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Quantification and prediction of jet macro-mixing times in static microwell plates

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Abstract

Automated experimentation in microwell plate formats is widely used in high throughput drug discovery. Such approaches are now being considered for the study of bioprocess unit operations in order to speed the delivery of new medicines to market. The generation of useful design data from microwell formats requires an understanding of the engineering environment within individual microwells. Rapid and efficient macro-mixing is crucial in this respect to ensure the generation of quantitative and reproducible data. In this study, we have developed a high-speed video technique for the accurate quantification of jet macro-mixing times in static microwell plates which also enables visualisation of jet formation and liquid flow patterns within wells. Mixing times have been determined using both the fixed ($d_i = 0.54$ mm) and disposable ($d_i = 0.6$ mm) tips of a Perkin Elmer MultiProbe II^{TM} liquid handling robot for a range of jet Reynolds numbers ($Re_j = 1000$–3960) and liquid addition volumes ($V_A = 10$–859 μl). Three microwell geometries have been investigated; one that is identical to a single well from a standard 96-round well plate ($V_i = 200$ μl) and two novel designs based upon theories of jet mixing ($V_i = 200$ and 1720 μl). For conditions where macro-mixing was complete within the lifespan of the jet, t_{95} mixing times for the standard round well were in the range 0.033–0.121 s while for the larger of the two designed wells they were in the range 0.228–0.705 s. The rapid mixing times in the standard round well are a consequence of increased energy dissipation as the liquid jet impinges on the base of the well. For the two designed wells maximising the jet length to nozzle diameter ratio (X/d_i) is shown to promote the most efficient macro-mixing due to entrainment and circulation of the bulk liquid in the well. For low volume additions and short jet lifespans it is also shown that mixing times can be of the order of minutes. Finally, the t_{95} results for each of the well geometries have been correlated to the conditions used for jet formation using a correlation of the form first proposed by Baldyga and co-workers [Baldyga, J., Bourne, J.R., Dubuis, B., Etchells, A.W., Gholap, R.V., Zimmermann, B., 1995. Jet reactor scale-up for mixing controlled reactions. Chemical Engineering Research & Design 73, 497–502]. This enables good prediction of the experimentally determined mixing times and estimation of the minimum liquid addition volume (V_{Crit}) that will ensure rapid and efficient macro-mixing. The correlation therefore enables automation users to optimise or control macro-mixing times in microwell experiments.

Keywords: Biochemical engineering; Jet mixing; Visualisation; Process automation; Microscale processing; High throughput screening

1. Introduction

The financial pressures to deliver new medicines quickly and economically to market are increasing while the regulatory hurdles necessary to ensure product safety lengthen the development time and inflate costs (Littlehales, 1999). To help speed drug discovery, automation and miniaturisation technologies have been widely implemented by companies over the last decade. The impact of these, however, has been an explosion in the number of potential new drug candidates (Balkenhohl et al., 1996) and a shift in the product development bottleneck from discovery activities to process development (Pollard, 2001; Weinmann et al., 1999).

Automated microscale processing techniques (Lye et al., 2003), the combination of automated experimentation and bioprocess studies carried out in microplate formats, have the potential to overcome this new development bottleneck. Already a range of bioprocess unit operations have been

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demonstrated in microwell formats most notably microbial fermentation (Doig et al., 2002; Elmahdi et al., 2003; Maharbiz et al., 2004; Minas et al., 2000; Welch et al., 2002). The potential for parallel experimentation and the operation of automated whole process sequences has also been shown (Lye et al., 2003; Ferreira-Torres et al., 2005).

Ensuring the reproducibility of data collected at the microwell scale is crucial. To date, microwell experimentation has mainly been applied to high throughput drug discovery (Delvin, 1997; Lamsa et al., 2004; Wegener et al., 2003) and the routine performance of laboratory assays (Kolb, 1994; Wang et al., 2003). Here the focus has been on understanding the reliability and operation of the liquid handling robots used to perform experiments (Astle and Akowitz, 1996; Berg et al., 2001; Stevens et al., 1998). For bioprocess studies it is equally important to understand how the physical environment within a microwell impacts on the reproducibility and scalability of the data obtained (Berg et al., 2001; Lye et al., 2003). Recent engineering studies have therefore begun to address gas–liquid mass transfer and mixing phenomena in microwells (Duetz and Witholt, 2001; Hermann et al., 2001; John and Heinzle, 2001; Weiss et al., 2002; Doig et al., 2005), how to control the environment within microwells (Elmahdi et al., 2003) and the speed of robot operation relative to the processes being studied (Nealon et al., 2005).

The rapid attainment and maintenance of liquid phase homogeneity is fundamental to the generation of reproducible data. At present, observations on mixing within microwells have mainly been reported for the types of shaken system used in fermentation studies (Duetz and Witholt, 2001; Hermann et al., 2001; John and Heinzle, 2001; Weiss et al., 2002). Many applications though, particularly those involving the study of enzyme kinetics and stability or biological assays are best carried out using static microwell plates (Berg et al., 2001; Lamsa et al., 2004; Lye et al., 2002; Aucamp et al., 2005). Here, rapid and complete macro-mixing must be achieved by the liquid additions made to each well by the pipette tips of the liquid handling robot.

1.1. Jet macro-mixing theory and its application to microwells

Jet mixing is well established in the chemical industries as a cost-effective method of mixing on the large scale (Fossett and Prosser, 1949; Fox and Gex, 1956; Lane and Rice, 1981; Baldyga et al., 1995). In jet mixing part of the bulk fluid is normally continuously recirculated by drawing it through a pump and returning it to the vessel through a nozzle. The resulting jet entrains the surrounding liquid and creates a circulating flow pattern within the vessel. It is this fluid motion and entrainment of fluid in the jet itself that leads to macro-mixing of the vessel contents.

The early experiments by Fossett and Prosser (1949) proposed a simple correlation for the mixing time (t_m) which was independent of the jet Reynolds number as shown in Eq. (1):

$$t_m = 9.0D^2/(u_o d_i). \tag{1}$$

Fox and Gex (1956) then extended this study to cover both the laminar and the turbulent flow regimes. They showed that the most important parameter in determining the mixing time was the momentum flux added to the vessel by the jet. They also determined that the mixing time is closely correlated to the jet Reynolds number in the laminar regime but is only weakly correlated in the turbulent regime. A number of contactor designs have been investigated and include the use of a vertical jet mixer (Hiby and Modifell, 1978) and a vertical mixer in a vessel with a hemispherical base (Lane and Rice, 1981). Work with hemispherically based tanks has been continued (Baldyga et al., 1995) and has lead to a widely used correlation for estimating the mixing time in turbulent flow regimes

$$t_{95} = \frac{4.48D^{1.5}h^{0.5}}{u_o d_i}. \tag{2}$$

A number of studies have investigated the flow patterns produced within a jet-mixed vessel and have proposed that the mixing time is a function of the jet Reynolds number and the longest jet length (Maruyama et al., 1982; Maruyama, 1986; Revill, 1992). An alternative approach also has been recommended, suggesting that the local turbulent energy dissipation rate at the end of the jet path controls the mixing rate for the whole vessel (Grenville and Tilton, 1996). A number of CFD models have been proposed that have emphasised the need to minimise or eliminate dead zones within a large vessel (Jayanti, 2001). These predict overall mixing times (Patwardhan, 2002; Zughbi and Rakib, 2002) and have investigated the effects of the jet position and the number of jets (Zughbi and Rakib, 2004) on macro-mixing within a vessel.

Jet mixing theory can be applied to microwell experimentation by considering the system as a being a downward facing, vertical jet. However, instead of having a continuous recirculation of liquid, true jet mixing will only occur during the lifespan of the jet (t_j). This will be determined by the liquid addition volume (V_A) and the volumetric flow rate of addition. Ideally, complete macro-mixing should be achieved within the lifespan of the jet. Concerning novel designs of microwell to achieve effective jet macro-mixing there are two approaches that can be followed. The first seeks to maximise fluid entrainment by ensuring a long jet path (X). This results in a well with an aspect ratio of 3:1 (Revill, 1992). The second seeks to maximise the local turbulent energy dissipation rate at the end of the jet resulting in a recommended well aspect ratio of $1/\sqrt{2}$ (Grenville and Tilton, 1996).

In this work we have established a video-based technique for the accurate quantification of jet macro-mixing times in static microwell plates. The impact of well geometry on macro-mixing has been investigated using a standard microwell design and two novel geometries based upon jet mixing theory. The experimental data obtained has also been correlated with the conditions used for jet formation to enable accurate predictions of jet mixing times and their optimisation.

2. Materials and methods

2.1. Design of individual microwell mimics

Three designs of microwell were utilised during the course of this work as shown in Fig. 1. The first was an identical copy