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Genetic network driven control of PHBV copolymer composition

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Abstract

We developed a detailed mathematical model describing the coupling between the molecular weight distribution dynamics of poly(3-hydroxybutyrate-co-3hydroxyvalerate) (PHBV) copolymer chains with those of hydroxybutyrate (HB) and hydroxyvalerate (HV) monomer formation. Sensitivity analysis of the model revealed that both the monomer composition and the molecular weight distribution of the copolymer chains are strongly affected by the ratio between the rates at which the two-monomer units are incorporated into the chains. This ratio depends on the relative HB and HV availability, which in turn is a function of the expression levels of genes encoding enzymes that catalyze monomer formation. Regulation of gene expression was accomplished through the aid of an artificial genetic network, the patterns of expression of which can be controlled by appropriately tuning the concentration of an extracellular inducer. Extensive simulations were used to study the effects of operating conditions and parameter uncertainties on the range of achievable copolymer compositions. Since the predicted conditions fell in the range of feasible bioprocessing manipulations, it is expected that such strategy could be successfully employed. Thus, the presented model constitutes a powerful tool for designing genetic networks that can drive the formation of PHBV copolymer structures with desirable characteristics.

Keywords: Poly(3-hydroxybutyrate-co-3-hydroxyvalerate); PHBV; Control; Mathematical modeling; Genetic toggle; Population balance; Molecular weight distribution

1. Introduction

Polyhydroxyalkanoates (PHAs) represent a broad class of polyesters produced by many bacterial species under nutrient-limited conditions (Anderson and Dawes, 1990). Recombinant DNA technology has allowed expressing PHA genes in non-natural producers, thus enlarging the range of suitable microorganisms. PHAs are biodegradable in both aerobic and anaerobic conditions and in different environments (Mergaert et al., 1993, 1995; Tanio et al., 1982) and biocompatible with various human tissues and blood

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Nomenclature

$a_{HB}(x_{HB},t)$ active PHB homopolymer chains
$a_{HV}(x_{HV},t)$ active PHV homopolymer chains
$\hat{a}_{HB}(x_{HB})$ time invariant active PHB homopolymer chains
$\hat{a}_{HV}(x_{HV})$ time invariant active PHV homopolymer chains
$a_{0,HB}$ total number of active PHB chains
$a_{0,HV}$ total number of active PHV chains
$A(x_{HB},x_{HV},t)$ active PHBV copolymer chains
$\hat{A}(x_{HB}, x_{HV})$ time invariant active PHBV copolymer chains
A_0 total number of active PHBV chains
$A_{1,HB}$ HB concentration of active PHBV chains
$A_{1,HV}$ HV concentration of active PHBV chains
$C_{AcAcCoA}$ AcAcCoA intracellular concentration
C_{AcCoA} AcCoA intracellular concentration
C_{Ace} acetate intracellular concentration
C_{ATP} ATP intracellular concentration
C_{CoA} co-enzyme A intracellular concentration
C_{HB} HB intracellular concentration
C_{HV} HV intracellular concentration
C_{NADPH} NADPH intracellular concentration
C_{PrCoA} PrCoA intracellular concentration
C_{Pro} propionate intracellular concentration
C_{ValCoA} ValCoA intracellular concentration
$\hat{C}_{HB}$ time invariant HB intracellular concentration
$\hat{C}_{HV}$ time invariant HV intracellular concentration
e_1 Acs concentration
e_2 prpE concentration
E_{HB} time invariant elongation rate HB addition
E_{HV} time invariant elongation rate HV addition
F PHBV copolymer mass fraction
h_1 P_Ls1con-cIts repressor Hill cooperativity
h_2 Ptrc-2-LacI repressor Hill cooperativity
h_3 IPTG-LacI repressor Hill cooperativity
$i_{HB}(x_{HB},t)$ inactive PHB homopolymer chains
$i_{HV}(x_{HV},t)$ inactive PHV homopolymer chains
$I(x_{HB},x_{HV},t)$ inactive PHBV copolymer chains
$I_{1,HB}$ HB concentration of inactive PHBV chains
$I_{1,HV}$ HV concentration of inactive PHBV chains
IPTG IPTG inducer concentration
k_{Ace} acetate Michealis–Menten constant
k_{Acs} Acs specific activity
k_{ATP} ATP Michealis–Menten constant
k_{CoA} CoA Michealis–Menten constant
$k_{elo,HB}$ homopolymer PHB elongation rate constant
$k_{elo,HV}$ homopolymer PHV elongation rate constant
$k_{ini,HB}$ homopolymer PHB initiation rate constant
$k_{ini,HV}$ homopolymer PHV initiation rate constant
k_{NADPH} NADPH Michealis–Menten constant
k_{prpE} prpE specific activity
k_{Pro} propionate Michealis-Menten constant
K_{IPTG} IPTG-LacI repressor dissociation constant
k_{TCA} TCA cycle flux constant
M PHBV molecular weight distribution
k_{12} P_Ls1con-cIts repressor dissociation constant
k_{21} Ptrc-2-LacI repressor dissociation constant
N_A Avogadro's number
r_{HB} time invariant PHB initiation rate
r_{HV} time invariant PHV initiation rate
p_1 LacI repressor concentration
p_2 cIts repressor concentration
$R_{elo,HB}$ copolymer PHBV elongation rate HB addition
$R_{elo,HV}$ copolymer PHBV elongation rate HV addition
R_{ter} copolymer PHBV termination rate
t Time
t_d doubling time
tr_{HB} time invariant PHB transition rate
tr_{HV} time invariant PHV transition rate
T_1 first copolymer PHBV termination rate constant
T_2 second copolymer PHBV termination rate constant