Contents lists available at ScienceDirect

Food and Bioproducts Processing

journal homepage: www.elsevier.com/locate/fbp

Dynamic modelling of aqueous two-phase systems to quantify the impact of bioprocess design, operation and variability

Nehal Patel[a,b], Daniel G. Bracewell[a], Eva Sorensen[b,*]

[a] Department of Biochemical Engineering, University College London, Bernard Katz Building, Gordon Street, London, WC1E 6BT, UK

[b] Department of Chemical Engineering, University College London, Torrington Place, London, WC1E 7JE, UK

ARTICLE INFO

Article history:
Received 21 April 2017
Received in revised form 15 October 2017
Accepted 16 October 2017

Keywords:
Aqueous two-phase extraction
ATPS
Equilibrium stage modelling
Multi-cycle batch
Continuous
Parameter estimation

ABSTRACT

Aqueous two-phase extraction (ATPE) is a promising downstream separation technology as an alternative, or addition, to chromatography in the production of biological products. Increasing demand for therapeutic proteins have triggered manufacturers to consider continuous upstream technologies to achieve greater process efficiencies; however, such technologies have an inherent variability, resulting in output streams of varying compositions and properties. It is therefore important to understand how this variability impacts on the downstream separation processes.

Exploring all potential sources of variability is challenging due to resource and time constraints, however, the use of targeted mathematical modelling can significantly reduce the need for expensive and time consuming experimentation. In this work, we present a dynamic equilibrium stage process model, and a methodology for prediction of key process parameters from limited experiments, capable of describing ATPE separations under both multi-cycle batch and continuous counter-current modes of operation. The capabilities of the proposed methodology are demonstrated using a case study separation of the enzyme α-amylase from impurities in a PEG 4000–phosphate aqueous two phase system (ATPS) containing NaCl. The model can be used to predict the separation performance of the process, as well as for the investigation of suitable design and operating conditions.

1. Introduction

The market for therapeutic proteins is currently increasing at a remarkable pace (Ecker et al., 2015). To cope with the increasing product and patient demands, drug developers are looking to take advantage of continuous manufacturing technologies for greater efficiencies. A continuous process needs to consistently produce product of a high quality throughout its entire period of operation, and regardless of process disturbances and changes. This is, however, a difficult task to achieve as the biological complexity of the cells used in upstream culture/fermentation is inherently variable. For instance, Valente et al. (2015) recently showed that as Chinese hamster ovary (CHO) cells age, the profile of hard to remove host cell proteins (e.g. impurities with similar separation behaviour to the product) changes. In addition to such inherent variability, other sources of disturbances must also be evaluated, such as equipment failure, human error, contamination etc. Understanding the impact of process changes on whole bioprocess performance is important as product quality could be compromised if the process is not sufficiently robust. Unfortunately, it is often costly and time consuming to evaluate all sources of variability experimentally. One solution to this issue is to use predictive process models to simulate the behaviour of systems under varying conditions, such as

* Corresponding author.
E-mail address: e.sorensen@ucl.ac.uk (E. Sorensen).
https://doi.org/10.1016/j.fbp.2017.10.005

Nomenclature

Notation

$\beta_{i,i}$	Binary interaction parameter between component i and component i (kg mol^{-1})
$\beta_{i,j}$	Binary interaction parameter between component i and component j where i ≠ j (kg mol^{-1})
μ_i	Chemical potential of component i (J mol^{-1})
i	Component index
$C_{i,q,n}$	Concentration of component i in stream number q in stage n (kg m^{-3}) where q is top or bottom
$\rho_{q,n}$	Density in q in stage n (kg m^{-3}) where q is top or bottom
R	Gas constant (J mol^{-1} k^{-1})
$F_{q,n}$	Mass flow of stream q in stage n (kg s^{-1}) where q is stream 1–4
$x_{i,q,n}$	Mass fraction of i in q in stage n where q is either top, bottom, overall or stream 1–4
$M_{i,q,n}$	Mass holdup of i in q in stage n where q is overall, top or bottom (kg)
m_i	Molality of component i (mol kg^{-1})
V_3	Molar volume of water (m^3 mol^{-1})
NC	Number of components
NS	Number of stages
$K_{impurities,n}$	Partition coefficient of impurities in stage n
$K_{\alpha-amylase,n}$	Partition coefficient of α-amylase in stage n
$Ø_n$	Phase ratio in stage n
n	Stage index
μ_1^0	Standard chemical potential of i (J mol^{-1})
q	Location index i.e. top, bottom, overall or stream 1–4
T	Temperature (K)
$w_{i,q,n}$	Weight percent of component i in q in stage n where q is overall, top or bottom (wt%)

used in the chemical process industries for tasks such as troubleshooting, design, optimisation etc. (Chen and Mathias, 2002; Biegler and Grossmann, 2004; Klatt and Marquardt, 2009; Hendriks et al., 2010; Mathias, 2014).

ATPE is a promising alternative technology to the chromatographic technologies normally used for the separation of proteins in biopharmaceutical processing (Rito-Palomares, 2004; Soares et al., 2015). ATPE is a liquid–liquid extraction technology where two phases are formed when either two hydrophilic polymers, or a polymer and a salt, are mixed together in the presence of water above a critical concentration. Proteins and other solutes partition between the two phases based on their thermo-physical properties (Asenjo and Andrews, 2011).

Soares et al. (2015) recently conducted a SWOT (strengths, weaknesses, opportunities and threats) analysis of ATPE. A key weakness identified was the lack of predictive design, as well as expertise, in validation and operation of such two-phase processes. This weakness can be partly addressed by effective process models, but only if thermodynamic behaviour is captured adequately. Thermodynamic models allow for properties, such as densities and equilibrium phase compositions, to be determined without the need to perform extra experiments, provided thermodynamic model parameters are accurate. Often, these parameters are derived from experimental phase equilibria data using parameter estimation protocols. The application of thermodynamics to describe the non-ideal ATPS phase equilibria has been summarised well by Cabezas (1996), however, there are more recent advances, such as the use of ePC-SAFT equations of state (Reschke et al., 2014). Thermodynamic models used for ATPS phase equilibria calculations often ignore the contributions of complex proteins due to the complexity in representing large biomolecules. Empirical correlations describing protein partitioning for such systems therefore have to be obtained partly from experimental data to ensure the required model accuracy. Unfortunately, the use of detailed thermodynamics in process models used to simulate the ATPS processes has, to the best of our knowledge, so far not been considered in the literature.

Secondly, and of equal importance, the dynamic behaviour of a system must be taken into account in order to consider process variability and disturbances. Such issues are important when attempting to understand and control continuous processes which operate for long periods of time. To date, process modelling of ATPSs has been limited mainly to continuous steady-state systems (Mündges et al., 2015; Prinz et al., 2014; Samatou et al., 2007; Huenupi et al., 1999; Mistry et al., 1996). Although the dynamics of continuous operation for control of such continuous systems has been investigated by Simon and Gautam (2004), their study resulted in a system description which was not uniquely defined (−2 degrees of freedom) and where phase equilibria was characterised by empirical relationships.

To tackle these issues, we present an approach based on a dynamic stage-by-stage equilibrium model that can be used to simulate ATPE processes under a variety of configurations and operating policies. Liquid–liquid equilibria data available from literature is used to estimate interaction parameters for the thermodynamic equations used in the process model. The approach permits a fast, yet systematic, investigation of both process design and process operation for the separation of biomolecules using ATPE. The approach is demonstrated by considering dynamic continuous counter-current, as well as multi-cycle batch, modes of operation for a case study involving the separation of enzyme α-amylase from impurities in a PEG 4000–phosphate ATPS in the presence of NaCl. The level of modelling detail is deliberately kept low, partly to reduce the complexity of the equation system and partly due to the limited availability of experimental data from literature, however, we discuss in detail how the level of modelling complexity can be increased.

2. Mathematical methods

The modelling approach used in this work is illustrated in Fig. 1. A general single stage dynamic equilibrium process model is used to describe both the multi-cycle batch and the counter-current mode of operation as the fundamental physical and chemical behaviour is the same in each mode. The system consists of a number of components: (1) those characterising the two phases, either two hydrophilic polymers, or a polymer and a salt, together with water, and (2) the desired protein and other biological material (in the following denoted impurities) from the upstream fermentation stage. Chemical potential is used to describe the phase equilibria between the two aqueous phases and the thermodynamic parameters required are obtained from experimental data. Empirical correlations are used to represent the more complex behaviour of protein partitioning, as currently no thermodynamic prediction methods exist which can accurately describe this behaviour. The use of correlations to describe the behaviour of the protein and that of the impurity reduces the complexity in describing the system when considering mixtures composed of a single desired protein product plus many impurities, as is often the case when dealing with primary recovery after cell culture/fermentation. The overall equation system is solved simultaneously using gPROMS ModelBuilder 4.1 (Process Systems Enterprise, 2017).

2.1. Assumptions

In developing the process model, a number of assumptions had to be made. Most of these assumptions are similar to those