Optimization of Fermentation Medium for Extracellular Lipase Production from Aspergillus niger Using Response Surface Methodology

Jia Jia, Xiaofeng Yang, Zhiliang Wu, Qian Zhang, Zhi Lin, Hongtao Guo, Carol Sze Ki Lin, Jianying Wang, and Yunshan Wang

1Shenzhen Leveking Bio-Engineering Co., Ltd., Guangdong, Shenzhen 518055, China
2School of Energy and Environment, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong
3Shenzhen Institute of Technology, Guangdong, Shenzhen 518116, China

Correspondence should be addressed to Qian Zhang; qianz@yeah.net

Received 31 May 2015; Revised 8 August 2015; Accepted 10 August 2015

1. Introduction

Lipases (triacylglycerol ester hydrolase EC 3.1.1.3) catalyze the hydrolysis of triglycerides into fatty acids and glycerol [1, 2] and under certain conditions can also catalyze the synthesis of esters through transesterification, thioesterification, and aminolysis [3–5]. Lipases exist widely in nature environment, especially in bacteria, yeasts, and filamentous fungi. In recent years, the interest in microbial lipases production has been increasing, because of their large potential in industrial applications for the synthesis of biopolymers and biodiesel, the production of enantiopure pharmaceuticals, agrochemicals, and flavour compounds [2, 6]. Both high-level production and the safety of the process and products are required to broaden the industrial application of lipases [6].

Aspergillus niger is one of the most important industrial microbes, which can produce more than 30 species of enzymes such as lipase [7–11], amylase [12, 13], cellulase [14, 15], pectinase [16], and glucose oxidase [17, 18]. It has been widely used to produce extracellular enzymes and organic acid for decades [19, 20]. The microorganism is Generally Recognized as Safe (GRAS) by the United States Food and Drug Administration [19]. Because of its excellent protein secretion capability, mature fermentation, and posttreatment process and safety, A. niger becomes one of the most important species in industrial application [21]. A. niger enzymes such as lipase could be utilized as auxiliary material or additive in food, medicine, and feed industry [19]. Maximization of the lipase yield is a prerequisite for the immense potential of A. niger lipase. The composition of fermentation medium has significant effect on microorganism metabolism [22], which is desired to optimize for the maximum yields of lipase.

Response Surface Methodology (RSM) is a mathematical and statistical method that can overcome some drawbacks like time consumption and high cost. It is widely used to solve multiple variable problems in different biotechnological
processes [13, 23–27]. RSM has been successfully applied to evaluate and optimize the effect of process parameters in the production of lipase. Hatzinikolaou et al. [24] used RSM to study the effect of carbon and nitrogen sources on the extracellular lipase production from A. niger. A maximum lipase activity of 42.4 U/mL was obtained in the optimum medium with a combination of corn oil and peptone. Also using RSM, Kaushik et al. [23] reported that sunflower oil, glucose, peptone, agitation rate, and incubation temperature were the most influential parameters in the production of extracellular lipase from A. caroneus. A 1.8-fold increase in production, with the final yield of 12.7 IU/mL, was obtained under the optimum medium and operation. Box-Behnken Design (BBD) is a RSM suitable for fermentation optimization in shake flasks, in which the most influential parameters can be determined by the minimum numbers of experiments [25]. In this study, we aimed to improve the yield of lipase production through optimization of the fermentation medium composition for A. niger G783, which is an industrial production strain. In this paper, the influential parameters were determined and optimized by a series of single-factor experiments and BBD method, respectively, which can also evaluate the effect and relationship of different medium components.

2. Materials and Methods

2.1. Strains and Chemicals. A. niger G783, a genetic stability lipase high-yielding industrial production strain, was genetically engineered by physical and chemical mutagenesis from A. niger CICC 2475 (China Center of Industrial Culture Collection), which was preserved in Shenzhen Leveking Bio-Engineering Co., Ltd.

Glucose, sucrose, corn starch, dextrin, soybean meal, peptone, beef extract, yeast extract, salt, and agar were obtained at analytical grade from chemical suppliers in China. Olive oil and soybean oil were purchased from local markets (Shenzhen, China).

2.2. Medium. Yeast extract peptone dextrose (YPD) medium contained (per liter) 10 g yeast extract, 20 g peptone, 20 g glucose, 15.0 g agar, and pH 7.0.

Seed medium (SM) contained (per liter) 30.0 g glucose, 3.0 g NaNO₃, 0.5 g NaH₂PO₄, 0.1 g K₂SO₄, and pH 7.2.

Fermentation medium I (FMI, per liter) contained 20.0 g glucose, 10.0 g corn starch, 40.0 g soybean meal, 10.0 g olive oil, 10.0 g NaNO₃, 2.0 g NaH₂PO₄, 0.2 g K₂SO₄, 5.0 g CaCO₃, and pH 7.2.

2.3. Strain Cultivation. The inoculum was produced in YPD medium in slant. Shake flasks of 250 mL containing 25 mL of seed medium were incubated in a shaker (220 rpm) at 30°C, for 20–32 h. Lipase production was carried out with a 2% (v/v) inoculum of A. niger G783 in 500 mL shaken flasks with 50 mL of FMI and incubated at 30°C in a shaker (220 rpm) for about 48 h. The broth was refrigerated at 4°C and centrifuged at 10,000 rpm for 10 minutes, and the supernatant was obtained as crude enzyme solution that was used for lipase activity analysis.

2.4. Single-Factor Experiment. To determine the influential parameters on the lipase production, we carried out a single-factor experiment. The value of the factors was set based on FMI medium. The medium was divided into five components, primary carbon sources, secondary carbon sources, nitrogen sources, oils, and inorganic salts. The composition of components and the experimental sequence for the single-factor experiment are shown in Table 1. Fermentation was performed in 500 mL shake flasks with 50 mL of medium and incubated at 30°C in a shaker (220 rpm) for about 48 h. The broth was refrigerated at 4°C and then centrifuged at 10,000 rpm for 10 minutes. The supernatant was obtained as crude enzyme solution that was used for lipase activity analysis. All components were analyzed independently, and every test was performed in triplicate. The influential factors and levels for the enzyme activity were evaluated.

2.5. BBD Design. Box-Behnken Design (BBD), one of RSM design, with a three-level factorial design was used as the experimental design model to optimize the influential parameters for enhancing lipase production. According to the results of single-factor experiment, the levels of the variables and the experimental design (according to Design-Expert

### Table 1: The composition of components and the experimental sequence for the single-factor experiment.

| Sequence | Nutrients     | Components                                          | Concentration (g/L) |
|----------|---------------|-----------------------------------------------------|---------------------|
| 1        | Primary carbon sources | Glucose, maltose, sucrose, glycerol, hydrol<sup>a</sup> | 15, 20, 25          |
| 2        | Secondary carbon sources | Cornstarch, modified starch, dextrin               | 8.5, 10, 11.5       |
| 3        | Nitrogen sources | Soybean meal, yeast extract, beef extract, peptone, sodium nitrate<sup>b</sup> | 35, 40, 45          |
| 4        | Oils | Olive oil, soybean oil, lard oil, peanut oil, sunflower seed oil | 5, 10, 15          |
| 5        | Inorganic salts | NaH₂PO₄, K₂SO₄, CaCO₃ | 0, 1.5, 2.0, 2.5 (for NaH₂PO₄), 0, 0.15, 0.2, 0.25 (for K₂SO₄), 0, 4, 5, 6 (for CaCO₃) |

<sup>a</sup>Corn starch hydrolyzate (hydrol), a less expensive nutrient source for industrial medium, contains 50–60 g/L glucose and is rich in trace elements. The value of hydrol was 30, 40, and 50 g/L.

<sup>b</sup>40 g/L soybean meal was supplemented in the analysis of sodium nitrate. The concentration of sodium nitrate was set as 8, 10, and 12 g/L, respectively.
was preceded at 36°C. Lipase activity in the fermentation and predict the optimal processing parameters [28].

According to our previous works and the characteristics of A. niger G783, the levels of the variables were set to be close to the central point in the single-factor experiments. As shown in Table 1, the levels of the variables were designed and the single-factor experiments were performed as the following sequence: primary carbon source, secondary carbon source, nitrogen source, oil, and inorganic salt. The optimum variable and level that were determined in every step would be used in the next steps.

As carbon catabolite repression (CCR) existed in Aspergillus sp. [31, 32], two types of carbon source, primary and secondary carbon sources, are thought to play different roles in the metabolism of Aspergillus sp. Therefore, these carbon sources were analyzed independently. Glucose, maltose, corn starch hydrolyzate (hydrol), sucrose, and glycerol were selected as the primary carbon source, while corn starch, modified starch, and dextrin were selected as secondary carbon source (Table 1). As shown in Figure 1, higher lipase activity was obtained when glucose was used as primary carbon source. Using glucose as the primary carbon source was significantly higher than using maltose, sucrose, or glycerol. However, the lipase activity obtained from hydrol was not significantly different than using glucose, because the major content of hydrol was glucose. This result suggested that glucose was the best primary carbon source for lipase production. Moreover, the lipase production was similar to each other between 15 and 25 g/L glucose. To make sure the carbon source is enough, 25 g/L glucose was used as the primary carbon source in the following studies. For the three types of secondary carbon source, corn starch was the best secondary carbon source on the lipase production (Figure 2). Among the concentration range of 5–15 g/L corn starch, the best condition was 10 g/L corn starch. Thus, the amounts of carbon sources were designed as 25 g/L glucose and 10 g/L corn starch in FMI.

During fermentation, the availability of precursors for protein synthesis [33] and the nitrogen source [34] are

### Table 2: Coded levels and real values (in parentheses) for the BBD and lipase activity achieved after fermentation by A. niger G783.

| Run | A: Corn starch (g/L) | B: Soybean meal (g/L) | C: Soybean oil (g/L) | Lipase activity (U/mL) |
|-----|----------------------|-----------------------|----------------------|------------------------|
| 1   | 0 (10)               | 0 (35)                | 0 (10)               | 1698                   |
| 2   | 1 (12)               | 0 (35)                | 0 (10)               | 1866                   |
| 3   | 0 (10)               | 0 (35)                | 0 (10)               | 2017                   |
| 4   | 0 (10)               | 0 (35)                | 0 (10)               | 2093                   |
| 5   | −1 (8)               | 0 (35)                | −1 (5)               | 1575                   |
| 6   | 0 (10)               | 0 (35)                | 0 (10)               | 2014                   |
| 7   | 0 (10)               | −1 (30)               | −1 (5)               | 1600                   |
| 8   | 0 (10)               | 0 (35)                | 0 (10)               | 2113                   |
| 9   | 0 (10)               | 0 (35)                | 0 (10)               | 2122                   |
| 10  | 1 (12)               | −1 (30)               | 0 (10)               | 1744                   |
| 11  | 0 (10)               | 1 (40)                | 1 (15)               | 1970                   |
| 12  | −1 (8)               | 0 (35)                | 1 (15)               | 1837                   |
| 13  | −1 (8)               | −1 (30)               | 0 (10)               | 1353                   |
| 14  | 0 (10)               | 1 (40)                | −1 (5)               | 1807                   |
| 15  | −1 (8)               | 1 (40)                | 0 (10)               | 1778                   |
| 16  | 1 (12)               | 1 (40)                | 0 (10)               | 1754                   |
| 17  | 1 (12)               | 0 (35)                | 1 (15)               | 1955                   |

**Figure 1:** The effect of primary carbon sources on lipase activity obtained by A. niger G783. The medium was modified based on FMI. The values are average of three independent experiments and the error bars represent standard deviation.

**2.6. Lipase Activity Assay.** Lipase activity in the fermentation broths was analyzed according to a slightly modified NaOH titration method [29]. The assay mixture contained 5 mL of the olive oil emulsion, 4 mL of 50 mmol/L glycine-NaOH buffer (pH 9.4), and 1 mL of enzyme solution. The reaction was preceded at 36°C for 15 minutes and stopped by adding 20 mL of 95% ethanol and 10 mL 30% NaCl. One unit of the lipase activity was defined as the amount of enzyme required to release 1 μmol of fatty acid per minute under assay condition [30].

### 3. Result and Discussion

#### 3.1. Single-Factor Optimization of Fermentation Media

According to our previous works and the characteristics of A. niger G783, the levels of the variables were set to be close to the central point in the single-factor experiments. As shown in Table 1, the levels of the variables were designed and the single-factor experiments were performed as the following sequence: primary carbon source, secondary carbon source, nitrogen source, oil, and inorganic salt. The optimum variable and level that were determined in every step would be used in the next steps.

As carbon catabolite repression (CCR) existed in Aspergillus sp. [31, 32], two types of carbon source, primary and secondary carbon sources, are thought to play different roles in the metabolism of Aspergillus sp. Therefore, these carbon sources were analyzed independently. Glucose, maltose, corn starch hydrolyzate (hydrol), sucrose, and glycerol were selected as the primary carbon source, while corn starch, modified starch, and dextrin were selected as secondary carbon source (Table 1). As shown in Figure 1, higher lipase activity was obtained when glucose was used as primary carbon source. Using glucose as the primary carbon source was significantly higher than using maltose, sucrose, or glycerol. However, the lipase activity obtained from hydrol was not significantly different than using glucose, because the major content of hydrol was glucose. This result suggested that glucose was the best primary carbon source for lipase production. Moreover, the lipase production was similar to each other between 15 and 25 g/L glucose. To make sure the carbon source is enough, 25 g/L glucose was used as the primary carbon source in the following studies. For the three types of secondary carbon source, corn starch was the best secondary carbon source on the lipase production (Figure 2). Among the concentration range of 5–15 g/L corn starch, the best condition was 10 g/L corn starch. Thus, the amounts of carbon sources were designed as 25 g/L glucose and 10 g/L corn starch in FMI.

During fermentation, the availability of precursors for protein synthesis [33] and the nitrogen source [34] are
both important in the production of extracellular enzymes. Nitrogen source would also significantly affect the pH of the medium. To obtain an insight for the effect of different nitrogen sources, various inorganic and organic nitrogen sources were investigated in lipase production. As shown in Figure 3, the activity of lipase has no significant difference when using peptone, beef extract, and soybean meal with or without NaNO\(_3\), respectively. However, lipase activity decreased significantly when yeast extract was used as nitrogen source. Thus, soybean meal was selected as nitrogen source because of its low cost and the ease of accessibility. Since the soybean meal was assumed to contain most of the necessary nutrients that lipase production needs, no other nitrogen supplements were necessary [26]. Herein, the lipase activity was not significantly different when supplemented with sodium nitrate, which suggested that sodium nitrate can be omitted.

Oils could be served as carbon source and inducer of lipase synthesis during \textit{A. niger} fermentation [35]. Figure 4 showed that olive oil and soybean oil were both outstanding for lipase production, compared to lard oil, peanut oil, and sunflower oil. As it is of low cost and easily acquired, soybean oil was selected to replace olive oil. Although the effect of sodium phosphate monobasic and calcium carbonate on the lipase production was greater than potassium sulfate (Figure 5), all inorganic salts chosen in the single-factor experiments are essential for the basic metabolism of \textit{A. niger}. The results indicated that the concentration of inorganic salts should be 1.5 g/L Na\(_2\)HPO\(_4\), 0.2 g/L K\(_2\)SO\(_4\), and 5 g/L CaCO\(_3\), respectively. Based on the results of single-factor experiments, a new fermentation medium (FM2) was established as follows (per liter): 25 g glucose, 10 g corn starch, 40 g soybean meal, 10 g soybean oil, 12 g NaNO\(_3\), 1.5 g NaH\(_2\)PO\(_4\), 0.2 g K\(_2\)SO\(_4\), 5 g CaCO\(_3\), and pH 7.2.

3.2. BBD Model Fitting and Data Analysis. According to the single-factor experiments, three influential factors \((A: \text{corn starch}, B: \text{soybean meal}, \text{and } C: \text{soybean oil})\) in FM2 for G783 fermentation were selected for BBD design and quadratic models analysis (Table 2). Parameters of the BBD design were set as follows: factor = 3, level = 3, and runs = 17 (including 5 replications of the center points), and lipase activity was set as response value \((Y)\). After BBD, the result was then analyzed by standard analysis variance (ANOVA) according to the simulated quadratic equation:

\[
Y_{\text{coded}} = 2071.8 + 97.00A + 114.25B + 76.50C - 103.75AB - 43.25AC + 16.25BC - A^2 - 227.03B^2 - 76.02C^2.
\]
The variance analysis results were listed in Table 3. This model processes high reliability, high fitting degree, and deviation with coefficient of determination \( R^2 = 0.9826 \) and adequate precision of 22.523. Furthermore, the \( P \) value of this model was significant \((P < 0.01)\) and the lack of fit was not significant \((P = 0.9057 > 0.05)\), which indicated that the residual might be caused by random error and this model is adequate. On the whole, this model could be used for evaluation, optimization, and prediction of lipase fermentation process. The variance analysis of three factors \((A, B, \text{ and } C)\) showed that \( A, B, C, A^2, B^2, \text{ and } C^2 \) have significant effect on enzyme production \((P < 0.05)\) as listed in Table 3. This model predicted that these three factors were significant affecting the lipase production. In the medium, corn starch, soybean meal, and soybean oil are the major source for supply carbon or nitrogen resource to maintain the normal microbe metabolism and protein synthesis. Additionally, various amounts of these three factors also affect the carbon to nitrogen ratio \((C/N)\), which could affect the metabolic pathway of the microorganism. We discussed about the effect of \(C/N\) ratio thereinafter. Therefore, the concentrations of corn starch, soybean meal, and soy oil bean in the medium have significant effect on enzyme production.

Earlier studies by Gombert et al. \[36\] and Dinarvand et al. \[37\] have optimized \(C/N\) ratio in the medium for enzyme production. Comparing to metabolite production \((C/N \text{ ratio } = 300)\ [38]\, lower \(C/N\) ratio medium is beneficial to enzyme production \((C/N \text{ ratio } < 14)\ [36, 37]\. Dinarvand et al. \[37\] indicated that both organic and inorganic nitrogen sources can improve cell growth and synthesis of enzymes. High productivity of enzymes was obtained under low \(C/N\) ratio condition, carbon limitation, and rich nitrogen. The \(C/N\) ratio in this BBD study was set to 6.6–8.3 within an appropriate range for enzyme production. As shown in Figure 6(a), the interaction between factors \(A\) (corn starch) and \(B\) (soybean meal) was significant, which indicated that \(C/N\) ratio (corn starch/soybean meal) was important for lipase production. This result suggested that more attention should be paid to \(C/N\) ratio in the optimization of enzyme production. As shown in Figures 6(b) and 6(c), the interactions of \(A \text{ and } C\) or \(B \text{ and } C\) were not significant. Although soybean oil not only induces the lipase synthesis but also is used as carbon source \[35\], our results suggested soybean oil did not significantly affect the \(C/N\) ratio.

### Table 3: ANOVA for lipase activity obtained by \(A. \ niger\) G783.

| Source | Degrees of freedom | Sum of squares | Mean square | \(F\) value | Prob. > \(F\) |
|--------|-------------------|---------------|-------------|-------------|--------------|
| Model  | 9                 | 704679.1      | 78297.7     | 43.89       | <0.0001      |
| A      | 1                 | 75272.0       | 75272.0     | 42.20       | 0.0003       |
| B      | 1                 | 104424.5      | 104424.5    | 58.54       | 0.0001       |
| C      | 1                 | 46818.0       | 46818.0     | 26.24       | 0.0014       |
| AB     | 1                 | 43056.2       | 43056.2     | 24.14       | 0.0017       |
| AC     | 1                 | 7482.2        | 7482.2      | 4.19        | 0.0798       |
| BC     | 1                 | 1056.2        | 1056.2      | 0.59        | 0.4667       |
| A\(^2\) | 1               | 148065.8      | 148065.8    | 83.01       | <0.0001      |
| B\(^2\) | 1               | 217012.0      | 217012.0    | 121.66      | <0.0001      |
| C\(^2\) | 1               | 24336.0       | 24336.0     | 13.64       | 0.0077       |
| Residual | 7             | 12485.8       | 1783.7      |             |              |
| Lack of fit | 3          | 1475.0        | 491.7       | 0.18        | 0.9057       |
| Error  | 4                 | 11010.8       | 2752.7      |             |              |
| Total  | 16                | 717164.9      |             |             |              |

\(^a\)Coefficient of determination \((R^2) = 0.9828, CV = 2.29\%\). A model with an \(F\) value of 43.89 implies that the model is significant, which could occur due to noise. Values of “Prob. > \(F\)” less than 0.01 indicate that model terms are significant. In this case, \(A, B, C, AB, A^2, B^2, \text{ and } C^2\) are significant model terms. The “Predicted \(R^2\)“ of 0.9431 is close to the “Adj. R-Squared” of 0.9602, which corrects the \(R^2\) values for the number of terms and for the sample size in the model. The “adequate precision” value of 22.523 indicates an adequate signal. The lack of fit is insignificant and the model is adequate. This model can be used to navigate the design space.

![Figure 5](image-url)
After derivation of the quadratic equation and calculation (according to Design-Expert 8.0), the concentrations of three factors in the optimum medium for maximum lipase activity production were predicted as 10.5 g/L corn starch, 35.4 g/L soybean meal, and 10.9 g/L soybean oil. Therefore, the optimum fermentation medium (FM3) was predicted as follows (per liter): 25 g glucose, 10.5 g corn starch, 35.4 g soybean meal, 10.9 g soybean oil, 12 g NaNO₃, 1.5 g NaH₂PO₄, 0.2 g K₂SO₄, and 5 g CaCO₃, pH adjusted to 7.2. The maximum lipase activity was predicted at 2,096 U/mL.

3.3. Validation Experiments. Validation experiments were carried out in triplicate to confirm the predicted optimal conditions. Initial fermentation medium (FM1), single-factor optimum medium (FM2), and BBD optimum medium (FM3) were investigated and evaluated to validate the predicted optimal conditions (Table 4). The final lipase activity of A. niger G783 in FM3 was 2,171 ± 41 U/mL with a slight increase compared to the predicted value. Considering the experimental error, this result was consistent with predicted value, which suggested that the model built by BBD in this study is valuable for optimization of the fermentation medium. Moreover, the lipase activity was significantly improved in

| Medium   | Batch 1 | Batch 2 | Batch 3 | Mean ± SD | CV (%) |
|----------|---------|---------|---------|-----------|--------|
| FM1      | 1863 ± 27 | 1835 ± 30 | 1896 ± 62 | 1865 ± 45 | 2.18   |
| FM2      | 2046 ± 34 | 1996 ± 63 | 2030 ± 59 | 2024 ± 52ᵃ | 2.27   |
| FM3      | 2174 ± 46 | 2161 ± 54 | 2177 ± 39 | 2171 ± 41ᵇ | 1.7    |

ᵃ Lipase activity in FM2 and FM3 increased by 8.5% and 16.4%, respectively, compared to FM1.

Table 4: Validation experiments of the optimum medium (n = 9).
FM3 compared to the other two media, especially the initial medium (FM1). This statistical optimization study on A. niger fermentation medium is important for industrial lipase production. This study also provided helpful experiences for industrial production strain improvement.

4. Conclusions

Medium component significantly affects the revenue in industrial scale fermentation, due to the effect on feedstock cost and product yield. In this study, we successfully demonstrated sequential single-factor experiments and BBD strategy could be used for optimization of fermentation medium for the industrial production strain. Due to its effect on the C/N ratio, corn starch, soybean meal, and soybean oil were selected as factors in BBD design to predict the optimal conditions. Statistical analysis from BBD suggested that the C/N ratio was important for A. niger lipase production. Using the predicted condition, the optimum lipase activity of A. niger G783 was up to 2,171 ± 41 U/mL, which was 16.4% higher than using the initial medium. This optimal fermentation medium formula could be used for the future upscale lipase production using A. niger. This study also provided helpful experiences for industrial production improvement.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

This work was supported by the Technical Innovation Projects through the foundation by Innovation of Science and Technology Commission of Shenzhen (CXZZ20130425111508558).

References

[1] R. K. Saxena, A. Sheoran, B. Giri, and W. S. Davidson, “Purification strategies for microbial lipases,” Journal of Microbiological Methods, vol. 52, no. 1, pp. 1–18, 2003.
[2] K.-E. Jaeger and T. Eggert, “Lipases for biotechnology,” Current Opinion in Biotechnology, vol. 13, no. 4, pp. 390–397, 2002.
[3] Y. Watanabe, S. Sato, S. Sera et al., “Enzymatic analysis of positional distribution of fatty acids in solid fat by 1,3-selective Transesterification with Candida antarctica lipase B,” Journal of the American Oil Chemists’ Society, vol. 91, no. 8, pp. 1323–1330, 2014.
[4] N. Weber, K. Bergander, E. Fehling, E. Klein, K. Vosmann, and K. D. Mukherjee, “Copolymeric polythioesters by lipase-catalyzed thioesterification and transthioesterification of alpha, omega-alkanedithiols,” Applied Microbiology and Biotechnology, vol. 70, no. 3, pp. 290–297, 2006.
[5] S. van Pelt, R. L. M. Tieuwen, M. H. A. Janssen et al., “Pseudomonas stutzeri lipase: a useful biocatalyst for aminolysis reactions,” Green Chemistry, vol. 13, no. 7, pp. 1791–1798, 2011.
[6] L. R. Gerits, B. Pareyt, K. Decamps, and J. A. Delcour, “Lipases and their functionality in the production of wheat-based food systems,” Comprehensive Reviews in Food Science and Food Safety, vol. 13, no. 5, pp. 978–989, 2014.
[7] N. D. Mahadik, U. S. Puntambekar, K. B. Bastawde, J. M. Khire, and D. V. Gokhale, “Production of acidic lipase by Aspergillus niger in solid state fermentation,” Process Biochemistry, vol. 38, no. 5, pp. 715–721, 2002.
[8] N. G. Edwinoliver, K. Thirunavukarasu, R. B. Naidu, M. K. Gowthaman, T. N. Kambe, and N. R. Kamini, “Scale up of a novel tri-substrate fermentation for enhanced production of Aspergillus niger lipase for tallow hydrolysis,” Bioresearch Technology, vol. 101, no. 17, pp. 6791–6796, 2010.
[9] P. Ellaiath, T. Prabhakar, B. Ramakrishna, A. Thaer Taleh, and K. Adinarayana, “Production of lipase by immobilized cells of Aspergillus niger,” Process Biochemistry, vol. 39, no. 5, pp. 525–528, 2004.
[10] N. C. Mhetras, K. B. Bastawde, and D. V. Gokhale, “Purification and characterization of acidic lipase from Aspergillus niger NCIM 1207,” Bioresource Technology, vol. 100, no. 3, pp. 1486–1490, 2009.
[11] G. Fernández-Lorente, C. Ortiz, R. L. Segura, R. Fernández-Lafuente, J. M. Guisán, and J. M. Palomo, “Purification of different lipases from Aspergillus niger by using a highly selective adsorption on hydrophobic supports,” Biotechnology and Bioengineering, vol. 92, no. 6, pp. 773–779, 2005.
[12] A. Bagheri, R. Khodarahmi, and A. Mostafaei, “Purification and biochemical characterisation of glucoamylase from a newly isolated Aspergillus niger: relation to starch processing,” Food Chemistry, vol. 161, pp. 270–278, 2014.
[13] S. Djekrif-Dakhmouche, Z. Gheribi-Aoulmi, Z. Meraiali, and L. Bennamoun, “Application of a statistical design to the optimization of culture medium for α-amylase production by Aspergillus niger ATCC 16404 grown on orange waste powder,” Journal of Food Engineering, vol. 73, no. 2, pp. 190–197, 2006.
[14] S. W. Kang, Y. S. Park, J. S. Lee, S. I. Hong, and S. W. Kim, “Production of cellulosases and hemicellulases by Aspergillus niger KK2 from lignocellulosic biomass,” Bioresource Technology, vol. 91, no. 2, pp. 153–156, 2004.
[15] H. Noreen, M. A. Zia, S. Ali, and T. Hussain, “Optimization of bio-polishing of polyester/cotton blended fabrics with cellulases prepared from Aspergillus niger,” Indian Journal of Biotechnology, vol. 13, no. 1, pp. 108–113, 2014.
[16] D. Ibrahim, H. Welosoamy, and L. Sheh-Hong, “Potential use of nylon scouring pad cubes attachment method for pectinase production by Aspergillus niger HFD5A-1,” Process Biochemistry, vol. 49, no. 4, pp. 660–667, 2014.
[17] A. A. L. Tribst, J. Cota, M. T. Murakami, and M. Cristianini, “Effects of high pressure homogenization on the activity, stability, kinetics and three-dimensional conformation of a glucose oxidase produced by Aspergillus niger,” PLoS ONE, vol. 9, no. 7, Article ID e103410, 2014.
[18] J.-Z. Liu, L.-P. Weng, Q.-L. Zhang, H. Xu, and L.-N. Ji, “Optimization of glucose oxidase production by Aspergillus niger in a benchtop bioreactor using response surface methodology,” World Journal of Microbiology and Biotechnology, vol. 19, no. 3, pp. 317–323, 2003.
[19] E. Schuster, N. Dunn-Coleman, J. Frisvad, and P. Van Dijck, “On the safety of Aspergillus niger—a review,” Applied Microbiology and Biotechnology, vol. 59, no. 4–5, pp. 426–435, 2002.
[20] C. Krishna, “Solid-state fermentation systems—an overview,” Critical Reviews in Biotechnology, vol. 25, no. 1-2, pp. 1–30, 2005.
[21] Y. Guo, P. Zheng, and J. Sun, “Aspergillus niger as a potential cellular factory: prior knowledge and key technology,” Chinese Journal of Biotechnology, vol. 26, no. 10, pp. 1410–1418, 2010.

[22] P. F. Stanbury, A. Whitaker, and S. J. Hall, “Media for industrial fermentations,” in Principles of Fermentation Technology, P. F. Stanbury, A. Whitaker, and S. J. Hall, Eds., chapter 4, pp. 93–122, Pergamon Press, New York, NY, USA, 2nd edition, 1995.

[23] R. Kaushik, S. Saran, J. Isar, and R. K. Saxena, “Statistical optimization of medium components and growth conditions by response surface methodology to enhance lipase production by Aspergillus carneus,” Journal of Molecular Catalysis B: Enzymatic, vol. 40, no. 3-4, pp. 121–126, 2006.

[24] D. G. Hatzinikolaou, J. B. Macris, P. Christakopoulos, D. Kekos, F. N. Kolisis, and G. Fountoukidis, “Production and partial characterisation of extracellular lipase from Aspergillus niger,” Biotechnology Letters, vol. 18, no. 5, pp. 547–552, 1996.

[25] S. M. Rafigh, A. V. Yazdi, M. Vossoughi, A. A. Safekordi, and M. Ardjmand, “Optimization of culture medium and modeling of curdlan production from Paenibacillus polymyxa,” International Journal of Biological Macromolecules, vol. 70, pp. 463–473, 2014.

[26] G. D. L. P. Vargas, H. Treichel, D. de Oliveira, S. C. Beneti, D. M. G. Freire, and M. Di Luccio, “Optimization of lipase production by Penicillium simplicissimum in soybean meal,” Journal of Chemical Technology and Biotechnology, vol. 83, no. 1, pp. 47–54, 2008.

[27] G. T. Dobrev, I. G. Pishitiisky, V. S. Stanchev, and R. Mircheva, “Optimization of nutrient medium containing agricultural wastes for xylanase production by Aspergillus niger B03 using optimal composite experimental design,” Bioresource Technology, vol. 98, no. 14, pp. 2671–2678, 2007.

[28] Z. C. Lai, M. Z. Zhu, X. F. Yang, J. F. Wang, and S. Li, “Optimization of key factors affecting hydrogen production from sugarcane bagasse by a thermophilic anaerobic pure culture,” Biotechnology for Biofuels, vol. 7, pp. 119–129, 2014.

[29] T.-C. Lin and C. Chen, “Enhanced mannanase production by submerged culture of Aspergillus niger NCH-189 using defatted copra based media,” Process Biochemistry, vol. 39, no. 9, pp. 1103–1109, 2004.

[30] M. C. T. Damaso, M. A. Passionoto, S. C. de Freitas, D. M. G. Freire, R. C. A. Lago, and S. Couri, “Utilization of agroindustrial residues for lipase production by solid-state fermentation,” Brazilian Journal of Microbiology, vol. 39, no. 4, pp. 676–681, 2008.

[31] M. Dinarvand, M. Rezaee, M. Masomian et al., “Effect of C/N ratio and media optimization through response surface methodology on simultaneous productions of intra- and extracellular inulinase and invertase from Aspergillus niger ATCC 20611,” BioMed Research International, vol. 2013, Article ID 508968, 13 pages, 2013.

[32] G. J. G. Ruijter and J. Visser, “Carbon repression in Aspergilli,” FEMS Microbiology Letters, vol. 151, no. 2, pp. 103–114, 1997.

[33] N. V. Kote, A. G. G. Patil, and V. H. Mulimani, “Optimization of the production of thermostable endo-β-1,4 mannanases from a newly isolated Aspergillus niger gr and Aspergillus flavus gr,” Applied Biochemistry and Biotechnology, vol. 152, no. 2, pp. 213–223, 2009.

[34] T.-C. Lin and C. Chen, “Enhanced mannanase production by submerged culture of Aspergillus niger NCH-189 using defatted copra based media,” Process Biochemistry, vol. 39, no. 9, pp. 1103–1109, 2004.

[35] M. C. T. Damaso, M. A. Passionoto, S. C. de Freitas, D. M. G. Freire, R. C. A. Lago, and S. Couri, “Utilization of agroindustrial residues for lipase production by solid-state fermentation,” Brazilian Journal of Microbiology, vol. 39, no. 4, pp. 676–681, 2008.