Chemical Engineering Science 144 (2016) 58–74

Contents lists available at ScienceDirect

Chemical Engineering Science

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

Characterizing bubble column bioreactor performance using computational fluid dynamics

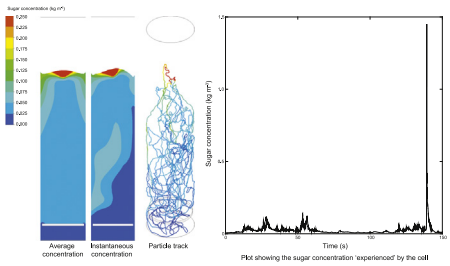
Dale D. McClure [a], John M. Kavanagh [a], David F. Fletcher [a,*], Geoffrey W. Barton [a]

[a] School of Chemical and Biomolecular Engineering, The University of Sydney, NSW 2006, Australia

HIGHLIGHTS

- Kinetics of microbial growth implemented in CFD model.
- Lagrangian particle tracking used to quantify impact of reactor design.
- A range of metrics were used to quantify impact of bioreactor design on performance.
- Impact of substrate addition point on yield examined.
- Approach developed can be used for design and optimisation of bioreactors.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 April 2015
Received in revised form
11 December 2015
Accepted 1 January 2016
Available online 22 January 2016

Keywords:
Bubble column
CFD
Bioreactors
Bioprocess
Scale-up

ABSTRACT

In this work we have coupled microbial kinetics with a Computational Fluid Dynamics (CFD) model of the hydrodynamics within a bubble column. *Saccharomyces cerevisiae* was used as a model organism due to its well characterized kinetics. A range of methodologies was used to quantify the impact of reactor design on performance. These methodologies included those based on the average substrate concentration, those based on the instantaneous substrate concentration, as well as those based on Lagrangian particle tracking. By using the particle tracking approach it was possible to quantify the duration of any oscillations in substrate concentration that the cells experience, a question of key physiological relevance. It was found that the relative yield as calculated using both approaches depended on the sugar addition location. Values of the relative yield between 75% and 93% were calculated based on the average concentration approach, 73–81% based on the instantaneous concentration approach; while values of 93–97% were calculated using particle tracking. Overall, the results from this work clearly demonstrate the potential of using CFD to characterize the complex and highly dynamic behaviour occurring in bubble column bioreactors.

1. Introduction

Bubble columns are gas-liquid contactors which are widely used in the bio-processing industry to produce a range of products (Kantarci et al., 2005). A key consideration in any industrial bioprocess is maximising the yield (typically defined as the mass of product produced per mass of substrate added). It has been observed that as a process is scaled-up the yield decreases; the magnitude of this reduction has been reported to be 6–7% for the production of baker's yeast (*Saccharomyces cerevisiae*) (George et al., 1998) and approximately 20% for the production of recombinant protein using *Escherichia coli* (Enfors et al., 2001).

This difference in yield has been attributed to substrate gradients which are caused by poor mixing. Such gradients can lead to the cells being exposed to high substrate concentrations which can trigger overflow metabolism in many industrially relevant microorganisms. For example, in the case of *S. cerevisiae* production,

* Corresponding author. Tel.: +61 2 9351 4147.
E-mail address: david.fletcher@sydney.edu.au (D.F. Fletcher).

http://dx.doi.org/10.1016/j.ces.2016.01.016
0009-2509/

exposure to high sugar concentrations leads to the production of ethanol (the Crabtree effect), which in turn leads to a reduction in yield (George et al., 1998). Similar behaviour occurs with *E. coli* with acetate being the typical product of overflow metabolism (Xu et al., 1999). As noted elsewhere (Kavanagh and Barton, 2008), acetate can inhibit the production of recombinant protein; hence, it is desirable to avoid conditions which lead to acetate production.

Exposure to zones where the substrate concentration is very low may lead to starvation, which also negatively affects the yield. Additionally, it has been shown (Hewitt and Nienow, 2007) that exposure to fluctuating environmental conditions can induce the stress response in *E. coli*, which again results in a reduction in the yield.

A number of authors (Bylund et al., 1999; Sweere et al., 1988; Xu et al., 1999) have examined the impact of exposing cells to fluctuating conditions; work that has been reviewed by Lara et al. (2006) and Neubauer and Junne (2010). Such approaches provide insight into the problems associated with substrate gradients; however, they offer little insight as to how the design or operation of large-scale reactors can be improved so as to minimise yield loss.

In order to begin to address this industrially important issue, several authors (Morchain et al., 2014; Vetter, 2009; Vrábel et al., 2001) have examined coupling a Computational Fluid Dynamics (CFD) model with relevant microbial kinetics, with the aim of developing a meaningful model of bioprocesses.

A key difficulty in any bioprocess modelling is the need to account for a wide range of time-scales. For example a typical fed-batch fermentation for the production of *S. cerevisiae* is of the order 12–18 h (Vetter, 2009); while the maximum specific growth rate of yeast is around 0.5–0.6 h^{-1} (Blanch and Clark, 1997) (meaning that the cell concentration will double approximately every 70 min). Scale-down studies performed at the laboratory scale have shown that exposing yeast to fluctuating environmental conditions with circulation times of the order 30–60 s is sufficient to cause an increase in by-product formation, as well as a drop in biomass (Lara et al., 2006). In comparison, characteristic relaxation times for bubbles in two-phase flow are of the order 1×10^{-3} s (Braun, 2012). Simulating behaviour across such a range of time-scales (seven orders of magnitude) represents a significant challenge.

One possible method of addressing this issue has been used by Vetter (2009) where a CFD model is coupled with a separate model of biological growth. In this 'sequential co-simulation' approach, the flow-patterns predicted by the CFD model are used to calculate the local concentrations of all relevant species (e.g. oxygen, glucose, ethanol, etc.). This information is then used by the biological model to calculate the rates of growth and consumption, with the resultant information being returned to the CFD model. A major advantage of this approach is that it is possible to simulate the entirety of a fed-batch fermentation, something which is too computationally demanding if the growth kinetics are incorporated directly into the CFD model. The reason for this is that relatively small (of the order 1×10^{-3} s) time-steps are needed to simulate the fluid flow, while typical fermentations can be 12–18 h in duration. Vetter (2009) divided an 18 h fermentation into four increments, obtaining excellent agreement with his experimental results. It was also noted that computational efficiency was a key consideration in the simulation of industrial systems; such a conclusion having also been reached by others (Noorman, 2011).

An alternative approach has been used by Morchain et al. (2014) to model growth in a stirred-tank bioreactor. In their work, they have introduced a population balance model for the biomass to account for the different concentrations experienced by the cells. In this approach, the local specific growth rate is decoupled from the population specific growth rate, meaning that the impact of poor substrate distribution can be accounted for in the model. By calculating the difference between local and global specific growth rates, it is possible to identify zones where the cells are exposed to excess or insufficient substrate concentrations.

In their work Lapin et al. (2006) developed an Euler–Lagrange model of *E. coli* growth in a stirred tank where the movement of cells throughout the reactor is tracked. The key advantage of this approach is that it is possible to quantify the degree to which the cells are exposed to fluctuating environmental conditions, such knowledge being very useful in quantifying the effect of reactor design on performance (i.e. yield).

A range of methodologies exist by which the performance of a bioreactor can be quantified. Hence, the key aim of the present work is to build upon these existing approaches and examine their potential as tools for quantifying the impact of reactor design on performance. We will focus on examining a *S. cerevisiae* fermentation; for the reason that it is a commonly used microorganism with well characterized genetics and kinetics.

2. Model set-up

2.1. CFD set-up

Here we have extended an existing CFD model of bubble column hydrodynamics that has been validated against a comprehensive experimental data set including measurements of local hold-up profiles, profiles of the gas and liquid velocity, bubble size distribution and mixing time (as a function of tracer addition and measurement location) (McClure et al., 2014b, 2014c, 2015a).

The system modelled was a pilot-scale bubble column 0.39 m in diameter and 2 m in height as shown schematically in Fig. 1. Air was introduced through a 'tree' type sparger, the centerline of which is located at a height (z) of 0.135 m, at a superficial velocity of 0.16 m s^{-1}.

In order to quantify the impact of reactor design, substrate was introduced in three different ways. The first substrate addition location (injection point 1) was located at the column centerline at a height (z) of 1.5 m; this position being chosen as substrate addition at the free surface is commonly employed. Injection point 2 was positioned at the column centerline, 0.05 m above the base of the column (i.e. below the sparger), with this position being selected on the basis that this is the most poorly mixed region of the column (McClure et al., 2015a). Addition below the sparger is unlikely to be used in any practical set-up, however, this location was chosen as it was felt that it would provide an interesting point of comparison with the more commonly used approach (i.e. adding substrate at the free surface). Finally, sugar was introduced as a volume source uniformly throughout the column, such an approach corresponding to perfect mixing whereby the substrate introduced is instantaneously and uniformly dispersed throughout the column. In all cases, the sugar was introduced at the same rate ($S_{Feed} = 1.1 \times 10^{-3}$ kg s^{-1}). Concentrations (i.e. of sugar, cells, etc.) are calculated on the basis of the liquid-phase volume (and not the two-phase volume) as is physically correct. The three sugar tracers have been introduced as separate scalars, with the kinetics of uptake being calculated for each individual scalar. All of the scalars share the same flow field; hence any predicted differences in sugar concentration and product yield are only a function of the addition point.

The approach used to solve the fluid-flow inside the reactor will be described here briefly; a more detailed description (including the relevant equations) has been presented in Appendix A. Here, we have used the Euler–Euler approach to model the two-phase flow; with ANSYS CFX 15.0 being used to solve the