DOI: 10.1002/jctb.1207

Analysis of acetic acid productivity in a continuous two-stage bioreactor with cell recycling

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Abstract: The performance of a two-stage system with cell recycling and fresh feed at each stage is studied numerically for continuous acetic acid production. In this system, both filtrate and bleed broth from the first stage are supplied to the second fermenter. At high substrate conversions, this configuration is found to provide higher acetic acid productivities than either a previous configuration where only the first-stage bleed broth is fed to the second stage or a single recycle chemostat at the same bleed ratios.

Keywords: acetic acid productivity; two-stage fermenter; cell recycle; model simulation

NOTATION

- a Parameter (kg kg^{-1} h^{-1})
- b Parameter (kg kg^{-1})
- B Bleed ratios, defined such that the bleed flow rate (m^3 h^{-1}) leaving the first stage is B_1F_1 and that leaving the second stage is $B_2(F_1 + F_2)$, as seen in Fig 1.
- c Parameter (kg kg^{-1} h)
- D Dilution rate (h^{-1})
- f Flow rate ratio (F_2/F_1)
- F Volumetric flow rate of fresh medium feed (m^3 h^{-1})
- k_1 Parameter (h^{-1})
- k_2 Parameter (h)
- n Parameter
- P Product (acetic acid) concentration (kg m^{-3})
- Pr Volumetric productivity of acetic acid (kg m^{-3} h^{-1})
- q_S Specific consumption rate of substrate (kg kg^{-1} h^{-1})
- S Substrate (ethanol) concentration (kg m^{-3})
- V Working fermenter volume (m^3)
- x_S Substrate conversion
- X Viable cell concentration (kg m^{-3})
- X_t Total cell concentration (kg m^{-3})
- $Y_{P/S}$ Yield of acetic acid based on substrate consumed (kg kg^{-1})
- α Fermenter volume ratio (V_2/V_1)
- γ Specific death rate of viable cells (h^{-1})
- μ Specific growth rate of cells (h^{-1})
- ν Specific production rate of acetic acid (kg kg^{-1} h^{-1})

Subscripts

- f Feed
- h Highest
- i ith stage or fermenter
- m Maximum
- ov Overall

1 INTRODUCTION

As a useful method to enhance production rates in continuous acetic acid fermentation processes involving severe product inhibition, membrane cell-recycle bioreactors have been used on a laboratory scale.[1–5] In a typical bioreactor, a continuous stirred tank fermenter is equipped with a membrane filter for cell separation, and the concentrated cells are returned to the fermenter for recycling. To obtain high productivity and high concentration of acetic acid, two-stage recycle systems have also been investigated experimentally, with a membrane module attached to only the second fermenter and the fermentation broth from the second stage being recycled back to the first or second fermenter.[5,6] A modified two-stage configuration with cell recycle and fresh feed at each stage has been previously considered.[7] In this configuration, high concentration of viable cells in the bleed broth withdrawn from the first

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(*Received 18 May 2003; revised version received 9 September 2004; accepted 14 September 2004*)
Published online 17 February 2005

stage can be reused in the subsequent fermenter to raise overall acetic acid productivity. In a previous paper,[7] this two-stage configuration with the first-stage bleed broth supplied to the second fermenter was studied numerically using the kinetic model from the literature.[2] It was found to yield a higher acetic acid productivity than a two-stage fermenter with a separator after the final stage and a single recycle chemostat.

The present paper considers another modified configuration for staging so that both the filtrate and bleed broth are introduced from the first stage into the second fermenter with fresh feed. This may be expected to improve overall substrate conversion, because the consumption of the residual substrate in the first-stage filtrate proceeds in the second fermenter, unlike the above type. In this work, acetic acid productivities at high substrate conversions in this two-stage system are modeled and solved numerically using the same kinetic model as above and are compared with those in the above type and in the single-stage system.

2 BIOREACTOR MODEL

A schematic diagram of a two-stage fermenter with cell recycle for continuous production of acetic acid is shown in Fig 1. This two-stage system consists of two nonequal-volume chemostats with cell separators after each stage. Fresh and sterile medium is supplied to each fermenter, and both filtrate and bleed broth from the first stage are fed to the second fermenter. It is assumed that each fermenter is ideally mixed, the residence time in each recycle loop is negligible, each filtrate contains no cells, and mass transfer resistance is negligible. The processes taking place in each fermenter are cell growth, cell death, substrate consumption and acetic acid production. For their specific rates, eqns (1)–(4) are assumed here to be applicable:

$$\mu = \mu_m\{1 - (P/P_m)^n\} \tag{1}$$

$$\gamma = k_1 \exp(k_2 D) \tag{2}$$

$$q_S = \nu / Y_{P/S} \tag{3}$$

$$\nu = a + b\mu - c\mu^2 \tag{4}$$

where $\mu_m = 0.26\,\text{h}^{-1}$, $P_m = 63.5\,\text{kg m}^{-3}$, $n = 3.61$, $k_1 = 0.059\,\text{h}^{-1}$, $k_2 = 0.325\,\text{h}$, $a = 1.92\,\text{kg kg}^{-1}\,\text{h}^{-1}$, $b = 386.8\,\text{kg kg}^{-1}$, $c = 1347\,\text{kg kg}^{-1}\,\text{h}$ and $Y_{P/S} = 1.18\,\text{kg kg}^{-1}$. These kinetic expressions and parametric values were determined by Park and Toda[2] in their experimental study on the production of acetic acid from ethanol as a substrate by *Acetobacter aceti* in a cell-recycle chemostat. The saturation constant in the Monod equation was measured as $0.005\,\text{kg m}^{-3}$, a low value that makes μ independent of S.[2]

Based on the above assumptions, steady-state mass balances for total and viable cells, substrate and product around the first fermenter with the first cell separator are expressed by eqns (5)–(8), respectively:

$$-B_1 D_1 X_{t1} + \mu_1 X_1 = 0 \tag{5}$$

$$-B_1 D_1 + \mu_1 - \gamma_1 = 0 \tag{6}$$

$$D_1 S_{f1} - D_1 S_1 - q_{S1} X_1 = 0 \tag{7}$$

$$D_1 P_{f1} - D_1 P_1 + \nu_1 X_1 = 0 \tag{8}$$

where $D_1 = F_1/V_1$. These eqns (5)–(8) are identical to those for a single chemostat with cell recycle.

Mass balances for the four components around the second stage are represented by eqns (9)–(12):

$$B_1 D_1 X_{t1} - (1+f) D_1 B_2 X_{t2} + \alpha \mu_2 X_2 = 0 \tag{9}$$

$$B_1 D_1 X_1 - (1+f) D_1 B_2 X_2 + \alpha(\mu_2 - \gamma_2) X_2 = 0 \tag{10}$$

$$f D_1 S_{f2} + D_1 S_1 - (1+f) D_1 S_2 - \alpha q_{S2} X_2 = 0 \tag{11}$$

$$f D_1 P_{f2} + D_1 P_1 - (1+f) D_1 P_2 + \alpha \nu_2 X_2 = 0 \tag{12}$$

where $\alpha = V_2/V_1$ and $f = F_2/F_1$. μ_i, γ_i, q_{Si} and ν_i in eqns (5)–(12) are given by the kinetic model used.

Operating parameters in the two-stage system are D_1, B_1, B_2, S_{f1}, S_{f2}, P_{f1}, P_{f2}, α and f. The bleed ratios are defined such that the bleed flow rate ($\text{m}^3\,\text{h}^{-1}$) leaving the first stage is $B_1 F_1$ and that leaving the second stage is $B_2(F_1 + F_2)$, as seen in Fig 1. The dilution rate in the second stage is fixed

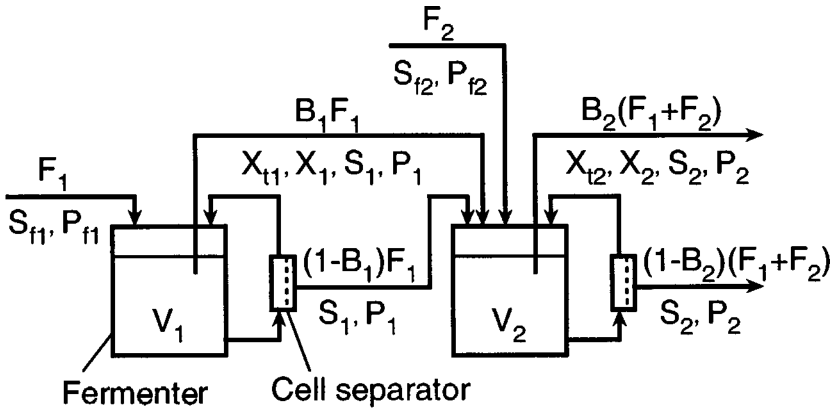

Figure 1. Schematic of a two-stage cell-recycle bioreactor.

by $(1+f)D_1/\alpha$. For a given set of these operating parameters, the concentrations of total and viable cells, substrate and product in each stage can be obtained by solving eqns (5)–(12) numerically with eqns (1)–(4). For the numerical solution, the Newton–Raphson method was used.

The overall productivity of acetic acid (Pr_{ov}) and the overall substrate conversion (x_{Sov}) for the two-stage system in Fig 1 are expressed by:

$$Pr_{ov} = D_1\{(1+f)P_2 - P_{f1} - fP_{f2}\}/(1+\alpha) \quad (13)$$

$$x_{Sov} = 1 - (1+f)S_2/(S_{f1} + fS_{f2}) \quad (14)$$

The productivity Pr and the substrate conversion x_S for the single-stage system are:

$$Pr = D(P - P_f) \quad (15)$$

$$x_S = 1 - S/S_f \quad (16)$$

In eqns (13) and (15), acetic acid in each bleed is recoverable as a product. A comparison of the total fermenter volume ($V_1 + V_2$) for the two-stage system with the volume V for the single-stage system can be made by using the relationship $(V_1+V_2)/V = (1+\alpha)D/\{(1+f)D_1\}$. This relationship is derived on the basis of $F = (F_1 + F_2)$ and using the definitions $\alpha = V_2/V_1, f = F_2/F_1, D_1 = F_1/V_1$ and $D = F/V$.

In the following analysis, $47.4\,\mathrm{kg\,m^{-3}}$ is mainly used for S_{f1} and S_{f2}. Under the condition of this concentration, the above-mentioned kinetic model[2] was determined. $P_{f1} = P_{f2} = 0$ since fresh medium contains no product in a usual fermentation process. To make a comparison of efficiency between the two-stage system and the conventional single-stage system at the same bleed ratios in both systems, a condition of $B_1 = B_2$ in the former system is also employed.

3 RESULTS AND DISCUSSION

3.1 Comparison of different fermenter configurations

In Fig 2 results are given for an example of the effect of the first-stage dilution rate D_1 on the predicted values of the overall acetic acid productivity Pr_{ov} and the overall substrate conversions x_{Sov} at fixed bleed ratios $B_1(=B_2) = 1$, 0.2 and 0.1. Here the substrate concentrations in the feeds of $S_{f1} = S_{f2} = 47.4\,\mathrm{kg\,m^{-3}}$ were used, as in cited kinetic studies. The solid lines indicate the operation of the two-stage system as shown in Fig 1. When D_1 increases at a constant B_1, Pr_{ov} increases, attains its maximum $Pr_{ov,m}$ at a certain dilution rate D_{1m} and then decreases, while x_{Sov} decreases gradually with increasing D_1 and then rapidly. For smaller values of B_1, both maxima $Pr_{ov,m}$ and D_{1m} are higher, and the values of x_{Sov} at a constant D_1 are greater, being similar to those for $B_1 = 1$ if stretched out along the abscissa D_1. The values of the flow ratio f and the volume ratio α used for this figure are chosen to give the highest $Pr_{ov,m}$ attainable when

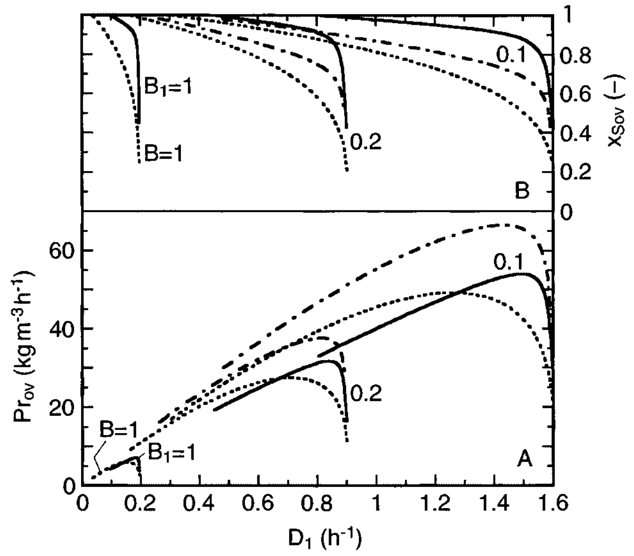

Figure 2. Predicted values of Pr_{ov} (A) and x_{Sov} (B) as a function of D_1 with B_1 as a parameter. $B_2 = B_1$ and $S_{f1} = S_{f2} = 47.4\,\mathrm{kg\,m^{-3}}$. In the case of solid lines (Fig 1), $f = 0.466$, $\alpha = 0.868$ for $B_1 = 1$, $f = 0.455$, $\alpha = 0.893$ for $B_1 = 0.2$, and $f = 0.462$, $\alpha = 0.994$ for $B_1 = 0.1$. Dot-dash lines indicate the case where the filtrate from the first stage in Fig 1 is not supplied to the second fermenter. Dotted lines refer to a single-stage system.

changing D_1 at a constant B_1 for various combinations of f and α. These highest values of $Pr_{ov,m}$ and the corresponding D_{1m} yielding it are denoted hereafter by $Pr_{ov,h}$ and D_{1h}, respectively. This result is compared with those in other cases. The dot-dash lines indicate the case where the filtrate from the first stage in Fig 1 is not supplied to the second fermenter, as studied in a previous paper.[7] In this case, $f = 0.785$ and $\alpha = 0.598$ for $B_1 = 0.2$, and $f = 0.775$ and $\alpha = 0.561$ for $B_1 = 0.1$. These combinations of f and α give the respective values of $Pr_{ov,h}$. The dotted lines refer to the case of a single-stage system. The values of $Pr_{ov,h}$ for the configuration in Fig 1 fall between those in these two cases at the same bleed ratios. However, x_{Sov} at a constant D_1 is higher for the system of Fig 1 than those in the other cases over a wide range of D_1 for the same bleed ratios. The reason for this is that the residual substrate in the filtrate from the first stage is consumed in the second fermenter, at the expense of reducing Pr_{ov} (due to the dilution by the filtrate).

Under the conditions of other bleed ratios, examining the effect of D_{1h} on the predicted values of $Pr_{ov,h}$ and x_{Sov} at D_{1h}, the results obtained are found to be similar to the above results. For $B_1(=B_2) = 0.05$–1, the values of x_{Sov} at D_{1h} for the system of Fig 1 are 0.85–0.89, which decrease only slightly with decreasing B_1 and are larger even at low bleed ratios than those in the other two cases. By choosing appropriate combinations of f and α, therefore, high Pr_{ov} at higher x_{Sov} may be expected to be achieved in the two-stage system of Fig 1.

3.2 Productivities at high substrate conversions

In the following, the values of Pr_{ov} were examined under various operating parameters which give high

x_{Sov} values of 95–99%. Since $S_{f1} = S_{f2}$ and $P_{f1} = P_{f2} = 0$ are set and the yield $Y_{P/S}$ is constant, the second-stage concentration of acetic acid in the case of the two-stage system shown in Fig 1 is about 53.1 and 55.4 kg m^{-3}, independent of f and α, for 95 and 99% x_{Sov}, respectively. Figure 3 shows an example of the predicted values of Pr_{ov} when x_{Sov} is fixed at 95%. Here are given the effect of α on Pr_{ov} and D_1 at $B_1 (= B_2) = 0.1$ with f as a parameter for the system of Fig 1. As depicted by the solid lines in the lower region, at a constant f, the values of Pr_{ov} increase, up to their maxima $Pr_{ov,m}$, and then decrease gradually with an increase in α. These maxima are higher than the corresponding Pr for the single-stage system as shown by the open circles on the $\alpha = 0$ axis. In this example, among these maxima, $Pr_{ov,m}$ for $f = 0.166$ is highest at $\alpha = 0.721$ as shown by the closed circle, although this $Pr_{ov,h}$ is very close to $Pr_{ov,m}$ for the cases of $f = 0$, 0.1 or 0.3. The dot-dash lines in Fig 3 indicate the case where the filtrate from the first stage in Fig 1 is not fed to the second fermenter. As shown by the closed square at $f = 0.723$ and $\alpha = 0.622$, $Pr_{ov,h}$ in this case is attained; but both this $Pr_{ov,h}$ and the corresponding D_{1h} yielding it are lower than the respective ones for the configuration of Fig 1.

From these results, the system of Fig 1 is considered to be useful for obtaining high Pr_{ov} and high x_{Sov} compared with the other cases. Therefore the values of $Pr_{ov,h}$ in this type were examined at other bleed ratios for various combinations of f and α. Figure 4 gives results showing the combination values of f and α giving $Pr_{ov,h}$ attainable when x_{Sov} is fixed at 95 and 99%, changing f, α and D_1 at a constant $B_1 (= B_2)$. The subscripts 95 and 99 indicate the percentage x_{Sov}. As shown by the solid lines, the combination values of f and α depend on B_1 and x_{Sov}. In the ranges of $B_1 = 0.026$–1 for 95% and $B_1 = 0.1$–1 for 99%, the values of f decrease gradually with decreasing B_1, and are greater for a lower x_{Sov} at a constant B_1. Such

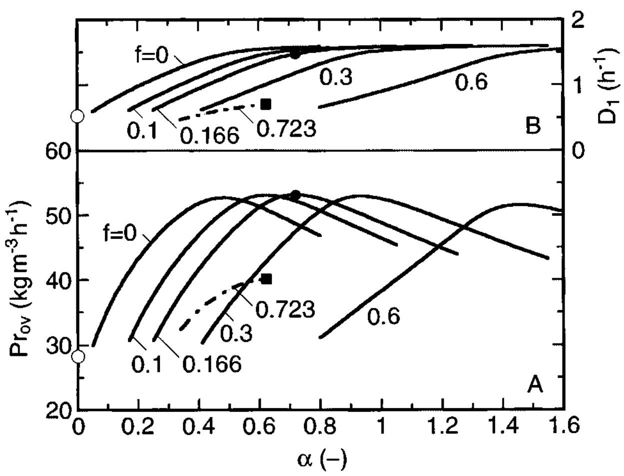

Figure 3. Effect of α on Pr_{ov} (A) and D_1 (B) yielding it with f as a parameter when x_{Sov} is fixed at 95%. $B_1 = B_2 = 0.1$ and $S_{f1} = S_{f2} = 47.4$ kg m^{-3}. Lines indicate the same configurations as in Fig 2. Closed circles and squares indicate the highest $Pr_{ov,m}$. Open circles refer to the single-stage system.

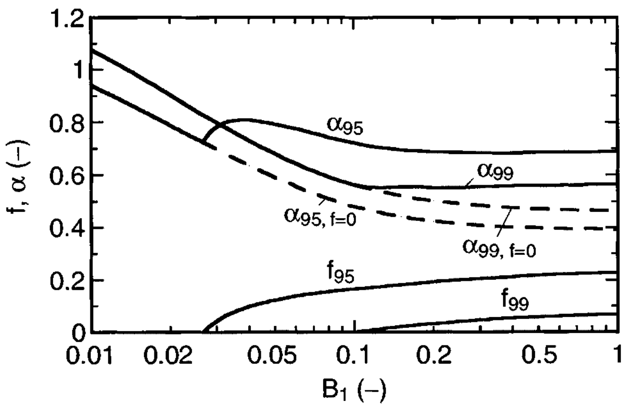

Figure 4. Combination values of f and α giving $Pr_{ov,h}$ attainable when x_{Sov} is fixed at 95 or 99% changing D_1, f and α at a constant B_1 in Fig 1 operation. $B_2 = B_1$ and $S_{f1} = S_{f2} = 47.4$ kg m^{-3}. Dashed lines indicate α for $Pr_{ov,m}$ when $f = 0$. Subscripts 95 and 99 indicate percentage substrate conversions.

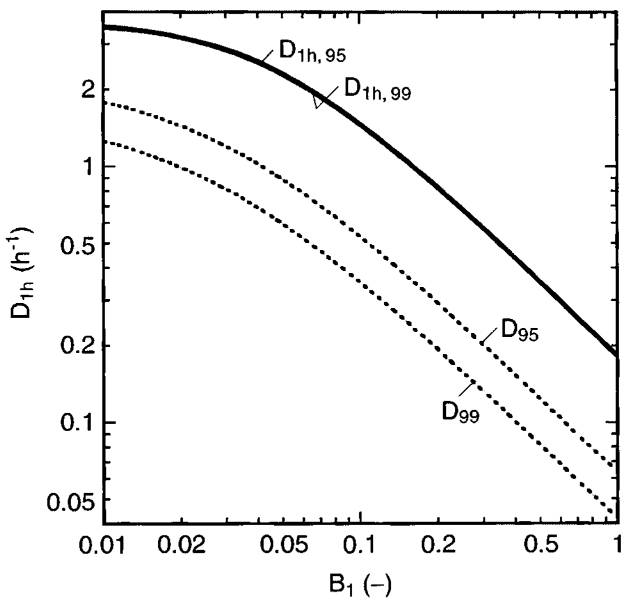

Figure 5. D_{1h} as a function of B_1, giving $Pr_{ov,h}$ when $x_{Sov} = 95$ or 99% with f and α given in Fig 4 at a constant B_1. $B_2 = B_1$ and $S_{f1} = S_{f2} = 47.4$ kg m^{-3}. Lines and subscripts indicate the same configurations and percentages as in Figs 2 and 4.

changes in these ranges are roughly the same for the values of α except those when $B_1 = 0.035$–0.3 for 95%. When $B_1 < 0.026$ for 95% and $B_1 < 0.1$ for 99%, $f = 0$, while α increases with decreasing B_1. The dashed lines indicate the values of α for $Pr_{ov,m}$ when $f = 0$, which increase gradually with a decrease in B_1, and are larger for a higher x_{Sov}.

The results presented in Fig 5 show the values of D_{1h} as a function of B_1 for yielding $Pr_{ov,h}$ with f and α as in Fig 4 for 95 and 99% substrate conversions. With decreasing B_1, the values of D_{1h} increase roughly linearly to ${B_1}^{-1}$ and then gradually, as shown by the solid lines. These values of D_{1h} for 99% are very close to those for 95%. Also, the values of D_{1m} when $f = 0$ are almost the same as those of the corresponding D_{1h}. These values for both conversions are greater with factors of 2.0–2.9 times for 95% and 2.7–4.3

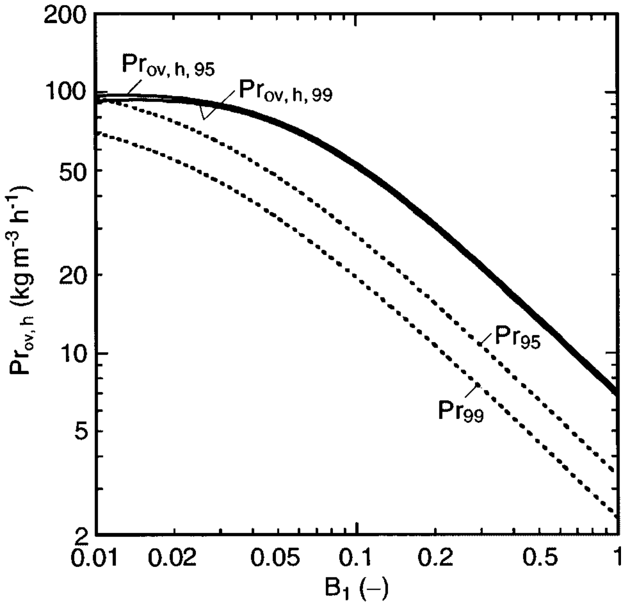

Figure 6. Relationship between $Pr_{ov,h}$ and B_1 when $x_{Sov} = 95$ or 99% with f and α in Fig 4 and D_{1h} in Fig 5 at a constant B_1. $B_2 = B_1$ and $S_{f1} = S_{f2} = 47.4\,kg\,m^{-3}$. Lines and subscripts indicate the same configurations and percentages as in Figs 2 and 4.

times for 99% compared with those of D in the single-stage system at the same $B_1 (= B)$, which are depicted by the dotted lines.

Figure 6 presents the relationship between $Pr_{ov,h}$ and $B_1 (= B_2)$ when the values of f and α from Fig 4 and D_{1h} from Fig 5 are used for 95 and 99% x_{Sov} in the two-stage system of Fig 1. As shown by the solid lines, at a constant B_1, the values of $Pr_{ov,h}$ for 99% x_{Sov} are slightly less than those for 95% x_{Sov}. These $Pr_{ov,h}$ values are larger than the corresponding Pr values, as shown by the dotted lines, in the single-stage system at the same bleed ratios, especially for 99% substrate conversion. When B_1 becomes small, the values of $Pr_{ov,h}$ increase similarly in the above case of D_{1h} and then decrease through their maxima at $B_1 = 0.0126$ for 95% x_{Sov} and 0.0145 for 99% x_{Sov}, while the changes in Pr by B for 95 and 99% x_S in the single-stage system are similar to those in D by B in Fig 5. The maxima of $Pr_{ov,h}$ are about 97.1 and $93.2\,kg\,m^{-3}\,h^{-1}$, respectively. The values of the ratio of $Pr_{ov,h}$ to Pr at the same $B_1 (= B)$ in the range of 0.01–1 are 1.02–2.10 for 95% conversion and 1.32–2.96 for 99% conversion; they gradually decrease with decreasing bleed ratio. In the range of low bleed ratios, with decreasing bleed ratio, such gradual decreases of the increases in $Pr_{ov,h}$, D_{1h}, Pr and D and finally the appearance of the maxima in $Pr_{ov,h}$ seem to be caused by a strong influence of the increasing cell death rate, as seen in eqn (2). When B_1 is about 0.04, the viable cell concentrations X_2 at D_{1h} for both conversions and at D_{1m} with $f = 0$ for 95% conversion are found to be higher than those at other B_1 values and are $2.9–3.0\,kg\,m^{-3}$. In this case, the ratio of $Pr_{ov,h}$ to the corresponding Pr takes values of 1.53 and 2.13 for 95 and 99% conversions, respectively.

Examining the ratios $(V_1 + V_2)/V$ calculated from Figs 4 and 5, their values in the range of $B_1 (= B) = 0.01–1$ are found to be 0.48–0.98 and 0.34–0.76 for 95% and 99% conversions, respectively. The shape of their changes with B_1 is similar to that found for α with $f = 0$ in Fig 4, and their values do not depend much on B_1 when $B_1 > 0.1$.

4 CONCLUSIONS

Using the experimentally-determined product-inhibition kinetic model,[2] the steady-state performance of a two-stage cell-recycle bioreactor having the configuration shown in Fig 1 was analyzed numerically for continuous acetic acid production. The effects of the operating parameters on overall acetic acid productivities and overall substrate conversions were examined. The values of the flow rate ratio, the fermenter volume ratio and the first-stage dilution rate which gave the highest acetic acid productivity attainable at high substrate conversions were determined, as shown in Figs 4 and 5. For the acetic acid productivity at the substrate conversion close to complete consumption, this two-stage system was found to be more efficient compared with the previous configuration[7] and the single-stage system at the same bleed ratios in the range of 0.01–1, as shown in Fig 6.

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