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Effect of process parameters on lipase production by *Candida cylindracea* in stirred tank bioreactor using renewable palm oil mill effluent based medium

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ABSTRACT

A two-level full factorial design (FFD) was employed to determine the effects of process parameters on lipase production by *Candida cylindracea* ATCC 14830 in palm oil mill effluent (POME)-based medium. Ten experimental runs based on three parameters (temperature, agitation and aeration) as indicated by the FFD were carried out in a stirred-tank bioreactor. On statistical analysis of the results, the optimum temperature, aeration and agitation rates were found to be 30 °C, 1.0 vvm and 400 rpm respectively, with a maximum activity of 41.46 U/ml after 36 h of fermentation. Analysis of variance (ANOVA) showed a high coefficient of determination (R^2) value of 0.999, indicating a satisfactory fit of the model with the experimental data. All the three parameters were statistically significant at $p < 0.05$. The validation experiment also confirmed that apart from lipase production, there was an increase in chemical oxygen demand (COD) removal throughout the fermentation period.

1. Introduction

Lipases (E.C. 3.1.1.3) have the ability to catalyze several reactions of industrial interest in addition to hydrolysis, such as esterification and transesterification [1]. The unique properties of lipase catalyzed reactions such as chemoselectivity, regioselectivity, stereoselectivity, non-requirement of cofactors and stability in organic solvents make microbial lipase a versatile biocatalyst in many industrial applications [2,3] such as foods, cosmetics, detergents, biosensors, pharmaceuticals [4,5], and recently in the field of bioenergy especially in biodiesel production [6]. Thus, the demand for a highly active preparation of lipolytic enzymes has led to an increase in the research especially on the development of culture and operational strategies for enhanced lipase production [7].

Microbial lipases are receiving more attention because of the cheaper cost of production and use of renewable resources. *Candida cylindracea* is among the most extensively studied microorganisms by biotechnologists with respect to its powerful lipase production capacity [8]. Several factors have been reported to affect the extracellular lipase production such as pH, temperature, aeration and medium composition [9]. In addition, other factors include the type and volume of inoculum, agitation and parameters related to bioreactor design such as impeller configuration, impeller spacing and diameter [8]. Therefore, the improvement in productivity of lipases can be achieved through manipulation of nutritional as well as physical parameters [10]. This can serve as an inference for commercial success of any industrial production process.

Several strategies have been used to model the fermentation process and to optimize the process parameters using conventional and statistical experimental designs. Lipase production by a Brazilian wild strain of *Yarrowia lipolytica* was studied at different stirring speeds and airflow rates; the results revealed that the stirring speed determines the pronounced effect of oxygen for lipase production [1]. In case of *Rhodotorula mucilaginosa* MTCC 8737, lipase production using molasses as a sole production medium was influenced by aeration and agitation; suggesting that mixing is very crucial for the maximum productivity [11]. Similarly, Valero et al. [7] discovered that effective aeration was necessary for lipase production by *Candida rugosa* and a shortage of oxygen during the fermentation restricted its production. In addition, Sokolovska et al. [12] found out that there was need for efficient air flow to maintain the oxygen saturation level for *C. cylindracea* CBS 6330 during lipase production. Moreover, Wu et al. [13] revealed that temperature is essential in regulating the synthesis of several enzymes, including both intracellular and extracellular enzymes. For extracellular enzymes, temperature influences their secretion possibly by changing the physical properties of the cell membrane.

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It is noteworthy that during fermentation, the operating conditions interact and influence their respective effects on the response. Thus, it is important that the techniques to be used account for these interactions so that a set of optimal process conditions can be determined. Factorial designs have been successfully used to optimize and evaluate the effects of operating parameters in the production of enzymes and other metabolites [14,15]. This is because industrial fermentation is moving away from traditional and largely empirical operation towards a knowledge based and a better-controlled process.

Considering the abundance of palm oil mill effluent (POME) as a cheap renewable residue especially in Malaysia where palm oil production is the major agricultural industry. The present investigation is directed towards large scale production using factorial experimental design to evaluate the effects of operational parameters on the production of lipases from *C. cylindracea* in POME-based medium. Several potential low-cost fermentation substrates have been reported in the literature, such as cheese whey [16], olive mill waste water [17], molasses [11], biodiesel by-product in form of glycerol [18] and vegetable oil processing waste [19], among others. However, in order to exploit the economy of lipase fermentation, this study was aimed at enhancing the lipase production besides using a renewable raw material (POME-based medium) by significant reduction in fermentation time. This is due to the fact that using agro-industrial residues as cheap substrates coupled with the optimization of process parameters (which favors the reduction in fermentation time) will undoubtedly decrease the total production costs as well as increased lipase productivity. A proper formulation and optimization of the POME-based medium was earlier reported in shake flask cultures by *C. cylindracea* ATCC 14830 based on statistical experimental design; involving Plackett–Burman (PB) design, one-factor-at-a-time (OFAT) method and face-centered central composite design (FCCCD); which led to a maximum lipase production of 20.26 U/ml [20].

2. Materials and methods

2.1. Sample collection

Palm oil mill effluent (POME) was collected from West Oil Mill of Sime Darby Sdn Bhd. Carey Island, Malaysia in clean containers and immediately brought to the laboratory and stored at 4 °C.

2.2. Microbial culture and inoculum preparation

C. cylindracea ATCC 14830 was obtained from American Type culture collection. The strain was grown on the potato dextrose agar (PDA) plates at 28 °C for 4 days and subcultured every two weeks. It was then maintained and preserved at 4 °C. Four-day old PDA-plate culture of *C. cylindracea* ATCC 14830 was suspended in 10 ml of sterile distilled water and this suspension was used as the inoculum for pre-cultures. Incubations were carried out at 28 °C for 48 h at 150 rpm in 100 ml of seed medium containing Tween-80 (0.65%, v/v), olive oil (0.20%, v/v) and peptone (0.45%, w/v) to yield a microbial density of about 10^8 cells/ml.

2.3. POME based medium preparation

The fermentation medium was optimized based on our previous study [20]. The optimized POME based medium contains POME sample of 1.0% (w/v) total suspended solids (TSS) supplemented with 0.2% (v/v) olive oil, 0.65% (v/v) Tween-80 and 0.45% (w/v) peptone, adjusting the initial pH to 6.0.

2.4. Bench-top stirred tank bioreactor

Experiments were conducted in 2-L Biostat (Sartorius BBI Systems) bioreactor filled with 1-L of POME based medium and sterilized in situ at 121 °C and 15 psi for 15 min. The reactor was equipped with four baffles, two six-flat-blade impellers and the fermentation parameters were controlled by a digital control system. Standard operating conditions (temperature, agitation and aeration) were set according to the experimental matrix of full factorial design and the pH was not controlled during the fermentation. The reactor was inoculated with 2.2% (v/v) of actively growing cells of *C. cylindracea* from 48 h-Erlenmeyer flask cultures. All fermentations were repeated to check the reproducibility of the experimental results and samples were analyzed in triplicate.

2.5. Design of experiment by full factorial design

In order to evaluate the factors that influence lipase production in POME based medium, a two-level and three-factor full factorial experiment was designed with two center points. Temperature, agitation and aeration were chosen as independent variables and the lipase activity (U/ml) was taken as the response of the design. The levels and the range of the independent variables were examined in two levels: '−1' for low level and '+1' for high level. Thus ranges of 25–35 °C, 300–500 rpm and 0.5–1.5 vvm were considered for temperature, agitation and aeration respectively using the average of these ranges as the center point.

Practical considerations were used in selecting the three factors and the range in which they were varied. Thus, aeration ensures the supply of necessary oxygen for the growth and performance of microbial cells, while agitation is important for uniform mixing of the medium components within the bioreactor (dispersion of cells and nutrients) as well as mass transfer phenomena. In the same way, temperature selection was based on the mesophilic nature of *C. cylindracea*.

MINITAB release 14 statistical software was used to create and analyze the experimental data, in order to measure the effect of various factors on lipase production. A total of 10 experiments with 2 center points were generated and the detailed experimental design with coded and actual values of the three factors was shown in Table 1. The factorial experimental design was based on the first order model:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC \quad (1)$$

where Y is the dependent variable (lipase production); β_0 is an intercept term; β_1, β_2 and β_3 are linear coefficients; β_{12}, β_{13} and β_{23} are the interaction coefficients; and A, B and C are the independent variables representing temperature, agitation and aeration respectively.

The coefficients of all effects, the ANOVA, coefficient of determination (R^2), three-dimensional (3D) and two-dimensional (2D) plots were examined to evaluate the model as well as to determine the best operating conditions for the production.

2.6. Validation of the model and kinetics of lipase production

The accuracy of the predicted model was verified with respect to all the three parameters using some sets of experiments both within and outside the design space. The experiments were carried out in a 2-L Biostat (Sartorius BBI Systems) bioreactor with a working volume of 1.0 L. The run that showed the highest lipase production was further studied by sampling every six hours to determine the lipase activity trend, pH, biosolids, protein content and chemical oxygen demand (COD).

Table 1
Full factorial design of three independent variables with two center points showing the actual and coded values along with the experimental and predicted response.

Run order	Temperature (°C)	Agitation (rpm)	Aeration (vvm)	Lipase activity (U/ml)	
				Experimental	Predicted
1	25 (−1)	500 (+1)	1.5 (+1)	5.503	5.342
2	35 (+1)	300 (−1)	0.5 (−1)	25.167	25.328
3	30 (0)	400 (0)	1.0 (0)	40.908	40.972
4	30 (0)	400 (0)	1.0 (0)	41.035	40.972
5	35 (+1)	500 (+1)	1.5 (+1)	8.492	8.653
6	25 (−1)	300 (−1)	0.5 (−1)	12.399	12.238
7	35 (+1)	500 (+1)	0.5 (−1)	14.201	14.040
8	25 (−1)	500 (+1)	0.5 (−1)	6.722	6.883
9	35 (+1)	300 (−1)	1.5 (+1)	16.609	16.448
10	25 (−1)	300 (−1)	1.5 (+1)	7.043	7.204

2.7. Analytical analyses

The total suspended solid as biosolids and chemical oxygen demand (COD) were determined according to the standard methods [21]. The protein concentration of the samples was estimated according to Bradford's dye binding assay method using bovine serum albumin as a standard [22]. Lipase activity assay was carried out according to the method described by Gopinath et al. [23], where 10 ml isopropanol containing 30 mg *p*-nitrophenyl palmitate (Sigma) was mixed with 90 ml of 0.05 M sodium phosphate buffer (pH 8.0) containing 207 mg sodium deoxycholate and 100 mg gum arabic. The substrate solution mixture was stirred until all the constituents were dissolved completely. A total amount of 2.4 ml of freshly prepared substrate solution was dispensed into each test tube. Thereafter, 0.02 ml of enzyme solution was added to initiate hydrolysis. After 15 min of incubation at 37 °C, the optical density at 410 nm was measured against an enzyme free control. One lipase unit (U) was defined as the amount of enzyme that liberated 1 μmol *p*-nitrophenol per milliliter per minute under the standard assay conditions. All the enzyme assays were carried out in triplicate and the average values were calculated.

3. Results and discussion

3.1. Optimization of process parameters by full factorial design for the production of lipase

In order to achieve high lipase production, the present work was targeted towards the optimization of process parameters (temperature, agitation and aeration) using factorial design so as to improve the lipase production in 2-L bioreactor utilizing the renewable low cost medium which enables the reduction of production cost at industrial scale.

The full factorial design was selected as it was insufficient to analyze how the process parameters might influence the lipase production by considering only individual factor effect at different levels. Since biological processes are highly complex and the enzyme production depends on the interaction of several processes influencing microbial cellular metabolism.

Apart from the selected three parameters (temperature, agitation and aeration), the influence of initial pH on lipase production was studied from pH 4 to 8 (data not shown), and pH 6.0 was found to be crucial for growth and lipase production of *C. cylindracea* in POME-based medium. Thus, initial pH in this study was set at pH 6.0, and the fermentation was carried out under uncontrolled pH conditions. This followed several reports in the literature asserting that lipase production occurs under uncontrolled pH conditions. Sokolovska et al. [12] used *C. cylindracea* CBS 6330 for lipase production in 2-L fermentor containing a synthetic medium, adjusting the initial pH to 4.5 and the process was carried out at uncontrolled pH. Lipase production by a Brazilian wild strain of *Y. lipolytica* at different stirring speeds and air flow rates was studied by Alonso et al. [1] using an initial pH of 6.0 and the production was obtained despite the pH fluctuations during the period of fermentation.

The selected process parameters were used to identify the optimum conditions that have influence on the lipase production using full factorial design. For each run, the experimental along with the predicted lipase activity obtained for the 10 experiments were shown in Table 1.

Based on this, lipase production was found to be strongly dependent on all the three process variables (temperature, agitation and aeration). The lipase activity (U/ml) widely varied from 5.50 U/ml to 41.04 U/ml and the best production was realized at the center points (runs 3 and 4) followed by run 2. The least activity was found in run 1, where the temperature was at its low level and the other two factors were at their high levels. The experimental results obtained were then analyzed by the model consisting of the effects of linear and interaction which give the following equation with lipase production (U/ml) as a function of temperature (A), agitation (B) and aeration (C).

$$\text{lipase activity (U/ml)} = -34.374 + 2.391A + 0.039B - 0.659C - 0.003AB - 0.385AC + 0.017BC \quad (2)$$

Estimation of the main variable effects and their interactions according to the *t*-test were represented in Table 2. It can be noted that all the variables have played a critical role on lipase production within the studied experimental range. The *p*-value is the probability value that is used as a tool to check the significance of each of the coefficients, which in turn may indicate the pattern of interactions between the variables. The significance of the data is judged by its *p*-value being closer to zero (0.00). For a 95% confidence level, the *p*-value should be less than or equal to 0.05 for the effect to be statistically significant [24]. Thus, both the linear and interaction coefficients in this study were statistically significant.

The statistical model was checked by *F*-test, and the analysis of variance (ANOVA) indicated in Table 3 showed the model *F*-value of 850.92, which implies that the model is highly significant with a very low probability value (p model, $F < 0.001$); suggesting that there is only 0.1% chance that the model *F*-value this large could occur due to noise. Values of Prob > *F* less than 0.05 indicate that

Table 2
Estimated effects and coefficients for lipase activity (U/ml).

Term	Net effect	Coefficient	Standardized effect (T)	p-value
Constant		12.02	103.51	0.000
Temperature (A)	8.20	4.10	35.32	0.001
Agitation (B)	−6.58	−3.29	−28.32	0.001
Aeration (C)	−5.21	−2.61	−22.44	0.002
AB	−2.97	−1.49	−12.78	0.006
AC	−1.92	−0.96	−8.28	0.017
BC	1.75	0.87	7.52	0.017

Standard error coefficient for all cases = 0.116.

Table 3
Analysis of variance for lipase activity (U/ml).

Source	Degrees of freedom	Sum of squares	Mean squares	*F*-value	*p*-value
Main effects	3	275.26	91.75	850.92	0.001
2-Way interaction	3	31.10	10.37	96.13	0.010
Curvature	1	1341.39	1341.39	12,439.97	0.000
Residual error	2	0.22	0.21		
Total	9	1647.97			

R^2 = 99.99%, R^2(adj) = 99.94% and lack of fit (*F*-value = 25.74 and *p*-value = 0.124).

model terms are significant. Also, lack of fit *F*-value of 25.74 implies its non significance relative to the pure error and there is only 12.4% chance that thelack of fit *F*-value could occur due to noise.

To further validate the model, the goodness of fit was evaluated by the coefficients of determination (R^2). The higher coefficient of determination (R^2 = 99.99% and adjusted R^2 = 99.94%) revealed that there is good agreement between the experimental and the theoretical values predicted by the model and almost all the variation could be accounted for by the model equation. The graphical representations of the regression equation (3D response surface and 2D contour plots) were obtained using MINITAB release 14, which indicated that the interactions between the independent variables were significant. In both cases, the maximum lipase production was located at the center of the experimental region. Similarly, Alam et al. [25] used 2D and 3D plots based on two-level fractional factorial design to describe the pattern of interaction of six factors affecting *Trichoderma harzianum* enzyme production utilizing domestic waste water sludge.

Lipase production varied significantly upon changing the temperature and agitation. Both 3D and 2D plots (Fig. 1) showed that the increase in temperature and agitation cause an increase in the lipase activity to optimum values, and further increase leads to the decrease in overall lipase production. Pandey et al. [4] indicated that high temperature affects the lipase activity especially due to low thermal stability of *C. rugosa* lipase when the temperature level was raised from 37 °C to 50 °C. Thus in this study the temperature range (25–35 °C) showed that 30 °C was the best for lipase production in POME based medium. This followed the findings of Lopez et al. [26] who reported the production and activity of different isoforms of *C. rugosa* lipase at 30 °C. Also, the temperature range of 28–30 °C led to maximum lipase production by mutant *Candida* sp. 99-125 [27]. This is because enzyme synthesis and energy metabolism in microorganisms are influenced by temperature and oxygen uptake [13].

The shapes of the response surface, circular or elliptical, indicate if interactions between variables are significant or not. The elliptical nature of the contour plots between temperature and aeration indicates that mutual interaction between these set of variables has a significant effect on the lipase yield (Fig. 2). Also, the interaction between agitation and aeration was significant as represented in Fig. 3, and this agrees with its significant *p*-value (Table 2). Thus, the smallest ellipse in the contour plot indicated the region of maximum response and in this case, interactions between all the independent variables showed elliptical contours. Muralidhar et al. [28] indicated that elliptical contours suggest perfect interactions between the independent variables.

Agitation and aeration have been indicated as key factors influencing lipolytic enzyme production by mesophilic microorganisms [29]. Increased secretion by *Aspergillus wentii* in liquid media was found when agitated due to availability of dissolved oxygen for its growth and lipase production [30]. At an agitation speed of 400 rpm in 30-L fermenter, *Candida* sp. grew quickly and the lipase production reached its peak within a short fermentation period;

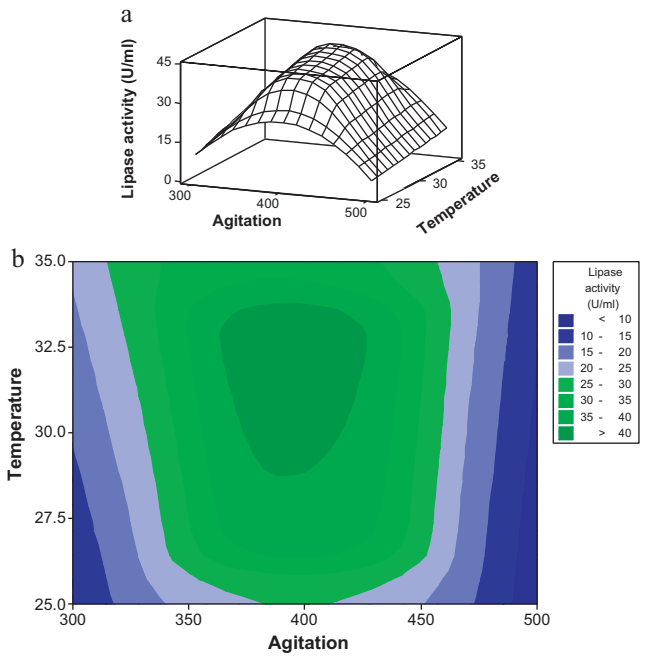

Fig. 1. Response surface and contour plots showing the effect of temperature (°C) and agitation (rpm) on the production of lipase (U/ml) by *C. cylindracea* in POME-based medium: (a) 3D response surface and (b) 2D contour plot.

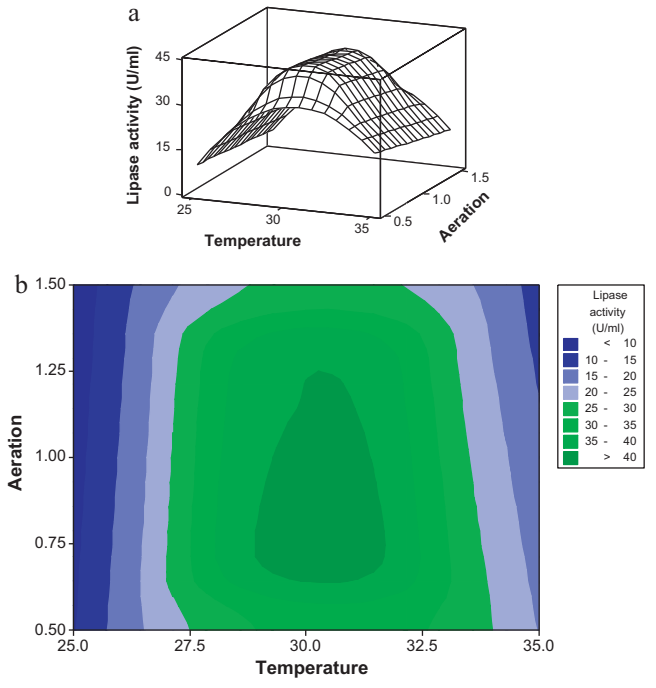

Fig. 2. Response surface and contour plots showing the effect of temperature (°C) and aeration (vvm) on the production of lipase (U/ml) by *C. cylindracea* in POME-based medium: (a) 3D response surface and (b) 2D contour plot.

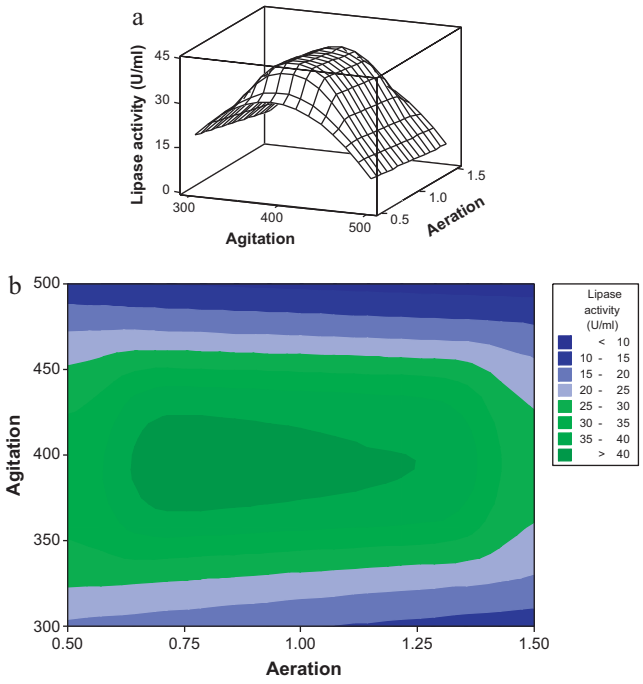

Fig. 3. Response surface and contour plots showing the effect of agitation (rpm) and aeration (vvm) on the production of lipase (U/ml) by *C. cylindracea* in POME-based medium: (a) 3D response surface and (b) 2D contour plot.

confirming that the metabolic pathway for lipase biosynthesis requires oxygen [27]. This lipase production was realized at aeration rate of 0.5 vvm, and further increase in aeration rate had no stimulatory effects on the production. Dominguez et al. [31] reported on lipase production by *Thermus thermophilus* HB27 in a bioreactor under different air flow conditions (0, 0.5, 1.0, 1.5 and 2.5 L air/min), and obtained the highest levels of intracellular lipase production (120 U/L) at 1.5 L air/min, and the highest levels of extracellular lipase production (60 U/L) at 1.0 L air/min.

Brozzoli et al. [17] showed that lipase production by *C. cylindracea* NRRL Y-17506 in olive mill wastewater was significantly affected by stirring speed using fixed aeration rate of 1.0 vvm, where low activity was observed at 300 rpm and several fold increment in activity was observed at high agitation rates of 500 rpm and 700 rpm. However, high levels of aeration were also correlated with hydrogen peroxide production, which was inhibitory to microbial growth and metabolism [32]; while higher agitation rates may cause shear effects on microbial cells resulting in reduced biomass concentration and lipase production [11]. Thus, an optimum balance between the aeration (oxygen tension) and agitation (shear) is necessary for maximum cell growth and lipase production [11].

Optimization of medium components influencing the extracellular lipase production by *C. cylindracea* in shake flask cultures was earlier reported, indicating that an incubation period of 144 h is required for maximum lipase activity of 20.26 U/ml [20]. However, following the process optimization in 2-L bioreactor; the lipase production was significantly improved under these optimized conditions and the duration of fermentation was significantly reduced to 36 h.

Several researchers found similar trend when moving from shake flask cultures to reactors. Bussamara et al. [33] reported that batch fermentation process in the 14-L bioreactor (soy oil, 28 °C and agitation rate 200 rpm) by *Pseudozyma hubeiensis* HB85A resulted in lipase production of 1.232 U/ml with significant decrease in fermentation time from 48 to 18 h when compared to their earlier

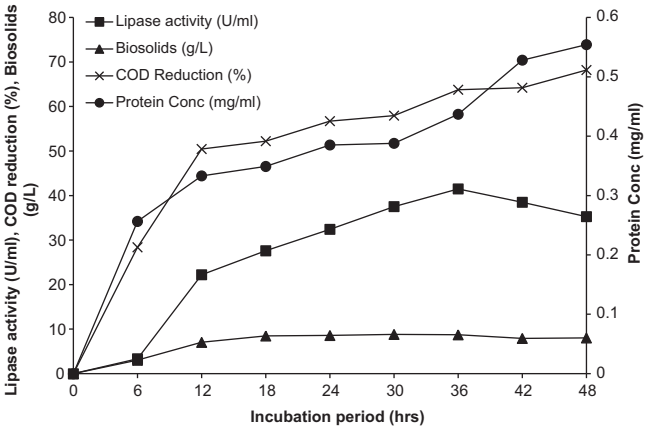

Fig. 4. Variation of different parameters with incubation period during lipase production at the optimum conditions (30 °C, 400 rpm and 1.0 vvm).

studies in Erlenmeyer flasks where an activity of 0.386 U/ml was obtained under the same conditions.

In case of *Aspergillus terreus*, 10% improvement in lipase production in 10-L bioreactor was observed and duration of fermentation was reduced from 96 to 54 h; suggesting that oxygen transfer rates caused by the agitation in bioreactors contributed to the observed effects [34]. Also, lipase activity improvement in 2-L bioreactor from 0.14 U/ml to 0.8 U/ml, which was five times higher than that obtained in the shake flask cultures was reported by Volpato et al. [18]. *Burkholderia multivorans* demonstrated a reduction in the peak of lipase production from 36 to 15 h of cultivation when scaling up from the shake flask to the bioreactor [35].

Based on this, the increase in lipase production as well as the reduction in fermentation period in POME-based medium by *C. cylindracea* could be related to the combined actions of process conditions which ensure oxygen transfer rates, dispersion of cells and nutrients, and growth of the organism. Bussamara et al. [33] also suggested that increased contact of the medium components with microorganisms and the amount of oxygen available in the system by continuous injection of air lead to higher and faster lipase production.

3.2. Validation of the model and kinetics of lipase production

To confirm the validity of the statistical experimental strategies and gain a better understanding of the lipase production; four sets of experiments were performed within the design space (Table 4). Good agreement between the predicted and experimental results verified the validity of the model and the existence of the optimal conditions. The specified optimum conditions (temperature 30 °C, aeration 1.0 vvm, and agitation 400 rpm) showed the lipase production of 41.46 U/ml. This was further studied to determine the trend of lipase production, biosolids accumulation, protein concentration, pH and removal of COD throughout the fermentation period.

Lipase production was examined after every 6 h and it was found to increase with fermentation time. The highest production of 41.46 U/ml was observed after 36 h (Fig. 4). This value was slightly higher than what was obtained during the full factorial runs. Protein concentration correlated with lipase activity (Fig. 4). However, the decrease in lipase production after 36 h could be attributed to higher protein concentration in the medium by hydrolytic action of proteases as indicated by Valero et al. [7]. In case of COD, there was a decrease in concentration throughout the fermentation period. This is due to the consumption of soluble and insoluble organic components of POME by *C. cylindracea* for growth and metabolism

Table 4
Validation of the model.

Experiment	Temperature (°C)	Agitation (rpm)	Aeration (vvm)	Lipase activity (U/ml)	
				Experimental	Predicted
1	30	400	1.0	41.460 ± 0.05	40.972
2	25	400	1.0	34.705 ± 0.10	36.942
3	35	350	0.5	23.021 ± 0.12	22.506
4	35	300	1.0	22.112 ± 0.07	20.888

as well as lipase production. In Fig. 4, the percentage of COD removal reached 69.1% after 48 h of fermentation process. This agrees with the findings of Brozzoli et al. [17], who reported the ability of *C. cylindracea* NRRL Y-17506 to produce lipase and to reduce COD on a variety of olive mill waste water. After the initial increase in biosolids concentration, its increment became minimal with fermentation time; indicating that lipase production by *C. cylindracea* in the medium was partially growth associated (Fig. 4). Volpato et al. [18] indicated that cells generate lipases in order to obtain the energy required for replication from the available carbon sources, and at the same time maintaining their normal metabolic activities irrespective of growth.

During the fermentation, pH drops from 6.0 to 4.99 (data not shown) and this could be attributed to the release of fatty acids in the presence of lipases. Similar behavior was observed during lipase production by *Y. lipolytica* in triturated nut which resulted in pH drop from 6.0 to a value of 4.5–5.0 [9]. Also, in view of the close relationship between lipase synthesis and use of the medium components, pH variation during fermentation may reflect the kinetic changes during the lipase production such as the start and end of the production period [32]. Brozzoli et al. [17] reported that higher lipase production by *C. cylindracea* in olive mill waste water was observed under uncontrolled pH conditions. Thus, the lipase production level obtained in this study appears to be interesting taking into account that it was attained using a cheaply and available raw material, POME.

4. Conclusion

From the results of this study, it could be concluded that temperature, agitation and aeration have significant effects on lipase production by *C. cylindracea* ATCC 14830 in POME-based medium. The maximum lipase production of 41.46 U/ml was obtained through the statistical method, full factorial design at 30 °C, 400 rpm and 1.0 vvm of temperature, agitation and airflow rates in a stirred tank reactor respectively with a significant reduction in fermentation time. This study showed a good benefit for the enzyme attainment, denoting that industrial-scale lipase production can be achieved in an economically attractive manner and the optimized process parameters pave away to focus on the product upscaling strategies using pilot-plant scale batch fermentation.

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