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Improved Poly-ε-Lysine Biosynthesis Using *Streptomyces noursei* NRRL 5126 by Controlling Dissolved Oxygen During Fermentation

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The growth kinetics of *Streptomyces noursei* NRRL 5126 was investigated under different aeration and agitation combinations in a 5.0 l stirred tank fermenter. Poly-ε-lysine biosynthesis, cell mass formation, and glycerol utilization rates were affected markedly by both aeration and agitation. An agitation speed of 300 rpm and aeration rate at 2.0 vvm supported better yields of 1,622.81 mg/l with highest specific productivity of 15 mg/l.h. Fermentation kinetics performed under different aeration and agitation conditions showed poly-ε-lysine fermentation to be a growth-associated production. A constant DO at 40% in the growth phase and 20% in the production phase increased the poly-ε-lysine yield as well as cell mass to their maximum values of 1,992.35 mg/l and 20.73 g/l, respectively. The oxygen transfer rate (OTR), oxygen utilization rate (OUR), and specific oxygen uptake rates (qO_2) in the fermentation broth increased in the growth phase and remained unchanged in the stationary phase.

Keywords: Growth kinetics, oxygen transfer rate, oxygen utilization rate, poly-ε-lysine, *Streptomyces noursei*

Poly-ε-lysine (ε-PL), (*S*)-poly(imino(2-amino-1-oxo-1,6-hexanediyl)), is a naturally occurring homopolymer of L-lysine with a degree of polymerization of 25–35, and characterized by peptide bonds between the carboxyl and ε-amino groups of L-lysine [13]. ε-PL is reported to be an effective antimicrobial agent against a wide spectrum of microorganisms including yeasts, fungi, Gram-positive and Gram-negative bacteria, and bacteriophages [28, 31]. Moreover, it is water soluble, biodegradable, heat stable, edible, and nontoxic. Therefore, it has multifarious applications as food preservatives, emulsifying agent, dietary agent, biodegradable fibres, highly water absorbable hydrogels, drug carriers, and anticancer agent enhancer, and in biochip coatings [25].

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ε-PL production has been achieved in batch and fed-batch systems by *Streptomyces albulus* [14, 15, 29] and *Kitasatospora* sp. MY 5-36 [32] with aerobic fermentation using a two-stage pH control strategy. It was produced by Hiraki and Suzuki [12] with cells immobilized in gel, embedded in porous ceramic and other supports, but without a significant increase in production. Shih *et al*. [26] developed a process of ε-PL production using waste from levan fermentation by *S. albulus*. Strategies for enhancing the production of ε-PL is continually being developed.

Aeration and agitation in the fermentation broth normally satisfy the oxygen requirements of a fermentation process. Agitation is important for adequate mixing, mass transfer, and heat transfer. It also maintains homogenous chemical and physical conditions in the culture by continuous mixing [6]. Dissolved oxygen (DO) in the broth is the result of a balance of its consumption rate in the cells and the rate of oxygen transfer from the gas to the liquid phase. Monitoring DO in the broth is mandatory because often oxygen becomes the factor governing the metabolic pathways in microbial cells. The oxygen transfer rate (OTR) in a bioreactor depends on the liquid side mass transfer coefficient (k_L), the total specific surface area available for mass transfer (a), and the driving force in terms of concentrations. Many empirical correlations have been proposed for k_La estimation [5, 7, 8, 16].

The oxygen uptake rate (OUR) is one of the fundamental physiological characteristics of culture growth and has been used for optimizing the fermentation process [19, 33]. Usually, the specific oxygen uptake rate (qO_2) is calculated from OUR, which is determined experimentally. OUR measurement has recently received due attention in different bioprocess studies [4, 9, 11, 21, 23].

The current study focuses on developing a scale-up process in a stirred tank reactor for ε-PL production from *S. noursei* NRRL 5126. The commercial importance of ε-PL demands not only a search for newer and better yielding microbial strains, but also economically viable bioprocesses for its large-scale production. ε-PL is currently produced

industrially by *S. albulus*. Our previous work explicated the possibility to use *S. noursei* as a better alternative for *S. albulus*, which is as yet not reported in the literature [1, 2]. The optimum pH for production of ε-PL is below 4.0. *S. noursei* showed significant growth between pH 4.5–3.5 and ε-PL production at this pH range was comparable with the other studies mentioned in the literature [14, 15, 27, 22]. The agitation speed, aeration rate, and percent oxygen required were optimized in the present study. Some relationships between the ε-PL production, k_La, OTR, OUR, and other growth and production related parameters were also evaluated through appropriate mathematical equations.

Materials and Methods

Materials

All the chemicals used in the present study were of AR grade and were purchased from Hi-Media Limited, Mumbai, India. Standard ε-PL was generously provided by Handary Bio-Engineering B.V., The Netherlands.

Microorganism and Medium

The bacterial strain, *S. noursei* NRRL 5126, was a gift sample from ARS Culture Collection, USA. M3G medium containing (g/l) proteose peptone (10), glucose (50), $(NH_4)_2SO_4$ (10), $FeSO_4{\cdot}7H_2O$ (0.03), $MgSO_4{\cdot}7H_2O$ (0.5), $ZnSO_4{\cdot}7H_2O$ (0.04), K_2HPO_4 (0.8), and KH_2PO_4 (1.36) was used as a seed medium. For production, medium previously optimized in our laboratory containing (g/l) proteose peptone (10), glycerol (30), $(NH_4)_2SO_4$ (8.0), $FeSO_4{\cdot}7H_2O$ (0.03), $MgSO_4{\cdot}7H_2O$ (0.5), $ZnSO_4{\cdot}7H_2O$ (0.04), K_2HPO_4 (0.8), and KH_2PO_4 (1.36) was used. Citric acid and aspartic acid were added after 24 h and 36 h fermentation with 5.0 mM and 2.0 mM, respectively [1, 2]. The initial pH of the medium was adjusted to 6.8 with 1 M NaOH before autoclaving at 121°C for 20 min.

Cell Growth and ε-PL Production in Batch Fermentation

A 4.0% (8.3×10^8 cells/ml) precultured seed of *S. noursei* NRRL 5126 was used for production of ε-PL (96–144 h, 30°C) in a 5.0 l stirred tank bioreactor, BIOSTAT B_{plus} (Sartorius, Germany), with a 2.0 l working volume equipped with a pH probe (Type InPro 3030; Mettler Toledo) and a DO probe (Hamilton, Switzerland). A fermenter vessel (UniVessels) having a height-to-diameter ratio of 2:1 with an internal concave bottom was used for optimum mixing. It was equipped with four baffles with six-flat-blade disc impellers (two) measuring 64 mm diameter, spaced 9.0 cm from each other. During the fermentation, the pH, DO, and aeration (gas flow) were monitored online, and ε-PL titer, cell growth, and residual glycerol were analyzed offline.

Effects of Aeration, Agitation, and Percent DO in the Fermentation Medium

The effects of aeration and agitation on ε-PL production were systematically studied in batch fermentation with different combinations of aeration rates of 1.0, 2.0, and 3.0 vvm and agitation speeds of 200, 300, and 400 rpm. Optimum percent DO in the fermentation medium was studied by varying the constant DO concentration at 30%, 40%, and 50% in the growth phase and 10%, 20%, and 30% in the production phase of the microorganisms at constant agitation speed of 300 rpm.

Determination of k_La

The determination of k_La in a bioreactor is essential in order to establish aeration efficiency and to quantify the effects of the operating variables on the provision of DO. A simple, fast, and advantageous method was used to determine k_La based on step change of oxygen concentration as explained by Dorresteijn *et al.* [5]. The method does not need an equilibrium concentration of oxygen in liquid phase (C*) and hence eliminates the need to await a new steady state. The change in DO concentration caused by a step change of the oxygen concentration in the bioreactor is described by the following mass balance equation:

$$\frac{dc}{dt}=k_La.(C^*-C)-qO_2 \quad (1)$$

where dc/dt represent O_2 accumulation in the liquid phase, C represents the DO concentration in the liquid medium (mol/m^3), C* is the DO concentration in the liquid phase at equilibrium with the oxygen concentration in the gas phase (mol/m^3), k_La is the volumetric O_2 transfer coefficient (1/h), and qO_2 is the oxygen consumption rate of the biomass (mol/m^3.h) To determine k_La, it was assumed that for the duration of the measurement, qO_2 remains constant. Nitrogen gas was passed until the oxygen concentration became close to zero. Air was then used to replace nitrogen, and the rate of increase of DO concentration in the broth was recorded. Both during the degassing and oxygen enriching steps, the broth was agitated as per specified conditions in the fermenter. k_La was calculated using the following formula.

$$k_La=-\frac{1}{t_i}\ln\frac{C_{L,t_2}-C_{L,0}\pm\sqrt{C^2_{L,0}+4.C^2_{L,t_1}+C^2_{L,t_2}-4.C_{L,0}.C_{L,t_1}+2.C_{L,0}.C_{L,t_2}-4.C_{L,t_1}.C_{L,t_2}}}{2.\left(C_{L,t_1}-C_{L,0}\right)} \quad (2)$$

Kinetic Analysis of Cell Growth, ε-PL Formation, and Glycerol Utilization

The rational design and optimization of industrial fermentations requires an understanding of production kinetics. In order to optimize the production of ε-PL for industrial applications, a fundamental understanding of fermentation parameters is necessary. This is enabled by developing a kinetic model for fermentative production of ε-PL, which is a vital part of overall investigations of ε-PL fermentation. A useful analytical model for biopolymer fermentation kinetics includes temporal variations of substrate (S), biomass (X), and biopolymer products (P).

Microbial growth: the logistic equation. The microbial processes may not always follow the classical kinetic model of substrate-limited biomass growth and product formation proposed by Monod [18]. Therefore, the logistic equation, a substrate-independent model, was used as an alternative empirical function [30]. The logistic equation [Eq. (3)] can be described as follows:

$$\frac{dx}{dt}=\mu_m\left(1-\frac{X}{X_m}\right).X \quad (3)$$

where μ_m is the maximum specific growth rate (1/h) and X_m is the maximum attainable biomass concentration (g/l). The integrated form of Eq. (3) using $X=X_o$ (t=0) gives a sigmoid variation of X as a

function of t, which may represent both an exponential and a stationary phase. Logistic equations are a set of equations that characterize growth in terms of carrying capacity (k). The usual approach is based on formulation in which specific growth rate is related to the amount of unused carrying capacity.

$$X=\frac{X_0 e^{kt}}{1-X_0/X_m(1-e^{kt})} \quad (4)$$

k can be estimated from the log–log plot of 1/X.dX/dt and $1-X_0/X_m$ as an intercept [30].

Product formation: Luedeking–Piret equation. The production of many microbial metabolites follows the classical equation of Luedeking and Piret [17]. According to this model, the product formation rate (r_P) depends on both the instantaneous biomass concentration and the growth rate in a linear manner.

$$r_p=\alpha.r_X+\beta.X \quad (5)$$

where r_p and r_x are the rates of the formation of P and X, respectively; α and β are the parameters determined experimentally, which provides the framework for classifying microbial metabolites into primary ($\beta=0$), secondary ($\alpha=0$), and mixed ($\alpha\neq0$ and $\beta\neq0$). The integration of Eq. (5) (where $P=P_o$ at $t=0$) with Eq. (4) yields

$$P_{(t)} = P_o+\alpha X_o\left(\frac{e^{kt}}{1-(X_o/X_\infty)(1-e^{kt})}-1\right)+\beta\frac{X_m}{\mu_m}\ln\left(1-\frac{X_o}{X_m}(1-e^{kt})\right) \quad (6)$$

Equation (6) can be used to determine the concentration of product formed at time t during the fermentation. The model employs rate equations for biomass (X), the product (ε-PL), and the substrate (glycerol) to describe the fermentation process. Equation (5) can be modified to Eq. (7) as follows:

$$P=\alpha.X+\beta X.t+P_0+\Phi \quad (7)$$

where Φ is the product of α and X_0 in numerical value

Glycerol uptake: the modified Luedeking–Piret equation. Glycerol was used in the present study to form cell components and metabolic products as well as for the maintenance of cells. The consumption of glycerol can be explained by the modified Luedeking–Piret equation. A simplified equation for glycerol consumption can be written as

$$-\frac{ds}{dt}=\gamma\frac{dX}{dt}+\delta.X \quad (8)$$

where γ is the sum of $1/Y_{X/S}$ and $\alpha/Y_{P/S}$, and δ is the sum of $\beta/Y_{P/S}$ and m_s. Similarly, integration of Eq. (8) with $S=S_0$ at $t=0$ yields

$$S_{(t)} = S_0-\gamma.X_0\left\{\frac{e^{kt}}{[1-(X_0/X_m)(1-e^{kt})]}-1\right\}-\delta\frac{X_m}{\mu_m}\ln\left[1-\frac{X_0}{X_m}(1-e^{kt})\right] \quad (9)$$

Equation (9) can be used to determine unutilized glycerol in the medium.

Determination of OTR, OUR, and qO_2

Accurate estimation of the OTR at different scales and under different operational conditions has a relevant role for the prediction of the metabolic pathway for both growth and production of any wished metabolite in aerobic cultures. It is of critical importance for the selection, design, and scale-up of bioreactors. The static gassing-out method based on an unsteady-state DO concentration material balance, which consists of the rate of oxygen transfer from gas to liquid, was used to calculate the OTR. The medium DO level was decreased to close to zero and then increased up to saturated level by supplying nitrogen and air in the medium respectively. The rate of change in DO concentration was recorded by using a polarographic electrode.

$$OTR=k_L.\alpha(C^*-C) \quad (10)$$

OTR was easily calculated from known values of k_La from Eq. (2).

OUR was calculated with the help of known OTR and the oxygen concentration profile in the liquid phase. The OUR during the fermentation medium was calculated using equation 11 [10].

$$OUR=k_L.a(C^*-C)-\left(\frac{dc}{dt}\right) \quad (11)$$

The following mass balance equation for the DO in batch fermentation can be established to calculate qO_2.

$$\left(\frac{dc}{dt}\right)=k_La(C^*-C)-q_{O_2}.C_X \quad (12)$$

where C_x is the cell concentration

Analytical Methods

Analysis of ε-PL. The culture broth was centrifuged (8,000 ×*g*, 10 min), and the ε-PL concentration was measured in the supernatant using the method of Shen *et al.* [24]. Briefly, 1.0 ml of supernatant was added to 2.88 ml of 0.1 mM phosphate buffer (pH 7.0) and 120 µl of trypan blue solution (1.0 mg/ml), mixed thoroughly, and incubated at 37°C for 60 min. Absorbance was measured after centrifugation (10,000 ×*g*, 10 min) at 580 nm on a Helios (α) UV–VIS spectrophotometer (Thermo Electron, Germany).

Dry cell weight (DCW) determination. The relationship between the optical density at 660 nm and the DCW (70°C until constant weight) was established with initial experiments, and the resulting equation was used to determine the DCW for further experiments by measuring the optical density after suitable dilutions.

Glycerol estimation. Glycerol in the fermentation broth was estimated by a colorimetric procedure described by Bok and Demain [3]. To 1.0 ml of fermentation broth, 1.0 ml of 0.015 M sodium metaperiodate in 0.12 M HCl and 2.0 ml of 0.1% rhamnose were added. Addition of 4.0 ml of Nash reagent produced a yellow-colored product after incubating the reaction mixture at 53°C, which had a strong absorbance at 412 nm.

RESULTS AND DISCUSSION

Optimum aeration and agitation are required for an aerobic bioprocess for homogeneous and better distribution of gas. The low solubility of oxygen in aqueous solutions makes DO to be a limiting nutrient in the broth. Pinches and Pallent [20] described the fall of DO concentration in broths due to the high demand during the exponential phase of the microorganism until it reaches the stationary phase, and the subsequent increase due to lower demand. In the present study, DO concentration started decreasing after 6.0 h of fermentation and reached close to zero during its growth phase (12–36 h) (data not shown). It was observed that the pH of the culture broth also decreased

from its initial value of 6.8 to 4.0 by 36 h fermentation and remained constant around 3.0 at 48 h and thereafter (data not shown). Cell growth in the medium increased biomass, decreased pH, and ultimately decreased the DO. After 36 h of fermentation, the pH decreased below 4.0, which is highly favorable for ε-PL production [14, 22, 31]. Thereafter, in the production phase, cell growth slowed down, DO concentration reached its saturation level, and pH remained constant at around 3.0.

Effects of Aeration and Agitation on Cell Growth, ε-PL Production, and Glycerol Consumption

Batch fermentations were carried out with different combinations of aeration rates at 1.0, 2.0, and 3.0 vvm and agitation speeds of 300, 400, and 500 rpm. A total of nine experiments were performed and their effects on cell growth are shown in Fig. 1. Batch fermentation by *S. noursei* NRRL 5126 showed a classical growth trend. After the inoculation, the cells entered the exponential growth phase (after 12 h) with a very short lag phase followed by the stationary phase after 84–96 h, and remained constant thereafter. The maximum cell mass reached was 18.84 g/l with 400 rpm and 2.0 vvm. A logistic equation was used to express and describe the cell growth and analyze the parameters for model fit. The model predictions for cell growth rate were basically consistent with all the experimental combinations from the exponential phase to the stationary phase (the correlation coefficients were above 0.96) (data not shown), which demonstrated the suitability of this model for predicting the growth of *S. noursei* NRRL 5126 under the given conditions. The highest biomass formation was observed at 2.0 vvm with 300 and 400 rpm, which confirmed the importance of aeration in *S. noursei* NRRL 5126 fermentation.

The fermenter run conditions, specific growth rate, specific ε-PL formation rate, specific glycerol consumption rate, carrying capacity factor (k), and various factors affecting growth, ε-PL production, and glycerol consumption (*viz.*, α, β, γ, and δ) are summarized in Table 1. k_La, maintenance coefficient (m), productivity (1/h), and yield coefficients ($Y_{x/s}$, $Y_{p/s}$, and $Y_{p/x}$) for different combinations of aeration and agitation are also illustrated in Table 1. The growth rate reflected as maximum specific growth rate (μ_{max}) shows that cell growth rate was not largely affected by aeration but it did decrease slightly with an increase in agitation speed (Table 1). The specific maximum ε-PL formation rate varied between the values of 0.023–0.034/h, whereas the specific maximum glycerol consumption rate remained unaltered within the range of 0.014–0.020/h at the different aeration and agitation conditions. These results again re-signifies that the ε-PL biosynthesis is dependent on biomass formation and ultimately on the glycerol consumption. The carrying capacity factor (k), which is a measure of maximum cell mass and rate of change of cell mass formation, remained constant in the range of 0.042–0.056 (1/h).

ε-PL biosynthesis is reported to be a highly aerobic fermentation. Fig. 2 clearly depicts that ε-PL production was maximum (1,622.81 mg/l) with highest productivity of 15.026 mg/l.h (Table 1) at 2.0 vvm and 300 rpm, and it remained constant at 400 rpm and 2.0 vvm. Its reproducibility was tested and verified by keeping all the parameters same. From the result, it can be seen that ε-PL formation is linearly related to cell growth up to 96 h. The kinetics of product formation was based on the Luedeking–Piret equation. From the experimental data, values for parameters of the Luedeking–Piret model for ε-PL formation were calculated (Table 1). The growth-associated parameter α, calculated for the production of ε-PL, showed diverse values at different aeration rates at constant rpm. The value of α increased for 200 rpm, remained constant for 300 rpm, and decreased for 400 rpm with 1.0, 2.0, and 3.0 vvm respectively. Contrary to this, the β value remained constant for all the aeration and agitation combinations in the range between 1.5 to 2.5 mg ε-PL/g cell.h. The larger values of α in all the operational parameters proved the growth-associated biosynthesis of ε-PL in the fermenter. In our previous studies at shake-flask level, both α and β values were large

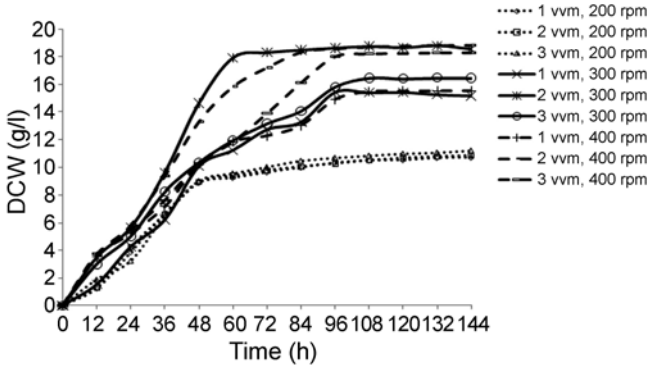

Fig. 1. Effects of aeration rate and agitation speed on biomass formation using *S. noursei* NRRL 5126 fermentation.

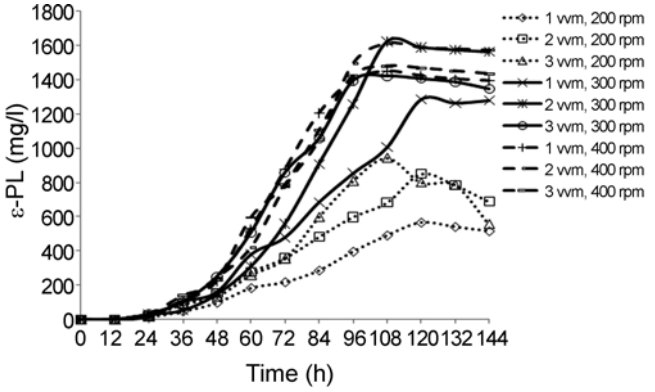

Fig. 2. Effects of aeration rate and agitation speed on ε-PL production using *S. noursei* NRRL 5126 fermentation.

Table 1. Effects of aeration rate and agitation speed on various kinetic parameters.

Agitation speed	200 rpm			300 rpm			400 rpm		
Aeration rate	1 vvm	2 vvm	3 vvm	1 vvm	2 vvm	3 vvm	1 vvm	2 vvm	3 vvm
μ_m (1/h)	0.058	0.052	0.059	0.0460	0.052	0.056	0.039	0.0494	0.0394
Sp ε-PL formation rate (1/h)	0.028	0.032	0.032	0.034	0.023	0.029	0.028	0.028	0.029
Sp glycerol consumption rate (1/h)	0.014	0.014	0.016	0.020	0.013	0.017	0.019	0.018	0.019
k (per h)	0.0521	0.0512	0.567	0.050	0.045	0.0487	0.046	0.0427	0.0423
α (mg ε-PL/g cell)	−20.59	−65.75	−65.26	−31.51	−42.11	−56.97	−116.3	−77.84	−40.15
β (mg ε-PL/ g cell.h)	0.791	2.187	1.69	1.466	1.78	2.36	2.67	2.53	1.7123
γ (g glycerol/g cell)	0.1022	0.0773	0.1251	0.079	0.048	0.06	0.126	0.071	0.088
δ (g glycerol/g cell .h)	5.14×10^{-4}	5.75×10^{-4}	3.47×10^{-4}	4.38×10^{-4}	4.35×10^{-4}	5.29×10^{-4}	3.45×10^{-4}	3.56×10^{-4}	2.93×10^{-4}
m (g glycerol/ g cell.h)	0.0076	0.0031	0.0048	0.0064	0.0060	0.0088	0.00632	0.00786	0.008364
$Y_{x/s}$ (g cell/ g glycerol)	6.407	6.679	7.327	9.485	11.911	10.626	8.830	10.953	11.091
$Y_{p/s}$ (mg ε-PL/ g glycerol)	359.253	547.897	641.054	769.228	1111.512	917.645	827.611	1025.745	941.567
$Y_{p/x}$ (mg ε-PL/ g cells)	53.956	79.741	86.933	83.852	86.504	86.360	93.621	86.073	81.178
k_La (1/h)	0.422	0.862	1.186	0.445	0.895	1.315	0.473	0.963	1.411
Productivity (mg/l.h)	4.730	7.077	8.725	10.705	15.026	13.170	13.410	14.911	13.688

and showed significant effect and revealed that the ε-PL production was mixed growth-associated (unpublished data). This shift in values is probably due to the change in operational conditions, especially oxygen concentration, in the fermenter level. Higher oxygen supply at the fermenter level resulted in higher cell mass formation and ε-PL biosynthesis as compared with the shake-flask level.

The titers of ε-PL are known to decrease either by increase in medium pH and/or by digestion with a peptide hydrolase(s) (ε-PL degrading enzyme) produced by an ε-PL producer, which act as self-protectors for microorganisms [14, 22, 25]. Although an experimental evaluation for ε-PL degrading enzyme was not performed, the production of this enzyme in *S. noursei* NRR 5126 appeared to be low at the fermenter level, which resulted in no degradation of the ε-PL in the fermentation broth, as seen from Fig. 2.

A comparison of the Luedeking–Piret model predicted and experimental values for ε-PL production showed good agreement, with a correlation coefficient more than 0.98 (data not shown). It is obvious that this model is very suitable for describing ε-PL yield and specific product formation rate. After fitting the experimental data to Eq. (7), the following equation was used to describe the relationship between ε-PL formation (2 vvm, 300 rpm) and cell concentration:

$$P=42.11\ X+1.78\ X.t+175.38$$

Utilization of glycerol by *S. noursei* NRRL 5126 at different aeration rates and agitation speeds is illustrated in Fig. 3. The rate of glycerol utilization was almost similar for all the combinations and it was slightly higher at 300 and 400 rpm. It was observed that an increase in biomass concentration was accompanied by a gradual decrease in residual glycerol concentration after 60 h of fermentation. Glycerol was consumed for cell growth, cell maintenance, and product formation. The model prediction data agreed well with the experimental results and showed a correlation coefficient >0.97 (data not shown). The values for γ and δ remained constant in the range of 0.05 to 0.12 g glycerol/g.cell and 3×10^{-4}–5.7×10^{-4} g glycerol/g.cell.h, respectively (Table 1)

The values for maintenance coefficient (g glycerol/ g.cell.h) and yield coefficient are illustrated in Table 1. Aeration at 2.0 vvm and agitation at 300 rpm gave highest values for yield coefficient with respect to cell mass, ε-PL, and glycerol consumption. The k_La value was determined as a function of aeration rate and agitation speed, and it was found to increase with an increase in the agitation and aeration rates. The aeration rate had a more prominent effect on the k_La change (Table 1). A linear relationship was observed between k_La and aeration rate upto 6.0 vvm

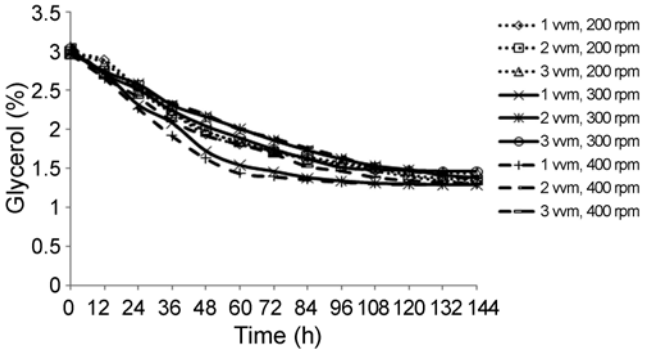

Fig. 3. Effects of aeration rate and agitation speed on glycerol consumption using *S. noursei* NRRL 5126 fermentation.

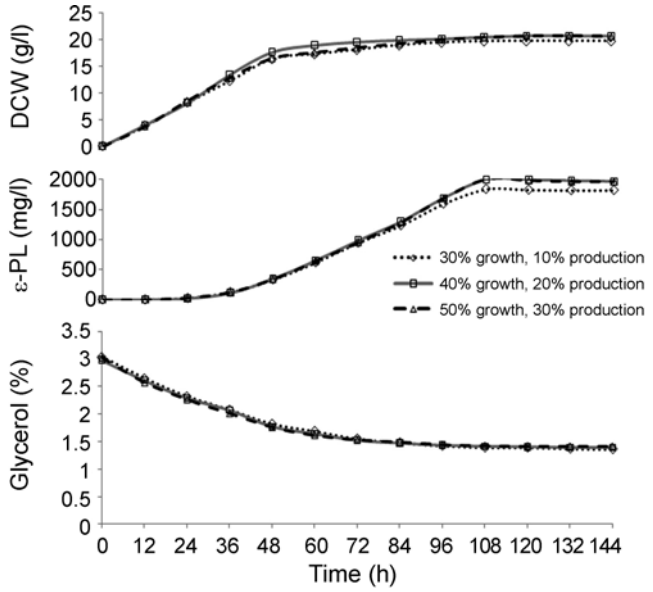

Fig. 4. Effect of constant percent DO in the growth and production phase using *S. noursei* NRRL 5126 fermentation.

(data not shown), which was used for further calculations of OTR, OUR and qO_2.

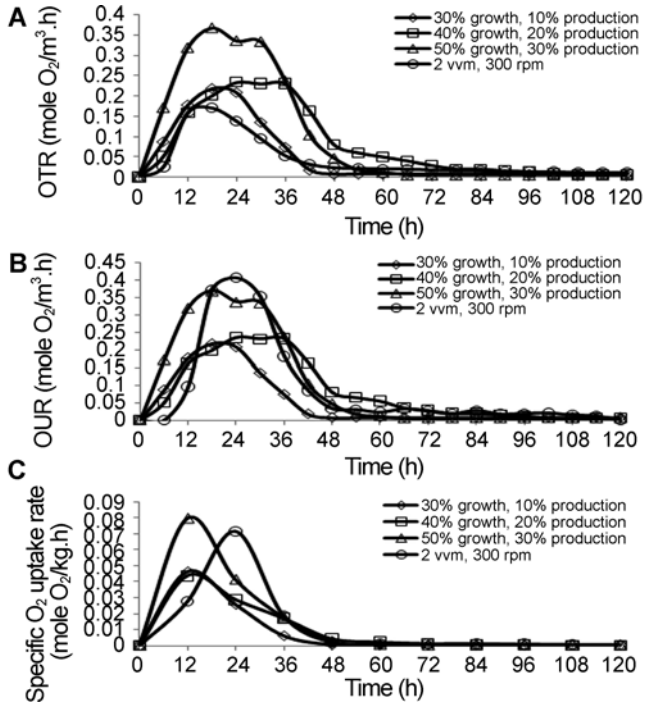

Fig. 5. Effects of different percent DO in the fermentation medium on OTR (**A**), OUR (**B**), and qO_2 (**C**) with *S. noursei* NRRL 5126.

Effects of Percent DO in Growth and Production Phase on ε-PL Production, OTR, OUR, and qO_2

A decrease in the DO concentration was observed in all the previous combinations during the exponential phase (growth phase) of the microorganism, due to the high specific oxygen demand by the cells, and reached a minimum. The DO concentration gradually increased in the stationary phase of the cells and remained constant until the end of fermentation. This enabled an alternative strategy of controlling the DO concentration at a constant value for growth and production phase of *S. noursei* NRRL 5126 for further improving the ε-PL yield. The fermentation medium was subjected to different percentage of DO (30%, 40%, and 50%) in the growth phase (upto 36 h) and lower percent DO (10, 20, and 30%) in production phase (after 36 h) at 300 rpm, as shown in Fig. 4. Cell mass reached to its maximum value of 20.73 g/l with 40% and 20% constant DO in growth and production phase, respectively, which in turn resulted in the highest ε-PL yield as 1,992.35 mg/l. Glycerol consumption rates were almost constant for all the three different parameters (Fig. 4).

During the aerobic bioprocess, oxygen is transferred from a rising gas bubble into a liquid phase and ultimately to the site of oxidative phosphorylation inside the cell. The OTR can control the overall rate of the bioprocess, and consequently, it can determine the capacity of a bioreactor. Scale-up of a fermenter using a constant OTR has been often chosen as a main criterion from the last few years. In the present study, OTR varied with different operational conditions. The OTR increased up to 24 h, decreased thereafter up to 48 h, and remained unchanged (close to zero) throughout the rest of the fermentation (Fig. 5). The maximum possible OTR observed with the present system was at 50% DO in the growth phase (0.37 mol O_2/m^3.h).

The OUR is consecutive to the OTR, and is the rate-controlling step in the fermentation. However, the consumption of oxygen can affect the OTR. The OUR increased in the exponential growth phase owing to a very high rate of substrate consumption, whereas in the stationary phase it decreased because of a decrease in the metabolic activity of cells (Fig. 5). A time profile of qO_2 obtained at different operational conditions is shown in Fig. 5. As with the OUR, the qO_2 reached its maximum up to 24 h and then decreased after 48 h.

Moderate conditions for *S. noursei* NRRL 5126 (2.0 vvm, 300 rpm) favored better ε-PL production and cell mass formation. To provide optimum oxygen demand to the microorganism for synthesis of ε-PL, 40% DO in the growth phase and 20% DO in the production phase gave the best results in terms of ε-PL biosynthesis and for cell mass formation. k_La value was found to have a linear correlation with aeration rate (vvm). OTR, OUR and qO_2 are the key parameters involved in the aerobic biosynthesis of ε-PL, which gave 1,992.35 g/l as the highest titer reported to date with *S. noursei* NRRL 5126.

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