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Investigating Effects of pH on Microbial Growth in Continuous Stirred Tank Bioreactors

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ABSTRACT

This paper presents the importance of pH on microbial growth and its control strategies in Continuous Stirred Tank Bioreactors (CSTBR) The CSTBR involves growth of a pure culture *Pediococcus acidilactici*, a lactic acid bacterium and media containing glucose as carbon and energy source whereas, a continuous flow of a base stream is fed in order to control pH. The mathematical model is developed for analyzing the parametric sensitivity of operational variables. The normalized objective sensitivity of pH-minimum with respect to various input variables, such as feed stream concentration and its dilution rate, base stream concentration and its dilution rate for pH control was calculated. A new criterion for parametric sensitivity or occurrence pH-runaway is proposed. pH-runaway condition is referred to a condition at which microbial growth is ceased beyond a certain range of pH and bioreactor becomes susceptible to destabilized. This criterion determines particular critical points where normalized objective sensitivity is maximum with respect to input parameters. The criterion is applicable to a range of similar bioreactors. The region of sensitivity with respect to any system input parameters may also be defined as generalized criterion of parametric sensitivity or pH-runaway was determined in this study.

Keywords: CSTBR, Normalized objective sensitivity, *Pediococcus acidilactici*, pH-runaway

INTRODUCTION

In recent years, Continuous Stirred Tank Bioreactors (CSTBRs) have gained much importance due to their high flexibility in operation. They are widely used in bioprocess industries, such as, brewing, fermentation, Pharmaceuticals, sewage and wastewater treatment etc. CSTBRs are relatively simple and commonly used for continuous production in large quantities. Although, CSTBRs are simple type of reactors but they can exhibit parametrically sensitive behavior when biochemical reactions are carried out. Biochemical reactions, which are involved during microbial growth, are very complex in nature due to nonlinear nature of their growth kinetics. The output variables such as, conversion, temperature and pH in the reactor undergo large variation in response to small variations of one or more of the reactor operating conditions. The stability and uniformity of a CSTBR is essential to maintain the product quality as well as to constrain any catastrophic situation in case of processing hazardous material. In order to understand, optimize and control CSTBRs, it is very important to have a sufficient information on system dynamics.

Microbial growth in CSTBRs is susceptible to change in the microorganism's living environment such as, temperature, dissolved O_2 and pH. Whereas pH plays primary role in the operation of CSTBR. Particularly, microbial growth is hindered, if the system pH goes beyond the optimum pH range. This kind of phenomenon is defined as 'pH-runaway' condition in CSTBR (Das et al., 2016). pH is typically controlled by monitoring the dosage of acid or base.

Comprehensive studies are available on the occurrence of multiple steady state with respect to product generation in CSTBRs and other similar bioreactors (Sadana et al., 1980, Patnaik et al., 1994, Dutta et al., 2001, Dutta et al., 2007), however, sufficient information on parametric sensitivity of bioreactors with respect to pH is not analyzed. Sayar et al., 2009a, 2009b observed presence of parametric sensitivity in enzymatic and microbial bioreactors. Dutta et al., 2001 analyzed the parametric sensitivity of pH in batch reactors using phenol degrading bacteria using a generalized sensitivity criterion proposed by Mobidelli and Verma 1988. Nonlinear behavior in CSTBRs was studied and parametric sensitivity as well as multiplicity of steady state was shown by Das et al., 2016. The unique and multiple steady state zones were identified in CSTBRs by applying the technique by Kauschus et al., 1978. However, in case of CSTBRs, the generalized criterion of parametric sensitivity, does not depend upon the topology of pH profile for identifying the

parameter space where the reacting system exhibits sensitive behavior, is lacking.

In the present study we investigate, growth of Lactic acid bacteria in a CSTBR, where pH is controlled by a continuous flow of an alkaline stream. The model kinetics developed by Das et al., 2016 for the growth of a lactic acid bacterium *Pediococcus acidilactici* in a media containing glucose as substrate was selected. The Mathematical model consists of a set of ordinary differential equations is developed to predict the change of concentration of different species and pH with respect to the propagation of time. The normalized objective sensitivity of pH-minimum was evaluated with respect to different input variables, such as initial substrate concentration, dilution rate of glucose feed stream, initial concentration of alkaline stream and dilution rate of alkaline stream. This kind of sensitivity analysis is useful to identify the parametric sensitivity regions and control strategies for reactor operations.

MATHEMATICAL MODELLING

Process Model

We consider a continuous stirred tank bioreactor (CSTBR) as define by Das et al., 2016**,** which is fed continuously with microbial growth media shown in Figure 1. Single microbial population, *Pediococcus Acidilactici*. (X) is capable of utilizing the substrate, i.e., glucose (S). Lactic acid is producing as a product during this microbial reaction. In order to maintain the optimum pH level for microbial growth, an alkaline stream of NaOH fed simultaneously. The reactions occurring in CSTBR are as followed:

$$x + \text{Glucose} \rightarrow nx + CH_3CH(OH)COOH \quad \text{(Lactic acid)} \tag{1}$$

$$CH_3CH(OH)COOH + NaOH \rightarrow CH_3CH(OH)COONa + H_2O \tag{2}$$

By using the Henderson-Hasselbalch equation (Atkins P & de Paula J. 2002) the pH of the solution medium can be calculated

$$pH = pK_a + \log\frac{s_A}{p} \tag{3}$$

Where, K_a is the equilibrium dissociation constant for lactic acid and s_A, p are the molar concentrations of sodium lactate and lactic acid respectively.

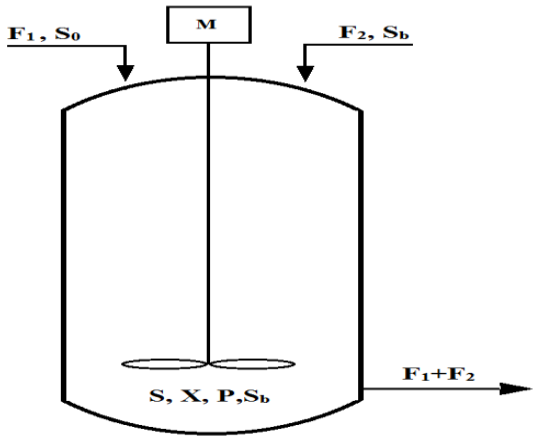

Figure 1. Continuous Stirred Tank Bioreactor

The governing equations around the unit for different species is established in the following

The mass balance for biomass x, is given by

$$\frac{dx}{dt} = (\mu - d - d_1)x \tag{4}$$

Substrate, s

$$\frac{ds}{dt} = d(s_0 - s) - d_1 s - \frac{1}{Y_{x/s}}\mu x \tag{5}$$

Where, $D=F_1/V$, dilution rate of glucose feed stream and $D_1=F_2/V$, dilution rate of alkaline stream, and V is the working volume.

Mole Balance for lactic acid, p

$$\frac{dp}{dt} = -dp + \frac{Y_{p/x}}{M_a}\mu x - d_1(s_b + p) \tag{6}$$

Mole Balance for sodium lactate, s_A

$$\frac{ds_A}{dt} = d_1(s_b - s_A) - ds_A \tag{7}$$

pH balance can be obtained by differentiating equation (3) with respect to time, t

$$\frac{dpH}{dt} = \frac{1}{s_A}\frac{ds_A}{dt} - \frac{1}{p}\frac{dp}{dt} \tag{8}$$

The dependence of pH on specific growth rate of microorganism can be represented by coupling the polynomial functionality of system pH with Monod kinetics (Das et al., 2016) as follows:

$$\mu = \frac{\mu_m S}{K_s + S}\left(c_1 pH^2 + c_2 pH + c_3\right) \quad (9)$$

The kinetic parameters are taken from validated experimental system (Das et al., 2016) which are provided in Table 1.

Table 1. Values of Kinetic Parameters

Kinetic parameter	Value
μ_m (h^{-1})	1.0775
K_s (gL^{-1})	4.5017
$Y_{x/s}$ (gg^{-1})	0.1
$Y_{p/x}$ (gg^{-1})	5.85
c_1	-0.10835
c_2	1.4368
c_3	-3.76195

The mass and molar balances are suitably transformed dimensionless using the variable given in Table 2. The non-dimensional equations for the different constituents are then

$$\frac{dX}{d\tau} = (U - D - D_1)X \quad (10)$$

$$\frac{dS}{d\tau} = D(S_0 - S) - UX - D_1 S \quad (11)$$

$$\frac{dP}{d\tau} = -DP + UX - D_1(MS_b + P) \quad (12)$$

$$\frac{dS_A}{d\tau} = D_1(NS_b - S_A) - DS_A \quad (13)$$

$$\frac{dpH}{d\tau} = \frac{1}{S_A}\frac{dS_A}{d\tau} - \frac{1}{P}\frac{dP}{d\tau} \quad (14)$$

On the other hand, the non-dimensional expression of the specific growth rate is given by

$$U = \frac{S}{S+1}\left(c_1 pH^2 + c_2 pH + c_3\right) \tag{15}$$

These five ordinary differential equations (10) to (14) form the model of above defined CSTBR. The kinetic and operating parameters are taken from validated experimental system (Das et al., 2016) which are provided in Table 1

Table 2. Definitions of dimensionless variable used in model

Parameter	Definition	Parameter	Definition
X	$x/(K_s Y_{x/s})$	U	μ/μ_m
S	s/K_s	S_0	s_0/K_s
P	$pM_a/(K_s Y_{p/s})$	S_b	$s_b M_b/K_s$
S_A	$s_A M_c/K_s$	D	d/μ_m
τ	$t\mu_m$	D_1	d_1/μ_m
M	$M_a/(M_b Y_{p/s})$	N	M_c/M_b

SENSITIVITY ANALYSIS

The pH-sensitivity analysis of the present system has been done using previous method adopted by Morbedelli and Varma 1988 and Dutta et al. 2001 for which the governing pH and substrate concentration equation can be written as by dividing equation (14) by equation (11)

$$\frac{dpH}{dS} = \frac{D_1 S_b\left[\frac{N}{S_A} + \frac{M}{P}\right] - \frac{UX}{P}}{D(S_0 - S) - D_1 S - UX} = f\left(\phi, X, S, P, S_A, pH\right) \tag{16}$$

Where, ϕ is the vector of model input parameters namely, S_0, D, D1 and S_b
Similarly, dividing equations (10) and (12) to (14) by equation (11), we have

$$\frac{dX}{dS} = \frac{(U - D - D_1)X}{D(S_0 - S) - D_1 S - UX} \tag{17}$$

$$\frac{dP}{dS} = \frac{-DP + UX - D_1(MS_b + P)}{D(S_0 - S) - D_1 S - UX} \tag{18}$$

$$\frac{dS_A}{dS} = \frac{D_1(NS_b - S_A) - DS_A}{D(S_0 - S) - D_1 S - UX} \tag{19}$$

With the initial conditions,

$pH=pH_0$, $X= X_0$, $P=P_0$ and $S_A=S_{A0}$ *at* $S= S_0$ (20)

By differentiating equation (16) with respect to parameter, ϕ we can evaluate the expression for first-order local sensitivity s_ϕ as,

$$\frac{d}{dS}\left(\frac{\partial pH}{\partial \phi}\right) = \frac{\partial f}{\partial \phi} + \frac{\partial f}{\partial pH}.\frac{\partial pH}{\partial \phi} \qquad S \in (S_0, 0) \tag{21}$$

$$\frac{ds_\phi}{dS} = \frac{\partial f}{\partial \phi} + \frac{\partial f}{\partial \gamma}.s_\phi \tag{22}$$

Equation (22) can be rewritten as,

$$L[s_\phi] \equiv \frac{ds_\phi}{dS} + H.s_\phi = \frac{\partial f}{\partial \phi} \equiv \sigma \tag{23}$$

Where, $s_\phi = \frac{\partial pH}{\partial \phi}$ (24)

And,

$$H = -\frac{\partial f}{\partial pH} = \frac{J.X}{D(S_0 - S) - D_1 S - U.}\left[\frac{dpH}{dS} - \frac{1}{P}\right] \tag{25}$$

Where,

$$J = \frac{S}{1+S}\left(2.c_1.pH + c_2\right) \tag{26}$$

The adjoined equation takes the following form:

$$\frac{d\rho}{dS} = H.\rho \tag{27}$$

Where,

$$\rho = \frac{\bar{s}_\phi(S)}{\bar{s}_\phi(0)} \qquad S \in (S_0, S^*) \tag{28}$$

S^* refers to the value of which $pH = pH_{min}$

The initial conditions are,

At $S = S_0$ (29)

$$\rho = 1,\ s_\phi(0) = \frac{\partial pH}{\partial \phi_i} = 0 \tag{30}$$

Where,

ϕ_i is one element of parameter vector, **ϕ.**

Calculation of sensitivities

The objective sensitivities have been evaluated as follows:

1. Equations (16) to (19) and (27) have been solved simultaneously with the initial conditions equations (20) and (30) until the γ attains its minimum. The corresponding values of $\rho_S{}^*$ and S^* have been determined.
2. $\bar{s}_\phi(0)$ given by equation (32) has been calculated using the value of $\rho_S{}^*$ with the help of following equation:

$$\bar{s}_\phi(0) = \frac{\bar{s}_\phi(S^*)}{\rho_{S^*}} = \frac{1}{\rho_{S^*}} \tag{31}$$

3. The objective sensitivity is then evaluated using the following equation:

$$s^*_{\phi_i} = s_{\phi_i}(0)\bar{s}_\phi(0) + \int_{S_0}^{S^*} \sigma_i \bar{s}_\phi(S)dS \tag{32}$$

The expressions for σ_i and corresponding to each ϕ_i have been shown in Table 4.

4. The normalized objective sensitivity $S^*_{\phi i}$ or $S(pH^*, \phi_i)$ for each ϕ_i has been evaluated using following equation:

$$S^*_{\phi i} = \frac{d(\ln pH^*)}{d(\ln \phi_i)} = \frac{\phi_i}{pH^*}\bar{s}_{\phi i} \tag{33}$$

Table 4. Expressions used for evaluating σ_i for various parameters ϕ_i as defined in equation (23)

ϕ_i	σ_i
D	$\dfrac{D_1 S_b\left(\dfrac{N}{S_A}+\dfrac{M}{P}\right)}{\left(DS_0-(D+D_1)S-UX\right)^2}$
D_1	$\dfrac{S_b\left(\dfrac{N}{S_A}+\dfrac{M}{P}\right)}{\left(DS_0-(D+D_1)S-UX\right)}+\dfrac{S\left[D_1 S_b\left(\dfrac{N}{S_A}+\dfrac{M}{P}\right)-\dfrac{UX}{P}\right]}{\left(DS_0-(D+D_1)S-UX\right)^2}$
S_0	$-\dfrac{D\left[D_1 S_b\left(\dfrac{N}{S_A}+\dfrac{M}{P}\right)-\dfrac{UX}{P}\right]}{\left(DS_0-(D+D_1)S-UX\right)^2}$
S_b	$\dfrac{D_1\left(\dfrac{N}{S_A}+\dfrac{M}{P}\right)}{\left(DS_0-(D+D_1)S-UX\right)}$

RESULTS

The numerical simulation of model equations (21) to (24) and (31) were solved simultaneously using Runga-Kutta 4th order method and equation (36) was solved using the trapezoidal numerical integration method. Behavior of a CSTBR is presented here in terms of normalized objective sensitivities coefficients as function of input parameters D, D_1, S_0 and S_b. The results of the present study are shown in Figures 2 to 3.

In Figure 2, $S(pH^*, \phi_i)$ are plotted as varying dimensionless dilution rate of feed stream (D) and dilution rate of alkaline stream (D_1) respectively. It can be observed from Figure 2a, the normalized objective sensitivities, $S(pH^*, \phi_i)$ exhibit a sharp maximum and a minimum at D= 0.056 whereas, in Figure 2b, at D_1= 0.047, $S(pH^*, \phi_i)$ show a sharp maximum and a minimum. On both cases, other input parameters are remained constant.

In Figure 3, $S(pH^*, \phi_i)$ are plotted as varying dimensionless initial substrate concentration (S_0) and concentration of base stream (S_b) respectively. Figure 3a shows at S_0 = 0.65, $S(pH^*, \phi_i)$ exhibit a sharp maximum and a minimum and in Figure 3b, $S(pH^*, \phi_i)$ exhibit a sharp maximum and a minimum when S_b = 0.778,. On both cases, other input parameters are remained constant.

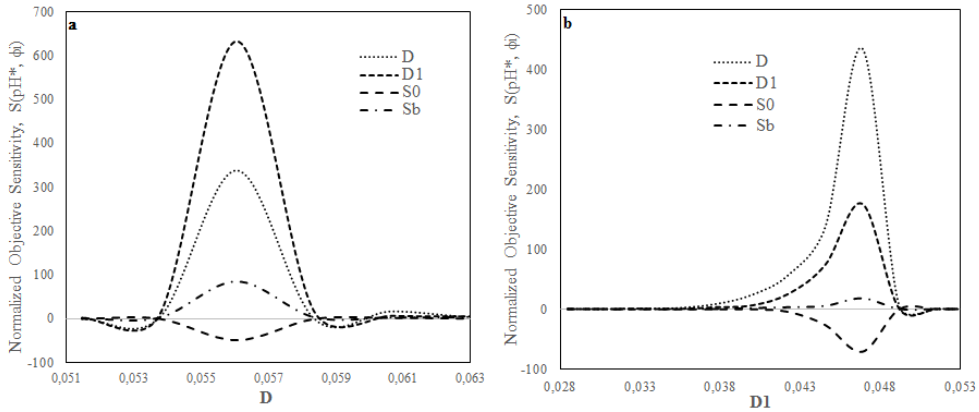

Figure 2. Normalized objective sensitivity $S(pH^*, \phi_i)$ a. with variation of D, at fixed D_1=0.0092, S_0 =1.0 and S_b= 0.0089. b. with variation of D_1, at fixed S_0 =1.0, D=0.0046 and S_b= 0.0444.

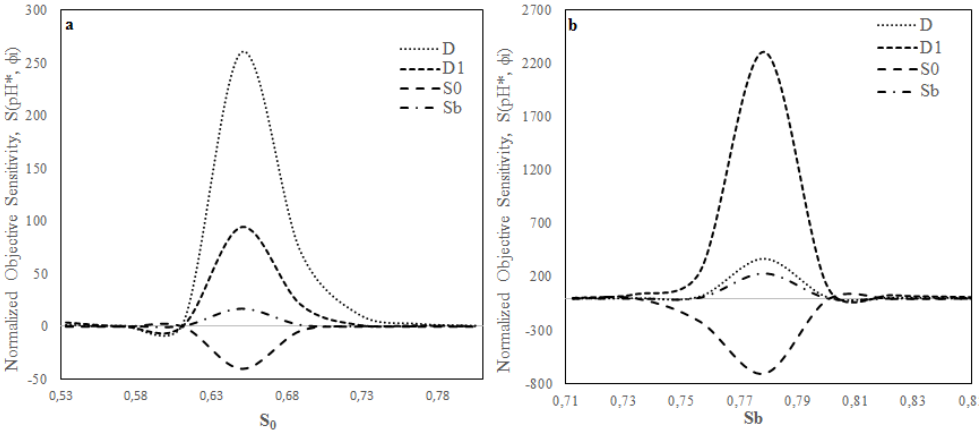

Figure 3. Normalized objective sensitivity $S(pH^*, \phi_i)$ a. with variation of S_0, at fixed D=0.00092, D_1=0.00092 and S_b= 0.0089. b. with variation of S_b, at fixed, D=0.0092, D_1=0.00092 and S_0 =1.0.

DISCUSSION

The parametric value at which the normalized objective sensitivity of pH minimum with respect to input parameters shows a maximum or minimum is identified as the critical point. At the critical point, the pH-minimum becomes simultaneously sensitive to small changes in all the parameters. It appears from the results that in each curve the critical point is same for any choice of parameter. This observation ensures the generalized nature of the adopted criterion because the system pH becomes sensitive to all the input parameters simultaneously.

Positive values of the objective normalized sensitivity of the pH-minimum with respect to input parameters D, D_1 and S_b indicate that the pH-minimum increases i.e., the CSTBRs will be more pH sensitive as the magnitude of this parameter increase. The negative value of objective normalized sensitivity of the pH-minimum with respect to S_0 indicates that the pH-minimum increases as S_0 decreases. Thus, if the sensitivity is positive, the transition from stable pH system to pH-runaway behavior occurs as this parameter is increased, while if the sensitivity is negative, the same transition occurs when the corresponding parameter is decreased.

CONCLUSION

This study presents behavior of continuous stirred tank bioreactor (CSTBR) using lactic acid bacteria, through parametric sensitivity analysis. The parametric range of inputs for maintaining optimal pH range favorable for the microbial growth during reactor operation are theoretically determined. It is observed that the productivity of CSTBRs is dependent upon maintaining the optimum pH within the reactor. The impact of basic input parameters D, D_1, S_b and S_0 that are directly involve in maintaining the pH of the system are investigated by determining normalized objective sensitivity of pH. A generalized criterion i.e., the specific range of input parameters at which the system found to be sensitive is also determined. This information is useful for developing control strategies for CSTBRs operation, irrespective of microorganism type following different growth kinetics or the system type.

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NOMENCLETURES

c_1, c_2, c_3	constant used in Equation (2)
d	dilution rate of substrate feed stream (h^{-1})
d_1	dilution rate of base stream (h^{-1})
K_a	equilibrium dissociation constant for lactic acid
k_S	substrate saturation constant (g L^{-1})
M_a	molecular weight of lactic acid (g mol^{-1})
M_b	molecular weight of sodium hydroxide (NaOH) (g mol^{-1})
M_c	molecular weight of sodium lactate acid (g mol^{-1})
p	product concentration (mol L^{-1})
s_A	concentration of sodium lactate (mol L^{-1})
s_b	concentration of base (NaOH) (mol L^{-1})
s	substrate concentration (g L^{-1})
s_0	initial substrate concentration (g L^{-1})
$s_{\phi i}/s(pH^*,\phi_i)$	Objective sensitivity function
$S_{\phi i}/S(pH^*,\phi_i)$	Normalized objective sensitivity function
t	time (h)
x	concentration of microorganism (g L^{-1})
$y_{x/s}$	yield coefficient of biomass to substrate (g g^{-1})
$y_{p/x}$	yield coefficient of product to biomass (g g^{-1})
μ	specific growth rate (h^{-1})
μ_m	Maximum specific growth rate (h^{-1})

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