW, such as stems, can be recycled and used as a friendly substrate for biofertilizer, further reducing costs.

Furthermore, very little research was conducted on the use of MW and Bokashi fermentation to produce high N biofertilizers. N is essential for the growth of mushrooms and their metabolic processes, especially in the synthesis of proteins and enzymes. During the growth stage, the fungal structure known as mycelium needs N to aid in colonization and to lower the risk of contamination during the development of the fruiting body[2]. After the biofertilizer is produced, it is essential to analyze the N content of the MW to guarantee its quality.

In this study, it is required to optimize the high N content in MW for biofertilizer production. The green leaves growing on plants are a result of N, which is essential for the health and growth of all plants. N helps in the process of photosynthesizing, in which plants use the sun's energy to split water and carbon dioxide into sugar[3]. Providing plants with sufficient N is like providing them with access to food since N helps with photosynthesis, leading to larger and stronger plants. In addition to promoting plant growth, biofertilizers reduce the expense of chemical fertilizers like phosphorus, N and potassium[3].

This study focused on two manipulated factors including MW content and drying temperature with three other parameters kept constant including Bokashi fermentation time, agitation of MW and MW size to develop biofertilizers with a high N content using MW through Bokashi fermentation. The study examined the optimized condition of these two parameters on N content using Design Expert (DE) software. Through the optimization of the operational factors, CCD is used to optimize the condition to achieve higher N content of biofertilizer production using MW.

Material and Methods

Mushroom waste sample collection and preparation: For this experiment, MW or *Pleurotus* spp. was collected from the industrial farm. The MW was fermented by using Bokashi fermentation method using Bokashi bran, provided by Ada Fresh Farm from Batu Pahat, Johor, Malaysia.

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Process of Bokashi fermentation method: During the Bokashi fermentation process, the process condition of the sample was subjected to several parameters. Initially, the MW was divided into two categories which were uncut and cut into 5 mm thick pieces. After that, the MW was dried at a specific temperature. Bokashi bran, obtained from Ada Fresh Farm was then mixed with MW in a tightly closed container, according to the appropriate weight composition, to maintain the anaerobic environment required for Bokashi fermentation. The biofertilizer samples were fermented for either 5 or 10 days, with agitation applied when necessary[22].

Experimental Set-up for factorial analysis: Five factors were selected and categorized into categorical and numerical factors, as shown in table 1. Three factors including the drying temperature, MW content and reaction time for Bokashi fermentation, were classified as numerical factors. The categorical factors included the size of MW whether the sample needed to be cut or left uncut.

The parameter for each sample was shown in table 3. After Bokashi fermentation was completed, the biofertilizer sample was diluted before undergoing the persulfate digestion method. The N content was measured by a HACH Spectrophotometer (DR1900) and was analyzed with Analysis of Variance (ANOVA) from Design Expert Software V7. The factors were examined using a 2^{5-1} fractional factorial design with a total of 16 runs generated by the Design Expert software. Two-Level factorial analysis (TLFA) was used in this study to identify factors that have a high significant impact on N content[17]. The most significant factors and optimum conditions for biofertilizer production using Bokashi method were analyzed using Design Expert software.

The optimum conditions points suggested by the Design Expert software were validated to verify the reliability of the model. The error between experimental values and predicted values was calculated for reliability test. Equation 1 was used to calculate the error.

$$Error\ (\%) = \frac{Experimental - predicted}{predicted} \times 100\% \qquad (1)$$

Experimental Set-up for optimization analysis: Table 2 shows the range of factors selected through factorial analysis, with the preference for only the two most significant factors. Low and high factors were coded as −1 and +1 in the experimental design, the center point was coded as 0 and the α value was set to 2. ANOVA was used to analyze the model's validity statistically[5]. The size of mushroom was kept constant at 1 mm in length, with daily agitation and reaction time of 10 days.

Table 5 shows optimization factors design analysis using Design Expert software. The two most important variables to be optimized in this study were drying temperature and the amount of MW (MW: Bokashi bran), as determined from preliminary studies. The fixed parameters also were set which were reaction time for Bokashi fermentation, size of MW and agitation of MW. After the Bokashi fermentation process was completed, the biofertilizer sample was mixed with deionized water and filtered using filter paper. The N content was measured using a HACH Spectrophotometer (DRB1900) with the persulfate digestion method and then was analyzed using Analysis of Variance (ANOVA) in Design Expert software. Restricted randomization was used to eliminate bias.

To conduct the experiment, a central composite design (CCD) was employed, resulting in a total of 13 runs. All data obtained was recorded in Design Expert software and the responses were analyzed to further optimize significant factors and interaction between the factors. Furthermore, optimization analysis was utilized to identify the condition of the factors that exerted a significant impact towards N content in this study[9]. The optimum conditions points suggested by Design Expert software were validated to verify the software model's reliability. The error between experimental values and predicted values was calculated for reliability test. Equation 1 was used to calculate the error.

Sample analysis using HACH Spectrophotometer: The N content in the MW were determined using a HACH Spectrophotometer. N content was analyzed using the total nitrogen hydroxide reagent with the persulfate digestion method 2.0 to 150 mg/L N (High Range).

Alkaline persulfate digestion converts all forms of N to nitrate. The organic N compounds present as organically bound N in the trinegative state are determined in this test. Pretreatment of the sample with acid and heat to hydrolyze condensed inorganic forms was performed by heating the sample in the DRB200 for 30 minutes at 105°C. N in this form is converted into ammonium salts by the action of sulfuric acid and hydrogen peroxide.

Table 1
Factors and actual values of coded levels

S.N.	Parameters	Coded	Type of factor	Actual values of coded levels		Units
				-1	+1	
1	Drying temperature	A	Numeric	60	105	℃
2	Size of mushroom waste	B	Categoric	Cut	Uncut	
3	Mushroom waste content	C	Numeric	30	70	%
4	Reaction time	D	Numeric	5	10	Day
5	Agitation of mushroom waste	E	Categoric	Yes	No	

Table 2
Factor selected and their level and range in CCD

Independent factors	Level and range				
	-2.00 (-α)	**-1**	**0**	**+1**	**+2.00 (+α)**
Drying Temperature (°C)	60	70	80	90	100
MW content (%)	50	60	70	80	90

Table 3
The result of the 2^5 fractional factorial experiments

Standard Order	Coded values of variables					N (mg/L)
	A	**B**	**C**	**D**	**E**	
1	60	Cut	30	5	No	787
2	105	Cut	30	5	Yes	953
3	60	Uncut	30	5	Yes	928
4	105	Uncut	30	5	No	898
5	60	Cut	70	5	Yes	1145
6	105	Cut	70	5	No	592
7	60	Uncut	70	5	No	1128
8	105	Uncut	70	5	Yes	1064
9	60	Cut	30	10	Yes	444
10	105	Cut	30	10	No	1228
11	60	Uncut	30	10	No	424
12	105	Uncut	30	10	Yes	1528
13	60	Cut	70	10	No	308
14	105	Cut	70	10	Yes	1551
15	60	Uncut	70	10	Yes	905
16	105	Uncut	70	10	No	1281

The intensity of the yellow color is proportional to the N concentration[15]. The test was then conducted using the DRB1900 HACH Spectrophotometer, which reads in a range from 2 mg/L to 150 mg/L.

Results and Discussion

Statistical analysis of factorial analysis: Table 3 presents the results obtained from the N analysis, which ranged from 308 to 1551 mg/L. The highest N content of 1551 mg/L was obtained under specific Bokashi fermentation conditions. The R^2 value derived from the ANOVA is utilized to evaluate the alignment of the data with the regression line. Generally, a model that fits well has an R^2 value exceeding 80%. In this analysis, an R^2 value of 0.9998 was achieved, demonstrating that the model accurately predicts both experimental and expected values. The mathematical equations, represented in terms of coded factors, are as follows:

$$N\left(\frac{mg}{L}\right) = 947.75 + 189.13A + 71.75B + 49C + 10.88D - 117E - 15.87AB - 63.87AC + 249.25AD - 20.12AE + 26BC + 30.25BE - 52.5CE - 31.37DE \quad (2)$$

where A is drying temperature, B is size of MW, C is MW content, D is reaction time and D is agitation of MW. The main factors are referred to A, B, C, D and E while interaction effects are referred to AB, AC, AD, AE, BC, BE, CE and DE.

The significance of the regression model for analyzing N content was determined using an Analysis of Variance (ANOVA), as shown in table 4. F-values were used to evaluate the statistical significance of the regression equation, while p-values assessed the significance of each coefficient. In this model, the F-value is 659.74, with a very low p-value ($p < 0.0015$), indicating only a 0.15% likelihood that such a high F-value is due to random variation. A robust model is typically characterized by a calculated F-value that is significantly higher than the tabulated value, occurring multiple times[12]. Smaller p-values indicate the greater significance of the respective variable[12]. According to the analysis, the model terms A, B, C, E, AC, AD, AE, BC, BE and CE significantly influence N content. Conversely, the model terms D was deemed insignificant, as their respective p-values were larger than 0.05.

Effect of individual and interaction factors: Figure 1 displays the Pareto chart illustrating the main and interaction effects of the factors involved in the process. This chart effectively highlights the most significant individual parameter, with the height of the bars representing their impact. The t-values for the bars are derived from the square root of the F-values obtained from the ANOVA. The t-value of the effects is displayed by two limit lines: the t-value limit line and the Bonferroni limit line. The two lines have values of 17.277 and 4.303 respectively. The factors of A, B, C, E, AC, AD, CE, DE, BE, BC and AE clearly surpass the t-value

limit of 4.303 in the Pareto chart and have a noteworthy impact on the N content during biofertilizer manufacturing.

A coefficient is considered significant when its t-value lies between the t-value limit line and the Bonferroni line while coefficients with t-values above the Bonferroni line are regarded as highly significant. Among the main factors, drying temperature lies above the Bonferroni limit line signifying the significances of the factors on N content. In the production of biofertilizer from MW, the drying process is important to ensure that no mushrooms popped up all around the garden when it was applied[16,21]. The interaction between drying temperature and reaction time during Bokashi fermentation was found to be the most significant. For effective biofertilizer production, it is crucial to maintain an optimal temperature during fermentation. Higher drying temperatures enhance N content in the biofertilizer by promoting the breakdown and decomposition of organic matter in MW.

Figures 2 shows the impact of main factors on N contents in the production of biofertilizer using MW through the Bokashi fermentation method. Figure 2 (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[10]. 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 2 (b) illustrates that using larger, uncut MW positively influenced N contents in the biofertilizer.

Table 4
Significance of regression coefficient for nitrogen content analysis

Source	Coefficient Estimate	Sum of Squares	F values	p-value Prob > F	
Model	947.75	2.069E+006	659.74	0.0015	Significant
A-Drying Temperature	189.13	5.723E+005	2372.20	0.0004	
B-Size of MW	71.75	82369.00	341.43	0.0029	
C-MW Content	49.00	38416.00	159.24	0.0062	
D-Reaction time	10.88	1892.25	7.84	*0.1074	
E-Agitation of MW	-117.00	2.190E+005	907.87	0.0011	
AB	-15.87	4032.25	16.71	0.0549	
AC	-63.87	65280.25	270.59	0.0037	
AD	249.25	9.940E+005	4120.24	0.0002	
AE	-20.12	6480.25	26.86	0.0353	
BC	26.00	10816.00	44.83	0.0216	
BE	30.25	14641.00	60.69	0.0161	
CE	-52.50	44100.00	182.80	0.0054	
DE	-31.38	15750.25	65.29	0.0150	

R^2 = 0.9998. *The model was not significant if the p-values were greater than 0.05.

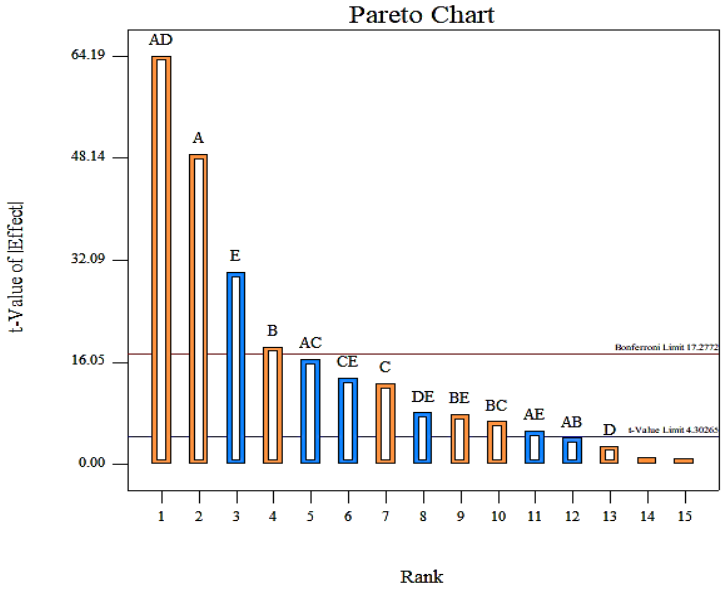


Figure 1: The effect of factors on N content analysis

The greater size of the MW, 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, providing more substrate for microbial decomposition and the released of N content[19].

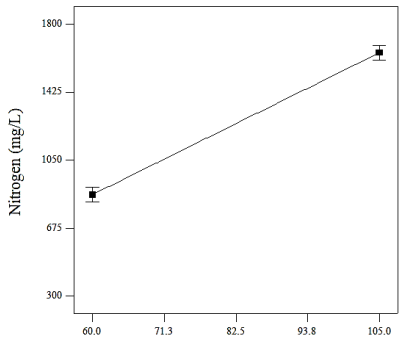
The increased surface area and volume of the larger MW promoted greater microbial activity and nutrient conversion, ultimately resulting in elevated N content in the biofertilizer[4]. Figure 2 (c) shows that as the MW content increased, there was an increase in microbial decomposition of the substrate content, resulting in higher contents of N in the biofertilizer[19]. Meanwhile figure 2 (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 MW.

The interaction effect between drying temperature and reaction time was examined under specific conditions of 66 % MW content, uncut mushroom size and agitation during Bokashi fermentation. Figure 3 (a) 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 °C. This can be attributed to the increased breakdown and decomposition of organic matter at higher temperatures[1,10].

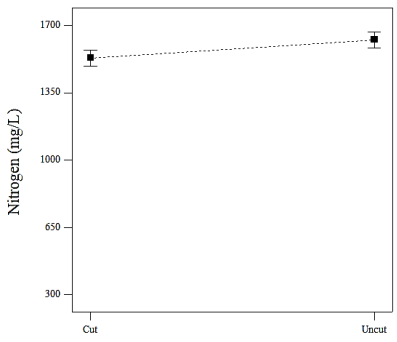
Meanwhile, at a lower drying temperature of 60 °C, the N content for 5 days reaction time was higher than temperature at 105 °C. This difference could be influenced by microbial activity and specific fermentation conditions at lower temperatures, which may favor a shorter reaction time for high N released and conversion.

Figure 3 (b) shows the interaction effect between drying temperature and MW content. The best conditions for factors were set at uncut MW. The figure also shows that N content was observed to increase at high drying temperature (105 °C) for both MW contents but it was higher at 70 % MW content than 30%. This can be attributed to increase microbial activity and the subsequent release of N in the biofertilizer process[11].

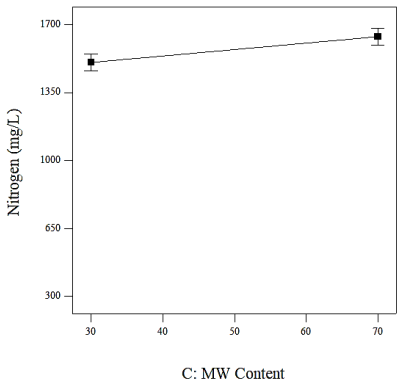
Figure 3 (c) illustrates the interaction effect between drying temperature and agitation on N contents. The results showed that as the drying temperature increased and agitation was presented during the Bokashi fermentation process, N contents increased. Agitation played a role in mixing the N content, 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 resulted in high N contents in biofertilizers[4].



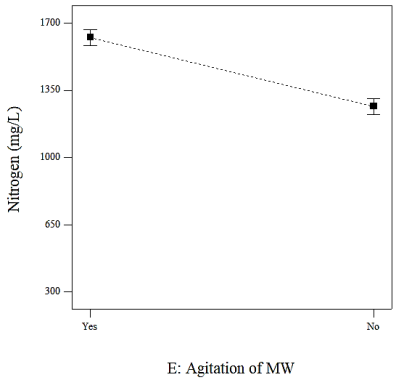
(a) Effect of drying temperature on N content



(b) Effect of size of MW on N content



(c) Effect of MW content on N content



(d) Effect of agitation of MW on N content

Figure 2 : Individual effect between parameter on N content

Figure 3 (d) depicts the interaction effect between the size of MW and MW content on N contents. The results indicated that a higher MW content (70 %) and a larger size of MW (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 MW content, allowed for enhanced nutrient conversion. The larger size of the MW provided a greater surface area for microbial colonization, promoted efficient nutrient processed and resulted in higher N contents[6,19]. Conversely, at lower MW content (30 %), the overall organic material is limited, diminishing the impact of waste size on N contents.

Figure 3 (e) shows the interaction effect between MW content and agitation. The result showed the increasing of N content as the MW content increased when agitation occured. However, without agitation, the N content decreased. Agitation provided a more homogeneous environment, ensuring equal exposure to microbial activity and facilitated the breakdown of organic matter[8]. So, when there was no agitation during Bokashi fermentation process, microbial activity decreased and produced low content of N.

In figure 3 (f), the interaction effects between MW size and agitation on N contents are observed. The results demonstrated that higher MW size, coupled with the presenced of agitation during the Bokashi fermentation process, led to increased N contents in the biofertilizer. The higher MW size 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[4,19]. The combined effect of increased waste content and agitation created favorable conditions for nutrient release and utilization, resulting in higher N contents.

In figure 3 (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[18,19]. 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.

Statistical analysis of Optimization study: Table 5 reports the amount of nitrogen obtained from the 13 sets of samples following each of the drying temperature and mushroom waste content as per the table setup. According to the table, the N contents ranged from 750 to 1305 mg/L. The significance of the N content model was analyzed using Analysis of Variance (ANOVA), as illustrated in table 6. F-values assessed the statistical significance of the regression equation, while p-values examined the significance of each coefficient.

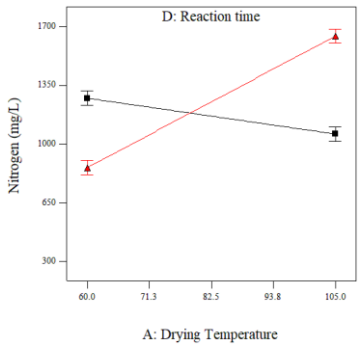
ANOVA evaluated the variables and their interactions, with "Prob > F" values less than 0.05 indicate significance of model terms[7]. Table 6 revealed a significant lack of fit with an F-value of 156.61, suggesting that the replicates had less variation around the mean than the design points around the predicted value.

This was confirmed as the predicted data were lower than the actual experimental results, despite the model's overall significance. The model's F-value was 10.65 with a p-value of 0.0036, indicating a 0.36% chance that such a large F-value occurred due to noise. The significant model was supported by a calculated F-value much greater than the tabulated value. Smaller p-values indicated greater significance of the variables, with the effects of A, B and AB being statistically significant in influencing N content[7].

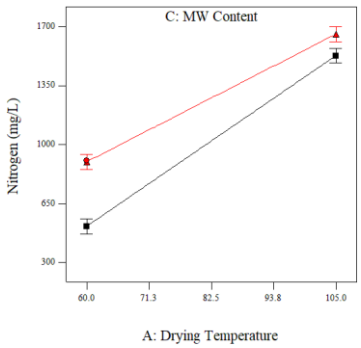
The R^2 value derived from the ANOVA is utilized to evaluate the alignment of the data with the regression line.

Table 5
Experiment Data for N Content Analysis

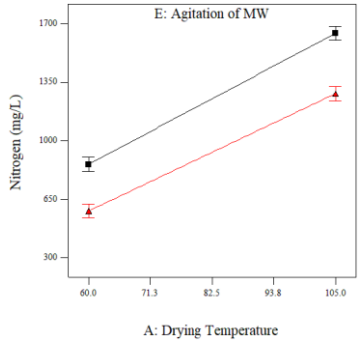
Std	Drying Temperature (ºC)	MW content, % (MW: Bokashi bran)	N content (mg/L)
1	70	60	750
2	90	60	1040
3	70	80	1070
4	90	80	795
5	60	70	750
6	100	70	976
7	80	50	850
8	80	90	795
9	80	70	1285
10	80	70	1275
11	80	70	1275
12	80	70	1305
13	80	70	1290



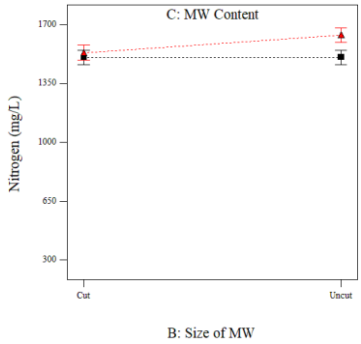
(a) Drying temperature and reaction time on N content



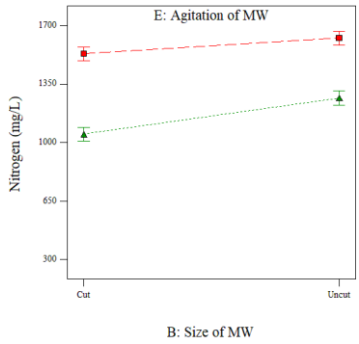
(b) Drying temperature and MW content on N content



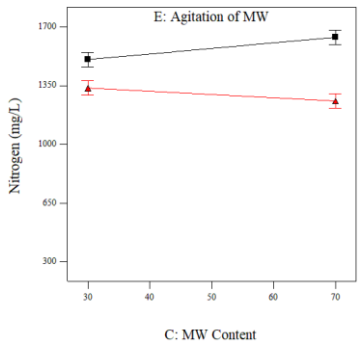
(c) Drying temperature and agitation on N content



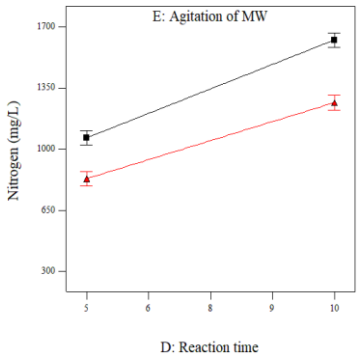
(d) Size of MW and MW content on N content



(e) Size of MW and agitation on N content



(f) MW content and agitation on N content



(g) Reaction time for Bokashi fermentation and agitation on N content

Figure 3: Interaction effect between parameter on N content

Generally, a model that fits well has an R^2 value exceeding 80%. The obtained R^2 value was 0.8838, indicating that the model effectively predicts the experimental and expected results. The mathematical model equations in terms of factors were determined in equation 3 which is used as an equation for predicted N content Design Expert value of N content.

$$N = -19695.80244 + (276.12184\ A) + (278.56911\ B) - (1.41250\ AB\ ) - (1.08347\ A^2) - (1.18472\ B^2) \quad (3)$$

where N is the analysis results of N in unit of mg/L, A is drying temperature (℃) and B is MW content (%).

As portrayed by figure 4 (a), the N content increased with drying temperature from 70 ˚C to 80 ˚C with 1070 mg/L and 1305 mg/L respectively. However, as the drying temperature increased from 80 ˚C to 90 ˚C, the N content seemed to level off. This was due to the excessive temperature that affected the moisture content of mushroom too low and the development of bacteria needed for the sample components decreased. According to researchers[1,21], too much heat generated from drying can harm the microorganism in the MW and can decrease the viability and efficiency of the biofertilizer where the N content decreased in the MW. It had happened due to the sudden rise in microbe interactions and activity. From the experimental result, we can conclude that the drying temperature at 80 ˚C was the optimum temperature for higher N content in MW.

Conversely, the N content presented an upward trend for factor B as the MW to Bokashi bran ratio increased from 60% to 70%, as shown in figure 4 (b). The N content reached its highest value at 1305 mg/L. The impact of MW content on the generation of Bokashi bran has been examined in several research. The quality of Bokashi bran was investigated in a study[13] using various ratios of MW to rice bran. According to the research[20], the amount of lactic acid bacteria, yeasts and fungi in the Bokashi bran increased as the ratio of MW to rice bran increased. This implies that MW, when used as a component in the Bokashi bran, contributes valuable nutrients to the overall mix.

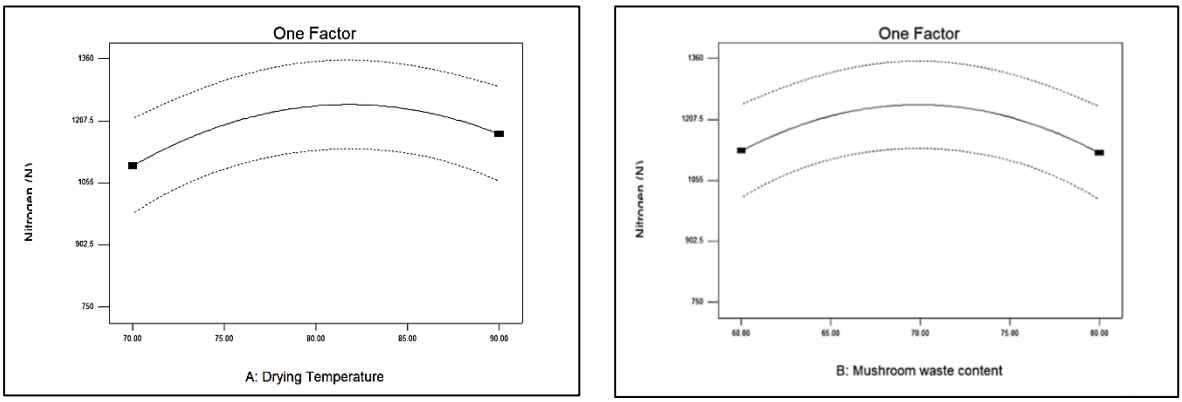
Hence, the N content decreased when the MW content was higher than 70% which indicates that the optimum MW content to achieve high N content is at 70%. The excessive amount of MW can create conditions that encourage N volatilization, particularly if it was not well-balanced with other organic components. During the breakdown process, ammonia, a kind of N, may be released into the environment. A drop in N content may result from this loss of volatile N molecules.

The effects of process factors and responses are also shown through contour plots and 3D-response surface in figures 5 (a) and 5 (b) respectively. As shown in figure 5 (a), the curve indicates that drying temperature and MW content had a quadratic effect on N content, suggesting that the optimal response occurred near the center point[4]. Meanwhile, the elliptical contour plot presented in figure 5 (b) reveals a significant interaction between the factors, with the peak response shown by the red area close to the center point. These demonstrate that N content increased with rising drying temperature and MW content but began to decrease once these factors exceeded the optimal conditions.

Table 6
Significance of regression coefficient for nitrogen content analysis

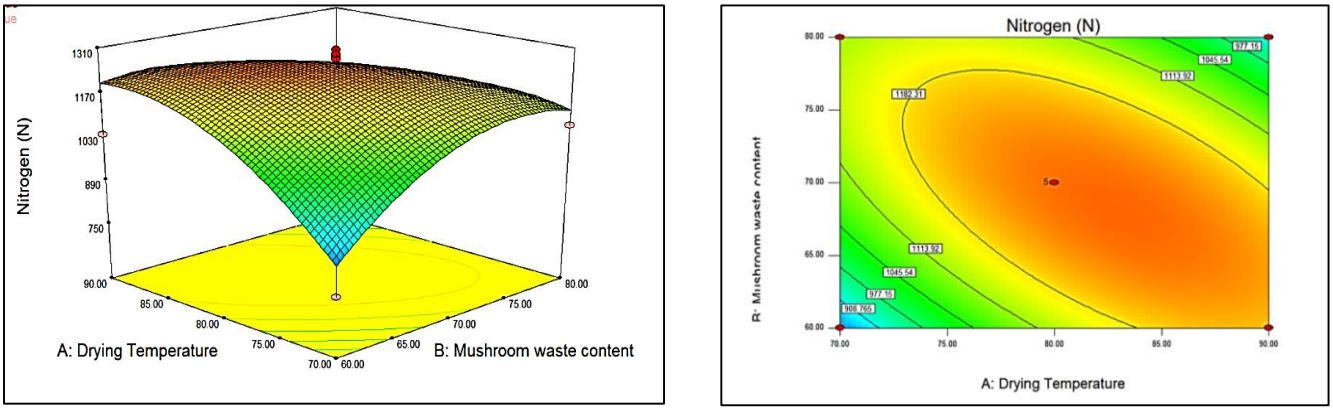
Source	Sum of values	df	Mean Square	F values	P-value Prob>F		R-Squared
Model	5.59E+05	5	1.12E+05	10.65	0.0036	Significant	0.8838
A-Drying Temperature	*18174.08*	1	*18174.08*	1.73	0.2296		
B-MW content (%)	*102.08*	1	*102.08*	*9.73E-03*	0.9242		
AB	*79806.25*	1	*79806.25*	7.61	0.0282		
A2	*2.69E+05*	1	*2.69E+05*	25.64	0.0015		
B2	*3.22E+05*	1	*3.22E+05*	30.65			
Residual	73444.12	7	10492.02				
Lack of Fit	*72824.12*	2	*24274.71*	156.61	<0.0001	Significant	

R^2 = 0.8838. *The model was not significant if the p-values were greater than 0.05.



(a) Drying temperature **(b) MW content**

Figure 4: Effect of two individual parameters on N content.



(a) Graphs of 3D-response surface curve. **(b) Contour plot**

Figure 5: Effect of two individual parameters on N content.

Optimization and Validation: The optimization and validation were performed by the numerical optimization technique through the DE software. Both drying time and MW content were adjusted within their respective ranges while N content was set to the highest level to maximize the model's desirability. The design expert software recommended a single solution: 83.06°C cycles and 68.02% MW content. However, for practical and precise application, these values were rounded to 80°C and 70% MW content in the actual experiment. This model had a desirability of 0.902, indicating its suitability. Nevertheless, the selected conditions only achieved 1250.7 mg/L, whereas the maximum N content measured in the experiment was 1305 mg/L at 80°C and 70% MW content.

Therefore, 1305 mg/L was chosen as the predicted value because it was higher than the N content predicted by the suggested optimal conditions. In the validation studies, percentage error was obtained by evaluating the difference between actual and predicted values. The error percentage was found to be within an acceptable range of less than 30% which is a standard threshold[14]. The actual N content obtained under the selected experimental conditions was 1320 mg/L, the highest recorded among the three validation studies. Therefore, the proposed conditions of 80°C drying temperature and 70% MW content were confirmed to be effective for maximizing N content in biofertilizer made from MW, aligning with the highest N content achieved in the validation experiment.

The two-level factorial design (TLFD) was used to identify the most significant factors in the analysis. These significant factors were then further optimized using the Central Composite Design (CCD) for improved results. While both TLFD and CCD examined the same factors, the ranges for each factor differed between the two analyses. The mathematical model derived from the TLFD was found to be significant for achieving optimization results when the same factor values from the optimization analysis were applied. These results suggested that using optimization methods with design expert software will increase the performance of mushroom waste as new alternatives for biofertilizers production.

Conclusion

The study focuses on the effects of several factors involved in biofertilizer production using MW. Based on full factorial design analysis (FFD), the results showed that the most significant factors in producing biofertilizer with high N content were drying temperature, MW content, size of MW and agitation. The best conditions for the biofertilizer processing factors using MW to achieve the maximum amount of N content were drying temperatures at 104 °C, size of MW uncut, 66 % of MW content, 10 days of reaction time of Bokashi fermentation with agitation. Based on the best conditions proposed, the N 1624 mg/L was achieved. The major factors contributing to N were further optimized using central composite design (CCD).

The mathematical model involved in N was best fitted to predict biofertilizer production. CCD revealed that the optimum condition for drying temperature is in the experimental region centre point as 83.06 °C and 68.05% MW content. The results of this study suggest that Bokashi fermentation was the new effective method of biofertilizer using MW fermentation. At this condition, the predicted value of N content can be achieved up to 1250.7 mg/L.

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