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A pulsatile flow bioreactor

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We report preliminary experiments on the viability of using pulsatile flow in a baffled tube as a bioreactor. Small-scale liquid phase laboratory bioreactions are normally carried out in either shakeflasks or cell roller bottles and when the reaction volume exceeds of order 1 l, stirred tanks or sparged air lift fermenters are preferred (see, for example, Bailey and Ollis, 1986). We are able to show in this paper that pulsatile flow in baffled tubes offers a viable alternative to the above established methods and in addition should be applicable to both small and large volume bioreaction.

Recent experiments have shown that it is possible to generate efficient eddy mixing within a periodically baffled tube if an oscillatory fluid motion is applied (Brunold *et al.*, 1989). The baffle geometry and batch bioreactor configuration we have chosen is shown in Fig. 1. The baffles, when present, represent an area constriction of 75% and they are spaced 1.4 × the tube diameter along the vertical tubes. The tube was made from glass and the baffle inserts from PTFE. Fluid oscillation is provided by means of a flexible silicone rubber diaphragm which is externally driven by an electrical oscillator.

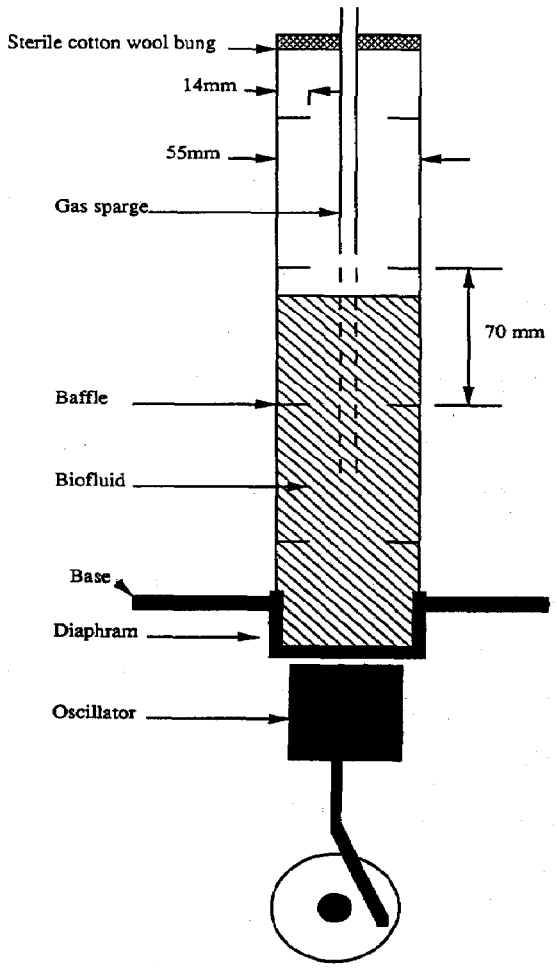

Fig. 1. Schematic diagram of pulsatile bioreactor.

For this given geometry, the fluid mechanics within this tube is controlled by two dimensionless groups, the oscillatory Reynolds number Re_o and the Strouhal number St, where

$$Re_o = \frac{x_0 \omega D}{\nu}, \quad St = \frac{D}{4\pi x_0}$$

where x_0 is the centre-to-peak amplitude of oscillation, ω the angular frequency of oscillation, D the tube diameter and ν the kinematic viscosity of the fluid.

It has been shown experimentally (Dickens *et al.*, 1989) that it is possible to generate a range of mixing conditions for oscillatory flow in baffled tubes. If $Re_o \sim 300$ and $St \sim 1$, gentle uniform mixing can be achieved where the fluid oscillation is just sufficient for each interbaffle region to operate as a stirred tank. If, in particular, the oscillatory Reynolds number Re_o is increased to say 10^3–10^4, very intense eddy mixing can be achieved. It is, therefore, potentially possible for this baffled configuration to be used for both delicate and intense agitation situations.

EXPERIMENTAL PROCEDURE AND TEST BIOREACTION

The micro-organism chosen for use in this study was *Alcaligenes eutrophus* H16, currently of commercial interest for the production of the biodegradable plastic, poly-β-hydroxybutyrate (PHB) (Byrom, 1987; Holmes, 1985). This Gram-negative bacterium is characterised by rapid growth, having a doubling time of approximately 100 min when grown in minimal media (Sonnleitner *et al.*, 1979; Harrison, 1990); hence, it taxes the mass transfer capability of the bioreactor employed. The bacterium was grown under aspetic conditions on a minimal medium of composition (kg/m^3): $(NH_4)_2SO_4$, 1.80; K_2HPO_4, 1.90; NaH_2PO_4, 1.56; $MgSO_4 \cdot 7H_2O$, 0.80; $FeSO_4 \cdot 7H_2O$, 7.5×10^{-3}; $CuSO_4 \times 5H_2O$, 4.8×10^{-4}; $ZnSO_4 \cdot 7H_2O$, 2.4×10^{-4}; $MnSO_4 \cdot 4H_2O$, 2.4×10^{-4}; $CaCl_2 \cdot 2H_2O$, 0.0312 and glucose, 20.0. A starting pH of 7.0 was employed but constant pH was not maintained during incubation. The reaction temperature was maintained at 30°C by use of a constant temperature room and the reaction was initiated by an inoculum of 5% by volume in the late exponential phase.

The kinetics of bacterial growth were compared using conventional baffled Erlenmayer flasks at a 10 to 40% liquid volume, a stirred tank reactor (LH Fermentation 2000 Series Fermenter) and a pulsatile baffled reactor (shown in Fig. 1) in which a 50% liquid volume was used. Mass transfer was provided in the shakeflasks by orbital agitation at 1.3 s^{-1}. The pulsatile bioreactor could be operated in the presence of baffles, pulsation and air sparging or in their absence. Where present, oscillation was produced at a frequency of 4.5 s^{-1} and a centre-to-peak amplitude of 3.5 mm, giving $Re_o = 5230$ and $St = 1.1$.

Bacterial growth was monitored by an increase in optical density at 660 nm. This has been shown to exhibit a linear relationship with the biomass concentration under the culture conditions employed (Harrison, 1990) and is described by the equation

$$Y = 0.387X + 2.016$$

where Y is the dry biomass concentration (kg/m^3) and X is the absorbance measured at 660 nm. Throughout the growth phase monitored, neither glucose nor any other ionic component of the mineral medium became limiting. However, owing to the absence of pH control, growth was finally inhibited by acidic conditions.

The kinetics of bacterial growth is readily analysed under the balanced growth conditions described by an unstructured model in which the cell mass or cell number alone is used to characterise the biophase. The specific growth rate μ, is defined by the equation

$$\frac{dx}{dt} = \mu x$$

where x is a measure of cell mass and t the time of incubation. μ, shows a dependence on the limiting substrate concentration and this is represented by the well-known Monod equation:

$$\mu = \frac{\mu_{max} S}{K_s + S}$$

where S is the limiting substrate concentration and K_s that concentration corresponding to a specific growth rate of $0.5\mu_{max}$. This equation is of the same form as both the Langmuir adsorption isotherm and the Michaelis–Menten rate equation for single substrate enzyme-catalysed reactions (Bailey and Ollis, 1986). It is readily seen that μ will simplify to the constant μ_{max} when S greatly exceeds K_s and the concentration of all other essential nutrients remains unchanged. These conditions are adhered to during the exponential growth of a well-adjusted population, which ceases on a deficient limiting substrate concentration or on inhibition by a toxin or the biomass concentration attained. Analysis of the exponential growth found between 0 and 10 h after inoculation in this study allows the determination of μ_{max} for a particular reactor configuration from the linear relationship

$$\ln_e x = \mu_{max} t + \ln_e x_0.$$

RESULTS

The fluid mechanics of the pulsatile flow bioreactor can be seen from Fig. 2. Flow pattern photographs were taken as described in Dickens *et al.* (1989). In Fig. 2(a) the intense mixing of the flow can be seen due to the coupled effect of the fluid oscillation and sharp edges of the baffles. The flow pattern is observed to continuously change with time during each oscillation. In Fig. 2(b) identical oscillation conditions are used but in the absence of the internal PTFE baffles. Little fluid mixing is observed in the bulk of the tube although an asymmetric oscillatory surface wave is set up.

In Fig. 3 we have shown data relating to biomass kinetics for three different growth conditions. In each case exponential growth is confirmed from the linearity of the natural logarithm of biomass to time plot. The slopes of the curves give values of the maximum specific growth rate. From the data shown it can be seen that the pulsatile flow bioreactor has a comparable performance to the shakeflask and that if fluid oscillation is not present, little or no growth occurs.

Table 1 summarises a range of results that have been carried out under different conditions. Comparison of growth in Erlenmeyer flasks at a 10 and 40% liquid volume yielded a μ_{max} of 0.36 h^{-1} in both cases and this independence of liquid volume suggests that oxygen limitation does not result in either case. Additionally, the result approximates to that of 0.34 h^{-1} previously obtained on batch exponential growth in an aerated stirred tank reactor under similar conditions (Harrison, 1990).

An equivalent growth rate was determined in the pulsatile bioreactor in the presence of pulsation and the sparging of air. In the absence of air sparging, the growth rate fell by 75%. It was unnecessary, however, to sparge the air into the fluid but sufficed to add it to the headspace. This strongly suggests that, while the baffled oscillator provides sufficient liquid–gas mass transfer, diffusion of oxygen into the headspace of the cylinder may be limiting.

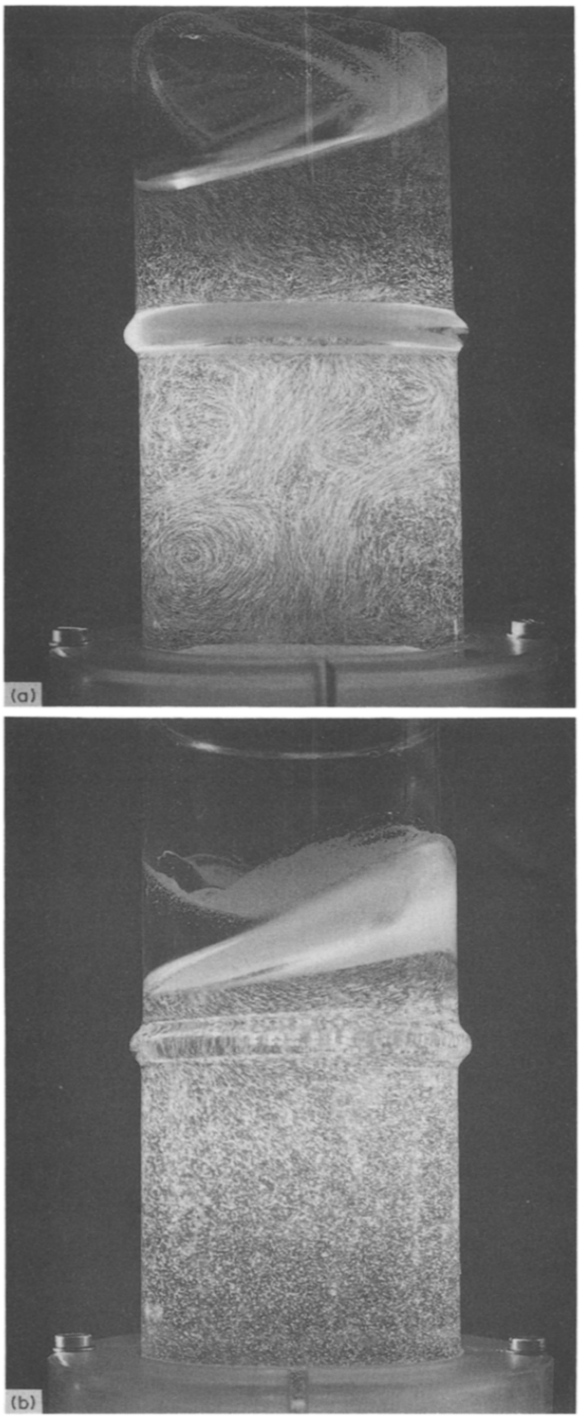

Fig. 2. Photographs of flow patterns within tube: (a) with baffles present; (b) without baffles. $Re_o = 5230$, $St = 1.1$.

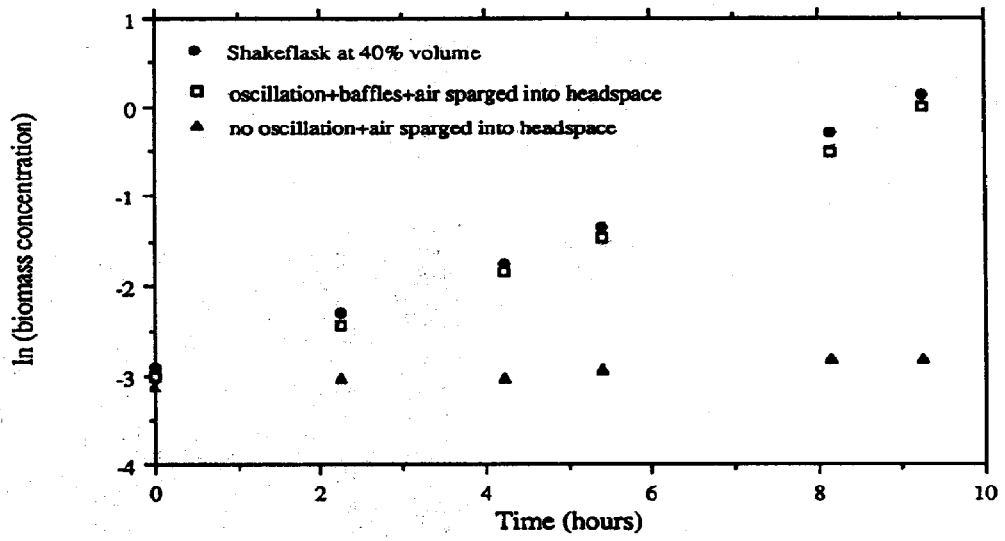

Fig. 3. Graph of ln (biomass concentration) as a function of time.

Table 1. Variation of maximum specific growth rate with reactor configuration

Growth conditions	μ (h^{-1})	Deviation (%)	Number of trials
Shakeflask, 40% volume	0.36	6.1	10
Shakeflask, 10% volume	0.35		
Oscillation + baffles	0.27	6.8	2
Oscillation + baffles + air sparging into fluid	0.39		
Oscillation + baffles + air sparging into headspace	0.36		
Oscillation + no baffles + no air sparging	0.11		
No oscillation + air sparging (large bubbles)	0.18	29.6	2
No oscillation + air sparging (small bubbles)	0.33	2.2	2
No oscillation + air sparging into headspace	0.04		
No oscillation + no air sparging	0.03		

To ensure that the small scale of the experimental apparatus did not negate the mass transfer loading on the system, control experiments were performed in the absence of oscillation in which growth rates were reduced by up to an order of magnitude. The significant reduction in growth rate ($\mu = 0.11\ h^{-1}$) on the removal of the baffles from the pulsatile bioreactor is accounted for by the reduction in mixing and, hence, in transfer. This is readily illustrated by the mixing patterns shown in Fig. 2. The sparging of small air bubbles into the pulsatile bioreactor in the absence of oscillation is seen to provide adequate mixing on the scale used. However, the diffuser employed to disperse the gas into the liquid phase may dramatically influence the results obtained. This is seen on comparison of the glass sinter used to produce small bubbles and the metal sinter used to produce larger bubbles in this study. In addition, mass transfer provided by air sparging is confined to small-scale application in practice.

CONCLUSIONS

The baffled pulsatile bioreactor has been shown to be suitable for the cultivation of rapidly growing, oxygen demanding micro-organisms. The results obtained compare favourably with shakeflask experiments and laboratory-scale stirred tank reactors, illustrating sufficient gas–liquid mass transfer in the absence of active aeration of the liquid by sparging. Diffusion of oxygen into the headspace of the cylindrical reactor may require attention. While the experiments performed to illustrate the appropriate use of the pulsatile flow reactor as a bioreactor have been confined to a small scale, studies of the pulsatile configuration have previously illustrated their applicability over a wide scale of use and the pulsatile bioreactor might be scaled to grow biomass from mg to kg quantities. The main practical advantage of the device is that the flexible diaphragm is the only moving part of the vessel and this enables easy access for on-line monitoring and controlling of growth conditions together with a simple and robust design.

In addition to scaling of this reactor design, the system can be operated to yield gentle, uniform mixing and the pulsatile flow bioreactor described in this paper has been shown to be viable for the growth of animal cells (Jarvis, 1991) which are generally restricted to roller–bottle culture. The combination of gentle mixing and efficient mass transfer may also be of interest for the cultivation of filamentous micro-organisms although the response of the fluid mechanics to non-Newtonian and high viscosity fluids has not yet been explored.

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NOTATION

D	internal tube diameter, m
Re_o	oscillatory Reynolds number ($\omega x_0 D/\nu$)
S	limiting substrate concentration, kg/m^3
St	Strouhal number ($D/4\pi x_0$)
x	cell mass, kg
x_0	centre-to-peak amplitude of oscillation, m
X	absorbance
Y	biomass concentration, kg/m^3

Greek letters

K_s	concentration when $\mu = 0.5\mu_{max}$, kg/m^3
μ	specific growth rate, h^{-1}
μ_{max}	maximum specific growth rate, h^{-1}
ν	kinematic viscosity, m^2/s
ω	angular frequency of oscillation, rad/s

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Intensification and selectivity modification through the use of a microphase: simultaneous absorption of two gases with chemical reaction

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INTRODUCTION

A microphase essentially interacts with a diffusing solute by physically solubilizing it and/or by reacting with it, near the interface formed by the reactant-bearing phases, so as to remove it from the vicinity of the interface. This causes the diffusional gradients to become steeper, resulting in an enhanced value of the interphase flux of the diffusing species.

The strategy of using microphase for intensification of multiphase reactions was first suggested by Sharma (1983). Subsequently, the use of a variety of microphases (micelles microemulsions, suspended fine catalyst particles, etc.) for enhancing the rates of absorption/extraction/dissolution have been reported by different workers (Janakiraman and Sharma, 1985; Bruining *et al.*, 1986; Bhagwat and Sharma, 1988; Mehra *et al.*, 1988). A comprehensive theory of mass transfer with chemical reaction in the presence of a microphase has been developed using the unsteady-state mass transfer models (Mehra, 1988).

The relative affinity of a microphase for a given solute in relation to other solutes in a mixture has also been exploited in effecting the selective absorption of one of the solutes by utilizing the different extent of enhancement in the specific absorption rates of the respective solute gases. Tinge *et al.* (1987) have reported the absorption selectivity between propane and ethylene in aqueous slurries of fine, activated carbon particles. Mehra and Sharma (1988) have analyzed the problem theoretically and have shown that the selective absorption of gases can be obtained through the use of microphases such as a nonreactive microphase, which, by virtue of its solubilizing power alone can increase the selectivity for a chosen solute (A) over other solutes (B) substantially, provided the ratio of the distribution coefficients of the solutes (m_A/m_B) is greater than the ratio of the homogeneous phase pseudo-first-order rate constants (k_{1A}/k_{1B}).

In the case of simultaneous absorption of two gases with chemical reaction, if the microphase favors a particular reaction then this may lead to the increased yield of the product corresponding to that reaction. The presence of a microphase, therefore, can also change the selectivity for a particular product. Some preliminary theoretical considerations on this phenomenon have appeared recently in the literature (Mehra, 1990). In the present work, the intensification in the rates of absorption of two gases as well as the change in product selectivity through microphase catalysis has been examined theoretically. The model system taken was the