Comparison of MEK utilization and growth kinetics between batch and fixed-film processes

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ABSTACT. A methodology is described for measurement of immobilized and suspended cell growth and substrate utilization kinetic parameters. Substrate utilization and growth kinetics were compared between immobilized and suspended cells for a bacterial consortium acclimatized to methyl ethyl ketone. Biofilm specific growth rate was estimated based on a zero-order consumption assumption to determine the effect of immobilization on cellular growth. The effective diffusivity and depth of penetration of MEK in the biofilm was estimated to assess the effect of internal diffusion resistances within the biofilm. Finally, theoretical maximum O_2 requirements were calculated based on cellular substrate oxidation and experimentally determined MEK utilization rates for suspension and fixed-film cells.

1 INTRODUCTION

Few studies have explicitly compared the performance of attached and free-living organisms. Suspended organisms grow in easily controlled conditions (pH, nutrient availability, etc.) while attached ones are confronted to a number of constraints (immobilization, restricted nutrient availability, concentration gradients, etc.). This work aims to compare growth kinetics and substrate utilization between immobilized cells and cells growing in suspension culture as well as the substrate diffusion coefficients in both processes.

Previous work dealing with a novel bioreactor type was carried out by Agathos *et al*. (1997). This bioreactor was designed to operate in almost total absence of water in order to favor better mass transfer. It was equipped with a venturi-type atomizer for independent input and control of the substrate gas and of an aqueous nutritive medium in the form of a thin mist. Such a mist contained an extremely low liquid volume (less than 0.1 vol. %) of micro-droplets with a diameter less than 50 μm. The droplets small size led to an extremely large gas-liquid exchange area which was estimated to be within 60-200 m^2 per liter of liquid injected (Thalasso *et al*., 1993). Moreover, despite the relatively low specific exchange area of 27 m^2 $m^{-3}_{reactor}$, the removal of MEK was shown to be comparable to results reported by Deshusses *et al*. (1995) who studied the aerobic biodegradation of MEK vapors in a biofilter with an interfacial area of 150 m^2

m^{-3} and those of Kirchner *et al.* (1987) who used a trickle bed reactor to remove MEK with specific strains which were immobilized on activated carbon.

2 MATERIALS AND METHODS

2.1 Tubular biofilm bioreactor

Figure 1 shows the experimental set-up. Compressed air is passed through a nozzle at the top of the bioreactor in order to generate a mist of liquid medium whose fine drops do not exceed 50 µm in diameter. The pollutant containing air stream flows downwards and very rapid saturation of the mist by the solvent takes place owing to an exceptionally high gas-to-liquid mass transfer rates (Thalasso *et al.*, 1993).

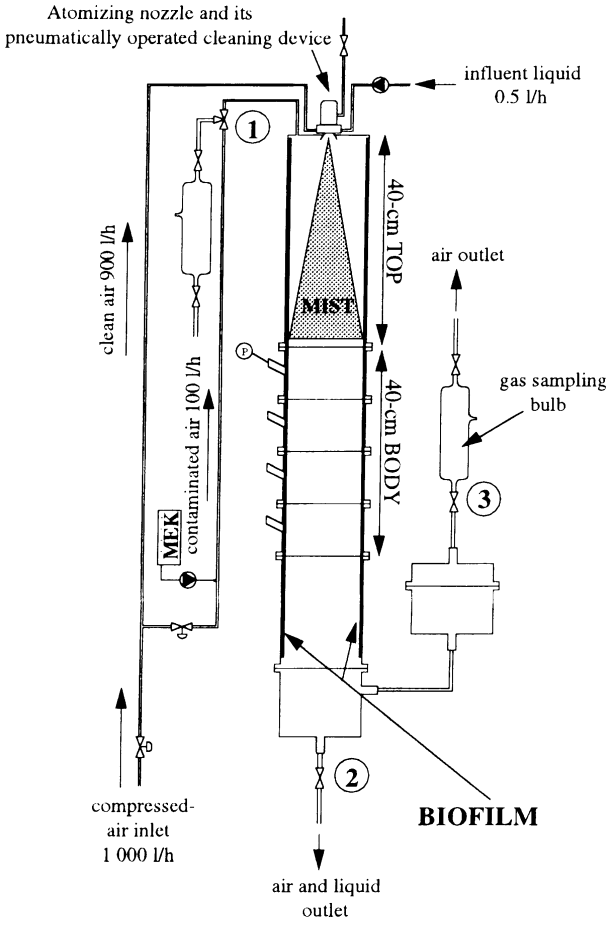

Figure 1. Experimental setup of the gas-phase bioreactor.

The bioreactor was constructed from Pyrex glass and was 1.12 m high and 14.7 cm in internal diameter. It included four 0.1 m high sections and a 0.4 m top linked together with tightening flanges and Teflon seals. The liquid atomizer used was fitted with an atomizing cone which functioned according to the external mixing principle.
Compressed air met the nutrient solution at the cone level producing a fine mist inside the bioreactor.

2.2 Gas feed
MEK was introduced with a precision feeding pump (Prominent Mikro gamma/5a 400150TT). MEK vaporized in a stainless steel tubing in which compressed air flowed at 100 l h^{-1}. This pollutant laden gas was introduced into the reactor and mixed with the air leaving the atomizer at 900 l h^{-1}. Therefore, 90 % of the total airflow rate was used to atomize the liquid solution and the remaining 10 % served to vaporize MEK.

2.3 Inoculum
The inoculum was taken from the bioreactor when methanol was previously tested as a model pollutant. It was constituted of *Alcaligens denitrificans*. It was then acclimatized to MEK in batch cultures in which the solvent concentrations of 300, 1000 and 3000 ppm w/v were successively applied. After the acclimation period, the bacterial consortium contained, apart from the dominant original strain of *Alcaligenes denitrificans*, three new bacterial strains representing 8% of total colonies on solid media, of which two were gram-negative rods and one was a gram-positive microorganism (Agathos *et al*., 1997).

2.4 Medium
The liquid medium was fed at the top of the reactor to give a mist at a flow rate of 0.5 l h^{-1} corresponding to a volumetric liquid flow rate of 0.632 m^3 $m_r^{-3}.d^{-1}$. It contained $Na_2HPO_4.2H_2O$ 6.9 g l^{-1}, KH_2PO_4 2.5 g l^{-1}, $(NH_4)_2$ SO_4 3.63 g l^{-1}, $MgSO_4.7H_2O$ 0.05 g l^{-1}, EDTA.2Na.$2H_2O$ 10 mg l^{-1}, $FeSO_4.7H_2O$ 5 mg l^{-1}, $MnCl_2.4H_2O$ 1.22 mg l^{-1}, $CoCl_2.6H_2O$ 0.4 mg l^{-1}, $ZnSO_4.7H_2O$ 0.25 mg l^{-1}, $CuSO_2.5H_2O$ 0.2 mg l^{-1}, $CaCl_2.2H_2O$ l mg l^{-1},$Na_2MoO_4.2H_2O$ 0.2 mg l^{-1}, biotine 0.02 mg l^{-1}, choline-Cl 1 µl l^{-1}, Ca-pentothenate 0.5 mg l^{-1}, inositol 2 mg l^{-1}, nicotinic acid 0.5 mg l^{-1}, pyridoxine-Cl 0.5 mg l^{-1}, thiamine-HCl 0.5 mg l^{-1}, p-aminobenzoïc acid 0.2 mg l^{-1}, cyanocobalamin 0.01 mg l^{-1} and folic acid 0.1 mg l^{-1}. The high phosphate concentration was used in order to neutralize the acidity resulting from the biological degradation of the solvent. The pH of the liquid was kept constant at a value of 7.3 even in the lower parts of the system due to the sufficient buffering capacity of the medium used.

2.5 Analysis
MEK concentrations in the influent and effluent gas phase were determined on a Delsi gas chromatograph provided with a flame ionization detector. A CP-Sil SCB capillary column of 25 m was used at 50°C with nitrogen as a carrier gas. Gas samples were taken into 0.5 l bulbs which were thermostatted at 35°C for 30 min. A gas-tight syringe was then used to inject a 500 µl gas sample into the chromatograph. The gas concentrations were derived from an external standard curve. The inlet and outlet gas flow rates were measured with gas flow meters (Schlumberger types 1 and G4).
Cell free aqueous sample (1 µl) was injected into an Intersmat gas chromatograph fitted with a Porapak Q-80-100 mesh column of 2 m at 185°C with nitrogen as a carrier gas. Ethanol served as an internal standard. Detection was with a flame ionization detector (FID). From the inlet gas phase and outlet gas and liquid phase concentrations, a

pollutant mass balance was carried out in order to determine the volumetric MEK degradation rate and the degree of conversion.

3. RESULTS

3.1 Measurement of growth kinetics in the essentially gas phase bioreactor

The rate of biofilm growth on the inner walls of the bioreactor can be established by setting a mass balance on the microorganisms present in the reactor. Assuming that film density is constant, i.e., that film mass is proportional to film volume, the rate of film growth is directly proportional to film volume (La Motta, 1976a) according to the following equation:

$$r_c = \mu V \quad \text{(i)}$$

The mass balance equation during the early stages of film growth, i.e., when erosion effects are still negligible, would be according to La Motta (La Motta, 1976b):

$$\frac{dV}{dt} = \mu V \quad \text{(ii)}$$

Integrating equation (ii) yields:

$$\ln\frac{V}{V_0} = \ln\frac{\delta}{\delta_0} = \mu t \quad \text{(iii)}$$

This phase is characterized by a logarithmic accumulation rate which continues until a "critical" film thickness is attained (Characklis *et al*., 1982). At the critical thickness, MEK removal reaches a steady-state maximum value and is not affected by additional film accumulation (Hoen and Ray, 1973). The logarithmic phase is then followed by a near-constant accumulation which is terminated when the shearing of the biofilm prevents further accumulation (Belkhadir *et al*., 1988; Characklis and Trulear, 1982). Kornegay and Andrews (1969) state that there exists an active layer of organisms at the film's surface that is responsible for all the nutrient removal that occurs. As pointed out in our previous study of biofilm composition evolution in the different stages of biofilm growth (Agathos *et al*., 1997), a fungal component was in part responsible of the pollutant degradation and would compensate the inhibition of the bacterial part following biofilm acidification preserving therefore the global bioreactor degradation performances. The biofilm formed between days 1 and 3 of a seven to nine-day detachment-colonization cycle was essentially bacterial and gave way from day 4 onwards to a film with an important fungal part that is more resistant to acidic conditions. The acidic conditions have, in fact, been attributed to the release of hydrogen ions from ammonium sulfate used in the nutritive medium following nitrogen consumption. The determination of the maximum biomass specific growth rate within the biofilm requires considering the evolution of biofilm thickness during the initial logarithmic biofilm growth phase comprised between days 1 and 3. This phase was characterized by an increasing degradation performance reaching its maximum on day 3 while only 32% of the wall surface was colonized by visible biofilm. Since experimental measurements of the biofilm growth are difficult to perform owing to a lack of growth heterogeneity on the reactor wall, we used measurements of the biological degradation activity to characterize the logarithmic phase (Characklis and Trulear, 1982).

According to La Motta (1976b), two stages occur during the growth cycle of a biological film. First, during the accumulation of the first layers of microorganisms, the intrinsic rate will be observed. Second, when a critical film thickness is exceeded, the substrate does not penetrate to the inner boundary. Under these conditions, the intrinsic rate is no longer observed and internal diffusion affects the rate of substrate removal. Supposing that the rate of reaction is equal to the rate of mass transfer across the film surface, the rate expressions are, in the case of complete substrate penetration (La Motta, 1976b; Bailey and Ollis, 1986):

$$r = Ak_v\delta \qquad \text{(iv)}$$

and in the case of incomplete substrate penetration :

$$r_p = A(2\,D_{eff}\,k_v)^{1/2}(C_1)^{1/2} \qquad \text{(v)}$$

According to equation (iv), the depth of substrate penetration inside the biofilm in the case of zero order kinetics can be written as follows:

$$\delta = \frac{r}{Ak_v} \qquad \text{(vi)}$$

Assuming that the microorganisms are present on the entire reactor wall even in the absence of a visible biofilm and that the cellular density stays constant during biofilm growth between days 1 and 3, equations (iii) and (vi) lead to the expression:

$$\ln\frac{\delta}{\delta_0} = \ln\frac{r}{r_0} = \mu t \qquad \text{(vii)}$$

The ratio of the average biofilm thicknesses on days 1 and 3 can therefore be evaluated through the use of the maximum degradation rates measured on those days assuming complete substrate penetration that would occur in the case of thin films, according to equation (vii).

3.2 Estimation of the effective diffusivity and depth of penetration of MEK in the biofilm.

The MEK diffusion coefficient can be derived from equation (v):

$$D_{eff} = \frac{r_p^2}{2A^2k_vC_1} \qquad \text{(viii)}$$

We have already pointed out that the fungal proliferation has been attributed to the biofilm acidification. Owing to the strong fungal development and the much bigger size of fungi as compared with bacteria, we could predict that the non bacterial volumetric fraction was predominant. Picologlou *et al.* (1980) have noted that the predominance of filamentous microorganisms leads to a decrease of biofilm density (< 40 $kg_{DW}\ m^{-3}$). It was therefore evident that the cellular mass concentration was higher during the initia1 so-called logarithmic phase of biofilm growth. The substrate diffusivity D_{eff} is based, among other things, on the mass concentration of cells according to the expression (Diks and Ottengraf, 1991):

$$k_v = \frac{\mu_{max}\,X_b}{Y} \qquad \text{(ix)}$$

Its value should therefore increase with the biofilm development. Since the value of X_b represented the total biofilm which sloughed at the end of the colonization-detachment cycle, we could therefore only estimate the MEK diffusivity corresponding to that biomass. Moreover, the mass balances carried out around the reactor indicated the absence of external diffusion limitation. At any MEK load, the ratio between the effluent gas and liquid concentrations was close to the Henry constant 5.10^{-3} determined experimentally at 35°C. It could therefore be easily derived from equation (viii) that the effective MEK diffusion coefficient amounted to:

$$D_{eff} = \frac{r_p^2 H_c}{2 A^2 k_v C_g} \quad \text{(x)}$$

Once the effective diffusitivities for MEK were computed for different loads (1.5, 3.5 and 5.5 $kg_{MEK}\ m_{reactor}^{-3}\ d^{-1}$), it was possible to estimate the penetration depths according to:

$$\delta_c = \left(\frac{2 D_{eff} C_l}{k_v} \right)^{1/2} \quad \text{(xi)}$$

In the absence of any data on the evolution of the biofilm composition with time and its influence on the biomass concentration, it was not possible to describe the time course variation of the biofilm density. The biofilm density that could be determined was representative of the biomass collected at the end of a cycle of colonization-detachment. It was therefore only possible to estimate the depth of substrate penetration on day 7, i.e., at the end of the cycle for the three loads applied if we assume that the active biomass was evenly distributed and that the biofilm density was uniform. If equation (v) is combined with equation (xi), the following result is obtained (La Motta, 1976b):

$$r_v = A k_v \delta_c \quad \text{(xii)}$$

This equation shows that when there is incomplete penetration, the observed rate depends on the magnitude of the depth of penetration and is independent of the total film thickness.

3.3 Comparison of external mass transfer and utilization rates by the biofilm

The MEK diffusivity at experimental conditions was estimated as $14\ 10^{-10}\ m^2\ s^{-1}$ using the Wilke-Chang correlation (La Grega *et al.*, 1994). The mass transfer coefficient for MEK within the liquid boundary layer can be obtained from:

$$k_l = \frac{D_l}{\delta_l} \quad \text{(xiii)}$$

The average liquid velocity on a planar surface is given by equation (xiv) (Giot, 1990) and can be applied to the case of a cylindrical wall if the thickness of the flowing liquid film is negligible compared to the radius of the tube:

$$\bar{u}_l = \frac{\rho_l g \delta_l^2 \cos\beta}{3\mu_l} \quad \text{(xiv)}$$

Considering a perfectly vertical wall ($\cos\beta = 1$) and that the liquid flow rate is related to the liquid film thickness and the perimeter of the reactor, we can write:

$$Q_l = \bar{u}\delta_l 1 = \frac{\rho_l g 1 \delta_l^3}{3\mu_l} \quad \text{(xv)}$$

Thalasso (1993) has shown that in the case of liquid and gas phases flowing co-currently in this novel type bioreactor, the effect of the gas on the thickness of the liquid film is negligible.

In order to define the type of mass-transfer regime and assuming steady state, we can make use of the Damköler number which is defined as the ratio of the maximum reaction rate to the maximum mass-transfer rate as follows (Bailey and Ollis, 1986):

$$Da = \frac{Vm}{k_l a\, C^*} \quad \text{(xvi)}$$

where C* is the liquid-phase concentration which is in equilibrium with the bulk gas phase.

3.4 Maximum O_2 and MEK utilization rates for suspension and fixed-film cells

Theoretical metabolic oxygen requirements by the suspension and immobilized cells were calculated based on cellular substrate oxidation stoichiometry utilizing theoretical cell yields and assuming complete methyl ethyl ketone oxidation.

4 DISCUSSION

The maximum volumetric MEK degradation rates obtained during a cycle of biofilm detachment-recolonization on days 1 and 3 were 1.2 and 3.5 $kgm^{-3}_{reactor}.d^{-1}$. These intrinsic rates allow calculating a specific biofilm growth rate of 0.0223 h^{-1}. This latter value corresponds to a biomass doubling mass of 31 hours. The ratio of the specific growth rates between suspended and fixed-film processes was about 7.2 since a value of 0.16 h^{-1} was obtained in batch cultures for an initial MEK concentration of 3 g/l.

The biomass concentration of the biofilm collected at the end of a seven-day cycle amounted to 46.1 $kg_{DW}\ m^{-3}_{biofilm}$. Indeed, a total wet biomass of 2.82 kg led to a dry weight of 130 g. Since a mass of 247 g of MEK was consumed during the cycle of detachment-colonization, a yield coefficient of 0.53 was derived. These data permit to compute a true rate of substrate uptake of 0.01 $g_{MEK}\ g^{-1}_{DW}\ h^{-1}$. In the case of suspension cultures, the cellular yield was equal to 0.43, while the maximum specific biodegradation rate in liquid batch experiments was 0.26 $g_{MEK}\ g^{-1}_{DW}\ h^{-1}$ for an initial MEK concentration of 3g l^{-1} (Agathos *et al*., 1997). It is evident that the biodegradation kinetics was profoundly altered upon attachment since the behavior of the microorganisms growing in suspension culture was not directly applicable for immobilized cell applications.

Table 1. Observed values of effective diffusivity and depth of penetration of MEK in the biofilm for three loads applied on day 7 of a detachment-recolonization cycle.

volumetric influent MEK load ($kgm^{-3}_{reactor}.d^{-1}$)	Influent gas-phase Concentration ($g\ m^{-3}$)	volumetric degradation rate ($kgm^{-3}_{reactor}.d^{-1}$)	Effective diffusivity (m^2s^{-1})	Depth of penetration (mm)
1.5	1.1	1.0	$8.39\ 10^{-10}$	0.82
3.5	2.5	1.6	$9.45\ 10^{-10}$	1.32
5.5	4.1	2.3	$11.91\ 10^{-10}$	1.90

The effective diffusitivities reported in Table 1 for three loads applied on day 7 of a cycle seem to be reasonable. If they are compared with the molecular diffusivity of MEK in water at the same temperature, i.e., $D_w = 14 \times 10^{-10}$ m^2 s^{-1}, they vary from 60% to 85% of the latter, a representative va1ue being 72.5% (average $D_{eff} = 10.15 \times 10^{-10}$ m^2 s^{-1}). These results agree with those of Characklis and Marshall (1990), Kornegay and Andrews (1969) and La Motta (1976b) who all note a decrease of diffusivities inside biofilms. On the basis of these effective diffusivities, the depths of substrate penetration on the last day of a seven-day colonization-detachment cycle were computed using equation (xi) (Table1). Since the maximum volumetric degradation rate obtained was equal to 3.5 kg_{MEK} $m_{reactor}^{-3}$ d^{-1} (Agathos *et al*., 1997), equation (xi) allows also computing a substrate depth of penetration of 2.89 mm for a total biofilm thickness of 5.5 mm obtained when a mass of 2.82 kg of wet biomass was collected. These results agree with those of Tomlinson and Snaddon (1966) who consider that thickness to be of the order of 2.1 mm in a biofilm composed essentially of *Geotrichum*. Such internal diffusion limitations will reduce the actual substrate concentrations inside the biofilm leading to a reduction of the specific growth rate within the immobilized cells.

As far as the batch cultures are concerned, it is unlikely that internal substrate limitations occurred within the suspension cells owing to their small diameter. Such limitations have been reported in the case of large floc particles (Matson and Characklis, 1976) but can be assumed to be absent in the reported suspended cell experiments.

The prediction of the external mass transfer required to compute the thickness of the liquid film layer which was estimated from Equation (xv) as 40 µm on the basis of a liquid flow rate of 0.5 l h^{-1}. Assuming a uniform distribution of the liquid we can deduce from equation (xiii) the value of k_l as $3.5\ 10^{-5}$ m s^{-1}. Therefore, it is possible to compute an overall $k_l a$ *e*stimate that is related to the bioreactor volume of $9.55\ 10^{-4}$ s^{-1}. The magnitude of the Damkoler number was 0.002 for MEK since a maximum volumetric degradation rate of 3.5 $kgm^{-3}{}_{reactor}.d^{-1}$ was obtained for a volumetric influent MEK load of 12.5 $kgm^{-3}{}_{reactor}.d^{-1}$. It must be noted that the treatment of the liquid layer as the unmixed liquid region associated with the biofilm confers to the latter a maximum value. It follows that the Damköler number determined according to equation (xvi) bears a maximum value. Thus, since Da was much smaller than unity, the maximum mass-transfer rate was much larger than the maximum rate of reaction. The mass transfer resistance was thus negligible and the system was controlled by the biological reaction rate. Similarly, we can assume that MEK concentration gradients in the bulk liquid within the batch reactors are minimized and that mass transfer resistances are small in this region owing to sufficient mixing of the broth (Doran, 1995).

The oxygen rates required for the observed substrate uptake were equal to 0.40 g O_2 g_{DW}^{-1} h^{-1} and 0.015 g O_2 g_{DW}^{-1} h^{-1} for suspension and immobilized cells respectively. These values were derived from the maximum observed cellular and fixed-film substrate utilization rates $Q_{s,max}$ = 0.26 g_{MEK} g_{DW}^{-1} h^{-1} and $r_{v,max}$ = 0.01 g_{MEK} $g^{-1}{}_{DW}$ h^{-1}.

As has been pointed earlier, the maximum elimination capacity of MEK in the dry tubular bioreactor was reported to be equal to 3.5 $kgm^{-3}{}_{reactor}.d^{-1}$. An estimation of the maximum elimination capacity of MEK in the tubular bioreactor was attempted as follows. If we consider the observed total dry biomass weight of 130 g over the whole surface of the bioreactor walls, then a dry mass of 6500 g_{DW} $m_{bioreactor}^{-3}$ active biomass contained in the bioreactor can be calculated. This value, when multiplied with the reported specific maximum biodegradation rate of 0.26 g_{MEK} $g^{-1}{}_{DW}$ h^{-1} should equal the maximum elimination capacity of the tubular bioreactor. Hence, the calculated

maximum elimination capacity for MEK is estimated to be 1690 g $m_{bioreactor}^{-3}$ h^{-1} or 40.5 kg $m_{bioreactor}^{-3}$ d^{-1}. This value does not reflect the observed maximum degradation rate in the bioreactor (3.5 kg m_r^{-3} d^{-1}). Such calculation shows the limits for the comparison of suspended growing biomass with immobilized cultures in the gas-phase bioreactor.

5 CONCLUSIONS

Growth and pollutant biodegradation characteristics as well as effective substrate diffusion coefficients in suspended and fixed-film processes were compared to find out whether biodegradation kinetics by suspended cultures could be related to the biodegradation kinetics in the gas-phase bioreactor. Results indicated the limits for such comparison. Immobilized cells exhibit a slower measured growth rate than suspension culture organisms. The maximum specific growth rate (μ_{max}) decreased by 6-fold upon immobilization. A parallel decrease by 26-fold of the experimental maximum MEK biodegradation rates was measured. Substrate diffusion limitations have been shown to occur within the biofilm at the end of a cycle of detachment-recolonization. High discrepancies relating to theoretical oxygen consumption and experimental MEK degradation rates were also observed between the two systems. Such calculation shows the limits for the comparison of suspended growing biomass with resting cultures in biofilters.

NOMENCLATURE

r_c is the rate of film accumulation (m^3 s^{-1})
μ is the specific film growth rate constant (s^{-1})
$V = A.\delta$ is the film volume (m^3), δ is the film thickness (m) and A is the film area (m^2)
r, r_p = degradation rates corresponding to zero and half order kinetics respectively (kg s^{-1})
A = reactor surface colonized by microorganisms (m^2)
k_v = true rate of substrate uptake (kg $m_{biofilm}^{-3}$ s^{-1})
δ = biofilm thickness (m)
D_{eff} = effective diffusion coefficient of substrate within the biofilm (m^2 s^{-1})
C_l = substrate concentration at the liquid-biofilm interface (kg m^{-3})
X_b = biomass concentration (kg_{DW} $m^{-3}_{biofilm}$)
Y = substrate to biomass yield coefficient
C_g = influent MEK gas-phase concentration (kg m^{-3})
H_c = Henry's law constant
k_l = mass transfer coefficient across the liquid boundary layer
δ_l = liquid boundary layer thickness
D_l = MEK diffusivity across the liquid boundary layer
μ_l is the dynamic viscosity of the liquid (Pa s)
ρ_l is the mass density of the liquid (kg m^{-3})
β is the angle of flow to the vertical
g is the gravitational acceleration
l is the perimeter of the tube (m)

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