OPTIMIZATION OF LIPASE PRODUCTION BY MICROORGANISM ISOLATED FROM WHEY

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ABSTRACT

Whey is the main by-product of milk processing. Although used in low proportions, its composition is rich in sugars, proteins, and fats, which favors the development of different types of microorganisms, mainly bacteria. The requirement to reuse this byproduct, combined with its nutritional composition, favors its use as a nutrient for microbial metabolites production. Among these metabolites, lipases, enzymes responsible for the hydrolysis of lipid molecules, have several applications in wastewater treatment and energy. In this sense, this work aimed to optimize the conditions for lipase production from a whey isolate. Previously bioprospection resulted in the selection of ML-15 bacterial isolate, which showed lipase activity of 10.5 $U.mL^{-1}$ in a medium composed of whey. Optimization tests for lipase production were carried out using a central composite rotational (DCCR) design. The optimized parameters of the submerged cultivation were pH, temperature, and agitation. The highest lipolytic activity identified was 65.08±0.1 $U.mL^{-1}$ at pH 9.0, 37°C, and 250 rpm, using *p*-nitrophenol palmitate as substrate. This condition has been experimentally validated. Considering these results, the importance of optimizing cultivation conditions is revealed attaining a 6,5-fold increase in lipase production.

Keywords: Lipase. Optimization. Cultivation Conditions. Isolated strain.

1 INTRODUCTION

In the pursuit of process sustainability and to reduce waste generation during and after industrial processes, the use of enzymes could be an alternative to meet this demand. They act as biological catalysts and find applications in a diverse range of industrial areas, including pharmaceutical, health, energy, food, and biomaterials segments [1]. Among the most industrially used groups of enzymes, lipases stand out. The main reaction catalyzed by lipases is the conversion of substrates, such as oils and fats, mainly triacylglycerols, into fatty acids and glycerol [2].

Lipases have high versatility in terms of applications, such as in the treatment of effluents and bioremediation, in biofuel obtention, in biodiesel production, and in the manufacture of food, cosmetics, aromas, pharmaceutical formulas, and detergents, among others. However, the lack of information on the specific conditions for lipase production is one of the bottlenecks in the enzyme synthesis process [2].

The production process of this enzyme by fermentative route requires mild production conditions [3]. Therefore, it is necessary to use statistical tools that allow the prediction of the production process, such as temperature, pH, and agitation to obtain superior enzymatic activity [4]. In this context, the present work aimed to optimize the submerged cultivation parameters for lipase production by the whey isolate and considering pH, agitation, and temperature parameters.

2 MATERIAL & METHODS

The culture ML-15 was previously isolated from a whey sample collected in a dairy industry situated in Vale do Taquari-RS. To evaluate the potential of this isolate in lipase production, qualitative methods were used using selective media (tributyrin and Rhodamine B), in addition to evaluation of lipase formation during submerged cultivation [5,6].

In order to optimize the cultivation conditions for lipase production, a central composite rotational design (DCCR) was performed with 3 independent variables, 5 levels and 3 repetitions in the central point totaling 17 tests. The orthogonality alpha used was 1.68. The independent variables considered were temperature ranging from 31°C to 43°C (x_1), initial cultivation pH ranging from 7.0 to 11.0 (x_2), and agitation from 150 to 350 rpm (x_3). The culture of isolate ML-15 was incubated for 48 hours until being attained an optical density (OD_{600nm}) equal to 1.0. At the end of the fermentation process, the lipolytic activities of each experiment were analyzed.

The enzymatic activity of lipases was determined using a spectrophotometric technique, using *p*-nitrophenol palmitate (*p*NPP) as substrate. One unit (U) was defined as the amount of enzyme required to release 1µmol of free fatty acids per minute under the assay conditions [6,7]. The data were processed in the statistical software Minitab 18 with a confidence level of 5% ($p < 0.05$). In order to validate the model predictions, the fermentation tests were carried out under conditions predicted by the models [7].

3 RESULTS & DISCUSSION

The isolate ML-15 was previously selected considering their lipase production in tributyrin and Rhodamine B media and subsequently evaluated in submerged cultivation. In this first evaluation of cultivation, lipolytic activity of 10.20 ± 0.41 $U.mL^{-1}$ was achieved [8]. The results obtained in this step show the versatility of whey as a source for the bioprospection of microorganisms and also as a nutrient for microbial growth and lipase production [9].

Table 1 shows the results of lipolytic activity considering the effects of initial pH, temperature, and cultivation agitation. Volumetric activity results ranged from 12.27 $U.mL^{-1}$ to 65.08 $U.mL^{-1}$. Conditions for maximum production were found to be temperature of 37°C, pH equal to 9.0, and stirring at 250 rpm. It is worth mentioning that this condition of the central point is similar that reported by other authors, which indicate that in more extreme conditions the parameters evaluated impair lipase production [5,7].

This result represents a 6.5-fold increase in enzymatic activity compared to than obtained from previous cultivation of ML-15 isolate (10.20 ± 0.41 $U.mL^{-1}$). Similar results were obtained with the optimization of cultivation conditions for lipase production by *Nocardiopsis alba* [5]. According to the authors, from the definition of the parameters of temperature (40°C), agitation (130 rpm), and pH 8.0, the highest lipase production of 65.78 $U.mL^{-1}$ was obtained, resulting in an increase of 5.7-fold compared to the medium without optimization.

Table 1 Enzymatic activity of lipase from strain ML-15 as a function of initial pH, cultivation temperature, and agitation. DCCR with real and encoded variables (in parentheses).

Test	x_1	x_2	x_3	Lipolytic activity ($U.mL^{-1}$)
	pH	T (°C)	Agitation(rpm)	
1	8 (-1)	34 (-1)	200 (-1)	24,17
2	10 (1)	34 (-1)	200 (-1)	24,94
3	8 (-1)	40 (1)	200 (-1)	20,75
4	10 (1)	40 (1)	200 (-1)	24,74
5	8 (-1)	34 (-1)	300 (1)	17,45
6	10 (1)	34 (-1)	300 (1)	20,91
7	8 (-1)	40 (1)	300 (1)	46,87
8	10 (1)	40 (1)	300 (1)	53,54
9	9 (0)	37 (0)	250 (0)	64,40
10	9 (0)	37 (0)	250 (0)	64,44
11	9 (0)	37 (0)	250 (0)	65,08
12	7 (-1,68)	37 (0)	250 (0)	40,51
13	11 (1,68)	37 (0)	250 (0)	46,77
14	9 (0)	31(-1,68)	250 (0)	12,27
15	9 (0)	43 (1,68)	250(0)	36,81
16	9 (0)	37 (0)	150 (-1,68)	16,36
17	9 (0)	37 (0)	350 (1,68)	34,91

Figure 1 depicts the Pareto diagram that allows evaluation of the effects of each parameter on the lipase production. The agitation showed the greatest effect on lipase production. This could be due to the assurance of interaction between enzyme and substrate in a directly proportional way. The second parameter that showed a significant effect was the temperature since it is related to the velocity of an enzyme-catalyzed reaction. However, depending on the temperature range it can also lead to denaturation of the enzyme. Thus, the definition of the biocatalyst to be used depends on temperature and pH conditions. According to the results obtained in this work, among the pH values evaluated, no significant effect of this parameter on the enzymatic activity of lipases was observed.

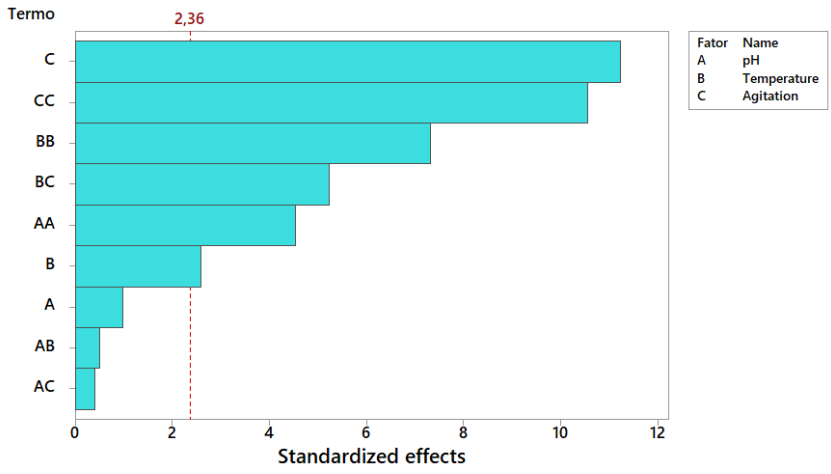

Figure 1 Pareto chart of standardized effects for lipase production by ML-15 strain.

The values obtained for the coefficient of determination (R^2) of 0.9990 and Fcal > F tab (13742.3 > 5.08 x10^{-14}) showed that the model is reliable and statically significant ($p \leq 0.05$). In Equation 1, shown below, significant terms are highlighted in bold. According to Eq. 1, it was observed that quadratic coefficients of the parameters are negative, indicating the existence of an optimal limit that would maximize lipolytic activity. Above this limit any additional increase in the values of these parameters would result in a reduction on enzyme activity.

$$f(x) = \mathbf{-4,47x_1^2 - 0,92x_2^2 - 0,08x_3^2} + 68{,}91\,x_1 + \mathbf{54,40\,x_2} + \mathbf{2,394x_3} + \mathbf{0,27x_1x_2} + 0{,}01x_1x_3 + 0{,}05x_2x_3 - 1619 \quad (1)$$

Although they all have a significant effect on lipase production, agitation showed the major influence on enzyme production, as mentioned before, followed by temperature and finally pH. Agitation above 300 rpm decreases the lipolytic activity, as identified in Figure 2. The effects of agitation were emphasized by others authors. Agitation frequencies higher than 130 rpm increased the production of metabolites toxic to *N. alba* and, consequently, interfere on lipase production [5]. The production of toxic metabolites, such as hydrogen peroxide was directly related to the frequency of agitation of the cultivation of *Pseudomonas*, being observed a decrease in the production of lipases under agitation superior to 200 rpm [10].

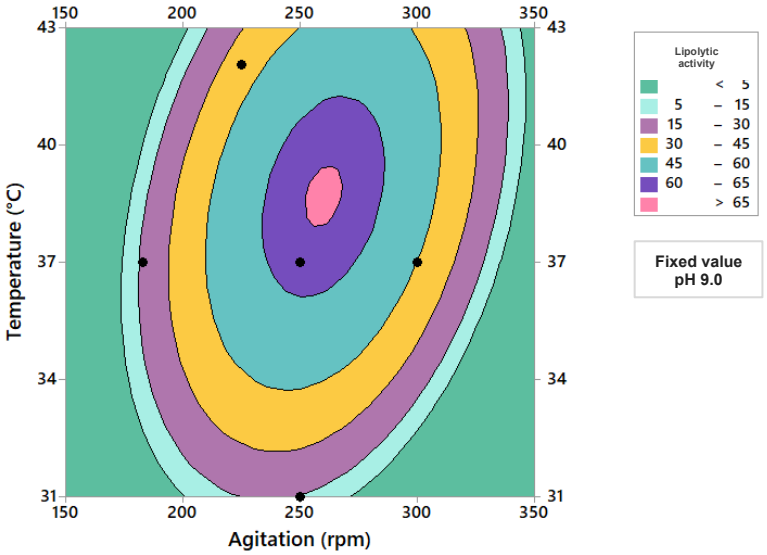

Figure 2 Contour curve of lipase activity of isolate ML-15 and the interaction of independent variables (agitation and temperature) at pH 9.0.

As mentioned before, the temperature is an important parameter to be evaluated because of denaturation effects on protein molecules. Furthermore, most of the lipases act at more basic pH levels and, in this case, the cultivation conditions defined in this work corroborate to the improvement of lipase production. Finally, due to the strong correlation between the predicted values, the empirical results obtained and the analysis of variance of the generated models, the model was considered valid to describe the behavior of lipase production.

4 CONCLUSION

This study aimed to optimize the cultivation conditions for lipase production by ML-15 strain isolated from whey. The parameters pH, temperature, and agitation were evaluated against lipase production. Using a central composite rotational design (DCCR), settings were optimized to maximize the lipolytic activity of the ML-15 isolate. The results revealed that agitation and temperature have the most significant effect on the production of this enzyme. Considering the optimization step, the maximum lipolytic activity increased approximately 6.5-fold about the initial conditions, highlighting the importance of each factor in optimizing lipase production.

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