Vaccine 33 (2015) 6752–6756

Contents lists available at ScienceDirect

Vaccine

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

The use of dissolved oxygen-controlled, fed-batch aerobic cultivation for recombinant protein subunit vaccine manufacturing

Patrick Farrell*, Jacob Sun, Paul-Philippe Champagne, Heron Lau, Meg Gao, Hong Sun, Arno Zeiser, Tony D'Amore

Bioprocess Research and Development, Sanofi Pasteur, 1755 Steeles Avenue West, Toronto, Canada M2R 3T4

ARTICLE INFO

Article history:
Received 20 April 2015
Received in revised form
24 September 2015
Accepted 14 October 2015
Available online 27 October 2015

Keywords:
Recombinant protein
Fed-batch cultivation
Scale-down

ABSTRACT

A simple "off-the-shelf" fed-batch approach to aerobic bacterial cultivation for recombinant protein subunit vaccine manufacturing is presented. In this approach, changes in the dissolved oxygen levels are used to adjust the nutrient feed rate (DO-stat), so that the desired dissolved oxygen level is maintained throughout cultivation. This enables high *Escherichia coli* cell densities and recombinant protein titers. When coupled to a k_La-matched scale-down model, process performance is shown to be consistent at the 2 L, 20 L, and 200 L scales for two recombinant *E. coli* strains expressing different protein subunit vaccine candidates. Additionally, by mining historical DO-stat nutrient feeding data, a method to transition from DO-stat to a pre-determined feeding profile suitable for larger manufacturing scales without using feedback control is demonstrated at the 2 L, 20 L, and 200 L scales.

1. Introduction

Escherichia coli based expression systems are widely used for the production of recombinant proteins for therapeutic applications and vaccines. Typically, fed-batch cultivation approaches are used to grow the recombinant *E. coli* biomass as they enable higher cell densities compared to those obtained from batch cultivations. The growth phase is then followed by an induction phase for the expression of the target recombinant protein.

A variety of fed-batch approaches have been employed in order to achieve high *E. coli* cell densities and recombinant protein titers, including pre-determined feeding profiles (e.g. constant feed-rate, exponential feed-rate, or making stepwise changes in the feed-rate) and feedback control based on real-time measurements of pH (i.e. pH-stat method), dissolved oxygen levels (i.e. DO-stat method), glucose concentration, or CO_2 evolution rate [1–3].

When developing fed-batch cultivation processes for different recombinant *E. coli* strains, a key disadvantage of methods that do not use feedback control is that they require an iterative approach to cultivation process development in order to generate a successful feeding profile. In contrast, fed-batch cultivation using feedback control can be applied as an "off-the-shelf" cultivation process that does not require extensive tailoring for different strains, media, or the recombinant protein target.

We previously reported the use of a scaled-down model coupled with a pH-stat fed-batch *E. coli* cultivation process to supply recombinant protein vaccine candidates for Phase I and II clinical studies [4]. However, when the biomass reached a cell density where its oxygen uptake rate (OUR) exceeded the bioreactor's oxygen transfer rate (OTR), pH-stat was unable to maintain a constant DO level. In another study, we found that the oxygen limitation resulting from the use of pH-stat had a detrimental impact on the quality of the recombinant protein expressed from an *E. coli* K12 host strain [5].

Considering the importance of maintaining a constant DO level during cultivation, a DO-stat fed-batch control strategy was investigated. DO-stat offers the advantage of maintaining the DO level at the set-point during cultivation, even at high cell densities, by controlling the nutrient feed rate to ensure that the biomass OUR does not exceed the OTR of the bioreactor. Furthermore, maintaining the DO level through the use of DO-stat was shown to improve the quality of the recombinant protein expressed from an *E. coli* K12 host strain in a previous study [5]. In the current study, we demonstrate the advantage of DO-stat over pH-stat for fed-batch cultivations for the early-stage clinical supply of 2 different vaccine candidates at the 2 L, 20 L, and 200 L scales. All cultivations used the same volumetric oxygen mass transfer coefficient (k_La) to improve the process performance consistency across scales [4].

Abbreviations: OUR, oxygen uptake rate (mmol O_2/L/s); OTR, oxygen transfer rate (mmol O_2/L/s); DO, dissolved oxygen (% of air saturation); k_La, volumetric mass transfer coefficient, (s^{-1}); OD_{600}, optical density at 600 nm (units); PID, proportional, integral, derivative.

* Corresponding author.
E-mail address: patrick.farrell@sanofipasteur.com (P. Farrell).

http://dx.doi.org/10.1016/j.vaccine.2015.10.071
0264-410X/

In larger-scale bioreactors, a combination of greater environmental heterogeneities and longer mixing times [6] may not provide sufficiently accurate real-time bulk DO measurements required for stable DO-stat control. Similarly, other fed-batch approaches relying on accurate real-time bulk environmental measurements for feedback control, such as pH or glucose concentration, may also be unable to maintain stable control of the culture. Instead, a robust pre-determined feeding profile would be preferable for large-scale cultivations since it does not rely on feedback control. Here, an approach is demonstrated to leverage a pre-determined feeding profile from historical small-scale DO-stat cultivation data, which can be scaled-up with minimal iterations of process development.

2. Materials and methods

2.1. Expression strains

The two expression strains that were used in this study were in different stages of clinical production. For the initial development of the DO-stat fed-batch control process, a recombinant *E. coli* BL21(DE3) strain harboring a pET28a plasmid was used for the expression of a 92 kDa recombinant protein vaccine candidate. For further development of DO-stat and the establishment of pre-determined continuous feeding profiles in preparation for larger-scale manufacturing, a recombinant *E. coli* K12 strain harboring an IPTG inducible expression plasmid was used for the production of a 41 kDa vaccine candidate.

2.2. Cultivation conditions

Cultivation conditions and pH-stat fed-batch control were described previously for the 2 L, 20 L, and 200 L scales [4].

For cultivations using DO-stat, cultures were operated in batch mode until a rise in pH, following the exhaustion of the initial supply of glucose, triggered the initiation of a supplemental nutrient feed comprising glucose, salts, and yeast extract. pH was controlled by the addition of 5 M NH_4OH. The nutrient feed rate was adjusted by a proportional, integral, derivative (PID) controller to maintain the DO level at the set-point according to the following closed-loop control equation in the Batch Expert control software (ILS Automation):

$$u(t) = K\left(e(t) + \frac{1}{T_i}\int_0^t e(t)\,\mathrm{d}t + T_d\frac{\mathrm{d}e(t)}{\mathrm{d}t}\right)$$

where u is the control signal (i.e. nutrient feed rate), e is the control error ($e = \mathrm{DO}_{\text{set-point}} - \mathrm{DO}_{\text{measured}}$), and K, T_i, and T_d are the proportional gain, integral time, and derivative time, respectively. The PID controller was tuned according to the frequency response variant of the Ziegler-Nichols method [7].

Recombinant protein expression was typically induced at an optical density (OD_{600}) of 40 units by the addition of 1 mM IPTG, after which cells were incubated for an additional 6 h. Temperature was controlled at 30 °C for the growth phase, while the expression temperature was set at either 36 °C or 30 °C for the strains expressing the 92 kDa and 41 kDa vaccine candidates, respectively. Air flow rates were described previously [4] and, in order to match the k_La at the different bioreactor scales, constant impeller rotational speeds of 880 rpm, 580 rpm, and 500 rpm were used with DO-stat at the 2 L, 20 L, and 200 L scales, respectively.

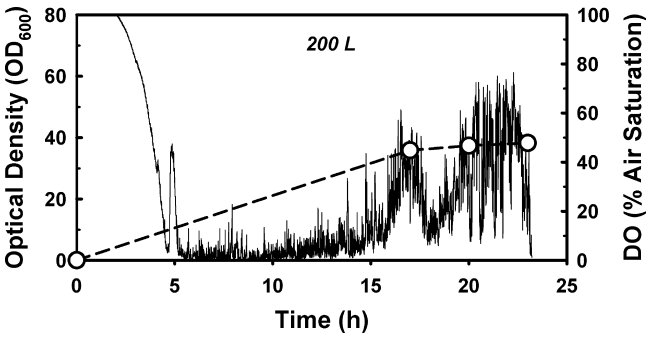

Fig. 1. Cell density (OD_{600}) and dissolved oxygen (DO) concentration for a 200 L cultivation of recombinant *E. coli* BL21(DE3) strain expressing the 92 kDa vaccine candidate when operated using a pH-stat fed-batch control scheme.

2.3. Analytical methods

Culture OD_{600} were measured offline using a spectrophotometer set at a wavelength of 600 nm. In one experiment, an online biomass probe (Optek) was used to continuously record optical densities directly in the bioreactor at near-infrared wavelengths and was calibrated to convert the measurements to the equivalent OD_{600}. Reversed-phase HPLC was used to determine the titer of the 92 kDa vaccine candidate. A Caliper LabChip® GXII system (PerkinElmer) was used to determine the titer of the 41 kDa vaccine candidate.

3. Results and discussion

3.1. pH-stat fed-batch control is unable to maintain DO levels throughout a cultivation

In a previous study using the recombinant *E. coli* K12 strain expressing the 41 kDa vaccine candidate, it was shown that the pH-stat fed-batch control scheme could not effectively control the DO level in a 200 L bioreactor in order to maintain an aerobic environment throughout the cultivation period [4]. In agreement with these earlier findings, the pH-stat control scheme struggled to supply sufficient oxygen to a 200 L cultivation of the recombinant *E. coli* BL21(DE3) strain expressing the 92 kDa vaccine candidate after approximately 5 h (Fig. 1). The impact of reduced oxygen availability is a lower growth rate and possibly cell death. As a result of a reduction in growth rate, a putative loss in cell viability, and the subsequent reduction in oxygen demand by the culture, the DO levels increased after approximately 15 h. This allowed the culture to maintain the ability to produce recombinant protein at approximately 17 h for a 6 h duration following induction with IPTG.

3.2. DO-stat fed-batch control can maintain DO-levels throughout the cultivation period at a scale up to 200 L

Considering the importance of maintaining a constant DO level throughout the culture period, a scale-down *E. coli* cultivation model designed to obtain consistent cultivations from small- to moderate- scales by k_La matching [4] was used to explore two different fed-batch approaches: profile feeding and DO-stat. Following a preliminary evaluation, DO-stat was selected for its ability to maintain a consistent physiological environment in the bioreactor with minimal experimentation, while the pre-determined feeding profile strategy was found to be an inefficient process development approach due to the iterative requirement associated with developing a robust feeding profile.

After an initial batch phase that allowed the biomass to reach a sufficient cell density in the bioreactor, a signature pH increase that