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Optimization of culture media for enhancing gamma-linolenic acid production by *Mucor hiemalis*

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Abstract

Introduction: γ-linolenic acid is an essential fatty acid in human nutrition. In the present study, production of γ-linolenic acid by *Mucor hiemalis* PTCC 5292 was evaluated in submerged fermentation.

Materials and methods: The fermentation variables were chosen according to the fractional factorial design and further optimized via full factorial method. Four significant variables, glucose, peptone, ammonium nitrate and pH were selected for the optimization studies. The design consisted of total 16 runs consisting of runs at two levels for each factor with three replications of the center points.

Results: The analysis of variance and three-dimensional response surface plot of effects indicated that variables were regarded to be significant for production of γ-linolenic acid by *Mucor hiemalis*. Results indicated that fermentation at the optimum conditions (100 g/l glucose concentration; 1 g/l peptone; 1 g/l ammonium nitrate, and pH of 4.5) enhanced the γ-linolenic acid production up to 709 mg/l.

Discussion and conclusion: The results of this study indicated that higher γ-linolenic acid yield can be achieved in a simple medium at high glucose and ammonium nitrate, low peptone concentrations and acidic pH by *Mucor hiemalis* PTCC 5292. This simple and low cost optimization condition of culture media can be applied for γ-linolenic acid production at higher scale for pharmaceutical and nutritional industries.

Key words: Factorial Design, Fermentation, γ-linolenic acid, *Mucor hiemalis*, Optimization

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Introduction

Recently, microbial production of lipids containing polyunsaturated fatty acids (PUFA) such as omega-3 and omega-6 compounds have attracted much attention. γ-Linolenic acid (GLA) (6, 9, 12, cis, cis, cis-octadecatrienoic acid), an omega-6 PUFA, is an essential fatty acid in human nutrition, which has been suggested for pharmacological and dietetic purposes. The numerous advantages mentioned for GLA can be summarized as suppressing human T-cell proliferation and activation, decreasing cardiovascular risk factors, impressing against rheumatoid arthritis, normalizing nerve conduction velocity and sciatic endoneurial blood flow (1- 9).

γ-Linolenic acid conventionally produces from plant sources such as seeds of evening primrose, borage and blackcurrant. However, to meet the increasing demand, extensive research is being carried out for its production from microbial sources as an alternative to the plant based production (10- 11).

The cells of some algae and fungi species have been identified to contain GLA. For example, producing substantial quantities of GLA by fungi the order Mucorales especially *Mucor*, *Mortierella*, and *Cunninghamella* genus has been reported. The first trials of PUFA production by *Mucor* fungi with the target being γ-Linolenic acid were carried out in the UK and Japan. Thereafter, microbial production of various PUFAs has been investigated with the aim of understanding effective production (12- 14).

Various studies have been conducted to optimize the production of fatty acids by fungi. In this way, the traditional one-at-a-time optimization strategy is relatively simple, and the individual effects of medium factors can be graphically depicted without statistical analysis. It has a low efficiency in experimental runs and does not consider the interactions. Moreover, it frequently fails to determine the region of optimum response. Consequently, factorial based designs have been suggested as efficient approaches to optimization. In fact, factorial is a useful experimental design to investigate the effect of several variables influencing the responses by varying them simultaneously and carrying out a limited number of experiments (15).

The main objective of this study was to screen the significant variables by the fractional factorial design, and further to optimize the screened variables exactly for GLA production by employing *Mucor hiemalis* PTCC 5292 in submerged fermentation using full factorial design.

Materials and methods

Microorganism, inoculum preparation and culture conditions: *Mucor hiemalis* PTCC 5292 was purchased from Iranian Research Organization for Science and Technology (IROST). *Mucor hiemalis* was kept on potato dextrose agar (PDA) slants by incubating at 28˚C for four days. Then, 2 ml of sterile distilled water was added to each sporulated slant and the spores were harvested by scrapping them from the PDA surface. 1ml of spore suspension (around1×10^7 spores) was used to inoculate 250 ml Erlenmeyer flasks containing 50 ml of basal fermentation medium according to the experimental design method (Table 1) and incubated in a rotary shaker-incubator at 180 rpm and 28˚C for 3 days (16).

The basal fermentation medium included the following salt components (g/l): NH_4NO_3 0.286, KH_2PO_4 0.75, $MgSO_4{\cdot}7H_2O$ 0.4 and $CaCl_2$ 0.4.

Cell dry mass determination and lipid extraction: The mycelia were harvested from medium through Whatman No. 1 filter paper and thoroughly washed with distilled water, then dried at 95 ± 2˚C for 24 hours and the dried biomass was weighed (17).

The lipid production from mycelia was assayed by extraction according to the modified procedure of Bligh and Dyer (18) using chloroform/methanol (1:1). Chloroform phase was recovered. The same process was repeated thrice. The whole solvent was evaporated and dried.

Methyl ester preparation and analysis of GLA: Free fatty acids and o-acyl lipids were esterified by heating with a large excess of anhydrous methanol in the presence of an acidic catalyst. Fatty acids were methyl esterified according to the procedure of Christie (19). The sample of lipid (50 mg) was dissolved with 1ml toluene in a test tube fitted with a condenser. Then 2ml of 1% sulfuric acid in methanol was added to it and maintained at 50°C for 24 h. The methylated fatty acids were separated from the aqueous layer by adding 5ml of 5% NaCl and then dissolved in 2×5ml n-hexane. The separated n-hexane layer using Pasteur pipettes washed by 4 ml of water containing 2% potassium bicarbonate and dried over anhydrous sodium sulfate. The solution is filtered and the solvent removed under reduced pressure in a rotary film evaporator or in a stream of nitrogen.

Gas chromatographic conditions: GC[1] was performed on Agilent 19091J-413 Series Gas Chromatograph equipped with a FID[2] and the capillary column HP5 (30 m ´ 0.25 mm i. d., 0.25 µm film thickness; USA). Injector and detector temperatures were maintained at 260°C and 300°C, respectively. The oven was programmed for 2 min at 100°C, then was increased to 160°C at 3 min, was maintained for 2 min at 215°C, was increased further to 217°C at 2 min, then was maintained for 2 min at 218°C and finally was increased to 260°C at 2 min. The carrier gas, nitrogen, was used at a flow rate of 1.5 mL/ min. The injection volume was 1 µL, with a split ratio of 50:1.

Experimental design and data analysis: In order to select the significant variables for GLA production, the independent variables such as carbon sources (glucose), mineral nitrogen sources (ammonium nitrate), organic nitrogen sources (peptone) and pH were considered and screened via 1/2 fractional factorial design. A total of four variables were included for screening and each independent variable was tested at two levels, high level (+1) and low level (-1) (Table 1). For a 1/2 fractional factorial with the 4 variables, 11 experimental runs with three replications of the center points are required (Table 2). The variables and their experimental matrix are listed in Tables 2 and 3 respectively. The main effect of each variable on GLA production was estimated as the difference between the two averages of measurements made at the higher and lower levels. The analysis of variance (ANOVA[3]) was used to determine the significance of each variable (Table 4). The p-value, as the most common means of assessing significance, was also determined for each variable. The *p-value* is the probability that the magnitude of a parameter estimate is due to random process variability. The present study was accepted only confidence levels above 95%. Therefore, it is possible to gradate the variables with respect to their effects on the GLA concentration.

Table 1- Experimental variables at different levels used for the fractional and full factorial designs.

		Experimental values		
Variable	Unit	Low level (-1)	Center point	High level (+1)
Glucose	g/l	20.00	60.00	100.00
Peptone	g/l	1.00	5.50	10.00
Ammonium nitrate	g/l	0.20	0.60	1.00
pH	-	4.50	6.00	7.50

Table 2- Lipid production, experimental design and results of the 1/ 2 fractional factorial method for GLA concentration by *Mucor hiemalis* 5292.

Run no.	Glucose	Peptone	Ammonium nitrate	pH	X (g/l)	$Y_{X/S}$ (g/g)	L_{max} (g/l)	$Y_{L/S}$ (g/g)	$Y_{L/X}$ (%)	GLA (mg/l)	C/N[a] (mol/mol)
1	1	1	-1	-1	3.60	0.04	0.60	0.01	16.66	42.10	28
2	1	-1	1	-1	5.40	0.05	1.20	0.01	22.22	709.0	127
3	0	0	0	0	3.60	0.06	0.40	0.01	11.11	112.0	28
4	-1	-1	1	1	3.60	0.20	0.60	0.03	16.66	69.3	27
5	1	1	1	1	3.80	0.04	0.64	0.01	16.84	55.60	26
6	-1	-1	-1	-1	3.80	0.19	0.70	0.04	18.42	80.2	47
7	1	-1	-1	1	5.80	0.06	1.20	0.01	20.69	339.9	214
8	0	0	0	0	3.60	0.06	0.42	0.01	11.66	108.3	28
9	-1	1	1	-1	7.60	0.38	0.20	0.01	2.63	44.6	7
10	0	0	0	0	3.60	0.06	0.42	0.01	11.66	121.9	28
11	-1	1	-1	1	4.40	0.22	0.60	0.03	13.64	32.0	8

Strain PTCC 5292 was cultivated at 28°C for 72h. Representation of biomass (X, g/L), biomass yield on the consumed glucose ($Y_{X/S}$, g/g), cellular lipid in biomass ($Y_{L/X}$, %) and lipid yield on the consumed glucose ($Y_{L/S}$, g/g) when the maximum quantity of cellular lipids (L_{max}, g/L) was achieved. Data are presented as mean values from duplicate experiments conducted by using different inocula. a For the determination of the initial C/N ratio in the medium, it was assumed that peptone contained 45.95% wt/wt, of carbon and 15.5% wt/wt, of nitrogen.

Table 3- Lipid production, experimental design and results of the full factorial method for GLA concentration by *Mucor hiemalis* 5292.

Run no.	Glucose	Peptone	Ammonium nitrate	pH	X (g/l)	$Y_{X/S}$ (g/g)	L_{max} (g/l)	$Y_{L/S}$ (g/g)	$Y_{L/X}$ (%)	GLA (mg/l)	C/N[a] (mol/mol)
12	1	1	1	-1	10.40	0.10	0.40	0.00	3.85	28.0	26
13	-1	-1	1	-1	5.00	0.25	0.40	0.02	8.00	138.2	27
14	1	-1	1	1	3.80	0.04	0.20	0.00	5.26	68.2	127
15	-1	1	1	1	7.80	0.39	0.16	0.01	2.05	37.6	7
16	-1	-1	-1	1	3.00	0.15	0.24	0.01	6.66	49.30	47
17	-1	1	-1	-1	8.40	0.42	0.06	0.00	0.71	0.00	8
18	1	1	-1	1	8.80	0.09	0.20	0.00	2.27	58.50	28
19	1	-1	-1	-1	5.40	0.05	1.40	0.01	25.90	257.4	214

Strain PTCC 5292 was cultivated at 28°C for 72h. Representation of biomass (X, g/L), biomass yield on the consumed glucose ($Y_{X/S}$, g/g), cellular lipid in biomass ($Y_{L/X}$, %) and lipid yield on the consumed glucose ($Y_{L/S}$, g/g) when the maximum quantity of cellular lipids (L_{max}, g/L) was achieved. Data are presented as mean values from duplicate experiments conducted by using different inocula. [a] For the determination of the initial C/N ratio in the medium, it was assumed that peptone contained 45.95% wt/wt, of carbon and 15.5% wt/wt, of nitrogen.

Table 4- ANOVA showing the effect of factors and their interactions for GLA concentration in the 1/2 fractional factorial design.

Variable	DF	Seq SS	Adj SS	Adj MS	F	P
R^2= 99.88 %						
Glucose concentration (g/l): A	1	105915	105915	105915	2142.15	0.000
Peptone concentration (g/l): B	1	131098	131098	131098	2651.47	0.000
Ammonium nitrate concentration (g/l): C	1	18461	18461	18461	373.37	0.003
pH : D	1	17965	17965	17965	363.34	0.003
A×B	1	96426	96426	96426	1950.24	0.001
A×C	1	18136	18136	18136	366.80	0.003
A×D	1	13786	13786	13786	278.83	0.004
Pure Error	2	99	99	49		
Total	10	409104				

DF refers to degrees of freedom, SS refers to sum of squares, MS refers to mean square, F and p refers to F and p values respectively.

The next step was to determine the optimum levels of the significant independent variables for GLA production. Therefore, the full factorial design was used for the completion of previous experiments (1/2 fractional factorial design) and was effective to improvement the time and cost of experiments. The levels of variables were similar to 1/2 fractional factorial design, as is shown in Table 1. The experimental design and the results of them are listed in Table 3.

All of the experiments were repeated. The sum of experiments was carried out in the 35 runs. The statistical software Minitab 16 was used to design and analysis of all experiments and statistical software Design-Expert 8.0.7.1 was applied to depict a three-dimensional response surface plot for investigating the effect of variables on the GLA production.

Results

Screening of the significant variables using the fractional factorial design: The results of the 1/2 fractional factorial design for GLA concentration in the screening of the significant variables step has been presented in Table 2.

The variables showing the p-values less than 0.05 were determined to have a significant effect on the response. Therefore, the variables of glucose, peptone, ammonium nitrate and pH were selected for further optimization through a full factorial design (Table 3). Table 4 indicates the analysis of variance of the variables on the response in the screening step.

Optimization of the significant variables using full factorial design: The design and the matching outcomes of full factorial experiments to evaluate the effects of four independent variables are presented in Table 3.

Variables and responses were evaluated for the GLA production consequently by Minitab version 16 to create a model based on design, which comprised main effects and interaction effects. The model planed model based on the analysis related GLA production with variables of the experiments. The subsequent equation was acquired from the coded data:

Where, Y is GLA concentration; A, glucose concentration; B, peptone concentration; C, ammonium nitrate concentration and D, pH. The analysis of variance of this fitting is showed in Table 5.

(1)

$$Y=123.35 + 67.62A - 87.03B + 18.58C - 36.32D - 58.91AB + 2.20AC - 27.31AD - 14.48BC + 44.63BD - 48.20CD - 10.49ABC + 29.99ABD - 38.99ACD + 45.02BCD + 45.40ABCD$$

Table 5- ANOVA showing the effect of factors and their interactions for GLA concentration in the full factorial design.

Variable	DF	Seq SS	Adj SS	Adj MS	F	P
R^2= 99.88%						
Glucose concentration (g/l): A	1	146300	146300	146300	4333.80	0.000
Peptone concentration (g/l): B	1	242365	242365	242365	7179.50	0.000
Ammonium nitrate concentration (g/l): C	1	11045	11045	11045	327.17	0.000
pH : D	1	42202	42202	42202	1250.15	0.000
A×B	1	111050	111050	111050	3289.61	0.000
A×C	1	154	154	154	4.57	0.046
A×D	1	23866	23866	23866	706.97	0.000
B×C	1	6714	6714	6714	198.87	0.000
B×D	1	63751	63751	63751	1888.49	0.000
C×D	1	74334	74334	74334	2201.98	0.000
A×B×C	1	3522	3522	3522	104.32	0.000
A×B×D	1	28782	28782	28782	852.60	0.000
A×C×D	1	48649	48649	48649	1441.10	0.000
B×C×D	1	64845	64845	64845	1920.89	0.000
A×B×C×D	1	65948	65948	65948	1953.56	0.000
Pure Error	18	608	608	34		
Total	34	934370				

DF refers to degrees of freedom, SS refers to sum of squares, MS refers to mean square, F and p refers to F and p values respectively.

The calculated determination coefficient (R^2) for the obtained model was equal to 0.9993 (the value>0.75 shows a good fitness of the model). This is an assessment of the fraction of overall variation in the data evaluated by the model and thus the model is capable of illustrate 99.93% of the variation in response. The adjusted-R^2 was found to be 0.9988 demonstrating that the model has a good prediction in terms of the significant variables. The three-dimensional response surface plot based on the suggested model was made to investigate the effect of variables on the response and to understand the optimum level of four variables for maximum GLA production from *Mucor hiemalis.* Fig. 1 demonstrates the surface plot of the effects of glucose and peptone on GLA concentration. In addition, effects of ammonium nitrate and pH on GLA concentration have been shown in Fig. 2. Fig. 1 revealed the zone for maximum GLA yield within 84 g/l to 100 g/l glucose and 2g/l to 1 g/l peptone. Fig. 2 showed this zone within 0.80 g/l to 1g/l ammonium nitrate and 5.10 to 4.5 pH.

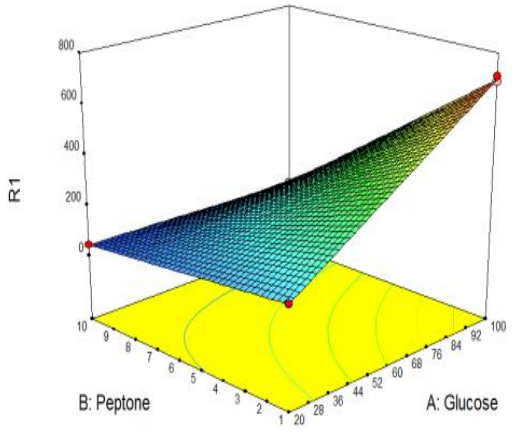

Fig. 1- Response surface plot describing effect of glucose (g/l) and peptone (g/l) on GLA concentration (R_1).

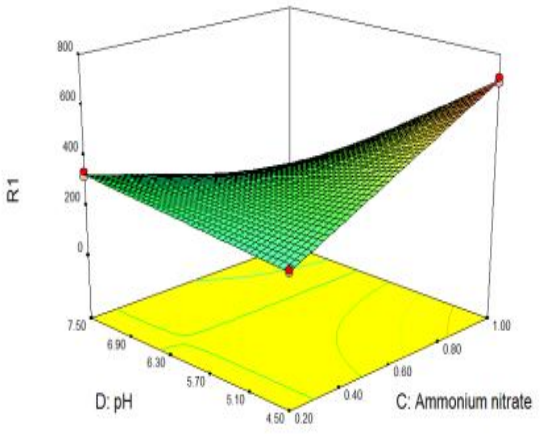

Fig. 2- Response surface plot describing effect of ammonium nitrate (g/l) and pH on GLA concentration ($R_{1)}$

The highest amounts of produced biomass (X, g/l), biomass yield on the consumed glucose ($Y_{X/S}$, g/g) and lipid yield on the consumed glucose ($Y_{L/S}$, g/g) were achieved in run numbers 12, 17 and 6 respectively. Additionally, both maximum quantity of cellular lipids (L_{max}, g/L) and cellular lipid in biomass ($Y_{L/x}$, %) were acquired in run number 19 (Tables 2 and 3).

The results of this study indicated that the highest GLA yield of 709 mg/l was discovered at 100 g/l glucose, 1 g/l peptone, 1 g/l ammonium nitrate and pH of 4.5, (run number 2, Table 2). The minimum GLA yield of 32 mg/l was detected at 20 g/l glucose, 10 g/l peptone, 0.2 g/l ammonium nitrate and 7.5 pH (run number 11, Table 2). Although, the maximum concentration of GLA was observed in run number 2 (Table 1), the percentage of total lipid to biomass was highest amount in run number 19 (Table 2). Regarding the estimated P-value from the ANOVA results (Table 5), the effects of culture conditions on GLA concentration were considered to be significant. The values of *R2* showed a good agreement between the experimental and predicted data for the regression. The statistical value of F-test probability showed that the model was accurate in describing the experimental data.

Discussion and conclusion

Production of single cell oils enriched with polyunsaturated fatty acids, such as GLA, is one of the main tasks for biotechnological production of nutritionally important lipids. Certain fungi, microalgae, and mosses synthesize GLA, and may represent potential microbial sources of this essential fatty acid. Fermentation physiology of oleaginous microorganisms is based on application of media rich with carbon source and restricted amounts of other nutrients, nitrogen especially. Biotechnological production of GLA was the first process developed for microbial polyunsaturated fatty acids (14, 20- 22).

Rhizopus arrhizus effectively converted saccharides to biomass rich in GLA and extracellular L(+)-lactic acid (23 & 24) based on calculations constructed fungal model and it was successfully applied it in large-scale fermentations. Furthermore, experimental design using statistical approach has been successfully applied to determine optimal parameters including medium composition and culture condition for GLA production in oleaginous fungi. Using the central composite design in conjunction with response surface methodology, the optimal medium components (glucose, yeast extract, and ammonium nitrate) for maximizing GLA

production in *Mucor rouxii* CFR-G15 were derived yielding about 18.6% GLA of total fatty acid and 35% lipid in biomass (25). Although GLA content in the fungal oil was 2 times higher than that of evening primrose oil, uncompetitive price, and other marketing difficulties with such specialized microbial oil were the major reasons for deferment of this industrial production in the Europe (26).

The highest amount of GLA produced in the present study was 709 mg/L when it was flask-cultured on high glucose concentration media (around 100 g/L). Similarly, Koike et al. reported that fatty acids content increase with the concentration of glucose from 2 to 12% in *Mortierella alpine* CBS 754.68 (27). Also, A *Mucor racemosus* strain, when it was flask-cultured in a medium that contained olive oil (2.5% wt/vol) and corn steep liquor (1% wt/vol), produced 725 mg/L GLA (28).

The aggregation of lipid inner the microbial cells or mycelia is activated by depletion of nitrogen from the culture medium, which permits the change of sugar to storing lipid. Therefore, high concentration of glucose (as a carbon source) and low concentration of peptone (as a nitrogen source) could enhance γ-linolenic acid production (29- 32). Microorganisms require mineral materials for their growth and the production of lipids. As a result, high concentration of ammonium nitrate could be effective to enhancement of γ-linolenic acid production.

Previous reports demonstrated that C/N ratio has great influence on lipid accumulation for oleaginous microorganisms and that inoculum level can significantly affect cell growth (17, 33 & 34).

In the present study, *Mucor hiemalis* was cultured on glucose in different initial C/N molar ratio (Table 2 and 3) media. The cellular lipid in biomass (%) was increased in higher quantities (25.9%) in the medium in which carbon and nitrogen were supplied in higher and lower excess respectively (run number 19 in Table 3, C/N molar ratio: 214). Similarly, Papanikolaou et al. reported that *Mortierella isabellina* utilizing glucose with a C/ N ratio of 237 produced the maximal level of cellular lipid in biomass (35). The production of GLA increased significantly to the maximal level (709 mg) at a C/N ratio of 127 in this study (run number 2 in Table 2). Gema et al. also reported that the fungus *Cunninghamella echinulata* utilizing glucose with a C/N ratio of 160 produced up to 720 mg GLA after lipid accumulation was completed (36).

Furthermore, the production of γ-linolenic acid was maximum amount in pH of 4.5. Also, this condition was increased lipid production according to reports of Ahmed et al and Xia et al. (3 & 37). The GLA production decreased sharply as the pH was raised from 4.5 to 7.5. It is possible that interactions between media components and pH reduce the ability of the strain to utilize the available nutrients in the medium (38). Many investigators claimed that different morphology of fungal

mycelia under a different initial pH value was a critical factor in biomass accumulation and metabolite formation (39 & 40).

The confirmation studies with 100 g/l glucose, 5.5 g/l peptone, 0.75 g/l ammonium nitrate and a pH of 6 caused to GLA concentration of 148.71 mg/l. This outcome was very nearby to model anticipated concentration of 148.93 mg/l from optimal response area.

The ability of mold to utilize baker's yeast may make the GLA production more economic. All these findings suggest that the present *Mucor* isolate is a potential candidate for further strain improvement for enhanced GLA content. The suitability of model predicted based on study demonstrated that factorial method was useful in evaluating the effects of four important fermentation variables; glucose concentration, peptone concentration, ammonium nitrate concentration and pH for the production of GLA using the *Mucor hiemalis* PTCC 5292. Also, the results show that high glucose and ammonium nitrate, low peptone concentrations and acidic pH exhibited significant effects to enhancement of GLA production. This simple and low cost optimization condition of culture media can be applied for large-scale production of GLA-rich oils, which is suitable for pharmaceutical and nutritional industries.

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[1]- Gase Chromatography
[2]- Flame Ionization Detector
[3]- Analysis of variance

بهینه‌سازی محیط کشت برای افزایش تولید گاما- لینولنیک اسید توسط *موکور همیلیس*

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چکیده

مقدمه: گاما- لینولنیک اسید یک اسید چرب ضروری در تغذیه انسان است. در پژوهش حاضر، تولید گاما لینولنیک اسید توسط قارچ *موکور همیلیس* PTCC ۵۲۹۲ در تخمیر غوطه‌وری ارزیابی شد.

مواد و روش‌ها: متغیرهای تخمیر بر اساس طراحی فاکتوریال نسبی انتخاب و سپس، از طریق روش طراحی فاکتوریال کامل بهینه‌سازی شدند. چهار متغیر معنادار گلوکز، پپتون، آمونیوم نیترات و اسیدیته برای مطالعات بهینه‌سازی انتخاب شدند. طراحی به طور کلی ۱۶ مرحله را شامل شد که مراحل در دو سطح برای هر متغیر با ۳ تکرار در نقطه مرکزی انجام شد.

نتایج: تحلیل واریانس و نقشه پاسخ سطح ۳ بعدی اثرات نشان داد که متغیرها عامل‌های معنادار همراه با سهم معناداری برای پاسخ بودند. نتایج نشان داد که انجام تخمیر در شرایط اپتیمم (غلظت ۱۰۰ گرم بر لیتر گلوکز، ۱گرم بر لیتر پپتون، ۱ گرم بر لیتر آمونیوم نیترات و اسیدیته ۴/۵) تولید گاما- لینولنیک اسید را تا ۷۰۹ میلی‌گرم بر لیتر افزایش می‌دهد.

بحث و نتیجه‌گیری: نتایج این پژوهش مشخص کرد که بازده بالای گاما- لینولنیک اسید می‌تواند در محیط کشت ساده در غلظت‌های بالای گلوکز و آمونیوم نیترات، غلظت پایین پپتون و اسیدیته اسیدی توسط قارچ *موکور همیلیس* PTCC ۵۲۹۲ به‌دست آید. شرایط ساده و کم هزینه بهینه‌سازی محیط کشت برای تولید گاما- لینولنیک اسید می‌تواند در مقیاس بالاتر برای صنایع دارویی و غذایی به کار برده شود.

واژه‌های کلیدی: بهینه‌سازی، تخمیر، طراحی فاکتوریال، گاما- لینولنیک اسید، *موکور همیلیس*

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