DESIGN OF CONTINUOUS FLOW IMMOBILIZED BACTERIAL CELLS REACTOR FOR PRODUCTION OF L-MALIC ACID

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An experimental study was carried out, aimed at establishing an engineering data base for the optimum design of a pilot-plant fixed bed reactor for continuous biotransformation of natrium fumarate to L-malic acid catalysed by immobilized bacterial cells. Cells *Corynebacterium sp.* immobilized in calcium alginate were used for experiments. Effective reaction kinetics including the effect of transport phenomena in the bed was determined and conditions of the safe conversion data scale up were defined. Regarding the requirements of continuous production, long-term stability of the biocatalytic system was tested at operation conditions corresponding to constant degree of substrate conversion at the reactor outlet. Quantitative evidence on changes of the effective reaction kinetics (i.e. on the decrease of the effective rate constant value) over the three-months production period was obtained from this experimental run. Decisive design parameters of a pilot-plant reactor were calculated for estimated production of 20 t of L-malic acid per 90 days period (maximum time of economic biocatalyst utilization).

EXPERIMENTAL

The experimental set-up is shown schematically in Fig. 1. Three cylindrical glass-wall reactors with thermostated jacket were used for experiments. Internal diameters of reactors were 0·026, 0·048, and 0·108 m, the height of all reactors was constant and equaled 0·6 m. Reactor bottom was formed by sintered-glass plates (in reactors 0·026 and 0·047 m I.D.) or by a stainless-steel perforated plate (holes diameter 0·001 m) covered by stainless-steel micromesh screens. The reactors were operated in the downflow regime. Substrate was fed by a peristaltic pump to the reactor top and the reaction product was withdrawn from the bottom via the overflow (see Fig. 1) to keep sufficiently high substrate layer above the bed of particles. Such an arrrangement was proved to ensure uniform initial distribution of the substrate feed over the bed cross-section. The experiments were carried out with 1M aqueous solution of natrium fumarate at pH 7, reaction temperature was 37°C. Spherical particles of the biocatalyst were prepared from the mixture containing 15 wt. % (wet weight) of cells *Corynebacterium sp.*, 2 wt. % of natrium alginate and 83 wt. % of the physiological solution. The perfectly mixed suspension was then dropped by capillaries into the aqueous solution of calcium chloride (1 vol. %). Resulting spherical particles were left for one hour in the stirred $CaCl_2$ solution for curing purposes and then rinsed by the physiological solution. To increase their mechanical strength and stability, the particles were dried for 24 h at 37°C and subsequently activated by a detergent[1]. The size

of such prepared biocatalyst particles varied between 2 and 3·5 mm. Conversion of the natrium fumarate was determined by an UV analyzer[2,3] at the wavelength 254 nm and pH 8. The content of dicarboxylic acids in the product was determined by liquid chromatography, the chromatographic column was packed by Katex H^+ Ostion LKGS 0800. The substrate conversion at the reactor outlet, x, was then evaluated as a function of the volumetric rate of substrate feed related to a unit of biocatalyst mass, $\dot{V}_L/m$.

RESULTS AND DISCUSSION

The Effective Reaction Kinetics

Dependence of substrate conversion, x, on the reciprocal value of specific substrate feed rate (related to a unit of biocatalyst mass), $m/\dot{V}_L$, was investigated in reactors 0·026 and 0·048 m in diameter at values of fixed bed height and biocatalyst mass 0·50 m, 0·225 kg and 0·48 m, 0·68 kg, respectively. Linear dependence of experimental data (Fig. 2) proved that within the whole experimental range of substrate conversion ($x \leqq 0{\cdot}735$) the reaction was of the first order to substrate. Its reaction rate can be thus expressed[4] as

$$-\frac{\mathrm{d}c_A}{\mathrm{d}(m/\dot{V}_L)} = k_{ef} c_A \,, \qquad (1)$$

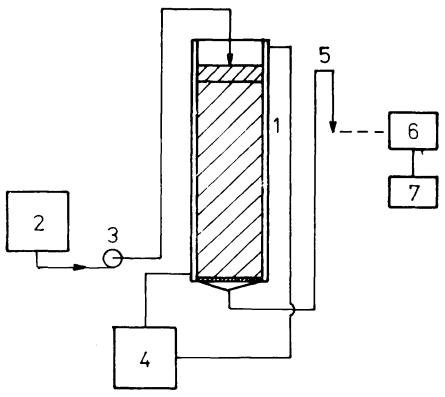

FIG. 1

Experimental set-up: 1 reactor, 2 substrate-storage vessel, 3 pump, 4 thermostat, 5 liquid overflow, 6 UV analyzer, 7 digital voltmeter

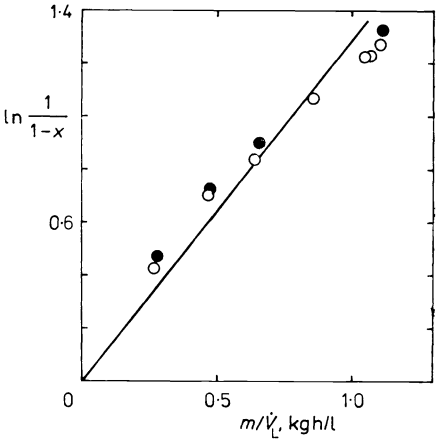

FIG. 2

Reaction kinetics at 37°C for fresh biocatalyst. ○ $D = 0{\cdot}026$ m, $H = 0{\cdot}50$ m; ● $D = 0{\cdot}048$ m, $H = 0{\cdot}48$ m; full line: data calculated for $k_{ef} = 1{\cdot}28$ l/kg h

or in terms of conversion ($x = 1 - c_A/c_A^0$)

$$\frac{dx}{d(m/\dot{V}_L)} = k_{ef}(1 - x) . \qquad (2)$$

Integration of the latter equation then yields

$$\ln (1/(1 - x)) = k_{ef}(m/\dot{V}_L) . \qquad (3)$$

The effective rate constant, k_{ef}, includes the effect of intraparticle transport and its value is thus characteristic for given particle size distribution. Experimental data yielded value $k_{ef} = 1{\cdot}28$ l/kg h. Formation of succinic acid was negligible within the whole range of substrate conversion studied. It can be assumed that its formation is completely suppressed during the long-term reactor performance.

According to literature sources[2,5], biotransformation of fumaric acid to L-malic acid catalysed by suspended or immobilized bacterial cells follows the Michaelis–Menten kinetics. Our kinetic data plotted in Fig. 2 have nevertheless proved that, for purposes of rector design, the effective reaction rate can be appropriately expressed by a simple power-law kinetic equation within the whole range of substrate conversion studied (at negligible succinic acid formation).

The Effect of Reactor Scale-Up

Dependences of substrate conversion on parameter $m/\dot{V}_L$ were determined in reactors 0·026, 0·048, and 0·108 m I.D. at different bed heights (i.e. for different biocatalyst mass). Corresponding values H/D varied between 1 and 19·2. Complete set of experimental data is given in Fig. 3. As can be seen from the figure, no appreciable conversion decrease occured at low bed heights (H/D values). It can thus be concluded that in downflow arrangement used (when sufficiently high substrate layer was kept above the particles bed) uniform liquid distribution over the bed cross-section was achieved in the whole region of experimental conditions. Apparently, this is in full agreement with analogical data obtained for the downflow arrangement in our previous study[6] devoted to the biotransformation of fumaric acid to L-aspartic acid catalysed by immobilized cells *Escherichia coli* entrapped in carageenan. It is however apparent from Fig. 3, that slightly higher conversion values were observed for larger relative bed heights and larger reactor diameters. Apparently these results can be ascribed to the additional substrate redistribution along the bed height and/or to decreasing importance of "wall-flow" contribution with increasing reactor diameter. It can be thus assumed that for the downflow arrangement studied, reactor scale up has favorable effect on uniformity of substrate distribution across the bed (i.e. on uniformity of particles wetting).

Long-Term Testing of Immobilized Cells Activity

Long-term activity of immobilized cells was tested in the reactor 0·026 m I.D. at bed height 0·5 m and substrate flowrate 0·210 l/h. Conversion values were evaluated during three-month period (92 days) and appropriate data were plotted in Fig. 4. Decrease of immobilized cells activity was clearly apparent during this experimental run and correspondingly conversion values decreased during the 92 days period from $x = 0{\cdot}706$ to $x = 0{\cdot}414$ (at constant experimental conditions). With respect to the long time scale, this decrease of cells activity can be considered to be linear. It has to be pointed out that mixing of the reactor content and its subsequent rinsing by fresh substrate always brought conversion increase (see arrow-heads in Fig. 4). Apparently, this phenomenon can be ascribed to the removal of impurities deposited on the particles surface (e.g. traces of Fe were clearly apparent on particles surface). At steady-state operation conditions corresponding to high substrate conversion demands, the lower part of particles bed remains in contact with substantially exhausted substrate (i.e. with concentrated reaction product) which also influences negatively cells activity.

The Effect of Long-Term Reactor Performance on the Effective Rate Constant

After the three-month performance period, reaction kinetics was determined in the reactor 0·026 m I.D. using the biocatalytic system tested. Experimental data are presented in Fig. 5 in comparison with those obtained at identical conditions for the fresh biocatalyst.

It is apparent that the dependence $\ln(1/(1 - x))$ vs $m/\dot{V}_L$ remained linear, corresponding thus to the first order reaction to substrate. Value of effective rate constant determined from the slope of the graph was 0·74 l/kg h. Such a value is thus by 42% lower than the effective rate constant obtained at identical conditions for the fresh biocatalyst which clearly corresponds to the substrate conversion decrease observed during the long-term testing of biocatalyst activity (see Fig. 4). Apparently, such a significant decrease of the reaction rate (i.e. large decrease of the cells activity) during the long-term reactor performance has to be taken into consideration in the process of production reactor design.

Pilot-Plant Reactor Design

Decisive design parameters of a pilot-plant reactor with production capacity 20 t of L-malic acid per 90 days of reactor performance were calculated for required substrate conversion, $x = 0{\cdot}7$. Standard activity and size distribution of the biocatalytic system was assumed, corresponding to conditions of our laboratory experiments, and constant value of packed bed density, $\varrho_c = 850\ \mathrm{kg/m^3}$, was used in calculations. Mass of the biocatalyst bed corresponding to given rate of substrate feed

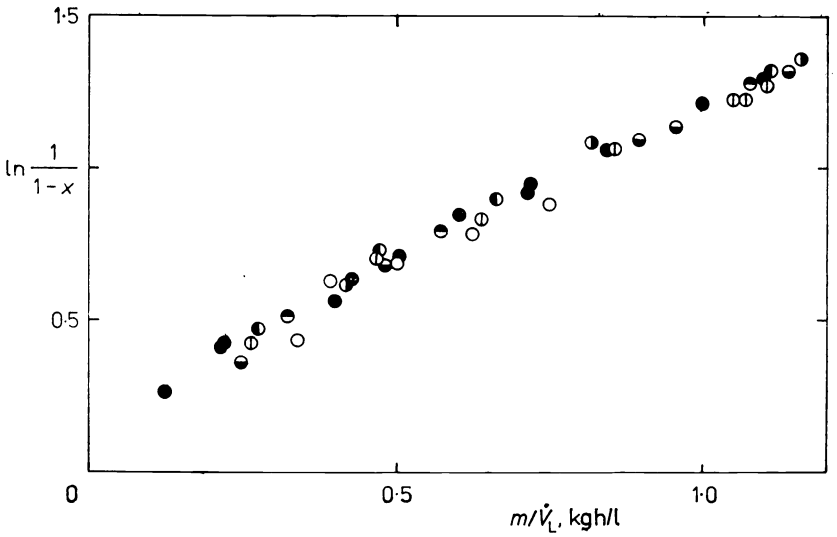

FIG. 3

Dependence of conversion data on packed-bed geometry. ○ $D = 0{\cdot}026$ m, $H = 0{\cdot}13$ m; ◒ $D = 0\ 048$ m, $H = 0\ 096$ m; ● $D = 0{\cdot}026$ m, $H = 0{\cdot}26$ m; ◓ $D = 0{\cdot}048$ m, $H = 0{\cdot}240$ m; ⦶ $D = 0{\cdot}026$ m, $H = 0{\cdot}50$ m; ◐ $D = 0{\cdot}048$ m, $H = 0{\cdot}480$ m; ◑ $D = 0{\cdot}108$ m, $H = 0{\cdot}108$ m

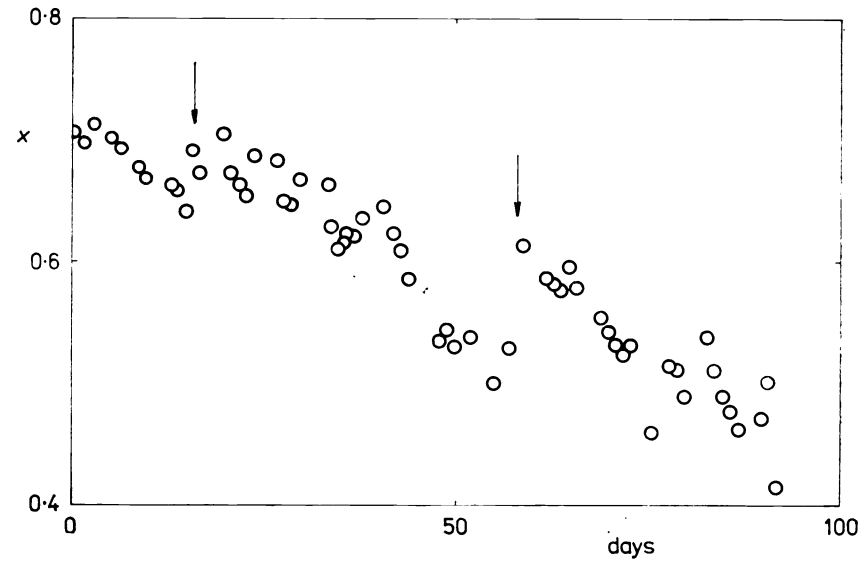

FIG. 4

Long-term activity of immobilized cells

and to the required substrate conversion was determined from the equation

$$m = \frac{\dot{V}_{L,av}}{k_{ef,av}} \cdot \ln\left(1/(1 - x)\right), \qquad (4)$$

where $k_{ef,av}$ represents value of the effective rate constant averaged over the whole production period. Appropriate substrate feed rates were 122·1 l/h and 75·3 l/h at the beginning of reactor performance (k_{ef} = 1·28 l/kg h) and after three-month performance (k_{ef} = 0·79 l/kg h) respectively, the average substrate feed rate used for calculation thus equaled to 98·7 l/h. Eq. (*4*) then yielded value m = 114·8 kg corresponding to the particles bed volume, V = 135·4 l or, assuming the application of commercially available glass cylinders 0·29 m in diameter, to the bed height 2·05 m. To avoid possible particles deformation which could occur in high beds, two reactors in series, with identical bed height 1·025 m, should be preferentially used instead of a single apparatus. Similarly, further increase of the reactor production capacity should be always achieved by the addition of another reactor modulus (i.e. by the increase of the series stages number) rather than by the increase of the total bed height in a single stage reactor. In cases when the operation time of a biocatalyst batch should be increased beyond the experimentally verified three-month period of biocatalyst efficiency (supposing that such prolonged reactor operation period would be economically justified), an appropriate average value of the rate constant for the whole operation period could be estimated from the extrapolation of linear decrease of biocatalyst activity observed in our experiments. The decrease of substrate feed rate corresponding in such cases to the biocatalyst desactivation is, however, generally limited by the minimum value of substrate flow rate, $(u_{oL})_{min}$ still ensuring uniform wetting of the particles bed[6,7]. As can be seen from experimental data plotted in Fig. 3, it has been proved that even the lowest flow rate used in experi-

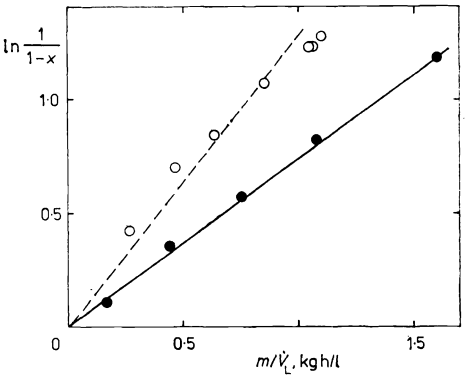

FIG. 5

Comparison of conversion data obtained at 37°C for fresh biocatalyst and for the same biocatalyst after 92 days of continuous reactor performance; D = 0·026 m, H = = 0·50 m; ○ fresh biocatalyst, ● biocatalyst after the long-term run; data calculated for k_{ef} = 1·28 l/kg h (dashed line) and k_{ef} = = 0·74 l/kg h (full line)

ments, $u_{oL} = 1{\cdot}83 \,.\, 10^{-5}$ m/s ($D = 0{\cdot}048$ m, $H = 0{\cdot}096$ m, $\dot{V}_L = 0{\cdot}119$ l/h), ensured uniform contact of substrate with biocatalyst particles, witnessed by the position of an appropriate experimental point on the linear dependence $\ln(1/(1 - x))$ vs $m/\dot{V}_L$. No conversion data were obtained in this work at lower substrate flow rates ($u_{oL} < 1{\cdot}83 \,.\, 10^{-5}$ m/s). The character of flow in this region (corresponding for given reactor configuration to conversion requirement $x > 0{\cdot}7$) and its possible impact on the reactor performance would thus have to be further investigated once the requirement of higher substrate conversion would arise.

SYMBOLS

A	reactor cross-section
c_A	concentration of natrium fumarate
c_A^o	concentration of natrium fumarate at the reactor inlet
d_p	particle diameter
D	reactor diameter
H	height of packed bed
k_{ef}	effective rate constant
$k_{ef,av}$	effective rate constant averaged over the whole production period
m	mass of particles in the bed
$\dot{V}_L$	volumetric substrate feed rate
$\dot{V}_{L,av}$	average value of the substrate feed rate
u_{oL}	$= \dot{V}_L/A$ substrate flow rate related to a unit of reactor cross-section
x	substrate conversion
ϱ_c	packed bed density

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