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On-Line Detection of Acetate Formation in *Escherichia coli* Cultures Using Dissolved Oxygen Responses to Feed Transients

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Abstract: Recombinant protein production in *Escherichia coli* can be significantly reduced by acetate accumulation. It is demonstrated that acetate production can be detected on-line with a standard dissolved oxygen sensor by superimposing short pulses to the substrate feed rate. Assuming that acetate formation is linked to a respiratory limitation, a model for dissolved oxygen responses to transients in substrate feed rate is derived. The model predicts a clear change in the character of the transient response when acetate formation starts. The predicted effect was verified in fed-batch cultivations of *E. coli* TOPP1 and *E. coli* BL21(DE3), both before and after induction of recombinant protein production. It was also observed that the critical specific glucose uptake rate, at which acetate formation starts, was significantly decreased after induction. On-line detection of acetate formation with a standard sensor opens up new possibilities for feedback control of substrate feeding. *Biotechnol Bioeng* **64**: 590–598, 1999.
Keywords: *Escherichia coli;* acetate; on-line detection; dissolved oxygen; transients; mathematical model

INTRODUCTION

Escherichia coli is one of the most frequently used host organisms for recombinant protein production. Fed-batch cultivation is a common method to obtain high cell densities and thereby high productivity. One of the problems encountered is the formation of byproducts such as acetate. Accumulation of acetate has been reported to inhibit growth (Luli and Strohl, 1990) and to reduce recombinant protein production (Bauer et al., 1990; Bech Jensen and Carlsen, 1990). To reduce or avoid acetate formation, a number of substrate feeding strategies have been developed; see Lee (1996) and Yee and Blanch (1992).

A typical problem in monitoring and control of microbial cultivations is that many important process variables cannot be measured on-line. This has triggered much research and development concerning new sensors, see for instance (Schügerl et al., 1996). Another way of addressing the problem is to improve and to extend the use of existing sensors (Wang et al., 1977; Stephanopoulos and San, 1984). In this paper we will demonstrate how a standard dissolved oxygen sensor can be used for on-line detection of undesirable acetate formation. The key idea is to exploit a characteristic change in the relation between oxygen uptake and glucose uptake at the onset of acetate formation. This change can be detected by superimposing short pulses in the glucose feed rate. An attractive feature is that no assumptions are required on parameters like stoichiometric coefficients.

ACETATE PRODUCTION

Formation of acetate, when *E. coli* is grown under fully aerobic conditions, typically occurs at high growth rates and/or high glucose uptake rates. The acetate production is thought of as an overflow phenomenon where flux of AcetylCoA is directed to acetate, via acetylphosphate, instead of entering the TCA cycle. In batch and continuous cultivations, it was observed that the specific oxygen uptake rate reached an apparent maximum at the onset of acetate formation (Andersen and von Meyenburg, 1980; Reiling et al., 1985). It was suggested that the respiratory system, where NADH is reoxidized, has a limited capacity. As flux to the TCA cycle results in NADH production and as flux to acetate does not, redirection of AcetylCoA flux to acetate would be necessary to avoid accumulation of NADH when the respiration saturates. Another explanation that has been suggested is that the TCA cycle has a limited capacity and that this limitation is reached before that of the respiration (Majewski and Domach, 1990). When the TCA cycle saturates, increasing glucose uptake will again result in flux from AcetylCoA to acetate. In this case, NADH production and respiration can increase further until the maximum res-

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piration capacity or the maximum glucose uptake is reached. The experiments presented in Paalme et al. (1997), however, indicate that a respiratory limitation is more likely.

In Majewski and Domach (1990), a flux network over parts of the central metabolic pathways was used to derive relations between triose flux and acetate production for the two explanations mentioned above. Assuming that the cells tend to maximize ATP production, a constrained optimization problem was formulated. Acetate production was predicted when constraints in the respiration or the TCA cycle were reached. These ideas were extended in (Ko et al., 1993, 1994; Varma et al., 1993) where the flux models cover larger parts of the metabolism and also describe acetate formation due to oxygen limitation.

SIMULATION MODEL

We will now derive a model for how dissolved oxygen in a bioreactor responds to transients in the feed rate. The purpose is to obtain a good description in a time scale of seconds to minutes. First, considerations on the cell level are used to derive relations between glucose uptake, acetate production, growth rate, and oxygen consumption. These relations are then incorporated into a macroscopic model of a bioreactor.

Metabolic Relations

From the analysis in Majewski and Domach (1990), it is straightforward to compute also the corresponding NADH flux for a respiratory limitation. Assuming that the oxygen consumption is proportional to the NADH production and that the glucose flux is proportional to the triose flux, relations between glucose uptake, acetate production, growth rate, and oxygen consumption can be obtained. Qualitative results are shown in Fig. 1. When the glucose uptake, q_g, exceeds a critical level, q_g^{crit}, acetate formation starts and the oxygen uptake saturates. Concomitantly, there is a decreased yield from glucose to cell mass. Similar relations result from the models in (Ko et al., 1993, 1994; Varma et al., 1993).

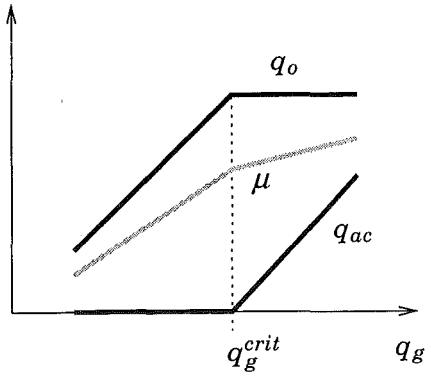

Figure 1. Relations between specific glucose uptake, q_g, specific oxygen uptake, q_o, specific growth rate, μ, and specific acetate production, q_{ac}.

The relation between specific glucose uptake, q_g, and specific oxygen uptake, q_o, can be represented as

$$q_o(G) = \begin{cases} q_g Y_{og}, & q_g < q_g^{crit}, \\ q_g^{crit} Y_{og}, & q_g \geq q_g^{crit}, \end{cases}$$

with the yield constant Y_{og}. Similarly, the specific growth rate, μ, can be described as

$$\mu(G) = \begin{cases} q_g Y_{xg}^{ox}, & q_g < q_g^{crit}. \\ q_g^{crit} Y_{xg}^{ox} + (q_g - q_g^{crit}) Y_{xg}^{fe}, & q_g \geq q_g^{crit}. \end{cases}$$

The specific glucose uptake, q_g, is taken to be of Monod type

$$q_g(G) = q_g^{max} \frac{G}{k_s + G},$$

which describes a smoothly saturating glucose uptake.

Bioreactor Model

Assuming that the expressions for oxygen uptake, growth rate, and glucose uptake are valid in a time scale of seconds, these are inserted in a dynamic model of a bioreactor in fed-batch mode. Component-wise mass balances for the bioreactor give the following equations:

$$\frac{dV}{dt} = F,$$

$$\frac{d(VX)}{dt} = \mu(G) \cdot VX,$$

$$\frac{d(VG)}{dt} = FG_{in} - q_g(G) \cdot VX,$$

$$\frac{d(VC_o)}{dt} = K_L a(N) \cdot V(C_o^* - C_o) - q_o(G) \cdot VX,$$

where V, X, G, and C_o are, respectively, the liquid volume, the cell concentration, the glucose concentration, and the dissolved oxygen concentration. Further, F, G_{in}, and C_o^* denote the feed rate, the glucose concentration in the feed, and the dissolved oxygen concentration in equilibrium with the oxygen in gas bubbles. To obtain good mixing in a reactor, the stirrer speed, N, is in practice never below a minimum value. For the considered range of stirrer speeds, the volumetric oxygen transfer coefficient, $K_L a$, is approximated as an increasing linear function of the stirrer speed.

In practice, most sensors do not measure the oxygen concentration but rather the dissolved oxygen tension. The dissolved oxygen tension O is related to the dissolved oxygen concentration through Henry's law

$$O = H \cdot C_o.$$

It is also important to consider the dynamics in the dissolved oxygen probe. It is here modeled as a first-order system with time constant T_p,

$$T_p \frac{dO_p}{dt} + O_p = O,$$

which is a reasonable approximation under normal turbulence levels (Dang et al., 1977).

KEY IDEA AND SIMULATIONS

The simulation model is now employed to illustrate that a standard dissolved oxygen probe can be used to detect acetate formation. The values of the model parameters that are used are found in Table I together with initial values for cell mass and volume. To obtain constant specific glucose uptake rates, exponentially increasing feed rates are used in the simulations. This also causes an increased oxygen demand and the stirrer speed is therefore increased exponentially to avoid trends in dissolved oxygen.

The key idea in the detection method is to exploit the characteristic change in the relation between oxygen uptake and glucose uptake at the onset of acetate formation. This change, and hence acetate formation, can be detected by superimposing short pulses in the glucose feed rate. Under glucose-limited conditions the feed pulses give rise to changes in the glucose uptake. These changes imply variations in the oxygen uptake that can be seen in the dissolved oxygen measurement.

Figure 2 shows simulations for increasing specific glucose uptake rates: below, at, and above the onset of acetate formation. Below acetate formation, a clear response in dissolved oxygen is seen to both up and down pulses in the glucose feed rate F, see Fig. 2a. When the onset of acetate formation is reached (see Fig. 2b), the response to an up pulse in F is absent due to the saturation in the specific oxygen uptake. For a glucose uptake above acetate formation, there is also a reduction in the response to a down pulse, Fig. 2c. When the glucose uptake is increased even further, the respiration is completely saturated and no oxygen response will be seen.

It is clear that the pulse responses would reveal if q_g is above q_g^{crit}, and thus if acetate is produced. The validity of the simulation results will now be examined experimentally.

Table I. Parameter values used in simulations.

Parameter	Value	Parameter	Value
q_g^{max}	1.34 g/(gh)	Y_{og}	0.5 g/g
Y_{xg}^{ox}	0.50 g/g	Y_{xg}^{fe}	0.25 g/g
k_s	10 mg/L	H	14,000 (L%)/g
q_g^{crit}	1.0 g/(gh)	G_{in}	500 g/L
O^*	100%	T_p	20 s
$VX(0)$	20 g	$V(0)$	2.0 L

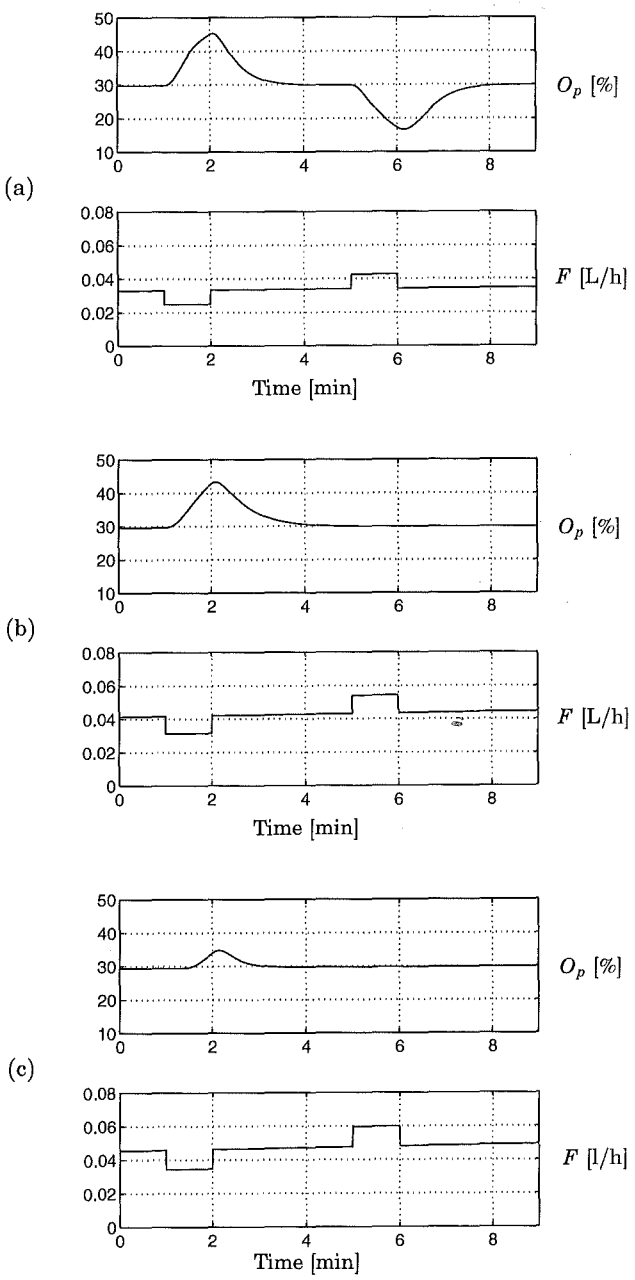

Figure 2. Simulation of responses in measured dissolved oxygen O_p to pulses in feed rate F. (a) Below onset of acetate formation, $q_g = 0.8$ g/(gh) $< q_g^{crit}$. (b) At the onset of acetate formation, $q_g = 1.0$ g/(gh) $< q_g^{crit}$. (c) Above onset of acetate formation, $q_g^{crit} < q_g = 1.1$ g/(gh).

MATERIALS AND METHODS

Microorganisms

Two recombinant *E. coli* strains with different plasmids were used. Experiments without induction of recombinant protein were performed with *E. coli* TOPP1 (Stratagene, La Jolla, CA) carrying a plasmid pHD389 with a protein L gene fragment inserted (Tocaj et al., 1995). The second strain employed was *E. coli* BL21(DE3) (Studier and Moffatt,

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