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Original Article

STUDY THE PRODUCTION OF PEDIOCIN BY PEDIOCOCCUS ACIDILACTICI IN REPEATED FED-BATCH FERMENTATION MODE ON MEAT PROCESSING WASTE

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ABSTRACT

Objective: The production of pediocin has been studied in a repeated fed-batch reactor with intermittent feeding. The producer strain of pediocin was *Pediococcus acidilctici*. Meat processing waste was used as nutrient in growth media.

Methods: Repeated fed-batch fermentation has been conducted in a bioreactor with three recycle ratio, such as 0.1, 0.2 and 0.3. A mathematical model has been developed for the prediction of reactor behaviour. The antimicrobial activity of pediocin was expressed as arbitrary units per millilitre (AU/ml). The concentrations of biomass, lactic acid, glucose and protein have also been measured with respect to time.

Results: The experimental results were matched well with predicted data. The correlation coefficient (R^2) was in between 0.95 to 0.97. The maximum productivity of pediocin was obtained 457,200 AU/h for 0.2 recycle ratio.

Conclusion: The model was validated for high-accuracy prediction. The study should be applicable for large-scale pediocin production.

Keywords: Pediocin, *Pediococcus acidilactici,* Meat processing waste, Repeated fed-batch fermentation, Recycle ratio

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INTRODUCTION

Several chemicals are usually used for the preservation of processed foods. These additives have harmful effects on human health. In order to avoid the application of detrimental chemicals, modern trends are attempting through bio-preservation of food materials. Bacteriocins are such bio-preservatives gaining attention to improve shelf life and quality of foods [1, 2]. Nisin and pediocin are most studied bacteriocins. Pediocin is mainly produced from several *Pediococcus* species shows anti-microbial activity towards a variety of pathogenic food spoiler micro-organism. Pediocin is food grade proteinaceous compounds that easily digestible in human gastrointestinal tract [3, 4].

The production of pediocin is highly dependent on cell growth of producer micro-organisms. Optimum fermentation conditions such as pH, temperature and nutrient in growth medium are necessary to maximize microbial growth as well as pediocin production. Varieties of waste materials have been used as nutrient media in pediocin production for the reduction of production cost [5, 6].

Many fermentation techniques, such as batch, continuous, fed-batch, etc. have been reported for pediocin production. However, these operations have different drawbacks like substrate lacking, product inhibition, less productivity, etc [7, 8]. Repeated fed-batch fermentation is an innovative process that can be implemented to enhance the pediocin productivity [9]. The fed-batch process is repeated through simultaneous withdrawal of growth medium and replacement of nutrient broth by saving the time required for cleaning and sterilization of bioreactor.

Under the study, the production of pediocin by *Pediococcus acidilctici* has been carried out in repeated fed-batch fermentation process using meat processing waste as nutrient media. The productivity of pediocin has been compared by maintaining different recycle ratio with intermittent nutrient feeding.

MATERIALS AND METHODS

Microorganism and growth media

Pediococcus acidilactici NCIM 2292 has been used for production of pediocin. The micro-organism was collected from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (Pune, India). The activity of pediocin has been determined against *Listeria monocytogenes* MTCC 839. The indicator strain was procured from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (Chandigarh, India). Slant cell cultures were kept at −4 °C in the solid agar medium.

Goat meat processing waste supplemented with glucose has been used for microbial propagation. The preparation of growth media containing 13 g/l protein and 20 g/l glucose was described in previous studies [10]. The media was used after sterilization at 121 °C for 15 min.

Analytical determinations

Microbial cell concentration has been determined through measuring absorbance of growth media by a UV-visible spectra-photometer at 600 nm wavelength. A standard curve has been prepared by plotting dry cell concentration against absorbance. Microbial cell was separated from a known volume of growth media by centrifugation (1200 rpm for 15 min). The solution obtained from centrifugation was known as cell free supernatant (CFS).

The antimicrobial activity of pediocin has been measured by agar well diffusion method [11] using CFS. Sterilized 1.5 % nutrient agar inoculated with indicator strain (*Listeria monocytogenes* MTCC 839) was solidified in a petri dish. CFS solution (70 µl) of serial dilution was poured into the well of 5 mm diameter made on the solid agar. Clear inhibition zone was found around the well after overnight incubation of the petri dish at 30 °C. Pediocin activity was expressed as reciprocal of the maximum serial dilution of CFS giving inhibition zone. The unit of activity was arbitrary units per millilitre (AU/ml) [12]. Where, AU/ml = $(1000D/d)$, d is dose (volume of CFS in each well), D is the dilution factor.

The concentration of lactic acid in CFS was measured by spectro-photometer, at 560 nm using p-hydroxydiphenyl [13]. Glucose concentration of CFS was determined by a spectrophotometer at wavelength of 575 nm using dinitrosalicylic (DNS) acid [14]. The protein concentration of CFS was estimated by spectrophotometer, at 550 nm using folin phenol reagent [15]. Suitable calibration curves were prepared for the estimation of lactic acid, glucose and protein concentration. Each analytical determination has been repeated in triplicate.

Mode of experiment

Fed batch fermentation for pediocin production by *Pediococcus acidilactici* was conducted in 5 l fermenter of 3 l working volume adjusting pH at 6.5. The reactor was intermittently fed with nutrient broth containing 150 g/l glucose and 22 g/l protein as described in previous studies [16]. The fed batch operation was switched to repeated fed batch mode once the pediocin concentrations reached a saturation level. At the end of fed batch fermentation, culture media was withdrawn and subsequently the fermenter was filled with fresh growth media to original reactor volume (3l). The reactor was again operated with intermittent feeding until the pediocin concentration reaches the saturation level. The cycle was repeated maintaining three recycle ratio, such as 0.1, 0.2 and 0.3. The volume of fermented medium withdrawn from the reactor were 2.7 l, 2.4 l and 2.1 l respectively in three recycle ratio of 0.1, 0.2 and 0.3. The same volume of fresh medium has been recharged into the reactor respectively for three recycle ratio. The performance of the repeated fed batch fermenter with intermittent feeding was studied using fractional volume remaining after each cycle. The experiments have been repeated in three times.

Mathematical expression

From the analysis of the performance of the fed batch operation mentioned [16], a repeated fed-batch strategy with intermittent feeding was adopted. On attainment of the saturation level the reactor was emptied up to different fractional volume γ (V_0/V) where, V_0 is the volume up to which the reactor is emptied, the V is the working volume (3 l) and γ is recycle ratio. Immediately the reactor volume was made up to the maximum level using fresh growth media. The reactor was operated under fed batch mode until the saturation level of pediocin concentration was achieved. The time required to reach the saturation has been defined as a critical period (t_c). The fed batch operation with intermittent feeding was run for three recycle ratio (0.1, 0.2 and 0.3). Emptying up to V_0 and filling up to V with fresh medium was repeated after each t_c. The change of volume with respect to time has been shown in fig. 1.

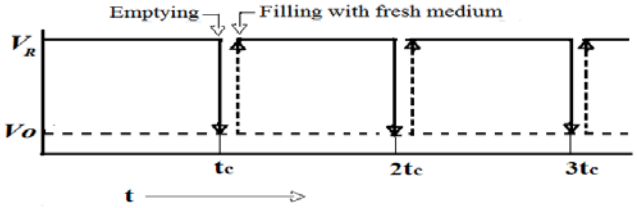

Fig. 1: Volume change of repeated fed batch reactor with intermittent feeding

The initial conditions at the beginning of each cycle are as follows,

$$X_{0(n+1)} = \frac{V_0 X_{fcn} + (V - V_0) X_0}{V} = \gamma X_{fcn} + (1-\gamma) X_{0c1} \quad \text{..... (1)}$$

Where subscripts cn = at the end of n^{th} critical period, 0 = at the beginning of the first cycle, 0(n+1) = at the beginning of cycle corresponding to $(n+1)^{th}$ critical period, fcn = At the end of n^{th} critical period, X is biomass concentration (g/l).

The initial condition for first, second and third cycles are as follows, at t = 0

$$\begin{bmatrix} X = X_0 \\ S_1 = S_{10} \\ S_2 = S_{20} \\ LA = 0_0 \\ P = 0_0 \end{bmatrix} \quad \begin{bmatrix} X = \gamma X_{fc1} + (1-\gamma) X_0 \\ S_1 = \gamma S_{1,fc1} + (1-\gamma) S_{10} \\ S_2 = \gamma S_{2,fc1} + (1-\gamma) S_{20} \\ LA = \gamma LA_{fc1} \\ P = \gamma P_{fc1} \end{bmatrix} \quad \begin{bmatrix} X = \gamma X_{fc2} + (1-\gamma) X_0 \\ S_1 = \gamma S_{1,fc2} + (1-\gamma) S_{10} \\ S_2 = \gamma S_{2,fc2} + (1-\gamma) S_{20} \\ LA = \gamma LA_{fc2} \\ P = \gamma P_{fc2} \end{bmatrix}$$

1st cycle 2nd cycle 3rd cycle ..... (2)

Where, S_1 is glucose concentration (g/l), S_2 is protein concentration (g/l), LA is lactic acid concentration (g/l) and P is pediocin activity (AU/ml).

The model equations were developed for biomass, pediocin, lactic acid, glucose and protein concentration in fed batch reactor mentioned in previous studies [16]. The values of kinetic parameters were determined by experimental results also shown in previous work [17].

RESULTS AND DISCUSSION

The experimental and simulated time histories of biomass, pediocin, lactic acid, glucose and protein concentration are shown in fig. 2 (a-e), 3 (a-e) and 4 (a-e) in the bioreactor with γ (V_0/V) of 0.1, 0.2 and 0.3 respectively. The model equations used for each cycle were shown in previous work [16]. The equations have been solved by MATLAB 7.0 with 4th order Runge-Kutta method for the simulation. The experimental results reveal that the time courses of biomass, pediocin, lactic acid, glucose and protein of each of three cycles of repeated fed batch process, as shown in fig. 2(a, b, c, d and e), 3(a, b, c, d and e) and 4 (a, b, c, d and e) have similar patterns. From the comparison of the experimental and simulated results, it is clear that the model is able to predict the behavior of the fermenter under the repeated fed batch mode of operation very well. The value of R^2 was 0.95 to 0.97.

The results shown in fig. 2(a), (b), (c), 3(a), (b), (c) and 4 (a), (b), (c) indicate that the concentrations of biomass, lactic acid and pediocin are saturated after 22, 24 and 28 h for γ of 0.1, 0.2 and 0.3 respectively.

The values of saturation concentrations of biomass, pediocin and lactic acid have been provided in table 1 for γ of 0.1, 0.2 and 0.3. From the analysis of the data in the table 1, it has been observed that although saturation concentration of pediocin remains constant at 4572 AU/ml, the concentration of lactic acid shows a decreasing trend with the increase of γ. On the other hand, saturation concentration of biomass passes through a maximum at 2.66 g/l at γ = 0.2. The critical period t_c is inversely varying with γ shown in table 1. Average productivity of pediocin in three cycles for γ of 0.1, 0.2 and 0.3 has been shown in table 2. Average volumetric productivity of pediocin (457,200 AU/h) is higher for γ of 0.2 compared to γ that at 0.1 or 0.3 as shown in table 2.

Table 1: The concentrations of biomass, pediocin and lactic acid in repeated fed-batch fermentation for γ of 0.1, 0.2 and 0.3

Recycle ratio (γ)	Biomass (X) (g/l)	Pediocin (P) (AU/ml)	Lactic acid (LA) (g/l)	Cycle time (t_c) (h)
0.1	2.64	4572	8.7	28
0.2	2.66	4572	7.8	24
0.3	2.24	4572	7.2	22

Table 2: Average volumetric productivity of pediocin for γ of 0.1, 0.2 and 0.3 in repeated fed-batch operation

Recycle ratio(γ)	Pediocin (P) (AU/ml)	Cycletime (t_c) (h)	Volume withdrawn (V-V_o)(ml)	Pediocin productivity (AU/h)
0.1	4572	28	2700	440872
0.2	4572	24	2400	457200
0.3	4572	22	2100	436418

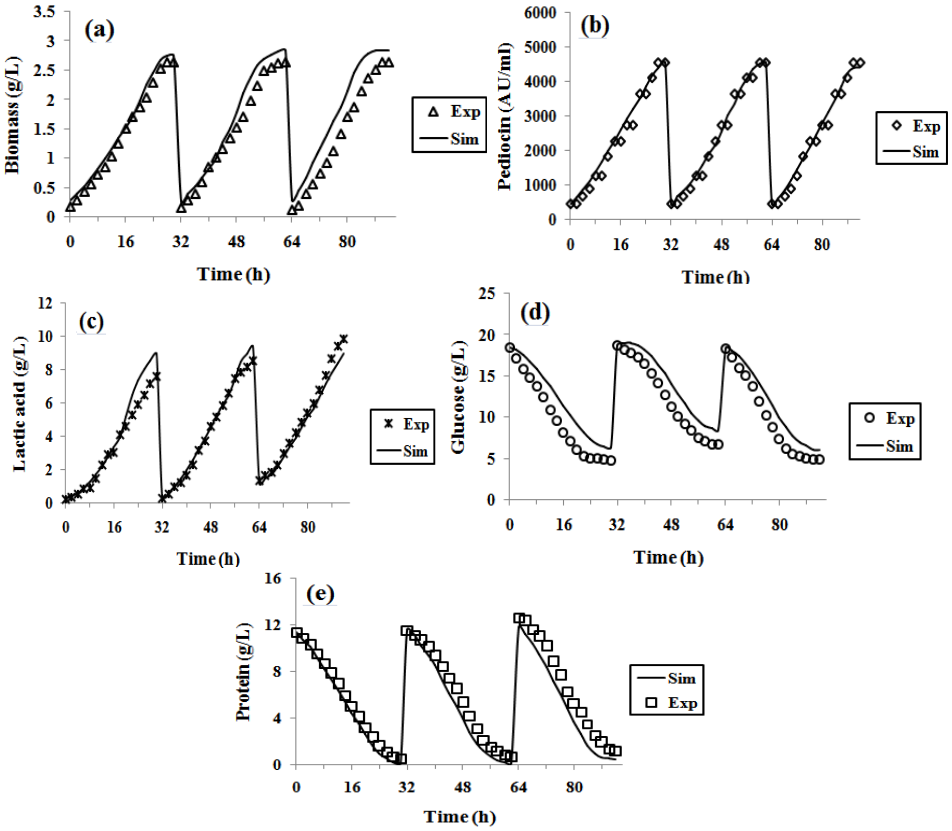

Fig. 2: Repeated fed-batch fermentation for γ =0.1. Continuous lines represented simulated values corresponding to the experimental results (points)

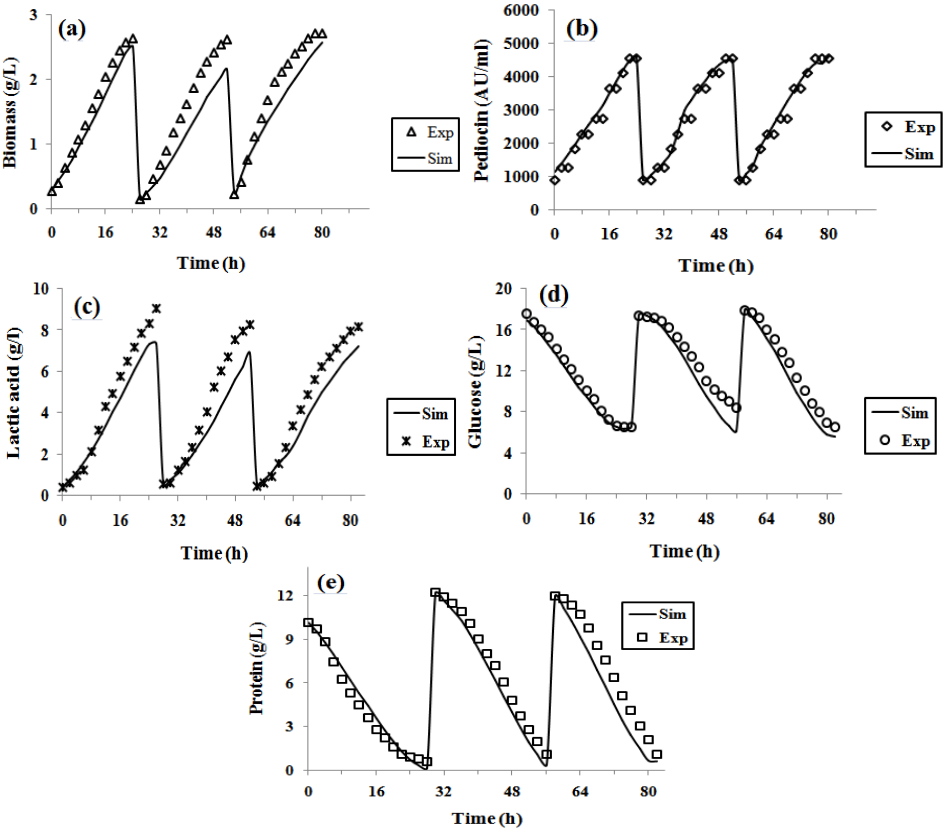

Fig. 3: Repeated fed-batch fermentation for γ =0.2. Continuous lines represented simulated values corresponding to the experimental results (points)

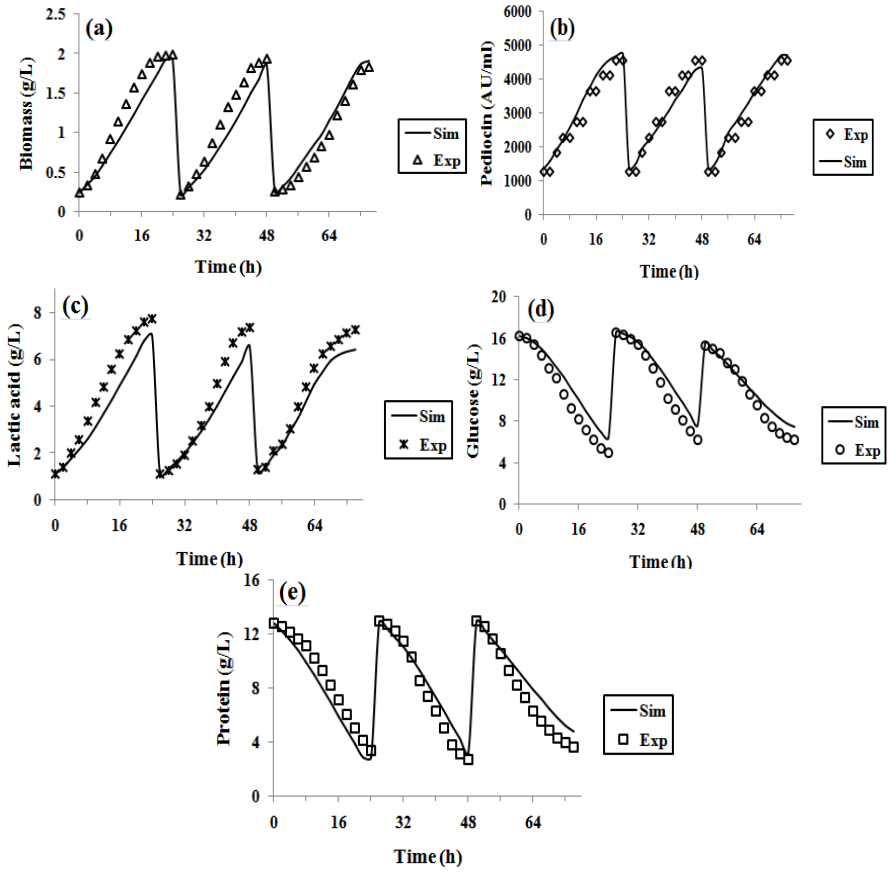

Fig. 4: Repeated fed-batch fermentation for γ =0.3. Continuous lines represented simulated values corresponding to the experimental results (points)

CONCLUSION

The present study focuses on the production of pediocin from the effluent of meat processing industries using *Pediococcus acidilactici* in repeated fed batch operation with intermittent feeding. The proposed model was able to match experimental results with the predicted ones very well. The productivities for γ of 0.1, 0.2 and 0.3 have been compared. With respect to productivity of pediocin repeated fed batch with intermittent feeding at γ = 0.2 seems to be the best-performing ones along the respective categories. The highest pediocin productivity is 457,200 AU/h for γ = 0.2. The experimental results indicate promising industrialization prospect of repeated fed batch operation with intermittent feeding for large scale production of pediocin. It is expected that the outcome of the present study will be helpful for the production of pediocin or similar biomolecules on a commercial scale.

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AUTHORS CONTRIBUTIONS

This is author's sole research work and not contributed by other ones.

CONFLICT OF INTERESTS

Declared none

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