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Statistical optimisation of radial growth rates of *Ganoderma neo-japonicum* (KLUM61076) in low cost solid agar plate's medium using full factorial design and central composite design

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Abstract

The combination of low cost medium sources (brown sugar, spent yeast, molasses and corn steep liquor) for growth of *Ganoderma neo-japonicum* (KLUM61076) in solid agar plates was studied. The mathematical model of Full Factorial Design and Response Surface Methodology were used to select the optimum combination of carbon/nitrogen sources for the cultivation of *G. neo-japonicum*. The combination medium of brown sugar and spent yeast was the best for growth of *G. neo-japonicum* at 5.74% (w/v) carbon content in brown sugar and 0.06% carbon content (w/v) in spent yeast. The combination of two constituents contributed to a low requirement of C/N ratio 1.74 and gave the fastest mycelial growth rates (20.74 mm/day). This combinations of spent yeast/brown sugars showed an increment in mycelia yield, which is up to two-fold as compared with the standard commercial medium (yeast extract-glucose). The low cost medium has the potential to contribute to cost-effective cultivation for *G. neo-japonicum* large-scale production.

Keywords: medium optimisation, response surface, mycelial growth rates, *Ganoderma neo-japonicum*

Introduction

Ganoderma sp. is the most popular medicinal mushroom for curing various diseases worldwide which includes hypertension, hepatitis, cardiovascular problems and hypercholesterolaemia (Wasser 2005). This Malaysian species of *Ganoderma neo-japonicum* (KLUM61076) or formally known as *cendawan senduk* has been frequently used in herbal preparation for natural healing and even as alternative medication. Its common usage serves as a positive indicator that this valuable natural resource might have some potential medicinal properties such as polysaccharides which can be proven scientifically (Lee et al. 2008).

The different strain of *Ganoderma* species showed different morphology and physical characteristic (Brendan and Sivasithamparam 2003). The growth of *Ganoderma* species is greatly influenced by the types and concentration of substrates as well as the condition of cultivation. All

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these factors can directly dictate the levels of growth rates and therefore, influence the performance of microbes.

Among diverse types of medium, the solid medium is the most frequently used in experiments to cultivate microorganisms (Song et al. 2007). Glucose and yeast extract have been widely used as common pure commercial carbon and nitrogen sources for fungal medium growth in both solid and liquid fermentations (Kim et al. 2002; Hsieh and Yang 2004). Besides that, malt extract agar (MEA) and potato dextrose agar (PDA) have been commonly used as carbon and nitrogen sources for *Ganoderma lucidum* in solid medium. The growth trials of *Ganoderma* isolate using PDA agar plates showed that the optimal growth has been achieved at temperature of 30 °C and pH 6 (Song et al. 2007). Meanwhile, all these carbon and nitrogen sources are expensive and not competitive for large scale production.

Few studies have been conducted by previous researchers to examine the preliminary growth patterns of fungi on different solid medium. For instance, Bilay et al. (2000) has investigated the growth rate of 30 mushroom cultures by exploiting the modified varieties of media preparations. While it is relatively easy to determine the biomass of a fungus growing in a liquid medium, it is more difficult to estimate biomass when a fungus is growing in all three dimensions over and through a solid medium. The growth of a colony can be expressed in terms of the radial extension of the colony.

According to Miller (2000) and Wu et al. (2004), the suitable carbon/nitrogen ratio is very important for producing the best medium for fungal growth. The suitable fungal carbon to nitrogen fractions suggested by Miller (2000) was 4:1 to 10:1. The ratios were varied based on the source of medium and types of fungi or microorganisms (Miller 2000). Hsieh and Yang (2004) showed the highest growth rate of 7.5 mm/day was observed in the medium with 80 C/N ratios in soy residue of solid state fermentation of *G. lucidum*. This finding, however, contradicts the report by Babistkaya et al. (2005) which suggested that the best C/N ratio for *G. lucidum* cultivation medium is 25. On the other hand, Baojing et al. (2012) also asserted the different views, and stated that the C/N ratio of 5 result in an optimum growth of *G. lucidum* with the production of 7.235 g/litre mycelial biomass and 1.723 g/litre of exopolysaccharide (EPS).

Therefore, the accurate determination of suitable carbon to nitrogen concentration is urgently required. Since this appears to be a very difficult task, various statistical experimental design strategies to choose the carbon and nitrogen sources which could optimise the C/N fraction in the fermentation media, subsequently maximised the growth yield have been explored (Chang et al. 2006b).

The statistically based experimental design was proved to be effective tools to optimise the medium components for maximal yield production. Response Surface Methodology (RSM) is a collection of statistical techniques for designing experiments, building models, evaluating the effects of factors and searching for the optimum conditions. RSM has been successfully applied in the optimisation of bioprocesses (Jesus et al. 2001).

The components of brown sugar, spent yeast and corn steep liquor are rich in complex organic and inorganic nutrients as well as vitamin and minerals (Steckley et al. 1979; Zabriskie et al. 1982; Hubert 2006). Therefore, the objective of this study was to investigate the suitable concentration of low cost medium sources (brown sugar, spent yeast and corn steep liquor) for *G. neo-japonicum* (KLUM61076) cultivation in solid agar plate.

Materials and methods

The strain of *G. neo-japonicum* (KLUM61076) was collected from Kenaboi Tropical Reserves Forest, Negeri

Plate 1. The fruit body of Ganoderma neo-japonicum (KLUM61076)

Plate 2. The mycelia of Ganoderma neo-japonicum (KLUM61076)

Table 1. Total carbon and nitrogen content in low cost medium

Medium	Total carbon (% w/v)	Total nitrogen (% w/v)
Brown sugar (BS)	98.99	N.D (<0.2)
Spent yeast (SY)	25.63	2.0
Molasses (MLS)	85.77	N.D (<0.2)
Corn steep liquor (CSL)	48.22	2.9

Sembilan and deposited to University of Malaya Culture Collection (Kuala Lumpur, Malaysia) (*Plate 1*). The stock culture was maintained on malt extract agar (MEA, Oxoid). The cultures were inoculated in petri dishes and incubated at 26 ± 2 °C for 7 days. These cultures were then used as inocula (5-mm discs containing mycelium) for the subsequent experiments (*Plate 2*).

Spent yeast (SY) is a by-product of the brewery industry from Carlsberg Brewery (Malaysia) Bhd., Selangor, while the brown sugar (BS) is a product of the cane industry from CSR Sugar Refinery Sdn. Bhd., Selangor. Corn steep liquor (CSL) is a by-product of the corn industry from Sigma-Aldrich (Sendirian Berhad) and molasses (MLS) is a by-product from sugar refinery industry from Malayan Sugar Manufacturing Co. (Bhd.), Pulau Pinang.

The total carbon and nitrogen contents in the different medium sources (molasses, corn steep liquor, spent yeast and brown sugar) were analysed. The total carbon content was determined using furnace method (AOAC 1980) while the total nitrogen content was analysed using Kjeldhal Method (AOAC 1980).

The four combinations of medium sources (SY-BS, SY-MLS, CSL-BS and CSL-MLS) were used in this study. The carbon and nitrogen contents in the sources (*Table 1*) were used for calculation of medium combinations as designed by mathematical model of full factorial design and response surface methodology. The combination of the sources were dissolved in basal medium containing 4% (w/v) bactoagar, 0.05% (w/v) KH_2PO_4, 0.05% (w/v) KH_2PO_4 and 0.05% (w/v) $MgSO_4.7H_2O$. The pH was adjusted to 6 with 0.1% NaOH and 0.1% HCl. The medium sources were mixed and autoclaved at 121 °C for 20 min. The sterilised medium sources were poured into petri dishes and allowed to solidify at room temperature. The cultures were incubated at 26 °C for 6 days in static condition. The mycelial extension rates were measured every 24 h.

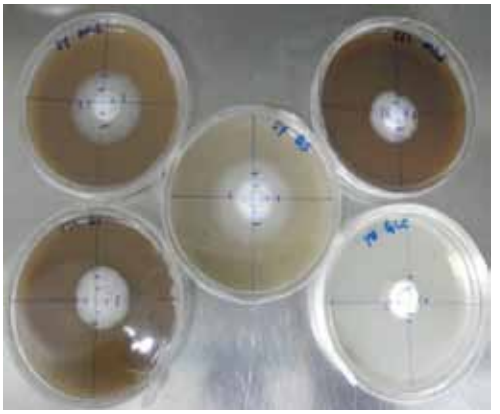

Plate 3. The radial growth of Ganoderma neo-japonicum (KLUM61076) in different medium sources

Radial extension rate was measured daily (every 24 h) as a rate of change in the colony's radius (mm/day). The size was defined as the average of four diameter measurements along lines crossing at right angles. The readings of colony radius were measured starting from the centre of the disk to the periphery of the colony until one of the strains reached the edge of the petri dish. The colony radius was plotted via time to obtain linear region of the graph for specific growth rate. Radial growth rate was determined by the slope and intercept of a linear graph of the linear model according to Baumer 2008 (*Plate 3*).

The yeast extract and glucose was used as positive control to represent the commercial medium for *G. neo-japonicum* (KLUM61076). The radial extension rate was assumed to be the mycelial growth rate according to Baumer (2008).

Two level of full factorial designs were constructed using MINITAB® software (Minitab Inc. USA). A total of 60 runs with four levels of factors were used in the experiment. The FFD was designed in four combinations of medium sources (SY- BS, SY-MLS, CSL-BS and CSL-MLS). Each factor was examined at high level (+1) and low level (–1) factors. The experiments were conducted in one block with three centre points and three replicates. The response variable was the mycelial growth rate (mm/day).

Response surface analysis was run using Central Composite Design (CCD) for two factors consisting of 39 runs with five centre points and three replicates. The response surface model was fitted to the response variable namely mycelial growth rate. The second order response function for the two quantitative factors is given by equation [1].

$$Y = ß_0 + ß_1X_1 + ß_2X_2 + ß_{11}X_1^2 + ß_{22}X_2^2 + ß_{12}X_1X_2 \quad [1]$$

where Y = response, X_1, X_2 = represents the levels of the factors according to *Table 6*, $ß_k$ = regression coefficients.

Five formulation mixtures were selected to test the reliability of the model. Samples were produced using the method described earlier. Validation was carried out under conditions predicted by the model. A close correlation was seen between the experimental and predicted values.

Statistical analysis

The statistical analysis data for the mycelial growth rate in solid media was performed using MINITAB Statistical Software (Minitab Inc, USA). Details for the factorial design and response surface were according to Myers and Montgomery (2001).

Results and discussion

Carbon and nitrogen contents of different substrates

The total carbon and nitrogen content in the low cost medium sources namely molasses, brown sugar, corn steep liquor and spent yeast were analysed. Results showed that the total carbon in brown sugar and molasses were 98.99% (w/v) and 85.77% (w/v) respectively (*Table 1*). Spent yeast and corn steep liquor, correspondingly has 25.63% (w/v) and 48.22% (w/v) carbon contents, which is almost two and threefold lower in comparison with brown sugar. As it is clear, *G. lucidum* was reported to produce

polysaccharides using brown sugar (Chang et al. 2006b) and molasses (Hsieh et al. 2005). Therefore, it was proved that both brown sugar and molasses have potential to replace glucose as low cost carbon sources.

However, despite their high carbon contents, brown sugar and molasses were determined to have a low nitrogen content, which is less than 0.2% w/v, and almost undetected. The spent yeast and corn steep liquor on the other hand were respectively indicated with high concentration of total nitrogen at 2.0% w/v and 2.9% w/v. The amount of carbon and nitrogen in the right proportion will maximise the growth of *G. neo-japonicum*, subsequently increase the production. Therefore, a statistical experimental design was used to calculate the percentage of carbon and nitrogen required.

Selection of substrate using full factorial design (FFD)

The concentration effects of carbon (molasses and brown sugar) and nitrogen sources (corn steep liquor and spent yeast) were investigated using statistical approach of two level FFD (*Table 2*). This design can be applied to identify and quantify the interaction between variables (Chang 2002). In this study, the ratio of carbon to nitrogen was set in the range of 0.1 to 9.84 (as designed by RSM) and the combination of spent yeast, brown sugar, corn steep liquor and molasses was used as growth medium for *G. neo-japonicum* (KLUM61076).

Of the carbon and nitrogen sources examined, the combination of brown sugar and spent yeast which supplies 0.06% C and 6% N respectively, promoted the most effective mycelial growth of 20.86 ± 0.015 mm/day (*Table* 3). The FFD of experimental data indicated that the variability of R^2 was 92.98% for mycelial growth rate (*Table 4*). Thus, this combination was selected for further optimisation analysis RSM and consequently be applied to develop the cultivation process for efficient production of *G. neo-japonicum* (KLUM61076).

Table 2. Levels of factors used in the two level full factorial design

Factor	Name	Level	
		–1	+1
X1	% C	2	10
X2	% N	0.02	0.1
X3	Carbon source	MLS	BS
X4	Nitrogen source	CSL	SY

Optimisation of carbon and nitrogen concentrations in a selected combination source (spent yeast-brown sugar) using response surface methodology (RSM)

The variable of selected constituent (BS and SY) at levels -1 (low), 0 (middle) and +1 (high) is given in *(Table 5*). A total of 39 trial runs were conducted using CCD for the investigation of two factors with five centre points and three replicates (*Table 6*). The response surface model suited the response variables, namely mycelial growth rate and C/N ratio. The concentration ranges of the variables tested were between 2 – 10% of carbon in BS and 0.02 – 0.1% of nitrogen from SY. These two variables are the most essential elements for the growth of *G. neo-japonicum*. The proportions of other organic and inorganic components were kept constant throughout the experiment.

The optimal C/N ratio was predicted to be 1.74 with 20.23 ± 0.023 g/litre of mycelial growth rate for *G. neo-japonicum* (KLUM61076) (*Figure 1*). Post data of regression analysis was performed to fit the response function (mycelial growth rate) with the experimental data. R^2 indicated 78.14% of variability for mycelial growth rate while 98.11% of variability for C/N ratio (*Table 7*).

The polynomial equation showed positive quadratic effect on mycelial growth rate, which indicated that mycelial growth rate increased as the level of spent yeast and brown sugar increased. On the other hand, if the parameter of spent yeast and brown sugar were decreased, the mycelial growth rate also decreased and continued

Table 3. Experimental design and responses for mycelial growth rate of *G. neo-japonicum* (KLUM61076) using FFD

Run	Carbon sources	Nitrogen sources	Nitrogen concentration	Carbon concentration	Mycelial growth rate
	X_1	X_2	X_4 (% w/v)	X_3 (% w/v)	(mm/day)
1	MLS	CSL	0.02 (–1)	2 (–1)	8.76 ± 0.079
2	MLS	CSL	0.02 (–1)	10 (+1)	6.62 ± 0.098
3	MLS	CSL	0.06 (0)	6 (0)	12.17 ± 0.032
4	MLS	CSL	0.1 (+1)	2 (+1)	15.33 ± 0.030
5	MLS	CSL	0.1 (+1)	10 (+1)	7.79 ± 0.006
6	BS	CSL	0.02 (–1)	2 (–1)	18.33 ± 0.017
7	BS	CSL	0.02 (–1)	10 (+1)	13.60 ± 0.026
8	BS	CSL	0.06 (0)	6 (0)	15.93 ± 0.051
9	BS	CSL	0.1 (+1)	2 (+1)	14.72 ± 0.040
10	BS	SY	0.1 (+1)	10 (+1)	17.83 ± 0.032
11	BS	SY	0.02 (–1)	2 (–1)	17.33 ± 0.083
12	BS	SY	0.02 (–1)	10 (+1)	13.96 ± 0.015
13	BS	SY	0.06 (0)	6 (0)	20.86 ± 0.015
14	BS	SY	0.1 (+1)	2 (+1)	16.90 ± 0.055
15	BS	SY	0.1 (+1)	10 (+1)	18.03 ± 0.032
16	MLS	SY	0.02 (–1)	2 (–1)	18.20 ± 0.026
17	MLS	SY	0.02 (–1)	10 (+1)	11.27 ± 0.019
18	MLS	SY	0.06 (0)	6 (0)	17.33 ± 0.025
19	MLS	SY	0.1 (+1)	2 (+1)	17.63 ± 0.015
20	MLS	SY	0.1 (+1)	10 (+1)	13.40 ± 0.027

MLS = Molasses, SY = Spent yeast, BS = Brown sugar, CSL = Corn steep liquor
Sample runs were in triplicates and three samples at centre points
Standard deviation was calculated from three independent samples

Table 4. Analysis of Variance (ANOVA) of Full Factorial Design (FFD)

Analysis of Variance (ANOVA) for growth rate (coded units)						
Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main effects	4	1.81783	1.76057	0.440142	62.73	0.000
2-Way interactions	6	1.50497	1.50475	0.250791	35.74	0.000
Curvature	1	0.32450	0.32450	0.324496	46.24	0.000
Residual error	42	0.29471	0.29471	0.007017		
Lack of fit	3	0.26863	0.26863	0.089544	133.92	0.000
Pure error	39	0.02608	0.02608	0.000669		
Total	58	4.20037				

S = 0.0837669, R – Sq = 92.98%, R – Sq (adj) = 90.31%

Table 5. Levels of factors used for SY and BS in CCD

Abbreviation	Factor		Level		
			–1	0	+1
X_1	Carbon content (BS)	(% w/v)	2	6	10
		g/litre	1.76	5.23	8.8
X_2	Nitrogen content (SY)	(% w/v)	0.02	0.06	0.1
		g/litre	1	3	5

Calculation for carbon and nitrogen in spent yeast-brown sugar (SY-BS)
The carbon content (% w/v) = BS (% w/v) + SY (% w/v)
Carbon content (g/litre) = [BS (g/litre) x 98.99%] + [SY (g/litre) x 25.63%]
The nitrogen content (% w/v) = SY (w/v)
Nitrogen content (g/litre) = SY (g/litre) x 2%

to decline until it reached a certain value. This phenomenon might be due to the fact that the cultivation medium was closely associated with the growth of mycelial growth rate production. The positive second order polynomial equation was found to provide explanation about C/N ratio of *G. neo-japonicum* (KLUM61076). The positive linear-square effect indicated that the C/N ratio increased as the amount of combination of spent yeast and brown sugar increased.

Hence, the term is omitted from the polynomial equation [1] utilised for the model which represents mycelial growth rate (mm/day) (*Y)* as function of BS (*X1*) and SY (*X2*) concentrations. The C/N ratio in equation [2] shows an opposite effect on the mycelial growth rate in equation [1]. The regression was statistically significant (p <0.05) at 95% of confidence level (*Table 7*) with high regression coefficient (R^2 = 0.7814) in mycelial growth rate and (R^2 = 98.11) The model of C/N ratio is expressed in equation [3] which represents C/N ratio (*R*) as function of BS (*X1*) and SY (*X2*) concentrations.

$$Y = 1.397 + 9.895(X1) + 0.1311(X1)^2 - 117.385(X2) - 0.01486(X2)^2 + 0.7031(X1X2) \quad [2]$$

$$R = 5.26357 + 0.0354(X1) + 0.6496(X2) + 0.0394(X2)^2 - 10.1031(X1X2) \quad [3]$$

The probability of C/N ratio can be observed in *Table 7* (p <0.05). Regression analysis was conducted to suit the response function (mycelial growth rate) to data from the experiment. The models were generated using quadratic and linear-square type and mentioned in equation [2] and [3]. Reduced models were generated for the significant variables. The surface and contour plot in *Figure 1* shows that the maximum mycelial growth rate at 20.74 mm/day can be achieved with 5.74% (w/v) carbon content in brown sugar and 0.06% (w/v) nitrogen content in spent yeast the C/N ratio of 1.74.

Medium verification

To verify the model prediction of the response, five independent experiments were performed under the optimal carbon and nitrogen as predicted by the model at 5.74% C and 0.06% N. The results of the verification experiments showed that by using an optimised medium formulation, a higher mycelial growth rate of 20.2 mm/day has been achieved and this was more precise to the predicted value of 20.7 mm/day in contrast with un-optimised medium, which was 20.0 mm/day.

Comparison between selected medium with standard commercial medium (yeast extract and glucose)

The most common carbon and nitrogen sources as reported to be a superior substrate by most researchers for the *G. lucidum* growth are glucose and yeast extract (Lee et al. 1999; Kim et al. 2002; Tang and Zhong 2003). Therefore, this comparison was conducted to prove the alternative sources

Table 6. Experimental design and responses for spent yeast and brown sugar of *G. lucidum* (KLUM61076) using CCD

Run order	PtType	Blocks	Spent yeast (% nitrogen)	Brown sugar (% carbon)	C/N ratio	Mycelial growth rate (mm/day)
1	1	1	0.02	10	9.84	1.38
2	–1	1	0.06	10	3.11	1.95
3	1	1	0.02	10	9.84	1.4
4	0	1	0.06	6	1.74	2.1
5	–1	1	0.1	6	0.954	1.7
6	1	1	0.02	2	1.76	1.8
7	–1	1	0.02	6	5.8	2.04
8	–1	1	0.06	2	0.413	1.83
9	0	1	0.06	6	1.74	2.09
10	1	1	0.1	10	1.76	1.84
11	1	1	0.1	10	1.76	1.78
12	0	1	0.06	6	1.74	2.07
13	0	1	0.06	6	1.74	2.06
14	0	1	0.06	6	1.74	2.05
15	–1	1	0.1	6	0.954	1.67
16	1	1	0.1	2	0.146	1.7
17	1	1	0.1	10	1.76	1.79
18	0	1	0.06	6	1.74	2.1
19	0	1	0.06	6	1.74	2.09
20	0	1	0.06	6	1.74	2.04
21	–1	1	0.02	6	5.8	2.06
22	0	1	0.06	6	1.74	2.08
23	0	1	0.06	6	1.74	2.1
24	–1	1	0.1	6	0.954	1.71
25	1	1	0.1	2	0.146	1.74
26	–1	1	0.06	10	3.11	1.81
27	–1	1	0.06	2	0.413	1.74
28	1	1	0.02	2	1.76	1.76
29	1	1	0.02	2	1.76	1.64
30	0	1	0.06	6	1.74	2.07
31	–1	1	0.06	10	3.11	1.86
32	–1	1	0.02	6	5.8	2.05
33	–1	1	0.06	2	0.413	1.74
34	1	1	0.02	10	9.84	1.41
35	1	1	0.1	2	0.146	1.63
36	0	1	0.06	6	1.74	2.09
37	0	1	0.06	6	1.74	2.1
38	0	1	0.06	6	1.74	2.11
39	0	1	0.06	6	1.74	2.05

Table 7. Response Surface Regression for mycelial growth rate and C/N ratio

7(a) Estimated Regression Coefficients for mycelial growth rate (mm/day)

Term	Coef	SE Coef	T	P
Constant	2.07414	0.02592	80.029	0.000
Spent yeast	0.00111	0.02548	0.044	0.965
Brown sugar	–0.02000	0.02548	–0.785	0.438
Spent yeast*spent yeast	–0.18782	0.03756	–5.001	0.000
Brown sugar*brown sugar	–0.23782	0.03756	–6.332	0.000
Spent yeast*brown sugar	0.11250	0.03121	3.605	0.001

S = 0.108110 PRESS = 0.606022 R-Sq = 78.14%
R-Sq (pred) = 65.66% R-Sq (adj) = 74.83%

7(b) Estimated Regression Coefficients for C/N ratio

Term	Coef	SE Coef	T	P
Constant	1.74303	0.08973	19.425	0.000
Spent yeast	–2.42333	0.08823	–27.468	0.000
Brown sugar	2.06517	0.08823	23.408	0.000
Spent yeast*spent yeast	1.62638	0.13004	12.507	0.000
Brown sugar*brown sugar	0.01088	0.13004	0.084	0.934
Spent yeast*brown sugar	–1.61650	0.10805	–14.960	0.000

S = 0.374308 PRESS = 7.25864 R-Sq = 98.11%
R-Sq (pred) = 97.03% R-Sq (adj) = 97.82%

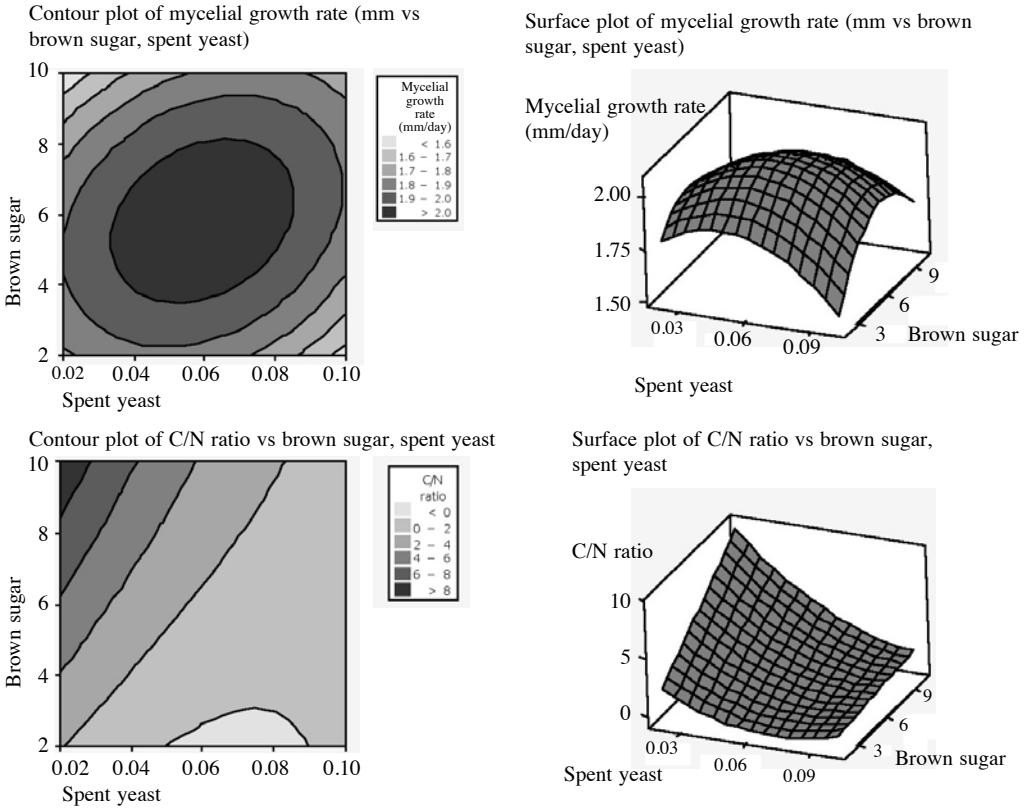

Figure 1. Three-dimensional plots and corresponding contour plots of the effect of mycelial growth rate of G. neo-japonicum (KLUM61076)

for the production of mycelial growth rate of *G. neo-japonicum* (KLUM61076). The highest growth rate (17.67 mm/day) was obtained at the concentration of 0.06% C with 2% N in standard commercial medium containing glucose as carbon source and yeast extract as nitrogen source (*Table 8*). The optimum growth rate of the combination of low cost medium (brown sugar-spent yeast) showed an increase to 2 fold compared to standard commercial medium (glucose-yeast extract) (*Figure 2*). Hence, from these results it could be concluded that, the medium of brown sugar-spent yeast combination with less expensive sources is likely to replace glucose-yeast extract medium since it is has a high potential to be applied in an economically viable industrial process.

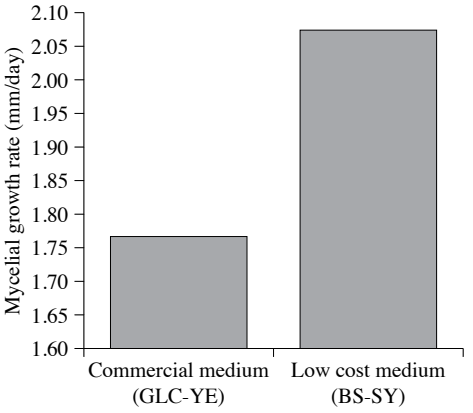

Figure 2. Comparison between standard commercial medium (GLC-YE) and low cost medium (BS-SY) of G. neo-japonicum (KLUM61076) cultivation

Conclusion

The combination of low cost media sources between spent yeast, brown sugar, corn steep liqour and molasses were identified using Full Factorial Design as growth medium for *Ganoderma neo-japonicum* (KLUM61076). Combination medium of brown sugar and spent yeast was the best for growth of *G. neo-japonicum* resulted at 5.74% (w/v) carbon content in brown sugar and 0.06% carbon content (w/v) in spent yeast. Optimal mycelial growth rate was determined to be at 20.74 mm/day with a low C/N ratio of 1.74. The optimisation data (C/N ratio) obtained from the statistical design has been applied throughout experimentation and the results verified to be in concurrence.

Table 8. The mycelial growth rate in commercial medium (GLC-YE) at different concentration of *G. neo-japonicum* (KLUM61076)

	YE	GLC	g/100 ml N	g/100 ml C	Mycelial growth rate (mm/day)
A	0.02%N	2%C	0.18	1.85	1.0393
B	0.02%N	6%C	0.18	5.85	1.0333
C	0.02%N	10%C	0.18	9.85	0.6619
D	0.06%N	2%C	0.54	1.55	1.7667
E	0.06%N	6%C	0.54	5.55	1.2171
F	0.06%N	10%C	0.54	9.55	0.7095
G	0.1%N	2%C	0.91	1.26	1.5333
H	0.1%N	6%C	0.91	5.26	1.49
I	0.1%N	10%C	0.91	9.27	0.7794

Calculation for carbon and nitrogen in glucose (GLC) and yeast extract (YE)

1. The carbon content (% w/v) = GLC (% w/v) + YE (% w/v)
   Carbon content (g/litre) = [GLC (g/litre) x 98.99%] + [YE (g/litre) x 25.63%]
2. The nitrogen content (% w/v) = YE (w/v)
   Nitrogen content (g/litre) = YE (g/litre) x 2%

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Abstrak

Kombinasi sumber medium berkos rendah (gula perang, yis terguna pakai, molas dan cuka peraman jagung) untuk percubaan pertumbuhan *Ganoderma neo-japonicum* (KLUM61076) telah dijalankan pada piring agar medium pepejal. Model matematik reka bentuk faktorial penuh dan reka bentuk komposit berpusat telah digunakan untuk memilih kombinasi sumber karbon/nitrogen yang optimum untuk pertumbuhan *G. neo-japonicum*. Kombinasi medium gula perang dan yis terguna pakai adalah yang terbaik untuk pertumbuhan *G. neo-japonicum* dengan keputusan didapati 5.74% kandungan karbon dalam gula perang dan 0.06% kandungan nitrogen dalam yis terguna pakai. Kombinasi dua elemen ini menyumbang kepada keperluan nisbah karbon/nitrogen (C/N) yang rendah dan penghasilan pertumbuhan miselium yang terpantas (20.74 mm/hari). Kombinasi gula perang/yis terguna pakai ini menunjukkan peningkatan dalam penghasilan miselium sebanyak dua kali ganda jika dibandingkan dengan standard medium komersial (ekstrak yis-glukosa). Medium berkos rendah ini berpotensi untuk menyumbang kepada kos yang efektif dalam penanaman *G. neo-japonicum* berskala besar.