

# Investigation of Biological Reactor Designs for Treatment of Methanol and Thiodiglycol Waste Streams

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## ABSTRACT

Biological reactor designs for the degradation of the toxic compounds methanol and thiodiglycol are compared to determine the smallest volume. Both compounds exhibit substrate-inhibited cell growth behavior. Design equations were used to simulate a continuous stirred tank with cell recycle, continuous stirred tanks in series, and an optimized repeated fed-batch reactor. Thiodiglycol is the primary hydrolysis product of sulfur mustard (2,2'-dichlorodiethyl sulfide), commonly referred to as "mustard gas." Experimental data for the growth of *Alcaligenes xylosoxidans xylosoxidans* (SH42) on thiodiglycol was fit by an Andrews type inhibition equation, while the data and model for the growth of methanol was taken from the literature. The simulation results indicate that the repeated fed-batch reactor leads to significant volume reduction compared to the other two reactors configurations.

**Index Entries:** Biodegradation; reactor design; substrate inhibition; thiodiglycol; repeated fed-batch.

## NOMENCLATURE

$D$ , dilution rate (l/h);  $F$ , substrate feed flow rate (L/h);  $F_{ave}$ , average substrate feed rate for fed-batch reactor (L/h);  $k_s$ , monod constant (mM/L or mg/L);  $k_i$ , inhibition constant (mM/L or mg/L);  $m$ , maintenance coefficient (l/h);  $r_s$ , rate of substrate utilization (mg/L h or mM/L

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h);  $r_x$ , rate of cell growth (mg/L h or mM/L h);  $s$ , substrate concentration (mg/L or mM/L);  $t$ , time (h);  $t_0$ , initial time (h);  $t_{\text{fill}}$ , time required for exponential fill (h);  $T_f$ , total processing time required for fed-batch reactor (h);  $V$ , reactor volume (L);  $V_{\text{max}}$ , maximum volume for fed-batch reactor (L);  $V_0$ , optimum drawdown volume (V);  $V_i$ , volume to which rapid fill is terminated (V);  $x$ , cell mass concentration (mg/L or OD);  $x_i$ , cell concentration during exponential phase of cell growth (mg/L or OD);  $x_f$ , final cell concentration (mg/L or OD);  $Y_{x/s}$ , constant yield (mg cells/mg methanol or OD cells/mM/L thiodiglycol);  $Y_{x/s}(s)$ , variable yield (mg cells/mg methanol or OD cells/mM/L thiodiglycol).

## Greek Letters

$\alpha$ , recycle ratio based on flow rates (dimensionless);  $\beta$ , ratio of cell concentration in the cell recycle stream to the cell concentration in the feed stream (dimensionless);  $\gamma$ , death rate (1/h);  $\mu$ , specific cell growth rate (1/h);  $\mu_{\text{max}}$ , maximum specific cell growth rate (1/h);  $\delta(t)$ , impulse function for rapid fill (1/hr).

## INTRODUCTION

Bioremediation or biodegradation, the use of microorganisms for degrading organic pollutants into harmless substances, is one of the most promising innovative technologies for waste treatment (1,2). In many cases, bioremediation is the most cost-effective and efficient method of treatment for toxic wastes (1). For example, incineration often costs \$250–\$500/t of waste, whereas biological methods of treatment can cost as little as \$40–\$70/t (1). In addition to attractive cost-effectiveness, another significant advantage of bioremediation compared to other conventional processes is that of minimal environment impact and liability (1). Bioremediation is a natural process that has the potential of degrading toxic chemicals into innocuous substances, such as carbon dioxide, water, and fatty acids, on completion of the process (2).

Biological reactor designs for the biodegradation of the toxic compounds methanol and thiodiglycol are compared to determine the smallest reactor volume. Reactor volume is directly related to the cost of the process. The design equations for biodegradation of toxic compounds with substrate-inhibited growth behavior are formulated. These equations are then used to compare the volume requirement of a continuous stirred-tank reactor (CSTR) with cell recycle (the conventional activated sludge process), with two stirred tanks in series configuration, and with a repeated fed-batch reactor. All three reactor types were applied to the biodegradation of thiodiglycol by *Alcaligenes xylosoxidans xylosoxidans* (SH42) by a simulation study. The biodegradation of this compound is the focus of a study occurring in our laboratory. Thiodiglycol is the primary hydrolysis product of sulfur mustard (2,2'-dichlorodiethyl sulfide), commonly referred to as "mustard

gas." Experimental growth and yield data are used for the thiodiglycol biodegradation analysis. These data were analyzed and found to exhibit substrate inhibition behavior. More extensive experiments are currently under way in our laboratory to determine model parameters that were not available from the original data. Data from the literature from prior studies by one of the authors are used for the methanol biodegradation analysis (3,4). Methanol studies are not currently a part of our work. The methanol simulations only involve a comparison of the CSTR with recycle with the two stirred tanks in series. These limited results are included to show a similar trend with respect to these reactor designs. The fed-batch analysis for methanol was not performed since their system is not of continued interest in our laboratory. However, a similar trend with respect to improvements by fed-batch operation would be expected, since the system also has substrate-inhibited growth.

## MODEL FORMULATION

### Kinetic Cell Growth Model

*A. xylosoxidans xylosoxidans* (SH42) and *Methylobacter* (L3) exhibit inhibitory cell growth using the substrates thiodiglycol and methanol, respectively, as a nutrient source present in high concentrations. The kinetics of this cell growth phenomena are satisfactorily described by the Andrews' kinetic growth model for substrate inhibition. Substrate inhibition is the decline in specific growth rate at high substrate concentrations. Substrate inhibition occurs with many toxic compounds. The specific growth rate is therefore given as:

$$\mu = \mu_{\max} S / (K_s + S + (S^2 / K_i)) \quad (1)$$

where  $\mu_{\max}$  is the maximum specific growth rate,  $S$  is the concentration of substrate,  $K_s$  is the Monod constant, and  $K_i$  is the inhibition constant.

An equation developed by Pirt (5) that describes the variable yield as it varies with the specific cell growth rate, and therefore substrate concentration, is

$$Y_{x/s}(s) = (Y_{x/s}\mu / \mu + m) \quad (2)$$

where  $Y_{x/s}$  is the constant value for the yield and  $m$  is the maintenance coefficient. The kinetic growth parameters for methanol utilization by *Methylobacter* (L3) (3,4) are  $\mu_{\max} = 0.504$  l/h,  $K_s = 8.59$  mg/L,  $K_i = 24,630$  mg/L,  $Y_{x/s} = 0.383$  g cell/g methanol,  $m = 0.055$  l/h, and  $\gamma = 0.007$  l/h (estimated), where  $\gamma$  is the cell death rate. The kinetic growth parameters for thiodiglycol utilization for *A. xylosoxidans xylosoxidans* (SH42) are  $\mu_{\max} = 0.0576$  l/h,  $K_s = 37.56$  mM/L,  $Y_{x/s} = 0.125$  OD cells/mM/L thiodiglycol,  $K_i = 334$  mM/L,  $m = 0$  l/h (unknown), and  $\gamma = 0$  l/h (unknown). See the appendix for the data and analysis for these parameters.

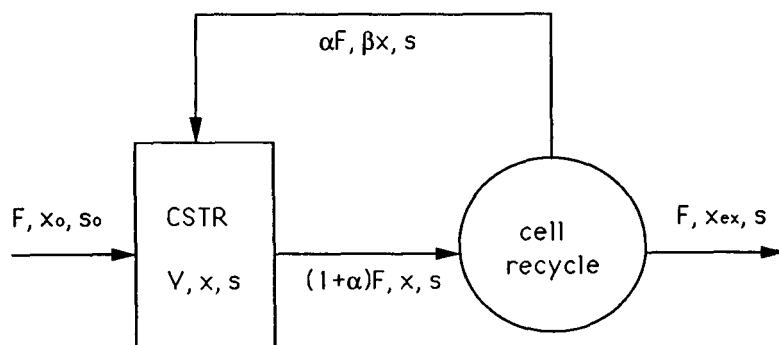


Fig. 1. Schematic diagram of a CSTR with cell recycle.

## CSTR with Cell Recycle

Processes involving microbial conversions are autocatalytic, i.e., the rate of conversion increases with cell concentration. In order to maintain the cell mass at a high concentration, cell can be recycled back to the reactor. By using a cell recycle, the rate of conversion can be increased. In addition, a cell recycle can increase the stability of the system by suppressing or minimizing the effects of disturbances. The cells in the effluent can be separated by centrifugation, filtration, or settling (6,7). The exit cell concentration,  $x_{ex}$ , of the cell recycle is given by  $x_{ex} = [1 - (\beta - 1)\alpha]x$ .

A flow diagram of a CSTR with cell recycle is illustrated in Fig. 1. A material balance on the cell concentration around the CSTR is given by the following equation:

$$Fx_0 + \alpha F\beta x - (1 + \alpha)Fx + V\mu x - V\gamma x = V(dx/dt) \quad (3)$$

where  $F$  is the process flow rate,  $x_0$  is the inlet cell concentration, which is set to zero in this analysis for sterile feed,  $\alpha$  is the recycle ratio based on flow rates,  $\beta$  is the ratio of cell concentration in the cell recycle stream to cell concentration in the reactor effluent system,  $x$  is the cell concentration of the reactor effluent,  $\gamma$  is the cell death rate, and  $V$  is the volume of the reactor.

At steady state,  $(dx/dt) = 0$ , the reactor volume is given by:

$$V = \{F[1 + \alpha(1 - \beta)] / \mu - \gamma\} \quad (4)$$

## Two CSTRs in Series

A flow diagram for two CSTRs in series is illustrated in Fig. 2. A material balance on cell concentration around the first CSTR is given by:

$$Fx_0 - Fx_1 + V_1\mu_1x_1 - V_1\gamma_1x_1 = V_1(dx_1/dt) \quad (5)$$

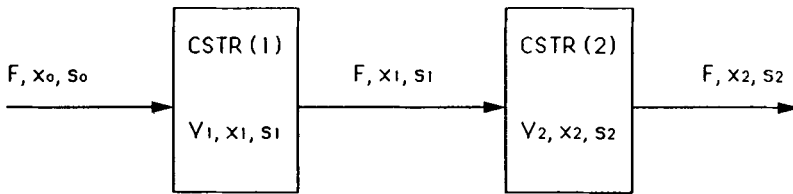


Fig. 2. Schematic diagram of two CSTRs in series.

where  $\mu_1$ ,  $x_1$ , and  $V_1$  are the specific growth rate, cell concentration, and reactor volume of the first reactor, respectively. A material balance on the substrate concentration around the first CSTR is given by:

$$Fs_0 - Fs_1 - V_1 (\mu_1 x_1 / Y_{x/s}) = V_1 (ds/dt) \quad (6)$$

where  $s_0$ , and  $s_1$  are the inlet substrate and substrate effluent concentrations of the first reactor, respectively. At steady state,  $(ds/dt) = 0$ , the following equations can be derived:

$$D_1 = (F/V_1) = \mu_1 - \gamma \quad (7)$$

$$x_1 = [D_1 Y_{x/s}(s_1)(s_0 - s_1) / \mu_1] \quad (8)$$

where  $D_1$  is the dilution rate. A material balance on the cell concentration around the second CSTR is given by:

$$Fx_1 + Fx_2 + V_2 \mu_2 x_2 - V_2 \gamma x_2 = V_2 (dx_2/dt) \quad (9)$$

where  $\mu_2$ ,  $x_2$ , and  $V_2$  are the specific growth rate, cell concentration, and volume of the second reactor, respectively. At steady state,  $(dx_2/dt) = 0$ , therefore:

$$x_2 = (D_2 x_1 / D_2 + \gamma - \mu_2) \quad (10)$$

where  $D_2$  is the dilution rate of the second reactor. A material balance on the substrate concentration around the second CSTR is given by:

$$Fs_1 - Fs_2 - V_2 (\mu_2 x_2 / Y_{x/s}) = V_2 (ds_2/dt) \quad (11)$$

where  $s_2$  is the substrate concentration in the second reactor. At steady state,  $(ds_2/dt) = 0$ , therefore:

$$x_2 = [D_2 Y_{x/s}(s_2)(s_1 - s_2) / \mu_2] \quad (12)$$

where  $D_2$  can be obtained by simultaneous solution of Eqs. (8), (10), and (12).

$$D_2 = (F/V) = [\mu_2 Y_{x/s}(s_1)(s_0 - s_1)(\mu_1 - \gamma) / \mu_1 Y_{x/s}(s_2)(s_1 - s_2)] \quad (13)$$

The combined volume of the two CSTRs in series is given by:

$$V_{\text{total}} = V_1 + V_2 = (F/D_1) + (F/D_2) \quad (14)$$

where  $D_1$  and  $D_2$  are given by Eqs. (7) and (13), respectively. The minimum total reactor volume is found by a numerical trial and error solution of these equations for the optimum  $s_1$ , denoted by  $s_1^m$ , when the effluent concentration,  $s_2$ , is fixed. In the special case where the maintenance coefficient,  $m$ , and the death rate,  $\gamma$ , are both zero, an analytical solution for  $s_1^m$  can be found. It is:

$$s_1 = \{k_s / [1 / k_i] + [\mu_{\max} / \mu_2(s_0 - s_2)]\}^{1/2} \quad (15)$$

### Fed-Batch Reactor

A repeated fed-batch reactor is a fed-batch culture in which the substrate or nutrients are continuously or semicontinuously added through the course of cultivation, and where the effluent is removed only at the end of the process and the cycle is then repeated (6). If the substrate feed limits the growth of microorganism, the substrate feeding policy can provide an effective means of controlling the growth and metabolic rate of the microorganism (8). Fed-batch operation has been determined to be particularly effective for controlling processes to achieve high cell concentrations, which subsequently result in higher production rates where phenomena, such as substrate inhibition, catabolite repression, auxotrophic mutation, and glucose effect, are significant (9). The fed-batch mode of operation has been successfully applied to the industrial manufacture of a wide range of biological products. These products include antibiotics, amino acids, enzymes, vitamins, single-cell proteins, biomass, and various other organic compounds of commercial importance (8).

Considering the fed-batch culture, the following equations represent cell concentration, substrate concentration, and the reactor volume, respectively:

$$(dx / dt) = r_x (Fx / V) \quad (16)$$

where  $x(0) = x_0$  and  $r_x = \mu x$

$$(ds / dt) = (F / V) (s_F - s) + r_s \quad (17)$$

$s(0) = s_0$ ,  $s_F > 0$  and  $r_s = - (r_x / Y_{x/s})$

$$(dV / dt) = F \quad (18)$$

where  $V(0) = V_0 > 0$  and where  $V$  is the reactor volume,  $x$  is the cell mass concentration,  $s$  is the substrate concentration,  $s_F$  is the substrate concentration in the feed stream,  $Y_{x/s}$  is the constant cell yield,  $F$  is flow rate of the substrate feed stream,  $r_x$  is the rate of cell growth,  $r_s$  is the rate of substrate utilization, and  $\mu$  is the specific growth rate. The following constraints are imposed on  $V$  and  $F$ , respectively:

$$0 < V_0 \leq V \leq V_{\max} \quad (19)$$

$$0 \leq F \leq F_{\max} \quad (20)$$

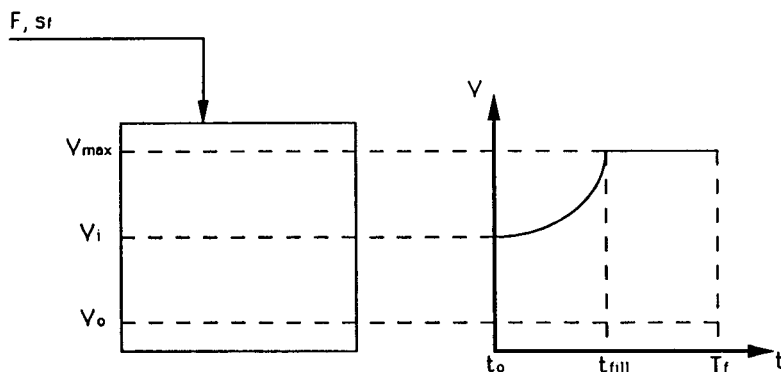


Fig. 3. Schematic diagram of a fed-batch reactor and volume profile for optimal substrate feeding involving initial rapid fill.

In this analysis, because of the nature of the slow growth of *A. xylosoxidans xylosoxidans* (SH42), the optimal substrate feeding policy of rapid fill previously developed will be used to determine the required volume for the fed-batch reactor (10). This previous work determined the optimal operation of a cycling or repeatedly fed-batch reactor by application of the maximum principle for the constant cell yield case. The substrate feeding policy was determined for the objective of maximum cell productivity that minimized the required processing time for a specified final cell concentration. In the feeding policy involving rapid fill, an assumption made is that even though there is a finite limit to the filling rate, the cell growth is so slow that a finite volume change can rapidly occur with essentially no cell growth. A finite volume change during a time period where essentially no cell growth occurs can be approximated mathematically by an impulse input on the cell growth time scale. The findings of the previous work (10) determined that the minimum processing time required involves an initial rapid fill to the maximum value of the specific growth rate, followed by an exponential filling portion in which the maximum specific growth rate is maintained until the reactor vessel is filled where batch concentration is then implemented until the desired final cell concentration is reached. The following relation, assuming constant yield, holds throughout the process:

$$x = Y_{x/s} (s_F - s) \quad (21)$$

The schematic diagram for a repeated fed-batch reactor and the associated filling policy is illustrated in Fig. 3. The filling policy consists of three phases. Starting from the initial volume  $V_0$  at  $t_0 = 0$ , an instantaneous input of substrate feed is initiated until  $V = V_i$

$$F = \Delta V \delta(t) = (V_i - V_0) \delta(t) \quad t = 0 \quad (22)$$

where  $\delta(t)$  is the form of the impulse function, and  $V_i$  is the volume that corresponds to the minimum processing time which is related to the optimum drawdown volume,  $V_0$ , by the following relation:

$$V_i x_i = V_0 x_f \quad (23)$$

where  $x_f$  is the final cell concentration, and  $x_i$  is the cell concentration during the exponential phase of cell growth, where the specific cell growth rate is a maximum for substrate inhibition.

When  $V_i$  is reached, the filling policy then becomes exponential in nature in which the cell concentration,  $x_i$ , held constant and the specific growth rate is at the maximum value. The substrate feeding rate is given by the following equation until the maximum volume  $V_{\max}$  is attained:

$$F = \mu(x_i)V(t) \quad 0 \leq t < t_{\text{fill}} \quad (24)$$

where  $t_{\text{fill}}$  is the time required to fill the reactor to  $V_{\max}$ . The required filling time is given by:

$$t_{\text{fill}} = [1 / \mu(x_i)] \ln [(V_{\max} x_i / V_0 x_f)] \quad (25)$$

where  $V_{\max}$  is given by the following equation:

$$V_{\max} = V_0 + \int_0^{t_{\text{fill}}} F(t) dt \quad (26)$$

On reaching  $V_{\max}$ , a batch mode of operation is implemented where the filling policy then becomes:

$$F = 0 \quad t_{\text{fill}} < t < T_f \quad (27)$$

until the desired final cell concentration,  $x_f$ , is reached. This cell concentration corresponds to the desired substrate effluent concentration through Eq. (21). The total processing time or cycle time required,  $T_f$ , is given by the following equation:

$$T_f = t_{\text{fill}} + t_{\text{batch}} = [1 / \mu(x_i)] \ln [(V_{\max} x_i / V_0 x_f)] + \int_{x_f}^{x_i} [dx / x \mu(x)] \quad (28)$$

On completion of the cycle, the reactor volume is then rapidly drawn down to the optimal drawdown volume,  $V_0$ , and the cycle is then repeated.

The optimal drawdown volume,  $V_0$ , that maximizes cell productivity is given by the following equation:

$$V_0 \{ \ln [(V_{\max} x_i / V_0 x_f)] + \mu(x_i) \int_{x_f}^{x_i} [dx / x \mu(x)] + 1 \} = V_{\max} \quad (29)$$

In this article, for the purpose of comparison with the CSTR with cell recycle and the two CSTRs in series configuration, a specified average substrate feed flow rate,  $F_{\text{ave}}$ , for the interval  $0 \leq t \leq T_f$  will be used for the fed-batch reactor where:

$$F_{\text{ave}} = [(V_{\max} - V_0) / T_f] = \{ V_{\max} [1 - (V_0 / V_{\max})] / T_f \} \quad (30)$$

It should be noted that under specified conditions, an exponentially fed-batch culture can be approximated by an extended continuous-flow



culture with a constant dilution rate (11). The volume requirement for the fed-batch reactor can then be found from Eq. (30) above

$$V_{\text{fed-batch}} = V_{\text{max}} = \{F_{\text{ave}}T_f / [1 - (V_0 / V_{\text{max}})]\} \quad (31)$$

## DISCUSSION

The design equations for each of the three reactor configurations—CSTR with cell recycle, two CSTRs in series, and the repeated fed-batch reactor—were used to compare their performance for the treatment of a thiodiglycol waste stream. The biodegradation of this compound is currently under study in our laboratory, and experimental comparison of these configurations are currently under way. Since methanol degradation is currently not part of our studies, a more limited simulation study was performed. The basis of the comparison was the required volume that was necessary to process a specified substrate feed flow rate of  $F = 50$  L/h with specified influent and effluent concentrations of the waste stream. Using reactor volume as the major basis of comparison is important because of the direct economic implications. However, other factors, such as feasibility, are also important when comparing reactor configurations for toxic waste treatment. The recycle ratio,  $\alpha$ , was set at 0.25, which is the normal optimum, and the recycle concentration ratio,  $\beta$ , was set high at 4. Experimental growth and yield data were used to model the biodegradation of thiodiglycol by *A. xylosoxidans xylosoxidans* (SH42). Data from the literature were used to model the biodegradation of methanol by *Methylomonas* (L3) (3,4). Substrate inhibition is found to occur with the utilization of these compounds by both of the microorganisms used. Substrate inhibition quite often occurs with toxic compound utilization.

For the methanol case, the fed-batch reactor analysis was not applied. The findings indicate that the two stirred tanks in series required a lower volume than the conventional CSTR with cell recycle configuration. These results are included in Tables 1–3 and Fig. 4. There is an increase in this trend of lower volume for the series configuration as compared to the CSTR with cell recycle configuration as the effluent methanol concentration is decreased and the influent methanol concentration is increased as can be seen in Fig. 4. Selected values for the trends in Fig. 4 are shown in Tables 1–3.

For the thiodiglycol case, the findings indicate that the fed-batch reactor has the advantage of lower reactor volume over both the two CSTRs in series and CSTR with cell recycle configurations. These results are included in Tables 4–6, and Figs. 5–7. In comparison to the CSTR with recycle configuration, an increase in the influent and a decrease in the effluent concentrations enhances the superiority of the fed-batch reactor design, i.e., lower volume required, as can be seen from Fig. 5. Compared

Table 1  
Volume (L) Comparison for an Effluent  
Methanol Concentration of 0.5 mg/L

Influent, mg/L	Recycle	Series	Recycle
			Series
100	603.2	268	2.3
200	603.2	216.3	2.8
400	603.2	181.1	3.3
600	603.2	165.9	3.6
1000	603.2	150.9	4.0
1500	603.2	141.5	4.3
2000	603.2	136	4.4

Table 2  
Volume (L) Comparison for an Effluent  
Methanol Concentration of 0.218 mg/L

Influent, mg/L	Recycle	Series	Recycle
			Series
100	2283	288.4	7.9
200	2283	229.8	9.9
400	2283	190.3	12
600	2283	173.2	13.2
1000	2283	156.4	14.6
1500	2283	146.0	15.6
2000	2283	139.8	16.3

Table 3  
Volume (L) Comparison for an Effluent  
Methanol Concentration of 0.15 mg/L

Influent, mg/L	Recycle	Series	Recycle
			Series
100	7576	294.6	25.7
200	7576	233.9	32.4
400	7576	193.1	39.2
600	7576	175.5	43.2
1000	7576	158.1	47.9
1500	7576	147.4	51.4
2000	7576	141	53.7

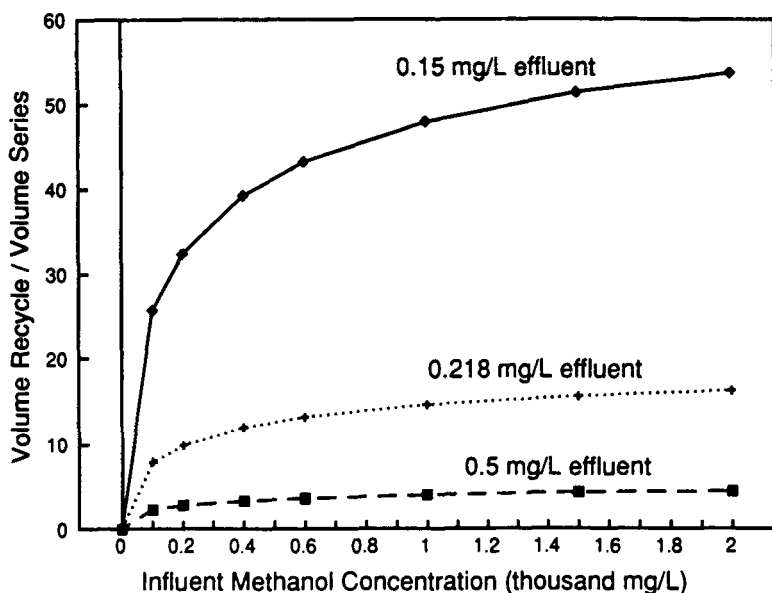


Fig. 4. Effect of methanol influent and effluent concentration on methanol volume ratio.

Table 4  
Volume (L) Comparison for an Effluent Thiodiglycol Concentration of 1 mM/L

Influent, mM/L				Recycle	Series	Recycle
	Recycle	Series	Fed-batch	Series	Fed-batch	Fed-batch
150	8368	6087	4571	1.37	1.71	2.34
300	8368	4621	2700	1.81	1.71	3.09
600	8368	3573	2222	2.34	1.61	3.77

Table 5  
Volume (L) Comparison for an Effluent Thiodiglycol Concentration of 0.1 mM/L

Influent, mM/L				Recycle	Series	Recycle
	Recycle	Series	Fed-batch	Series	Fed-batch	Fed-batch
150	81,727	17,524	4328	4.66	4.05	18.88
300	81,727	12,696	3160	6.44	4.02	25.86
600	81,727	9263	2509	8.82	3.69	32.57

Table 6  
Volume (L) Comparison for an Effluent Thiodiglycol Concentration of 0.01 mM/L

Influent, mM/L				Recycle	Series	Recycle
	Recycle	Series	Fed-batch	Series	Fed-batch	Fed-batch
150	815,321	53,905	5029	15.12	10.72	162.12
300	815,321	38,417	3576	21.22	10.74	227.99
600	815,321	27,445	2764	29.71	9.92	294.97

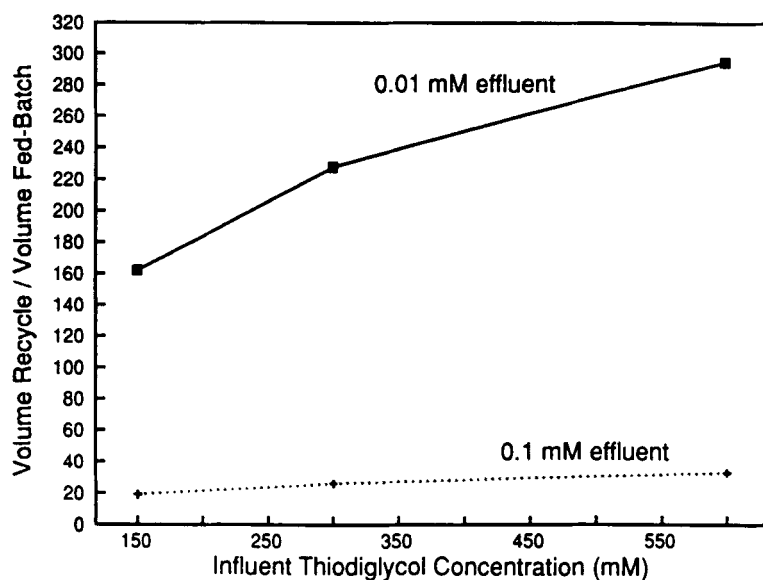


Fig. 5. Thioglycol volume ratio (recycle/fed-batch) vs influent and effluent concentration.

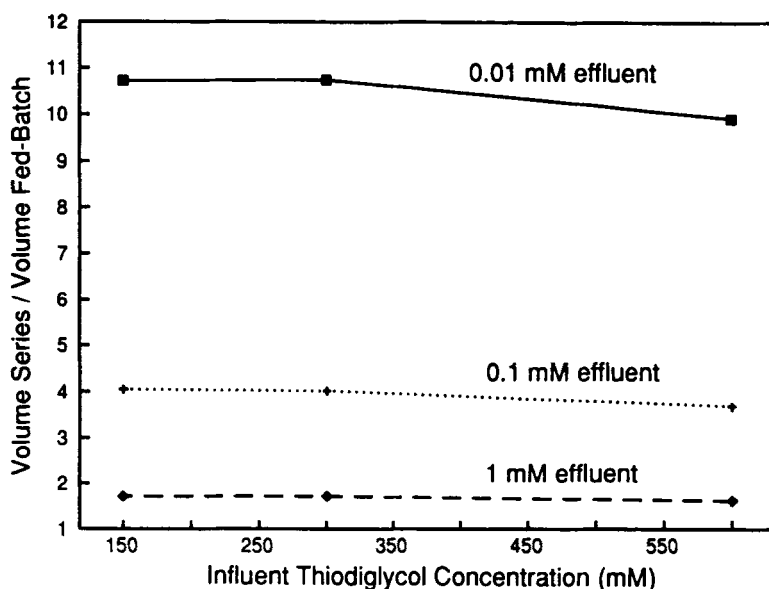


Fig. 6. Thioglycol volume ratio (series/fed-batch) vs influent and effluent concentration.

to the two CSTRs in series configuration, a decrease in the effluent and a decrease in the influent concentrations lead to an advantage for the fed-batch reactor design as can be seen in Fig. 6. As can be seen from Figs. 5 and 6, the fed-batch reactor design has a clear advantage over both configurations. In addition, the same trend that was found in the methanol case is found in the thioglycol case. Figure 7 shows that the two CSTRs

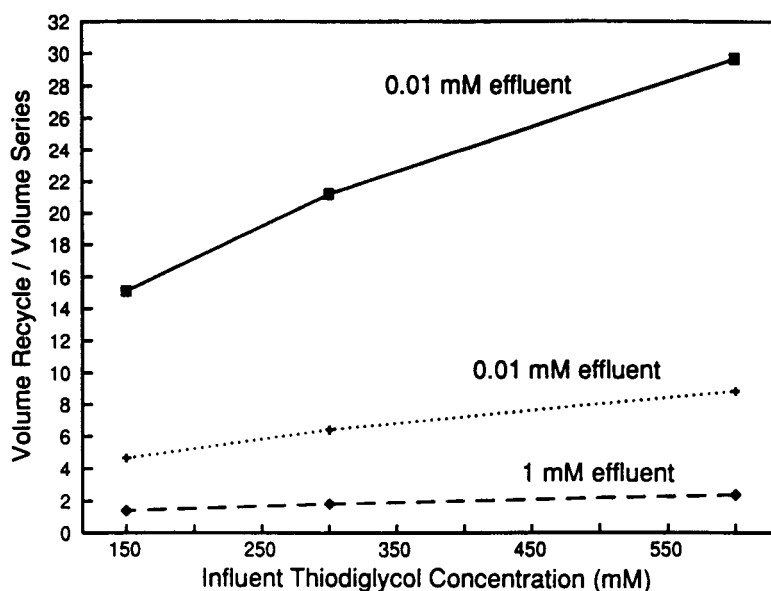


Fig. 7. Thiodiglycol volume ratio vs influent and effluent concentration.

in series have the advantage of lower volume with high-influent and low-effluent concentrations compared to the CSTR with cell recycle configuration. Selected numerical values for the trends shown in Figs. 5-7 are presented in Tables 4-6.

The superiority of the fed-batch reactor can be explained in that this design offers an optimal substrate feeding policy that the other reactor configurations lack and that can offer better control for the process. The concentration of the substrate can be more effectively controlled, i.e., maintained at the optimum value that keeps the specific growth rate at the maximum value, to increase culture productivity, therefore making this process much more efficient than the other configurations. This increased efficiency is reflected in the fed-batch reactor volume. Of course, the quantitative behavior found in the plots depends on the particular kinetics of the system under study. However, the qualitative trends as shown in these plots, as related to influent and effluent concentrations and the kinetic parameters, will be valid in general as long as substrate inhibition kinetics apply. Therefore, if kinetic experiments currently under way in our laboratory determine that maintenance and death terms cannot be ignored, we still expect the same trend for fed-batch.

## CONCLUSIONS

The findings indicate that the fed-batch reactor requires a significantly smaller volume in most cases of practical interest for the thiodiglycol biodegradation case. For example, for an influent thiodiglycol concentration

of 300 mM/L and a feed rate of 50 l/h, with a 0.1 mM/L effluent, the stirred-tank reactor with cell recycle requires a volume of 81,727 L, the two stirred tanks in series configuration require a total volume of 12,696 L, and the repeated fed-batch reactor requires a volume of 3160 L. In addition, the findings indicate that for the methanol case, for which the fed-batch reactor analysis was not applied, the two continuous stirred tanks in series configuration requires a smaller volume in most cases of practical interest. For example, for an influent methanol concentration of 200 mg/L with 0.15 mg/mL effluent, the CSTR with cell recycle requires a volume of 7576 L, whereas the two continuous stirred tanks in series configuration requires a volume of 236 L.

Currently, the kinetics of thiodiglycol degradation are under study in our laboratory in order to obtain an accurate growth model over a broad range of substrate concentrations. Also, experimental comparisons of these three reactor types are under way for thiodiglycol.

The simulated reduction in reactor volume for the fed-batch reactor case appears to justify the more complicated operating procedures required. In addition, the suboptimal case of constant feed rate fed-batch will be compared by both simulation and experiment. This last case has a simplified operating policy, but is expected to require some increase in reactor volume. The final choice of reactor type will consider both reactor volume and operational requirements.

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## REFERENCES

1. Thayer, A. M. (1991), *Chem. Eng. News* **69**(34), 23–44.
2. Hinchee, R. E. and Olfenbuttel, R. F. (1991), *On Site-Bioreclamation: Processes for Xenobiotic and Hydrocarbon Treatment*, Butterworth-Heinemann, Stonham, MA.
3. Hirt, W. E., Papoutsakis, E., Krug, E., Lim, H. C., and Tsao, G. T. (1978), *Appl. Environ. Microbiol.* **36**, 56–62.
4. Dibiaso, D., Lim, H. C., and Weigand, W. A. (1981), *AIChE J.* **27**, 284–291.
5. Pirt, S. I. (1965), *Proc. of Royal Soc., London*, series **B**, **163**, 224–231.
6. Shuler, M. L. and Kargi, F. (1992), *Bioprocess Engineering: Basic Concepts*, Prentice-Hall, Inc., Englewood Cliffs, NJ.
7. Bailey, J. E. and Ollis, D. F. (1986), *Biochemical Engineering Fundamentals*, 2nd ed., McGraw-Hill, Inc., New York.
8. Pirt, J. S. (1979), *Ann. NY Acad. Sci.* **326**, 119–125.
9. Parulekar, S. J. and Lim, H. C. (1985), *Biochem. Eng. Biotechnol.* **32**, 207–258.
10. Weigand, W. A. (1981), *Biotechnol. Bioeng.* **23**, 249–266.
11. Lim, H. C., Chen, B. J., and Creagan, C. C. (1977), *Biotechnol. Bioeng.* **19**, 425–433.

Table 7  
Thiodiglycol Raw Growth Data,  
OD vs Time at Different Initial Thiodiglycol Concentrations

Initial thiodiglycol concentration, mM/L					
Time, h	20	40	60	80	100
0	0.018	0.019	0.019	0.02	0.019
26.25	0.021	0.021	0.022	0.02	0.021
48.5	0.038	0.038	0.041	0.038	0.038
71.75	0.062	0.079	0.087	0.095	0.103
96.5	0.086	0.132	0.164	0.201	0.242
Calculated $\mu$ , $\text{h}^{-1}$	0.020094	0.026659	0.028953	0.033501	0.035634
Time	120	140	160	180	200
0	0.019	0.018	0.017	0.022	0.019
26.25	0.018	0.02	0.016	0.022	0.015
48.5	0.041	0.035	0.03	0.025	0.03
71.75	0.097	0.097	0.087	0.072	0.054
96.5	0.256	0.246	0.221	0.177	0.113
Calculated $\mu$ , $\text{h}^{-1}$	0.037729	0.036589	0.038253	0.031401	0.028397

## APPENDIX

Data for the growth of *A. xylosoxidans xylosoxidans* (SH42) on the waste compound thiodiglycol are shown below. These data consisted of the batch culture's changing optical density over time for different thiodiglycol initial concentrations (see Table 7). A yield coefficient was quoted as being 8 mM thiodiglycol consumed for every OD ( $Y = .125$  (OD/mM/L substrate)). It was assumed that cell mass density corresponded directly to optical density. Specific growth rate values at each substrate concentration level were determined by finding the slope of  $\ln(X/X_0)$  vs time. These values for specific growth are only good if the substrate concentration is constant during growth (7), a valid assumption given the low-yield factor, e.g., at 100 mM/L thiodiglycol, the concentration only changes 1.77 mM/L after 96.5 h.

The declining growth rate after 140 mM/L thiodiglycol indicates that the culture is inhibited by the substrate. Nonlinear regression was used to fit this curve to the Monod-like inhibition model (Eq. [1]). The coefficients obtained were;  $\mu_{\max} = .0576 \text{ h}^{-1}$ ,  $K_s = 37.56 \text{ mM/L}$ , and  $K_i = 334 \text{ mM/L}$ . Because of a lack of data, values for the death rate and maintenance coefficients,  $\gamma$ ,  $m$ , were set to zero.