Journal of Biotechnology 164 (2013) 531–542

Contents lists available at SciVerse ScienceDirect

Journal of Biotechnology

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

Short-term dynamic behavior of *Escherichia coli* in response to successive glucose pulses on glucose-limited chemostat cultures

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ARTICLE INFO

Article history:
Received 11 October 2012
Received in revised form
22 December 2012
Accepted 14 January 2013
Available online 30 January 2013

Keywords:
Repeated glucose pulses
Dynamic behavior
Transient responses
Escherichia coli yciG::luxCDABE fusion
Chemostat

ABSTRACT

The effect of repeated glucose perturbations on dynamic behavior of *Escherichia coli* DPD2085, *yciG::LuxCDABE* reporter strain, was studied and characterized on a short-time scale using glucose-limited chemostat cultures at dilution rates close to 0.18 h^{-1}. The substrate disturbances were applied on independent steady-state cultures, firstly using a single glucose pulse under different aeration conditions and secondly using repeated glucose pulses under fully aerobic condition. The dynamic responses of *E. coli* to a single glucose pulse of different intensities (0.25 and 0.6 g L^{-1}) were significantly similar at macroscopic level, revealing the independency of the macroscopic microbial behavior to the perturbation intensity in the range of tested glucose concentrations. The dynamic responses of *E. coli* to repeated glucose pulses to simulate fluctuating environments between glucose-limited and glucose-excess conditions were quantified; similar behavior regarding respiration and by-product formations was observed, except for the first perturbation denoted by an overshoot of the specific oxygen uptake rate in the first minutes after the pulse. In addition, transcriptional induction of *yciG* promoter gene involved in general stress response, σ^S, was monitored through the bioluminescent *E. coli* strain. This study aims to provide and compare short-term quantitative kinetics data describing the dynamic behavior of *E. coli* facing repeated transient substrate conditions.

1. Introduction

With the increasing size of bioreactors, it has been stated that mass and heat transfers often become limiting phenomena due to poor mixing efficiency, leading to heterogeneities within large-scale bioreactors (Enfors et al., 2001; Hewitt and Nienow, 2007; Palomares et al., 2010). Substrate gradients are then commonly encountered, which can create and/or enlarged other gradients such as dissolved oxygen, pH and temperature (Amanullah et al., 2001; Bylund et al., 1998; Enfors et al., 2001). In such complex conditions, cell populations would have different metabolic behaviors depending on the nature, the intensity and the frequency of the microenvironment encountered by the cells circulating inside the bioreactor. Consequently, the resulting distribution of cell phenotypes can then engender performance variability in terms of yield, titer and/or productivity in industrial bioreactors comparing to laboratory-scale bioreactors (Enfors et al., 2001; Lara et al., 2006a).

Up to now, studies dealing with cell behavior in response to defined environmental stress have been carried out, using mainly the scale-down approach due to reduced experimental costs and to efficient control of the physico-chemical parameters inside laboratory-scale bioreactors. Recent reviews present the different "scale-down" configurations implemented to study the impact of environmental heterogeneities on microbial behavior cultivated in bioreactors (Lara et al., 2006a; Neubauer and Junne, 2010; Takors, 2012). Various nature of environmental stresses have been investigated, e.g. changes in dissolved oxygen concentrations (Kar et al., 2010; Lara et al., 2006b; Oosterhuis and Kossen, 1984), in pH (Amanullah et al., 2001), and substrate concentrations (Bylund et al., 1999; Delvigne et al., 2009; Hoque et al., 2005; Larsson et al., 1996; Taymaz-Nikerel et al., 2011). Among these studies, pulse-based experiments (substrate or pH) are usually carried out inside or outside the bioreactor in a mixing chamber as being reviewed in detail by Schädel and Franco-Lara (Schadel and Franco-Lara, 2009). Single substrate pulse experiments are commonly conducted in order to investigate metabolic shifts regarding intracellular and extracellular metabolites as well as gene expression (Hoque et al., 2004; Lara et al., 2009; Taymaz-Nikerel et al., 2009).

Only few studies have been conducted to assess the frequency effect of successive substrate perturbations on cell responses.

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http://dx.doi.org/10.1016/j.jbiotec.2013.01.014

Pickett and co-workers, using square-wave glucose perturbations on chemostat cultures have studied the impact of frequency and amplitude on growth and macromolecular composition of *E. coli*. The time-scale of both perturbations and analyses of the microbial response were in the range of few hours contrary to pulse-based perturbations. Furthermore, no kinetic values regarding growth, respiration, substrate uptake and metabolites production were determined (Pickett et al., 1979, 1980). More recently, substrate oscillation experiments were performed using a stirred tank reactor operating in fed-batch mode coupled or not to a plug flow reactor (Delvigne et al., 2009; Jazini and Herwig, 2011; Lin and Neubauer, 2000; Neubauer et al., 1995b). In such experimental design, the characterization of transient response of the microorganisms is subjected to both the cell history and to the presence of subpopulation at different physiological states.

In a previous study (Sunya et al., 2012a), we investigated the effect of the intensity of single glucose perturbation on *E. coli* behavior at both macroscopic and transcriptional levels. We demonstrated that the macroscopic response of *E. coli* cells to glucose perturbation was independent on the glucose pulse intensities within the tested range (0.08, 0.4 and 1 g L^{-1}). On the contrary, the response at the transcriptional level was affected by the intensity of perturbation.

To our knowledge, the characterization, pulse after pulses, of the transient dynamic behavior to repeated substrate perturbations, of a physiologically homogenous population, has not been documented in literature. In the present study, we then focused on the characterization of the dynamic behavior of the bioluminescent *E. coli* DPD2085 strain by carrying out successive glucose pulses into independent steady-state glucose-limited chemostat cultures. This study aims (i) to describe the dynamics of *E. coli* responses when they are exposed for the 1st, 2nd, 3rd and 4th times to the same perturbation, and (ii) to elucidate the impact of substrate oscillation on a longer period on *E. coli* behavior, and (iii) to give access to precise kinetics data on a timescale of seconds to hours.

2. Materials and methods

2.1. Bacterial strain, medium and growth conditions

The strain *E. coli* DPD2085 which contains an *yciG::luxCDABE* plasmid-based fusion was obtained from DuPont company (USA). Plasmid/strain construction was previously described (Sunya et al., 2012b; Van Dyk et al., 1998b; Van Dyk and Rosson, 1998a; Van Dyk et al., 2001; Zanzotto et al., 2006). Culture conditions and medium composition were performed as previously reported (Sunya et al., 2012a). All chemical products used in this study (glucose, salts, oligo-elements, orthophosphoric acid and NH_3) were of the highest grade commercially available.

2.2. Fermentation

Batch and chemostat experiments were performed in a 1.6 L stainless-steel stirred tank bioreactor with a 1 L working volume (BIOSTAT® Bplus, Sartorius, Germany). Reactor configuration and sensor equipments as well as pre-culture steps were as previously described (Sunya et al., 2012a). During chemostat runs, the stirring rate was fixed at 1000 rpm and the aeration rate at 0.5 L min^{-1} with dry air through a sparger placed at the bottom of the reactor in order to ensure a fully aerobic condition. Neither agitation nor aeration rates were modified during chemostat runs. The pressure in the bioreactor headspace was regulated at 0.06 bars (relative pressure) for facilitating the rapid sampling system as previously described (Sunya et al., 2012a). pH value was maintained at 6.70 and regulated by addition of 7% (v/v) NH_3 solution. 1 mL antifoam (Polypropylene glycol) and 150 mg ampicillin were periodically added (pulse-based addition) by means of a peristaltic pump to maintain a constant concentration in the bioreactor for a given residence time. All continuous cultures were carried out at dilution rate close to 0.18 h^{-1}.

2.3. Glucose pulse experiments

Shifts in the glucose concentration from glucose-limited to glucose-excess conditions were achieved by injecting sterilely, within a second, a concentrated glucose solution inside the bioreactor to 0.25 and 0.6 g L^{-1} final concentrations. The injected volume was less than 0.5% of the total working volume. For each concentration, the experiments were carried out with single, two- and four-successive glucose pulses into independent steady-state runs. The time between successive pulses (pulse period) corresponded to 2.7 and 5.1 min for the glucose pulses of 0.25 and 0.6 g L^{-1} respectively. This pulse period corresponds to the time required for the prior pulse to be dissipated and the prior baseline to be retained. The choice of frequency was dictated by the time necessary for the complete depletion of the glucose from the former pulse in order to be able to characterize the kinetic parameters in well-controlled environment avoiding the concomitant presence of residual and newly added substrate. Rapid sampling of culture broth for glucose and organic acid analyses was initiated before and after the glucose pulse. Rapid sampling system and quenching were performed as previously described (Sunya et al., 2012a). For the successive pulse experiments, the sampling frequency was reduced in order to avoid a significant change in the culture volume inside the bioreactor.

2.4. Glucose oscillating experiments

In order to create fluctuating environmental conditions between glucose-limited and glucose-excess conditions, two intermittent feed strategies were applied by means of an external peristaltic pump (functioning with a timer) in two independent chemostat cultures: (i) to create glucose oscillations of 0.05 g L^{-1}, the peristaltic pump was settled to turn "on" for 2 s every 3.5 min; (ii) to create glucose oscillations of 0.5 g L^{-1}, the peristaltic pump was settled to turn "on" for 20 s every 20 min. A 300 g L^{-1} glucose-concentrated solution was rigorously prepared and sterilized for these both experiments. The peristaltic pump was beforehand calibrated and the established flow rate was 5.0 mL min^{-1}. During the glucose oscillations, the medium feed was maintained in order to avoid glucose starvation.

2.5. Analytical methods

Glucose and organic acids were analyzed and quantified by high-performance ionic chromatography. The composition of inlet/outlet gas (O_2 and CO_2) was quantified using an acoustic gas analyzer (INNOVA 1313). All procedures and precisions of these apparatus were followed, according to previously described analytical methods (Sunya et al., 2012a).

Biomass concentration was determined from both optical density measurement at 620 nm (OD_{620nm}) and dry cell weight measurement (X). Biomass composition was determined by elemental analysis of C, H, O and N on *E. coli* DPD2085 cells during steady states at 0.16 h^{-1}. This analysis was subcontracted to the Central Service of Analysis (SCA) of the CNRS (http://www.sca.cnrs.fr) which meets the requirements of ISO9001 and ISO17025. In addition, the numbers of plasmid-bearing cells and total cells were also quantified using a plate count technique onto Plate Count Agar (PCA) with and without 150 mg L^{-1} ampicillin (Sunya et al., 2012a), in order to check for a homogeneous population of plasmid-bearing cells along chemostat runs. Fraction