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Application of a two-dimensional disposable rocking bioreactor to bacterial cultivation for recombinant protein production

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ABSTRACT

Disposable rocking bioreactors (RBs) are widely employed for cultivation of recombinant mammalian and insect cell lines, although the perception of inadequate mass transfer has prevented their application to bioprocesses based on microbial platforms. In this study, one-dimensional (1D) and two-dimensional (2D) RBs were assessed and compared with the conventional stirred tank reactor (STR) for recombinant therapeutic protein production in *Escherichia coli*. The comparison involved: (1) physical characterization of oxygen mass transfer efficiency and mixing intensity, (2) growth characteristics in batch cultivation, and (3) culture performance for the production of recombinant protein. Our results show that oxygen mass transfer was comparable between the 1D RB and STR at low working volume (WV), declining linearly with increasing WV, and was highest in the 2D RB for all tested WVs with the maximum mass transfer coefficient (k_La) at 3 L WV. Well mixing behavior was observed in all three systems for water and aqueous carboxymethylcellulose (CMC) solutions. Batch growth characteristics were similar in all bioreactor systems, although metabolite accumulation was significant in the 1D RB. Culture performance for the production of recombinant GST-hCD83ext (glutathione *S*-transferase-hCD83ext fusion protein) was similar in terms of soluble protein yield and inclusion body formation for all bioreactor systems.

1. Introduction

Due to the highly competitive nature of today's biopharmaceutical market and the high failure rates associated with biopharmaceuticals [1], flexible and cost-effective manufacturing facilities are prerequisite for survival of biopharmaceutical producers. Over the last decade, disposable bioreactors have become integral components in the production of many high-value biopharmaceuticals [2]. Advantages of disposable bioreactors compared to conventional tank reactors include high flexibility, reduced occurrence of cross-contamination, lower capital investment, reduced labor costs associated with validation and cleaning, and shorter turnover times between production runs [1,3]. Disposable bioreactors are classified by mode of power input as mechanically driven (wave-mixed, stirred, orbitally shaken or vertically oscillating), pneumatically driven, or hybrid systems [3].

Wave-mixed rocking bioreactors (RBs) were introduced in the late 1990s [4], and are commonly employed in the production of biopharmaceutical products (e.g. monoclonal antibodies, vaccines, therapeutic and diagnostic proteins) in mammalian [5,6], plant [7], insect [8], and human cell cultures [9]. These cultivation systems typically consist of an oscillating or sectional platform, supporting one or more pre-sterilized cultivation bags made of biologically inert polymers such as polyethylene, polypropylene, or polytetrafluoroethylene. Temperature is controlled through the moving platform or external cabinet. Bags are equipped with disposable or multiuse online pH and dissolved oxygen (DO) sensors. Gas–liquid mass transfer occurs through surface aeration and turbulent air entrainment via wave propagation and is controlled by adjustment of vertical displacement (VD) and rocking rate (expressed as rocks per min, rpm). While reports of cultivation of microorganisms in disposable RBs [10–13] exist, it is commonly assumed that these disposable systems are not capable of meeting the high oxygen demand of microbial cultures. Toward the end of 2011, 66 out of 211 biopharmaceuticals receiving regulatory approval in the United States and European Union are produced in *Escherichia coli* while mammalian cells represent the most common host system for biopharmaceutical production [14]. However, few reports exist

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on the application of disposable RBs to recombinant therapeutic protein production in *E. coli*.

The oscillation trajectory for most RB platforms is one-dimensional (1D), as shown in Fig. 1, limiting the extent of wave development and, in turn, the efficiency of mass transfer. The CELL-tainer®, a RB with an innovative design of two-dimensional (2D) oscillation trajectory, moves around the axis of rotation in a closed loop facilitating simultaneous vertical and horizontal displacement (Fig. 1) [15]. 1D systems tilt along a central pivot axis which only a portion of the fluid is able to pass with each oscillation [16]. In the CELL-tainer®, the fluid completely passes the center of the bag due to the additional horizontal movement, which may partially explain increased mass transfer due to additional film formation along the bottom of the bag [13]. Additionally, the CELL-tainer® provides more efficient mass transfer with respect to specific power input, and is capable of processing larger working volumes (WV) and higher gas flow rates compared to 1D RBs [17]. Previous studies of oxygen transfer efficiency in 1D RBs report k_La values in range of 38–55 h^{-1} [11,12], while k_La exceeded 500 h^{-1} in the CELL-tainer® at maximum WV [13]. In this study, the CELL-tainer® was used for recombinant therapeutic protein production in *E. coli* and the culture performance was compared with the traditional stirred-tank reactor (STR) and 1D RB. The target protein of GST-hCD83ext is a protein fusion of glutathione *S*-transferase and the extracytoplasmic domain of human CD83 (hCD83ext), and the bioprocess for its expression and purification was previously developed [18]. We specifically investigated oxygen transfer efficiency and mixing intensity of 1D and 2D disposable RBs to evaluate performance relative to the STR. RBs are believed to provide an environment suitable for shear-sensitive and fragile microbial recombinant cells. Batch growth characteristics, glucose consumption, and metabolite profiles of non-recombinant *E. coli* were compared to elucidate suitability of RBs for cultivation under typical culture conditions. Also, culture performance for recombinant GST-hCD83ext expression was evaluated under previously optimized culture conditions, and soluble and insoluble fractions of cell lysates were analyzed to assess titer and inclusion body formation.

2. Materials and methods

2.1. Physical characterization and OUR estimation

10 L and 20 L cultivation bags were used for physical characterization and cultivation in the 1D and 2D (CELL-tainer®, CELLution Biotech, The Netherlands) disposable RBs, respectively. Different WVs were tested under conditions providing maximum oxygen transfer, i.e. 40 rpm and 12° VD (1D RB), and 40 rpm and 17° VD (CELL-tainer®). Trials were conducted at 28 °C and 0.4 vessel volume per minute (vvm) aeration rate. Measurements of k_La were performed using the dynamic "gas out–gas in" method [19]. The cultivation chamber was filled with an appropriate volume of deionized water, which was subsequently stripped of oxygen by nitrogen purging until the DO level fell below 5% of air saturation. The headspace was evacuated with a vacuum pump (GAST, MI, USA) and then filled with air. Once the headspace was full, agitation resumed and DO measurements were recorded at appropriate time intervals. An optical DO sensor was used without the oxygen permeable membrane resulting in a time constant of <6 s. k_La estimates were obtained from the mass balance equation:

$$\ln = \left(\frac{DO* - DO(t)}{DO* - DO(t_0)}\right) = -k_La(t - t_0) \quad (1)$$

where DO^* is the saturation reading of the probe. The volumetric oxygen uptake rate (OUR) was measured during exponential growth by temporarily stopping the supply of gas to the cultivation chamber, evacuating the headspace, and recording DO

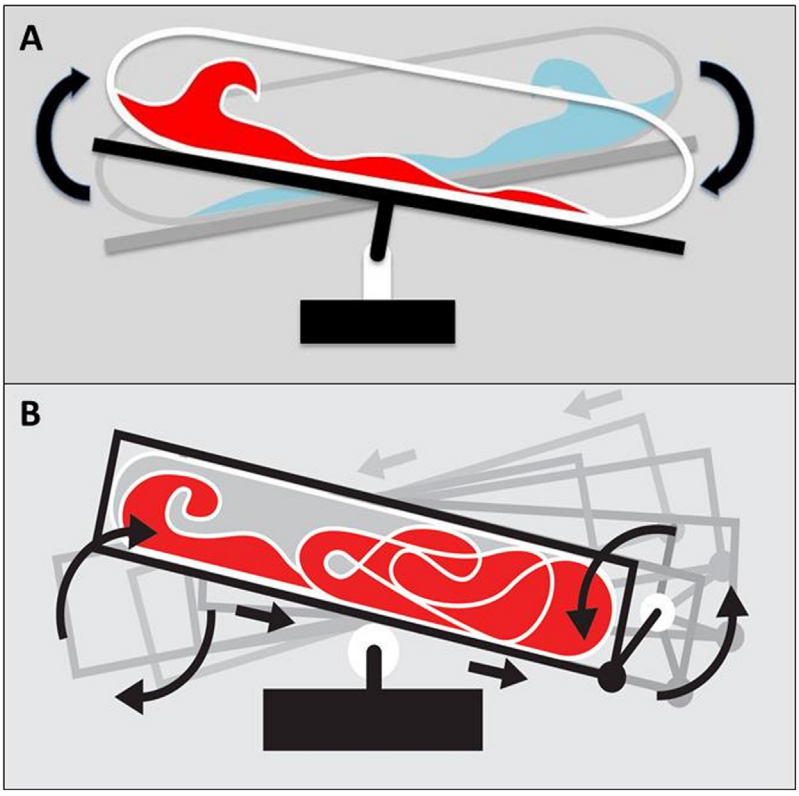

Fig. 1. Schematic representation of the oscillation trajectories of 1D disposable RBs (Panel A) and the CELL-tainer® (Panel B). 1D RBs tilt along a central pivot axis causing vertical displacement (5–10°) of the fluid at rocking rates of 6–40 rpm. The CELL-tainer®, a 2D disposable RB, moves around the axis of rotation in a closed loop facilitating simultaneous vertical (5–17°) and horizontal (60–200 mm) displacement at rocking rates of 5–50 rpm.