

Fed-batch propionic acid production by *Propionibacterium acidipropionici*

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Abstract

The batch fermentations were conducted using lactose as the substrate at pH 6.5 and temperature 30°C. Average batch kinetic data was eventually used to develop an unstructured mathematical model. The kinetic parameters of the model were determined by non-linear regression technique using the batch experimental results. Parametric sensitivity analysis showed the maximum specific substrate consumption rate (r_S^{\max}) and the maintenance energy constant (m_S) to be the most sensitive parameters. The experimental observations in batch fermentation were close to the model predictions. The batch model was extrapolated to identify nutrient feeding strategies, which were tested successfully for two different fed-batch fermentations. It demonstrated enhanced propionic acid productivity. The developed model was found suitable for the design of feeding strategies to increase propionic acid production in fed-batch mode of reactor operation.

Keywords: Batch/fed-batch propionic acid fermentation; Modelling of kinetics

1. Introduction

Propionic acid and its salts are widely used as raw material in different industries; e.g., esters of propionic acid are used in the perfume industry, and cellulose propionate finds its use as an important thermoplastic in plastic industry [1]. Recently, there has been increasing interest in producing propionic acid from whey lactose using *Propionibacterium* [2–6]. Fermented whey has been shown to be a natural ‘fungistatic’ agent when incorporated in bread or other bakery products. It can replace chemical preservatives and is of substantial commercial importance in the sale of ‘natural’ bakery products. The Na⁺, Ca⁺⁺ and K⁺ salts of propionic acid have also been listed as preservatives which are of the category known as generally recognized as safe (GRAS) food additives [5]. Therefore, there is an increasing interest in the production of propionic acid by the fermentation route.

A typical growth cycle of *Propionibacteria* features the consumption of substrate (e.g., lactose, glucose and lactate) under anaerobic conditions and the liberation of acids, mainly propionic, succinic and acetic acids as end products [1,7,8]. A significant amount of pyruvic acid is also accumulated in the fermentation broth initially, which is later on

consumed to produce succinic and acetic acids. The major bottlenecks in the fermentation are the slow growth of bacteria, strong end product inhibition [3,9,10] and difficulty in product recovery from the dilute fermentation broth in which they are produced [6]. Attempts are being made to eliminate product inhibition and improve reactor productivity by extractive fermentation [11–15]. Propionic acid tolerant strains [16], immobilized cell reactors [4,6,17–19] and cell recycle reactors [5,19] have also been developed to enhance the yield and productivity of fermentation. Fed-batch fermentations with and without extractive membranes have been utilized in the past for improving the production rates and overall yields of fermentation [13,15,18,20–22]. The commonly used feeding strategy was addition of sugar at frequent intervals. However, in these fermentations, the sugar concentration usually reached zero before the addition of the next feed. Therefore, fed-batch fermentations which ensure constant/continuous nutrient supply in the fermentation broth would be more useful. A simple feeding strategy could be the supplementation of nutrient feed at a constant feed rate, when the growth rate is high, to eliminate nutrient depletion and avoid the accumulation of inhibitory products. Application of such an approach for propionic acid production might result in the improvement of its productivity. Trial and error procedures had been adopted in the past for the development of suitable fed-batch design. For efficient exploitation of microbial metabolism, the quantification of metabolic pro-

cesses through empirical process models and material balancing techniques have been adopted for other culture systems. The mathematical models can be utilized intelligently to optimize the fresh nutrient feeding strategy in fed-batch propionic acid fermentation with minimum trial and error experiments. The application of such an approach has not been attempted so far. In the present work, the mathematical model of propionic acid fermentation was proposed and the model developed was used to predict fed-batch fermentations which demonstrated improved propionic acid productivity.

2. Materials and methods

2.1. Culture and media

Propionibacterium acidipropionici (ATCC 4875) was grown in a medium containing (per litre): 10 g yeast extract (Hi Media Laboratories, Bombay, India), 5 g trypticase (CDH, Bombay), 0.25 g K_2HPO_4 (Hi Media Laboratories), 0.05 g $MnSO_4$ (E. Merck, Bombay) and 40 g lactose (SRL, Bombay). The medium (without lactose) was heat sterilized in the bioreactor at $121^\circ C$ and 15 psig for 20 min. Lactose was autoclaved separately and added aseptically to the bioreactor.

2.2. Fermentation

The batch fermentations were carried out in a 7 l Bioengineering (Bioengineering AG, Switzerland) glass reactor at initial concentrations of lactose 45.2, 50.2 and 73 g l^{-1} . The average batch kinetics at $S_0 = 47.7 \text{ g l}^{-1}$ is, however, reported and used for modelling in this investigation. The temperature was controlled at $30 \pm 1^\circ C$ and the pH was maintained at 6.5 ± 0.1 by automatic addition of the sterile 6 N NaOH. A pH value of 6.5 has been reported to be suitable for propionic acid accumulation [23]. The head space of the reactor was flushed with nitrogen gas to maintain anaerobiosis throughout the fermentation. Inoculum culture was grown in custom made 500 ml serum flasks (headspace flushed with nitrogen gas). A bioreactor containing 4 l of medium was inoculated with 200 ml of 1 day old log phase culture. Samples were collected at appropriate time intervals. After measuring the optical density (OD), the remaining volume of the sample was centrifuged at $10,000 \text{ g}$ for 20 min. The supernatant was stored at $-20^\circ C$ for HPLC analysis.

Fed-batch fermentation was initiated as batch fermentation at $S_0 = 56.0 \text{ g l}^{-1}$. After 35–37 h, it was converted to fed-batch fermentation by adding concentrated nutrient feed (lactose concentration = 300 g l^{-1} and concentrations of other nutrients were increased proportionately) at a constant feed rate of $0.03\text{--}0.04 \text{ l h}^{-1}$. Feeding was stopped after 57–59 h and residual lactose in the fermentation broth was allowed to ferment by batch fermentation.

2.3. Analysis methods

OD of the cells was measured at 660 nm in a UVICON spectrophotometer in a cell having a 10 mm path length. A standard curve was plotted between cell dry weight and absorbance and was used to determine cell weight in the samples from their absorbance. Samples were diluted with distilled water if the OD was greater than 0.4.

High performance liquid chromatography (HPLC) was used to analyse the concentrations of lactose, propionic, acetic, succinic and pyruvic acids in the fermentation broth. Samples were filtered through $0.45 \mu\text{m}$ filters. Separation of organic acids and lactose took place in an HPX-87H column (BIORAD, Richmond, CA) operated at $58^\circ C$ with $0.005 \text{ M H}_2\text{SO}_4$ as the mobile phase at a flow rate of 0.6 ml min^{-1} . Peaks were detected with a Waters differential refractometer (model R401) and concentrations calculated as peak areas.

3. Results and discussion

3.1. Batch experimental

Fig. 1 describes the average batch fermentation kinetic data at an initial lactose concentration of 47.7 g l^{-1} . Fig. 1 shows that lactose was completely consumed by the end of 85 h to produce propionic acid (20.75 g l^{-1}) as the major end product. Succinic and acetic acids were also accumulated along with propionic acid, but pyruvic acid was produced upto 35 h and then consumed slowly.

The lactose consumption rate and biomass production rate were very high during 25–45 h. Accumulation of organic acids created unfavourable growth conditions. There was no further multiplication of cells, but acids were released

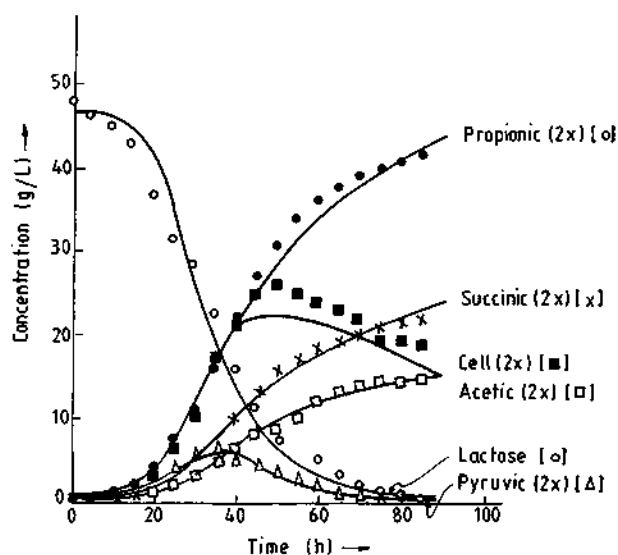


Fig. 1. Batch propionic acid fermentation at $S_0 = 47.7 \text{ g l}^{-1}$ and pH 6.5. Data points – experiment. Smooth curve – model simulation.

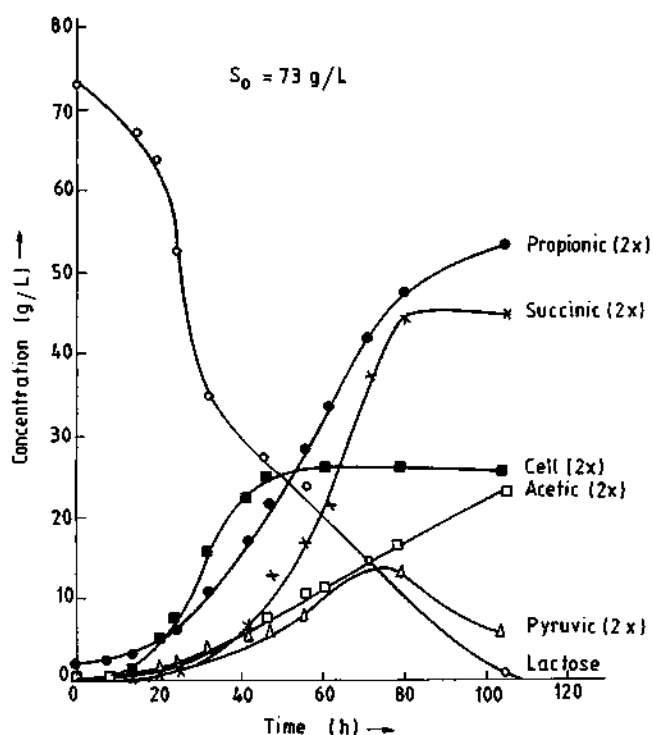


Fig. 2. Batch propionic acid fermentation at $S_0 = 73 \text{ g l}^{-1}$ and pH 6.5.

continuously in the fermentation broth till the depletion of lactose.

The initial concentration of lactose (S_0) was subsequently increased to 73 g l^{-1} to figure out if increase in S_0 would lead to increased propionic acid accumulation in batch mode.

Fig. 2 represents the batch culture kinetics at $S_0 = 73 \text{ g l}^{-1}$. The culture featured an accumulation of 26.3 g l^{-1} of propionic acid, 11.4 g l^{-1} of acetic acid and 22.5 g l^{-1} of succinic acid at the end of fermentation (104 h). The pyruvic acid peak (6.9 g l^{-1}) was observed at 70 h, which was eventually consumed to form acetic and succinic acids. In spite of the increase in S_0 from 47.7 to 73 g l^{-1} , propionic acid production in the fermentation broth was increased from 20.75 to 26.3 g l^{-1} only. Propionate yield was decreased from 0.435 g g^{-1} (for $S_0 = 47.7 \text{ g l}^{-1}$) to 0.364 g g^{-1} (for $S_0 = 73 \text{ g l}^{-1}$).

Unlike the previous fermentation (at $S_0 = 47.7 \text{ g l}^{-1}$), a lot of sugar was available at 80 h; however no significant improvement in propionic acid yield was observed. Apparently, all the available lactose was utilized for cell maintenance and acetic acid production. Production of acetic acid being an ATP (energy) generating activity during culture growth, on the availability of excess substrate, the culture seemingly produced high energy yielding products of metabolism.

Another distinct feature of this fermentation was high succinic acid accumulation (for example, 10.5 g l^{-1} for $S_0 = 47.7$ and 22.5 g l^{-1} for $S_0 = 73 \text{ g l}^{-1}$). This could be attributed to the changeover of culture metabolism at this unusually high substrate concentration.

The initial lactose concentration, $S_0 = 73 \text{ g l}^{-1}$, was found unsuitable for propionic acid production by batch fermentation because it did not result in significant increase in propionic acid concentration. However, other acids (succinic, acetic and pyruvic) were accumulated at a reasonably high concentration.

Therefore, instead of fermenting a high concentration of lactose in batch mode, it was essential to feed lactose such that it favours the accumulation of propionic acid. Subsequently, feeding strategies were designed with the help of a mathematical model for enhanced propionic acid production with minimum experimental trials.

3.2. Model development

For the development of the mathematical model, the batch kinetic data (average) of *P. acidipropionici* ATCC 4875 at $S_0 = 47.7 \text{ g l}^{-1}$ and pH 6.5 was utilized.

The development of the present fermentation model was based on the following assumptions which are basically derived from the knowledge of the fermentation biochemistry [24] and mass balance around the bioreactor.

1. Lactose is the only limiting substrate in batch fermentation.
2. There is no process limitation by the nitrogen source.
3. Culture inhibition is brought about by the accumulating metabolic products (propionic/acetic acids only).
4. The pH is known and controlled at a constant value throughout the modelled period.
5. Propionic acid production is by direct conversion of lactose as its intermediate succinic acid does not demonstrate accumulation and consumption like pyruvic acid.

The system of differential mass balances summarized below containing Eq. (1) to (7) represent a general mathematical model capable of describing the batch dynamics of propionic acid fermentation:

$$r_S = \frac{r_S^{\max} S}{(K_S + S)} \left(\frac{K_{I1}}{K_{I1} + P_1} \right) \left(\frac{K_{I2}}{K_{I2} + P_3} \right) \quad (1)$$

$$-r_S = \frac{1}{X} \frac{dS}{dt} \quad (2)$$

$$\mu = \frac{r_S Y_{X/S}^{\max} - m_S}{Y_{X/S}^{\max}} \quad (3)$$

$$q_{P1} = K_1 r_S + m_1 \quad (\text{propionic acid}) \quad (4)$$

$$q_{P2} = K_2 r_S + m_2 - K_3 \frac{P_2}{P_2 + K_{S1}} \quad (\text{pyruvic acid}) \quad (5)$$

$$q_{P3} = K_4 r_S + m_3 + K_5 \frac{P_2}{P_2 + K_{S1}} \quad (\text{acetic acid}) \quad (6)$$

$$q_{P4} = K_6 r_S + m_4 + K_7 \frac{P_2}{P_2 + K_{S1}} \quad (\text{succinic acid}) \quad (7)$$

Eq. (1) denotes the specific substrate consumption rate of the culture which features substrate limitation due to lac-

tose and inhibition due to propionic (P_1) and acetic acids (P_3).

Eq. (3) represents the equation for μ (specific growth rate) which was obtained by the Leudking and Piret expression. m_S refers to the maintenance requirement of the cells.

The first and second terms in Eq. (4) refer to growth associated and non-growth associated contribution of propionic acid accumulation rates in the reactor, respectively.

Eq. (5) accounts for the net pyruvic acid accumulation rate in the reactor. Pyruvic acid was assumed to be accumulated due to growth and non-growth associated product formation (due to the consumption of substrate). ($K_3(P_2/(P_2 + K_{S1}))$) term accounted for the disappearance of pyruvic acid during batch fermentation.

Eq. (6) accounts for the total acetic acid accumulation rate in the reactor. Acetic acid was assumed to be accumulated due to growth and non-growth associated product formation. The ($K_5(P_2/(P_2 + K_{S1}))$) term accounted for the conversion of the pyruvic acid to acetic acid as per the biochemistry of the fermentation [1,24].

Eq. (7) accounts for the total succinic acid accumulation rate in the reactor. Succinic acid was assumed to be accumulated due to growth associated and non-growth associated product formation. The ($K_7(P_2/(P_2 + K_{S1}))$) term accounted for the conversion of the pyruvic acid to succinic acid as per the biochemistry of the fermentation [1,24].

3.2.1. Evaluation of the batch mathematical model parameters

For optimal estimation of model parameters, a non-linear regression technique, [25] assisted by a computer program [26,27], was used to minimize the deviations between the model predictions, and the experimental batch results. For the calculation of the model predictions, the system of differential equations of the model were solved using an integration program based on the Runge Kutta method of fourth order. The optimization program for the direct search of the minimum of the multivariable function was based on the original method of Rosenbrock, 1960 [28]. The minimization criteria used in the program was as follows:

$$SSWR = \sum_{i=1}^n \sum_{j=1}^m \frac{\Delta_{ij}^2}{W_{ij}^2} \quad (8)$$

Where SSWR represents the sum of the square of weighed residues. 'i' and 'j' represent the number of experimental data points and the number of variables, respectively, W_{ij} represents the weight of each variable (usually the maximum value of each variable) and Δ_{ij} denotes the difference between the model and the experimental value.

The values of the optimized parameters are given in Table 1. The model equations described above in Eqs. (1)–(7) were simulated on the computer using the optimum values of the model parameters. The comparison of the model simulation (smooth line) and experimental data

Table 1
Model parameters and parametric sensitivity values

Model parameter	Units	Parameter value	APS ^a	RPS ^b
K_S	g l ⁻¹	47.101	325.477	0.054
r_S^{\max}	g g ⁻¹ h ⁻¹	1.294	0.451	2.059
$Y_{X/S}^{\max}$	g g ⁻¹	0.362	0.518	0.661
m_S	g g ⁻¹ h ⁻¹	0.038	8.286	1.111
K_1	—	3.952	-0.278	0.243
K_2	—	5.587	0.767	0.484
K_3	g g ⁻¹ h ⁻¹	0.054	-0.777	0.148
K_{S1}	g l ⁻¹	1.516	0.002	0.012
K_4	—	18.519	1.448	0.276
K_5	g g ⁻¹ h ⁻¹	0.008	7.749	0.219
K_6	—	11.628	0.401	0.122
K_7	g g ⁻¹ h ⁻¹	0.0085	1.465	0.044
K_{11}	g l ⁻¹	12.585	0.003	0.135
K_{12}	g l ⁻¹	6.077	-1.243	0.034
m_1	g g ⁻¹ h ⁻¹	0.015	0.002	0.066
m_2	g g ⁻¹ h ⁻¹	0.009	5.073	0.161
m_3	g g ⁻¹ h ⁻¹	0.005	14.935	0.263
m_4	g g ⁻¹ h ⁻¹	0.009	7.611	0.242

^a APS: absolute parametric sensitivity.

^b RPS: relative parametric sensitivity.

(points) is shown in Fig. 1. The agreement between the model simulation and the experimental data points is clearly reflected.

To further evaluate the degree of reliability of the model, a method recommended by Bard, 1974 [25] was used to test the hypothesis of a zero mean deviation of the model and experimental data. The mean residual of each variable ' Δ_j ' was calculated as follows:

$$\Delta_j = \frac{1}{n} \sum_{i=1}^n \Delta_{ij} \quad (9)$$

Where 'n' is the total number of experimental data points, and Δ_{ij} is the difference between the experimental value of a variable and its corresponding model simulation value. The variance of the error of a residual (S_j) was then estimated as follows:

$$S_j = \frac{1}{n-1} \sum_{i=1}^n (\Delta_{ij} - \Delta_j)^2 \quad j = 1, m \quad (10)$$

Where 'm' = the number of variables. The value of the statistics defined as

$$\lambda = \frac{(n-m)n}{(n-1)m} \sum_{j=1}^m \frac{\Delta_{ij}^2}{S_j} \quad (11)$$

was calculated. The statistics ' λ ' has the $F_{m,n-m}$ distribution. Its value was calculated as 0.654, which was less than the ' $F_{6,11}$ ' value (obtained from F tables) for 99 % confidence. for the whole experimental set. This made it possible to accept the hypothesis of zero mean deviation between experimental data and the model. This established the validity of the model.

The parametric sensitivity analysis [26] showed the maximum specific substrate consumption rate (r_S^{\max}) and the

maintenance energy constant (m_s) to be the most sensitive parameters indicating the maximum effect of the above parameters on the overall model simulation. These parameters should, therefore, be estimated/determined with extreme care and accuracy.

Experimental values of different process variables in Fig. 1 are described by data points where a smooth curve illustrates the model simulation. As indicated in Fig. 1, the model closely simulated the experimental trends of lactose consumption, biomass production and accumulation of organic acids.

3.3. Fed-batch fermentation design

From the batch fermentation kinetics (Fig. 1), it was found that, during 35–40 h of fermentation, the lactose consumption rate and the culture growth rate are very high. It was anticipated that slow feeding of concentrated lactose at this stage of fermentation might give rise to better propionic acid accumulation as opposed to fermenting lactose in batch mode. With this objective, a nutrient feeding strategy was designed and then simulated with the help of a model to determine its effect on propionic acid production.

In order to design a fed-batch experiment, the existing model was modified by adding the bioreactor mass balance terms in the batch mathematical model. The fed-batch model equations are described below by Eqs. (12)–(18):

$$r_s = \frac{r_s^{\max} S}{(K_S + S)} \left(\frac{K_{I1}}{K_{I1} + P_1} \right) \left(\frac{K_{I2}}{K_{I2} + P_3} \right) \quad (12)$$

$$\frac{dS}{dt} = -\frac{r_s^{\max} S}{S + K_S} \frac{K_{I1}}{K_{I1} + P_1} \frac{K_{I2}}{K_{I2} + P_2} X + D(S_0 - S) \quad (13)$$

$$\frac{dX}{dt} = r_s Y_{X/S}^{\max} X - m_s Y_{X/S}^{\max} X - DX \quad (14)$$

$$\frac{dP_1}{dt} = K_1 r_s X + m_1 X - DP_1 \quad (\text{propionic acid}) \quad (15)$$

$$\frac{dP_2}{dt} = K_2 r_s X + m_2 X - K_3 \frac{P_2}{P_2 + K_{S1}} X - DP_2 \quad (\text{pyruvic acid}) \quad (16)$$

$$\frac{dP_3}{dt} = K_4 r_s X + m_3 X + K_5 \frac{P_2}{P_2 + K_{S1}} X - DP_3 \quad (\text{acetic acid}) \quad (17)$$

$$\frac{dP_4}{dt} = K_6 r_s X + m_4 X + K_7 \frac{P_2}{P_2 + K_{S1}} X - DP_4 \quad (\text{succinic acid}) \quad (18)$$

D (dilution rate) = F/V , where F is the feed flow rate and V is the volume of the fermenter.

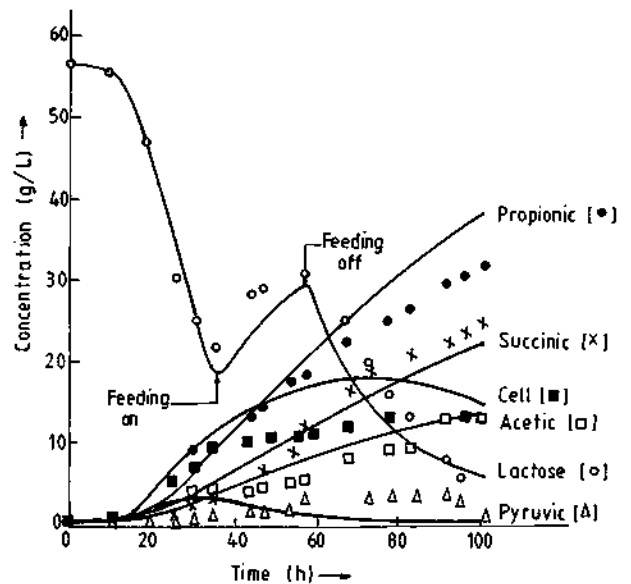


Fig. 3. Model based fed-batch propionic acid fermentation. 0–35 h – batch, 35–57 h – fed-batch with a constant feeding rate of 0.031 h^{-1} , 57–101 h of batch fermentation. Data points – experiment. Smooth curve – model simulation.

3.3.1. Fed-batch at constant feed rate (0.031 h^{-1})

3.3.1.1. Model simulation. The mathematical model was used to predict batch growth till 35 h of fermentation. During 35–57 h, a constant feed rate at 0.031 h^{-1} (with $S_0 = 300 \text{ g l}^{-1}$) was maintained and after 57 h again the mathematical model was used to simulate the batch growth till the end of the fermentation.

3.3.1.2. Experimental observations. Fig. 3 demonstrates the culture kinetics (experimental observations and model simulation) of fed-batch fermentation using the above-given nutrient feeding strategy. At the end of fermentation, 31.15 g l^{-1} of propionic acid was produced in the fermentation broth.

The experimental observations (data points) were close to the model simulations (smooth curves). The feeding strategy not only enhanced propionic acid accumulation but also improved its productivity ($0.31 \text{ g l}^{-1} \text{ h}^{-1}$) as opposed to batch fermentation ($0.23 \text{ g l}^{-1} \text{ h}^{-1}$). Therefore, the next fed-batch fermentation was conducted at a higher feed rate to further improve productivity. It was also simulated to establish the utility of the developed fed-batch model.

3.3.2. Fed-batch fermentation at constant feed rate (0.041 h^{-1})

3.3.2.1. Model simulation. Fig. 4 represents the model simulation (solid curves), which features batch fermentation (0–37 h), fed-batch fermentation (37–59 h) at a constant

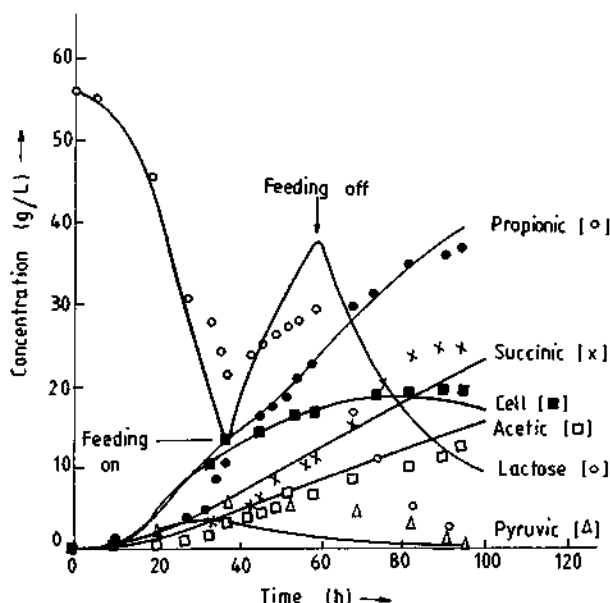


Fig. 4. Model based fed-batch propionic acid fermentation. 0–37 h – batch, 37–59 h – fed-batch with a constant feeding rate of 0.041 h^{-1} , 59–95 h of batch fermentation. Data points – experiment. Smooth curve – model simulation

feed rate (0.041 h^{-1}) and feed concentration (300 g l^{-1}), and batch fermentation (59–95 h).

3.3.2.2. Experimental. Fig. 4 also shows the experimental data (points) for lactose consumption, and biomass and organic acids production. The experimental observations were close to model predictions. Some deviations were observed because batch model parameters were used to simulate fed-batch culture kinetics. However, the simulated feeding strategy not only enhanced propionic acid concentration upto 37 g l^{-1} but also resulted in improved productivity ($0.4 \text{ g l}^{-1} \text{ h}^{-1}$) as compared to batch fermentation.

3.4. Comparison with the literature reports

The propionic acid accumulated (37 g l^{-1}) by fed-batch fermentation conducted at a constant feed rate 0.041 h^{-1} in this study was higher than 23 g l^{-1} , previously reported by fed-batch fermentation using another strain *Selenomonas ruminantium* [22]. Ozadali et al. [15] reported fed-batch fermentation with membrane extraction and achieved $30\text{--}37 \text{ g l}^{-1}$ of propionic acid after 150 h of fermentation. However, in the present investigation, within 95 h, 37 g l^{-1} of propionic acid was obtained, thereby giving rise to enhanced productivity. The maximum propionic acid productivity obtained in this investigation was $0.4 \text{ g l}^{-1} \text{ h}^{-1}$, which was higher than $0.3 \text{ g l}^{-1} \text{ h}^{-1}$ reported by Boyaval et al. [13] using glycerol as the substrate for propionic acid production by *Propionibacterium thoenii*. It was also found to be significantly higher than fed-batch fermentations with glucose

($0.23 \text{ g l}^{-1} \text{ h}^{-1}$) and corn steep liquor ($0.18 \text{ g l}^{-1} \text{ h}^{-1}$) when using propionic acid tolerant strain of *P. acidipropionici* [18].

As demonstrated in the above two cases, the model developed is able to reasonably simulate the fed-batch kinetics of propionic acid fermentation. The present model can also be used to generate a nutrient feeding profile which may ensure high propionic acid productivity with maximum utilization of reactor volume and no lactose left at the end of fermentation. Development and testing of such feed strategies will be the topic of our future investigations.

4. Conclusions

Batch fermentations were conducted at pH 6.5, temperature 30°C and initial concentration of lactose of 47.7 g l^{-1} . The average propionic acid accumulation was 20.75 g l^{-1} , with an overall productivity of $0.23 \text{ g l}^{-1} \text{ h}^{-1}$. Average batch kinetic data was used to develop a mathematical model. Model parameters were determined by the non-linear regression technique. Parametric sensitivity analysis indicated the higher sensitivity of the maximum specific substrate consumption rate (r_S^{max}) and maintenance energy constant (m_S) model parameters. The model successfully simulated the average batch kinetics. Statistical validity of the model was demonstrated. The batch model was used to design nutrient feeding strategy for two different fed-batch fermentations at constant feed rates (0.03 and 0.041 h^{-1}), which resulted in improved propionic acid productivity (0.31 and $0.4 \text{ g l}^{-1} \text{ h}^{-1}$). The model simulations were compared with the experimental fed-batch results to establish accuracy of the model developed. Fed-batch experimental observations were close to model predictions which proved that the developed model can be used to design fed-batch fermentation. It can also be used for the design of other feeding strategies of fed-batch fermentation.

5. Nomenclature

D	dilution rate (h^{-1})
dC_i/dt	rate of reaction for the component C_i ($C_i = X, S, P_1, P_2, P_3, P_4$)
F	feed flow rate (l h^{-1})
K_S	saturation constant for substrate (g l^{-1})
K_{S1}	constant with respect to pyruvic acid (g l^{-1})
K_{I1}	inhibition constant for propionic acid (g l^{-1})
K_{I2}	inhibition constant for acetic acid (g l^{-1})
K_1	growth associated contribution for propionic acid production
K_2	growth associated contribution for pyruvic acid production
K_3	constant in Eq. (5)
K_4	growth associated contribution for acetic acid production

K_5	constant in Eq. (6)
K_6	growth associated contribution for succinic acid production
K_7	constant in Eq. (7)
m_S	maintenance energy constant ($\text{g g}^{-1} \text{h}^{-1}$)
m_1	constant in Eq. (4) ($\text{g g}^{-1} \text{h}^{-1}$)
m_2	constant in Eq. (5) ($\text{g g}^{-1} \text{h}^{-1}$)
m_3	constant in Eq. (6) ($\text{g g}^{-1} \text{h}^{-1}$)
m_4	constant in Eq. (7) ($\text{g g}^{-1} \text{h}^{-1}$)
P	product concentration (g l^{-1})
P_1	propionic acid concentration (g l^{-1})
P_2	pyruvic acid concentration (g l^{-1})
P_3	acetic acid concentration (g l^{-1})
P_4	succinic acid concentration (g l^{-1})
q_{P_1}	specific propionic acid production rate ($\text{g g}^{-1} \text{h}^{-1}$)
q_{P_2}	specific pyruvic acid production rate ($\text{g g}^{-1} \text{h}^{-1}$)
q_{P_3}	specific propionic acid production rate ($\text{g g}^{-1} \text{h}^{-1}$)
q_{P_4}	specific pyruvic acid production rate ($\text{g g}^{-1} \text{h}^{-1}$)
r_S	specific substrate consumption rate ($\text{g g}^{-1} \text{h}^{-1}$)
r_S^{\max}	maximum specific substrate consumption rate ($\text{g g}^{-1} \text{h}^{-1}$)
S	substrate concentration (g l^{-1})
S_0	inlet substrate concentration (g l^{-1})
S_j	variance of error of a residual
SSWR	sum of squares of weighed residues
V	volume (l)
w_{ij}	weight of a process variable
X	biomass concentration (g l^{-1})
$Y_{X/S}^{\max}$	maximum yield of biomass with respect to substrate (g g^{-1})

6. Subscripts

n	number of data points
m	number of process variables

7. Greek letters

μ	specific growth rate of the cell population (h^{-1})
Δ_{ij}	difference between the experimental and model simulation values of a process variable
Δ_j	mean residual of each variable

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