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The development of an industrial-scale fed-batch fermentation simulation

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ABSTRACT

This paper describes a simulation of an industrial-scale fed-batch fermentation that can be used as a benchmark in process systems analysis and control studies. The simulation was developed using a mechanistic model and validated using historical data collected from an industrial-scale penicillin fermentation process. Each batch was carried out in a 100,000 L bioreactor that used an industrial strain of *Penicillium chrysogenum*. The manipulated variables recorded during each batch were used as inputs to the simulator and the predicted outputs were then compared with the on-line and off-line measurements recorded in the real process. The simulator adapted a previously published structured model to describe the penicillin fermentation and extended it to include the main environmental effects of dissolved oxygen, viscosity, temperature, pH and dissolved carbon dioxide. In addition the effects of nitrogen and phenylacetic acid concentrations on the biomass and penicillin production rates were also included. The simulated model predictions of all the on-line and off-line process measurements, including the off-gas analysis, were in good agreement with the batch records. The simulator and industrial process data are available to download at www.industrialpenicillinsimulation.com and can be used to evaluate, study and improve on the current control strategy implemented on this facility.

1. Introduction

Industrial-scale production of antibiotics was pioneered through the development of deep tank fermentation during the scaling up of penicillin in the 1940s (Shuler and Kargi, 2002). This technique transformed the biotechnology sector into a billion dollar industry with deep-tank fermentations at its core. Despite the reliance of major pharmaceutical and biotech companies on large-scale fermentations, regulatory restrictions have limited innovation and decreased R&D into advanced process control strategies, which includes the development of mathematical models at this scale (Grabowski et al., 1978; Yu, 2008).

The majority of the research conducted on fermentation processes has used laboratory-scale equipment, some of this research has focused on developing first principle mathematical models. As penicillin was the first antibiotic to be commercially scaled up, a considerable amount of research has focused on quantitatively describing this process. Models defined for this type of process, range from highly complex structured models that consider the internal structure of the *Penicillium chrysogenum* fungus (Paul and Thomas, 1996; Megee et al., 1970; Nestaas and Wang, 1983; Nielsen, 1993), to more simplistic unstructured models based on kinetic expressions of growth profiles of penicillin and biomass (Righelato et al., 1968; Birol et al., 2002; Bajpai and Reul, 1980). Over the last decade it has been the simpler unstructured models which have been applied more frequently to industrial applications (Menezes and Alves, 1994). The most notable unstructured model is that developed by Bajpai and Reul (1980) which has been extended by Birol et al. (2002) and used as the standard test bed for almost every aspect of bioprocess control including multivariate statistical process monitoring and control (Lee et al., 2004; Ündey and Ertunç, 2003), regression model analysis (Zhang and Lennox, 2004) and optimisation of feeding strategies (Ashoori et al., 2009). However, based on the limited application of models in industry, there is a clear division between a useful academic fermentation model and a practical industrial one. It has been highlighted by Patnaik (2001) that the more information a model contains the more complex it becomes, which reduces its "usefulness" for monitoring and control

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studies. However considering that simple unstructured models are not sufficient to capture complex process dynamics, structured models need to be utilised and applied to industrial processes to facilitate the development of enhanced control strategies.

The purpose of this research is to develop a realistic simulation of an industrial fermentation to be used as a benchmark in process control and optimisation studies. The work describes an extension of the structured penicillin fermentation model developed by Paul and Thomas (1996) and describes all the component balances relating to the process variables. The simulation was subsequently validated using the batch records from ten 100,000 L fed-batch penicillin fermentations. The simulator improves on previous fed-batch simulations as it considers the typical problems encountered on large-scale fermentations, including challenges associated with the control of the dissolved oxygen during highly viscous fermentations and controlling key nutrients using delayed off-line measurements. The simulator and industrial process data are available to download at www.industrialpenicillinsimulation.com. The simulator can also be used as a stand alone application which includes batch to batch variation, deviations relating to input concentrations and typical process faults including foaming, agitator tripping and problems relating to inaccurate sensors. The developed simulator provides a challenge for researchers to develop robust monitoring and control strategies applicable to a complex industrial-scale penicillin fermentation simulation.

2. Industrial-scale penicillin model

The structured model developed by Paul and Thomas (1996) was adapted and implemented here, as it has been shown to best describe the fermentation behaviour when compared with ten other penicillin models (Syndall, 1998). The simulation considers the growth, morphology, metabolic production and degeneration of the biomass during a submerged *P. chrysogenum* fermentation. The simulation divides the internal structure of the biomass or hyphae into four separate regions: actively growing regions (A_0), non-growing regions (A_1), degenerated regions (A_3) formed through vacuolation and autolysed regions (A_4), as illustrated in Fig. 1. This paper omits the description of the formation of vacuoles, which represent nutrient depleted regions of the hyphae whose growth is responsible for the formation of the degenerated regions (A_3). The vacuoles are defined by Paul and Thomas (1996) as vacuole regions (A_2). Paul and Thomas (1996) provide a full description of the formation and growth of these individual regions.

A component balance on the fermenter was performed and presented here. The parameters relating to the simulation presented are available in Table 1 and Table 2 and the parameters relating to the structured penicillin model are available in Table 1A.

Growing regions (A_0):

$$\frac{dA_0}{dt} = \underbrace{r_b}_{\text{branching}} - \underbrace{r_{diff}}_{\text{differentiation}} - \underbrace{\frac{F_{in}A_0}{V}}_{\text{dilution}} \quad (1)$$

Non-growing regions (A_1):

$$\frac{dA_1}{dt} = \underbrace{r_e}_{\text{extension}} - \underbrace{r_b}_{\text{branching}} + \underbrace{r_{diff}}_{\text{differentiation}} - \underbrace{r_{deg}}_{\text{degeneration}} - \underbrace{\frac{F_{in}A_1}{V}}_{\text{dilution}} \quad (2)$$

Degenerated regions (A_3):

$$\frac{dA_3}{dt} = \underbrace{r_{deg}}_{\text{degeneration}} - \underbrace{r_a}_{\text{autolysis}} - \underbrace{\frac{F_{in}A_3}{V}}_{\text{dilution}} \quad (3)$$

Table 1
Summary of model parameters.

Parameter	Description	Units
t	Batch time	h
A_0	Growing biomass concentration	g L^{-1}
A_1	Non-growing biomass concentration	g L^{-1}
A_3	Degenerated biomass concentration	g L^{-1}
A_4	Autolysed biomass concentration	g L^{-1}
X	Total biomass concentration	g L^{-1}
P	Penicillin concentration	g L^{-1}
s	Substrate concentration	g L^{-1}
V	Vessel Volume	L
V_m	Vessel Volume	m^3
F_{in}	Total flow in	L h^{-1}
F_{dis}	Discharge rate	L h^{-1}
F_s	Sugar flow rate	L h^{-1}
F_{PAA}	Phenylacetic acid flow	L h^{-1}
F_{oil}	Soybean oil flow rate	L h^{-1}
F_w	Water for injection flow rate	L h^{-1}
$F_{a/b}$	Acid/base flow rate	L h^{-1}
F_{evp}	Evaporation flow rate	L h^{-1}
DO_2	Dissolved oxygen concentration	mg L^{-1}
k_La	Volumetric mass transfer coefficient	h^{-1}
μ_{app}	Apparent viscosity	cP
F_g	Aeration rate	m^3 min^{-1}
P_{ag}	Agitator power	kW
P_{air}	Power dissipated by aeration	kW
P_1	Vessel back pressure	Pa
ρ_b	Density of broth	kg m^{-3}
P_0	Vessel bottom pressure	Pa
Z	Ungassed liquid height	m
T_b	Temperature of broth	K
H^+	Hydrogen ion concentration	mol L^{-1}
N_{shots}	Nitrogen shots	kg
PAA	Phenylacetic acid concentration	mg L^{-1}
N	Nitrogen concentration	mg L^{-1}
$O_{2_{out}}$	Oxygen off-gas concentration	%
$CO_{2_{out}}$	Carbon dioxide off-gas concentration	%
$CO_{2,L}$	Dissolved carbon dioxide concentration	g L^{-1}

Autolysed regions (A_4):

$$\frac{dA_4}{dt} = \underbrace{r_a}_{\text{autolysis}} - \underbrace{\frac{F_{in}A_4}{V}}_{\text{dilution}} \quad (4)$$

Total biomass (X):

$$X = A_0 + A_1 + A_3 + A_4 \quad (5)$$

Product formation (P):

$$\frac{dP}{dt} = \underbrace{r_P}_{\text{Production}} - \underbrace{r_h}_{\text{hydrolysis}} - \underbrace{\frac{F_{in}P}{V}}_{\text{dilution}} \quad (6)$$

Substrate consumption (s):

$$\frac{ds}{dt} = -\underbrace{Y_{s/X}r_e}_{\text{extension}} - \underbrace{Y_{s/X}r_b}_{\text{branching}} - \underbrace{m_s r_m}_{\text{maintenace}} - \underbrace{Y_{s/P}r_P}_{\text{production}} + \underbrace{\frac{F_s c_s}{V} + \frac{F_{oil}c_{oil}}{V}}_{\text{feedin}} - \underbrace{\frac{F_{in}s}{V}}_{\text{dilution}} \quad (7)$$

where, $r_{b,diff,e,deg,a,P,h,m}$ is the rate of branching, differentiation, extension, degeneration, autolysis, product formation, product hydrolysis and maintenance, respectively. The batch time is represented by t. $Y_{s/X}$ and $Y_{s/P}$ represents the substrate yield coefficients of biomass and penicillin, respectively, and m_s is the substrate maintenance term. F_s, F_{oil}, c_s and c_{oil} represents the sugar and soybean oil feed rate and concentrations, respectively. For simplicity the addition of oil was combined with sugar to form a representative single substrate, s. F_{in} represents all the process inputs