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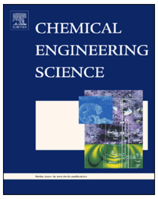

Effective shear rates in shake flasks

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HIGHLIGHTS

- A correlation for effective shear rates in shake flasks is developed.
- The correlation is derived from dimensional analysis and experimental data.
- Effective shear rates are calculated for three different shake flask fermentations.
- Apparent viscosities in shake flasks are now accessible for non-Newtonian broths.
- Shear rates and viscosities in shake flasks are compared to those in stirred tanks.

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ABSTRACT

In bioprocess development and scale-up and scale-down consistent fermentation conditions in shake flasks and stirred tank reactors are crucial. Fermentation broths at elevated viscosities generally show a non-Newtonian flow behavior. The effective shear rate is known to influence apparent viscosity, and, as a consequence, mixing as well as mass and heat transfer. Unknown effective shear rates pose the risk of screening and producing under unfavorable conditions. The present study is the first to systematically investigate the effective shear rate in shake flasks. Based on Buckingham's π-theorem a shear rate correlation for 50 mL to 1000 mL shake flasks as a function of viscosity and volumetric power input is developed. Viscosity and power input measurements for a wide range of pseudo-plastic flow behaviors and a broad spectrum of commonly applied operating conditions were applied. Effective shear rates in shake flasks cover a range from 20 s^{-1} to 2000 s^{-1}. To demonstrate the value of the obtained shear rate correlation, effective shear rates over time were calculated for three different exemplary fermentation systems in shake flasks generating elevated viscosities. With the aid of this new source of information the specific courses of the individual fermentations could now be interpreted. Depending on the broth's flow behavior, the effective shear rate in shake flasks is at least 1.55 times higher than that in stirred tank reactors operated at the same volumetric power input, leading to a potentially 50% lower apparent viscosity in shake flasks. The obtained shear rate correlation is a valuable tool to explain existing deviations in screening and production results, ultimately assisting bioprocesses development by means of consistent scale-up or scale-down procedures.

1. Introduction

Unknown shear rates and apparent viscosities pose the risk of screening and producing under unfavorable fermentation conditions leading to incorrect conclusions (Peter et al., 2004; Seviour et al., 2011). Although broth viscosity is known to influence mixing as well as gas/liquid mass and heat transfer (Büchs et al., 2001; Giese et al., 2014; McNeil and Harvey, 1993; Tan et al., 2011), the effective shear rate in shake flasks has never been systematically investigated before. Due to this lack of knowledge, engineering-based scaling from shake flasks to stirred tanks with consistent apparent viscosities on both scales is not yet possible—although shake flasks are the predominant screening tool employed for microbial strains, culture media and process parameters (Kumar et al., 2004). Peter et al. (2004) adapted a method introduced by Metzner and Otto (1957) to determine the effective shear rate in shake flasks and conducted preliminary measurements. The authors concluded: "Much more efforts have to be dedicated to

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this area to introduce a general model, which is valid for all kinds of pseudo-plastic fluids and operating conditions".

The aim of the present study is to quantify and correlate the effective shear rates in shake flasks for a wide range of pseudo-plastic flow behavior and operating conditions. To achieve this aim, the volumetric power input into filled shake flasks is measured using a special shaker with integrated power input measurement option (Büchs et al., 2001). Then, the viscous flow behavior of the pseudo-plastic liquids is determined via a rheometer enabling the effective shear rate to be determined for a specific shake flask operating condition (Metzner and Otto, 1957; Peter et al., 2004). This approach allows the formulation of a correlation based on the power input concept postulated by Henzler and Kauling (1985). In this study, an effective shear rate correlation is newly derived for shake flasks employing a dimensional analysis and Buckingham's π-theorem. The resulting correlation is applied for the calculation of the effective shear rates in shake flasks and to demonstrate the impacts of the nominal flask volume, filling volume, shaking frequency and flow behavior, respectively. On the basis of the developed shear rate correlation, three different exemplary fermentation systems leading to elevated viscosities are analyzed over time with respect to apparent viscosities and Phase numbers. Additionally, comparative calculations of the effective shear rate in shake flasks and stirred tank reactors are conducted.

2. Theory

2.1. Rheological behavior of fermentation broths

As a result of filamentous growth morphology or biopolymer formation, viscous fermentation broths usually show a pseudo-plastic flow behavior (Charles, 1978; McNeil and Harvey, 1993; Metz et al., 1979; Reuss et al., 1982). These broths are commonly described by the Ostwald-de Waele law, which is a simple and the most universal approach (Allen and Robinson, 1990; Blanch and Bhavaraju, 1976; Goudar et al., 1999; Kemblowski and Kristiansen, 1986):

$$\eta_{\text{app}} = K(\dot{\gamma}_{\text{eff}})^{m-1}. \tag{1}$$

The parameter K represents the consistency index and m the flow behavior index. As defined by Eq. (1), the apparent viscosity η_{app} is a function of the effective shear rate $\dot{\gamma}_{\text{eff}}$. Since the local shear rate in both, shake flasks and stirred tank reactors, varies within the liquid depending on position in the reactor, authors like Metzner and Otto (1957) (related to stirred tanks), Nishikawa et al. (1977), Schumpe and Deckwer (1987) (both related to bubble columns) as well as Shi et al. (1990) (airlift reactors) introduced respective correlations for an effective shear rate. The authors present the effective shear rate as the relevant mean shear rate affecting the apparent viscosity influencing mass and heat transfer phenomena.

2.2. Power input into shake flasks

Büchs et al. (2001) and Büchs et al. (2000b) evaluated the power input P into shake flasks during shaking (Eq. (2)), and found a correlation between the modified Newton number Ne' (Eq. (3)) and the Reynold's number Re (Eq. (4)):

$$Ne' = 70\ Re^{-1} + 25\ Re^{-0.6} + 1.5\ Re^{-0.2} \tag{2}$$

$$Ne' = \frac{P}{\rho\ n^3\ d^4\ V_L^{1/3}} \tag{3}$$

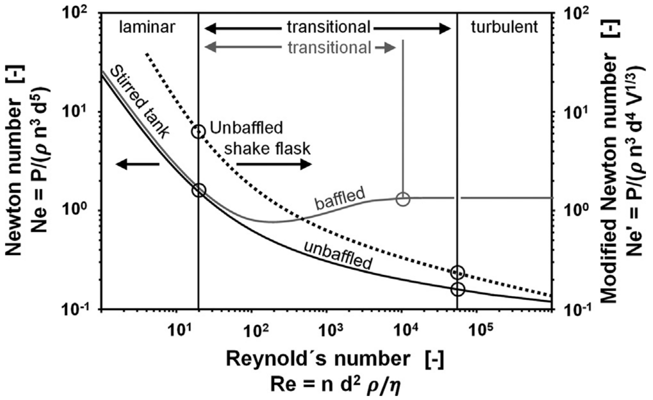

Fig. 1. Qualitative impact of the *Re* number on the power input into baffled and unbaffled stirred tanks with Rushton turbines (solid line; Rushton et al., 1950; Zlokarnik, 2001), as well as into unbaffled shake flasks (dotted lines; Büchs et al., 2000b). The power input is represented by the conventional *Ne* number for stirred tanks and the modified *Ne'* number for shake flasks, respectively. The critical *Re* number for turbulence is 10^4 in baffled stirred tanks, 5×10^4 in unbaffled stirred tanks (Rushton turbines; Zlokarnik, 2001), and 6×10^4 in unbaffled shake flasks (Peter et al., 2006).

$$Re = \frac{\rho\ n\ d^2}{\eta_{\text{app}}} \tag{4}$$

The three Re number terms in Eq. (2) are related to the laminar (Re^{-1}), transitional ($Re^{-0.6}$) and turbulent ($Re^{-0.2}$) flow regimes, respectively, which are indicated in Fig. 1 (dashed curve). In contrast to shake flasks and unbaffled stirred tanks, the Ne number of baffled stirred tanks remains constant within the turbulent flow regime (Fig. 1). Turbulence in baffled stirred tanks equipped with Rushton turbines is indicated by a Re number larger than 10^4 (Zlokarnik, 2001). By contrast, the critical Re number for turbulent fluid flow in unbaffled shake flasks and unbaffled stirred tanks is higher. With approx. 6×10^4 (Peter et al., 2006) and 5×10^4 (Zlokarnik, 2001) these critical Re numbers are in a similar range.

For shake flasks, Büchs et al. (2001) observed the so-called "out-of-phase" phenomenon, which leads to strongly reduced power input, mixing and mass transfer, because the largest part of the liquid bulk remains at the shake flask's bottom. A Phase number (Ph) larger than 1.26 is a proven indicator for "in-phase" operation, where the liquid bulk circulates "in-phase" with the shaker drive. It can be calculated according to Eq. (5) (Büchs et al., 2001).

$$Ph = \frac{d_0}{d}\left[1 + 3\log_{10}\left(Re \times \frac{\pi}{2}\left[1 - \sqrt{1 - \frac{4}{\pi}\left(V_L^{1/3}/d\right)^2}\right]^2\right)\right] > 1.26. \tag{5}$$

2.3. Effective shear rates in stirred tanks and shake flasks

Many approaches have been introduced in literature describing the effective shear rate in stirred tanks. Good summaries are presented by Herbst et al. (1992) and Sánchez Pérez et al. (2006). However, the effective shear rate in stirred tanks is most commonly still determined using the stirrer frequency concept devised by Metzner and Otto (1957) and Calderbank and Moo-Young (1959) (Eq. (6)). For laminar flow regimes ($Re < 10^1$) Metzner and Otto found a linear relation between stirrer frequency n and the average shear rate $\dot{\gamma}_{\text{eff}}$ in a stirred tank, which are correlated via the Metzner and Otto constant K_{MO}.

$$\dot{\gamma}_{\text{eff}} = K_{MO} \times n \tag{6}$$