สารอาหารและอุณหภูมิต่อการเจริญของ *Lentinus squarrosulus* Mont. LS-BUB-9

Effect of nutrients and temperature on growth of *Lentinus squarrosulus* Mont. LS-BUB-9

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บทคัดย่อ

เส้นใยของ *Lentinus squarrosulus* Mont. LS-BUB-9 ถูกคัดแยกได้จากดอกเห็ด ซึ่งเก็บได้ในพื้นที่จังหวัดอุบลราชธานี ประเทศไทย เส้นใยถูกเพาะเลี้ยงในอาหารเหลวเบซอล พบว่าเส้นใยสามารถเจริญและเข้าสู่ระยะการเจริญมากที่สุดที่ 25 วัน ความเข้มข้นของแหล่งคาร์บอน ไนโตรเจน ที่เป็นส่วนประกอบในอาหารและอุณหภูมิที่ใช้เพาะเลี้ยงเป็นปัจจัยในการศึกษา สร้างแบบการทำนายการเจริญเติบโตของสายใยเห็ดโดยใช้วิธีพื้นผิวตอบสนอง พบว่าอุณหภูมิการเพาะเลี้ยงมีปฏิสัมพันธ์เชิงบวกทั้งกับแหล่งไนโตรเจน และกับแหล่งคาร์บอน เมื่อเพิ่มอุณหภูมิการเพาะเลี้ยงจาก 25 เป็น 35 องศาเซลเซียส ส่งผลให้มีการสร้างชีวมวลเพิ่มขึ้น การสร้างชีวมวลที่มากที่สุดได้จากการเพาะเลี้ยงที่ 35 องศาเซลเซียส นาน 25 วัน ในอาหารที่มีการเติมซูโครสร้อยละ 3 และแอมโมเนียร้อยละ 0.3

คำสำคัญ: สารอาหาร อุณหภูมิ การเพาะเลี้ยงบนผิวหน้า *Lentinus squarrosulus*

ABSTRACT

The mycelia culture of *Lentinus squarrosulus* Mont. LS-BUB-9 was isolated from a mushroom fruiting body in Ubon Ratchathani Province, Thailand. The culture was grown on a liquid basal medium to reach maximum growth, reaching the stationary phase in 25 days. Concentrations of C-source, N-source in the medium and incubation temperature were the factors investigated. Surface response methodology was used to determine a prediction model for investigating changes of the growth of the culture. The incubation temperature had positive interactions with increasing both N-source and C-source. When increasing incubation temperature from 25 to 35°C, the biomass production increased. The highest biomass production was obtained by incubating at 35°C for 25 days with the addition of 3% (w/v) sucrose and 0.3% (w/v) ammonium sulfate in the basal medium.

Keywords: nutrient, temperature, surface culture, *Lentinus squarrosulus*

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INTRODUCTION

Mushrooms are cultivated for their taste and nutrition attributes and have potential industrial applications [1]. The importance of mushrooms in protein production and biodegradation has been reported by scientists [2, 3]. The world production of cultivated edible mushroom increased more than 30-folds during last 40 years [4]. The protein content in fungal mycelia was reported at 40-55% [5]. Moreover, the fungal mycelium as a protein food source is commercially produced in European countries. The exploration and study of local mushroom culture as a potential novel protein food source should be possible.

The study of mushroom as a single protein has advantages over the other cultures, especially bacteria. Problems related to nucleic acids and non-digestible cell wall have been reported with bacterial single cell protein. The protein from bacteria should be extracted prior to being utilized as human foods. The mushroom mycelium can be directly utilized as a food ingredient.

Lentinus squarrosulus Mont. (LSM) is one of the favorite edible mushrooms found and consumed in the North-Eastern part of Thailand. The mycelium of this mushroom was found to contain significant amounts of crude fiber [6]. The protein content had been reported to be about double that of fresh potato [7] while the essential amino acid content exceeds that of kidney beans. The water extract of this fungal contained high protein (57g/100g) and was rich in magnesium, potassium, vitamin B1 and B2; with total phenolic content of 39.16 mg/100g) [8]. *L. squarrosulus* contains 26.32% of protein and 10.68% of monosodium glutamate-like amino acid [9]. The nutritional evaluation of this mushroom protein as compared to standard casein diet, reported by Pi *et al*. [10], was lower in protein efficiency ratio (PER), while the net protein ratio (NPR) values were slightly higher. Other than the nutritional aspects, it has been reported to be a potential antagonist against some plant pathogenic fungi [11]. Ghate and Sridhar [12] reported the antioxidant properties of *L. squarrosulus* influenced by their bioactive principle and proximal properties.

Culturing of LSM was able to grow on submerged and solid substrates. The culture of LSM has been successful on a wide variety of solid substrates for production of fruiting body as food. It has been reported for growing on supplemented sawdust, rice straw, and also on wood logs. On solid culture, the mycelia of mushroom are associated with the substrate and hard to separate. Under submerged condition, a high biomass was reported when fructose was applied as a carbon source and yeast extract was applied as a nitrogen source [13]. Starch and yeast extract were identified as the most important nutrients in mycelia and crude exo-polysaccharide production [14]. The liquid culture has some advantages over the solid culture including easier mycelia harvesting, higher mycelia production in a more compact space and shorter time, with fewer chances of contamination [15, 16]. The objective of this study was to find the effect of nutrients and incubation temperature on production of LSM LS-BUB-9 biomass on a defined liquid culture

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using statistical design thorough response surface methodology.

MATERIALS AND METHODS

The culture of *Lentinus squarrosulus* Mont. LS-BUB-9 was isolated from a mushroom fruiting body found in Ubonrachatani Province, Thailand, and cultured on PDA (potato dextrose agar) at 30°C and transferred every 7 days.

1. Growth on surface culture with one-factor-at-a-time

A 5 day old culture on PDA was used as inoculum. The inoculums were prepared by punching 1 cm^2 of PDA plate culture with a sterile cylindrical cutter. The surface culture was carried on in a 150 ml Erlenmeyer flask containing 30 ml of basal medium, as described by Gbolagade *et al*. [17]. The one liter of basal medium (the control) was composed of 0.05g KH_2PO_4, 0.05g $MgSO_4$, 0.01g $FeSO_4$, 1.55g KNO_3 and distilled water. The basal medium added with 0.8 % sucrose as C-source was prepared to compare the growth and biomass production. Both media were sterilized at 121°C for 15 min prior to inoculation. The biomass was determined every two days to find the maximum growth at 30°C of incubation temperature.

Effects of 3 factors, incubation temperature, sucrose and ammonium sulfate addition, on growth of LSM were investigated. The incubation temperature was varied at 25, 30, 35 and 40°C in the basal medium added with 0.8 % sucrose. The concentration of ammonium sulfate added to the basal medium with 0.8% sucrose was varied at 0.0, 0.1, 0.2 and 0.3 %. The concentration of sucrose was varied at 1.0, 2.0 and 3.0 % to compare with 0.8 % sucrose. The biomass dry weight was determined after 25 days of incubation at 30°C. The experiment was repeated 2 times. The data was analyzed using Analysis of Variance (ANOVA) and means were compared by Duncan's new multiple range test (IBM SPSS Statistic, V. 17).

The biomass was measured by filtering the fungal mycelia through a Whatman No. 4 filter paper. The obtained mycelia was washed with distilled water and dried overnight to a constant weight at 80°C.

2. Determination of the prediction model using response surface methodology

The mycelia culture of LSM was inoculated in the basal medium with different conditions of temperature, sucrose and ammonium sulfate. The 2^3 factorial with a center point was applied for designing the experiment. There were a total of 9 treatment conditions as shown in Table 1. The biomass dry weight was determined after 25 days of incubation. Two replications were conducted for the 8 treatments at the corner points and 10 replications for the center point. The data was analyzed using Analysis of Variance (ANOVA) and means were compared by Duncan's new multiple ranges test. The prediction model for biomass dry weight production of the LSM was determined using Response Surface Methodology calculated by a computer program (Design-Expert V.7) to find the optimum conditions.

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Table 1 The level of variables of the 9 treatment combinations

Treatment combination	Coded level			Actual level		
	X_1	X_2	X_3	Temperature (°C)	Sucrose added (%)	Ammonium sulfate added (%)
1	-1	-1	-1	25	1	0.1
2	+1	-1	-1	35	1	0.1
3	-1	+1	-1	25	3	0.1
4	+1	+1	-1	35	3	0.1
5	-1	-1	+1	25	1	0.3
6	+1	-1	+1	35	1	0.3
7	-1	+1	+1	25	3	0.3
8	+1	+1	+1	35	3	0.3
9	0	0	0	30	2	0.2

RESULTS AND DISCUSSION

1. Growth on surface culture

The mycelia culture of LSM was prepared for inoculation from a stock culture to then follow the experimental procedure. The growth of this culture on the basal liquid medium is shown in the figure 1. The basal medium contained minimal nutrient for the culture to grow. It was found that the culture reached the stationary phase, after it was incubated around 20-25 days with cell dry weight about 0.01 mg/ml. This showed that this mushroom was able to grow on this basal medium, but provided very small amount of biomass. Compared to the culture with sucrose added, we found that the addition of some sucrose increased growth rate and the maximum growth was clearly determined. To find the optimum conditions by varying one factor at a time, we added sucrose at 0.8 % to the basal medium to lower experimental error in determination of biomass. For further experiments, the LSM was incubated for 25 day period and harvested to determine the biomass.

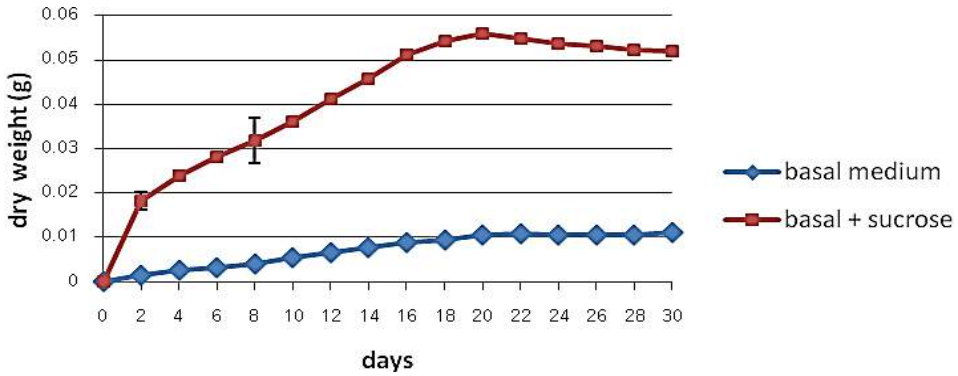

Figure 1 The growth curve of *L. squarrosulus* Mont. LS-BUB 9 on basal medium and basal medium with 0.8% sucrose, incubated at 30°C for 30 days

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The incubation temperature controlled biochemical reactions in *L. squarrosulus* [19], while the N-source and C-source were necessary for biosynthesis and energy [20]. When each factor was investigated individually, the incubation temperature and the concentration of ammonium sulfate showed significant effects ($p<0.05$) as shown in the Figure 2. When the carbon and nitrogen sources were limited, the temperature of 30°C gave the highest mass dry weight. At 30°C, addition of ammonium sulfate provided the highest biomass production. The addition of sucrose did not significantly affect the mass production of this culture. The 2% added sucrose tended to provide the highest average biomass production. A report described that a 2.0% concentration of C source provided the maximum growth of fungal mycelium [21]. Roy Das et al. [22] reported the best carbon source for surface culture to produce the highest mycelial dry weight was fructose and the best nitrogen source was yeast extract.

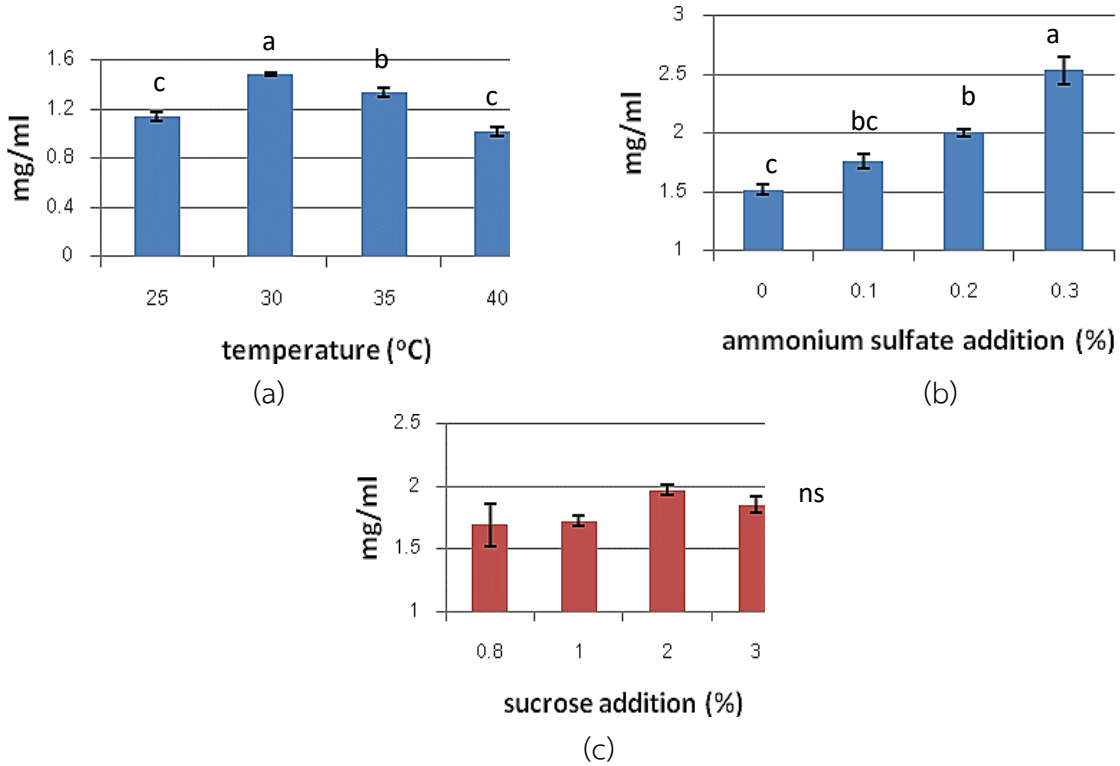

Figure 2 The biomass production of *L. squarrosulus* Mont.LS-BUB-9 at various (a) temperature in the basal liquid medium, (b) ammonium sulfate added, 30°C and (c) sucrose added, 30°C. Means with different letters are different ($p<0.05$) and ^{ns} denotes means were not significant ($p>0.05$) by the Duncan's New Multiple Range Test.

2. Determination of the prediction model using response surface methodology

The effect of 3 factors on the biomass production of LSM was investigated simultaneously in the 2^3 factorial with a center point design. The treatment combinations and mean responses are shown in Table 2. There were significant effects of the interaction between temperature and C-source and the interaction between temperature and N-source ($p< 0.05$).

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From table 2, it was found that the increase in temperature from 25 to 35°C increased biomass production, and an increment of the amount of sucrose in the medium also provided higher the mass production. The addition of ammonium sulfate did not affect the mass production at low incubation temperature, but it provided better mass production at 35°C.

Table 2 The treatment combinations of temperature, sucrose addition and ammonium sulfate addition and means with standard deviations of mass dry weight of the culture (mg/ml)

Temperature	1 % sucrose		2 % sucrose	3 % sucrose	
(°C)	0.1% $(NH_4)_2SO_4$	0.3% $(NH_4)_2SO_4$	0.2% $(NH_4)_2SO_4$	0.1% $(NH_4)_2SO_4$	0.3% $(NH_4)_2SO_4$
25	$1.385^{f} \pm 0.113$	$1.472^{f} \pm 0.252$	-	$2.46^{cd} \pm 0.146$	$2.310^{cd} \pm 0.127$
30	-	-	$2.13^{de} \pm 0.261$	-	-
35	$1.748^{ef} \pm 0.460$	$2.724^{c} \pm 0.274$	-	$3.632^{b} \pm 0.007$	$4.280^{a} \pm 0.127$

Note: Means with different letters are significantly different (P<0.05) by the Duncan's New Multiple Range test; average of three replications.

The estimated regression coefficients for dry weight are shown in Table 3. The relationship between the mass dry weights of this culture with temperature, amount of N-source and C-source were expressed in equation 1. The analysis of variance (ANOVA) of equation 1 is shown in Table 4. The adjusted R-sq and the Analysis of Variance for regression and Lack of Fit showed that the equation 1 was appropriate for the prediction. The predicted R-sq of 0.9389 was in reasonable agreement with the adjusted R-sq of 0.9151.

Table 3 Estimated Regression Coefficient

Term	Parameter estimate	p-value
constant	2.13	
temp	0.59	<0.0001
C-source	0.67	<0.0001
N-source	0.19	0.0043
temp*temp	0.37	0.0011
C-source*C-source	-	-
N-source*N-source	-	-
temp*C-source	0.19	0.005
temp*N-source	0.21	0.0022
C-source*N-source	-0.071	0.2458

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Final equation in terms of coded factors

$$Y = 2.13+0.59X_1+0.67X_2+0.19X_3+0.37X_1^2+0.19X_1X_2+0.21X_1X_3-0.071X_2X_3 \quad (1)$$

Y = dry mass of *L. squarrosulus* LS-BUB 9 (mg/ml)

X_1 = incubation temperature

X_2 = addition of sucrose

X_3 = addition of ammonium sulfate

Table 4 Analysis of Variance

Source of variance	DF	SS	MS	F	P
Regression	7	15.66	2.24	39.50	<0.0001
Residual Error	18	1.02	0.057		
Lack of Fit	1	1.806×10^{-3}	1.806×10^{-3}	0.03	0.8461
Error	17	1.02	0.06		
Total	12	8.679			

R-sq = 0.9389 adjusted R-sq = 0.9151

From Figures 3-5 were the 3D-suface plot and 2D contour plots; it was found that, at 25°C, the N-source (ammonium sulfate) did not affect the mass production. There was interaction between temperature and N source. It was demonstrated that at 0.3% N-source in the medium with the increase in the incubation temperature, the biomass production was increased from 1.5 to 2.7 mg/ml.

The biomass production was increased from 1.3 to 2.4 mg/ml when the concentration of sucrose in the medium was increased from 1 to 3%. There was an interaction between the concentration of ammonium sulfate and sucrose concentration in the medium. At 35°C the biomass increased from 2.4 to 4.2 mg/ml when the basal medium was supplemented with 0.3% ammonium sulfate and 3% sucrose. The mycelia mass production of LSM was comparable to 3.2 mg/ml of mass produced by *Lentinula edodes* in unshaken culture, incubated for 30 days, reported by Hassegawa *et al*. [18] .

LSM LS-BUB 9 can grow well in the liquid culture medium at high temperature. This might indicate that it has potential to be cultured without temperature control in Thailand's local climate. There is a potential for it to be used for many applications including single cell protein or liquid mushroom spawn.

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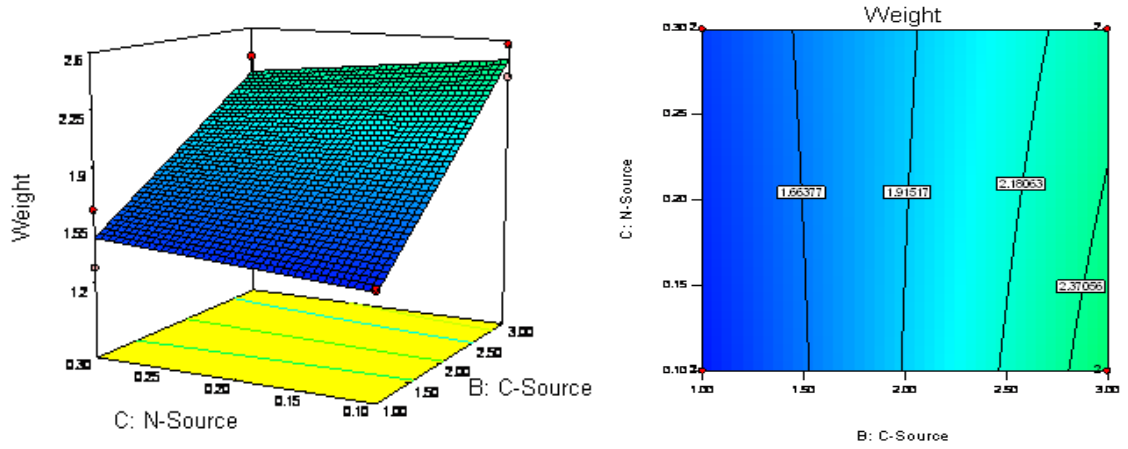
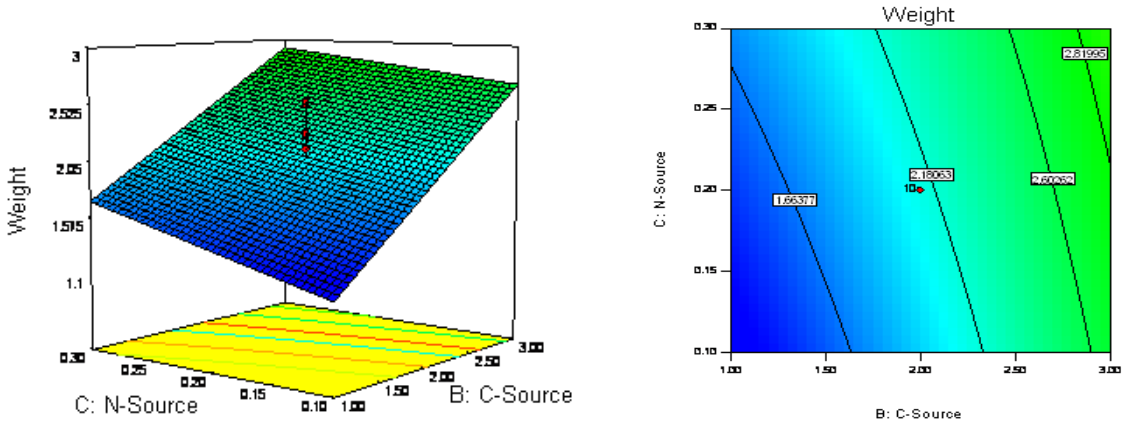


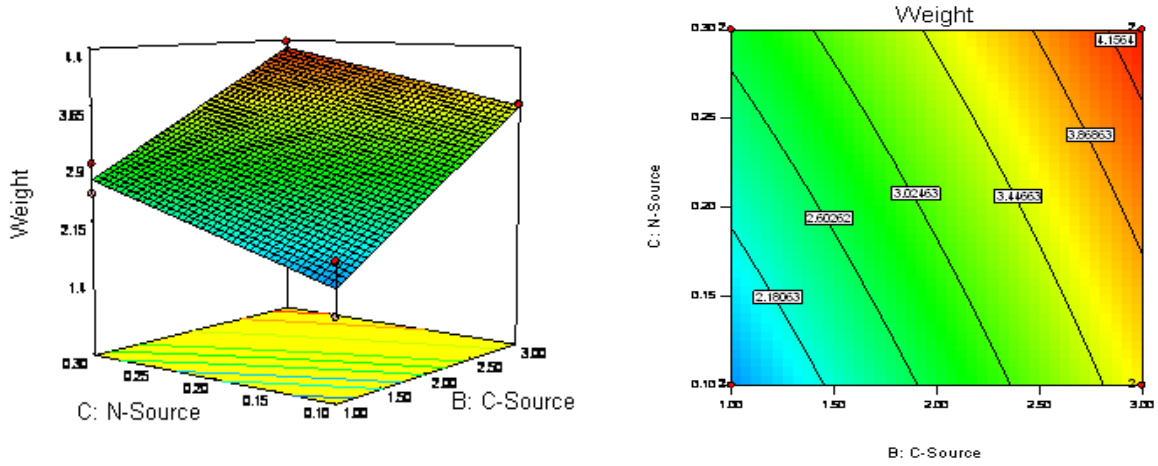
(a) (b)

Figure 3 The 3D-suface plot (a) and 2D contour plot (b) of *L. squarrosulus* LS-BUB 9 mass with various C (sucrose) and N (ammonium sulfate) source in basal medium, incubated at 25°C



(a) .... (b)

Figure 4 The 3D-suface plot (a) and 2D contour plot (b) of *L. squarrosulus* LS-BUB 9 mass with various C (sucrose) and N (ammonium sulfate) source in basal medium, incubated at 30°C



(a) (b)

Figure 5 The 3D-suface plot (a) and 2D contour plot (b) of *L. squarrosulus* LS-BUB 9 mass with various C (sucrose) and N (ammonium sulfate) source in basal medium, incubated at 35°C

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CONCLUSION

The culture of LSM LS-BUB-9 grown in basal medium could reach the maximum growth after 25 days of incubation in a surface culturing condition. It was found that incubation temperature of 30°C and the addition of 0.3% ammonium sulfate provided the highest biomass production, while the amount of sucrose as a carbon source did not affect the biomass production. The investigation of biomass production was applied with a 2^3 factorial with a center point design and surface response methodology. It was found that the incubation temperature had positive interactions with Increasing both N-source and C-source. When increasing incubation temperature from 25 to 35°C, the biomass production increased. The condition of 35°C with the addition of 3% sucrose and 0.3 % ammonium sulfate to the basal medium could yield mass production up to 4.28 $\pm$ 0.13 mg/ml, after 25 days of incubation.

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REFERENCES

[1] Mata, G., Hemande, D.M. and Andreu, L.G. (2005). Changes in ligocellulosic enzymes and activities in six strains cultivated on coffee pulp in confrontation with *Trichoderma* spp. World Journal of Microbology and Biotechnology. 21(2): 143-150.

[2] Jin, B., Yan, X.Q., Yu, Q. and van Leeuwen, H.J. (2002). A comprehensive pilot plant system for fungal biomass and protein production and waste water reclamation. Advances in Environmental Research. 6(2): 179-189.

[3] Estevez, E., Velga, M.C. and Kennes, C. (2005). Biodegradtion of toluene by the new fungal isolates *Paecilomyces varioli* and *Exophialaol digosperma.* Journal of Industrial Microbiology and Biotechnology. 32(1): 33-37.

[4] Royse, D.J., Baars, J. and Tan, Q. (2017). Current overview of mushroom production in the world. In Edible and Medicinal Mushrooms: Technology and Applications ed by Diego, C.Z. and Pardo-Giménez. John Wiley & Sons Ltd.

[5] Christias, C., Couvaraki, C., Georgopoulos, S.G., Macris, B. and Vomvoyanni, V. (1975) Protein content and amino acid composition of certain fungi evaluated for microbial protein production. Applied Microbiology. 29(2):250-254.

[6] Fasidiand, I.O. and Kadiri, M. (1991). Changes in nutrient contents of *Termitomyces robustus* (Geeli) Heim and *Lentinus subnudus* Berk during sporophore development. Acta Botanica Hungarica. 36: 167-172.

[7] Atikpo, M., Onokpise, O., Abazinge, M., Louime, C., Dzomeku, M., Boateng, L. and Awmbilla, B. (2008). Sustainable mushroom production in Africa: A case study in Ghana. African Journal of Biotechnology. 7(3): 249-253.

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[8] Mind Omar, N.A., Abdulah, N., Kuppusamy, U.R., Abdulla, M.A. and Sabaratnam, V. (2011). Nutritional composition, antioxidant activities and antiulcer potential of *Lentinus squarrosulus* (Mont.) mycelia extract. Evidence-based complementary and alternative medicine. Vol 2011 Article ID 53356, 8 pages [Online] Available from http://www.hindawi.com/journals/ecam/2011/539356/)

[9] Zhou, S., Tang, Q.J., Zhang, Z., Li., C.H., Yang, Y. and Zhang, J.S. 2015. Nutritional composition of three domesticated culinary-medicinal mushrooms: *Oudemanseilla sudmusida*, *Lentinus squarrosulus*, and *Tremella aurantialba*. International Journal of Medicinal Mushrooms. 17: 43-49.

[10] Pi, N., Au, K., JB, A. and VJ, U. 2006. Nutritional studies with *Lentinus squarrosulus* (Mont.) Singer and *Psathyrella atroumbonata* Pegler: I. Animal assay. African Journal of Biotechnology 5(5): 457-460.

[11] Idwan Sudriman, L., Lefebvre, G., Kiffer, E. and Botton, B. (1994). Purification of antibiotics produced by *Lentinus squarrosulus* and preliminary characterization of a compound active against *Rigidoporus lignosus.* Current Microbiology. 29: 1-6.

[12] Ghate, S and Sridhar, K. (2017). Bioactive potential of Lentinus squarrosulus and Termitomyces clypeatus from the Southwestern region of India. Indian Journal of Natural Products and Resources. 8(2): 120-131.

[13] Joshi, SR., Das, A.R., Borthakur, M., Saha, A.K., Joshi, S.R. and Das, P. (2015). Growth of mycelial biomass and fruit body cultivation of *Lentinus squarrosulus* collected from home garden of Tripura in Northeast India. Journal of Applied Biology & Biotechnology. 3(4): 17-19.

[14] Anike, F.N., Isikhuemhen, O.S., Blum, D. and Neda, H. (2015). Nutrient requirements and fermentation conditions for mycelia and crude exo-polysaccharides production by *Lentinus squarrosulus*. Advances in Bioscience and Biotechnology. 6: 526-536.

[15] Pokhrel, C.P. and Ohga, S. (2007). Submerged culture conditions for mycelia yield and polysaccharides production by *Lyophyllum decastes*. Food Chemistry. 105: 641-646.

[16] Yang, F.C., Huang, H.C. and Yang, M.J. (2003). The influence of environmental conditions on mycelia growth of *Antrodia cinnamomea* in submerged cultures. Enzyme Microbial Technology. 33: 395-402.

[17] Gbolagade, J., Sobowale, A. and Adejoye, D. (2006). Optimization of sub-merged culture condition for biomass production in *Pleurotus florida* (mont.) Singer, a Nigerian edible fungus. African Journal of Biotechnology 5(6): 1464-1469.

[18] Hassegawa, R.H., Kasuya M.C.M. and Vanetti, M.C.D. (2005). Growth and antibacterial activity of *Lentinula edodes* in liquid media supplemented with agricultural wastes. Electronic Journal of Biotechnology. 8(2): available at http://www.ejbiotechnology.info/index.php/ejbiotechnology/article/view/v8n2-3/464

[19] Chang, S.T. and Miles, P.G. (1989). In Edible mushrooms and their cultivation. CRC Press Inc., Florida, 345 p

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[20] Wu, J.Z., Cheungo, P.C.K., Wong, K. and Hang, N. (2003). Studies on submerged fermentation of *Pleurotus tuberregium* (fr.) Singer. Part I: physical and chemical factors affecting the rate of mycelia growth and bioconversion. Food Chemistry. 81: 389-393.

[21] Petcharatana, V. (1995). Culturing wild mushroom: Hed Korn Kao *(Lentinus squarrosulus* Mont*.).* Songklanakalin campus. Songklanakalin University.

[22] Roy Das, A., Borthakur, M., Saha, A.K., Joshi, S.R. and Das, P. (2015). Growth of mycelial biomass and fruit body cultivation of *Lentinus squarrosulus* collected from home garden of Tripura in Northest India. Journal of Applied Biology and Biotechnology. 3(4): 17-19.

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