Journal of the Serbian Chemical Society
JSCS@tmf.bg.ac.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. *87* (9) 997–1010 (2022)
JSCS–5573
Original scientific paper
Published 22 July 2022

Statistical optimization of lipase production from oil mill effluent by *Acinetobacter* sp. KSPE71

SELVAPRIYA KUMARASWAMY[1]* and JAYANTHI SINGARAM[2]

[1]*Department of Industrial Biotechnology, Government College of Technology, Coimbatore, Tamil Nadu-641013, India and* [2]*Department of Civil Engineering, Government College of Engineering, Bodinayakannur, Tamil Nadu-625582, India*

(Received 19 January, revised 26 April, accepted 28 April 2022)

Abstract: The present study investigated the valorisation of oil-rich residues of coconut oil mill effluent (COME) as a potential growth medium for the microbial production of extracellular lipase. The bacterial species isolated from oil mill effluent, *Acinetobacter* sp. KSPE71 was tested for its efficiency to grow and produce lipase in undiluted COME and 0.2 % yeast extract and 0.2 % NH_4Cl supplemented COME. In this connection, the process parameters such as pH, temperature, agitation speed, and inoculum size were optimized to maximize the production using a central composite design in the Response surface methodology. At the optimized state of pH 7.5, 35 °C, 150 rpm with 0.6 % inoculum size, a maximum of 3.95 U mL^{-1} activity was obtained, four-fold higher than the basal condition. At this stage, 73 % of the lipid content was degraded. The present work results imply that the oil mill effluent can be used as a cheaper production medium for lipase and the new isolate *Acinetobacter* sp. KSPE71 as a potential lipase producer. The degradation of oil waste along with the production of the valuable product has multiple advantages of cost reduction of lipase and environmental concern.

Keywords: waste valorisation; recycle of lipid waste; submerged fermentation; oil degradation; enzyme for fat hydrolysis; industrial waste.

INTRODUCTION

The vast interest in lipase is reflected in the numerous potential applications in food, detergent, chiral drug manufacturing, paper industry, biofuel and bioremediation.[1–14] The characteristics of the lipase such as catalyzing both hydrolysis and synthesis reaction and carrying reaction at the interface of the biphasic system, making it an universal enzyme with various applications in all fields. These characteristic features and wide applications of lipase show that it is an industrially potential enzyme, along with protease and amylase. The recent stu-

* Corresponding author. E-mail: selvapriyakumaraswamy@gct.ac.in
https://doi.org/10.2298/JSC220119038K

dies also point out that the market projection of this enzyme will reach 590.2 million USD by the end of 2023.[15] Since many commercial lipase enzymes are available, the selling price is high, mainly due to the increased production cost.[16] This can be reduced by using potential low-cost substrates as fermentation medium, and the productivity can also rise by optimizing the medium composition and physical conditions of fermentation. So, if it is chosen to ferment the medium with inexpensive agricultural and industrial waste, it could significantly reduce the cost of the lipase, while simultaneously increasing the valorisation of waste.

Among several industrial wastes, oil industry waste is considered one of the potential organic materials that can be used to produce a variety of value-added products like biodiesel, biosurfactant, bioplastic, lipase, bioactive compounds and organic acids.[4,17–21] For lipase production, many works were done on the solid--state fermentation of raw agricultural residues or oil cakes.[20,22,23] However, a liquid effluent as a potential medium has been limitedly studied so far, and most of the research on it was done about palm and olive oil effluent.[16,20,24–26] The report on coconut oil mill waste was evaluated by very few researchers[27] and the use of coconut oil in India is also higher than palm and olive oil. So, the present study took coconut oil mill effluent as a production medium.

Previous research on lipase production from oil mill effluent primarily focused on yeast species, with *Yarrowia lipolytica* being the most investigated. For example, after 5 days of incubation on unsupplemented olive oil mill effluent, Mofta *et al.*[24] reported activity of 160 IU dm^{-3} at the optimal conditions of 30 °C, 150 rpm, pH 4.5. Lopes *et al.*[26] investigated the production by *Y. lipolytica* W29 strain on an unsupplemented olive mill effluent and found it to be 26 U L^{-1}. Vera *et al.*[25] tested *Magnusiomyces capitatus* for the production in yeast extract supplement olive oil mill effluent and observed 0.11 U mL^{-1} of activity at pH 6.8, 180 rpm after 72 h. Despite numerous studies on yeast production, there have been no studies on bacteria's ability to produce lipase when cultured on oil refinery effluent. To the best of our knowledge, this is the first time *Acinetobacter* bacteria have been used to produce lipase from oil mill effluent.

Using some low-cost refinery effluent as a medium to reduce the cost of lipase production is a great way to recycle the oil waste, which benefits both the economy and the ecology. In light of this and the limited previous work on coconut oil refinery effluent and *Acinetobacter* sp., this study was planned with the aim to optimize the submerged fermentation of coconut oil mill effluent for lipase production using the newly isolated *Acinetobacter* sp. KSPE71.

EXPERIMENTAL

Materials

All reagents were purchased from HIMEDIA@, and double-distilled water was used in all experiments. Para nitrophenol palmitate was purchased from Alfa Aesar. *p*-Nitrophenol

was purchased from Isochem. The coconut oil mill effluent (COME) was collected from an oil mill in Coimbatore, Tamil Nadu, India, and stored correctly until further use.

Microorganism used

The organism *Acinetobacter* sp. KSPE71 used in this study was previously isolated from coconut oil mill effluent, and biochemical, and molecular characterization was done (not shown here). It was deposited in National Centre for Microbial Resources (NCMR), Pune, Maharashtra, India, with accession number MCC 4758. The culture was subcultured periodically in yeast extract, and peptone broth (YEP) supplemented with 1 % olive oil at 33 °C, 120 rpm, overnight.

Growth characteristics of the isolate in COME

The growth ability of *Acinetobacter* sp KSPE71 was studied. The centrifuged COME sample was diluted to 50 % with Yeast extract peptone broth. 100 % COME, 50 % diluted COME, and YEP were taken as a medium to study growth characteristics. 1 vol. % of overnight grown culture (optical density between 0.8–1) was added to the above COME culture medium and kept at 33±2 °C, 120 rpm for 96 h. Samples were withdrawn every 12 h, and the growth was measured regularly by estimating the biomass. The culture solution was centrifuged at 6000 rpm for 15 min at 4 °C, and the supernatant was used for analyzing the lipase activity. The pellet was recovered by repeated washing and drying in a hot air oven to estimate biomass.

Optimization of nitrogen source for submerged fermentation

COME composition was optimized by adding nitrogen compounds. One factor at a time experiment was done with various organic and inorganic nitrogenous compounds (peptone, yeast extract, malt extract, meat extract, NH_4Cl, $(NH_4)_2SO_4$) to a final concentration of 0.2 %.

Sampling and characterization of COME

Due to the high number of suspended solids, the sample was filtered and centrifuged before using as a medium. The primary characteristics of effluent, such as chemical oxygen demand, pH and total suspended solids, were studied as per the standard method.[28] The total lipid content (g L^{-1}) was determined by the gravimetric method using hexane as an extraction solvent.[27] The reducing sugar concentration was determined by the 3,5-dinitrosalicylic acid (DNS) method and was expressed as glucose equivalents (g L^{-1}).[29] The protein content was measured by Lowry's method and was expressed as bovine serum albumin (g L^{-1}).[30]

Lipase assay

Lipase activity was determined spectrophotometrically using *p*-nitrophenyl palmitate (*p*-NPP) as the substrate described by Winkler and Stuckman with slight modification.[31] The substrate solution was prepared by solvating 30 mg of p-NPP in 10 mL of isopropanol completely, and the solution was added to 90 mL of 50 mM of Tris-HCl buffer (pH 8). 250 µl of enzyme solution was added to 2.25 ml of substrate solution and incubated in a temperature--controlled water bath at 40 °C for 15 min. After 15 min, the assay solution was kept at –20°C for 10 min immediately to stop the reaction and centrifuged for 5 min at 10,000 rpm. The supernatant was used to determine the amount of para nitrophenol released from *p*-NPP by measuring the absorbance at 410 nm. The discharged para nitrophenol concentration was determined from the standard curve by the straight-line:

$$y = 20.285x - 0.0044 \quad (1)$$

One unit of enzyme activity was expressed as one µmol of *p*-nitrophenol released per minute under the assay condition.

Parameter interaction study by RSM

The synergistic effect of physical parameters such as pH, temperature, agitation speed, and inoculum size on lipase activity was optimized using central composite design (CCD) in response surface methodology, in the statistical software package Design-Expert version 13 (State Ease Inc., Minneapolis, MN). A COME supplement with 0.2 % yeast extract and 0.2 % ammonium chloride (NH_4Cl) was taken as a medium for this study. The full 2k composite design was performed to determine the response pattern and interaction of variables, and it gave 31 experiments where eight axial point experiments and seven replicated experiments at a centre point were done. The four factors at three levels (–1, 0, +1) were studied, and their minimum and maximum ranges in actual and coded values are listed in Table S-I (Supplementary material to this paper). These values were fixed based on the previous one-factor studies done for the organism in the chemical defined media and the physical condition of the oil mill effluent collected. The response value (*Y*) of each run was recorded in triplicate and represented in Table I.

TABLE I. Central composite design of four independent factors with the actual and predicted value

Run order	pH	Temperature, °C	Inoculum size, %	Agitation speed, rpm	Lipase activity, U mL^{-1}	
					Actual	Predicted
1	7	35	0.5	150	3.75	3.69
2	7	45	0.5	150	1.08	1.22
3	9	35	0.5	150	2.88	3.02
4	6	30	0.75	100	1.25	1.12
5	6	40	0.25	100	1.48	1.13
6	7	35	0.5	150	3.52	3.69
7	8	30	0.25	200	1.54	1.44
8	8	30	0.25	100	1.50	1.3
9	8	40	0.25	100	1.12	1.1
10	6	40	0.75	100	1.94	1.93
11	7	35	0.5	150	3.53	3.69
12	7	35	0.5	150	3.62	3.69
13	7	35	0.5	50	1.47	1.88
14	8	40	0.75	200	2.92	2.84
15	6	40	0.25	200	1.10	1.16
16	8	40	0.25	200	1.32	1.31
17	7	35	0.5	150	3.70	3.69
18	6	40	0.75	200	1.98	2.03
19	8	30	0.75	200	2.28	2.48
20	7	35	1	150	1.93	2.01
21	5	35	0.5	150	1.62	1.73
22	6	30	0.75	200	1.25	1.16
23	6	30	0.25	200	0.74	0.78
24	7	35	0.5	150	3.92	3.69
25	8	30	0.75	100	2.43	2.27
26	8	40	0.75	100	2.74	2.56
27	7	35	0	150	0	0.18
28	7	35	0.5	250	2.27	2.11

TABLE I. Continued

Run order	pH	Temperature, °C	Inoculum size, %	Agitation speed, rpm	Lipase activity, U mL^{-1}	
					Actual	Predicted
29	6	30	0.25	100	0.85	0.82
30	7	35	0.5	150	3.79	3.69
31	7	25	0.5	150	0.44	0.56

The experimental data were analyzed in the multiple regression procedure using the below second-order polynomial:

$$Y = \beta_0 + \Sigma\beta_i x_i + \Sigma\beta_{ii} x_i^2 + \Sigma\Sigma\beta_{ij} x_i x_j^2 \quad (2)$$

where Y is predicted response, β_0 is the intercept term, β_i is the linear coefficient, β_{ii} is the quadratic coefficient, β_{ij} is the interaction coefficient, and x_i and x_j are independent variables. The data obtained from CCD for lipase activity was subjected to ANOVA (analysis of variance) to assess the significance of the model (Table II).

TABLE II. ANOVA analysis for the response surface quadratic model for lipase activity

Source	Sum of squares	Degree of freedom	Mean square	F-value	p-value
Model	36.25	14	2.59	57.1	< 0.0001
A – pH	2.5	1	2.5	55.22	< 0.0001
B – Temperature	0.6817	1	0.6817	15.03	0.0013
C – Agitation speed	0.0823	1	0.0823	1.81	0.1968
D – Inoculum size	5.05	1	5.05	111.35	< 0.0001
AB	0.2609	1	0.2609	5.75	0.029
AC	0.0311	1	0.0311	0.6854	0.4199
AD	0.4372	1	0.4372	9.64	0.0068
BC	0.0049	1	0.0049	0.109	0.7456
BD	0.2442	1	0.2442	5.39	0.0338
CD	0.0057	1	0.0057	0.125	0.7283
A^2	3.07	1	3.07	67.74	< 0.0001
B^2	14.02	1	14.02	309.2	< 0.0001
C^2	5.12	1	5.12	112.83	< 0.0001
D^2	12.04	1	12.04	265.59	< 0.0001
Residual	0.7256	16	0.0453		
Lack of fit	0.6	10	0.06	2.87	0.1049
Pure error	0.1256	6	0.0209		
Corrected total	36.98	30			
R^2	0.9804				
Adjusted R^2	0.9632				
Predicted R^2	0.9019				
Adequate precision	23.7179				
Coefficient of variation, %	10.32				

RESULTS AND DISCUSSION

Growth characteristics of the isolate

The isolate's ability to grow in COME was studied by using 50 % diluted COME with YEP, 100 % COME, and comparing growth in synthetic YEP medium (Fig S-1). Since the isolate was subcultured in YEP broth, the same broth was taken for the dilution of the COME medium. Though the isolates can grow in 100 % COME, the final biomass concentration (4.1 g L^{-1}) was low in comparison to the 50 % diluted COME medium (6.48 g L^{-1}) and synthetic YEP medium (4.91 g L^{-1}). The lag phase was also extended from 6 h (YEP) to 12–24 h (YEP + 50 % COME and 100 % COME). The lipase activity of these three media was also investigated and the maximum activity of YEP, 50 and 100 % COME was 0.086 (14 h), 1.18 (60 h) and 0.34 U mL^{-1} (72 h), respectively. The lipase production was found to be highest when the cell population entered the stationary phase, implying that the enzyme secretion is growth-dependent. As a result, the increase in enzyme production was primarily related to an increase in cell mass.

These results clearly show that YEP + 50 % COME medium stimulates increased growth and lipase activity. Followed by 50 % COME, YEP medium demonstrated higher growth activity, while 100 % COME demonstrated higher lipase activity. These findings indicate that adding a nitrogen source increased both growth and lipase production. This could be due to a lack of nitrogen in the COME medium, as protein building blocks are needed for both cell proliferation as well as for enzyme production. Similar findings have been reported by Vera *et al.*[25] and Moftah *et al.*[24] who supplemented olive mil waste with nitrogen sources and reported increased growth and lipase activity, in comparison to the unsupplemented oil waste for the yeast *M. capitatus* and *Y. lipolytica*, respectively.

The growth in the 100 % COME medium suggests that the presence of sugars and residual lipids in COME may act as a carbon source. The decreased lipase activity in the YEP medium denotes that the lipase from *Acinetobacter* sp. is inducible and lipids in the COME medium act as inducers. Vera *et al.*[25] reported a 3-fold higher increase in the olive mil waste medium supplemented with yeast extract and olive oil, than in oil mill wastewater (OMW) supplemented with yeast extract alone for yeast *M. capitatus*.

Optimization of nitrogen source for submerged fermentation

From growth characteristics studies, it is evident that adding nitrogen sources to the COME medium increases the lipase activity along with growth. The COME medium was supplemented with several nitrogen sources to boost the lipase production, and the choice of nitrogen source is critical for inducing lipase production as well as economic considerations. 100 % COME medium was used

as control. The effect of various nitrogen sources on enzyme production by *Acinetobacter* sp. KSPE71 was shown in Fig. S-2 of the Supplementary material.

From the result, it is evident that both organic and inorganic nitrogen sources enhance the production of lipase. Among the organic nitrogen sources, yeast extract showed a two-fold increase in the activity followed by peptone with a 1.8--fold increase. Contrary to other studies that reported peptone gave better enzyme production, in this study yeast extract provided good growth and production.[32] This could be due to the presence of vitamins and other trace elements in the yeast extract that might complement nutrients which are lacking in the COME medium. Kanmani *et al.*[27] discovered that adding the basal salt medium, containing coconut oil mill waste with yeast extract and peptone, increased *Staphylococcus pasteuri* lipase production from 9.95 to 22.28 U mL^{-1}. However minimal supplements were not employed in this study. Inorganic sources such as ammonium chloride were found to increase activity by 53 % compared to control. NH_4Cl is very soluble in water and easily releases ammonium ions in aqueous conditions due to its high ionic strength. This readily available nitrogen enhances cell growth and can be quickly integrated into other amino acids, making it a crucial component of the protein synthesis process.

Malt extract, meat extract and ammonium sulphate gave lower activity compared to other nitrogen sources. So, the choice of nitrogen was left with yeast extract, peptone and ammonium chloride. Even though peptones have an inciting effect on the development of lipase enzymes, making them one of the most important organic nitrogen sources along with carbon sources, the cost is higher than other sources. Since this work was mainly focused on low-cost medium with wastewater, giving expensive nitrogen sources like peptone is not fulfilling the purpose. So further studies were carried out by the supplementation of COME with both yeast extract and ammonium chloride. The supplementation of both organic and inorganic nitrogen sources gave activity of 0.91 U mL^{-1} which is a 2.7-fold increase in the activity compared to 100 % COME.

Characterization of COME

The physiochemical characteristics of raw COME taken in this study were pH 8.2, with chemical oxygen demand 12.7 g L^{-1}, total suspended solids 18 g L^{-1}, reducing sugar 2.03 g L^{-1}, protein content was 0.57 g L^{-1} and lipid was 3.8 g L^{-1}.

Optimization of process parameters

The interaction between the variables pH, temperature, inoculum size and agitation speed were studied in the CCD method by conducting 31 experiments. The maximum lipase activity level was taken as the optimum value for the factors. The predicted and observed values, along with the design matrix was represented in Table I. An analysis of variance ANOVA was performed to assess the model's validity (Table II). The second-order regression equation provided the

levels of lipase activity as a function of pH, temperature, inoculum concentration, and agitation speed, which can be presented in terms of coded factors as in the following equation:

$$Y = 3.69 + 0.3230A + 0.1685B + 0.0585C + 0.4587\ \mathrm{D} - 0.1277AB + \\ + 0.0441AC + 0.1653AD + 0.0176BC + 0.1236BD + 0.0188CD - \\ - 0.3278A^2 - 0.7003B^2 - 0.4230C^2 - 0.6490D^2 \quad (3)$$

Where Y is the lipase activity (U) and A, B, C, D are coded factors for pH, temperature, agitation speed and inoculum size, respectively.

The significance of the model developed was examined by Fisher's statistics analysis (F value 57.01) and significance (p value < 0.05) value. The insignificance of lack of fit further confirms the model developed for lipase production.

The correlation coefficient (R^2) is a mark of variation in the response due to the model rather than an error. The value of 0.9807, which is close to 1, can account for up to 98.07 % of the variation in the response. The variation between the adjusted R^2 and predicted R^2 is less than 0.2, which implies the model's acceptability.

The model's suitability was also confirmed by the signal-to-noise ratio measurement of adequate precision, that is greater than 4, which is desirable. The value 23.744 denotes low noise in the design and indicates that the model can navigate the space. Based on the ANOVA results, it can be confirmed that the model is highly significant and appropriate to predict the response within the limit of the experimental factors.

In this study, ten model terms were found to be significant, and the factors with predominant effects were the linear coefficients A, B, D, the interaction coefficients AB, AD, BD and quadratic term coefficients A^2, B^2, C^2, D^2 with p value < 0.05.

Response surface plots

The graphical representation of the regression equation can be plotted as a three-dimensional surface representing the interactive effect of factors on lipase activity for two independent factors, while keeping the other factors at the central level (0). The interaction effect of the independent variable on lipase activity was shown as 3D surface plots in Fig. 3a–f.

Fig 3a shows the interaction of initial pH and temperature of the medium with respect to lipase activity. The activity increased with the pH and temperature, optimum values being 7 and 35 °C. Further, an increase in both factors reduces the activity. This could be due to: 1) growth restriction in the *Acinetobacter* sp. KSPE71 and 2) lipase instability. Any factors and levels of factors supporting the above two conditions would reduce the lipase activity. Both the initial pH and temperature play a crucial role in the growth and metabolism of cells, and the stability of lipase. Selection of the initial pH of the medium is more important,

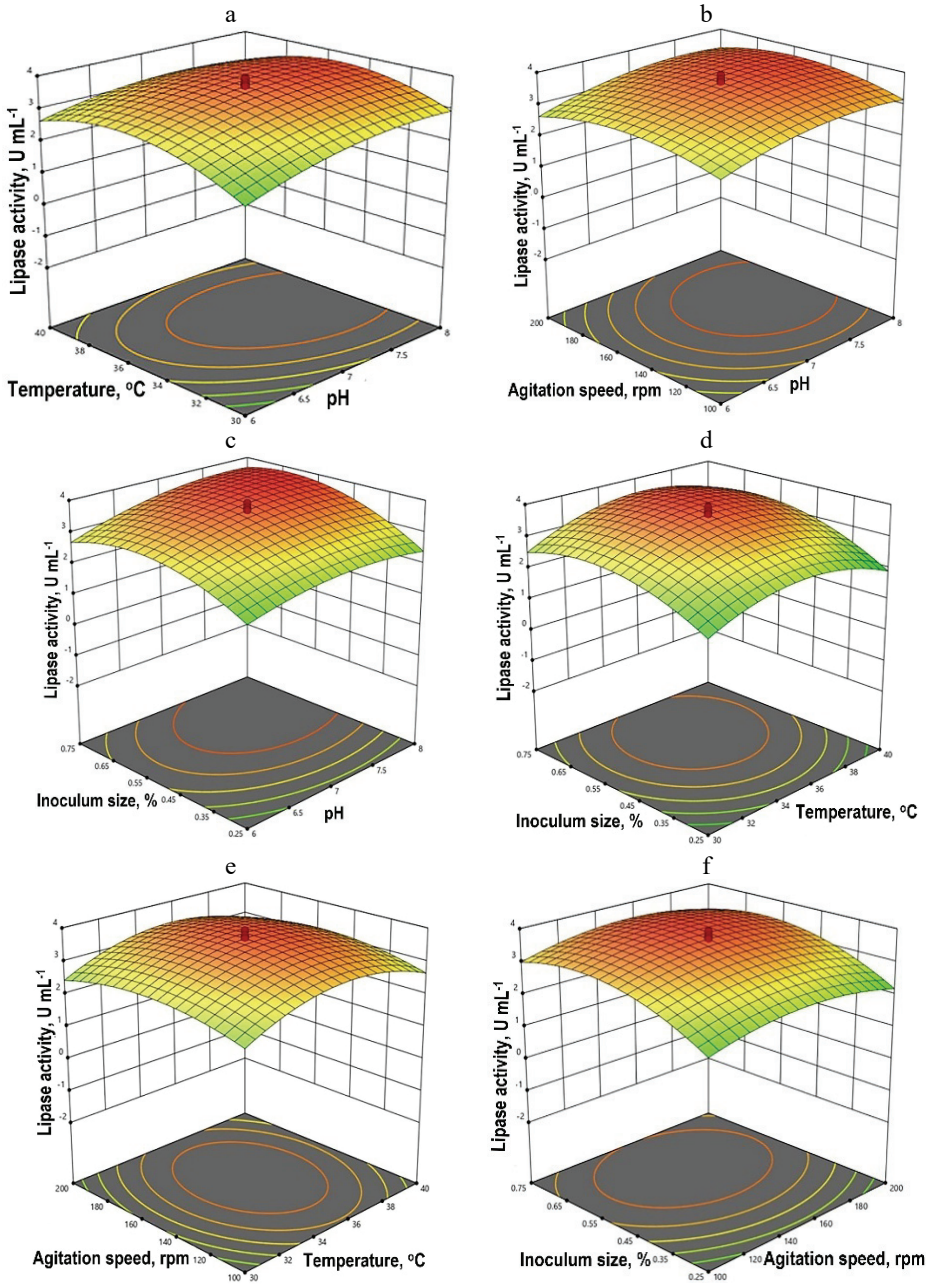

Fig. 3. 3D surface plot of lipase activity showing an interaction between pH and temperature (a), pH and agitation speed (b), pH and inoculum size (c), temperature and inoculum size (d), temperature and agitation speed (e) and agitation speed and inoculum size (f).

since the release of free fatty acids, or any other organic acid during the fermentation process, may lower the pH of the medium, impeding its growth and production.[21] Apart from this, most of the lipase production and stability are favoured by the neutral and slightly alkaline conditions than acidic pH.[33] In the same way the extreme levels of temperature affect enzymes involved in the metabolic activity, and also cause thermal instability in secreted lipase. From the results, it's clear that both growth conditions of *Acinetobacter* sp. KSPE71 (data not shown) and lipase production show optimum pH and temperature of 7±0.5, and 35±2 °C, respectively. This fact again proves that the lipase from *Acinetobacter* sp. KSPE71 was a growth-associated product. According to a few reports, the degree of lipase production by *Acinetobacter* sp. varies depending on the initial pH, with neutral pH being the most prevalent and the temperature in the range of 30 to 35 °C.[32–35] This variation could be due to the source of the isolated organism as well as to the climatic conditions of the location.

The interaction of inoculum size with pH and temperature is illustrated in Fig. 3c and d. The acclimation of the cell and the quantities of enzymes generated to assist the cell metabolism are affected by the number of bacteria in the media.

The enzyme activity rose concomitantly with inoculum size to 0.5 %, after that activity got reduced by increasing inoculum size. This could be caused by an overpopulated culture which would limit the nutrient availability and the dissolved oxygen concentration, resulting in a rapid death phase and reduction in lipase activity. In low inoculum size, the slow growth initiation and the long lag period is high, when compared to the large inoculum size. As a result, not only the affected growth diminishes the production, but it also lengthens the incubation time. So the optimal size of 0.5 % gave higher activity. Since we are using waste as a medium with limiting nutrients, the inoculum size should be proper for maximum benefits. The high F value in all linear, two-factor interaction, and quadratic terms clearly indicates the importance of inoculum size in enzyme production from waste media. A similar study was done by Lopes *et al.*[21] who reported a 36 % reduction in enzyme activity at higher cell concentration than low cell concentration in olive mill waste medium.

Fig. 3b, e and f illustrate the interaction of agitation speed with pH, temperature, and inoculum size. Even though the significance and F value show that the agitation speed has the least importance in the single and two-factor interactions, the quadratic term indicates the importance of agitation speed. It means that increasing and decreasing the speed to extreme conditions may result in a significant reduction in the production rate. This could be related to the fact that the proper mixing conditions are required to provide adequate lipid dispersion in the medium. It would make fatty carbon sources more accessible to organisms and promote oxygen transport.[21] At the same time, the faster the speed, the greater the shear stress on cells and the structure of the enzyme which reduces activity.

In the present work, there was a 2.5-fold increase and 1.6-fold decrease in the enzyme activity in optimum speed, when compared to low and high-speed conditions. These agree with the obtained results of Isiaka *et al.,* and Gururaj *et al.,*[32,36]

Validation of the model

The major goal of this research was to employ a central composite design model to find the best conditions of process parameters to obtain the maximum lipase production in the oil waste medium. So, the validity of the model was investigated further by carrying out an additional experiment using the conditions mentioned in the table S-II of the Supplementary material. The highest predicted activity was found to be 3.88 U mL^{-1} at optimized conditions of pH, temperature, inoculum size, and agitation speed respectively at 7.5, 35 °C, 0.6 % and 150 rpm. The activity of 3.95 U mL^{-1} was obtained, which was 4.3 times higher activity than the non-optimized condition. The obtained value was closely agreed with the predicted value and also shows a difference of 2.67 % between predicted and actual experimental values. The obtained response model demonstrates that a difference of less than 10 % makes the model valid. Based on the findings of this study, it is possible to conclude that the model developed can be trusted to forecast the lipase production from the oil mill waste.

Lipid degradation in COME

The oil in the wastewater is one important ingredient that must be adequately removed; otherwise, direct disposal will result in de-oxygenation of water, causing irreversible damage to aquatic life. Many studies have been conducted on the use of lipase enzymes in the pre-treatment of oil wastes, which results in good oil degradation.[11,12] However, because the enzyme is a delicate protein molecule that is sensitive to the field conditions, controlled shake flask experiments can't predict the results of enzymes for environmental applications. Instead, using the organic-rich oil waste as a medium to produce valuable products like in this study, is a more rational approach.

Totally 73 % reduction in oil was observed in the given condition of 0.6 % inoculum size, pH 7.5, at 35 °C, 150 rpm at 48 h. The obtained degradation value was low compared to the work of Kanmani *et al*.[27] and Annibale *et al.*[37] who reported 90 % degradation by the bacteria *Staphylococcus aureus* at 72 h and untraceable lipid levels after 96 h of fermentation by *Caulerpa cylindracea* NRRL Y-17506, respectively. This increased performance of the organism might be due to the high nutrient supplements in the oil waste medium, contrary to our work with limited supplements. These high nutrients have a positive influence on the growth of the organism as well as on the lipase production, both of which can decrease the oil content in the medium.

CONCLUSION

This study showed that COME is a potential organic factory, and it could be used for growth and lipase production for the isolate, which can imply for another organisms also. This production was further enhanced by supplying minimal nitrogen sources and optimizing the physical conditions of the fermentation, using response surface methodology. Since the optimized conditions used for the production are similar to physical conditions of the effluent collected, the need for pre-treatment of the waste before fermentation was not needed. From a perspective of large-scale production, this would make an enormous impact on the cost of the final product. The lipid degradation in wastewater, along with the high value-added product, may be an efficient solution for the environmental management of oil waste. It would be appreciable to consider industrial waste for further optimization of certain productions with minimal change.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/11575, or from the corresponding author on request.

Acknowledgment. This dataset is the outcome of the experiments carried out in the Department of Industrial Biotechnology, Government College of Technology, Coimbatore, India, and the authors are thankful for all the support the institution provides.

ИЗВОД

СТАТИСТИЧКА ОПТИМИЗАЦИЈА ПРОИЗВОДЊЕ ЛИПАЗЕ ИЗ ОТПАДА УЉАРЕ ПРИМЕНОМ *Acinetobacter* SP. KSPE71

SELVAPRIYA KUMARASWAMY[1] и JAYANTHI SINGARAM[2]

[1]*Department of Industrial Biotechnology, Government College of Technology, Coimbatore, Tamil Nadu 641013, India и* [2]*Department of Civil Engineering, Government College of Engineering, Bodinayakannur, Tamil Nadu – 625582, India*

У раду је описана евалуација отпада кокосовог уља из уљаре (COME) као потенцијалног медијума за раст микроорганизама који производе ванћелијску липазу. Из уљног отпада је изолована бактерија *Acinetobacter* sp. KSPE71 и тестирана је њена способност да расте и производи липазу у неразблаженом медијуму COME, у присуству 0,2 % екстракта квасца и 0,2 % NH_4Cl. Оптимизација процеса у погледу pH, температуре, брзине мешања и величине инокулума, а у циљу постизања максималне производње, је урађена користећи метод одзивних површина. Под оптималним условима: pH 7,5, 35 °C, 150 rpm и 0,6 % инокулума, постигнута је максимална производња, изражена као активност од 3,95 U mL^{-1}, што је четири пута више од активности у основним условима. Под наведеним условима разложено је 73 % липидног сарджаја. Добијени резултати упућују на примену уљног отпада из уљара као јефтиног медијума за производњу липазе, као и изолата *Acinetobacter* sp. KSPE71 као потенцијалног произвођача липазе. Истовремено разлагање уљног отпада и производња корисног састојка има вишеструки значај у смањењу трошкова производње липазе и смањењу загађења животне средине.

(Примљено 19. јануара, ревидирано 26. априла, прихваћено 28. априла 2022)

REFERENCES

1. C. E. C. de Souza, B. D. Ribeiro, M. A. Z. Coelho, *Appl. Biochem. Biotechnol.* **189** (2019) 933 (https://doi.org/10.1007/s12010-019-03047-5)
2. C. Bernal, F. Guzman, A. Illanes, L. Wilson, *Food Chem.* **239** (2018) 189 (https://doi.org/10.1016/j.foodchem.2017.06.105)
3. R. Balakrishnaraja, V. Nisha, S. Geethadevi, C. Monisha, S. Devi, M. Pravinkumar, J. Revathi, K. Selvapriya, *J. Adv. Chem.* **12** (2016) 5010 (https://rajpub.com/index.php/jac/article/view/1052)
4. L. C. Phukon, R. Chourasia, M. Kumari, T. K. Godan, D. Sahoo, B. Parameswaran, A. K. Rai, *Bioresour. Technol.* **309** (2020) 123352 (https://doi.org/10.1016/J.BIORTECH.2020.123352)
5. E. Vanleeuw, S. Winderickx, K. Thevissen, B. Lagrain, M. Dusselier, B. P. A. Cammue, B. F. Sels, *ACS Sustainable Chem. Eng.* **7** (2019) 15828 (https://doi.org/10.1021/acssuschemeng.9b03257)
6. G. B. Pinto, F. M. L. Mendes, A. M. de S. Antunes, *Mini-Rev. Org. Chem.* **17** (2020) 701 (https://doi.org/10.2174/1570193X16666190913181530)
7. F. J. Contesini, M. G. Davanço, G. P. Borin, K. G. Vanegas, J. P. G. Cirino, R. R. de Melo, U. H. Mortensen, K. Hildén, D. R. Campos, P. de O. Carvalho, *Catalysts* **10** (2020) 1032 (https://doi.org/10.3390/catal10091032)
8. A. Sharma, S. Balda, N. Gupta, N. Capalash, P. Sharma, *J. Cleaner Prod.* **271** (2020) 122573 (https://doi.org/10.1016/J.JCLEPRO.2020.122573)
9. S. Hama, H. Noda, A. Kondo, *Curr. Opin. Biotechnol.* **50** (2018) 57 (https://doi.org/10.1016/J.COPBIO.2017.11.001)
10. F. L. C. Almeida, B. M. Travália, I. S. Gonçalves, M. B. S. Forte, *Biofuels, Bioprod. Biorefin.* **15** (2021) 1141 (https://doi.org/10.1002/BBB.2183)
11. Z. Ning, J. Ji, Y. He, Y. Huang, G. Liu, C. Chen, *Energy Fuels* **30** (2016) 7326 (https://doi.org/10.1021/acs.energyfuels.6b01097)
12. C. H. Okino-Delgado, D. Z. do Prado, R. Facanali, M. M. O. Marques, A. S. Nascimento, C. J. da C. Fernandes, W. F. Zambuzzi, L. F. Fleuri, *PLoS One* **12** (2017) e0186246 (https://doi.org/10.1371/JOURNAL.PONE.0186246)
13. N. Sarmah, D. Revathi, G. Sheelu, K. Y. Rani, S. Sridhar, V. Mehtab, C. Sumana, *Biotechnol. Prog.* **34** (2017) 5 (https://doi.org/10.1002/BTPR.2581)
14. X. Li, N. Shimizu, E. R. Rene, M. C. Veiga, *Fermentation* **7** (2021) 284 (https://doi.org/10.3390/FERMENTATION7040284)
15. *Markets and Markets*, https://www.marketsandmarkets.com/PressReleases/microbial-lipase.asp (accessed October 24, 2021)
16. M. Lopes, S. M. Miranda, I. Belo, *Crit. Rev. Environ. Sci. Technol.* **50** (2020) 2583 (https://doi.org/10.1080/10643389.2019.1704602)
17. T. Ahmad, T. Belwal, L. Li, S. Ramola, R. M. Aadil, Abdullah, Y. Xu, L. Zisheng, *Trends Food Sci. Technol.* **99** (2020) 21 (https://doi.org/10.1016/j.tifs.2020.02.017)
18. H. Kamilah, A. Al-Gheethi, T. A. Yang, K. Sudesh, *Arab. J. Sci. Eng.* **43** (2018) 3453 (https://doi.org/10.1007/s13369-018-3118-1)
19. P. Carlozzi, M. Seggiani, A. Capperucci, D. Tanini, P. Cinelli, A. Lazzeri, *J. Biotechnol.* **295** (2019) 28 (https://doi.org/10.1016/j.jbiotec.2019.02.006)
20. T. Szymczak, J. Cybulska, M. Podleśny, M. Frąc, *Agriculture* **11** (2021) 540 (https://doi.org/10.3390/agriculture11060540)
21. M. Lopes, S. M. Miranda, J. M. Alves, A. S. Pereira, I. Belo, *Eur. J. Lipid Sci. Technol.* **121** (2019) 1800188 (https://doi.org/10.1002/ejlt.201800188)

22. R. K. Sahoo, A. Das, M. Gaur, A. Sahu, S. Sahoo, S. Dey, P. K. S. M. Rahman, E. Subudhi, *Prep. Biochem. Biotechnol.* **50** (2020) 578 (https://doi.org/10.1080/10826068.2020.1719513)
23. S. Steudler, A. Werner, T. Walther, *Solid State Fermentation, Advances in Biochemical Engineering/Biotechnology,* Springer, Switzerland AG, Cham, 2019, pp. 51–81 (https://doi.org/10.1007/10_2019_85)
24. O. A. S. Moftah, S. Z. Grbavcic, W. A. S. Moftah, N. D. Lukovic, O. L. Prodanovic, S. M. Jakovetic, Z. D. Kneževic-Jugovic, *J. Serb. Chem. Soc.* **78** (2013) 781 (https://doi.org/10.2298/JSC120905005M)
25. V. Salgado, C. Fonseca, T. Lopes da Silva, J. C. Roseiro, A. Eusébio, *Waste Biomass Valorization* **11** (2020) 3207 (https://doi.org/10.1007/S12649-019-00725-7)
26. M. Lopes, C. Araújo, M. Aguedo, N. Gomes, C. Gonçalves, J. A. Teixeira, I. Belo, *J. Chem. Technol. Biotechnol.* **84** (2009) 533 (https://doi.org/10.1002/JCTB.2075)
27. P. Kanmani, K. Kumaresan, J. Aravind, *Electron. J. Biotechnol.* **18** (2015) 20 (https://doi.org/10.1016/j.ejbt.2014.11.003)
28. A. Eaton, *Standard Methods for the Examination of Water and Wastewater*, 21st ed., APHA-AWWA-WEF, Washington DC, 2005
29. G. L. Miller, *Anal. Chem.* **31** (2002) 426 (https://doi.org/10.1021/ac60147a030)
30. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193** (1951) 265 (https://pubmed.ncbi.nlm.nih.gov/14907713/)
31. U. K. Winkler, M. Stuckmann, *J. Bacteriol.* **138** (1979) 663 (https://doi.org/10.1128/jb.138.3.663-670.1979)
32. P. Gururaj, S. Ramalingam, G. Nandhini Devi, P. Gautam, *Braz. J. Microbiol.* **47** (2016) 647 (https://doi.org/10.1016/j.bjm.2015.04.002)
33. M. O. Al-Limoun, K. M. Khleifat, K. Y. Alsharafa, H. N. Qaralleh, S. A. Alrawashdeh, *Biocatal. Biotransform.* **37** (2019) 139 (https://doi.org/10.1080/10242422.2018.1506445)
34. M. O. Allimoun, M. R. Ananzeh, K. M. Khleifat, *Int. J. Biosci.* **6** (2015) 44 (https://doi.org/10.12692/ijb/6.10.44-56)
35. R. Patel, V. Prajapati, U. Trivedi, K. Patel, *3 Biotech* **10** (2020) 508 (https://doi.org/10.1007/S13205-020-02501-0)
36. A. Isiaka Adetunji, A. Olufolahan Olaniran, *Biotechnol. Biotechnol. Equip.* **32** (2018) (https://doi.org/10.1080/13102818.2018.1514985)
37. A. D'Annibale, G. G. Sermanni, F. Federici, M. Petruccioli, *Bioresour. Technol.* **97** (2006) 1828 (https://doi.org/10.1016/J.BIORTECH.2005.09.001).