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Biochemical Engineering Journal 27 (2006) 287–294

Biochemical Engineering Journal

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# Optimization of lipase production in a triple impeller bioreactor

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Received 9 July 2004; received in revised form 3 August 2005; accepted 3 August 2005

**Abstract**

The fermentation kinetics for the synthesis of lipase by *Candida rugosa* has been studied in a batch system in a 2 l batch bioreactor. The studies illustrated the influence of gas–liquid mass transfer coefficient on the cell growth and hence the lipase production. In order to maintain sufficient oxygen concentration for the optimum cell growth and lipase activity, fermentation has been carried out using triple impeller system at an operating speed of 600 rpm (optimum operating speed as found in the earlier work) and at different aeration rates. Gas-flow rate of 50.34 cc/s has been observed to be optimum. Under optimized conditions of the bioreactor, cell production was enhanced and the lipase activity increased by 2.5 folds. The Monod's kinetics was fitted to the data of the operating parameters to understand the cell growth and substrate consumption. Luedking and Piret model was applied to the data to determine the relationship between the cell growth and lipase production. The lipase production was found to be microbial growth associated function.


*Keywords:* Bioreactor; Gas–liquid mass transfer; Fermentation; Triple impeller; Aerobic process; Growth kinetics

## 1. Introduction

*Candida cylindracea* (now named *Candida rugosa*) is recognized to be a good producer of lipase [1–3]. Lipase produced by *Candida rugosa* has been one of the most widely used enzymes in research owing to its high activity in hydrolytic reactions as well as synthetic chemistry. Research on lipases is focused particularly on structural characterization, elucidation of mechanism of action, kinetics of lipase catalyzed reactions, sequencing and cloning of lipase genes and general characterization of its performance. In comparison with this effort, very little literature is available about the effect of different fermentation conditions, the relationship between the processes involved in the consumption of the substrate and the lipase production and the development of lipase bioreactor system for a commercial use [4]. Microbial lipase fermentations are affected by the medium pH, temperature, medium composition, inoculation volume, aeration and agitation and many other factors related to bioreactor design such as impeller configuration, impeller spacing, impeller diameter, distance of the impeller from the bottom of bioreactor, *H/T* ratio, etc. Vadehra and Harmon [5] and Alford and Smith [6] observed that a presence of air was essential for lipase production by *Staphylococcus aureus*. Similar reports indicating the necessity of the aeration and agitation for lipase production are available in the literature though using different strains [7–10].

The initial shake flask study of *Candida rugosa* suggested that there should be an optimum balance between the aeration and agitation for maintaining proper oxygen concentration for the good growth of the *Candida rugosa* and the lipase activity [11]. Shaken flask appears to be a simple technique, however it can present a number of experimental difficulties [12–13] indicating that agitation is a preferable alternative. In the case of stirred bioreactors also, multiple impeller bioreactors are now becoming important due to efficient gas distribution, higher gas phase residence time, increased gas hold-up, superior liquid flow (plug flow) characteristics and lower power consumption per impeller as compared to the single impeller systems resulting into a substantial savings in the operational costs. The increase in the number of energy dissipation points due to redistribution, results into a higher volumetric gas–liquid mass transfer coefficients [14]. The aim of the present work was to determine the optimum oxygen tension favourable for the growth of *Candida rugosa* and the

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doi:10.1016/j.bej.2005.08.016

**Nomenclature**

| | |
|---|---|
| $D$ | diameter (m) |
| $K_s$ | Monod's constant (mg/l) |
| $N$ | impeller speed (rps) |
| $P$ | lipase activity (units/ml) |
| $P_g$ | power consumption in presence of gas (Watt) |
| $Q_G$ | volumetric gas-flow rate ($m^3$/s) |
| $q_p$ | specific rate of the lipase formation (units/mg-h) |
| $S$ | substrate concentration (mg/ml) |
| $S_0$ | initial substrate concentration (mg/ml) |
| $T$ | fermentation time (h) |
| $V$ | volume of liquid ($m^3$) |
| $V_g$ | the superficial gas velocity (m/s) |
| $W$ | blade width (m) |
| $X$ | cell concentration (mg/ml) |
| $X_m$ | maximum cell concentration (mg/ml) |

*Greek letters*

| | |
|---|---|
| $\alpha$ | growth associated product formation (g/g/h) |
| $\beta$ | non-growth associated product formation (g/g/h) |
| $\mu$ | specific growth rate ($h^{-1}$) |
| $\mu_{max}$ | maximum specific growth rate ($h^{-1}$) |

lipase activity. The parameters optimized by the shake-flask studies and the exponents obtained from the mass transfer correlations representing the dependence of $K_La$ (mass transfer coefficient) on process variables have been used in a 2 l bioreactor for the fermentation of *Candida rugosa* to obtain high lipase production [11,15].

## 2. Methods and materials

### 2.1. Microorganism

*Candida rugosa* (ATCC 14830) procured from Microbial Technology of Culture Collection, Chandigarh, India. The culture was preserved on MYPD agar slants (malt extract 0.3%, w/v, yeast extract 0.3%, w/v, peptone 0.5%, w/v and dextrose 1.0%, w/v) at 4 °C. Subculturing was done at the regular interval of 1 month.

### 2.2. Fermentation media

In the fermentation media, as per the initial optimization in the shake flaks studies [11] dextrose (20 g/l), urea (0.4 g/l) and olive oil (1 ml/l) were used as a carbon source, a nitrogen source and an inducer respectively. The modified basic mineral solution contained potassium dihydrogen phosphate (15 g/l), dipotassium hydrogen phosphate (5.5 g/l), magnesium sulphate heptahydrate (1 g/l), urea (4 g/l) and calcium chloride dihydrate (0.1 g/l). The micronutrient solution comprised of ferric chloride hexahydrate (10 mg/ml), inositol (0.004 mg/l), biotin (0.008 mg/l) and thiamine hydrochloride (0.2 mg/ml) [16].

### 2.3. Preparation of inoculum

Inocula were grown in small shake flasks (100 ml) that contained the same media as that in the final fermentation. The culture from the MYPD slants was suspended in saline solution (0.85%, w/v) and inoculated in 50 ml of the medium. The shake flasks were kept for incubation on an orbital shaker at 200 rpm for 36–40 h under ambient conditions. This culture was used as an inoculum for the bioreactor experiments.

### 2.4. Description of bioreactor design

A glass bioreactor was used for the fermentation of *Candida rugosa* constructed of glass (capacity 2.0 l with a height to diameter ratio of 1.692). The important design details of the bioreactor are described in our previous work [15] and the schematic representation of the experimental setup has been shown in Fig. 1. Impeller configuration used was triple impeller (the specifications of triple impeller i.e. disc turbine-pitched blade downflow turbine-pitched blade downflow turbine used in the experiments are illustrated in Table 1); location of the bottom impeller was 4.5 cm from the bottom. The ratio of the diameter of the impeller to the diameter of the tank was 0.333. Air was sparged inside the bioreactor with the air pump (Dolphin, India) and downward pumping spargers to avoid clogging. During fermentation, air at inflow

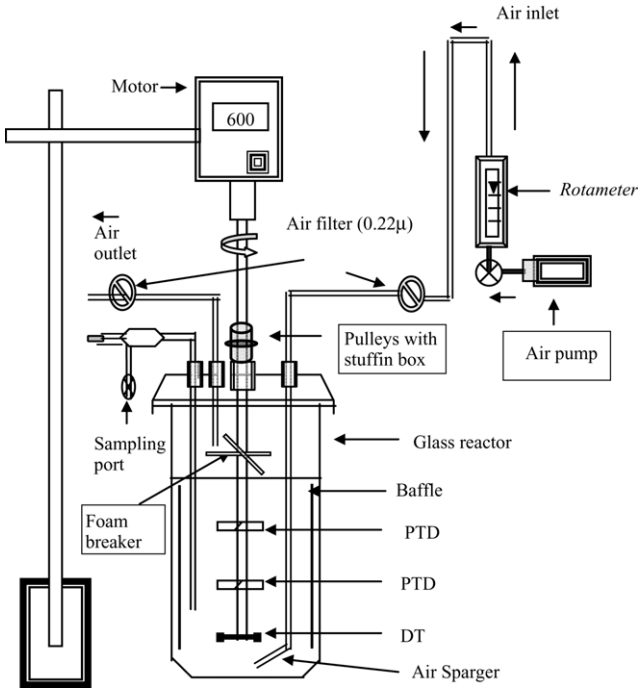


Fig. 1. Calibration curve of yeast cell count using haemocytometer.

Table 1
Triple impeller configuration

| Impeller configuration | Blades | Impeller diameter (cm) | Impeller blade length (cm) | Impeller blade width (cm) | Power number ($N_p$) |
|---|---|---|---|---|---|
| Triple impeller (distance between each impeller 5 cm) | Six bladed DT* | 4.33 | 1.4 | 1.2 | 7.8 |
| | 45° PTD• (number of blades 4) | 4.33 | 1.8 | 1.4 | |
| | 45° PTD (number of blades 4) | 4.33 | 1.8 | 1.4 | |

DT*: disc turbine; PTD•: pitched blade turbine down-flow.

and out flow was filtered through 0.22 μm filters (PTFE pall filters, Gelman laboratories, USA). In the gas space above the liquid level, one more attachment of two glass rods was made to serve as a foam breaker. The bioreactor was hermetically sealed and sampling was done under aseptic conditions at periodic intervals (4 h) via the sampling port and since the whole process is under positive pressure, chance of contamination during sampling is rare.

The basic mineral solution, urea, magnesium sulphate and substrates were autoclaved separately and incorporated into the medium under aseptic conditions (sterilization at 10 psig pressure for 20 min in an autoclave). Ferric chloride solution and vitamins were transferred after filtration through 0.22 μm cellulose nitrate filters (Pall filters, Gelman laboratories, USA) under aseptic conditions. Sterility testing of medium was performed on MYPD agar plates during each run to ensure complete sterilization. The dissolved oxygen (DO) was measured with oxygen probe of galvanic type (Oxygen electrode model no.: RL425, Russell Electrode, Scotland). The pH of the medium was checked with the glass pH electrode at regular intervals of time (Toshniwal Process Inst. Pvt., India). Samples were analyzed for the protein content, glucose consumption and lipase activity. At the time of the sampling, both the dissolved oxygen probe and the pH electrode probe were swabbed with alcohol in aseptic conditions and introduced in the fermentor aseptically after allowing for complete evaporation of alcohol, for the assessment of the oxygen concentration and pH of the medium, respectively.

### 2.5. Analytical methods

To check the reproducibility of the experimental data two samples were withdrawn at definite interval of time from the base of the fermentor. Fermentations were performed in duplicate cultures and analyses were also carried out in duplicate. The data was found to be reproducible within experimental limits of ±5% and the values reported in the figures are the average of the measurements.

The initial number of microbial cells added in the fermentation as an inoculum was estimated from the calibration curve prepared on digitalized image analyzer (Image Pro-plus) with haemocytometer (Neubour's chamber). Corresponding optical density of culture was measured on spectrophotometer (Chemito 2500 UV–vis spectrophotometer, calibration curve has been represented in Fig. 2). The inoculum of 1.21–1.34 × $10^6$ cells/ml (OD 0.35–0.45 absorbance

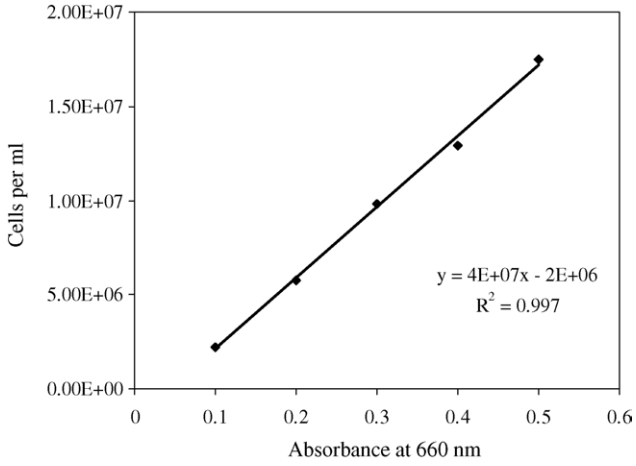


Fig. 2. A schematic representation of a laboratory scale bioreactor used for fermentation of *Candida rugosa*, (capacity 2 l).

units at 540 nm with 1:4 dilution in saline) was incorporated in the bioreactor. Cell growth was monitored by dry cell weight and correlated with turbidimetry. Samples were centrifuged in a centrifuge (Remi Motors, speed of 5000 rpm, at $g$ of 15000 m/s$^2$ for 15 min). The cell pellet was washed thrice with distilled water, further dried in an oven at 85–90 °C till constant weight, cooled in a desiccator and weighed. The supernatant separated after the centrifugation was assumed to be the source of the extracellular lipase enzyme and subjected to lipase activity assay, protein content analysis and glucose estimation.

Lipase activity assay was performed using olive oil as a substrate in a modified method [16]. Olive oil (1 ml) was added to Tris buffer (7 ml, 0.05 M, pH 7.0). Calcium chloride dihydrate (1 ml, 0.02 M) was added to the above reaction mixture subsequently to enhance the stability of the enzyme. On the addition of enzyme sample (1 ml), the mixture was further incubated at room temperature for 1 h. At the end of 1 h, the reaction was terminated with the addition of 20 ml of methanol and the contents were titrated against 0.05 M methanolic NaOH. Blank determinations were made for every sample separately. The blank contained the same constituents as test except the enzyme being denatured by the methanol (0 h reading). One unit of lipase activity was defined as the amount of enzyme necessary to hydrolyze 1 μmol of ester bond per minute under the assay conditions. Glucose was estimated using dinitrosalicylic acid method (DNSA)

[17]. The calibration curve was made with glucose (2 mg/ml). Protein concentration was determined by a modified Lowry method. The calibration curve was prepared using bovine serum albumin as a standard (1 mg/ml) [18]. Protease activity was measured using casein as a substrate using a modified method as proposed by Ota et al. [19].

## 3. Kinetic model

Fermentation is a very complex process and it is often difficult to obtain a complete picture of the fermentation process. A kinetic model can provide some insight with respect to the analysis, the design and the operation of the fermentor. The experimental data obtained in the present work was fitted to Monod's kinetics [20] and Luedking Piret model [21] to understand the fermentation process better and to analyze the effects of process variables on the fermentation results and thus optimize the process.

## 4. Results and discussion

### 4.1. Optimization studies for actual fermentation studies of Candida rugosa

From the shake flask studies it was inferred that there should be an optimum balance between the aeration (oxygen tension) and agitation (shear) to have maximum cell growth and lipase activity [11]. From the earlier work it was found that the maximum $K_L a$ value was obtained at an impeller speed of 600 rpm and constant gas-flow rate of 67.12 cc/s with triple impeller system, in a 2 l bioreactor [15]. With a view of optimizing the gas-flow rate, batches were performed in a bioreactor at a constant speed of agitation (600 rpm) and the gas-flow rate was varied in the range 19.45–67.12 cc/s (corresponding $V_g$ of 3.7–12.8 $\times 10^{-4}$ m/s). Variations in the gas-flow rates resulted in a change in the oxygen supply to the fermentor and hence changes in the oxygen transfer rates (OTR) as well as dissolved oxygen concentration, which in turn affected the cell growth and lipase production.

### 4.2. Performance comparison

A typical glucose consumption pattern of all the four batches is depicted in Fig. 3. The glucose utilization trajectory of the yeast is almost similar for all batches irrespective of the aeration rates, however a slightly faster glucose uptake has been seen in the case of Batch 3 (aeration rate 50.34 cc/s).

A comparison of the actual DO levels in all the four batches recorded during the course of these fermentations has been illustrated in Fig. 4. All these fermentation batches showed variable DO levels with respect to the corresponding gas-flow rates. Nevertheless, in all the four batches the initial DO level decreased during the initial phase of the fermentation process indicating the logarithmic phase of the *Candida rugosa* fermentation and high oxygen uptake rate. The DO levels in the case of Batch 1 (gas-flow rate 19.45 cc/s) were very low (approximately 0.5–1 mg/l) throughout the fermentation period, indicating a possibility of lower cell growth. For Batch 2 (gas-flow rate 33.81 cc/s) the DO was observed between 1 and 2 mg/l and then it reached about 6 mg/l after 90 h of fermentation. In the case of Batch 3, (gas-flow rate 50.34 cc/s) after an initial decrease in the DO value, it was found to be in the range of the 2–3 mg/l during its late logarithmic and initial stationary phase whereas in the case of Batch 4 (gas-flow rate 67.12 cc/s) the DO level was found to

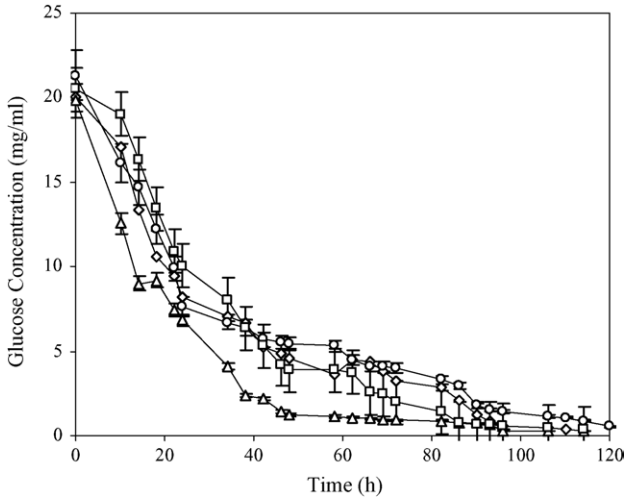


Fig. 3. Effect of oxygenation on glucose consumption during fermentation of *Candida rugosa* ((◊) gas-flow rate 19.45 cc/s, (◯) gas-flow rate 33.81 cc/s, (△) gas-flow rate 50.34 cc/s, (□) gas-flow rate 67.12 cc/s).

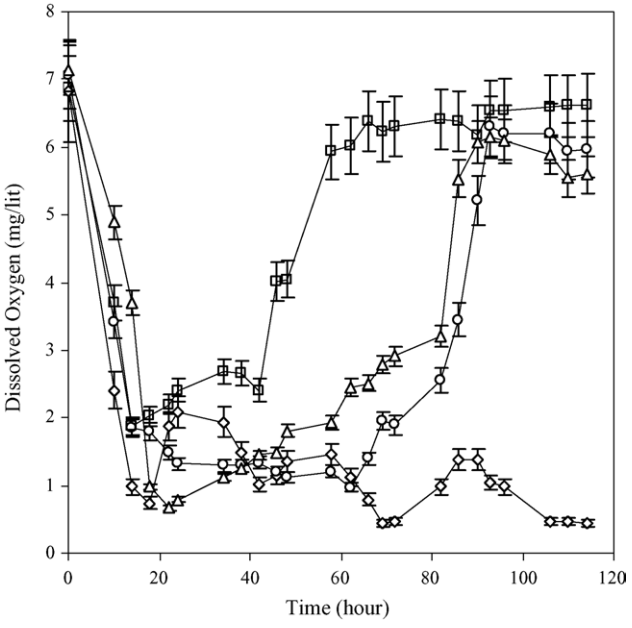


Fig. 4. Dissolved oxygen variation during fermentation of *Candida rugosa* at different gas flow rates ((◊) gas-flow rate 19.45 cc/s, (◯) gas-flow rate 33.81 cc/s, (△) gas-flow rate 50.34 cc/s, (□) gas-flow rate 67.12 cc/s).

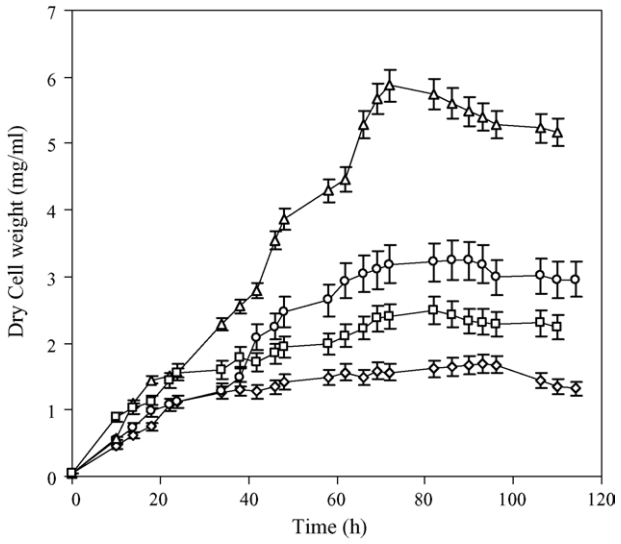


Fig. 5. Effect of oxygenation on cell growth of *Candida rugosa* during fermentation ((◊) gas-flow rate 19.45 cc/s, (◯) gas-flow rate 33.81 cc/s, (△) gas-flow rate 50.34 cc/s, (□) gas-flow rate 67.12 cc/s).

be in the range of 4–6 mg/l. There is a possibility that this variable DO levels would be influencing the cell growth and the resultant lipase activity. In order to see the effect of variable DO levels on cell growth and lipase production, their individual patterns obtained at various gas-flow rates were compared (Figs. 5 and 6).

As seen from the Fig. 5 showing cell growth pattern of all the four batches, it is very evident that change in the gas-flow rate has a definite effect on the growth of *Candida rugosa*. The cell mass increases with an increase in the gas-flow rate till the gas-flow rate of 50.34 cc/s (Fig. 5). At this gas-flow rate

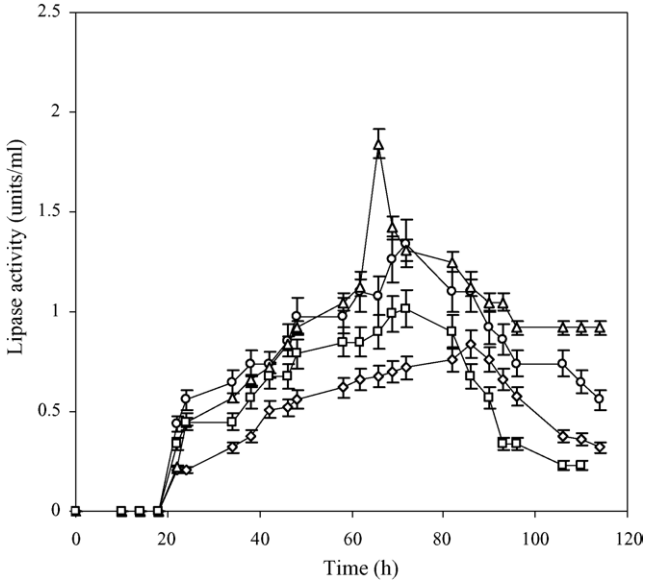


Fig. 6. Effect of oxygenation on lipase activity during fermentation of *Candida rugosa* ((◊) gas-flow rate 19.45 cc/s, (◯) gas-flow rate 33.81 cc/s, (△) gas-flow rate 50.34 cc/s, (□) gas-flow rate 67.12 cc/s).

maximum cell mass obtained was 5.87 mg/ml in 72 h. This could be attributed to the improved oxygenation in the bioreactor. However, as the gas-flow rate was further increased to 67.12 cc/s, the cell mass decreased significantly (2.49 mg/ml in 82 h). Improved gas-flow rate should have shown a proportionate increase in the cell growth. So it was expected that at this flow rate cell mass would be higher. However, the cell mass obtained at this flow rate was even lower than cell mass obtained with the gas-flow rate of 33.81 cc/s (3.17 mg/ml in 72 h) (Fig. 5). This indicated that dissolved oxygen concentration in the medium was probably more than the oxygen demand of the microorganism and this was probably responsible for the reduction in a cell mass. This indicates that an optimum oxygen concentration (optimum gas-flow rate is 50.34 cc/s as obtained in the present work) is essential for growth of *Candida rugosa* and any further increase in the oxygen supply or dissolved oxygen concentration results in a detrimental effect. Above results supported the observations obtained in the case of shake flask studies indicating that the cell mass gets affected with the increase in the surface area of the medium during fermentation [11].

Further examination of the growth curves of all four batches revealed that even lipase production was affected due to the change in the oxygen concentration of the fermentation medium (Fig. 6). At lower gas-flow rate (19.45 cc/s) the optimum lipase achieved was 0.84 units/ml in 86 h while at the gas-flow rate of 33.81 cc/s it was 1.34 units/ml in 72 h. At high gas-flow rates (50.34 cc/s) and 67.12 cc/s the lipase optima were 1.84 and 1.02 units/ml, respectively and were attained in 66 and 72 h, respectively. Thus, with an increase in the gas-flow rate the lipase production increases till the gas-flow rate of 50.34 cc/s (superficial gas velocity $9.5 \times 10^{-4}$ m/s) and the optimum fermentation time (66 h) was also found to be slightly improved as compared to other gas-flow rates. These observations indicated that DO has a significant effect on the lipase production as well. Thus, it can be proposed that the *Candida rugosa* fermentation being aerobic, oxygen is a necessity for the cell growth to obtain the optimum cell mass, however higher oxygen concentration beyond the optimum value is detrimental and decreases the biomass production, especially in the late logarithmic phase of growth cycle which in turn affected the overall lipase production.

### 4.3. Protease effect

There is a possibility, that besides oxygen, the lipase activity at the same fermentation time might be influenced by other enzymes (possibly protease enzyme) during the fermentation. Hence the batches were evaluated for the protease content of the medium. The samples withdrawn during the fermentation were analyzed for the presence of protease with casein as a substrate [19]. It was observed from the analysis of the samples that protease appeared in the broth at the end of exponential growth phase of the biomass i.e. around 62 h and then its concentration gradually increased [Table 2]. In the case

Table 2
The protease activity in a broth during fermentation

| Fermentation time (h) | Protease activity (units/ml) |
|---|---|
| 0 | 0 |
| 18 | 0 |
| 38 | 0 |
| 48 | 0 |
| 54 | 0 |
| 62 | 39.27 |
| 66 | 48.69 |
| 72 | 56.69 |
| 86 | 146.77 |
| 94 | 170.65 |
| 114 | 193.91 |
| 120 | 184.48 |

of Batch 3 (gas-flow rate of 50.34 cc/s) the optimum lipase activity occurred in 66 h and in the case of Batch 2 (gas-flow rate 33.81 cc/s) and Batch 4 (gas-flow rate 67.12 cc/s) the optimum enzyme release was found to be at the end of 72 h. In all these cases the effect of protease seemed marginal as the difference in protease concentration was insignificant between 66 and 72 h. But in the case of Batch 1 (gas-flow rate, 19.45 cc/s) where the lipase activity was optimum in 84 h, there is a possibility that protease was also responsible for the reduction in the lipase activity due to extended fermentation period. Nevertheless, the effect of high dissolved oxygen concentration appears to be a major cause in reducing the lipase release as well as in the reduction in the lipase activity during fermentation.

### 4.4. Model

#### 4.4.1. Application of Monod's kinetics to bioreactor study data

The specific growth rate of the biomass was calculated as a function of the rate of change of substrate consumption at a given substrate concentration. The values of $\mu_{max}$ and $K_s$ measured using Linweaver–Burk plot are depicted in the Table 3. The saturation constant ($K_s$) is the measure of the affinity of the organism for the growth-limiting substrate (i.e. glucose). Smaller the $K_s$ value, higher is the substrate affinity and greater is the capacity to grow rapidly at lower growth-limiting substrate concentration. The $K_s$ value was found to be very low for Batch 3 while the $\mu_{max}$ value was found to be highest at this gas-flow rate (Fig. 7). Thus, it can be inferred that the gas-flow rate of 50.34 cc/s was favourable for the growth of the cells as compared to the other gas-flow rates. Also, at this gas-flow rate the lipase production was observed to be higher and achieved relatively earlier as compared to other gas-flow rates. Favorable oxygenation at this gas-flow rate might have resulted in enhanced cell growth due to the precise uptake of the glucose substrate and the organism might have reached the stationary phase earlier. Lower $\mu_{max}$ value and higher $K_s$ value at the gas-flow rate of 67.12 cc/s as compared to gas-flow rate of 50.34 cc/s confirmed that the high oxygen concentration in the later stages of the exponential phase and early stationary phase have remarkably affected the cell growth and suggests the need of the optimum oxygen tension for the maximum cell growth. Thus, the estimation of kinetic parameters has quantitatively confirmed our earlier qualitative explanation related to the existence of optimum oxygen concentration corresponding to a gas-flow rate of 50.34 cc/s.

Table 3
Fitting of Monod's kinetics

| Gas-flow rate (cc/s) | $\mu_{max}$ ($h^{-1}$) | $K_s$ (mg/ml) |
|---|---|---|
| 19.45 | 0.059 | 13.78 |
| 33.81 | 0.06 | 12.71 |
| 50.34 | 0.10 | 6.08 |
| 67.12 | 0.063 | 16.29 |

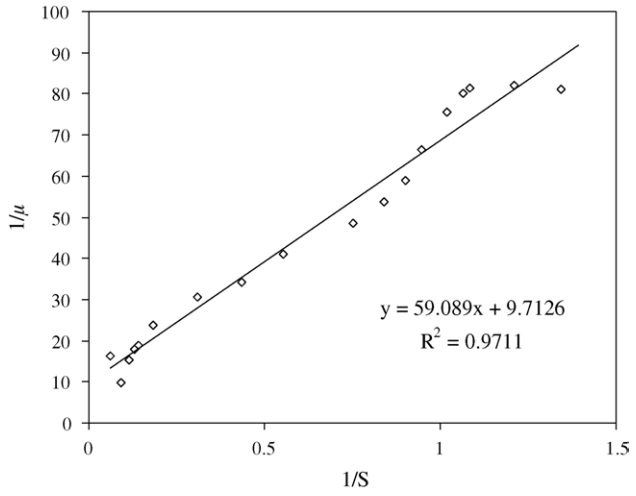


Fig. 7. Lineweaver Burk plot (gas-flow rate 50.34 cc/s).

#### 4.4.2. Application of Luedking and Piret model to bioreactor data

The mixed model of Luedking and Piret [21] has been employed to relate lipase production rate to the cell growth. The rate of change of lipase formation ($q_p$) values were calculated with respect to time at a given substrate concentration. These $q_p$ values are then plotted against specific growth values ($\mu$) in the mixed growth model. The plot of $q_p$ versus $\mu$ for the combined results of the above batches is shown in Fig. 8. In the case of growth-associated product, as the cell growth progresses, the product formation takes place and the product optimum is reached at a maximum cell growth (i.e. at the end of exponential phase or early stationary phase). In the case of non-growth associated product formation, the product concentration is independent of cell growth pattern. Following equation generally describes the relationship between lipase formation and the cell concentration.

$$q_p = \alpha\mu + \beta \quad (1)$$

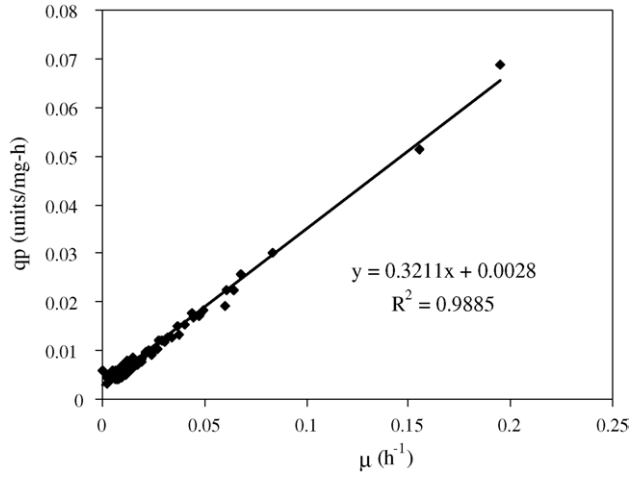


Fig. 8. Description of *Candida rugosa* lipase production by the mixed-growth model (Luedking and Piret model).

From the above equation, it can be seen that the lipase formation from *Candida rugosa* shows *a* linear relationship with the cell growth. The results showed that the biosynthesis of lipase can be attributed to the growth-associated type. In the present work $\alpha$ has been obtained to be equal to 0.3211 g/g/h and has been identified as the product formation coefficient while $\beta$ has been obtained to be equal to 0.0028 g/g/h and this denoted the level of lipase resulted from the inoculum with the $R^2$ value of 0.9885. The model suggested the possible explanation of the decrease of the lipase production with a decrease in the cell mass at late exponential phase in view of the release pattern of the lipase and its dependency on the cell growth.

### 4.5. Correlation

From the fitting of the Luedking and Piret model it was observed that there exists the possibility of dependency of the lipase production on cell mass. In an attempt to correlate these two parameters, the ratios of lipase activity to cell mass (measured at optimum lipase activity h) were calculated for the above batches. The ratios were found to be 0.51, 0.42, 0.43 and 0.42 units/mg for the batches with gas-flow rates of 19.45, 33.81, 50.34 and 67.12 cc/s, respectively. Since the lipase production is growth-associated, maintenance of this aforementioned ratio within 0.4–0.5 units/mg might prove an important monitoring tool in the fermentation of *Candida rugosa*. From these batches, the DO level of 2–3 mg/l as observed in Batch 3 is supposed to be an optimum for both the cell growth and the lipase production, especially when this DO level was maintained in the late logarithmic phase and early stationary phase. The DO levels less than the aforesaid limit might not be sufficient for the optimum cell mass and the lipase activity, while higher DO levels are found to be unfavorable for the cell growth and lipase activity during the course of fermentation.

## 5. Conclusions

At an impeller speed 600 rpm and the gas-flow rate 50.34 cc/s (superficial gas velocity $9.5 \times 10^{-4}$ m/s) optimum lipase activity and cell growth was achieved. The cell mass increased by 20% and lipase activity improved by 2.5-folds as compared to shake flask studies. Besides, lipase release was attained earlier (66 h as against 72 h) as compared to shake flask studies. The enhancement could be attributed to the balanced oxygenation and agitation in the fermentor during the exponential phase and early stationary phase to achieve maximum cell growth and subsequent improved lipase activity. It was also established in the study that protease enzyme released in the medium towards the end of the exponential phase of the cell growth did not significantly affect the lipase activity. Consequently high dissolved oxygen concentration (excessive) was found to be responsible for the decreased lipase production and an optimum oxygen concentration (2–3 mg/l) corresponding to gas-flow rate of 50.34 cc/s has been observed. The obtained qualitative results have also been quantitatively confirmed by fitting of the Monod' kinetics and Luedking Piret model to the experimental data. Thus, the present work has enabled us both qualitatively and quantitatively to establish the fact that optimum oxygen concentration is required for maximizing the cell growth and lipase production.

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