BIOTECHNOLOGY PROCESSES

Scale-up and Mixing

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TABLE 1
Experimentally Determined Diffusivities of Some Fermentation Substrates and Products ($\times 10^6$ cm^2/s).

Type of experiment	Glucose	Lactose	$NH_4\ Cl$	Patulin	6-MSA
Cell-free beads	6.68	6.4	55	6.28	2.92
With dead Cells	6.68	6.0	45	5.03	1.95
With live Cells	7.58	7.2	-	-	-

$$(\frac{\partial C_b}{\partial r})_{r=R} = (\frac{2}{R}) \sum_1^\infty \psi_n(t) \qquad \text{(A-13)}$$

Substitution in Eqn. A-1 yields:

$$\varepsilon \frac{dC_L}{dt} = (\frac{F}{V})(C_{in} - C_L) - 6(1-\varepsilon)(\frac{D_{eff}}{R^2}) \sum_1^\infty \psi_n(t) \qquad \text{(A-14)}$$

Evaluation of the infinite summation $\sum_1^\infty \psi_n(t)$

Equation A-12 can be written as

$$(\frac{1}{k_n}) \frac{d\psi_n}{dt} + \psi_n(t) = (\frac{1}{k_n}) \frac{d\phi}{dt} \qquad \text{(A-15)}$$

In Eqn. A-15, the dynamics of the functions ψ_n's depend of the value of the coefficient $\tau = (1/k_n)$; small values of τ indicate that the functions $\psi_n(t)$ follow the forcing function $(1/k_n)(d\phi/dt)$. It is therefore reasonable to assume that for $\tau < \tau_0$, the ψ_n's can be evaluated using the quasi-steady state approximation

$$\psi_n(t) = (\frac{1}{k_n}) \frac{d\phi}{dt} \qquad \text{(A-16)}$$

or, using dimensionless quantities

$$\psi_n(t_R) = \frac{1}{\varepsilon D_R \pi^2 n^2} (\frac{d\phi}{dt_R}) \qquad \text{(A-17)}$$

The value of n_0 is determined as the one corresponding to τ_0:

$$\frac{1}{\varepsilon D_R \pi^2 (n_0^2)} = \tau_0$$

or

$$n_0 = (\frac{1}{\pi}) (\frac{1}{\varepsilon D_R \tau_0})^{0.5}$$

It was found by numerical experimentation, that for values of τ_0 less than 0.01, the resulting response curves, for given D_R were identical to five significant figures. Subsequently, a value of $\tau_0 = 0.01$ was used in all the runs.

After the above considerations, the infinite summation $\sum_1^\infty \psi_n(t)$ is estimated as follows:

$$\sum_1^\infty \psi_n(t_R) = \sum_1^{n_0} \psi_n(t_R) + \sum_{n_0}^\infty \psi_n(t_R)$$

$$= \sum_1^{n_0} \psi_n(t_R) + \frac{1}{\varepsilon D_R \pi^2} \frac{d\phi}{dt_R} \sum_{n_0}^\infty (\frac{1}{n})^2$$

and finally

$$\sum_1^\infty \psi_n(t_R) = \sum_1^{n_0} \psi_n(t_R) + \frac{1}{\varepsilon D_R \pi^2} (\frac{d\phi}{dt_R}) \left[\sum_1^\infty (\frac{1}{n})^2 - \sum_1^{n_0} (\frac{1}{n})^2 \right]$$

Using the property $\sum_1^\infty (\frac{1}{n})^2 = \frac{\pi^2}{6}$, and defining:

$$S_n = \sum_1^{n_0} (\frac{1}{n})^2 \quad \text{and} \quad S_l = \sum_1^\infty (\frac{1}{n})^2 = \frac{\pi^2}{6}$$

we obtain

$$\sum_1^\infty \psi_n(t_R) = \sum_1^{n_0} \psi_n(t_R) + \frac{1}{6 \varepsilon D_R} (\frac{d\phi}{dt_R}) (1 - \frac{S_n}{S_l}) \qquad \text{(A-18)}$$

The concentration at the surface, $\phi(t)$, can be expressed in terms of the bulk concentration $C_L(t)$ using the partition coefficient (β), namely $\phi(t_R) = C_L(t_R)/\beta$

Hence Eqn. A-18 becomes:

$$\sum_1^\infty \psi_n(t_R) = \sum_1^{n_0} \psi_n(t_R) + \frac{1}{6 \beta \varepsilon D_R} (\frac{dC_L}{dt_R}) (1 - \frac{S_n}{S_l}) \qquad \text{(A-19)}$$

Subsequent substitution Eqn. A-19 into A-14 yields:

$$\frac{dC_L}{dt_R} = \frac{C_{in} - C_L - 6\varepsilon_p(1-\varepsilon) D_R \sum_1^{n_0} \psi_n}{1 + \frac{\varepsilon_p(1-\varepsilon)}{\beta \varepsilon} (1 - \frac{S_n}{S_l})} \qquad \text{(A-20)}$$

The normalized form of Eqn. A-12 is:

$$\frac{d\psi_n}{dt_R} = -\varepsilon (n \pi)^2 D_R \psi_n + (\frac{1}{\beta}) (\frac{dC_L}{dt_R}),$$

$$n = 1,2,3,\ldots,n_0. \qquad \text{(A-21)}$$

Solution by numerical integration of Equations A-20 and A-21 yields the dynamic response of the reactor.

rotating through an arc of two inches in diameter at 250 RPM. The entire contents of the two flasks (approximately 1400 ml after incubation) were used to inoculate 1900 liters of a secondary vegetative stage having the following composition (mg/ml): soybean flour, 5.0; yeast extract (Difco Laboratories, Detroit, Michigan), 5.0; calcium gluconate, 10.0; KCl, 0.2; $MgSO_4 \cdot 7H_2O$, 0.2; $FeSO_4 \cdot 7H_2O$, 0.004; Sag 471 antifoam (Union Carbide, Danbury, Connecticut). The potassium, magnesium, and ferrous salts were prepared separately as follows: 7.6 g $FeSO_4 \cdot 7H_2O$ was dissolved in 76 ml of concentrated HCl. 380 g of $MgSO_4 \cdot 7H_2O$ and 380 g of KCl and deionized water were added to bring the total volume to 3800 ml. The inoculated medium was incubated 24 hours in a stainless steel vessel at 30°C. The vessel was aerated at 0.85 v/v/m and stirred with conventional agitators.

The mature secondary seed (8.33% v/v) was used to inoculate a production medium of the following composition (mg/ml): soybean flour, 22.0; $Fe(NH_4)_2SO_4 \cdot 6H_2O$, 0.66; glucose monohydrate, 8.25; Sag 471, 0.22; potato dextrin, 33.0; and molasses (blackstrap), 2.75.

Two types of stirred reactors were used. The smaller vessel, operated at 120 liters, was agitated with two conventional flat Rushton type impellers at relatively high power input. The larger vessel, operated at 4550 liters, was equipped with impellers having curved paddles and was operated at relatively low power input. Air flow in both reactors was supplied at 0.5 v/v/m by large open tubes which were estimated to contribute very little to the overall mixing. Respiration rates were estimated by difference in inlet and exhaust gas concentration via a Perkin-Elmer mass spectrometer. Distribution of A21978C factors was estimated by high performance liquid chromatography as described previously (2).

Examination of the batch fermentation medium suggested that the growth limiting nutrient was carbon in the form of carbohydrate. It was then hypothesized that in a fed-batch operation a moderately toxic substrate, such as decanoic acid, could be fed continuously to the fermentation if the metabolic consumption rate exceeded the addition rate.

Delivery of decanoic acid to the culture presented a problem. With a melting point of 34°C the compound is a solid at the fermentation temperature of 30°C, and the compound has very low solubility in water. In order to avoid the obvious problems arising in supplying a limiting nutrient as a solid phase, the substrate was dispensed to the stirred reactor as a five percent solution dissolved in a fifty percent ethanol/water mixture. There was an immediate response in oxygen uptake to the onset of the decanoic acid feed, as illustrated in Figure 2. Also, a significant improvement in LY146032 concentration was immediately realized (Table 2).

TABLE 2
Distribution of A21978C Factors with Decanoic Acid Feed[a]

A21978C Factor	Concentration µg/ml	% of Total A21978C Complex
C1	72	19.8
C2	109	29.9
C3	42	11.5
C5	19	5.2
LY146032	122	33.5
	364	

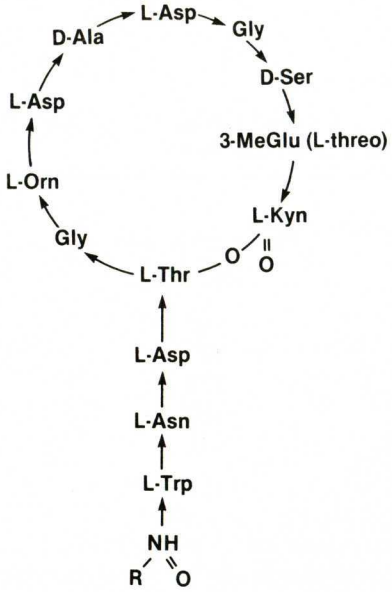
(a) N-decanoic acid/ethanol/water 1:2:2 fed 50 ml per hour to 120 L operating volume.

Material balances suggested that only a small portion of the decanoic acid that was fed could be accounted for by incorporation into the product. Thus, most of the fatty acid was apparently catabolized, presumably by the beta-oxidation pathway. In an attempt to increase the amount of decanoic acid available for the incorporation, the concentration of fatty acid in the

TABLE 3
Distribution of A21978C Factors with Increased Decanoic Acid Feed[a]

A21978C Factor	Concentration µg/ml	% of Total A21978C Complex
C1	131	10.4
C2	189	15.0
C3	107	8.5
C5	52	4.1
LY146032	784	62.1
	1263	

(a) N-decanoic acid/ethanol/water 1:2:2 fed 50 ml per hour to 120 L operating volume.



Factor	R
$A21978C_0$	C_{10} alkanoyl*
$A21978C_1$	8-methyl decanoyl
$A21978C_2$	10-methyl undecanoyl
$A21978C_3$	10-methyl dodecanoyl
$A21978C_4$	C_{12} alkanoyl †
$A21978C_5$	C_{13} alkanoyl †

* mixture of two isomers † structure uncertain

FIGURE 1. Naturally occurring A21978C factors.

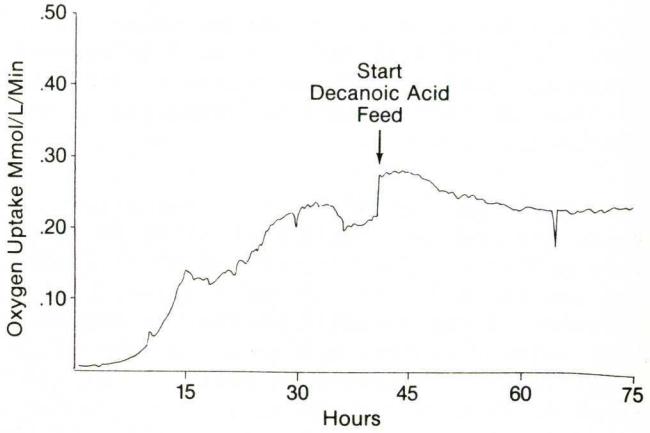


FIGURE 3. The toxic effect of overfeeding decanoic acid.

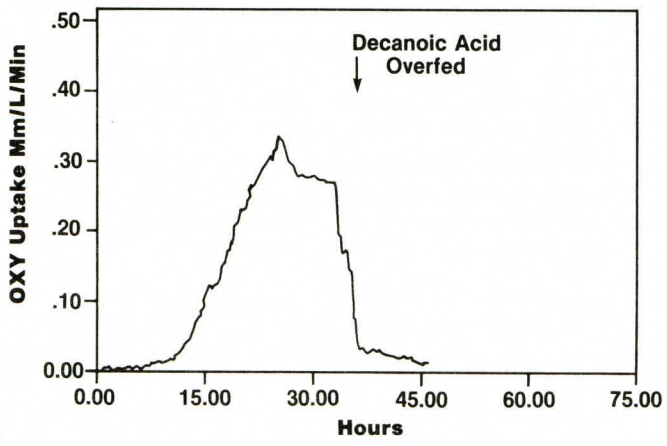


FIGURE 2. The effect of *n*-decanoic acid feed upon oxygen uptake.

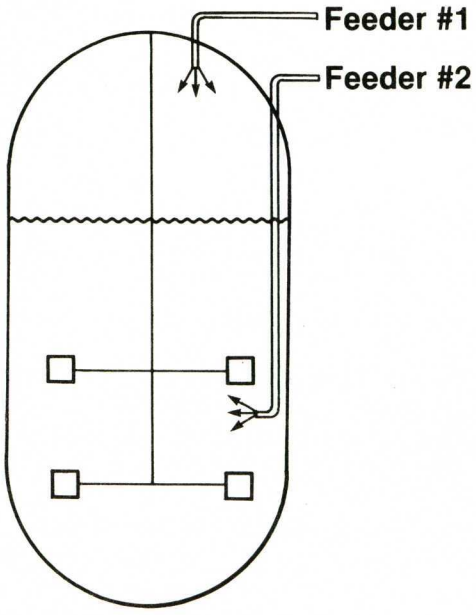


FIGURE 4. Feeding of *n*-decanoic acid/methyl oleate to a large reactor via two entry points.