Optimization of Continuous Bioconversion Process of Glycerol to 1,3-Propanediol

Gongxian Xu*, Dan Wang, Caixia Li

Department of Mathematics, Bohai University, Jinzhou, China
E-mails: gxxu@bhu.edu.cn, 844364936@qq.com, 1126668962@qq.com

**Corresponding author*

Received: November 25, 2016 **Accepted: August 15, 2018**

Published: September 30, 2018

Abstract: *This paper addresses the optimization of continuous bioconversion process of glycerol to 1,3-propanediol (1,3-PD) by Klebsiella pneumoniae. The studied bioprocess is a complex nonlinear system that involves the gene regulation for dha regulon, enzyme-catalytic kinetics on the reductive pathway, the active transport of glycerol and (passive) diffusion of 1,3-PD across the cell membrane, and the inhibition of glycerol dehydratase (GDHt) and 1,3-propanediol oxidoreductase (PDOR) by 3-hydroxypropionaldehy (3-HPA). We first propose a nonlinear optimization model that can maximize the production rate of 1,3-PD. Then the optimal solution of this optimization problem is obtained by using an interior point method. In this approach a sequence of barrier problems are solved iteratively. We finally obtain the maximum production rate of 1,3-PD increased more than 22.86 times its initial value.*

Keywords: *Optimization, Bioconversion process, Continuous bioprocess, Interior point method, 1,3-propanediol, Glycerol.*

Introduction

1,3-propanediol (1,3-PD) has a wide range of potential applications on a large commercial scale [1, 11, 20, 26]. Among all kinds of microbial production of 1,3-PD, bioconversion of glycerol to 1,3-PD has been studied extensively since 1980s due to its relatively high yield and productivity [9, 11, 15, 20, 27]. In recent years, much research has been made to improve the bioconversion process of glycerol [4-6, 8, 9, 11, 15-17, 19, 20-28]. For example, research on the quantitative description of the cell growth kinetics of multiple inhibitions by substrate and products, product formation in continuous culture has been made [22, 26, 27]. The methods of conversion of glycerol into 1,3-PD were reviewed and discussed in [11]. The 1,3-PD production directly from carbon dioxide was achieved by engineered *Synechococcus elongatus* PCC 7942 with a synthetic metabolic pathway in [8]. Wang et al. presented a nonlinear impulsive system for glycerol bioconversion to 1,3-PD in fed-batch cultures based on the dynamical system of batch cultures [19]. Yuan et al. reported the robust identification of enzymatic nonlinear dynamical systems for 1,3-PD transport mechanisms in microbial batch culture [25]. Xiu et al. investigated the optimal conditions of batch and continuous glycerol bioconversion by *Klebsiella pneumoniae* using the volumetric productivity of 1,3-PD as an optimization objective based on a five-dimensional nonlinear system that takes into account the growth kinetics of multiple inhibitions and the metabolic overflow of substrate consumption and product formation [20]. Xu designed a H_∞ controller for bioconversion process of glycerol to 1,3-PD through the H_∞ mixed sensitivity method [23]. Zhu et al. presented a μ robust technique to control the continuous bioconversion process of glycerol to 1,3-PD [28]. Sun et al. set up an eight-dimensional nonlinear system that considers enzyme-catalytic reductive pathway and transport of glycerol and 1,3-PD across cell membrane [16]. A fourteen-dimensional nonlinear dynamic system was proposed

to represent the continuous and batch fermentations of glycerol to 1,3-PD by *Klebsiella pneumoniae*, in which the enzyme-catalytic kinetics on the reductive pathway, the transport of glycerol and diffusion of 1,3-PD across cell membrane, and the inhibition of 3-hydroxypropionaldehyde (3-HPA) to glycerol dehydratase (GDHt) and 1,3-propanediol oxidoreductase (PDOR) are all taken into consideration [17]. But the optimization for this complex bioprocess involving gene regulation and enzyme catabolism has not yet been addressed.

The aim of this paper is to deal with the optimization of continuous bioconversion process of glycerol to 1,3-PD by maximizing the production rate of 1,3-PD. In the following, we first describe the continuous bioconversion of glycerol to 1,3-PD by *Klebsiella pneumoniae*. This is followed by a presentation of nonlinear optimization problem for this bioprocess under steady-state conditions. Then the proposed optimization problem is solved by an interior method. In Section 5, the attained optimization results are presented. Finally, brief conclusions of present work are given.

Continuous bioconversion process of glycerol

In this paper, we use a mathematical model with gene regulation for the *dha* regulon and enzyme-catalytic kinetics for continuous bioconversion process of glycerol to 1,3-PD by *Klebsiella pneumoniae*, in which the expression of gene-mRNA-enzyme-product, the active transport of glycerol and (passive) diffusion of 1,3-PD across the cell membrane, and the inhibition of GDHt and PDOR by 3-HPA are taken into consideration [17]. This mathematical model accounting for the main substances under anaerobic conditions at 37 °C and pH 7.0 is written as follows:

$$\frac{\mathrm{d}M_{\mathrm{R}}}{\mathrm{d}t} = K_{M_{\mathrm{R}}} G_{\mathrm{D}} \frac{[\mathrm{O_t}](C_{3\text{–HPA}} + K_{\mathrm{d}})}{(C_{3\text{–HPA}} + K_{\mathrm{d}}) + rC_{3\text{–HPA}}} - (k_{M_{\mathrm{R}}} + \mu) M_{\mathrm{R}}, \tag{1}$$

$$\frac{\mathrm{d}R}{\mathrm{d}t} = K_{\mathrm{R}} M_{\mathrm{R}} - (k_{\mathrm{R}} + \mu) R - k_{-1} \frac{R_{\mathrm{t}} C_{3\text{–HPA}}}{C_{3\text{–HPA}} + K_{\mathrm{d}}}, \tag{2}$$

$$\frac{\mathrm{d}M_{\mathrm{GDHt}}}{\mathrm{d}t} = K_{\mathrm{m}}^{\mathrm{GDHt}} G_{\mathrm{D}} \frac{[\mathrm{O_t}](C_{3\text{–HPA}} + K_{\mathrm{d}})}{(C_{3\text{–HPA}} + K_{\mathrm{d}}) + rC_{3\text{–HPA}}} - (k_{\mathrm{d}}^{\mathrm{mGDHt}} + \mu) M_{\mathrm{GDHt}}, \tag{3}$$

$$\frac{\mathrm{d}M_{\mathrm{PDOR}}}{\mathrm{d}t} = K_{\mathrm{m}}^{\mathrm{PDOR}} G_{\mathrm{D}} \frac{[\mathrm{O_t}](C_{3\text{–HPA}} + K_{\mathrm{d}})}{(C_{3\text{–HPA}} + K_{\mathrm{d}}) + rC_{3\text{–HPA}}} - (k_{\mathrm{d}}^{\mathrm{mPDOR}} + \mu) M_{\mathrm{PDOR}}, \tag{4}$$

$$\frac{\mathrm{d}[\mathrm{GDHt}]}{\mathrm{d}t} = K_{\mathrm{GDHt}} M_{\mathrm{GDHt}} - (k_{\mathrm{d}}^{\mathrm{GDHt}} + \mu)[\mathrm{GDHt}], \tag{5}$$

$$\frac{\mathrm{d}[\mathrm{PDOR}]}{\mathrm{d}t} = K_{\mathrm{PDOR}} M_{\mathrm{PDOR}} - (k_{\mathrm{d}}^{\mathrm{PDOR}} + \mu)[\mathrm{PDOR}], \tag{6}$$

$$\frac{\mathrm{d}X}{\mathrm{d}t} = (\mu - D) X, \tag{7}$$

$$\frac{\mathrm{d}C_{\mathrm{Se}}}{\mathrm{d}t} = D(C_{\mathrm{SF}} - C_{\mathrm{Se}}) - q_{\mathrm{S}} X, \tag{8}$$

$$\frac{\mathrm{d}C_{\mathrm{Si}}}{\mathrm{d}t} = \frac{1}{V_{\mathrm{S}}} \left[J_{\max} \frac{C_{\mathrm{Se}}}{C_{\mathrm{Se}} + K_{\mathrm{m}}} + \frac{1}{A_{\mathrm{S}}} (C_{\mathrm{Se}} - C_{\mathrm{Si}}) - q_{\mathrm{S}} \right] - \mu C_{\mathrm{Si}}, \tag{9}$$

$$\frac{dC_{3-HPA}}{dt} = k_1[GDHt]\frac{C_{Si}}{K_{mGDHt}\left(1+\frac{C_{3-HPA}}{K_i^{GDHt}}\right)+C_{Si}} - k_2[PDOR]\frac{C_{3-HPA}}{K_{mPDOR}+C_{3-HPA}\left(1+\frac{C_{3-HPA}}{K_i^{PDOR}}\right)}$$

$$-\mu C_{3-HPA} - nk_{-1}\frac{R_t C_{3-HPA}}{C_{3-HPA}+K_d}, \quad (10)$$

$$\frac{dC_{PDi}}{dt} = k_2[PDOR]\frac{C_{3-HPA}}{K_{mPDOR}+C_{3-HPA}\left(1+\frac{C_{3-HPA}}{K_i^{PDOR}}\right)} - K_{PD}\left(C_{PDi}-C_{PDe}\right)-\mu C_{PDi}, \quad (11)$$

$$\frac{dC_{PDe}}{dt} = q_{PD}X - DC_{PDe}, \quad (12)$$

$$\frac{dC_{HAc}}{dt} = q_{HAc}X - DC_{HAc}, \quad (13)$$

$$\frac{dC_{EtOH}}{dt} = q_{EtOH}X - DC_{EtOH}, \quad (14)$$

$$r = \frac{R_t}{K_0}, \quad (15)$$

$$A_S = \frac{\delta}{B\times D_f}, \quad (16)$$

$$\mu = \mu_m \frac{C_{Se}}{K_S+C_{Se}}\left(1-\frac{C_{Se}}{C_{Se}^*}\right)\left(1-\frac{C_{PDe}}{C_{PDe}^*}\right)\left(1-\frac{C_{HAc}}{C_{HAc}^*}\right)\left(1-\frac{C_{EtOH}}{C_{EtOH}^*}\right), \quad (17)$$

$$q_S = m_S + \frac{\mu}{Y_S^m} + \Delta q_S^m \frac{C_{Se}}{C_{Se}+K_S^*}, \quad (18)$$

$$q_{PD} = m_{PD} + \mu Y_{PD}^m + \Delta q_{PD}^m \frac{C_{Se}}{C_{Se}+K_{PD}^*}, \quad (19)$$

$$q_{HAc} = m_{HAc} + \mu Y_{HAc}^m + \Delta q_{HAc}^m \frac{C_{Se}}{C_{Se}+K_{HAc}^*}, \quad (20)$$

$$q_{EtOH} = q_S\left(\frac{0.025}{0.06+DC_{Se}} + \frac{5.18}{50.45+DC_{Se}}\right), \quad (21)$$

where t is the fermentation time, h; M_R and R are the concentrations of mRNA coding repressor and free repressor, respectively, mmol/L; K_{M_R} and K_R represent the rate constants for the formation of mRNA coding repressor and free repressor, respectively, h^{-1}; G_D denotes the gene dosage; $[O_t]$ denotes the concentration of total operator, mmol/L; C_{3-HPA} is the concentration of 3-HPA, mmol/L; K_0 is the dissociation constant of holorepressor-operator, mmol/L; μ is the specific growth rate of cells, h^{-1}; M_{GDHt} and M_{PDOR} represent the mRNA concentrations of coding enzymes GDHt and PDOR, respectively, mmol/L; [GDHt] and [PDOR] are the concentrations of enzymes GDHt and PDOR, respectively, mmol/L; K_m^{GDHt} and K_m^{PDOR} denote the rate constants for the formation of GDHt mRNA and PDOR mRNA, respectively, h^{-1}; K_{GDHt} and K_{PDOR} are the rate constants for formation of GDHt and PDOR, respectively, h^{-1}; X is the biomass, g/L; D represents the dilution rate, h^{-1}; C_{SF} denotes the substrate (glycerol) concentration in feed, mmol/L;

C_{Se} and C_{Si} are the extracellular and intracellular glycerol concentrations in reactor, respectively, mmol/L; q_S represents the specific consumption rate of substrate, mmol/(g·h); C_{PDi} and C_{PDe} denote the intracellular and extracellular 1,3-PD concentrations, respectively, mmol/L; q_{PD} is the specific formation rate of product 1,3-PD, mmol/(g·h); C_{HAc} represents the concentration of product acetate, mmol/L; q_{HAc} denotes the specific formation rate of product acetate, mmol/(g·h); C_{EtOH} is the concentration of product ethanol, mmol/L; q_{EtOH} represents the specific formation rate of product ethanol, mmol/(g·h); δ denotes the cell membrane thickness, mm; B is the surface area of per biomass, mm^2/g; D_f represents the diffusion coefficient of glycerol, L/(mm·h); and the definitions of the remain parameters used in (1)-(21) and their values are listed in Table 1 [17]. The expression for the specific formation rate of ethanol as shown in (21) is drawn from [20, 21].

To transform Eqs. (1)-(6) into the corresponding simple forms the following transformations are carried out:

$$m_R = \frac{M_R}{K_{M_R} G_D [O_t]},$$
$$K_\theta = K_R K_{M_R} G_D [O_t],$$
$$m_{GDHt} = \frac{M_{GDHt}}{K_m^{GDHt} G_D [O_t]},$$
$$K_{GDHt}^m = K_m^{GDHt} K_{GDHt} G_D [O_t],$$
$$m_{PDOR} = \frac{M_{PDOR}}{K_m^{PDOR} G_D [O_t]},$$
$$K_{PDOR}^m = K_m^{PDOR} K_{PDOR} G_D [O_t].$$

By substituting these transformations into Eqs. (1)-(6) we obtain the following forms:

$$\frac{dm_R}{dt} = \frac{(C_{3-HPA} + K_d)}{(C_{3-HPA} + K_d) + rC_{3-HPA}} - (k_{M_R} + \mu) m_R, \quad (22)$$
$$\frac{dR}{dt} = K_\theta m_R - (k_R + \mu) R - k_{-1} \frac{R_t C_{3-HPA}}{C_{3-HPA} + K_d}, \quad (23)$$
$$\frac{dm_{GDHt}}{dt} = \frac{(C_{3-HPA} + K_d)}{(C_{3-HPA} + K_d) + rC_{3-HPA}} - (k_d^{mGDHt} + \mu) m_{GDHt}, \quad (24)$$
$$\frac{dm_{PDOR}}{dt} = \frac{(C_{3-HPA} + K_d)}{(C_{3-HPA} + K_d) + rC_{3-HPA}} - (k_d^{mPDOR} + \mu) m_{PDOR}, \quad (25)$$
$$\frac{d[GDHt]}{dt} = K_{GDHt}^m m_{GDHt} - (k_d^{GDHt} + \mu)[GDHt], \quad (26)$$
$$\frac{d[PDOR]}{dt} = K_{PDOR}^m m_{PDOR} - (k_d^{PDOR} + \mu)[PDOR], \quad (27)$$

where the new parameters K_θ, K_{GDHt}^m, K_{PDOR}^m used in Eqs. (23), (26) and (27) present the values of 0.2004 mmol/(L·h^2), 10.8083 h^{-1}, 24.1030 h^{-1} [17].

Table 1. Definitions, values and units of parameters used in the model (1)-(21)

Parameter	Representation	Value	Unit
K_d	Dissociation constant of holorepressor	917.9722	mmol/L
r	Dimensionless parameter for *dha* regulon	13.1567	–
k_{M_R}	Degradation rate of mRNA coding repressor	0.1096	h^{-1}
k_R	Degradation rate of free repressor	1.1305×10^{5}	h^{-1}
k_{-1}	Dissociation rate constant	79.9150	h^{-1}
R_t	Concentration of total repressor	1.48×10^{-5}	mmol/L
k_d^{mGDHt}	Rate constant for the degradation of GDHt mRNA	1.5462	h^{-1}
k_d^{mPDOR}	Rate constant for the degradation of PDOR mRNA	11.4334	h^{-1}
k_d^{GDHt}	Rate constant for the degradation of GDHt	12.7837	h^{-1}
k_d^{PDOR}	Rate constant for the degradation of PDOR	20.2310	h^{-1}
V_S	Specific intracellular volume in biomass	0.151	L/g
J_{max}	Maximum specific transport rate of substrate	54.664	mmol/(g·h)
K_m	Michaelis–Menten constant of glycerol permease	1.340	mmol/L
A_S	–	1.896×10^{-4}	g/(L·h)
k_1	Catalyze coefficient of GDHt for glycerol	36.3625	h^{-1}
k_2	Catalyze coefficient of PDOR for 3-HPA	42.5261	h^{-1}
K_{mGDHt}	Michealis-Menten constant of enzyme of GDHt	0.53	mmol/L
K_{mPDOR}	Michealis-Menten constant of enzyme of PDOR	0.14	mmol/L
K_i^{GDHt}	Inhibitor constant for 3-HPA to enzyme of GDHt	220.319	mmol/L
K_i^{PDOR}	Inhibitor constant for 3-HPA to enzyme of PDOR	0.418	mmol/L
n	Binding sites	2.0421	–
K_{PD}	Diffusion coefficient for 1,3-PD	25.137	h^{-1}
μ_m	Maximum specific growth rate	0.67	h^{-1}
K_S	Monod saturation constant for glycerol	0.28	mmol/L
C_{Se}^*	Critical concentration of glycerol	2039	mmol/L
C_{PDe}^*	Critical concentration of 1,3-PD	939.5	mmol/L
C_{HAc}^*	Critical concentration of acetate	1026	mmol/L
C_{EtOH}^*	Critical concentration of ethanol	360.9	mmol/L
m_S	Maintenance term of substrate consumption	2.20	mmol/(g·h)
m_{PD}	Maintenance term of 1,3-PD formation	–2.69	mmol/(g·h)
m_{HAc}	Maintenance term of acetate formation	–0.97	mmol/(g·h)
Y_S^m	Maximum growth yield	0.0082	g/mmol
Y_{PD}^m	Maximum 1,3-PD yield	67.69	mmol/g
Y_{HAc}^m	Maximum acetate yield	33.07	mmol/g
Δq_S^m	Maximum increment of substrate consumption rate	28.58	mmol/(g·h)
Δq_{PD}^m	Maximum increment of 1,3-PD formation rate	26.59	mmol/(g·h)
Δq_{HAc}^m	Maximum increment of acetate formation rate	5.74	mmol/(g·h)
K_S^*	Saturation constant for substrate in kinetic equations	11.43	mmol/L
K_{PD}^*	Saturation constant for 1,3-PD in kinetic equations	15.50	mmol/L
K_{HAc}^*	Saturation constant for acetate in kinetic equations	85.71	mmol/L

Optimization problem of continuous bioconversion process of glycerol

In this work, we will propose a steady-state optimization problem of continuous bioconversion process of glycerol to 1,3-PD that can maximize the production rate of extracellular 1,3-PD. The performance index describing the production rate of extracellular 1,3-PD is given directly by DC_{PDe}. The resulting steady-state optimization problem is as follows:

$$\max \quad DC_{\mathrm{PDe}}, \tag{28}$$

subject to satisfying:

$$\frac{(C_{3\text{–HPA}}+K_{\mathrm{d}})}{(C_{3\text{–HPA}}+K_{\mathrm{d}})+rC_{3\text{–HPA}}}-(k_{M_{\mathrm{R}}}+\mu)m_{\mathrm{R}}=0, \tag{29}$$

$$K_{\theta}m_{\mathrm{R}}-(k_{\mathrm{R}}+\mu)R-k_{-1}\frac{R_{\mathrm{t}}C_{3\text{–HPA}}}{C_{3\text{–HPA}}+K_{\mathrm{d}}}=0, \tag{30}$$

$$\frac{(C_{3\text{–HPA}}+K_{\mathrm{d}})}{(C_{3\text{–HPA}}+K_{\mathrm{d}})+rC_{3\text{–HPA}}}-(k_{\mathrm{d}}^{\mathrm{mGDHt}}+\mu)m_{\mathrm{GDHt}}=0, \tag{31}$$

$$\frac{(C_{3\text{–HPA}}+K_{\mathrm{d}})}{(C_{3\text{–HPA}}+K_{\mathrm{d}})+rC_{3\text{–HPA}}}-(k_{\mathrm{d}}^{\mathrm{mPDOR}}+\mu)m_{\mathrm{PDOR}}=0, \tag{32}$$

$$K_{\mathrm{GDHt}}^{\mathrm{m}}m_{\mathrm{GDHt}}-(k_{\mathrm{d}}^{\mathrm{GDHt}}+\mu)[\mathrm{GDHt}]=0, \tag{33}$$

$$K_{\mathrm{PDOR}}^{\mathrm{m}}m_{\mathrm{PDOR}}-(k_{\mathrm{d}}^{\mathrm{PDOR}}+\mu)[\mathrm{PDOR}]=0, \tag{34}$$

$$(\mu-D)X=0, \tag{35}$$

$$D(C_{\mathrm{SF}}-C_{\mathrm{Se}})-q_{\mathrm{S}}X=0, \tag{36}$$

$$\frac{1}{V_{\mathrm{S}}}\left[J_{\max}\frac{C_{\mathrm{Se}}}{C_{\mathrm{Se}}+K_{\mathrm{m}}}+\frac{1}{A_{\mathrm{S}}}(C_{\mathrm{Se}}-C_{\mathrm{Si}})-q_{\mathrm{S}}\right]-\mu C_{\mathrm{Si}}=0, \tag{37}$$

$$k_1[\mathrm{GDHt}]\frac{C_{\mathrm{Si}}}{K_{\mathrm{mGDHt}}\left(1+\frac{C_{3\text{–HPA}}}{K_{\mathrm{i}}^{\mathrm{GDHt}}}\right)+C_{\mathrm{Si}}}-k_2[\mathrm{PDOR}]\frac{C_{3\text{–HPA}}}{K_{\mathrm{mPDOR}}+C_{3\text{–HPA}}\left(1+\frac{C_{3\text{–HPA}}}{K_{\mathrm{i}}^{\mathrm{PDOR}}}\right)}$$

$$-\mu C_{3\text{–HPA}}-nk_{-1}\frac{R_{\mathrm{t}}C_{3\text{–HPA}}}{C_{3\text{–HPA}}+K_{\mathrm{d}}}=0, \tag{38}$$

$$k_2[\mathrm{PDOR}]\frac{C_{3\text{–HPA}}}{K_{\mathrm{mPDOR}}+C_{3\text{–HPA}}\left(1+\frac{C_{3\text{–HPA}}}{K_{\mathrm{i}}^{\mathrm{PDOR}}}\right)}-K_{\mathrm{PD}}(C_{\mathrm{PDi}}-C_{\mathrm{PDe}})-\mu C_{\mathrm{PDi}}=0, \tag{39}$$

$$q_{\mathrm{PD}}X-DC_{\mathrm{PDe}}=0, \tag{40}$$

$$q_{\mathrm{HAc}}X-DC_{\mathrm{HAc}}=0, \tag{41}$$

$$q_{\mathrm{EtOH}}X-DC_{\mathrm{EtOH}}=0, \tag{42}$$

$$0.05\le D\le 0.5, \tag{43}$$

$$0\le C_{\mathrm{SF}}\le 2039, \tag{44}$$

$$0\le m_{\mathrm{R}}\le 1000, \tag{45}$$

$$0\le R\le 1000, \tag{46}$$

$$0\le m_{\mathrm{GDHt}}\le 1000, \tag{47}$$

$$0\le m_{\mathrm{PDOR}}\le 1000, \tag{48}$$

$$0 \le [\text{GDHt}] \le 1000, \quad (49)$$
$$0 \le [\text{PDOR}] \le 1000, \quad (50)$$
$$0.05 \le X \le 5, \quad (51)$$
$$0 \le C_{\text{Se}} \le 2039, \quad (52)$$
$$0 \le C_{\text{Si}} \le 2039, \quad (53)$$
$$0 \le C_{3-\text{HPA}} \le 500, \quad (54)$$
$$0 \le C_{\text{PDi}} \le 939.5, \quad (55)$$
$$0 \le C_{\text{PDe}} \le 939.5, \quad (56)$$
$$0 \le C_{\text{HAc}} \le 1026, \quad (57)$$
$$0 \le C_{\text{EtOH}} \le 360.9. \quad (58)$$

Obviously, the problem (28)-(58) is a nonconvex nonlinear programming with complex constraints. In this optimization problem, equality constraints (29)-(42) are the steady-state conditions, while the inequality constraints (43)-(58) are the lower and upper bounds for the optimized variables D, C_{SF}, m_{R}, R, m_{GDHt}, m_{PDOR}, [GDHt], [PDOR], X, C_{Se}, C_{Si}, $C_{3\text{-HPA}}$, C_{PDi}, C_{PDe}, C_{HAc} and C_{EtOH}, respectively. This set of equality and inequality constraints defines the feasible region of the optimization problem (28)-(58).

Solution method

There are several techniques to solve a bioprocess optimization problem [7, 10, 12-14]. In this work, we will use an interior point method [2, 3, 18] to efficiently solve the proposed nonlinear optimization problem (28)-(58). This approach replaces the optimization problem (28)-(58) by a sequence of barrier subproblems.

We first rewrite the problem (28)-(58) as the following formulation:

min $f(x)$

subject to satisfying:

$$h_j(x) = 0, \; j = 1, 2, ..., 14 \quad (59)$$
$$g_i(x) \le 0, \; i = 1, 2, ..., 32$$

where optimization variable x, objective function $f(x)$, equality constraint functions $h_j(x)$ (j = 1, 2, …, 14) and inequality constraint functions $g_i(x)$ (i = 1, 2, …, 32) have the following forms:

$$x = (x_1, x_2, x_3, x_4, x_5, x_6, x_7, x_8, x_9, x_{10}, x_{11}, x_{12}, x_{13}, x_{14}, x_{15}, x_{16})^{\text{T}}$$
$$= (D, C_{\text{SF}}, m_{\text{R}}, R, m_{\text{GDHt}}, m_{\text{PDOR}}, [\text{GDHt}], [\text{PDOR}], X, C_{\text{Se}}, C_{\text{Si}}, C_{3-\text{HPA}}, C_{\text{PDi}}, C_{\text{PDe}}, C_{\text{HAc}}, C_{\text{EtOH}})^{\text{T}},$$
$$f(x) = -x_1 x_{14},$$
$$h_1(x) = \frac{(C_{3-\text{HPA}} + K_{\text{d}})}{(C_{3-\text{HPA}} + K_{\text{d}}) + rC_{3-\text{HPA}}} - (k_{M_{\text{R}}} + \mu) m_{\text{R}},$$
$$h_2(x) = K_{\theta} m_{\text{R}} - (k_{\text{R}} + \mu) R - k_{-1} \frac{R_{\text{t}} C_{3-\text{HPA}}}{C_{3-\text{HPA}} + K_{\text{d}}},$$

$$h_3(x) = \frac{(C_{3-\mathrm{HPA}} + K_\mathrm{d})}{(C_{3-\mathrm{HPA}} + K_\mathrm{d}) + rC_{3-\mathrm{HPA}}} - (k_\mathrm{d}^{\mathrm{mGDHt}} + \mu)m_{\mathrm{GDHt}},$$

$$h_4(x) = \frac{(C_{3-\mathrm{HPA}} + K_\mathrm{d})}{(C_{3-\mathrm{HPA}} + K_\mathrm{d}) + rC_{3-\mathrm{HPA}}} - (k_\mathrm{d}^{\mathrm{mPDOR}} + \mu)m_{\mathrm{PDOR}},$$

$$h_5(x) = K_{\mathrm{GDHt}}^{\mathrm{m}} m_{\mathrm{GDHt}} - (k_\mathrm{d}^{\mathrm{GDHt}} + \mu)[\mathrm{GDHt}],$$

$$h_6(x) = K_{\mathrm{PDOR}}^{\mathrm{m}} m_{\mathrm{PDOR}} - (k_\mathrm{d}^{\mathrm{PDOR}} + \mu)[\mathrm{PDOR}],$$

$$h_7(x) = (\mu - D)X,$$

$$h_8(x) = D(C_{\mathrm{SF}} - C_{\mathrm{Se}}) - q_\mathrm{S}X,$$

$$h_9(x) = \frac{1}{V_\mathrm{S}}\left[J_{\max}\frac{C_{\mathrm{Se}}}{C_{\mathrm{Se}} + K_\mathrm{m}} + \frac{1}{A_\mathrm{S}}(C_{\mathrm{Se}} - C_{\mathrm{Si}}) - q_\mathrm{S}\right] - \mu C_{\mathrm{Si}},$$

$$h_{10}(x) = k_1[\mathrm{GDHt}]\frac{C_{\mathrm{Si}}}{K_{\mathrm{mGDHt}}\left(1 + \frac{C_{3-\mathrm{HPA}}}{K_\mathrm{i}^{\mathrm{GDHt}}}\right) + C_{\mathrm{Si}}} - k_2[\mathrm{PDOR}]\frac{C_{3-\mathrm{HPA}}}{K_{\mathrm{mPDOR}} + C_{3-\mathrm{HPA}}\left(1 + \frac{C_{3-\mathrm{HPA}}}{K_\mathrm{i}^{\mathrm{PDOR}}}\right)}$$

$$- \mu C_{3-\mathrm{HPA}} - nk_{-1}\frac{R_\mathrm{t}C_{3-\mathrm{HPA}}}{C_{3-\mathrm{HPA}} + K_\mathrm{d}},$$

$$h_{11}(x) = k_2[\mathrm{PDOR}]\frac{C_{3-\mathrm{HPA}}}{K_{\mathrm{mPDOR}} + C_{3-\mathrm{HPA}}\left(1 + \frac{C_{3-\mathrm{HPA}}}{K_\mathrm{i}^{\mathrm{PDOR}}}\right)} - K_{\mathrm{PD}}(C_{\mathrm{PDi}} - C_{\mathrm{PDe}}) - \mu C_{\mathrm{PDi}},$$

$$h_{12}(x) = q_{\mathrm{PD}}X - DC_{\mathrm{PDe}},$$

$$h_{13}(x) = q_{\mathrm{HAc}}X - DC_{\mathrm{HAc}},$$

$$h_{14}(x) = q_{\mathrm{EtOH}}X - DC_{\mathrm{EtOH}},$$

$$g_1(x) = D - 0.5,$$

$$g_2(x) = 0.05 - D,$$

$$g_3(x) = C_{\mathrm{SF}} - 2039,$$

$$g_4(x) = -C_{\mathrm{SF}},$$

$$g_5(x) = m_\mathrm{R} - 1000,$$

$$g_6(x) = -m_\mathrm{R},$$

$$g_7(x) = R - 1000,$$

$$g_8(x) = -R,$$

$$g_9(x) = m_{\mathrm{GDHt}} - 1000,$$

$$g_{10}(x) = -m_{\mathrm{GDHt}},$$

$$g_{11}(x) = m_{\mathrm{PDOR}} - 1000,$$

$$g_{12}(x) = -m_{\mathrm{PDOR}},$$

$$g_{13}(x) = [\mathrm{GDHt}] - 1000,$$

$$g_{14}(x) = -[\mathrm{GDHt}],$$

$$g_{15}(x) = [\mathrm{PDOR}] - 1000,$$

$$g_{16}(x) = -[\mathrm{PDOR}],$$

$$g_{17}(x) = X - 5,$$

$$g_{18}(x) = 0.05 - X,$$

$g_{19}(x) = C_{\mathrm{Se}} - 2039$,
$g_{20}(x) = -C_{\mathrm{Se}}$,
$g_{21}(x) = C_{\mathrm{Si}} - 2039$,
$g_{22}(x) = -C_{\mathrm{Si}}$,
$g_{23}(x) = C_{3-\mathrm{HPA}} - 500$,
$g_{24}(x) = -C_{3-\mathrm{HPA}}$,
$g_{25}(x) = C_{\mathrm{PDi}} - 939.5$,
$g_{26}(x) = -C_{\mathrm{PDi}}$,
$g_{27}(x) = C_{\mathrm{PDe}} - 939.5$,
$g_{28}(x) = -C_{\mathrm{PDe}}$,
$g_{29}(x) = C_{\mathrm{HAc}} - 1026$,
$g_{30}(x) = -C_{\mathrm{HAc}}$,
$g_{31}(x) = C_{\mathrm{EtOH}} - 360.9$,
$g_{32}(x) = -C_{\mathrm{EtOH}}$.

Then grounded on the similar thought of [18], we can replace the optimization problem (59) with a sequence of barrier equality constrained problems of the form:

$$\min \quad f_{\rho^{(k)}}(x) = f(x) - \rho^{(k)} \sum_{i=1}^{32} \ln(s_i),$$

subject to satisfying:

$$\begin{aligned} &h_j(x) = 0,\ j = 1, 2, ..., 14, \\ &g_i(x) + s_i = 0,\ i = 1, 2, ..., 32, \end{aligned} \tag{60}$$

where the integer k is the sequence counter, $s_i > 0$ (i = 1, 2, …, 32) are the slack variables, and $\rho^{(k)}$ are the barrier parameter with $\lim_{k\to\infty} \rho^{(k)} = 0$. As $\rho^{(k)} \to 0$, the minimum of $f_{\rho^{(k)}}(x)$ approaches the minimum of $f(x)$. The problem (60) is a sequence of equality constrained problems. These are easier to solve than the original constrained problem (59).

Now we present the following algorithm to solve the optimization problem (59):

Step 1. Choose an initial barrier parameter $\rho^{(0)}$, an initial point $x^{(0)}$ and the required solution accuracy. Set iterative counter $k = 0$.

Step 2. At the k-th ($k \geq 1$) iteration of the algorithm, solve the barrier problem (60) by using a technique of switching between a line search method that computes steps by factoring the primal-dual equations and a trust region method that uses a conjugate gradient iteration [18]. By default, the algorithm first attempts to take a direct factorization step. If it cannot, it attempts a trust region iteration that guarantees progress toward stationarity is invoked.

Step 3. If the problem (60) is solved to meet the required accuracy, then *stop*; else, reset the barrier parameter $\rho^{(k)}$ so that $\rho^{(k)} < \rho^{(k-1)}$, set $k = k + 1$ and go to *Step 2*.

Optimization results

In the optimization computation of the problem (28)-(58) we set the initial barrier parameter $\rho^{(0)}$ of interior point method to be 0.1. The initial values of optimized variables and objective are given in Table 2.

Table 2. Initial values of optimized variables and objective

Variable	Representation	Lower bound	Initial value	Upper bound	Unit
x_1	D	0.05	0.05	0.5	h^{-1}
x_2	C_{SF}	0	1000	2039	mmol/L
x_3	m_R	0	1	1000	h
x_4	R	0	1	1000	mmol/L
x_5	m_{GDHt}	0	1	1000	h
x_6	m_{PDOR}	0	1	1000	h
x_7	[GDHt]	0	1	1000	mmol/L
x_8	[PDOR]	0	1	1000	mmol/L
x_9	X	0.05	1	5	g/L
x_{10}	C_{Se}	0	1000	2039	mmol/L
x_{11}	C_{Si}	0	1000	2039	mmol/L
x_{12}	C_{3-HPA}	0	100	500	mmol/L
x_{13}	C_{PDi}	0	100	939.5	mmol/L
x_{14}	C_{PDe}	0	100	939.5	mmol/L
x_{15}	C_{HAc}	0	100	1026	mmol/L
x_{16}	C_{EtOH}	0	100	360.9	mmol/L
x_1x_{14}	DC_{PDe}	0	5	469.75	mmol/(L·h)

Table 3 shows the optimization result obtained by using the interior point method. In this table, it can be seen that the maximum rate of 1,3-PD production is 114.3005 mmol/(L·h), when the dilution rate D is 0.2857 h^{-1} and the initial glycerol concentration C_{SF} is 730.7987 mmol/L. We can also conclude that the achieved maximum rate of 1,3-PD production is increased more than 22.86 times its initial value. To check whether the obtained optimal solution meets the equality constraints of the problem (28)-(58), we substitute it into the left side of (29)-(42).

Table 4 presents the computed values of equality constraint functions in the problem (28)-(58) at the optimal solution. From this table, it can be seen that the maximum magnitude of equality constraint violation is 1.0×10^{-11}, which is close to zero. This concludes that the attained optimal solution has a very good feasibility for the problem (28)-(58).

Table 3. Optimal values of optimized variables and objective

Variable	Representation	Lower bound	Optimal value	Upper bound	Unit
x_1	D	0.05	0.2857	0.5	h^{-1}
x_2	C_{SF}	0	730.7987	2039	mmol/L
x_3	m_R	0	1.6678	1000	h
x_4	R	0	2.9561×10^{-6}	1000	mmol/L
x_5	m_{GDHt}	0	0.3599	1000	h
x_6	m_{PDOR}	0	0.0563	1000	h
x_7	[GDHt]	0	0.2976	1000	mmol/L
x_8	[PDOR]	0	0.0661	1000	mmol/L
x_9	X	0.05	2.8857	5	g/L
x_{10}	C_{Se}	0	98.0986	2039	mmol/L
x_{11}	C_{Si}	0	98.0962	2039	mmol/L
x_{12}	C_{3-HPA}	0	37.5360	500	mmol/L
x_{13}	C_{PDi}	0	395.6144	939.5	mmol/L
x_{14}	C_{PDe}	0	400.1021	939.5	mmol/L
x_{15}	C_{HAc}	0	116.5758	1026	mmol/L
x_{16}	C_{EtOH}	0	42.3268	360.9	mmol/L
x_1x_{14}	DC_{PDe}	0	114.3005	469.75	mmol/(L·h)

Table 4. Computed values of equality constraint functions in the problem (28)-(58) at the optimal solution

Equality constraint function	Value
Left side of (29)	-1.1×10^{-16}
Left side of (30)	4.6×10^{-17}
Left side of (31)	0
Left side of (32)	0
Left side of (33)	4.4×10^{-16}
Left side of (34)	2.2×10^{-16}
Left side of (35)	3.2×10^{-16}
Left side of (36)	-5.7×10^{-14}
Left side of (37)	-1.0×10^{-11}
Left side of (38)	-1.6×10^{-15}
Left side of (39)	-3.0×10^{-13}
Left side of (40)	4.3×10^{-14}
Left side of (41)	7.1×10^{-15}
Left side of (42)	3.6×10^{-15}

Conclusion

This paper has addressed the optimization of continuous bioconversion process of glycerol to 1,3-PD. The proposed nonlinear optimization model (28)-(58) can be transformed into a sequence of barrier subproblems that are easy to solve. The maximum rate of 1,3-PD production has been successfully achieved when the dilution rate is 0.2857 h^{-1} and the initial glycerol concentration is 730.7987 mmol/L. The approximation algorithm used in solving the proposed optimization problem (28)-(58) not only can obtain a maximum rate of 1,3-PD production, but also can yield an optimal operation condition that has a very good feasibility for the problem (28)-(58). This suggests that the interior method is a good choice for dealing with the optimization of continuous bioconversion process of glycerol to 1,3-PD.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Nos. 11101051 and 11371071), the Program for Liaoning Excellent Talents in University (No. LJQ2013115), Liaoning Provincial Natural Science Foundation of China (No. 2015020038) and the Program for Liaoning Innovative Research Team in University (No. LT2014024).

References

1. Biebl H., K. Menzel, A. Zeng, W. D. Deckwer (1999). Microbial Production of 1,3-Propanediol, Applied Microbial Technology, 52(3), 289-297.
2. Byrd R. H., J. C. Gilbert, J. Nocedal (2000). A Trust Region Method Based on Interior Point Techniques for Nonlinear Programming, Mathematical Programming, 89(1), 149-185.
3. Byrd R. H., M. E. Hribar, J. Nocedal (1999). An Interior Point Algorithm for Large-scale Nonlinear Programming, SIAM Journal on Optimization, 9(4), 877-900.
4. Celińska E. (2010). Debottlenecking the 1,3-Propanediol Pathway by Metabolic Engineering, Biotechnology Advances, 28(4), 519-530.
5. Celińska E. (2012). *Klebsiella Spp* as a 1,3-Propanediol Producer – the Metabolic Engineering Approach, Critical Reviews in Biotechnology, 32(3), 274-288.
6. Celińska E., A. Drożdżyńska, M. Jankowska, W. Białas, K. Czaczyk, W. Grajek (2015). Genetic Engineering to Improve 1,3-Propanediol Production in an Isolated *Citrobacter Freundii* Strain, Process Biochemistry, 50(1), 48-60.
7. Egea J. A., E. Vazquez, J. R. Banga, R. Martí (2009). Improved Scatter Search for the Global Optimization of Computationally Expensive Dynamic Models, Journal of Global Optimization, 43(2-3), 175-190.
8. Hirokawa Y., Y. Maki, T. Tatsuke, T. Hanai (2016). Cyanobacterial Production of 1,3-Propanediol Directly from Carbon Dioxide Using a Synthetic Metabolic Pathway, Metabolic Engineering, 34, 97-103.
9. Kumar V., M. Durgapal, M. Sankaranarayanan, A. Somasundar, C. Rathnasingh, H. Song, D. Seung, S. Park (2016). Effects of Mutation of 2,3-Butanediol Formation Pathway on Glycerol Metabolism and 1,3-Propanediol Production by *Klebsiella Pneumoniae* J2B, Bioresource Technology, 214, 432-440.
10. Kwon J.-H., M. Rögner, S. Rexroth (2012). Direct Approach for Bioprocess Optimization in a Continuous Flat-bed Photobioreactor System, Journal of Biotechnology, 162(1), 156-162.
11. Lee C. S., M. K. Aroua, W. M. A. W. Daud, P. Cognet, Y. Pérès-Lucchese, P-L Fabre, O. Reynes, L. Latapie (2015). A Review: Conversion of Bioglycerol into 1,3-Propanediol via Biological and Chemical Method, Renewable and Sustainable Energy Reviews, 42, 963-972.

12. Petrov M. (2008). Multiple Objective Optimization and Optimal Control of Fermentation Processes, International Journal Bioautomation, 10, 21-30.
13. Roeva O. (2012). Optimization of *E. coli* Cultivation Model Parameters Using Firefly Algorithm, International Journal Bioautomation, 16(1), 23-32.
14. Roeva O., S. Tzonkov (2009). A Genetic Algorithm for Feeding Trajectory Optimisation of Fed-batch Fermentation Processes, International Journal Bioautomation, 12, 1-12.
15. Silva J. P., Y. B. Almeida, I. O. Pinheiro, A. Knoelchelmann, J. M. F. Silva (2015). Multiplicity of Steady States in a Bioreactor during the Production of 1,3-Propanediol by *Clostridium Butyricum*, Bioprocess and Biosystems Engineering, 38(2), 229-235.
16. Sun Y., W. Qi, H. Teng, Z. Xiu, A. Zeng (2008). Mathematical Modeling of Glycerol Fermentation by *Klebsiella Pneumoniae*: Concerning Enzyme-catalytic Reductive Pathway and Transport of Glycerol and 1,3-Propanediol Across Cell Membrane, Biochemical Engineering Journal, 38(1), 22-32.
17. Sun Y., J. Ye, X. Mu, H. Teng, E. Feng, A. Zeng, Z. Xiu (2012). Nonlinear Mathematical Simulation and Analysis of *Dha* Regulon for Glycerol Metabolism in *Klebsiella Pneumoniae*, Chinese Journal of Chemical Engineering, 20(5), 958-970.
18. Waltz R. A., J. L. Morales, J. Nocedal, D. Orban (2006). An Interior Algorithm for Nonlinear Optimization that Combines Line Search and Trust Region Steps, Mathematical Programming, 107(3), 391-408.
19. Wang G., E. Feng, Z. Xiu (2008). Modeling and Parameter Identification of Microbial Bioconversion in Fed-batch Cultures, Journal of Process Control, 18(5), 458-464.
20. Xiu Z., B. Song, Z. Wang, L. Sun, E. Feng, A. Zeng (2004). Optimization of Dissimilation of Glycerol to 1,3-Propanediol by *Klebsiella Pneumoniae* in One- and Two-stage Anaerobic Cultures, Biochemical Engineering Journal, 19(3), 189-197.
21. Xiu Z., A. Zeng, L. An (2000). Mathematical Modeling of Kinetics and Research on Multiplicity of Glycerol Bioconversion to 1,3-Propanediol, Journal of Dalian University of Technology, 40(4), 428-433 (in Chinese).
22. Xiu Z., A. Zeng, W. D. Deckwer (1998). Multiplicity and Stability Analysis of Microorganisms in Continuous Culture: Effects of Metabolic Overflow and Growth Inhibition, Biotechnology and Bioengineering, 57(3), 251-261.
23. Xu G. (2010). Robust Control of Continuous Bioprocesses, Mathematical Problems in Engineering, 2010, Article ID 62703.
24. Xu G., C. Shao, W. Qian (2015). Optimization and Control for Nonlinear Biochemical Processes, Science Press, Beijing, China (in Chinese).
25. Yuan J., X. Zhu, X. Zhang, H. Yin, E. Feng, Z. Xiu (2014). Robust Identification of Enzymatic Nonlinear Dynamical Systems for 1,3-Propanediol Transport Mechanisms in Microbial Batch Culture, Applied Mathematics and Computation, 232(5), 150-163.
26. Zeng A., H. Biebl (2002). Bulk Chemicals from Biotechnology: The Case of Microbial Production of 1,3-Propanediol and the New Trends, Advances in Biochemical Engineering/Biotechnology, 74, 239-259.
27. Zeng A., W. D. Deckwer (1995). A Kinetic Model for Substrate and Energy Consumption of Microbial Growth under Substrate-sufficient Conditions, Biotechnology Progress, 11(1), 71-79.
28. Zhu X., J. Yuan, X. Wang, E. Feng, Z. Xiu (2014). μ-synthesis of Dissimilation Process of Glycerol to 1,3-Propanediol in Microbial Continuous Culture, World Journal of Microbiology and Biotechnology, 30(2), 767-775.

Assoc. Prof. Gongxian Xu, Ph.D.
E-mail: gxxu@bhu.edu.cn

Gongxian Xu is an Associate Professor in the Department of Mathematics at Bohai University. He obtained his Ph.D. degree in Department of Applied Mathematics at Dalian University of Technology in 2008. He also received a B.Sc. degree in Mechanical Engineering from Liaoning University of Technology in 2000. From 2013 to 2014, he worked at the Department of Chemical Engineering of Auburn University as a postdoctoral fellow. His research interests are in the fields of mathematical optimization (nonlinear optimization, global optimization, multi-objective optimization), operational research, optimization and control of (bio)chemical processes, and systems biology. He received several funds including the National Natural Science Foundation of China, Liaoning Provincial Natural Science Foundation of China, Liaoning Province Doctor Startup Fund of China and the Program for Liaoning Excellent Talents in University. He has authored or co-authored more than 40 articles and two monographs. Currently he is an editorial board member of several international journals (International Journal of Modeling and Optimization, etc.). He is also a reviewer of some journals (European Journal of Operational Research, Journal of the Operational Research Society, Engineering Optimization, Journal of Theoretical Biology, Applied Mathematical Modelling, and Biotechnology and Applied Biochemistry, etc.).

Dan Wang, M.Sc. Student
E-mail: 844364936@qq.com

Dan Wang has graduated from Bohai University in 2015. Currently, she is a M. Sc. student in Department of Mathematics at Bohai University. Her research interest is in the field of mathematical modelling and optimization of biochemical processes.

Caixia Li, M.Sc. Student
E-mail: 1126668962@qq.com

Caixia Li has graduated from Jining Normal University in 2015. Currently, she is a M. Sc. student in Department of Mathematics at Bohai University. Her research interest is in the field of mathematical modelling and optimization of biochemical processes.