

APPLICATION OF THE TAGUCHI METHOD TO OPTIMIZE *MONASCUS* SPP. CULTURE

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ABSTRACT

*The Taguchi method was applied to determine optimum conditions for submerged culture of *Monascus* spp. fermentation to produce a high yield of monacolin K (also known as lovastatin, mevinolin or mevacor). The control factors included carbon, nitrogen, oil, and salt sources and pH values. In the growth phase the optimum culture conditions are 1% whole wheat flour, 1% peptone, 0.01% olive oil and 0.01% potassium phosphate and a pH of 5.0 ($P < 0.05$). In the metabolic phase the optimal culture conditions are 1% whole wheat flour, 1% peptone, 0.01% soybean oil, 0.01% potassium phosphate and a pH of 3.0 ($P < 0.001$). Using optimal culture conditions in the growth and metabolic phases, the yield of monacolin K in the fermentation process was 151.06 ppm.*

PRACTICAL APPLICATIONS

The Taguchi method can be employed for all kinds of industries. By using orthogonal arrays, it reduces the number of experimental trials to a practical and effective size. It helps us to optimize manufacturing processes involving multiple factors with different numbers of levels, especially fermentation processes and new food product developments, simultaneously and economically. In this *Monascus* spp. fermentation process, five factors with four levels need only 16 experimental trials. A set of optimal conditions can be established after a calculation of signal-to-noise ratio and analysis of variance to produce

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a high yield of monacolic K, a functional compound. Significant factors are the main factors and need to be tuned as the fermentation process in the laboratory is scaled up in the future.

INTRODUCTION

Monascus spp. are a food fungus that has been widely used as an enzymatic agent to make wines and other fermented food products. It has also been reported in ancient Chinese literature to have especially beneficial effects, such as the improvement of food digestion and blood circulation (Ma *et al.* 2000). In Taiwan, *Monascus* spp. have also been regarded as a natural food additive source and it has been included in the Department of Health sanitation standard for edible natural colorants (DOH, Taiwan 2004) to change the color of foodstuff as part of the Chinese diet.

Based on morphology and taxonomy, *Monascus* spp. belong to an order of *Fungi*, *Eumycophyta*, *Ascomycota*, *Euascomycetes*, *Eurotiales*, *Monascaceae*, and *Monascus*, in which there are more than thirty kinds now recognized in the world (Hawksworth and Pitt 1983; Zhong and Fang 2003). *Monascus* spp. are stained easily, and need rigorous culture conditions in fermentation. Many recent studies show that this strain can produce several fungal metabolic derivatives, such as ethanol, monascus pigments, γ -aminobutyric (GABA), monacolins (including monacolin K, dehydromonacolin K, methyl ester of monacolin K hydroxyl-acid form, hydroxyl-acid form of monacolin K, monacolin L, methyl ester of monacolin L hydroxyl acid form, etc.) (Ma *et al.* 2000). Among them, Monacolin K (also known as lovastatin, mevinolin or mevacor) is a primary secondary metabolite of *Monascus* spp. with the molecular formula $C_{24}H_{36}O_5$ and a molecular weight of 404.55 amu (Endo 1979). It functions as an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, which is not only the regulatory enzyme of cholesterol biosynthesis, but also provides the ability to lower blood-lipid levels in animal models and in humans (Juzlova *et al.* 1996; Li *et al.* 1998; Su *et al.* 2003).

Traditionally, *Monascus* spp. have been cultivated using solid-state cultures such as rice grains or bread. However, solid-state cultures take at least 14 days (Su *et al.* 2003) and have scale-up and contamination problems, such as species variation, air contamination, microbe infection and oxygen transference (Wu *et al.* 2000). Consequently, many studies have attempted to enhance the production of *Monascus* spp. and their fungal metabolites by liquid fermentation. Such studies dealt with the mutation of strains (Lai *et al.* 2003; Wang *et al.* 2004), and changes in culture's physical conditions (Endo 1980; Negishi *et al.* 1986; Tseng *et al.* 2000; Chang *et al.* 2002).

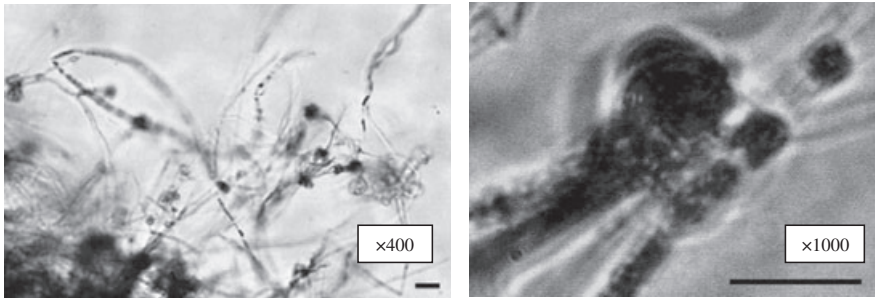


FIG. 1. MICROSCOPY SLIDES OF *MONASCUS* SPP. SCREENED FROM KITCHEN WASTE FROM PINGTUNG COUNTY, TAIWAN, AFTER 1 WEEK GROWTH IN SUBMERGED CULTURE. (SCALE BAR = 16 μ m)

In order to obtain a high yield of products, optimization of the existing process is necessary. The Taguchi method is a systematic application of analysis of experiments for the purpose of designing and improving product quality. It is used especially for evaluating several process factors at a time with the smallest number of experimental runs based on a Table, known as the orthogonal array. The conclusions drawn from small scale experiments are valid over the entire experimental region spanned by the control factors and their settings (Taguchi 1990). In recent years, the Taguchi method has become a powerful tool employed in industry for improving productivity during research and development, so that high quality products can be produced quickly and at very low cost. There are as yet few applications in food science and engineering, such as wine fermentation (Honda *et al.* 1998), and citric acid production from apple pomace in multi-layer packed bed solid-state bioreactor (Shojaosadati and Babaeipour 2002). The objective of the present study was to apply the Taguchi method to determine the optimum conditions for producing a high yield of monacolin K from *Monascus* spp. in submerged culture, using a rotary aerobic liquid culture in an incubator.

MATERIALS AND METHODS

Microorganism Culture and Medium Composition

The fungus was isolated from kitchen waste in a local area in Pingtung County, southern Taiwan. Cultures were maintained at 37C for 48 h in potato dextrose agar (PDA, Difco, Sparks, NV). Figure 1 shows optical Microscopy slides (Model PBM-834, Microtech M&T Optics Co., Hsinchu, Taiwan) of the fungus at 400X and 1,000X, which was isolated after 1 week growth in

submerged culture. The medium was prepared with 1% carbon, 1% nitrogen, 0.01% oil, and 0.01% salt sources (Chul *et al.* 1998). For carbon sources, there was waxy rice (TN 1, Taiwan), Japonica-type rice (TC 189, Taiwan), Indica-type rice (KS1, Taiwan) and whole wheat flour (Hard red wheat, TC 2, Taiwan); for nitrogen sources, there was gelatin (Logicmatic International Co., Ltd., Tainan, Taiwan), monosodium glutamate (MSG, Sigma-Aldrich Co., St. Louis, MO), peptone (Himedia Laboratories, PVT. Ltd., Mumbai, India) and ammonium nitrate (NH_4NO_3 , Nihon Shiyaku Industries, Ltd., Osaka, Japan); for oil sources, there was olive oil (Taiwan Sugar Co., Tainan, Taiwan), soybean oil (Taiwan Sugar Co.), glycerol (Yue Ba Enterprise Co., Ltd., Taipei, Taiwan) and none; for inorganic salt sources, there was magnesium sulphate (MgSO_4 , Nihon Shiyaku Industries, Ltd.), calcium carbonate (CaCO_3 , Nihon Shiyaku Industries, Ltd.), sodium chloride (NaCl , Nihon Shiyaku Industries, Ltd.) and potassium phosphate (KH_2PO_4 , Nihon Shiyaku Industries, Ltd.) and various pH values (from 3 to 9).

Seed cultures were prepared by transferring a loop of spores from a PDA agar slant into a 250 mL Hinton flask containing 20 mL of basal medium (Su *et al.* 2003). The fungus cultures were inoculated in 100 mL of the resultant medium in a 500 mL Hinton flask and aerobically cultured on a rotary shaking incubator (180 rpm, Hipoint 721SR, Jiun-Hsing Instrument Co. Ltd., Kaohsiung, Taiwan) at 37C for 11 days. The initial average weight in each unfermented flask was recorded. Culture broths were filtered through dry filter paper (No. 4, Whatman Biometra, Imprint, Maidstone, UK) and washed twice with distilled water to remove culture debris (Kim *et al.* 2002). The fermented materials were freeze-dried by the vacuum freezing dryer (Model 4.5, Labconco Co., Kansas City, MO) at -80C , 50×10^{-3} mBar for 12 h. The dried microorganism was weighed to determine the value of biomass (g/L).

Measurement of monacolin K

By following the procedures of Chang *et al.* (2002), after the 11th day of culture, culture cell broths were first filtered. Then they were vacuum freeze dried at -80C and 50×10^{-3} mBar for 12 h. Dried cells were broken by a cell disrupter (S-300, Misonix, Inc., Farmingdale, NY). Mycelia were then extracted twice from dried broken cells with 100% methanol at 30C for 12 h and separated from debris by a centrifugal machine at 4C for 20 min ($10,000 \times g$, Hitachi CR21E, Tokyo, Japan). The extracted mycelia were filtered again through a $0.45 \mu\text{m}$ membrane, and then analyzed by high performance liquid chromatography (HPLC, Hitachi L-7100, Tokyo, Japan) to quantify the intracellular monacolin K (Chang *et al.* 2002). The HPLC system used for analysis of monacolin K concentration was composed of two pumps, a solvent delivery controller, a UV-VIS detector (Hitachi L-7420, Tokyo,

TABLE 1.
CONTROL FACTORS AND LEVELS IN THE FERMENTATION PROCESS

Factors	A	B	C	D	E
	Carbon source	Nitrogen source	Oil source	Salt source	pH
Level 1	Waxy rice	Gelatin	Olive oil	MgSO ₄	3
Level 2	Japonica-type rice	Monosodium glutamate (MSG)	Soybean oil	CaCO ₃	5
Level 3	Indica-type rice	Peptone	Glycerol	NaCl	7
Level 4	Whole wheat flour	NH ₄ NO ₃	None	KH ₂ PO ₄	9

Carbon sources: waxy rice (TN 1, Taiwan), Japonica-type rice (TC 189, Taiwan), Indica-type rice (KS 1, Taiwan) and whole wheat flour (Hard red wheat, TC 2, Taiwan).

Nitrogen sources: gelatin (Logimatic International Co., Ltd., Tainan, Taiwan), monosodium glutamate (MSG, Sigma-Aldrich Co., St. Louis, MO, USA), peptone (Himedia Laboratories, PVT. Ltd., Mumbai, India), and ammonium nitrate (NH₄NO₃, Nihon Shiyaku Industries, Ltd., Osaka, Japan).

Oil sources: olive oil (Taiwan Sugar Co., Tainan, Taiwan), soybean oil (Taiwan Sugar Co., Tainan, Taiwan), glycerol (Yue Ba Enterprise Co., Ltd., Taipei, Taiwan) and none.

Salt sources: magnesium (MgSO₄, Nihon Shiyaku Industries, Ltd., Osaka, Japan), calcium carbonate (CaCO₃, Nihon Shiyaku Industries, Ltd., Osaka, Japan), sodium chloride (NaCl, Nihon Shiyaku Industries, Ltd., Osaka, Japan) and potassium phosphate (KH₂PO₄, Nihon Shiyaku Industries, Ltd., Osaka, Japan).

Japan), a computer and a hyperbond C18 column (LiChroCART 250-4, 250 × 4.6 mm, 5 μm, Merck Biosciences Ltd., Darmstadt, Germany). The injection sample volume was 20 μL. The sample was eluted with a mobile phase comprising 65% acetonitrile and 35% methanol at a flow rate of 1.0 mL/min, and the chromatogram was monitored at 238 nm. The monacolin K standard (Sigma-Aldrich Co.) was used to construct a calibration curve to quantify monacolin K concentration in the sample of extracted mycelia.

Design of Experiment

The control factors were the different kinds and levels of carbon, nitrogen, oil, and salt sources and pH values in the fermentation process (Table 1). The selected L₁₆(4⁵) orthogonal arrays in the experiments are shown in Table 2. It represents 16 experimental runs that can be included in the experiment, in which 5 columns (factors) and 4 levels are available within the orthogonal array. The signal-to-noise ratio (S/N ratio, η) was calculated from experimental data, which created a transformation function of the repetition data to another value and was used as a measure of the variation present in the experiment. The S/N equation depends on the criterion for the quality characteristic to be optimized. The objective in this study was to find the optimum cultural conditions for biomass in the growth phase and monacolin K metabolites in the metabolic phase, so the higher-the-better loss function was used to calculate S/N ratio as:

TABLE 2.
L₁₆(4⁵) ORTHOGONAL ARRAY OF THE EXPERIMENTAL DESIGN

L ₁₆ 4 ⁵	A	B	C	D	E
Trial no.	Carbon source	Nitrogen source	Oil source	Salt source	pH
1	1	1	1	1	1
2	1	2	2	2	2
3	1	3	3	3	3
4	1	4	4	4	4
5	2	1	2	3	4
6	2	2	1	4	3
7	2	3	4	1	2
8	2	4	3	2	1
9	3	1	3	4	2
10	3	2	4	3	1
11	3	3	1	2	4
12	3	4	2	1	3
13	4	1	4	2	3
14	4	2	3	1	4
15	4	3	2	4	1
16	4	4	1	3	2

Carbon sources: A₁ = waxy rice, A₂ = Japonica-type rice, A₃ = Indica-type rice, A₄ = whole wheat flour.
 Nitrogen sources: B₁ = gelatin, B₂ = MSG, B₃ = peptone, B₄ = NH₄NO₃.
 Oil sources: C₁ = olive oil, C₂ = soybean oil, C₃ = glycerol, C₄ = none; Salt sources: D₁ = MgSO₄,
 D₂ = CaCO₃, D₃ = NaCl, D₄ = KH₂PO₄; pH: E₁ = 3, E₂ = 5, E₃ = 7, E₄ = 9.

$$\eta = -10 \log \left(\frac{1}{n} \sum_{i=0}^n 1/y_i^2 \right) \tag{1}$$

where y_i is the i th quality parameter and n is the number of trials (Roy 2001).

The factors and process parameters that significantly affect the performance characteristic and confidence were investigated by analysis of variance (ANOVA) in SAS 8.2 (Statistical Analysis System, Cary, NC). The Duncan multiple range test was used to compare the difference between means at the probability level 0.05. Individual t -tests were used to determine the significance at different levels.

RESULTS

Fungus Appraisal

Based on methods of Hawksworth and Pitt (1983) and Cannon *et al.* (1995) for the classification of cultural and species keys, the fungus under

TABLE 3.
THE RESULTS OF THE $L_{16}(4^5)$ ORTHOGONAL ARRAY FOR THE
PRODUCTION OF BIOMASS

No.	W ₁ (g/100 mL)	W ₂ (g/100 mL)	Average	SD	S/N ratio (db)
1	1.498	1.531	1.515	0.023	5.365
2	1.538	1.546	1.542	0.006	5.523
3	1.483	1.488	1.486	0.004	5.198
4	1.394	1.422	1.408	0.020	4.732
5	1.512	1.517	1.515	0.004	5.366
6	1.653	1.776	1.715	0.087	6.427
7	1.463	1.596	1.530	0.094	5.427
8	1.532	1.537	1.535	0.004	5.480
9	1.561	1.604	1.583	0.030	5.745
10	1.353	1.539	1.446	0.132	4.910
11	1.842	1.885	1.864	0.030	7.166
12	1.667	1.681	1.674	0.010	6.236
13	1.366	1.381	1.374	0.011	4.517
14	1.488	1.539	1.514	0.036	5.357
15	1.822	1.889	1.856	0.047	7.126
16	1.925	2.013	1.969	0.062	7.639
		Average	1.595	0.037	5.763

study was initially determined and classified as *Monascus purpureus* (Fig. 1). The fungus colonies were about 20–25 mm diameter on potato dextrose agar (PDA) after 7 days of incubation at 25C. The secondary metabolites contained both red water-soluble pigments and monacolin K. This fungus is a purple pigment-producing fungus that belongs to a group of endthallial conidia. It is oval-shaped and greater than 5 μm in diameter (Hawksworth and Pitt 1983).

Optimal Cultural Conditions for the Production of Biomass

The process of fermentation includes two phases, growth and metabolic phases. The greater the amount of biomass produced in the growth phase, the more metabolite is generated in the metabolic phase.

The signal-to-noise ratio (S/N ratio) was calculated from the average experimental data (concentration, ppm) by a loss function for the higher-the-better performance (Eq. 1), as shown in Table 3. Table 4 shows results of ANOVA analysis, indicating that all of the selected factors are significant parameters with 95% confidence ($P < 0.05$) in submerged culture of *Monascus* spp. Figure 2 shows that the optimal conditions in the growth phase (circled) are A₄ (1% whole wheat flour), B₃ (1% peptone), C₁ (0.01% olive oil), D₄ (0.01% potassium phosphate) and E₂ (pH = 5). According to Table 4 and

TABLE 4.
ANOVA SUMMARY TABLE FOR THE PRODUCTION OF BIOMASS

Factor	SS	DOF	Var	F	Confidence	Significant
A	0.1685	3	0.0562	20.3341	100.0%	*
B	0.1752	3	0.0584	21.1493	100.0%	*
C	0.4824	3	0.1608	58.2161	100.0%	*
D	0.0301	3	0.0100	3.6376	96.4%	**
E	0.0420	3	0.0140	5.0664	98.8%	**
Error	0.0442	16	0.0028	0.0526		
Total	0.9424	31	0.0304			

* Significant at least at the 0.1% level ($P < 0.001$).

** Significant at least at the 5% level ($P < 0.05$).

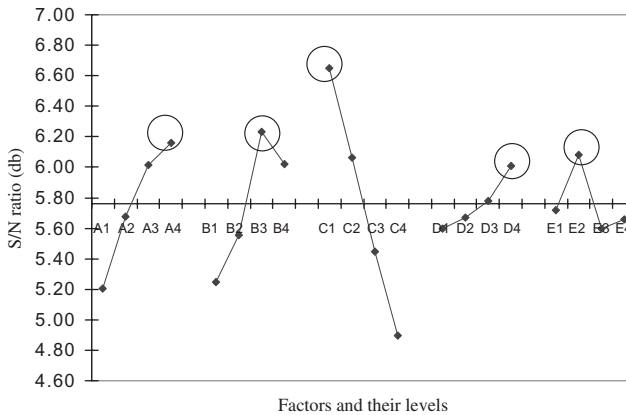


FIG. 2. THE RESPONSE GRAPH OF THE EXPERIMENTS FOR THE PRODUCTION OF BIOMASS

Carbon sources: A₁ = waxy rice, A₂ = Japonica-type rice, A₃ = Indica-type rice, A₄ = whole wheat flour; Nitrogen sources: B₁ = gelatin, B₂ = MSG, B₃ = peptone, B₄ = NH₄NO₃; Oil sources: C₁ = olive oil, C₂ = soybean oil, C₃ = glycerol, C₄ = none; Salt sources: D₁ = MgSO₄, D₂ = CaCO₃, D₃ = NaCl, D₄ = KH₂PO₄ pH: E₁ = 3, E₂ = 5, E₃ = 7, E₄ = 9.

Fig. 2, in the growth phase, carbon, nitrogen, and oil sources were the most significant factors of all five factors. A confirmation run was conducted based on these optimum conditions and the response was 8.0216 db, in which the corresponding production of biomass was 2.035 g/L.

Optimal Culture Conditions for the Production of Monacolin K

In order to calculate the concentration of monacolin K, the peak at 18.3 min retention time, a standard sample was used to identify the test sample

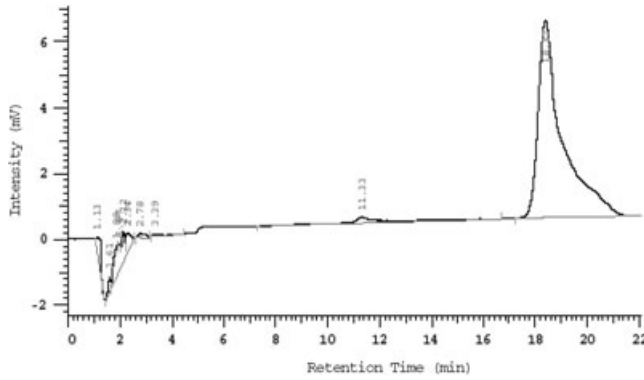
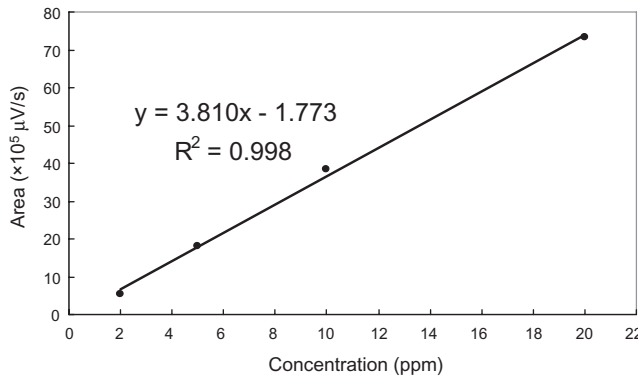


FIG. 3. HPLC TRACES OF MONACOLIN K AT 238 nm

FIG. 4. THE CORRELATION COEFFICIENT R^2 BETWEEN CONCENTRATIONS AND PEAK AREAS

detected by the HPLC system (Fig. 3). Regression of monacolin K concentration as a function of peak area was set up by polynomial regression analysis in SAS 8.2 (Statistical Analysis System, Cary, NC). The value of the coefficient of determination R^2 was 0.998 (Fig. 4). The first-order equation could be used to directly derive the corresponding concentration value for the peak area of each sample. Table 5 shows the signal-to-noise ratio (S/N ratio) from the average experimental data (concentration, ppm) as a loss function for the higher-the-better performance (Eq. 1). The summary results of ANOVA are shown in Table 6, indicating that all factors are significant parameters with 99.9% confidence. The optimum conditions, which can be obtained from Fig. 5 (circled), are A_4 (1% whole wheat flour), B_3 (1% peptone), C_2 (0.01%

TABLE 5.
THE RESULTS OF THE $L_{16}(4^5)$ ORTHOGONAL ARRAY FOR THE PRODUCTION
OF MONACOLIN K

No.	monacolin K (ppm)	monacolin K (ppm)	Average	SD	S/N ratio(db)
1	22.772	22.819	22.796	0.033	28.918
2	20.092	20.103	20.097	0.008	27.824
3	21.517	21.523	21.520	0.005	28.418
4	20.120	20.160	20.140	0.028	27.842
5	27.248	27.255	27.251	0.005	30.469
6	23.913	24.086	24.000	0.123	29.365
7	79.438	79.627	79.532	0.133	39.772
8	23.820	23.827	23.823	0.005	29.301
9	62.048	62.109	62.078	0.043	37.620
10	35.946	36.210	36.078	0.186	32.906
11	26.025	26.086	26.055	0.043	30.079
12	22.390	22.409	22.400	0.014	28.766
13	20.089	20.110	20.100	0.015	27.825
14	20.818	20.889	20.853	0.051	28.144
15	151.013	151.108	151.060	0.067	45.344
16	25.031	25.156	25.093	0.088	29.752
		Average	37.680	0.053	31.397

TABLE 6.
ANOVA SUMMARY TABLE FOR THE PRODUCTION OF MONACOLIN K

Factor	SS	DOF	Var	F	Confidence	Significant
A	4,408.6405	3	1,469.5468	265,731.4766	100.0%	*
B	11,283.3002	3	3,761.1001	680,102.6373	100.0%	*
C	4,113.9318	3	1,371.3106	247,967.8641	100.0%	*
D	8,360.7938	3	2,786.9313	503,948.1204	100.0%	*
E	7,655.7997	3	2,551.9332	461,454.4956	100.0%	*
Error	0.0885	16	0.0055	0.0744		
Total	35,822.5545	31	1,155.5663			

* Significant at least at the 0.1% level ($P < 0.001$).

DOF, degrees of freedom; SS, sums of squares.

soybean oil), D_4 (0.01% potassium phosphate) and E_1 (pH = 3). According to Table 6 and Fig. 5, in the metabolic phase, nitrogen, oil and salt sources were the most significant factors of all five factors. The result of the confirmation run was 45.38 db, and the most production of monacolin K under optimum culture conditions was 151.06 ppm, which is shown on the eighth day in Fig. 6. It is 15% higher than in the past study (131 ppm) (Chang *et al.* 2002).

It has been reported (Chang *et al.* 2002) that *Monascus* spp. fermentation with acid buffer solution at pH 5–5.5 could produce maximal yield of biomass

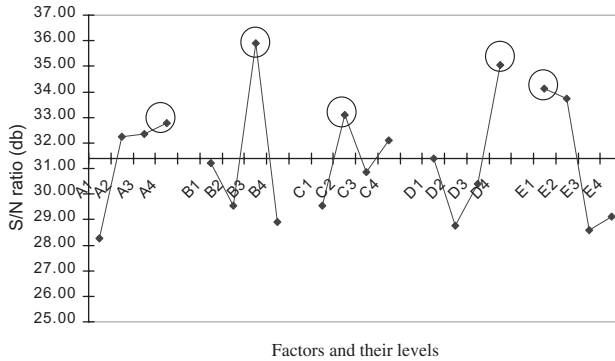


FIG. 5. THE RESPONSE GRAPH OF THE EXPERIMENTS FOR THE PRODUCTION OF MONACOLIN K

Carbon sources: A₁ = waxy rice, A₂ = Japonica-type rice, A₃ = Indica-type rice, A₄ = whole wheat flour; Nitrogen sources: B₁ = gelatin, B₂ = MSG, B₃ = peptone, B₄ = NH₄NO₃; Oil sources: C₁ = olive oil, C₂ = soybean oil, C₃ = glycerol, C₄ = none; Salt sources: D₁ = MgSO₄, D₂ = CaCO₃, D₃ = NaCl, D₄ = KH₂PO₄ pH: E₁ = 3, E₂ = 5, E₃ = 7, E₄ = 9.

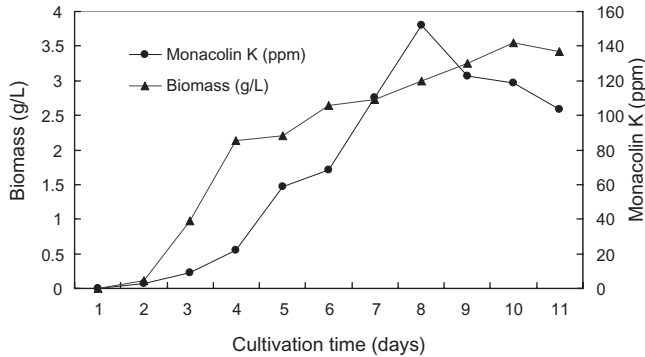


FIG. 6. PRODUCTION OF FUNGUS BIOMASS (TRIANGLES) AND MONACOLIN K (CIRCLES) UNDER THE OPTIMAL CONDITIONS: 1% WHOLE WHEAT FLOUR, 1% PEPTONE, 0.01% SOYBEAN OIL, 0.01% KH₂PO₄ AND pH = 3

utilized to produce monacolin K metabolite. The same result was confirmed by the optimal conditions in this work in which a pH of 5 was selected and used in the first growth phase of submerged culture to produce maximal yield of biomass. Also, a pH of 3 was selected to produce maximal yield of metabolite in the second metabolic phase, monacolin K. In other words, a weak acid condition (pH 5) can be set in the first 5 of the 11 days, the growth phase, mainly to produce a maximal yield of biomass (Fig. 6). Then, it shifts to a

strong acid condition (pH 3) starting on the sixth day in order to produce a maximal yield of monacolin K.

Olive and soybean oils were the optimum parameters contributing to production of biomass and monacolin K, respectively. In fact, foam is generated during the growth phase in which too much foam would result in the broth overflowing the fermentor. For a practical industry application it is usual to add antifoaming agent, Tween-60 (Top Rhyme International Co., Ltd., Taipei, Taiwan), a kind of chemical synthetic oil which may result in a food safety problem. On the contrary, edible olive oil and soybean oil can replace chemical oil Tween-60 as antifoamers to minimize the surface tension in the broth and avoid overflowing the fermentor.

Besides, it was found that a small amount of endogenous metabolite monacolin K existed in liquid medium during the submerged culture. It was inferred that shaking could break hyphae apart and release some monacolin K metabolite from fungi which had also been reported in previous study (Kim *et al.* 2002). Increased attention shall be given in future scale-up of the fermentation process to the strong possibility that stirring speed in the reactor may result in the breaking of fungi and affect the yield of monacolin K.

CONCLUSION

The Taguchi method of determining optimal culture conditions for a high yield of monacolin k was validated by running a five-factor and three-level experiment design. Herein, a weak acid (pH 5) condition in the growth phase and a strong acid (pH 3) condition in the metabolic phase led to the maximal yield of monacolin K in *Monascus* spp. submerged culture. It was observed that carbon, nitrogen, and oil sources in the growth phase; and nitrogen, oil, and salt sources in the metabolic phase will also have the largest contribution to the future scale-up *Monascus* spp. fermentation.

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