Unstructured Model for L-Lysine Fermentation under Controlled Dissolved Oxygen

Semsi Ensari,*,† Joon Ha Kim,‡ and Henry C. Lim‡

Biotechnology Development, Schering-Plough Research Institute, 1011 Morris Avenue, U-14-2-20, Union, New Jersey 07083, and Department of Chemical Engineering and Materials Science, University of California−Irvine, Irvine, California 92697

An unstructured model was developed for batch cultivation of *Corynebacterium lactofermentum* (ATCC 21799) under controlled dissolved oxygen. The model is capable of predicting batch experiments performed at various initial substrate concentrations. By extending the batch culture model to a fed-batch model and using a heuristic approach to optimize the fed-batch cultivation, it is shown that fed-batch cultivation is superior to batch operation due to increased productivity at high substrate concentrations.

Introduction

Various modes of operation have been reported for L-lysine, the second most produced amino acid worldwide. Yet, no study has developed an extensive model to predict the optimal productivity. Previously developed empirical fits predict experimental data; however, they cannot be used to further investigate the lysine fermentation due to omission of variables that affect the outcome (*1, 2*). Several studies dealt with the kinetic parameters in continuous and batch cultures but did not provide any kinetic model that is useful for optimization (*2*−*10*). Only Ohno et al. (*11*) in their optimization study developed an overly simplified model. Past studies investigated the dependence of L-lysine fermentation on oxygen (*12*−*15*). Some of the strains were shown to be strongly dependent on the oxygen. Limitation of oxygen caused decreased biomass yield, substrate consumption, and L-lysine production (*15*). In an earlier work, Kiss and Stephanopoulos maintained high yield and specific productivity for extended times through fed-batch cultivation using online respiratory measurements for feed rate control (*16*). In a recent study, an unstructured model was developed by incorporating the dependence of the specific rates of growth, substrate consumption, product formation, and oxygen uptake on the cell, the substrate, the product concentrations and dissolved oxygen (*17*). The model developed proved to be applicable to both steady and unsteady operations of continuous cultivation.

Batch experiments are simple to run and easy to control, but attaining consistent results routinely might become cumbersome. However, dynamic kinetic studies are essential for dynamic behavior of batch and fed-batch cultures. In the present study, batch experiments were run at 50% saturation dissolved oxygen using various initial substrate concentrations. An unstructured model is developed by using the specific rate expressions suggested previously (*17*). As an alternative to batch cultures, in industry, fed-batch cultures are found to be most effective in overcoming glucose effects, substrate inhibition, and catabolite repression. A fed-batch operation may be superior whenever the specific rates of cell growth and/or product formation are nonmonotonic functions of the substrate concentration (*18*). In this study, the specific rates developed using batch culture data are adapted for fed-batch cultures and an intuitive optimization technique is used to show through simulation the superiority of fed-batch operation.

Materials and Methods

Microorganism and Culture Conditions. A detailed description of materials and methods was previously described (*12, 17*). Briefly, *Corynebacterium lactofermentum* (ATCC 21799 AEC^R, Hom$^-$) was cultured in a BioFlo III (2 L working volume; New Brunswick Scientific Co., Inc., Edison, NJ) bioreactor with two six-bladed impellers and four baffles at 30 °C and the pH was maintained at 7.0. The dissolved oxygen level was maintained at 50% saturation by controlling the agitation speed between 450 and 700 rpm with an aeration rate of 1 vvm (2 L/min). The bioreactor was equipped with a condenser to minimize any evaporative losses due to off-gas. The dry cell density was determined from the optical density of culture broth; L-lysine and glucose concentrations were determined by enzymatic methods (*12, 17*).

Batch culture experiments were conducted at six different initial substrate concentrations with dissolved oxygen maintained at 50% saturation. To avoid any nitrogen limitation, the ratio of glucose to ammonium sulfate was kept constant for various initial substrate concentrations. While the batch fermentation is easy to carry out, it is difficult to replicate the same initial conditions from run to run. Therefore, the seed was added

* To whom correspondence should be addressed. E-mail: semsi.ensari@spcorp.com. Phone: (908) 820-6354. Fax: (908) 820-6995.

† Schering-Plough Research Institute.

‡ University of California−Irvine.

10.1021/bp034019s CCC: $25.00
Published on Web 05/31/2003