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Novel bioreactor design for the culture of suspended mammalian cells. Part I: Mixing characterization

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Abstract

Mammalian cells are extremely sensitive to mechanical stress. Ideally, a bioreactor design for mammalian cell culture should assure adequate mixing at low mechanical stress. This paper focuses on the mixing characterization of a novel stirred tank bioreactor configuration, proposed for the culture of mammalian cells, based on the principle of displacing the agitation shaft to an eccentric position and replacing the impellers normally used for mammalian cell culture with a disc impeller with no blades. Experiments in a 1.0 L prototype are conducted to study flow patterns using UV light visualization techniques. Three different impeller shaft positions are tested $E = 0.0, 0.21$, and 0.42. For the purposes of this work, eccentricity (E) is defined as the distance between the shaft and the vertical centerline of the tank/tank radius. The mixing performance of two different impeller disc diameters (3.0 and 5.0 cm) are compared. Experimental results show that adequate mixing conditions are achieved at very low *Re* numbers for some of the eccentric cases considered. Computations are used to illustrate mixing improvement caused by eccentricity, and to validate the existence of globally chaotic conditions for the eccentric cases tested experimentally.

Keywords: Mixing; Bioreactor; Mammalian cells; Eccentric agitation

1. Introduction

Biotechnology companies originally relied on recombinant bacteria to commercially produce the first examples of high added-value biopharmaceutical drugs (Robertson, 2006). However, most of the new processes developed for bio-drugs of therapeutic value use mammalian cell lines as vectors of expression (see Friedemann and Wagner, 2000; Chu and Robinson, 2001; Walsh, 2005; Kwaks and Otte, 2006). The metabolic machinery of mammalian cells allows for the correct folding of proteins, making them ideal candidates for the production of fully functional biodrugs of proteic nature. As an example, more than 70% of Genentech® products (Genentech® is the second largest Biotechnology Company worldwide) are manufactured using mammalian cell lines as expression vectors. For the industrial culture of mammalian cells, stirred tanks have been adopted as the preferred reactor configuration (Chu and Robinson, 2001). When designing stirred tank bioreactors for mammalian cell culture, mixing and agitation intensity should be important considerations (Boudreault et al., 2001; Haut et al., 2003; Micheletti et al., 2006). Mammalian cells are highly sensitive and responsive to mechanical stress (Cherry and Papoutsakis, 1988; Cherry, 1993; Elias et al., 1995; Aloi and Cherry, 1996; Saini and Wick, 2003), and their rate of growth (Motobu et al., 1998; Boudreault et al., 2001), biochemical behavior (Aloi and Cherry, 1996; Miyazawa et al., 2005; Kakisis et al., 2005), aggregation behavior (Moreira et al., 1995) and ultimately, their rate of survival (O'Connor and Papoutsakis, 1992; Zhang et al., 1995; Apenberg et al., 2003) may strongly depend on agitation conditions. For some industrial applications, mammalian cell lines engineered to grow in suspension and tolerate mechanical stress have been developed. In most manufacturing scale applications, shear-protecting agents such as Pluronic F68 are used to minimize shear damage to cells in the highly turbulent environment of stirred tank bioreactors (see for example Zhang et al., 1995). And yet, it seems obvious to recommend laminar (or at the most transitional)

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flow regime conditions for such cellular systems, provided that they guarantee adequate mixing for cell growth and product expression. A stirred tank geometry allowing to mix efficiently in the laminar regime at low mechanical stress will be a more cost effective solution than cellular engineering or the use of protecting additives. However, typical stirred tank bioreactor geometries (highly symmetric) when operated in the laminar regime, exhibit mixing pathologies such as the presence of segregated regions or flow separation planes (Lamberto et al., 1999; Álvarez, 2000; Peña et al., 2002; Arratia et al., 2004a,b; Álvarez et al., 2005).

In this contribution, we study the performance of a novel stirred tank design for culture of suspended mammalian cells. The new design aims to guarantee adequate mixing conditions and decrease shear stress as compared to classical bioreactor configurations by means of the relocation of the axes of agitation to an eccentric position. Eccentric stirred tank configurations are recommended to avoid strong vortex formation in turbulent unbaffled stirred tanks (see for example Joosten et al., 1977 and Cole-Parmer Catalog 1999–2000), but have been just recently studied from the point of view of mixing performance both in turbulent (Nishikawa et al., 1979; Medek and Fort, 1985; Hall et al., 2004; Hall et al., 2005a,b,c; Karcz and Szoplik, 2004; Szoplik and Karcz, 2005; Karcz et al., 2005; Montante et al., 2006), transitional (Szoplik and Karcz, 2004), and laminar regimes (Álvarez et al., 2002a; Ascanio et al., 2002; Rivera et al., 2004).

Álvarez et al. (2002a) studied the performance of eccentric stirred tank systems agitated by typical axial and radial impellers. The authors showed that chaos can be originated by means of eccentricity in stirred tank systems. At some eccentric stirring locations, segregated regions and separation planes recurrent in concentric tank configurations operated in laminar regime can be destroyed. In this contribution, we further explore the role of eccentricity and propose the use of stirred tank eccentric configurations agitated by disc impellers as basis for the design of low shear bioreactors for mammalian cell culture.

2. Materials and methods

2.1. Experimental system

A 1.0 L prototype bioreactor was constructed, and devised with three ports for agitation located at different eccentricity values. For the purpose of this work, eccentricity (E) is defined as the ratio of the distance of the rotation axes from the centerline of the vessel/radial distance of the vessel. The tank dimensions and other geometrical particularities are illustrated in Fig. 1. For the experiments presented here, the tank was filled with 0.750 L of liquid ($H/D = 1.25$). A Newtonian viscous liquid, glycerin (viscosity in the range of 750–900 cp), is used as model fluid. Its high viscosity allows to retard diffusion effects and to "slow down" convection in order to conduct good quality visualizations at low Re (see Lamberto et al., 1999). Mixing times were recorded for different agitation speed (100, 300, and 500 rpm) and degrees of eccentricity ($E = 0.0, 0.21$, and 0.42) in the novel system. Two different disc impellers diameters were tested: $d = 3$ and 5 cm.

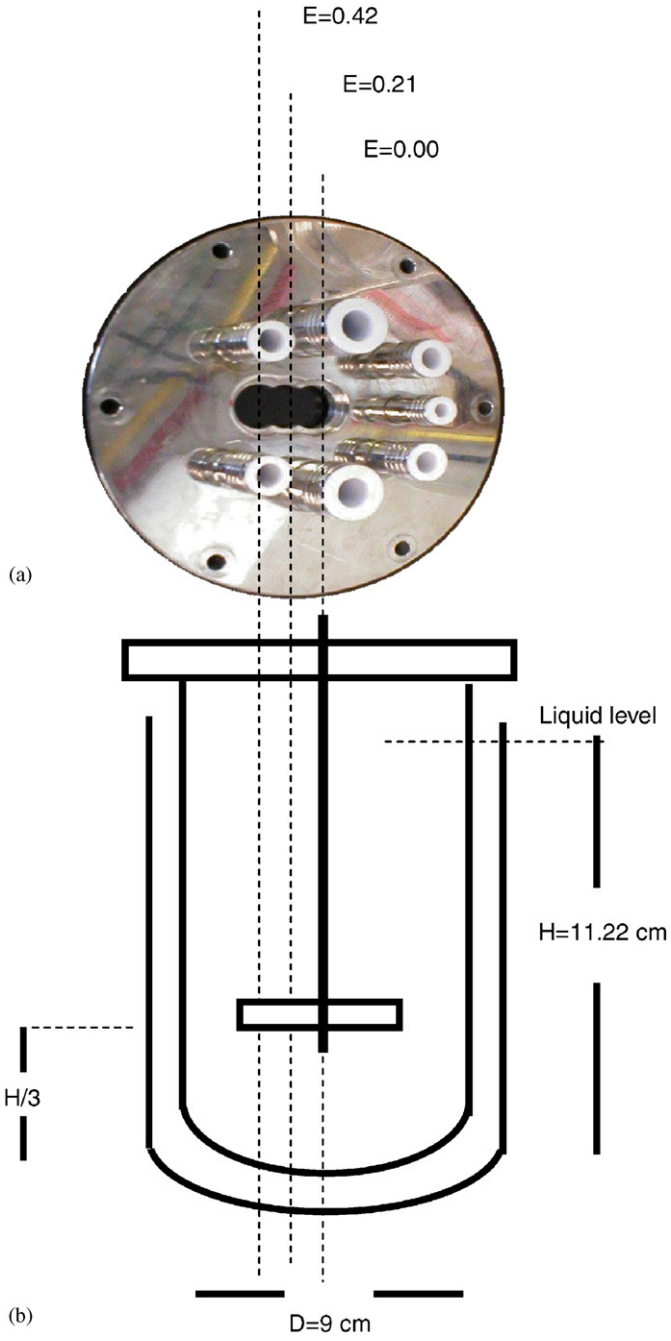

Fig. 1. Experimental bioreactor system. (a) Top view of tank lid, showing the disposition of shaft ports for the three different eccentricity values tested, (b) dimensions and general geometrical features of the bioreactor vessel.

2.2. Visualization techniques

Using UV visualization, and laser induced fluorescent techniques (LIF), the flow patterns characteristic of eccentric stirred tank configurations were revealed and contrasted to those observed in concentric systems. Fluorescent tracer injections