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Research Article

STUDY THE GROWTH KINETICS OF *PEDIOCOCCUS ACIDILACTICI* WITH ESTIMATION OF KINETIC PARAMETERS AND APPLIED IN LARGE SCALE PEDIOCIN PRODUCTION

BARNALI MANDAL*

Department of Chemical Engineering, University College of Science & Technology, University of Calcutta, Kolkata - 700 009, West Bengal, India. Email: bmandal20@yahoo.co.in

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ABSTRACT

Objectives: The aim of the study was to determine the growth kinetics of *Pediococcus acidilactici* using a mathematical model for large scale pediocin production.

Methods: Growth kinetics of *P. acidilactici* has been studied for pediocin production in small scale batch fermenter (Erlenmeyer flask) using meat processing waste medium. The experiments have been conducted with varying the concentrations of glucose, protein, and lactic acid. A mathematical model has been developed to describe growth rate, products (pediocin and lactic acid) formation rate, and substrates (glucose and protein) utilization rate. Monod model for dual substrates (glucose and protein) has been used with considering lactic acid inhibition. Luedeking-Piret model has been introduced to describe the production of pediocin and lactic acid.

Results: The values of kinetic parameters have been determined using experimental data and model equations. The model prediction has been compared satisfactorily with the experimental data for the validation of the model.

Conclusions: The developed model was satisfactorily validated to scale up the production of pediocin.

Keywords: *Pediococcus acidilactici,* Pediocin, Meat processing waste, Monod model, Luedeking-Piret model, Kinetic parameters.

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INTRODUCTION

In the recent years, biological preservation of foods, a novel approach has gained growing attention for the extension of the shelf-life and enhancement of the safety of foods using antagonistic microorganisms or their metabolic products to inhibit or destroy undesired microorganisms in foods [1,2]. This strategy aims at the reduction of health risks without changing the qualities of food products. Bacteriocins produced by different genera of lactic acid bacteria offer several desirable properties that make them suitable for preservation of food. They are potential peptides that can kill or inhibit pathogenic bacteria that compete for the same ecological niche or nutrient pool [3,4]. They are non-toxic, generally recognized as safe status substances and active at low concentrations [4]. Pediocins are those bacteriocins which are produced by *Pediococcus* species show broad inhibition activity against pathogenic and food spoilage bacteria [5].

In general, a kinetic model is used to understand the relationships between cell growth, substrate utilization, and product formation of the microorganism. The values of kinetic parameters are estimated using experimental data of small scale reactor. Modeling is an important strategy to understand the behavior of large scale reactor.

Under the present study, growth kinetics of *Pediococcus* strain has been determined in the small bioreactor (Erlenmeyer flask) at different initial glucose concentration, initial protein concentration, and lactic acid concentration. A mathematical model has been developed for cell growth, products formation, and substrates consumption. The values of kinetic parameters have been evaluated using experimental results. Validity of kinetic model has been checked through the experimental and simulated data in large scale pediocin production.

METHODS

Microorganisms and growth medium

Pediococcus acidilactici NCIM 2292 procured from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (Pune, Maharashtra, India) was used as the producer strain of pediocin. This bacterium is highly anaerobic, and its growth temperature is 30°C. Meat processing waste (MPW) hydrolysate containing optimum initial concentration of protein, and glucose was used as growth medium [6]. *Staphylococcus aureus* NCIM 2127 obtained from NCIM, National Chemical Laboratory, Pune, Maharashtra, India, was used as indicator organism to assay the pediocin activity. The indicator organism was grown in nutrient agar at 30°C.

Analytical methods

Pediocin activity was determined using well diffusion method, and the concentration of biomass, lactic acid, glucose, and protein was determined using the same protocol described in the previous article [6].

Batch experiments in Erlenmeyer flasks for determination of growth kinetics

To determine the growth kinetics of *P acidilactici*, a series of batch fermentations was conducted in Erlenmeyer flasks (250 ml with working volume 100 ml) at 30°C for 24 hrs incubation. Separate sets of experiments were conducted to study the effects of initial glucose concentration, initial protein concentration, and lactic acid concentration on specific growth rate and specific production rate of pediocin. The experimental methods are described below.

Exclusive glucose dependence

To determine the dependence of specific growth rate exclusively on glucose, its initial concentration was varied from 0 to 30 g/L, keeping the values of other parameters at optimum level, determined through response surface methodology (RSM) technique [6]. No lactic acid was added to the medium during initiation of the batch experiments.

Exclusive protein dependence

During these experiments, initial protein concentration was varied from 0 to 21.86 g/L while the optimum values of other parameters as determined using RSM were maintained at the beginning [6]. Similar

to the experiments for exclusive glucose dependence, initial lactic acid concentration was kept at zero.

Exclusive lactic acid dependence

For these experiments, lactic acid was deliberately added to the growth medium at the beginning. Initial concentration of lactic acid was varied in the range of 0-12 g/L. The values of other parameters were maintained at optimum.

Performance of large scale bioreactor under batch mode

Batch fermentation of *P. acidilactici* has been conducted in a 5 L stirred bioreactor (Eyela, Tokyo Rikakikai Co. LTD, Japan) with working volume of 3 L for the large scale production of pediocin using MPW medium. The fermenter was equipped with automatic temperature controllers that maintained constant temperature (30°C) by recirculation of water. Fermentation was performed without aeration and without controlling of pH. The agitation was kept at 50 rpm to maintain spatial homogeneity throughout the fermenter. Each experimental run was performed in triplicate. The samples were withdrawn at 2 hrs interval to determine pediocin activity and concentrations of lactic acid, biomass, residual glucose, and residual protein.

MATHEMATICAL MODEL

Growth kinetics

Under the present research, an attempt has been made to determine unstructured growth kinetics of *P. acidilactici*. Therefore, the exact intracellular reactions, as considered in structured models [7], are not incorporated, and cells are assumed to be constituted of uniform biomass without any demarcation as described in classical Monod type [8] growth models. As evident from experimental observation of the present study as well as from literature review [9], growth of *P. acidilactici* culture depends on two substrates, namely, protein and glucose. In addition, cell growth is inhibited by the production of lactic acid. From the literature survey, it appears that different types of growth kinetics have so far been attempted [10] to incorporate the effects of concentration of glucose, protein, and lactic acid on the growth of *P. acidilactici* strain [9]. For the simplicity of the model, a multiplicative type dual substrate (glucose and protein) model along with reciprocal correlation term for lactic acid inhibition has been attempted. This is as follows:

$$\mu = \frac{\mu_m S_1}{S_1 + K_{S_1}} \frac{S_2}{S_2 + K_{S_2}} \frac{K_{iLA}}{LA + K_{iLA}} \quad (1)$$

Where, μ is specific growth rate (h^{-1}), μ_m is the maximum specific growth rate (h^{-1}), t is time (h), S_1 is concentration of glucose (g/L), S_2 is protein concentration (g/L), LA is lactic acid concentration (g/L), K_{S_1} is saturation constant for glucose (g-glucose/L), and K_{S_2} is protein saturation constant (g-protein/L). K_{iLA} is inhibition constant for lactic acid formation (g-lactic acid/L).

$$\mu = \frac{1}{X}\frac{dX}{dt} \quad (2)$$

Where, X is biomass concentration (g/L).

Estimation of kinetic parameters for growth

Determination of K_{S_2}

The data of batch type experiments described in above section, exclusively varying initial concentration of protein have been used to determine K_{S_2}. As the initial glucose concentration was kept at the optimum level, the expression $\frac{S_1}{S_1 + K_{S_1}}$ in Equation (1) may be considered to be constant at the beginning of each experiment of the series. Since no lactic acid was present at the beginning, therefore, Equation (1) reduces to

$$\mu = \mu_m \phi \frac{S_2}{S_2 + K_{S2}} \quad (3)$$

Where,

$$\phi = \frac{S_1}{S_1 + K_{S_1}} \quad (4)$$

The values of $\mu_m \phi$ and K_{S_2} have been determined from the intercept and slope of the double-reciprocal plot obtained by graphing reciprocals of μ against those of S_2. The value has also been determined using non-linear regression analysis.

Determination of K_{S_1} and μ_m

The data obtained from batch type experiments in Erlenmeyer flasks varying only the initial glucose concentration have been used to determine the value of K_{S_1}. Since initial value of protein concentration was maintained at the optimum level, therefore, $\frac{S_2}{S_2 + K_{S_2}}$ remain constant.

Let $\frac{S_2}{S_2 + K_{S_2}} = \xi$ (5)

The value of ξ may be calculated using the values of K_{S_2} and S_2 is optimum concentration of protein [6].

The Equation (1) may be written as,

$$\frac{\mu}{\xi} = \frac{\mu_m S_1}{S_1 + K_{S_1}} \quad (6)$$

Let the LHS of the Equation (6) be denoted by ψ

i.e., $\frac{\mu}{\xi} = \psi$

Therefore,

$$\psi = \frac{\mu_m S_1}{S_1 + K_{S_1}} \quad (7)$$

Making double-reciprocal plot by graphing $\frac{1}{\psi}$ against $\frac{1}{S_1}$ the values of kinetic parameters μ_m and K_{S_1} have been determined from the intercept and slope, respectively. The values have also been cross-checked by non-linear regression analysis.

Determination of K_{iLA}

The batch type experimental data obtained by varying initial lactic acid concentration have been used to determine K_{iLA}. Using the values of μ_m, K_{S1}, and K_{S2}, the initial value of $\frac{\mu_m S_1}{S_1 + K_{S_1}} \frac{S_2}{S_2 + K_{S_2}}$ has been determined.

Referring to Equation (1),

$$\frac{\mu}{\frac{\mu_m S_1}{S_1 + K_{S_1}} \frac{S_2}{S_2 + K_{S_2}}} = \frac{K_{iLA}}{LA + K_{iLA}} \quad (8)$$

The LHS of Equation (8) may be denoted by "ω."

Therefore,

$$\omega = \frac{K_{iLA}}{LA + K_{iLA}} \quad (9)$$

By plotting $(\frac{1}{\omega} - 1)$ against LA, the value of K_{iLA} has been determined from the slope.

Product generation kinetics

Lactic acid generation

Lactic acid is a product which is partly related to growth of cells and partly independent of growth. Thus, Luedeking-Piret model [11] is applicable for the specific rate of generation, q_{LA}, of lactic acid.

Therefore,

$$q_{LA} = \alpha_1 \mu + \beta_1 \quad (10)$$

Where,

$$q_{LA} = \frac{1}{X}\frac{dLA}{dt} \quad (11)$$

Where, α_1 is the growth-associated constant (g-lactic acid/g-biomass), and β_1 is the non-growth-associated constant (g-lactic acid/g-biomass/h) for lactic acid. The values of q_{LA} and μ have been determined at different reaction times. A plot has been made using q_{LA} as ordinate and μ as the abscissa. The values of α_1 and β_1 have been determined from the slope and the intercept of the plot. Non-linear regression analysis using the values of q_{LA} and μ has also been done to evaluate α_1 and β_1.

Pediocin production

As observed by previous researchers [9,11] similar to lactic acid, pediocin production is also partly related to cell growth and partly independent of growth. Thus, Luedeking-Piret model [11] is applicable for the rate of pediocin production (q_p).

$$q_P = \alpha_2 \mu + \beta_2 \quad (12)$$

Where,

$$q_P = \frac{1}{X}\frac{dP}{dt} \quad (13)$$

Where, P is the pediocin activity (AU/ml), α_2 is growth-associated constant (AU/mg-biomass), and β_2 is the non-growth-associated constant (AU/mg-biomass/h) for pediocin. Values of α_2 and β_2 have been determined both graphically and through non-linear regressions analysis using the values of q_p and μ.

Substrates utilization

In the cell cycle, substrates contribute to biomass growth, energy source, extracellular product generation, maintenance and repairing of cells. The yield coefficients for substrates may be defined as follows:

1. Glucose

$$\frac{dS_1}{dt} = -\frac{1}{Y_{X/S_1}}\frac{dX}{dt} \quad (14)$$

2. Protein

$$\frac{dS_2}{dt} = -\frac{1}{Y_{X/S_2}}\frac{dX}{dt} \quad (15)$$

Determination of yield coefficient

The yield coefficient, Y_{X/S_1} of biomass with respect to glucose has been assumed to be constant determined using the correlation following:

$$Y_{X/S_1} = \frac{|\Delta X|_{24h}}{|\Delta S_1|_{24h}} \quad (16)$$

Where, $|\Delta X|_{24h}$ = mass of *P. acidilactici* (biomass) formed over 24 hrs batch time,

$|\Delta S_1|_{24h}$ = mass of glucose utilized over 24 hrs batch time.

The value of Y_{X/S_2}, i.e., the yield coefficient of biomass with respect to protein has also been determined by following the same method. Therefore,

$$Y_{X/S_2} = \frac{|\Delta X|_{24h}}{|\Delta S_2|_{24h}} \quad (17)$$

Where, $|\Delta S|_{24h}$ =mass of protein utilized over 24 hrs batch time.

Large scale batch reactor

The performance of large scale batch reactor has been attempted to be predicted by the development of a deterministic mathematical model using the kinetic parameters determined under the present study. The batch reactor has been schematically represented in Fig. 1.

The differential mass balance equations for biomass, glucose, protein, and pediocin under controlled pH conditions are as follows:

1. Biomass

$$\frac{dX}{dt} = \frac{\mu_m S_1 X}{S_1 + K_{S_1}} \frac{S_2}{S_2 + K_{S_2}} \frac{K_{iLA}}{LA + K_{iLA}} \quad (18)$$

2. Lactic acid

$$\frac{dLA}{dt} = \alpha_1 \frac{dX}{dt} + \beta_1 X \quad (19)$$

3. Pediocin

$$\frac{dP}{dt} = \alpha_2 \frac{dX}{dt} + \beta_2 X \quad (20)$$

4. Glucose

$$\frac{dS_1}{dt} = -\frac{1}{Y_{X/S_1}}\frac{dX}{dt} \quad (21)$$

5. Protein

$$\frac{dS_2}{dt} = -\frac{1}{Y_{X/S_2}}\frac{dX}{dt} \quad (22)$$

RESULTS AND DISCUSSION

Kinetic parameters for growth

Estimation of K_{S_2}

As described above section, a double-reciprocal plot has been made using inverse of μ and initial protein concentration as the ordinate and abscissa, respectively, in Fig. 2. The linear nature of the plot in Fig. 2 establishes the Monod type dependence of specific growth rate on initial protein concentration. The value of K_{S_2} has been determined using Equation (3) both graphically and through regression analysis (R^2=0.996). The value of K_{S_2} is determined as 0.77 g-protein/L from least square equation shown in Fig. 2.

Estimation of K_{S_1} and μ_m

As described above section, the values of μ_m and K_{S_1} have been determined by plotting $1/\psi$ versus $1/S_1$ using Equation (7) both graphically and through regression analysis. The value of ψ has been calculated using Equation (6). Protein concentration (S_2) has been

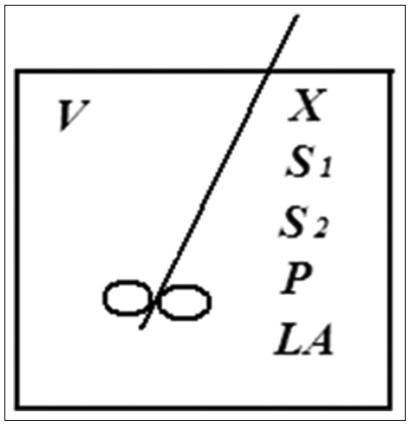

Fig. 1: Schematic of batch reactor

considered at optimum value, i.e., 12.67 g/L and the value of K_{S_2} of 0.77 g-protein/L as determined. The values of K_{S_1} and μ_m have been determined to be 1.34 g-glucose/L and 0.45/h, respectively, from regression equation shown in Fig. 3. The value of R^2 (coefficient of determination) is 0.987.

Estimation of K_{iLA}

As described, the value of K_{iLA} has been determined by plotting $(1/\omega-1)$ against initial lactic acid concentration using Equation (9). The values of $\mu m, K_{S_1}$ and K_{S_2} are used as obtained previously. Optimum values of S_1 and S_2 are considered. The value of K_{iLA} has been determined as 1.03 g-lactic acid/L from regression equation shown in Fig. 4. The value of R^2 is 0.899.

Estimation of α_1 and β_1

The values of α_1 and β_1 have been determined by plotting q_{LA} and μ as the ordinate and abscissa, respectively, in Fig. 5. The values of α_1 and β_1 have been determined using Equation (10) both graphically and through regression analysis (R^2=0.947). The values of α_1 and β_1 are determined as 2.661 and 0.36, respectively, from regression equation shown in Fig. 5.

Estimation of α_2 and β_2

The values of α_2 and β_2 have been determined by plotting q_P versus μ using Equation (12) both graphically and through regression analysis. The values of α_2 and β_2 have been to be 1732 and 0.004, respectively, from regression equation shown in Fig. 6. The value of R^2 was 0.988.

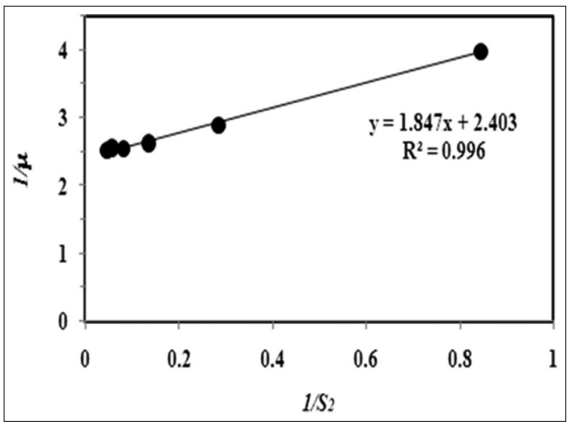

Fig. 2: Double-reciprocal (Lineweaver-Burk) plot for protein

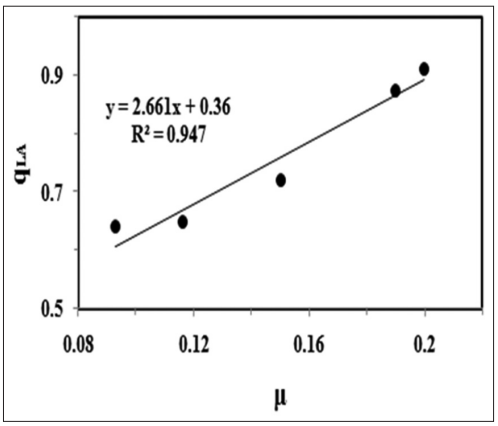

Fig. 5: q_{LA} versus μ plot

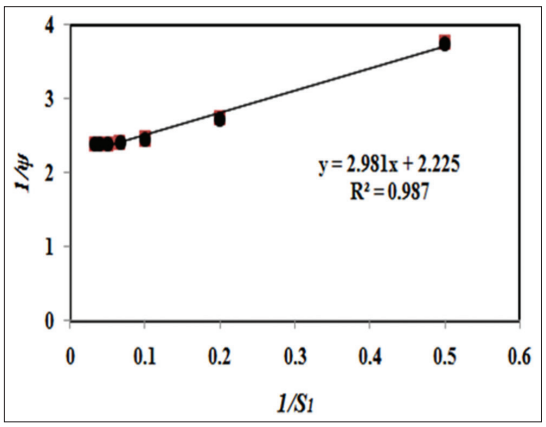

Fig. 3: $1/\psi$ versus $1/S_1$ plot

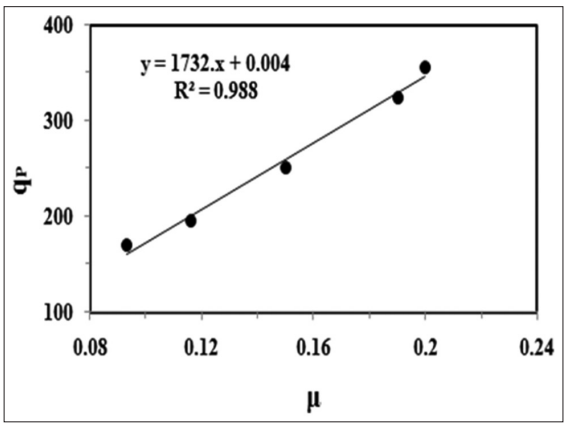

Fig. 6: q_p versus μ plot

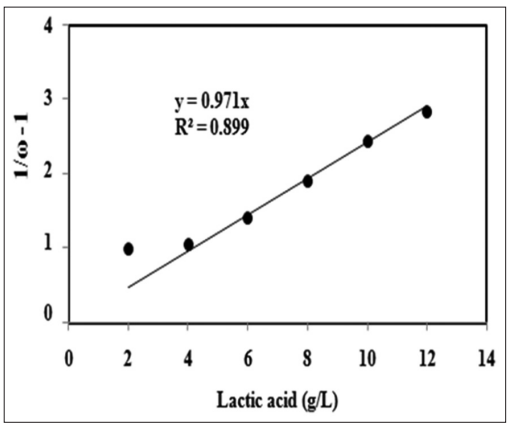

Fig. 4: $(1/\omega-1)$ versus lactic acid plot

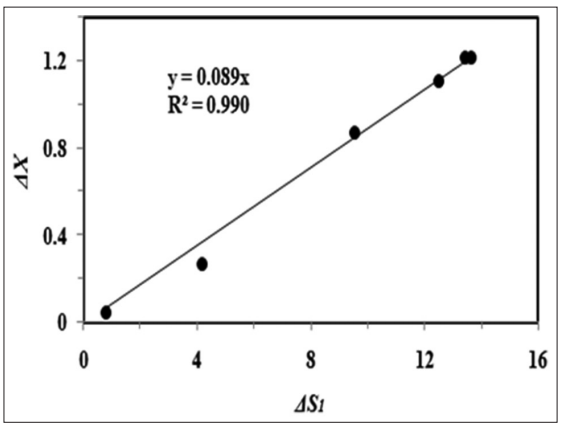

Fig. 7: ΔX versus ΔS_1 plot

Estimation of Y_{X/S_1}

The yield coefficient, Y_{X/S_1} of biomass with respect to glucose has been determined by plotting ΔX as ordinate and ΔS_1 as the abscissa using Equation (16). The value of Y_{X/S_1} has been determined as 0.089 from regression equation shown in Fig. 7. The value of R^2 was 0.99.

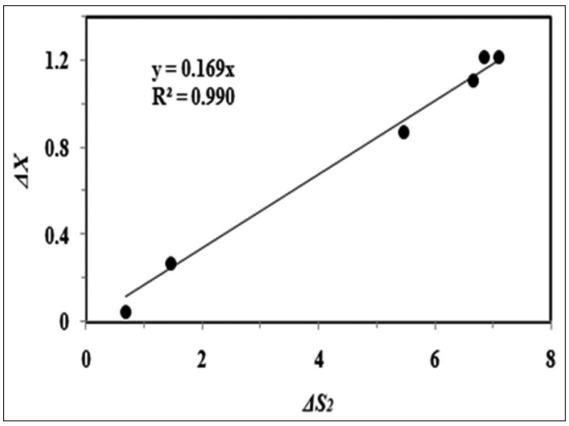

Fig. 8: ΔX versus ΔS_2 plot

Estimation of Y_{X/S_2}

The value of Y_{X/S_2}, i.e., the yield coefficient of biomass with respect to protein has also been determined by plotting ΔX as ordinate and ΔS_2 as the abscissa using Equation (17). The value of Y_{X/S_2} is determined as 0.169 from regression equation shown in Fig. 8. The value of R^2 is 0.99.

Performance of large scale bioreactor under batch mode

Simulated (Equations 18, 20, 19, 21, and 22) and experimental time histories of cell concentration, pediocin activity, lactic acid, residual glucose, and residual protein have been shown in Fig. 9a-e, respectively. After 24 hrs of incubation, the concentration of biomass is 1.26 g/L. It has been observed from experimental data shown in Fig. 9a that the exponential growth occurs up to 18 hrs. Pediocin activity reaches value of 2285 AU/ml at the end of the fermentation. As depicted by Fig. 9a and b, it may be inferred that pediocin is mostly growth associated as its activity gets saturated as the exponential phase of growth is over. The concentration of lactic acid peaks at 6.58 g/L. From the analysis of Fig. 9a and c, it is evident that production of lactic acid is both associated with growth and non-growth type because its concentration increases monotonically even after the cessation of exponential growth phase. Both the findings are comparable to the results of previous

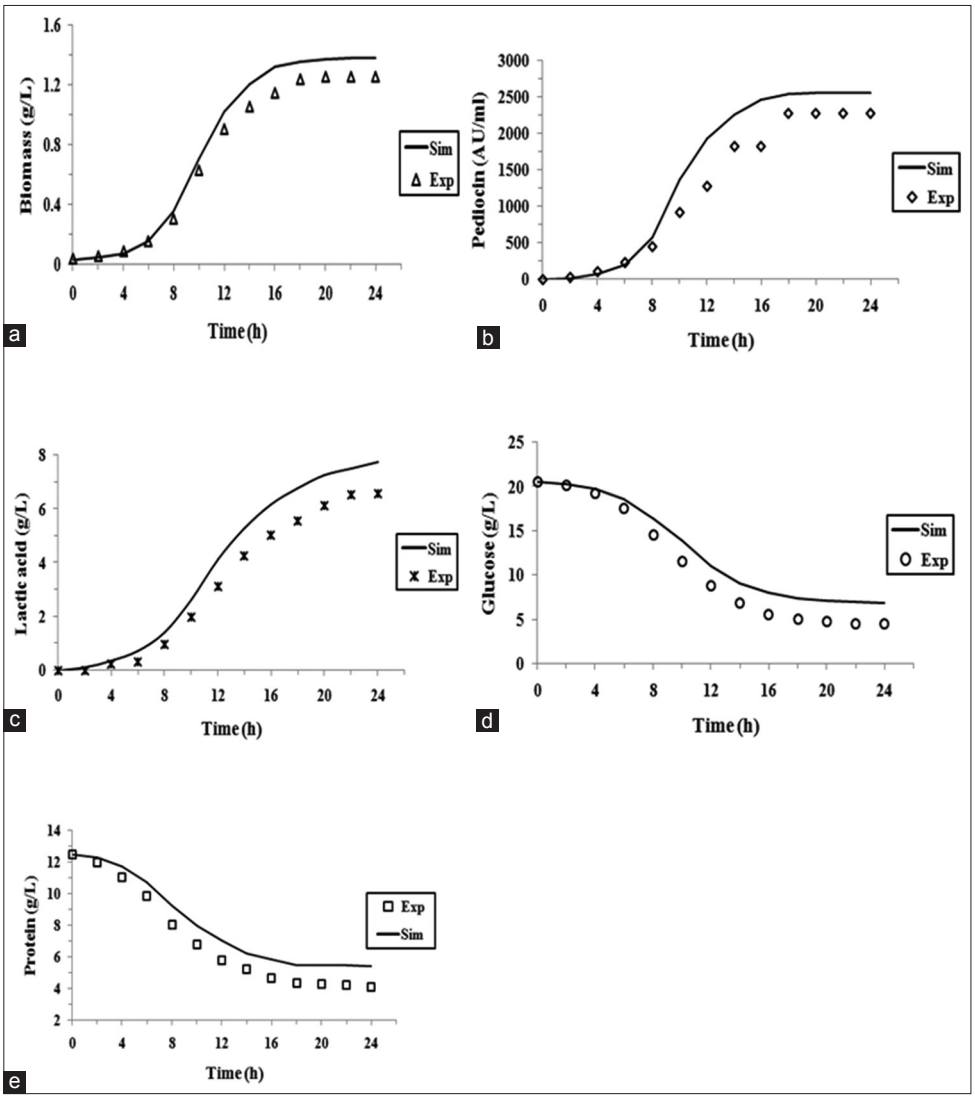

Fig. 9: Experimental data of batch fermentation of *Pediococcus acidilactici* NCIM2292 using meat processing waste medium without pH control: (a) Biomass; (b) pediocin activity; (c) lactic acid; (d) residual glucose, and (e) residual protein. Continuous lines represented simulated values corresponding to the experimental results (points)

researches [12]. The rates of glucose and protein consumptions are 0.46/gL/h and 0.24/gL/h[1], respectively. From Fig. 9a, d, and e, it is evident that bacterial growth enters the stationary phase before complete utilization of glucose and soluble protein, respectively, and suggests that both the substrates are being utilized simultaneously during the batch period. In all the cases, the simulated trends can explain the experimental reality. The figures indicate very good agreement between the simulated and the experimental results. It is evident that the mathematical model developed using the kinetic parameters derived from batch type experiments conducted in small scale reactor (Erlenmeyer flask) can predict the performance of a large-scale bioreactor and the model is considered validated.

CONCLUSION

The present study focuses on the production of pediocin from the effluent of meat industries using *P. acidilactici* NCIM 2292. The values of kinetic parameters have been determined by conducting batch studies in small bioreactors (Erlenmeyer flask) at varying initial concentration of glucose, protein, and lactic acid. A mathematical model based on the kinetic parameters obtained from batch data of small reactors (Erlenmeyer flask) has been developed for the large-scale bioreactor run on the batch modes. The model prediction has been compared satisfactorily with the experimental data for the validation of the model. From the experimental results of the large scale batch bioreactor, it may be concluded that although the production of pediocin is mainly growth associated, production of lactic acid is associated with both growth and non-growth.

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