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Critical evaluation and comparison of fluid distribution systems for industrial scale expanded bed adsorption chromatography columns

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ABSTRACT

The hydrodynamic properties of an expanded bed contactor with 30 cm or 150 cm internal diameter, which employs a rotating or oscillating fluid distributor, were compared to prototype columns of 60 cm or 150 cm diameter employing local stirring (fixed wall nozzles plus central bottom mounted stirrer) for fluid distribution. Fluid introduction *through* a rotating fluid distributor was found to give superior hydrodynamic characteristics in the 30 cm and 150 cm diameter column compared to using the local stirrer in both the 60 cm and 150 cm diameter columns. The shortcomings of the local stirring distributor at large scale were apparent: dead zones were present which could not be removed by increasing rotation rates or flow rates, and such changes led to a deterioration in hydrodynamic properties. In contrast, during fluid introduction through a rotating distributor no dead zones were observed, and residence time distribution tests showed that plate numbers remained constant or increased slightly as flow rate was raised from 200 cm h^{-1} to 470 cm h^{-1}. Under the conditions studied, oscillation of the rotating fluid distributor led to increased mixing and poorer performance than rotary movement. The results imply that further improvement in distributor design is needed and careful attention should be given to the trade off between turbulence and adequate fluid distribution.

1. Introduction

Expanded bed adsorption (EBA) has the potential to markedly simplify existing downstream processing purification trains by fusing three classical steps – clarification, concentration and initial purification – into one unit operation [1–5]. In EBA, adsorbent particles with a defined size and density distribution are fluidized by a mobile phase directed upwards to form a stable 'classified' fluidized bed, which is commonly termed an expanded bed [4,6–9]. Central to the performance of the EBA system is that axial mixing is low and that the void fraction is increased which allows the application of crude bioprocess liquors such as culture broths or cell homogenates without the risk of blocking the chromatographic bed [4,6,7,9,10]. Under ideal conditions the adsorbent beads move little relative to each other and fluid moves through the bed in a laminar flow type way, giving rise to a number of theoretical plates in the column and chromatographic behaviour that is analogous to that of a packed bed [7,10]. In order to maximize plate numbers, dead zones and channelling should be avoided and the amount of axial dispersion minimized. The type of distributor employed within a fluidized bed column can have very significant effects on the resulting chromatographic performance of the system given that the latter is inextricably linked to the prevailing mixing conditions [4,10]. Perforated plate distributors give suitable hydrodynamic properties in columns up to 60 cm in diameter [9,11] and larger [12], however, are prone to severe fouling and consequent cleaning in place (CIP) difficulties [4,13,14].

In view of these constraints on EBA, alternative types of fluid distributors have been sought and a rotating fluid distributor (RFD) prototype, has been demonstrated in expanded bed columns of up to 150 cm internal diameter [15–17]. In the only reported studies with such a large column, Hubbuch et al. [15,16] studied hydrodynamic performance under different operational conditions and showed by applying a rotating fluid distributor that a parabolic flow pattern suitable for EBA processing was obtained and up to 29 theoretical plates were measured. Those authors also showed that the hydrodynamic conditions within the column are very susceptible to small changes in rotation rate of the distributor. The optimal condition needed for the lowest value of axial dispersion was obtained

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at a rotation rate of 2.5 rpm. Since the report of Hubbuch et al. [16], Feuser et al. [18] and Nilsson [19] both studied the performance of EBA columns (Streamline Direct) with diameters of 2.4–60 cm equipped with a fluid distribution system which oscillated through ca. 180° (*i.e.* not rotating) and concluded that a robust scale up could be achieved as well as suitable CIP.

Despite the above successes, alternative fluid distribution systems have also been studied. After the report by Hubbuch et al. [16], Clemmitt and Chase [20] reported stable expansion was obtained by applying a stirred distribution system in a 1 cm column and compared the performance to that for a sintered distribution system. They showed that shear within the column created by the stirred distribution system was sufficient to prevent the formation of aggregates and bed collapse, whereas applying a sintered distribution system led to formation of aggregates and a large drop in *Saccharomyces cerevisiae* binding capacity (~85%) when a prototype resin made of perfluorocarbon coated by polyvinyl alcohol and functionalized by concanavalin A was used. Subsequently, Menkhaus and Glatz [21] compared the performance of different inlet designs for processing of corn endosperm extract by expanded bed adsorption. They showed that applying a localized mixing and stirring style of distributor allowed corn solids up to 550 μm to enter the column (FastLine 20 column, UpFront Chromatography, Copenhagen, Denmark) without clogging. However, Anspach et al. [4] as well as others [16,22] have suggested that the performance of a distributor based on local stirring for columns larger than those evaluated by Zafirakos and Lihme [23] (*i.e.* >40 cm diameter) is questionable due to the expectation of excessive mixed zones for columns of larger diameters. In the locally stirred design, flow is introduced into the column through one or more inlets located around the column wall [23]. Zafirakos and Lihme [23] argued that stable bed expansion above the mixed zone created by the stirrer led to efficient adsorption of *Lens culinaris* agglutinin by UFC MIMO (mixed mode ligand) beads from an extract of crushed red split lentils under the conditions tested. They also showed that increasing the column diameter from 2 cm to 40 cm increased the ratio of the mixed zone height to expanded bed height from 12.5% to 20%, which in turn led to lower numbers of theoretical plates [23]. To date, no examinations of the hydrodynamic properties, of EBA columns above 40 cm in diameter employing localized stirring appear to have been reported and no concrete data has been presented to support the contention that they cannot be scaled up.

Given that publications appeared after the introduction of the rotating fluid distributor by Hubbuch et al. [16], which suggested the suitability of locally stirred columns for EBA (*e.g.* [20,21]) we have conducted a systematic investigation of the hydrodynamic properties of industrial scale columns fitted with both types of distributors under the same conditions. Using dye and acetone tracers, the effects of rotation rate and flow rate in the columns on dead zones, turbulent and mixing zones and on hydrodynamic parameters are examined. The effect of distributor oscillation period is also examined in the 30 cm diameter commercial EBA column.

2. Experimental

2.1. Materials

Underivatized EBA chromatography matrix consisting of 1–4 silica beads surrounded by cross-linked agarose (density 1.3–1.5 kg L^{-1} and size distribution 100–300 μm) was donated by UpFront Chromatography. Bromophenol blue and acetone were supplied by Sigma (St. Louis, MO, USA). Tap water was used to fluidize the supports in all experiments.

2.2. Columns employing a rotating fluid distributor

A FastLine 300 EBA column (30 cm diameter) from UpFront Chromatography was used (designated here as RFD30). The glass column was equipped with a controllable rotating or oscillating fluid distribution system fixed to a stainless steel bottom. The drive shaft for the distributor was a tube and passed through the base plate and ended in a boss that screwed into the central distribution chamber, from which was connected eight cylindrical arms ~2 cm above the base plate, and with two holes (facing down) in each. Water was pumped by a peristaltic pump (624U, Watson-Marlow Bredel Pumps, Falmouth, UK) through a tube that was split in two parts: one connected to the drive shaft tube and another one to a space underneath the base surrounding the drive shaft. Thus the flow was introduced to the column through both the drive shaft tube (and the arms connected to that) and in the middle of the column around the drive shaft (Fig. 1A). In total, eight holes were located in the tips of the distributor arms and eight more holes were located either 3 cm or 7 cm from the distributor centre (Fig. 1A–C). Liquid was removed from the column via a floating adaptor and peristaltic pump.

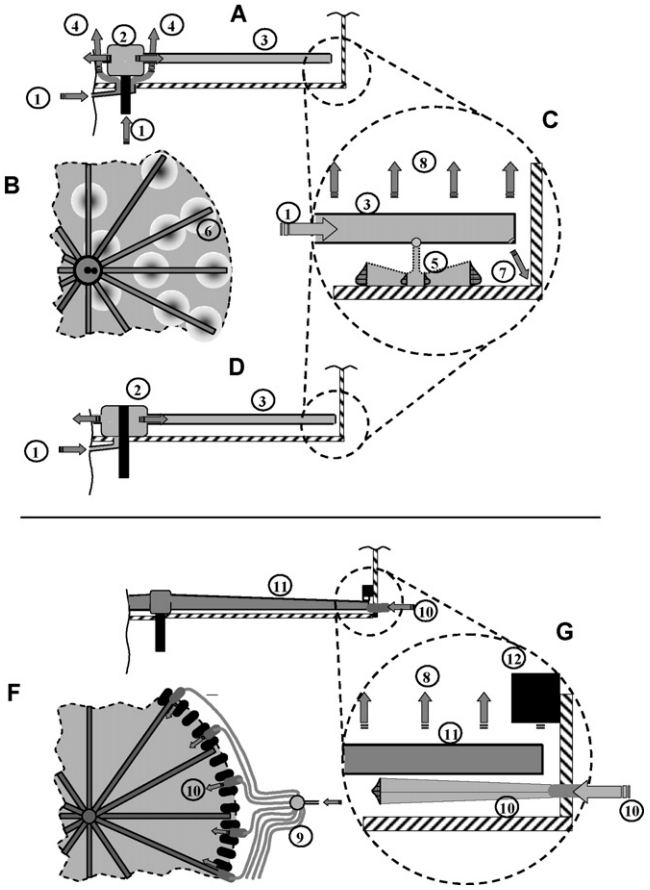

Fig. 1. Schematic of the fluid distribution systems examined. Upper panel: RFD30 and RFD150. Lower panel: LS60 and LS150. (A) Side view of the RFD30, (B) top view of the bottom of the columns (C) expanded side view of the distributor tips and column walls, (D) side view of the RFD150, (E) side view of the LS60 and LS150 columns, (F) top view of LS columns and manifold system, (G) expanded side view of LS distributor. Legend: 1, fluid entry into the distributor; 2, distributor central chamber; 3, hollow distributor arm; 4, liquid flow around the RFD30 central chamber; 5, liquid jet leaving the distributor blade and impacting column base; 6, zones from fluid jets impacting floor of the RFD150 column; 7, fluid jet from end of RFD150 distributor; 8, fluid rising up through the column; 9, manifold on LS150 column; 10, fluid jet entering the LS columns; 11, stirrer; 12, baffle.