Biological Journal of Microorganism

Received: November 22, 2017/ **Accepted:** April 15, 2018. **Page:**

Production of lipase by the deep-sea bacterium *Idiomarina zobellii*; Identification of effective factors and optimization of production process

Shahrbanoo Sadeghi-Dastjerdi
M.Sc student, Department of Biochemistry and Biophysics, Faculty of Biological sciences, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran, s_shahrbanoo@yahoo.com
Mehdi Ebrahimi *
Assistant Professor, Department of Biochemistry and Biophysics, Faculty of Biological sciences, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran, ebrahimi@iauvaramin.ac.ir
Rozbeh Yalfani
Assistant Professor, Department of Biochemistry and Biophysics, Faculty of Biological sciences, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran, yalfani@iauvaramin.ac.ir

Abstract

Introduction: In this study, the deep-sea bacterium, *Idiomarina zobellii,* was used as a new source for the production of lipase. Lipases from halophilic bacteria are attractive for use in diverse industrial and biotechnological applications.

Materials and methods**:** The optimal temperature and pH for the activity of *I. zobellii* lipase was determined using different temperature and pH ranging from 30-60 °C and 7-11, respectively. The growth conditions influencing lipase production were determined using two-level fractional factorial Plackett–Burman design. The optimum levels of effective factors for production process were determined by using the response surface central composite design.

Results: The optimum temperature and pH of *I. zobellii* lipase in the crude mixture were 7-8 and 50 °C, respectively. Among the screened 10 factors, $MgCl_2$, yeast extract, sodium bicarbonate, ammonium nitrate concentrations and temperature were found to be effectively important. The highest productivity level was achieved at 42°C temperature and $MgCl_2$ 7.18 (g/L), yeast extract 1.1 (g/L), NH_4NO_3 3.65 (g/L) and $NaHCO_3$ 0.11 (g/L) concentrations. When the predicted optimum levels of effective factors were employed, the enzyme activity almost doubled (0.8 comparing to 0.39 U/ml) of the initial.

Discussion and conclusion: The *Idiomarina zobellii* originated lipase is an alkalophile enzyme. A significant increase in enzyme production, as large as 2.09 folds, is achievable under optimized conditions that favours biotechnology and industrial applications.

Key words: Lipase, *Idiomarina zobellii*, Plackett–Burman Design, Response Surface Methodology (RSM), Deep-sea Bacterium

*Corresponding Author

Introduction

Lipases (triacylglycerol acylhydrolases, E.C. 3.1.1.3) catalyze the hydrolysis of fats into fatty acids and glycerol and also esterification reaction for the production of fats. Lipases can perform these reactions at the water-insoluble substrate interface (1). In addition, lipases have the ability to catalyze inter esterification, acidolysis, alcoholysis and aminolysis reactions. In addition to their broad substrate specificity, lipases carry properties such as good chemoselectivity, regioselectivity and enantioselectivity (2). This wide range of biochemical specifications has made lipases one of the indispensable components in different biotechnological fields including synthesis of biopolymers, biodiesel, the production of enantio-pure pharmaceuticals, agro-chemicals, and flavour compounds (3). These applications have made lipases one of the most consumed and economical important enzymes in the world enzyme market. This growth in daily applications and demands in new biotechnological and industrial applications have led to daily efforts to discover new lipases with different specificities. Therefore, characterization of more natural sources of lipases holding newer properties is increasingly demanded (4). Many microorganisms and higher eukaryotes can naturally produce lipases. Among microorganisms, bacteria, fungi, yeasts and actinomyces are the most important lipase producers. Microbial lipases are most commercially useful and widely used in various industries (4, 5).

Over the last few decades, microorganisms from extreme marine conditions such as high pressures, hypersaline habitats and extreme temperature have increasingly received interest for industrial processes due to their high potentials in the production of new bio-products including enzymes and other active metabolites (6-8). Therefore, studies focusing on optimization of growth conditions and production of bioactive products such as enzymes from marine microorganisms are attractive and on demand for biotechnological and pharmaceutical industries. In addition, enzymes from psychrophilic micro-organisms effectively function in the cold, and the biotechnological applications of these cold-adapted enzymes have been considered in the last few decades (9).

Idiomarina zobellii is a gram-negative, psychrotolerant, heterotrophic, aerobic, rod-shaped bacterium which was isolated from the seawater of north-western Pacific Ocean at the depths of 4000-5000 m. Growth occurs at temperature of 4−30°C with its optimum at 20−22°C. The pH range for growth is 5.5−9.5 with its optimum at 7.5−8. This deep-sea bacterium requires NaCl (0.6−15%) for growth, hence it is regarded as moderate halophile (10). Enzymes from halophilic microorganisms such as *I. zobellii* can survive at high salt concentrations (11). These adaptations besides the existing robust nature of lipases, make halophilic lipases more attractive for industrial and biotechnological applications. (12). The number of studies on lipase producing halophilic organisms however, is limited. To date, there is more attention to these halophilic microorganisms, especially halo-archaea and their applicability for lipase production (13-15). Submerged fermentation is commonly used for the production of enzymes and other bio-active compounds by micro-organisms (5, 6). Growth conditions such as availability of carbon and nitrogen sources, the presence of activators, stimulators, inhibitors, surfactants, incubation temperature, pH, and the level and source of inoculum can influence the production of lipase (16). Besides, these various factors, the dependency of a microorganism for lipase production varies

from one microorganism to another. Moreover, most of the culture mediums and conditions proposed for optimal growth of microorganisms may be improper for the production of lipase. Therefore, the optimization of production conditions is an important step in the development of economically feasible bioprocesses (17).

The one factor at a time method, is a time consuming and costly method to distinguish factors affecting the process; therefore, it has been replaced with Plackett–Burman design (PBD) method (18). After determining the effective factors, Response Surface Methodology (RSM) could be used to investigate the combinatorial interactions of medium components and define the optimum conditions of effective factors for appropriate responses (17).

In this study, for the first time, the nutritional and cultural parameters affecting the production of lipase from a deep-sea bacterium, *I. zobellii* were investigated using PBD. These factors were used for optimization of growth condition by applying RSM to gain higher lipase production. Some properties such as optimum pH and temperature of lipase from *I. zobelli* were also investigated.

Materials and Methods

Microorganism and Preparation of inoculum: Live active culture of *I. zobelli* in a glass plate was obtained from the Iranian Biotechnology Research Center (IBRC-M10598). For pre-culture, a loop full of the bacterial active colony was added aseptically to 25 ml of Marine broth pH 7.5 and the bacterial suspension was incubated at 22°C for 48 h with 120 rpm shaking. A volume of 50 ml of Marine broth as preparation medium collected in a 250 ml Erlenmeyer flask was inoculated with 1 ml of the 48 h old culture and incubated as previously described. The optical density of the culture at 600 nm was recorded every 24 h to obtain the growth kinetics of *I. zobellii.* Using fresh culture medium, load of the bacterial growth was adjusted at OD=1. This preparation was subsequently used for inoculation of the experimental flasks.

Lipase assay: p-Nitrophenyl palmitate was used as a substrate for the measurement of *I. zobellii* lipase activity (19). To prepare the substrate solution, 10 ml of p-nitrophenyl palmitate (0.1 M) ethanol solution was added to a mixture of 207 mg of sodium deoxycholate and 100 mg of gum Arabic in 90 ml phosphate buffer (0.05 M, pH 6.5). To start the reaction, 0.1 ml of crude enzyme extract or blank solution (50 mM phosphate buffer, pH 6.5) was added to 2.4 ml of substrate solution. After 15 min incubation in 50 mM phosphate buffer, absorbance at 410 nm was recorded. One enzyme unit (U) was defined as the lipase activity that liberates 1µmol of p-nitrophenyl palmitate per milliliter per minute under standard assay conditions (20).

Temperature and pH effect on the lipase activity: The optimal temperature for the activity of *I. zobellii* lipase was determined using different temperatures ranging from 30-60°C (with 5°C intervals). For this, the substrate solution was mixed with crude enzyme extract, incubated at the selected temperatures for 15 min with the absorbance level recorded at 410 nm. The pH dependency of *I. zobellii* lipase was determined using substrate solution with different pH ranging from 7-11 with 0.5 unit intervals in the following buffers: 0.5 M sodium phosphate buffer (7-8.5), 0.5 M Britton-Robinson Buffer (9-11).

Plackett–Burman design: Factors affecting the production of lipase by *I. zobellii* were determined with experimental designed using the two factorial Plackett–Burman (PB) methods. In this method, n+1 experimental design

for n variables and interactions between the different variables were explained statistically (21). Among medium components and culture conditions, 10 parameters including ferric citrate (g/L), ammonium nitrate (g/L), sodium bicarbonate (g/L), yeast extract (g/L), peptone (g/L), $MgCl_2$ (M), shaking (rpm), pH, incubation temperature (°C) and time (h) were investigated. All experimental patterns and analysis were designed using the statistical software package "Minitab 17" (Stat-Ease Inc., USA) in 2 levels for each factor, high (+) and low (–), with one center point. According to selected levels for each variable, a collection of 15 experiments were designed. All experiments were performed in triplicate and the average was analyzed using Minitab software. Table (1) shows variables and selected levels for each experimental design.

The PB design is based on the following first-order polynomial equation:

$Y = ßo + \Sigma\ ßi\ Xi$

Where, Y, is the lipase activity; ßo, is the model intercept; ßi, is the linear coefficient; Xi, is the level of the independent variable.

Optimization using response surface methodology (RSM): RSM methodology was used for further optimization of significant variables obtained by Plackett-Burman design. According to screening experiments, among investigated variables, $MgCl_2$ medium, yeast extract, sodium bicarbonate, ammonium nitrate and the temperature were determined as significant. These variables were used in a Central Composite Design (CCD) to explore their effect on lipase production. A collection of 32 different experiments for three variables each in 5 levels (Table 2) was designed. This design consists of 16 cube points, 6 center points in cube and 10 axial point runs with 6 replications at the center point to evaluate the pure error. The lipase activity (U/ml) of each run was taken as response and the following second order polynomial equation was used to determine the relationship among variables:

$Y = ßo + \Sigma\ ßi\ xi + \Sigma\ ßii\ xi2 + \Sigma\ ßij\ xixj,$
$i = 1, 2, 3... k$

Where, Y, is the predicted response; k, is the number of factor variables; ßo, is the model constant; ßi, is the linear coefficient; ßii, is the quadratic coefficient; ßij, is the interaction coefficient.

Model validation: The Fischer's F-test was used to determine the statistical significance of polynomial model and the coefficient of determination R^2 value was checked for the quality of represented model (17, 22).

Optimization of variable's level: The final goal of optimization process was to create an increment in lipase production by a combination of factor levels. To achieve this, predicted levels for each variable were used in an experiment. The predicted levels of variables were as follows: temperature 42 °C, $MgCl_2$ 7.18 (g/L), yeast extract 1.1 (g/L), NH_4NO_3 3.65 (g/L) and $NaHCO_3$ 0.11 (g/L). The predicted lipase activity by the model in 95% confidence level was 3.78 U/ml. The "Minitab 17" (Stat-Ease Inc., USA) software package was used for experimental design and statistical analysis of the responses.

Results

Inoculum preparation: Inoculum size and growth profile of bacterial culture can affect enzyme production. The number of bacterial cells in the production media determines the availability of nutrients. The concentration of growth inhibitors in culture increases, with increase in culture time (23). To overcome these effects in lipase production, growth profile of *I. zobellii* was obtained in order to realize a

constant amount of active bacteria for use as an inoculum in all experiments. According to Fig. 1, absorbance at 600 nm increased up to 1.7 for 48 h and then remained nearly constant. After 72 h, absorbance decreases as a result of the death of bacteria. About 1 ml of a 24 h bacterial culture was used with absorbance of 0.8 at 600 nm to inoculate culture medium in all experiments.

Optimization of enzyme assay: It is important to assay each enzyme in its optimum condition. Temperature and pH are two important assay conditions, which can affect enzyme activity. Lipase activities in different pH and temperatures were investigated to obtain the optimum condition of the assay (Fig. 2). The pH profile shows that *I. zobellii* lipase has the maximum activity at pH 7-8. According to Figure 2b, the optimum temperature for *I. zobellii* lipase was determined as 50°C.

Detection of significant factors using Plackett–Burman design: Different medium components and culture conditions including ferric citrate, ammonium nitrate, sodium bicarbonate, yeast extract, peptone, $MgCl_2$, shaking, pH and incubation temperature, and time were selected as independent variables and were investigated in PB design. Using this method, a set of 15 different experiments were designed. Results of these experiments with respect to lipase activity were shown in Table 1. The following equation shows the effect of each studied variable in lipase production from *I. zobellii* as a mathematical model:

Activity (U/ml) = -1.712 + 0.0277 temperature+ 0.01 pH - 0.00083 Incubation time - 0.00017 Shaking+ 0.0250 peptone + 0.708 yeast extract + 0.0918 $MgCl_2$ - 2.29 $NaHCO_3$ + 0.224 $[NH_4]NO_3$+ 1.08 ferric citrate + 0.017 Ct Pt

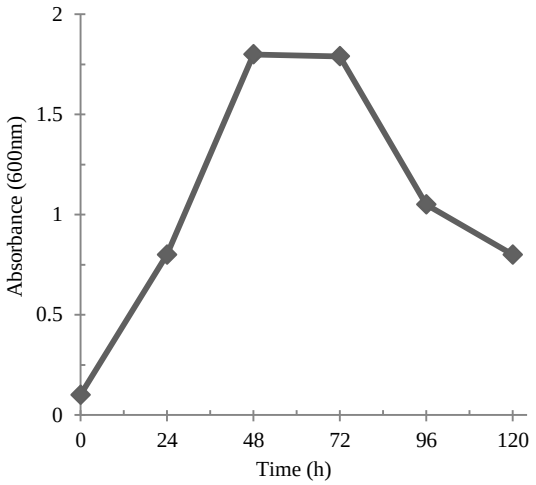

Fig. 1- Growth curve of *I. zobellii* during different incubation time

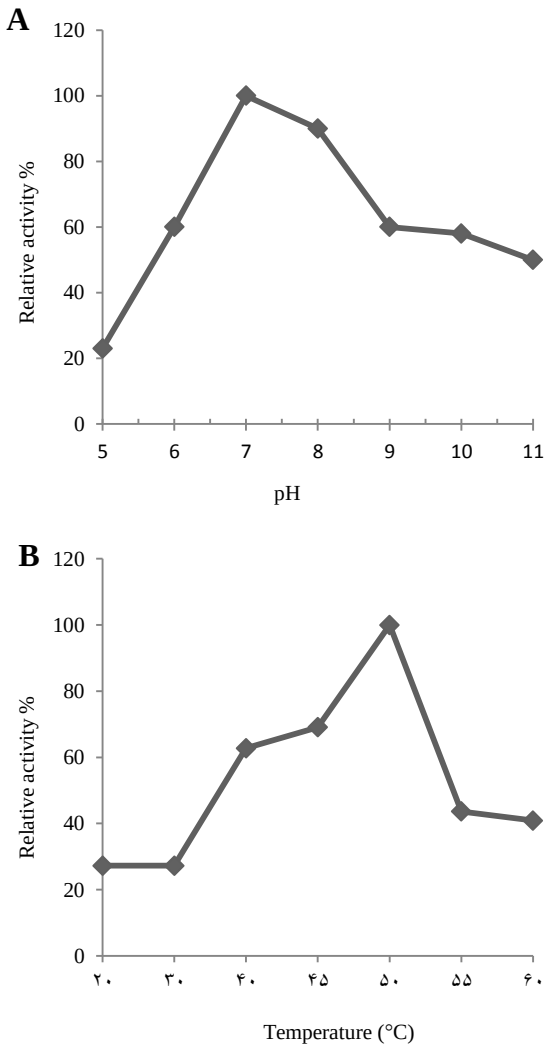

Fig. 2- Activity of *I. zobellii* lipase in different pH (a) and temperatures (b)

Table 1- The Placket-Burman design for detection of significant factors affecting lipase production in I. zobellii

	Variables										Lipase activity (U/ml)	
Run order	Temperature (°C)	pH	Shaking (rpm)	Incubation time (h)	NH_4NO_3 (g/L)	$MgCl_2$ (g/L)	Yeast extract (g/L)	Peptone (g/L)	Ferric citrate (g/L)	$NaHCO_3$ (g/L)	Observed	Predicted
1	26.4	6.5	80	72	1.92	7.08	0.8	6.25	0.08	0.192	6.50	10.13
2	26.4	8.5	160	24	1.92	4.72	0.8	3.75	0.12	0.192	8.62	5.43
3	17.6	8.5	80	72	1.92	4.72	0.8	3.75	0.12	0.128	2.74	4.30
4	26.4	6.5	160	72	1.28	4.72	1.2	3.75	0.12	0.128	11.21	14.07
5	26.4	8.5	160	24	1.28	7.08	0.8	6.25	0.08	0.128	8.93	11.41
6	26.4	8.5	80	72	1.28	4.72	1.2	3.75	0.08	0.192	12.62	9.84
7	17.6	8.5	160	72	1.92	7.08	1.2	5	0.08	0.128	18.26	16.37
8	17.6	6.5	160	72	1.28	7.08	0.8	6.25	0.12	0.192	4.94	1.82
9	17.6	6.5	160	24	1.92	4.72	1.2	6.25	0.08	0.192	4.54	7.44
10	26.4	6.5	80	24	1.92	7.08	1.2	3.75	0.12	0.128	25.39	21.73
11	17.6	8.5	80	24	1.28	7.08	1.2	5	0.12	0.192	7.29	9.19
12	17.6	6.5	80	24	1.28	4.72	0.8	5	0.08	0.128	3.37	1.35
13	22	7.5	120	48	1.6	5.9	1	3.75	0.1	0.16	9.17	9.42
14	22	7.5	120	48	1.6	5.9	1	6.25	0.1	0.16	8.54	9.42
15	22	7.5	120	48	1.6	5.9	1	6.25	0.1	0.16	9.25	9.42

The F- and P-values of the given model were 7.78 and 0.004, respectively, so the model seems significant. The confidence of determination (R^2) of PB design is 81.2%. This means that the represented mathematical model can fit 81.2% of total variables in the range of studied values.

The significance of each variable at the designed confidence level was determined with p-value and F-value of ANOVA analysis. The significant variables should have low p-value (less than 0.05) and high f-value (24). Standardized effects of variables were shown in Pareto chart (Fig. 3). According to this chart, five parameters including ammonium nitrate, sodium bicarbonate, yeast extract, $MgCl_2$, and incubation temperature were determined as significant. This implies that ferric citrate and peptone used in the culture of *I. zobellii* as carbon and nitrogen sources have no effects on lipase production and could be replaced by other components. Among three significant factors, yeast extract and ammonium nitrate have the highest and lowest effects, respectively.

Optimization of lipase production using RSM: The Response Surface Methodology (RSM) can be used to investigate interactions between significant parameters in the production of lipase. In this study, significant variables determined with PB design (temperature, yeast extract, sodium bicarbonate, ammonium nitrate and $MgCl_2$) were used to inquire the combined effects of these independent variables. A set of 32 experiments were designed using five levels for each parameter in the CCD.

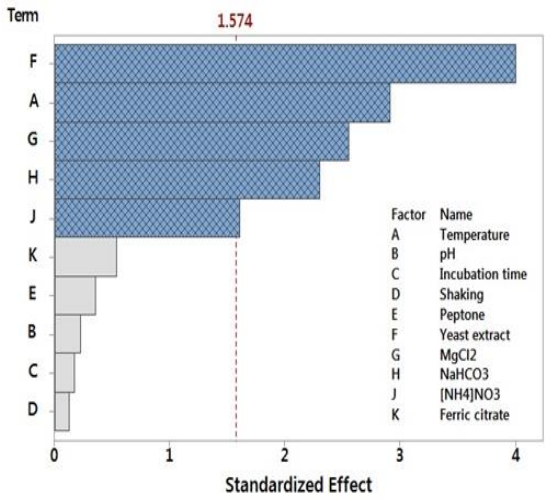

Fig. 3- Pareto chart of the standardized effect of variables for detection of significant factors for lipase production from *I. zobellii*.

Table 2 represents observed and predicted values for each experiment in the CCD. The observed lipase activity varied widely from 1.18 to 3.96 U/ml among different experiments. The following second order polynomial equation represents linear, square and 2-way interaction effects in the production of lipase.

Lipase activity (U/ml)= -19.11 + 0.054 A + 2.24 B + 15.55 C - 0.181 D + 37.8 E + 0.000205 A*A - 0.1409 B*B - 4.82 C*C - 0.03999 D*D - 212.5 E*E - 0.00233 A*B - 0.0312 A*C + 0.00164 A*D - 0.131 A*E - 0.477 B*C + 0.0748 B*D + 1.25 B*E - 0.186 C*D + 4.9 C*E + 0.63 D*E

Where, A; temperature, B; $MgCl_2$, C; yeast extract, D; $[NH_4]NO_3$ and E; $NaHCO_3$.

Table 2- Data for the central composite design of significant factors in the production of lipase from *I. zobellii.*

Std. Order	Variables					Lipase activity (U/ml)	
	Temperature (°C)	$MgCl_2$ (g/L)	Yeast (g/L)	$[NH_4]N0_3$ (g/L)	$NaHCO_3$ (g/L)	Observed	Predicted
1	27	6.49	1.1	1.76	0.14	1.78	2.02
2	37	6.49	1.1	1.76	0.11	2.73	2.94
3	27	7.67	1.1	1.76	0.11	1.96	2.21
4	37	7.67	1.1	1.76	0.14	1.39	1.78
5	27	6.49	1.3	1.76	0.11	2.88	2.88
6	37	6.49	1.3	1.76	0.14	2.07	2.21
7	27	7.67	1.3	1.76	0.14	1.45	1.63
8	37	7.67	1.3	1.76	0.11	1.62	1.76
9	27	6.49	1.1	4.96	0.11	1.63	1.78
10	37	6.49	1.1	4.96	0.14	1.54	1.82
11	27	7.67	1.1	4.96	0.14	2.28	2.61
12	37	7.67	1.1	4.96	0.11	2.84	3.14
13	27	6.49	1.3	4.96	0.14	1.18	1.26
14	37	6.49	1.3	4.96	0.11	1.65	1.70
15	27	7.67	1.3	4.96	0.11	1.75	1.83
16	37	7.67	1.3	4.96	0.14	1.57	1.79
17	22	7.08	1.2	3.36	0.125	3.52	3.24
18	42	7.08	1.2	3.36	0.125	3.96	3.47
19	32	5.9	1.2	3.36	0.125	2.49	2.29
20	32	8.26	1.2	3.36	0.125	2.89	2.33
21	32	7.08	1	3.36	0.125	3.43	2.73
22	32	7.08	1.4	3.36	0.125	1.99	1.92
23	32	7.08	1.2	0.16	0.125	1.86	1.46
24	32	7.08	1.2	6.56	0.125	1.45	1.09
25	32	7.08	1.2	3.36	0.095	2.94	2.72
26	32	7.08	1.2	3.36	0.155	2.49	1.94
27	32	7.08	1.2	3.36	0.125	3.01	3.26
28	32	7.08	1.2	3.36	0.125	2.89	3.26
29	32	7.08	1.2	3.36	0.125	3.31	3.26
30	32	7.08	1.2	3.36	0.125	2.86	3.26
31	32	7.08	1.2	3.36	0.125	3.36	3.26
32	32	7.08	1.2	3.36	0.125	3.36	3.26

The F- and P-values for the model were 2.9 and 0.037, respectively, implying that the model terms are significant. The coefficient of variations (R^2) always lies between 0 and 1 and it indicates the ability of the model to explain the variability in the response (25). The stronger model has R^2 close to 1 while in a weak model, it is close to zero (26). Considering R^2 value (0.8405), demonstrated model can explain 84.05% of the total variability within the range of studied values.

Analysis of Response Surface Methodology (RSM): Interactive effects of two variables at a time on lipase production in *I. zobellii* were investigated by 3D surface plots. In each plot, one variable remains constant while two other variables change according to defined values in CCD. The surface of the curved sheet shows the changes in the lipase production. According to the 3D surface plots, different temperatures have no effect on lipase production when other factors remain constant (Fig. 4a, 4b, 4c and 4d). In each given temperature, we can see a maximum lipase production in response to the variations in second factors. Moreover, there was no significant difference in the maximum lipase production in response to the different temperatures. In the presence of different concentrations of $MgCl_2$, a maximum point in lipase production can be seen in response to variations in other factors (Fig. 4e, 4f and 4g). In the presence of different concentrations of $NaHCO_3$ and $(NH_4)NO_3$, lipase production decreased when the concentration of yeast extract increased from 1.2 (Fig. 4h and 4i). According to Fig. 4j, there is an optimal concentration of $NaHCO_3$ and $(NH_4)NO_3$ for lipase production.

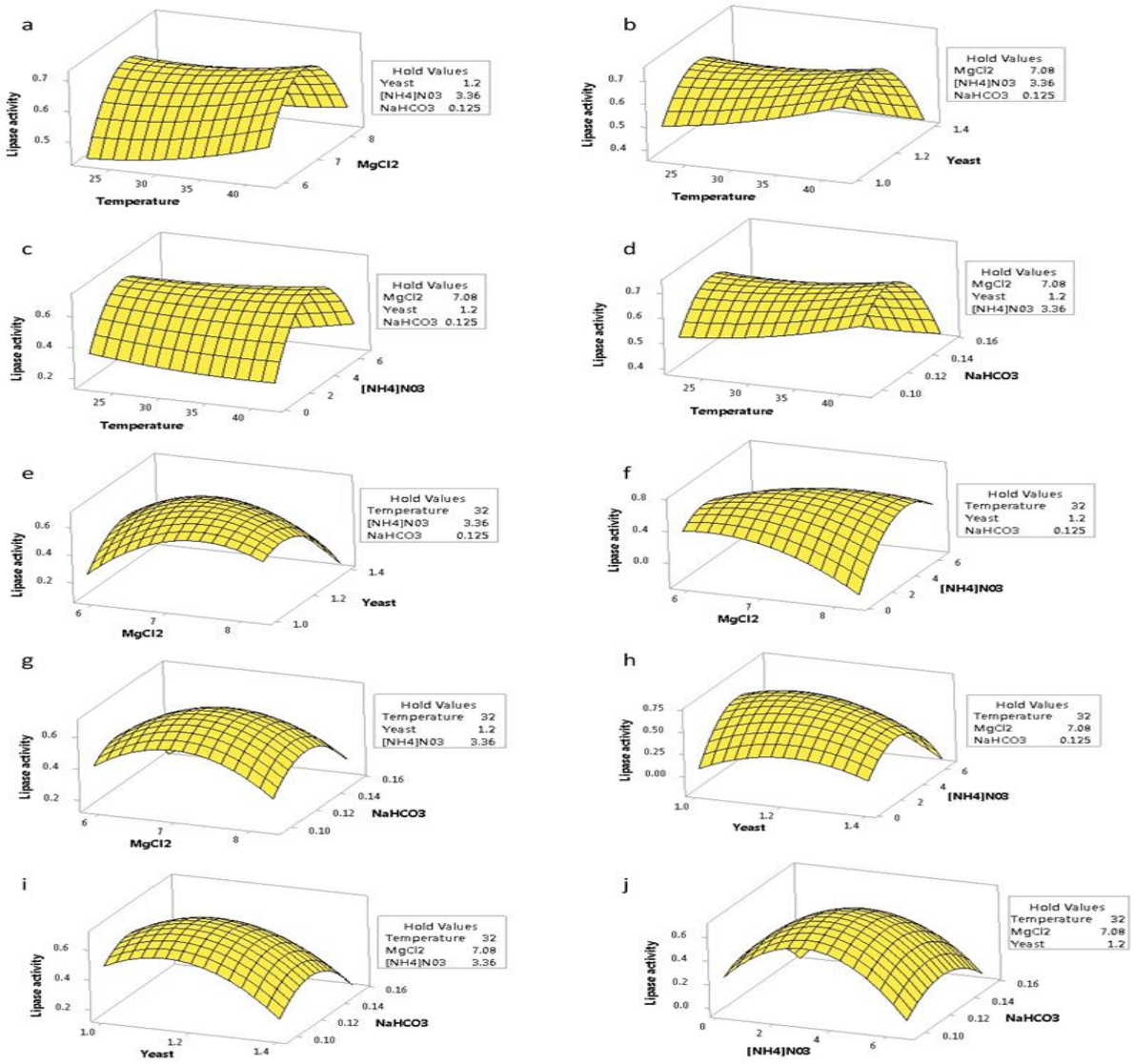

Fig. 4- Surface plot for investigation of interactive effects of variables on lipase production.

A confirmatory test was performed using the model predicted level of significant parameters and compared with the un-optimized condition. The lipase activity in optimized condition was 3.88 $U.ml^{-1}$ which was close to the predicted value (3.73 $U.ml^{-1}$) and was significantly more than un-optimized condition (1.89 $U.ml^{-1}$). This result shows that the lipase production under optimized condition increases by nearly 100% and the model can properly define optimal levels of determined variables to increase the lipase production.

Discussion and conclusion

Lipases are one of the most usable enzymes with wide applications from different industrial areas such as food, paper, detergent, leather, textile, organic synthesis, cosmetics to pharmaceutical and medical (27). These abundant applications have made lipases one of the most demanded enzymes in global enzyme market. Application of lipases in new processes requires enzymes with new proportional properties. Finding lipase enzymes with new properties is a daily demand, and for this reason, many efforts are focused to find new sources of lipases. Among different sources, microbial lipases are most valuable and widely used.

In this study, an optimization process for increase in the production of lipase enzyme from *I. zobellii*, a deep-sea bacterium, was investigated. Lipase activity was used to determine the production level of enzyme in each experiment. Optimum pH and temperature of the enzyme in crude mixture was determined to be 7-8 and 50°C, respectively. Most bacterial lipases have maximum activity at pH values higher than 7, and they are known as Alkalophilic (5, 28). The maximum activity of *I. zobellii* lipase (50°C) is similar to that of other moderate thermophile lipases from *Spirulina platensis* (45°C), *P. camaembertii* Thom PG-3 (48 °C) and *Aspergillus niger* F044 (45 °C) (20, 29, 30).

Submerged culture method is widely used for the production of lipases using different microbial sources (4). In each microbial source, optimal culture condition and nutritional requirements must be determined to increase lipase production. The most important parameters influencing lipase production includes the type and concentration of carbon and nitrogen sources, the growth temperature, the culture pH and agitation (31). Detection of effective parameters in the production of lipase involves the investigation of different levels of each parameter when other parameters are unchanged. This method is time-consuming and costly when a large number of factors need to be investigated. Nowadays, Plackett–Burman design is widely used as a powerful technique for the evaluation and detection of the variables that influence the response (17, 18, 24, 32-34). The strength of this method is a considerable reduction in the number of experiments required to determine significant variables. Culture conditions including incubation time, temperature, pH and shaking directly influence bacterial growth and lipase production. The effect of these factors on lipase production relates to bacterial species. Among these factors, temperature effect on lipase production by *I. zobellii* was determined as significant. In addition, using PB method, temperature, $MgCl_2$, yeast extract, $(NH_4)NO_3$ and $NaHCO_3$ were determined to be effective in lipase production by *I. zobellii*. Bacterial dependency on the medium component and optimal levels for lipase production differ among bacterial species. In *I. zobelli*, optimal lipase production temperature (42°C) is significantly more than growth temperature (22°C). In the study reported by Esmaeili, the optimum production and growth temperature for *B. subtilis* PTCC were 43.4 and 37°C, respectively (35). According to the results from a study on *Bacillus* sp., increase in temperature of

isolates from 37 to 40°C led to an increase in lipase production (35). Golani reported the same optimum growth and lipase production temperature at 37°C by *Staphylococcus chromogenes* O1A (36). In the study reported by Yele, 12 medium components including $MgCl_2$ and peptone were investigated by PB methods and were not determined as effective in the production of lipase by *Staphylococcus warneri* (18). Kai used PB method and reported that four factors including peptone and yeast extract have significant effects on lipase production by newly isolated *Thalassospira permensis M35-15* (37).

There are similar reports on optimization of production processes using response surface methodology. The increment level in these studies is different and related to the bacterial strain and medium components and cultural conditions. Wang reported the isolation of *T. permensis* M35-15 from deep-sea water and 1.8 fold increase in lipase production by optimization of glucose, peptone, yeast powder and olive oil (38). Salihu et al. used sequential optimization strategy based on statistical experimental design including one-factor-at-a-time (OFAT) method and enhanced the 5.19-fold increase in the production of lipase by *Candida cylindracea* ATCC 14830 using palm oil mill effluent as a basal medium in shake flask cultures (39). In another study, the Placket-Burman method was used for screening 10 medium factors and after optimization of effector factors using the Box–Behnken technique, a 9.1-fold enhancement in lipase production by *Stenotrophomonas maltophilia* was reported (40). In our study, lipase production by *I. zobellii* showed a 2.09-fold increase by using Response Surface Methodology (RSM) for effective factors. In comparison to mentioned studies, increment level gained in our study is acceptable. The next step for more increase in lipase production by *I. zobellii* could be the replacement of ineffective factors with factors reported to be effective in similar or closely related bacterial species.

The results of our study and similar studies showed that there are no constant requirements for lipase production among different bacterial strains. Optimization studies similar to this study can reveal requirements of new bacterial species in the production process of high-value products and in addition to increase in process efficiency can decrease process costs. According to the advantages of alkaline enzymes, *I. zobellii* seems promising for biotechnological and industrial applications, which are conducted at alkaline reaction conditions.

References

(1) Tan CH, Show PL, Ooi CW, Ng EP, Lan JC, Ling TC. Novel lipase purification methods - a review of the latest developments. *Biotechnology Journal* 2015; 10 (1): 31-44.

(2) Joseph B, Ramteke PW, Thomas G. Cold active microbial lipases: Some hot issues and recent developments. *Biotechnology Advances* 2008; 26 (5): 457-70.

(3) Jaeger K-E, Eggert T. Lipases for biotechnology. *Current Opinion in Biotechnology* 2002; 13 (4): 390-7.

(4) Sharma R, Chisti Y, Banerjee UC. Production, purification, characterization, and applications of lipases. *Biotechnology Advances* 2001; 19 (8): 627-62.

(5) Gupta R, Gupta N, Rathi P. Bacterial lipases: an overview of production, purification and biochemical properties. *Applied Microbiology and Biotechnology* 2004; 64 (6): 763-81.

(6) Dalmaso GZ, Ferreira D, Vermelho AB. Marine extremophiles: a source of hydrolases for biotechnological applications. *Marine Drugs* 2015; 13 (4): 1925-65.

(7) Ivanova EP, Romanenko LA, Chun J, Matte MH, Matte GR, Mikhailov VV, et al. Idiomarina gen. nov., comprising novel indigenous deep-sea bacteria from the Pacific Ocean, including descriptions of two species, Idiomarina abyssalis sp. nov. and Idiomarina zobellii sp. nov. *International Journal of Systematic and*

Evolutionary Microbiology 2000; 50 (2): 901-7.

(8) Zhang C, Kim S-K. Research and Application of Marine Microbial Enzymes: Status and Prospects. *Marine Drugs* 2010; 8 (6): 1920-34.

(9) Cavicchioli R, Charlton T, Ertan H, Mohd Omar S, Siddiqui KS, Williams TJ. Biotechnological uses of enzymes from psychrophiles. *Microbial Biotechnology* 2011; 4 (4): 449-60.

(10) Margesin R, Schinner F. Potential of halotolerant and halophilic microorganisms for biotechnology. *Extremophiles* 2001; 5 (2): 73-83.

(11) Karan R, Kumar S, Sinha R, Khare SK. Halophilic Microorganisms as Sources of Novel Enzymes. In: Satyanarayana T, Johri BN, editors. Microorganisms in Sustainable Agriculture and Biotechnology. Dordrecht: Springer Netherlands; 2012: 555-79.

(12) Hezayen FF, Rehm B, Tindall BJ, Steinbuchel A. Transfer of Natrialba asiatica B1T to Natrialba taiwanensis sp. nov. and description of Natrialba aegyptiaca sp. nov., a novel extremely halophilic, aerobic, non-pigmented member of the Archaea from Egypt that produces extracellular poly(glutamic acid). *International Journal of Systematic and Evolutionary Microbiology* 2001; 51 (3): 1133-42.

(13) Schreck SD, Grunden AM. Biotechnological applications of halophilic lipases and thioesterases. *Applied Microbiology and Biotechnology* 2014; 98 (3): 1011-21.

(14) Delgado-García M, Valdivia-Urdiales B, Aguilar-González CN, Contreras-Esquivel JC, Rodríguez-Herrera R. Halophilic hydrolases as a new tool for the biotechnological industries. *Journal of the Science of Food and Agriculture* 2012; 92 (13): 2575-80.

(15) Litchfield CD. Potential for industrial products from the halophilic Archaea. *J Ind Microbial Biotechnology* 2011; 38 (10): 1635-47.

(16) Hasan F, Shah AA, Hameed A. Methods for detection and characterization of lipases: A comprehensive review. *Biotechnology Advances* 2009; 27 (6): 782-98.

(17) Chennupati S, Potumarthi R, Gopal Rao M, Manga PL, Sridevi M, Jetty A. Multiple Responses Optimization and Modeling of Lipase Production by Rhodotorula mucilaginosa MTCC-8737 Using Response Surface Methodology. *Applied Biochemistry and Biotechnology* 2009; 159 (2): 317-29.

(18) Yele VU, Desai K. A New Thermostable and Organic Solvent-Tolerant Lipase from Staphylococcus warneri; Optimization of Media and Production Conditions Using Statistical Methods. *Applied Biochemistry and Biotechnology* 2015; 175 (2): 855-69.

(19) Vorderwülbecke T, Kieslich K, Erdmann H. Comparison of lipases by different assays. *Enzyme and Microbial Technology* 1992; 14 (8): 631-9.

(20) Demir BS, Tükel SS. Purification and characterization of lipase from Spirulina platensis. *Journal of Molecular Catalysis B: Enzymatic* 2010; 64 (3-4): 123-8.

(21) Plackett RL, Burman JP. The Design of Optimum Multifactorial Experiments. *Biometrika* 1946; 33 (4): 305.

(22) Sumrin A, Ahmed W, Ijaz B, Sarwar MT, Gull S, Kausar H, Shahid I, Jahan S, . Purification and medium optimization of α-amylase from Bacillus subtilis 168. *African Journal of Biotechnology* 2011; 10 (11): 2119-29.

(23) Thakur V, Tewari R, Sharma R. Evaluation of Production Parameters for Maximum Lipase Production byP. stutzeriMTCC 5618 and Scale-Up in Bioreactor. *Chinese Journal of Biology* 2014; 2014: 1-14.

(24) Pareek N, Singh RP, Ghosh S. Optimization of medium composition for enhanced chitin deacetylase production by mutant Penicillium oxalicum SAEM-51 using response surface methodology under submerged fermentation. *Process Biochemistry* 2011; 46 (8): 1693-7.

(25) Uma MRJ, Satyanarayana T. Statistical optimization of a high maltose-forming, hyperthermostable and Ca2+-independent alpha-amylase production by an extreme thermophile Geobacillus thermoleovorans using response surface methodology. *Journal of applied microbiology* 2003; 95 (4): 712-8.

(26) Haaland PD. *Experimental design in biotechnology*: CRC press; 1989.

(27) Andualema B, Gessesse A. Microbial lipases and their industrial applications. *Biotechnology* 2012; 11 (3): 100-18.

(28) Mobarak-Qamsari E, Kasra-Kermanshahi R, Moosavi-nejad Z. Isolation and identification of a novel, lipase-producing bacterium, Pseudomnas aeruginosa KM110. *Iranian Journal of Microbiology* 2011; 3 (2): 92-8.

(29) Tan T, Zhang M, Xu J, Zhang J. Optimization of culture conditions and properties

of lipase from Penicillium camembertii Thom PG-3. *Process Biochemistry* 2004; 39 (11): 1495-502.

(30) Shu Z-Y, Yang J-K, Yan Y-J. Purification and Characterization of a Lipase from Aspergillus niger F044. *Chinese Journal of Biotechnology* 2007; 23 (1): 96-101.

(31) Elibol M, Ozer D. Influence of oxygen transfer on lipase production by Rhizopus arrhizus. *Process Biochemistry* 2000; 36 (4): 325-9.

(32) Heydari R, Zahiri HS, Noghabi KA, Rad VV, Khaniki GB. A statistical approach to the optimization of cold-adapted amylase production by Exiguobacterium sp SH3. *Starch-Starke* 2012; 64 (12): 955-63.

(33) Prajapati VS, Trivedi UB, Patel KC. A statistical approach for the production of thermostable and alklophilic alpha-amylase from Bacillus amyloliquefaciens KCP2 under solid-state fermentation. *3 Biotech* 2015; 5 (2): 211-20.

(34) Liu J, Zhang Z, Liu Z, Zhu H, Dang H, Lu J, et al. Production of cold-adapted amylase by marine bacterium Wangia sp. C52: optimization, modeling, and partial characterization. *Marine Biotechnology (NY)* 2011; 13 (5): 837-44.

(35) Esmaeili M, Yolmeh M, Shakerardakani A, Golivari H. A central composite design for the optimizing lipase and protease production from Bacillus subtilis PTCC 1720. *Biocatalysis and Agricultural Biotechnology* 2015; 4 (3): 349-54.

(36) Golani M, Hajela K, Pandey G. Screening, Identification, characterization and production of bacterial lipase from oil spilled soil. *International Journal of Current Microbiology and Applied Sciences* 2016; 5 (3): 745-63.

(37) Kai W, Peisheng Y. Optimization of Lipase production from a novel strain Thalassospira permensis M35-15 using Response Surface Methodology. *Bioengineered* 2016; 7 (5): 298-303.

(38) de Morais WGJ, Kamimura ES, Ribeiro EJ, Pessela BC, Cardoso VL, de Resende MM. Optimization of the production and characterization of lipase from Candida rugosa and Geotrichum candidum in soybean molasses by submerged fermentation. *Protein Expression and Purification* 2016; 123: 26-34.

(39) Salihu A, Alam MZ, AbdulKarim MI, Salleh HM. Optimization of lipase production by Candida cylindracea in palm oil mill effluent based medium using statistical experimental design. *Journal of Molecular Catalysis B: Enzymatic* 2011; 69 (1): 66-73.

(40) Hasan-Beikdashti M, Forootanfar H, Safiarian MS, Ameri A, Ghahremani MH, Khoshayand MR, et al. Optimization of culture conditions for production of lipase by a newly isolated bacterium Stenotrophomonas maltophilia. *Journal of the Taiwan Institute of Chemical Engineers* 2012; 43 (5): 670-7.