Available online at www.sciencedirect.com
ScienceDirect
Bioresource Technology 98 (2007) 2599–2604
BIORESOURCE TECHNOLOGY

Substrate consumption and biomass growth of *Ralstonia eutropha* at various S_0/X_0 levels in batch cultures

Jin Wang, Fang Fang, Han-Qing Yu *

School of Chemistry, University of Science and Technology of China, Hefei 230026, China

Received 16 July 2006; received in revised form 9 September 2006; accepted 12 September 2006
Available online 27 October 2006

Abstract

The biomass growth, substrate consumption and polyhydrobutyrate (PHB) production of *Ralstonia eutropha* with butyric acid and fructose as the carbon and energy sources at various ratios of initial substrate concentration (S_0) to initial biomass concentration (X_0) were investigated in this study. Results indicated that the PHB content increased with the increasing S_0/X_0 ratio. Different substrates exhibited a similar trend for cell growth and substrates consumption with the changing S_0/X_0 ratio. The specific consumption rates of both butyric acid and fructose increased with the increasing S_0/X_0 ratio. An S_0/X_0-dependent kinetic model was modified to describe the kinetics of biomass growth and substrate consumption for *R. eutropha*. This model was verified with the experimental results from this work and in literature.

Keywords: Biomass growth; Polyhydroxybutyrate (PHB); *Ralstonia eutropha*; S_0/X_0; Substrate consumption

1. Introduction

Polyhydroxybutyrate (PHB) has attracted considerable interests because of its potential as a renewable and biodegradable plastics, and as a source of chiral synthons since the monomers are chiral (Kessler et al., 2001). On the other hand, it is also an intracellular storage compound that provides a reserve of carbon and energy in many microorganisms (Lee, 1996). *Ralstonia eutropha* is a bacterium used for PHB biosynthesis and prefers accumulating PHB under nitrogen-limited or unbalanced conditions (Gostomski and Bungay, 1996; Muller et al., 1999). Since PHB biosynthesis by *R. eutropha* is partially growth-associated (Yamane et al., 1996; Shahhosseini, 2004) and PHB could be produced from many substrates (Lee, 1996), the biomass growth and substrate selection become an important issue in the ultimate PHB production.

Batch cultures have been usually employed to optimize biological reactions and to evaluate kinetic parameters (Kovarova-Kovar and Egli, 1998; Gupthar et al., 2000; Muller-Feuga et al., 2004; Liu et al., 2005). As two important parameters, initial substrate concentration (S_0) and initial biomass concentration (X_0) have been investigated in many studies (Gaudy and Ramanathan, 1971; Chudoba et al., 1992). However, a great number of experimental results reported could not be compared because of the different operating conditions. To solve this problem, Pitter and Chudoba (1990) suggested that the ratio of S_0/X_0 should be a universal parameter. It is evident that the kinetic behavior of batch cultures could be governed by this ratio and that this ratio could be used to interpret different results obtained in batch experiments (Chudoba et al., 1992; Liu et al., 2005). However, in previous studies only effects of X_0 or S_0 on the PHB production and biomass growth were evaluated (Wang and Yu, 2001), the important roles of S_0/X_0 ratio were not given sufficient attention. Therefore, to describe the biomass growth and PHB production at different ratios of S_0/X_0, series of batch

* Corresponding author. Fax: +86 551 3601592.
E-mail address: hqyu@ustc.edu.cn (H.-Q. Yu).

0960-8524/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2006.09.005

experiments were conducted when butyric acid and fructose were used as the carbon and energy sources for *R. eutropha*. In addition, an S_0/X_0-dependent kinetic model developed by Liu et al. (2005) was modified to describe the kinetics of biomass growth and substrate consumption for *R. eutropha*.

2. Model development

An S_0/X_0-dependent kinetic model was proposed by Liu et al. (2005) to describe microbial growth in batch cultures from process thermodynamics, as shown in Eq. (1).

$$\mu = \mu_m \frac{S_0/X_0}{K_{S/X} + S_0/X_0} \tag{1}$$

in which μ (defined as $\mu = dX/dt/X$) and μ_m are the specific consumption rate of the microorganism and its maximum value, respectively; S_0/X_0 is the ratio of initial substrate concentration to initial biomass concentration; $K_{S/X} = e^{(\Delta G^0 - \Delta G)/RT}$ (ΔG^0 and ΔG are the change of the standard free energy and overall free energy, respectively). In this study, the intracellular polymer PHB was regarded as a part of the whole biomass. In addition, it was assumed that there was no substrate inhibition on cell growth. Thus, Eq. (1) was used to describe the microbial growth with varied S_0/X_0 values in batch cultures here.

However, in biochemical reactions describing microbial growth, if microorganisms and substrates could be regarded as reactants, and the biomass newly synthesized is treated as products, the microbial growth process could be determined by both microbial specific growth rate and substrate consumption rate. Thus, a model to describe the relationship between the substrate consumption kinetics and S_0/X_0 ratio is needed.

Maintenance energy is required by microorganisms to satisfy a multitude of cellular functions, including motility, maintaining ion gradients, actively transporting molecules, regulating internal concentrations, turning over enzymes and macromolecules (Turner et al., 1989) as well as supporting substrate cycles (Tempest and Neijssel, 1984). Furthermore, the free energy change of catabolic reactions is generally tightly coupled to the anabolic steps of cellular biosynthesis, and total energy flux can be partitioned into biomass growth and maintenance function (Russell and Cook, 1995). Since substrate consumption is mainly used for cell growth and maintenance, the specific substrate consumption rate can be expressed as follows:

$$q_S = \frac{1}{Y_{X/S}}\mu + m \tag{2}$$

where $Y_{X/S}$ is the true growth yield which accounts for the devotion of substrate consumed directly for biomass growth.

Substituting Eq. (1) into Eq. (2) yields:

$$q_S = \frac{\mu_m (S_0/X_0)}{Y_{X/S}(K_{S/X} + S_0/X_0)} + m \tag{3}$$

If $q_m = \mu_m / Y_{X/S}$, Eq. (3) could be expressed as:

$$q_S = q_m \frac{S_0/X_0}{K_{S/X} + S_0/X_0} + m \tag{4}$$

where m is the specific rate of substrate consumption for maintenance, and q_S is the specific microbial consumption rate (defined as $q_S = -dS/dt/X$).

Eq. (4) shows that the specific substrate consumption rate is indeed a function of the S_0/X_0 ratio.

In order to estimate the values of the two parameters, $K_{S/X}$ and μ_m, Eq. (1) can be rewritten as:

$$\frac{1}{\mu} = \frac{K_{S/X}}{\mu_m}\frac{1}{(S_0/X_0)} + \frac{1}{\mu_m} \tag{5}$$

μ was determined at the exponential growth phase in batch experiments. According to Eq. (5), a plot of $1/\mu$ against $1/(S_0/X_0)$ should gives a straight line. The slope of this line is equal to $K_{S/X}/\mu_m$, while the intercept is equal to $1/\mu_m$. Moreover, $1/Y_{X/S}$ and m in Eq. (2) could be obtained in a similar way. In addition, the value of the parameter q_m could be obtained readily.

3. Methods

3.1. Strain cultivation

R. eutropha ATCC 17699 was maintained in agar slants of a medium containing (g L^{-1}) 10 yeast extract, 10 peptone, 5 beef extract, and 5 $(NH_4)_2SO_4$. The same medium without agar was used for seed culture. The seed culture was cultivated in 500-ml flasks (100 ml medium in each) on a rotary shaker at 180 rpm. The temperature was kept at 30 °C for 24 h. The cells were harvested through centrifugation at 4000*g* for 10 min, washed with 0.9% NaCl solution and then re-suspended in a mineral solution, which contained carbon source at predetermined concentrations.

The compositions of the mineral solution were (g L^{-1}) 1.2 $MgSO_4$, 1.7 citrate, 13.3 KH_2PO_4, and 10 ml trace element solution [(g L^{-1}): ($FeSO_4 \cdot 7H_2O$ 10, $ZnSO_4 \cdot 7H_2O$ 2.25, $CuSO_4 \cdot 5H_2O$ 2.25, $MnSO_4 \cdot 5H_2O$ 0.5, $CaCl_2 \cdot 2H_2O$ 2, H_3BO_4 0.3, and $(NH_4)_6Mo_7O_{24}$ 0.1)]. The C/N molar ratio in the medium was 60:1. The batch cultures were initiated at various S_0/X_0 ratios by varying S_0 at a fixed X_0, or by changing X_0 while keeping S_0 constant. Different S_0/X_0 ratios were achieved as follows: (1) X_0 was fixed at 0.5 g L^{-1}, S_0 ranged from 0.5 to 8 g L^{-1}; (2) S_0 was kept at 2.5 g L^{-1}, while X_0 varied from 0.3 to 4.0 g L^{-1}. Fructose and butyric acid were used as carbon sources in the culture.

For a typical test, more than ten conical flasks with 80 ml of medium in each were incubated at 30 °C and 200 rpm for 48 h. Samples were taken at given time intervals to measure the concentrations of substrate and dry cell weight (DCW). PHB was determined when its production reached the stationary phase.