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End-to-end integrated fully continuous production of recombinant monoclonal antibodies

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1. Introduction

Historically, conversion from batch to continuous processing has resulted in lean, fully automated and agile manufacturing regardless of the nature of an industry (Anderson, 2001; Fletcher, 2010; Laird, 2007; Reay et al., 2013; Tanner, 1998; Thomas, 2008). This conversion has enabled these industries to overcome key business continuity challenges by reducing manufacturing OPEX, CAPEX, and enhancing portability (Utterback, 1994). The Biologics industry is facing similar challenges due to the emergence of competition from biosimilars, cost pressures due to declining industry growth (Kamarck, 2006; Warikoo et al., 2012), and the desire to gain access to emerging markets through standardization and portability. To leverage the lessons learned from other industries, there has been significant interest in the implementation of continuous bioprocessing (Godawat et al., 2012; Konstantinov, 2010; Vogel et al., 2002; Warikoo et al., 2012). This strategic shift is due to the convergence of both the business drivers and advances in downstream technology. Although, the Biologics industry has extensive experience in continuous (perfusion) upstream processing, there are limited examples of downstream continuous processing due to the lack of enabling disruptive technologies (Godawat et al., 2012; Heeter and Liapis, 1996; Konstantinov and Cooney, 2014a; Lacki and Bryntesson, 2004). However, the past few years have seen maturation of separation technologies such as Simulated Moving Bed (SMB) (Grabski and Mierendorf, 2009), Aqueous Two Phase Systems (ATPS) (Azevedo et al., 2009; Oelmeier et al., 2010; Rosa et al., 2013), single pass TFF (Dizon-Maspat et al., 2011), high capacity membrane absorbers (Orr et al., 2013), and continuous viral inactivation (Caillet-Fauquet et al., 2004; Lorenz et al., 2009). At present, the confluence of these emerging technologies and the business need to innovate is catalyzing the development of continuous bioprocessing.

The Biologics industry is highly regulated to ensure patient safety and, therefore, delivery of consistent product quality is the principal goal of biomanufacturing processes. Inherently, however, the dynamic nature of the Biologics process results in variable product quality as a function of culture days in both perfusion and batch cell culture processes (Pacis et al., 2011; Reid et al., 2010; Robinson et al., 1994). Mohan et al. have specifically shown degradation effects of protease being minimized in continuous-flow cultures as opposed to batch processes (Mohan et al., 1993). Previously, we have demonstrated that a steady state perfusion cell culture process integrated with a continuous capture step can minimize this variability (Warikoo et al., 2012) (Fig. 1); however, in that study, post-capture downstream processing was performed in batch mode. Further benefits of continuous bioprocessing can be realized by developing an end-to-end continuous bioprocess

Abbreviations: Brx, Bioreactor; ATF, Alternating tangential filtration; CAPEX, Capital expenditure; DS, Drug substance; OPEX, Operational expenditure; SMB, Simulating moving bed; ATPS, Aqueous two phase systems; PCC, Periodic counter-current chromatography; mAb, Monoclonal antibody.

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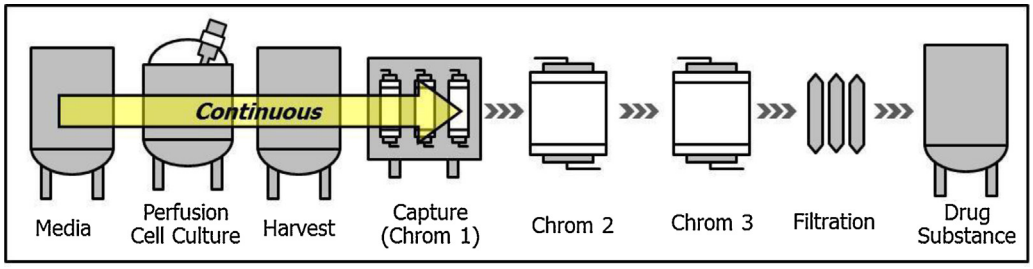

Fig. 1. Continuous upstream + capture, batch downstream.

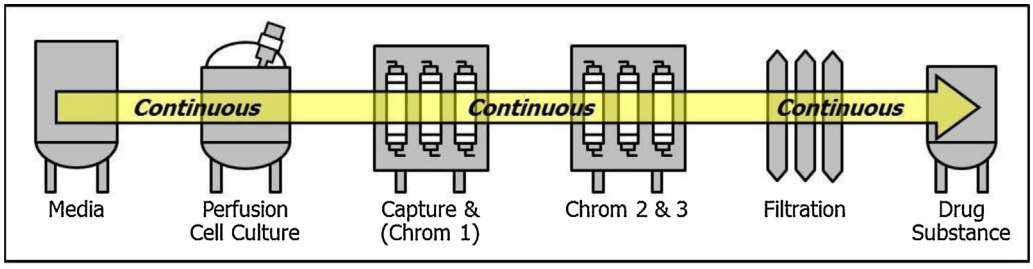

Fig. 2. End-to-end continuous upstream and downstream.

(Fig. 2, (Konstantinov and Cooney, 2014b)). We envision a fully integrated process from bioreactor to formulated drug substance (DS) that is closed, bioburden-free, fully automated with global process control, and operable for long durations. The process should consist of a high cell density perfusion bioreactor connected to a cell retention device, where the cell-free harvest from the bioreactor is continuously captured by a multi-column SMB chromatographic skid. Further, the output from the capture step will be processed downstream by one or more continuous purification steps that are all integrated with one another. The continuous process should also include a concentration and diafiltration step (for example single pass TFF), viral inactivation, viral filtration and sterile filtration. The inputs (raw materials) and outputs (eluates/filtrates) from each unit operation will be constantly monitored using Process Analytical Technology (PAT) to ensure sufficient traceability and in-process control.

We have used an incremental approach toward the development of fully integrated continuous and closed bioprocessing platform envisioned above. Previously, the continuous flow only included upstream and capture step (Fig. 1). This study demonstrates the feasibility of end-to-end continuous bioprocessing using a mAb as a model protein (Fig. 2). Fully continuous downstream processing can be achieved by any combination of technologies capable of facilitating continuous flow such as SMB in its various modes of operation, membrane adsorbers, precipitation, etc (Hammerschmidt et al., 2015; Sá Gomes and Rodrigues, 2011; Warikoo et al., 2012; Xie et al., 2002). In this study, to develop an integrated and end-to-end continuous process for the production

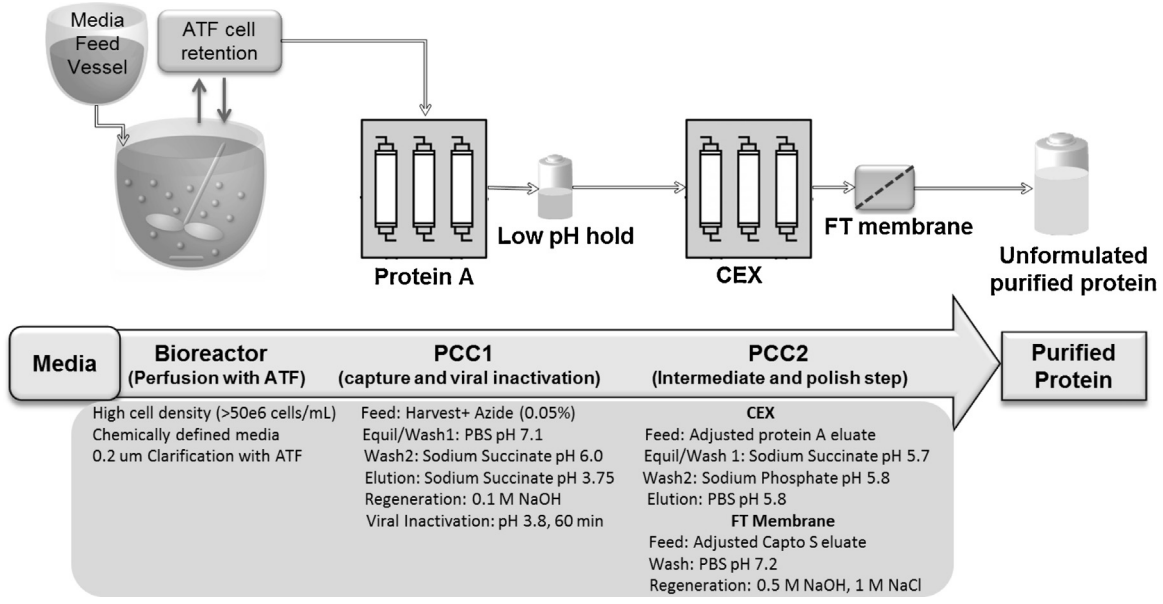

Fig. 3. Architecture of end-to-end continuous bioprocessing. The architecture includes a perfusion bioreactor with ATF as cell retention device for upstream processing. The downstream process included two 4-Column PCC systems. Each PCC system performed two distinct unit operations continuously. The upstream and downstream unit operations were sized and integrated to run in a continuous manner. The details of each unit operation are provided in the shaded region.

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