Model Based Constant Feed Fed-Batch L-Sorbose Production Process for Improvement in L-Sorbose Productivity

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Batch sorbose productions were done using *Gluconobacter oxydans* and initial sorbitol concentrations of (γ_{S0} = 200 kg m^{-3}). The average batch kinetic data was used to develop an unstructured mathematical model to represent the sorbose production system. Model parameters were identified using a non-linear regression technique assisted by a computer program which minimized the deviation between the model predictions and actual batch experimental data. F-test indicated a 99% confidence on the prediction of model using optimized parameters. Parametric sensitivity analysis indicated maximum specific growth rate (μ_m) to be the most sensitive model parameter. The batch model was then used to identify nutrient feeding strategies to maintain a constant noninhibitory and non-limiting supply of sorbitol for fed-batch cultivation, in order to improve the sorbose productivity. The adequacy of the fed-batch model was tested by comparing actual fed-batch cultivation data with the model predictions. Excellent agreement was found between experimental data and model simulation. The fed-batch model was found to be a powerful tool for designing feeding strategies for enhancing yield and productivity of sorbose production.

Keywords:
D-sorbitol, L-sorbose, *Gluconobacter oxydans*, modeling of kinetics, fed-batch fermentation

1. Introduction

L-sorbose is an intermediate in the industrial production of Vitamin-C. It is produced by the microbial oxidation of D-sorbitol by *Gluconobacter oxydans*[1,2]. Chemical oxidation of D-sorbitol produces both D and L-sorbose. Since biochemical oxidation yields only the L isomer, this process is industrially employed for the production of L-sorbose.

Sorbose production exhibits both substrate [3–5] and product inhibitions[6–8]. The rate of oxidation declines drastically with increasing initial sorbitol concentrations in a batch bioreactor[3]. Sorbitol concentration above 500 kg m^{-3} has also been known to decrease the water activity of cultivation medium resulting in reduced oxygen transfer rate through liquid-microbial biomass interface, which eventually gives rise toward cell growth\product formation rates[9] . Since the initial concentration of sorbitol is restricted due to its inhibition, it is extremely difficult to obtain high concentrations of sorbose in batch processing. Furthermore, a high concentration of sorbose in the fermentation broth can lead to the simultaneous crystallisation[10] and makes the sorbose recovery operations easy and economical. Batch processing also features very large unproductive downtime (for harvesting, cleaning, recharging etc.), leading to lower overall reactor productivities.

In order to obtain a high concentration of sorbose, it is absolutely necessary to biooxidise large quantities of sorbitol, keeping its concentration below inhibitory levels, with maximum utilization of full working capacity of the bioreactor. The fed-batch mode of processing provides an excellent means of regulating the nutrient feed rate in order to prevent over-and under-feeding of nutrients into the bioreactor. In fed-batch processing highly concentrated sorbitol along with other nutrients can be added when culture is in the actively growing log phase, and the sorbitol concentration in the fermenter becomes low. Thus, the ultimate concentration of sorbitol in the reactor can be kept below inhibitory levels[4]. Furthermore, the substrate concentration in the medium can be kept at a predetermined appropriate (non-limiting and non-inhibiting) level by controlling the inlet concentration, feed rate and feeding time of the nutrients in fed-batch cultivation. This eventually leads to maximum specific growth rate, which can be maintained throughout the cultivation resulting in faster oxidation of sorbitol to sorbose. Suitably designed fed-batch cultivation has been indicated to be beneficial for substrate inhibited kinetics for growth and product formation[11].

Continuous fermentation of sorbitol to sorbose with and without cell recycle of microbial cells were conducted[12]. High product formation rates to a level of 91 kg m^{-3}h^{-1} were obtained for the cell recycle continuous fermentation, however,

the practical application of the production strategy was not feasible due to unusual cultivation conditions (pure oxygen, high rpm, complex cell recycle device) used by the investigators[12]. It has been generally observed that product inhibition can be eliminated by continuous cultivation since the inhibitory product is continuously withdrawn from the bioreactor. However, literature reports[10] indicates that up to a sorbose mass concentration of 770 kg m^{-3} can be accumulated without considerable inhibition by sorbose. Since sorbitol to sorbose bioconversion is not a severely product inhibited system, fed-batch processes which can be easily adopted to industrial conditions, are ideal.

Attempts were made in the past to eliminate sorbitol inhibition and to improve reactor productivity by fed-batch cultivation.[4,6,10,13] Trial and error procedure for substrate feeding has been adopted for these fed-batch cultivation. Mathematical models can be used as a valuable tool for the design of feeding strategy to maximize the product concentration and reactor productivity.

In the present investigation, a batch kinetic model was developed for sorbose production using average batch kinetic data for an initial sorbitol concentration of 200 kg m^{-3}. The adequacy of the model was tested by conducting fed-batch sorbose production with constant flow rate of 0.2 dm^3 h^{-1} (inlet sorbitol concentration, 500 kg m^{-3}) and by comparing the observed fermentation kinetics with the model simulation.

2. Materials and Methods

2.1 Chemicals

D-sorbitol solution (w = 70 %) was supplied by M/s Anil starch products, Ahmedabad, India. Yeast extract powder, ammonium dihydrogen phosphate and magnesium sulfate were obtained from M/s Qualigens fine chemicals, Mumbai, India. All chemicals were of Analytical Reagent (AR) grade.

2.2 Media

2.2.1 Standard liquid medium

The composition of the standard liquid medium (in γ/kg m^{-3}) was as follows :

D-sorbitol, 5.0; yeast extract powder, 5.0; ammonium dihydrogen phosphate, 3.0; magnesium sulfate, 1.0; The pH of the medium was maintained at 6.0 by addition of 3 mol dm^{-3} HCl/NaOH.

2.2.2 Culture maintenance media

The culture was maintained on agar slants having composition similar to the standard liquid medium (as indicated in 2.2.1) + agar 20 kgm^{-3}. A 48 hour growth at 30 °C was preserved at 4 °C.

2.3 Microorganism and maintenance

Gluconobacter oxydans subsp. s*uboxydans* NRRL B-72 strain was used in this work.

2.4 Inoculum development

A loop of microorganism from the slant was transferred into 0.01 dm^3 standard liquid medium in test tubes having the same composition as above (as mentioned in 2.2.1). The test-tubes were incubated at 30 °C for 72 hours. The growth was characterised by the appearance of a thick pellicle on the surface and uniform turbidity. It was then transferred into 1.0 dm^3 capacity flasks containing 0.1 dm^3 standard liquid medium (as mentioned in 2.2.1). The flasks were incubated in a rotating shaker (Adolf Kuhner, Germany) at 30 °C and 250 rpm. Subsequent transfer into the bioreactor was done when the biomass concentration in the flasks was about 2.5 to 3.0 kg m^{-3}.

2.5 Inhibition studies

In order to establish the nature of relationship between μ and γ_{S0}, inhibition studies were conducted in shaking flask cultivation. The standard liquid medium as described in section 2.2.1 was used except that the sorbitol mass concentrations were (kg m^{-3}), 100, 150, 200, 250, 350, 450 and 510. The flasks were incubated in a rotating shaker (Adolf Kuhner, Germany) at 30 °C and 250 rpm.

The biomass concentration (γ_X) was estimated by measuring the optical density of the cell suspension at intervals of one hour during the initial phases of culture growth (8 hours) when there was very little sorbose formation and thereby less oxygen limitation. Specific growth rate was calculated from (ln γ_X vs t) plot. A graphical correlation between μ and γ_{S0} as shown in figure 1, was obtained.

The maximum substrate mass concentration, γ_{Sm}, at which the growth completely stopped and the dimensionless inhibition exponent *'α'* which indicated the type of inhibition by the substrate on specific growth rate (concave, linear or convex) were determined to be 510 kgm^{-3} as described by *Luong*[14].

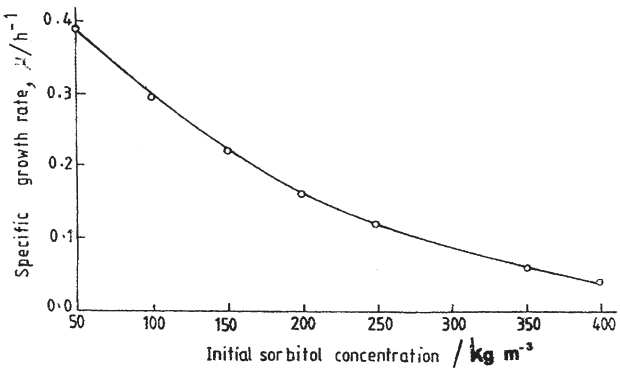

Fig. 1 – *Effect of increasing initial substrate concentratin on specific growth rate.*

2.6 Cultivation

2.6.1 Batch cultivation

Batch cultivations were carried out in a 7.0 dm^3 fermenter (Bioengineering AG, Switzerland) equipped with 2 sets of flat-blade turbine impellers under the following conditions: working volume 4.5 dm^3, airflow rate 2.2 $dm^3\ dm^{-3}\ kg^{-1}$, agitation speed 700 rpm, temperature 30 °C. The pH was maintained at 6.0 by a pH controller through the automatic addition of 3 mol dm^{-3} HCl/NaOH. The cultivation medium used was the standard liquid medium described in section 2.2.1 except that the initial sorbitol concentration was 200 kg m^{-3}.

2.6.2 Fed-batch cultivation with constant feed rate of 0.2 $dm^3\ h^{-1}$, 500 kg m^{-3} sorbitol

Fed-batch cultivations with constant feed flow rate of 0.2 $dm^3\ h^{-1}$, 500 kg m^{-3} sorbitol, were initiated as a batch with γ_{S0} = 225 kg m^{-3}. After 8 hours, when log phase growth with a biomass concentration of 1.8 kg m^{-3} was established, fresh nutrient medium containing 500 kg m^{-3} sorbitol was added at a constant feed rate of 0.2 $dm^3\ h^{-1}$. After completion of feed addition at 18 hours, the residual sorbitol in the reactor was allowed to ferment batchwise. The fermentation was terminated when the dissolved oxygen level in the reactor indicated an abrupt increase.

2.7 Analytical Techniques

2.7.1 Biomass concentration

Optical density (OD) of the suitably diluted samples was measured at 600 nm in a UVIKON 930 spectrophotometer (Kontron Instruments, USA). Biomass was estimated from a OD vs concentration (kg m^{-3}) correlation which was determined *a priori* as follows:

Biomass concentration of a known volume of the samples was determined gravimetrically after measuring the OD at 600 nm. A standard curve was plotted between OD of the samples and their respective biomass concentrations to arrive at the following correlation.

$$\text{Biomass (kg m}^{-3}) = 0.73 \times \text{OD}_{600}$$

2.7.2 Sorbitol and sorbose concentrations

Sorbitol and sorbose concentrations were estimated by HPLC (Waters Associates, USA) using a Supelcosil LC-NH_2 (Supelco, USA) column (25 cm × 4.6 mm ID) equipped with Refractive Index (RI) detector and using Ψ (acetonitrile:water) = 75:25 as eluent with a flow rate of 0.06 $dm^3\ h^{-1}$ at ambient temperature.

3. Results and Discussions

3.1 Batch experiments

Fig. 2 describes the average batch cultivation kinetic data at initial substrate concentration (γ_{S0}) = 200 kg m^{-3}. The cultivation was over in 14 hours and an overall sorbose productivity of 13.58 kg $m^{-3}h^{-1}$ was obtained. The biomass formation was 5.9 kg m^{-3} at hour 14 and the dissolved oxygen concentration value decreased from initial value of 100 % (8ppm) to 0 % (0 ppm) in 14 hours. This resulted in a biomass yield value of 737.5 based on oxygen consumption. Sorbitol fed to the reactor was converted to sorbose to the extent of 96.0 %. The observed batch kinetic data were utilized for the development of mathematical model.

3.2 Model development

The model development was based on the following assumptions.

3.2.1 Assumption

1. Sorbitol is the only limiting nutrient

2. There is no effect of oxygen on the sorbitol to sorbose bioconversion. This was ensured by selecting the maximum possible air flow rate (2.2 $dm^3\ dm^{-3}\ kg^{-1}$) under the available experimental conditions.

3. There is no process limitation by nitrogen, phosphorous and growth factors (ex. yeast extract) and they are in excessiv supply in the fermentation medium.

4. There is no change in temperature and pH of culture growth throughout the cultivation. Yeast extract has been indicated to be a rate limiting nutrient[5,15] in sorbose production. Therefore, optimal supply of yeast extract was taken alongwith assumption 1.

3.2.2 Model equations

The system of differential material balance equations which represented the general mathematical model capable of describing the batch dynamics of sorbose production is summarized below:

$$\mu = \left(\frac{\mu_m \gamma_S}{\gamma_S + K_s}\right)\left(1 - \left(\frac{\gamma_S}{\gamma_{S_m}}\right)^a\right)\left(\frac{K_p}{K_p + \gamma_p}\right) \quad (1)$$

$$\frac{d\gamma_X}{dt} = \left(\frac{\mu_m \gamma_S}{\gamma_S + K_s}\right)\left(1 - \left(\frac{\gamma_S}{\gamma_{S_m}}\right)^a\right)\left(\frac{K_p}{K_p + \gamma_p}\right)\gamma_X \quad (2)$$

Equation (2) represents the rate of biomass formation featuring Monod type sorbitol (substrate) limitation. Since the inhibition phenomena is not completely understood the empirical model suggested by *Luong*[14] for the inhibitory effects of

ethanol on yeast growth is extended to inhibition by sorbitol on the growth of *Gluconobacter oxydans*. Similarly the product inhibition is represented by the exponential decay model[16] of the specific growth rate with increasing product concentration.

The specific rate of substrate consumption (r_s) was best mathematically described by equation (3) which is a modified form of the *Leudking* and *Piret*[17] model to account for substrate consumption for product. The complex lumping of the parameters was done to better represent the specific substrate consumption rate.

$$r_s = -\left(\frac{1}{Y_x}\mu + \frac{1}{Y_p}r_p + m_s\right) \tag{3}$$

$$\frac{d\gamma_S}{dt} = -\left(\frac{1}{Y_{x/s}}\mu\gamma_x + \frac{1}{Y_p}r_p\gamma_X + m_s\gamma_X\right) \tag{3a}$$

Where m_s represent the maintenance energy requirement of the cell and Y_p and Y_x are respective yields of the metabolic product and biomass based on substrate. The specific rate of sorbose production (r_p) could be represented by growth-associated and non growth associated product formation terms[5,17,18]

$$r_p = \alpha\mu + \beta \tag{4}$$

where α and β are constants which include sorbose formation in exponential (growing) and in stationary (non growing phase) by bacterial growth[5,17] respectively.

$$\frac{d\gamma_p}{dt} = \alpha\mu\gamma_x + \beta\gamma_X \tag{4a}$$

$$\frac{d\gamma_p}{dt} = \alpha\left(\frac{\mu_m\gamma_S}{\gamma_S + K_s}\right)\left(1 - \left(\frac{\gamma_S}{\gamma_{S_m}}\right)^a\right)\left(\frac{K_p}{K_p + \gamma_p}\right)\gamma_X + \beta_X \tag{4b}$$

Substituting r_p in equation 3a from equation 4 yields the substrate consumption rate ($d\gamma_S/dt$) as expressed by equation (5)

$$\frac{d\gamma_S}{dt} = -(K_1\mu\gamma_X + K_2\gamma_X) \tag{5}$$

Where K_1 refers to the sum of reciprocals of the growth associated yield term for biomass and metabolic products.

$$K_1 = \frac{1}{Y_{x/s}} + \frac{\alpha}{Y_p} \tag{6}$$

and K_2 reflects the sum of substrate energy fraction channelled towards maintenance of cellular functions and that expended for total substrate energy for growth non-associated product formation.

$$K_2 = m_s + \frac{\beta}{Y_p} \tag{7}$$

The equations (2) (5) and (4b) represent the Model equations for rates of biomass, substrate and product concentrations for the batch sorbitol to sorbose biooxidation process.

3.3 Evaluation of model parameters

For optimal estimation of the model parameters, non-linear regression technique[19] assisted by a computer program[20,21], was used to minimize the deviations between the model predictions and batch exponential data. For calculation of the model predictions the system of differential equations (2), (5) and (4b) which describe the batch sorbose production kinetics were solved by an integration program based on Runge-kutta method of fourth order[20]. The optimization program for the direct search of the minimum of a multivariable function was based on the original method of *Rosenbrock*[22]. The minimization criteria used in the program were as follows:

$$\mathrm{SSWR} = \sum_{i=1}^{n}\sum_{j=1}^{m}\frac{\Delta_{ij}^2}{W_{ij}^2} \tag{8}$$

where SSWR represents the sum of the squares of weighed residues. (n) and (m) represents the number of experimental data points and the number of variables respectively, W_{ij} represents the mass of each variable (usually the maximum value of each variable) and Δ_{ij} denotes the difference between the model and the experimental value.

The value of the optimized parameters for fermentation conducted at γ_{S0} = 200 kg m^{-3} is given in Table 1. The value of μ_m, K_s and K_p were found to be 0.71, 37.32 and 160.83. The value of μ_m compares

Table 1 – *Model parameters and parametric sensitivity values for batch fermentation (γ_{S0} = 200 kg m^{-3})*

Model parameter	Units	Value	APS[a]	RPS[b]
K_s	kg m^{-3}	37.32	–108.45	3.3
K_p	kg m^{-3}	160.83	22.32	2.93
μ_m	h^{-1}	0.71	33,288.6	19.34
K_1	kg kg^{-1}	33.33	344.06	10.11
K_2	kg kg^{-1}h^{-1}	0	1,004.6	0
α	kg kg^{-1}	33.04	–84.37	2.27
β	kg kg^{-1}h^{-1}	0.0001	0	0
γ_{Smax}	kg m^{-3}	510	independently determined	
a	dimensionless	0.81		

[a] APS : absolute parameter sensitivity
[b] RPS : relative parameter sensitivity.

fairly well with 0.661 for *G. oxydans* (ATCC 621)[5], however μ_m, K_s and K_p were reported to be 0.3 h^{-1}, 14.4 & 205.0 for *A. suboxydans* (NBIMCC 902)[8]. The variation in the kinetic parameter values could be due to the variation in culture, growth medium and culture conditions. The model equations were simulated on the computer using the optimum value of the model parameters. The comparison of the model simulation (smooth curve) and experimental data (points) is shown in fig. 2. The experimental data and the model simulation demonstrated good agreement.

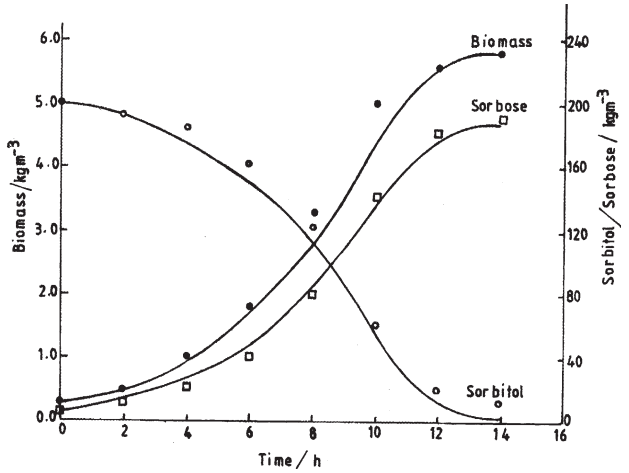

Fig. 2 – *Model simulation (smooth curve) and experimental data (points) for batch sorbose production (γ_{S0} = 200 kg m^{-3}) by Gluconobacter oxydans.*

In order to further evaluate the degree of reliability of the model, a method recommended by *Bard*[19] 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:

$$\Delta_j = \frac{1}{n}\sum_{i=1}^{n}\Delta_{ij} \tag{9}$$

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 model simulation value. The variance of the error of a residual (S_j) was then estimated as follows:

$$\gamma_{S_j} = \frac{1}{n-1}\sum_{n-1}^{n}(-\Delta_{ij})^2\Delta_j \qquad \text{for } j = 1, m \tag{10}$$

where m is the number of variables. The value of the statistics (λ) defined as follows:

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

'λ' was calculated. The statistics 'λ' has the $F_{m,\,n-m}$ distribution. It was calculated as 10.33 for fermentation using γ_{S0} = 200 kg m^{-3}, which was less than the '$F_{3,5}$' value (obtained from F tables) for 99 % confidence for the whole experimental set. This established the accuracy of the developed mathematical model.

3.4 Fed-batch model equations

In order to design suitable nutrient feeding strategies, the developed batch model was extrapolated to fed-batch conditions by incorporating the dilution term, D. The fed-batch model equations are summarised below:

$$\frac{d\gamma_x}{dt} = \left(\frac{\mu_m\gamma_S}{\gamma_S+K_s}\right)\left(1-\left(\frac{\gamma_S}{\gamma_{S_m}}\right)^a\right)\left(\frac{K_p}{K_p+\gamma_p}\right)\gamma_X - D\,\gamma_x \tag{12}$$

$$\frac{d\gamma_S}{dt} = -K_1\left(\frac{\mu_m\gamma_S}{\gamma_S+K_s}\right)\left(1-\left(\frac{\gamma_S}{\gamma_{S_m}}\right)^a\right)\left(\frac{K_p}{K_p+\gamma_p}\right)\gamma_X - K_2\gamma_X + D(\gamma_{S_r}-\gamma_S) \tag{13}$$

$$\frac{d\gamma_p}{dt} = \alpha\left(\frac{\mu_m\gamma_S}{\gamma_S+K_s}\right)\left(1-\left(\frac{\gamma_S}{\gamma_{S_m}}\right)^a\right)\left(\frac{K_p}{K_p+\gamma_p}\right)\gamma_X + \beta\gamma_X - D\gamma_p \tag{14}$$

$$\frac{dV}{dt} = Q \tag{15}$$

$$D = \frac{Q}{V} \tag{16}$$

The feeding strategies were designed as based on the computer simulation of equations (12) – (16) and implemented in actual fed-batch fermentations.

3.4.1 Fed-batch fermentation with constant feed glow rate of 0.2 dm^3 h^{-1}, using inlet sorbitol concentration of 500 kg m^{-3}

3.4.1.1 Model simulation

In order to test the adequacy of the fed-batch model (for γ_{S0} = 200 kg m^{-3}), a fed-batch cultivation with constant feed rate was simulated and implemented experimentally. The fed-batch cultivation was simulated at a feed rate of 0.2 dm^3 h^{-1} with an inlet sorbitol concentration of 500 kg m^{-3}. The nutrient feeding was done during hour 8 to hour 18. After completion of feed addition at 18 hours, the fermentation was simulated as batch till the residual sorbitol was consumed. The model demonstrated a sorbose accumulation of 321.17 kg m^{-3} with a productivity of 14.59 kg m^{-3} h^{-1}.

3.4.1.2 Experimental observations

The fed-batch cultivation with constant feed rate of 0.2 dm^3 h^{-1} (500 kg m^{-3} sorbitol) was initiated as a batch with γ_{S0} = 225 kg m^{-3}. At hour 8,

when the biomass concentration in the reactor was 1.8 kg m^{-3}, nutrient medium containing 500 kg m^{-3} sorbitol was added at a constant feed rate of 0.2 dm^3 h^{-1}. After completion of feed addition at 18 hours, the cultivation was continued as a batch to consume the residual sorbitol in the reactor.

The experimental observation (data points) and model simulation (smooth curves) for fed-batch cultivation with constant feed rate is shown in Fig. 3 (biomass), Fig. 4 (sorbose) and Fig. 5 (sorbitol).

The cultivation was over in 24 hours with a sorbose concentration of 336.24 kg m^{-3} against the simulated value of 321.17 kg m^{-3}. 96% of the sorbitol fed was converted to sorbose with a productivity of 14.01 kg m^{-3} h^{-1} as against the simulated value of 14.59 kg m^{-3} h^{-1}. Excellent agreement was found between experimental data and model simulation which indicated that the developed model can serve as a powerful tool for designing feeding strategies for enhancing yield and productivity of sorbose production.

Several researchers have attempted to increase the sorbose productivity by implementing different feeding strategies in fed-batch cultivation[4,6,13]. *Bošnjak* et al.[4] attempted gradient fed-batch cultivation and obtained a sorbose productivity of 11.62 kg m^{-3} h^{-1}. *Mori* et al.[6] used *G. oxydans* (ATCC 621) pure oxygen supply to the bioreactor and enhanced the sorbose productivity to 44.85 kg m^{-3} h^{-1}. *Bull* et al.[12] used partial recycling of microbial cells in chemostat and could enhance the product formation rates to a level of 91 kg m^{-3} h^{-1} . However, unusual design of cell retention device[12] pure oxygen[6,12] sorbitol powder us-

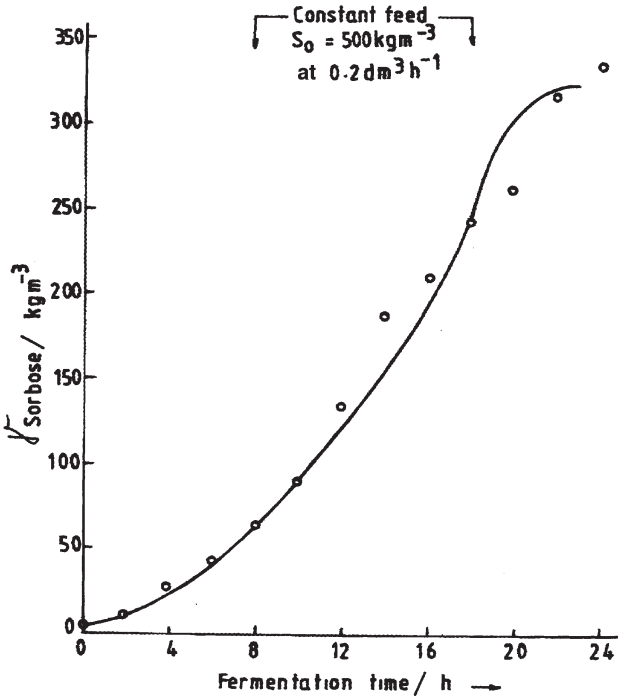

Fig. 4 – *Time course of sorbose concentration for model based fed-batch sorbose production at constant feed rate: smooth curve-model simulation; data points-experiment, 0 – 8 h batch (γ_{S0} = 225 kg m^{-3}), 8 – 18 h fed-batch (inlet sorbitol 500 kg m^{-3}, feed flow rate 0.2 dm^3 h^{-1}), 18 – 24 h batch cultivation.*

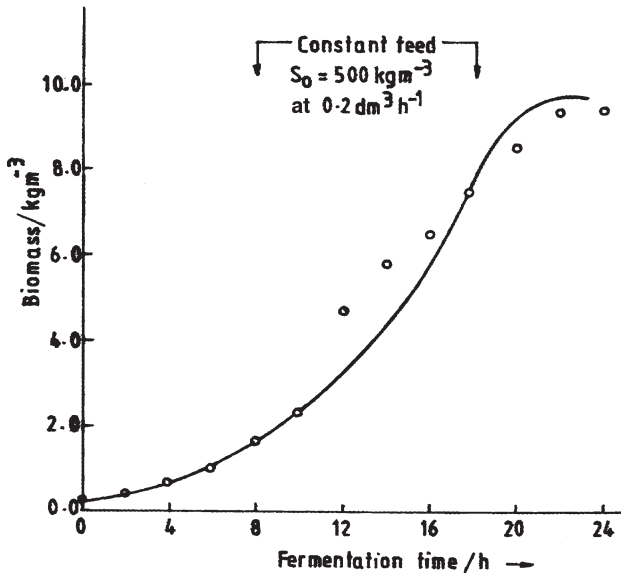

Fig. 3 – *Time course of biomass mass concentration for model based fed-batch sorbose production at constant feed rate: smooth curve-model simulation; data points-experiment, 0 – 8 h batch (γ_{S0} = 225 kg m^{-3}), 8 – 18 h fed-batch (inlet sorbitol 500 kg m^{-3}, feed flow rate 0.2 dm^3 h^{-1}), 18 – 24 h batch cultivation.*

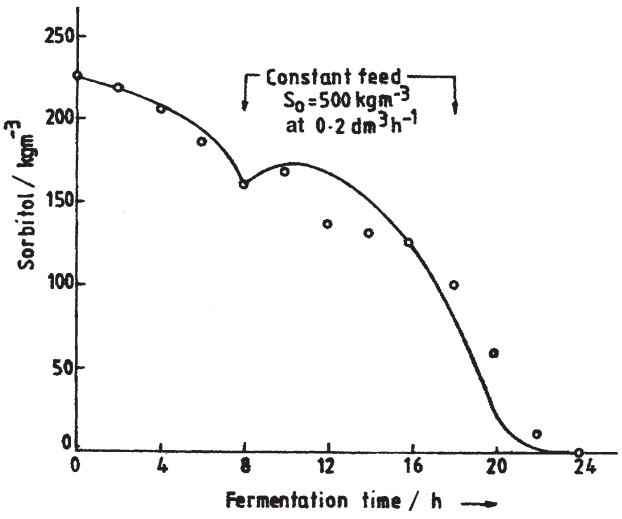

Fig. 5 – *Time course of sorbitol concentration for model based fed-batch sorbose production at constant feed rate: smooth curve-model simulation; data points-experiment, 0 – 8 h batch (γ_{S0} = 225 kg m^{-3}), 8 – 18 h fed-batch (inlet sorbitol 500 kg m^{-3}, feed flow rate 0.2 dm^3 h^{-1}), 18 – 24 h batch cultivation.*

age[6] and high rpm utilized[12] in their investigations, restricted the industrial applicability of their production strategies. *Srivastava* and *Lasrado*[13] cultivated *Gluconobacter oxydans* (NRRL B-72) in fed-batch cultivation by feeding sorbitol and fresh nutrients via exponentially increasing feed and obtained a productivity of 12.6 kg m^{-3} h^{-1}. The present work demonstrated a productivity of 14.01 kg m^{-3} h^{-1} which is higher compared to that reported by earlier workers except *Mori* et al.[6] and *Bull* et al.[12] .

4. Conclusions

The average kinetic data from batch cultivation conducted at γ_{S0} = 200 kg m^{-3} was used to develop the unstructured mathematical model. Model parameters were identified by the non-linear regression technique assisted by computer program. Parametric sensitivity analysis indicated μ_m to be the most sensitive model parameter. Excellent agreement was found between batch experimental data and model simulations. Statistical validity (F-test) of the model indicated 99% confidence on the predictions of the model for γ_{S0} = 200 kg m^{-3}. The batch model was extrapolated to identify nutrient feeding strategy for fed-batch cultivation with a constant feed rate of 0.2 dm^3 h^{-1}, 500 kg m^{-3} sorbitol. The fed-batch cultivation demonstrated increased sorbose concentrations of 336.24 kg m^{-3} and a productivity of 14.01 kg $m^{-3}h^{-1}$. Fed-batch model predictions were close to experimental observations which proved that the developed model can be used to design different feeding strategies for fed-batch sorbose cultivations.

Nomenclature

D – dilution rate, h^{-1}

K_s – monod's saturation constant for substrate, kg m^{-3}

K_p – inhibition constant for sorbose, kg m^{-3}

m_s – maintenance energy constant, kg $kg^{-1}h^{-1}$

r_s – specific substrate consumption rate, kg $kg^{-1}h^{-1}$

r_p – specific product formation rate, kg kg^{-1} h^{-1}

Q – feed flow rate, dm^3 h^{-1}

t – time, h

V – volume, dm^3

K_1 – Sum of reciprocal of growth associated yield term for biomass and metabolic products, kg kg^{-1}

K_2 – Sum of substrate energy fraction channelled towards maintenance of cellular-functions and for growth non associated sorbose production

w – mass fraction

Subscript

a – exponent

n – number of data points

m – number of process variables

p – product (sorbose)

s – substrate

x – biomass

Greek letters

α – coefficient of proportionality between the rate of product formation and growth rate, kg kg^{-1}

β – coefficient of proportionality between the rate of product formation and biomass fraction, kg $kg^{-1}h^{-1}$

μ – specific growth rate, h^{-1}

Δ_{ij} – difference between experimental and model simulated value of a process variable.

S_j – means residual of each variable

Ψ – volume ratio, dm^3 dm^{-3}

γ_p – sorbose concentration, kg m^{-3}

γ_s – substrate concentration, kg m^{-3}

γ_{Sm} – maximum value of substrate concentration at which there is no growth, kg m^{-3}

γ_{So} – initial substrate concentration, kg m^{-3}

γ_r – inlet substrate concenration, kg m^{-3}

γ_x – biomass concentration, kg m^{-3}

$d\gamma/dt$ – rate of reaction for the component X, S, P

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