Experimental Evaluation of Batch and Continuous Production of Baker's Yeast under Computer Controlled pH

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ABSTRACT: *Steady state and dynamic fermentations of baker's yeast in molasses based media were analyzed in a laboratory scale bioreactor. Sugar and biomass balances together with the Monod biokinetics were used to develop the process model. Parameters of the model were obtained using collected experimental data. Model predicted open loop responses to step changes in feed concentration as well as dilution rate were compared with experimental data and a good agreement was observed. Despite the nonlinear nature of pH in a biological system, it was controlled successfully using a special on-off strategy implemented on a personal computer. Results proved that productivity of the continuous process was at least twice that of the batch process.*

KEY WORDS: *Baker's yeast, Continuous production, Modeling, pH control.*

INTRODUCTION

Despite the successful application of continuous operations in numerous chemical processes, batch and fed-batch modes of operations still dominate the field of biochemical processes. This is while continuous operations is expected to have advantages such as lower consumption of raw materials, energy, time and demonstrating greater productivity [1,2,3,4]. However, application of continuous operation is possible only if one could manage to overcome the challenge of handling the strong dynamics of cell metabolism [5,6]. Quantification of the available metabolic knowledge of the living systems in terms of mathematical models could be a valuable means in this regard [7]. Simulations could have important applications in optimization, process design, parameter estimation and process control.

Modeling the behavior of biological systems is usually complicated as there are several parameters affecting the cell function in a nonlinear manner [8-12]. One such a system of commercially importance is production of the "Baker's yeast". At the present time, this product is commercially manufactured via large scale aerobic fed-batch fermentation of selected strains of *Saccharomyces cerevisiae.* The raw material used in this process is predominantly molasses, although there are other alternatives including by-products of potato and wheat starch industry [13]. Industrial scale batch and fed-batch fermentation of Baker's yeast has been modeled using mass balances on biomass, sugar, ethanol and

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1021-9986/05/2/43 6/$/2.60

oxygen [14,15]. The process has been also modeled for cases where both ethanol and glucose were present as carbon sources in the continuous operation [16].

Inspite of the efforts made to run the baker's yeast process continuously, no commercial success has been reported so far. As stated earlier, this is mainly due to difficulties associated with controlling the process. To challenge these difficulties, continuous process of baker's yeast production was analyzed in this research. The process was first modeled using simple material balances and its parameters were evaluated via experiments. Accuracy of the model was then tested by its application to predict dynamic responses of the system to impulses imposed in its input. It is known that pH has a nonlinear dynamics and difficult to be controlled. On the other hand, metabolism of microorganisms in general is limited to a narrow range of this parameter [17]. Thus it is an important task to keep the pH at the desired value and minimize its fluctuations in the fermenter broth. Therefore, pH change in the process was taken into consideration and a specially tuned on/off algorithm was devised for its control through the course of fermentation. The productivity of batch and continuous processes are compared through experimental studies.

MATERIALS AND METHODS

The microbial species was a commercial strain of *Saccharomyces cerevisiae* obtained from Iran-Mayeh factory, Tehran, Iran. As illustrated in Table 1, a carbon limited molasses based medium adjusted to an initial pH of 4.5 was used throughout this study. This medium was sterilized at 121 ^{o}C and used for both pre-culture and main culture inside the fermenter. Inoculums were prepared from slants in shake flasks at 30^{o}C.

Fig. 1 illustrates the experimental setup designed to run the main cultures. The fermenter was a 4 liter laboratory scale aerated one (CHEMAP, Switzerland). Two peristaltic pumps were used to transfer fluids into and out of the fermenter and maintain a constant volume in the fermenter during continuous operations. pH was monitored online throughout the fermentation and its values were recorded in the computer. pH was measured and the corresponding analog signal was sent to a personal computer equipped with an A/D converter hardware. The sampling frequency was one second. A computer program written in BASIC interpreter was used to control pH according to the proposed control strategy. The program uses the difference between the measured and set values to activate a peristaltic pump to introduce 0.5 molar sodium hydroxide base into the fermenter. The proper time span to add the base solution into the fermenter is calculated by the program.

Operating conditions are given in Table 2. To start the continuous operation, 4 litre of the medium was introduced into the fermenter initially and autoclaved in situ. Cooled solution was then inoculated with 300 ml of the pre-culture and cultivated in batch mode for 17 hours to obtain a proper amount of biomass concentration growing in the exponential growth phase. Then input and output flows were established and system became stable within a few hours. Foaming was suppressed by addition of anti foam.

At 3 hours time intervals, samples were taken from the fermenter and centrifuged to separate cells from the broth. Dry weight of biomass was measured by drying overnight in 105 ^{o}C [18]. Supernatant was analyzed to measure residual sugar concentration via the DNS method.

Simulation

The biological model was developed considering sugar and biomass balances and incorporating the Monod cell biokinetics [19]. Considering that the volume of fermenter is constant, equations describing the system are as follows:

$$\frac{dx}{dt} = \mu.x - D.x \qquad (1)$$

$$\frac{ds}{dt} = D.(s_f.s) - \frac{\mu}{Y_{XS}}x \qquad (2)$$

$$\mu = \mu_{mas}\frac{s}{K_s + s} \qquad (3)$$

Equation (1) is the result of biomass balance assuming a first order growth kinetics for the cells. Equation (2) was obtained from mass balance on sugar as the limiting substrate. Monod's specific growth rate equation was used as the cell biokinetic model in equation (3). Y_{xs}, μ_{max} and K_s parameters were obtained from the results of steady state experiments. The set of equations was numerically solved in MATLAB environment.

Table 1: Composition of the molasses based fermentation medium.

Clarified Molasses	114 ml
NaH_2PO_4	2.5 g
$(NH_4)_2SO_4$	8 g
$MgSO_4.7H_2O$	0.25 g
Total Volume	1000 ml

Table 2: The main operational parameters in fermentation runs.

Temperature	30 °C
pH	4.5
Stirrer Speed	500 rpm
Aeration Rate	1.25 v/v/m
Sugar Conc. in Feed	15 or 30 g/l
Working Volume	4 l

pH control strategy

Biological metabolisms usually lead to secretion of organic acids into the medium. Therefore, they are normally associated with a decline in pH. Considering this fact , in order to avoid a salt making operation, a precise addition of a basic solution could be applied to maintain an appropriate pH value during fermentation. Based on these facts, an On-Off control strategy was adopted to control the pH and it was implemented through the prepared computer program. Fig. 2 exhibits the flowchart of the prepared algorithm.As may be seen in Fig. 2, when pH falls down below the setpoint value and T_p is greater than T_c, the base pump is turned on for $T_{inj.}$ seconds and a specific amount of base is added to the broth. The program waits T_c seconds and if pH does not reach the set point value, next injection is carried out.

RESULTS AND DISCUSSIONS

Using the aforementioned control strategy, pH of the fermentation process was successfully controlled around 4.5 in all fermentations run in various modes of operations. For a continuous reactor with sterile feed, a steady state cell mass balance shows that the specific growth rate is equal to the dilution rate of the system [20]. This implies that in principle several steady state conditions may be obtained when the bioreactor is operated at dilution rates smaller than the maximum specific growth rate of the cell. At greater dilution rates, the wash out phenomenon happens and no steady state continuous operation could prevail. Therefore knowing that the yeast specific growth rate to be around 0.4 h^{-1}, the bioprocess was worked out to achieve steady state conditions at dilution rates ranging from 0.082 up to 0.185 h^{-1} at feed sugar concentrations of 15 and 30 g/l. Results obtained in these runs are presented in Table 3. By plotting $1/\mu$ versus 1/s and curve fitting, parameters of the Monod's growth rate kinetics namely μ_{max} and K_s were estimated as 0.341 h^{-1} and 1.0 g/l respectively.

Aside from estimation of the Monod biokinetic parameters, a comparison was made between continuous and batch mode fermentation productivities. Table 4 presents the outcome of this comparison. As may be seen from Table 4, the continuous process productivity was as much as 2.35 times greater than its preceding batch process on average. This figure was calculated without consideration of the required time for sterilization at the beginning of each batch and it is obvious that by taking this time into account, superiority of continuous operation over the batch process would be still more pronounced.

Figs. 3 and 4 show the results of dynamic studies. As illustrated in Fig. 3, introducing 200% step change in dilution rate from 0.148 to 0.298 h^{-1} to feed sugar with concentration 15 g/l, the steady state biomass concentration fells from 4.5 to a final value of about 2.5 g/l which is around 50% decrease. The consistency observed between the model prediction and experimental data indicates reasonable model accuracy. Fig. 4 shows another dynamic response of the system when subject to a 200% step increases in the feed sugar concentration from 15 to 30 g/l at a dilution rate of 0.133 h^{-1}. Here again model predicted trend is in agreement with the measured biomass concentration which increases twice, changing from an initial 5 g/l to a final value of about 10 g/l. The minor discrepancy between experi-mental and simulation results can be attributed to the large input changes which is of course not common in normal fermentations.

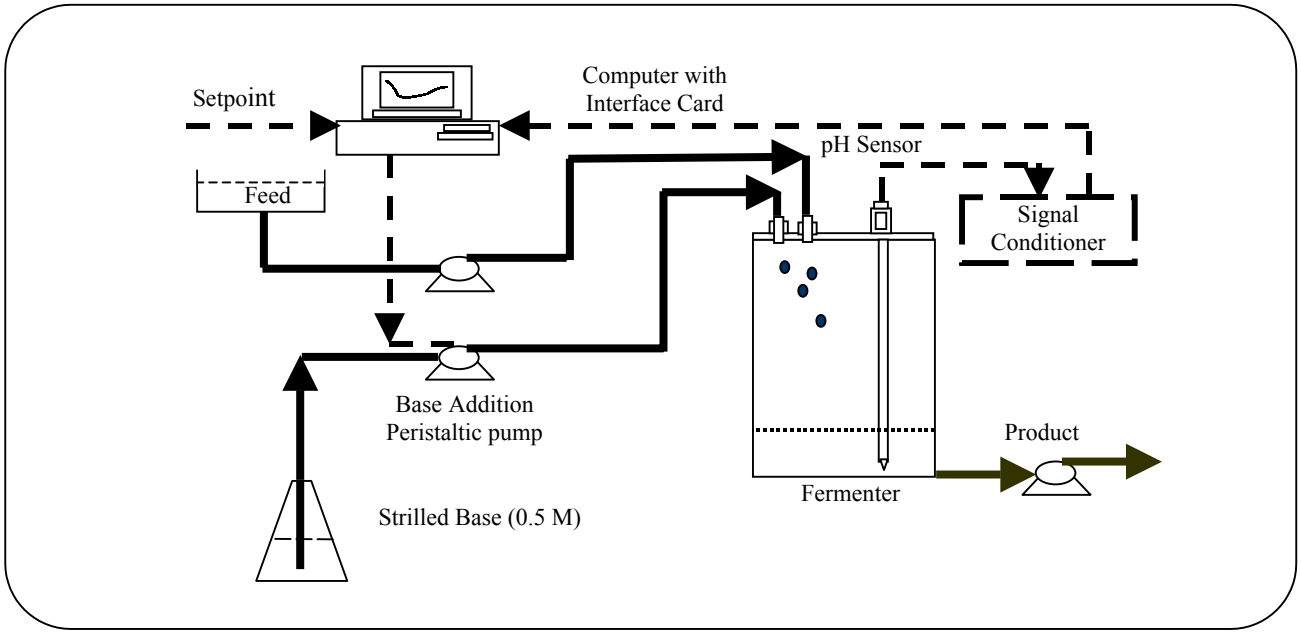

Fig. 1: Schematic diagram of the process under computer pH control.

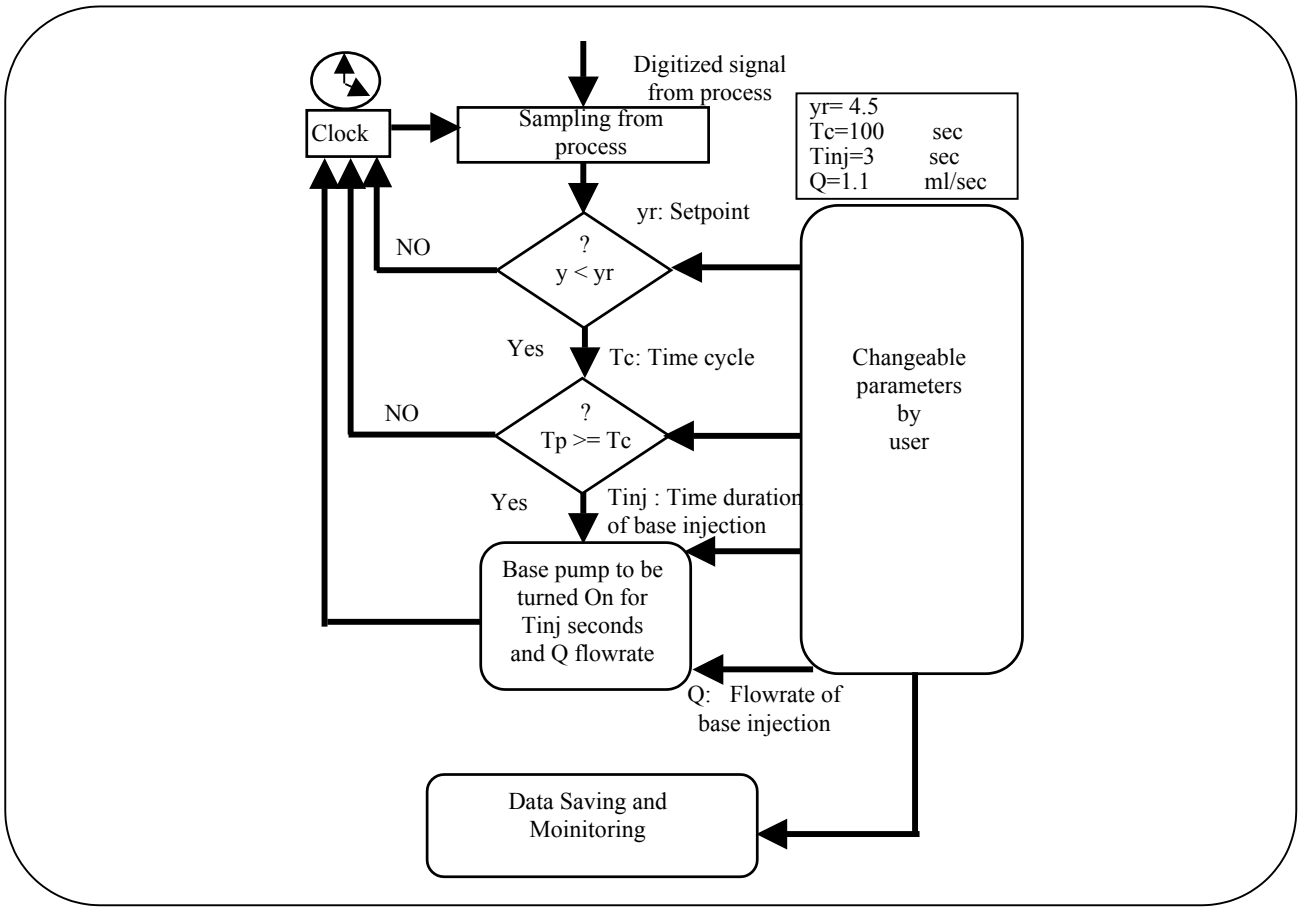

Fig. 2: The logical algorithm to manage the base addition in controlling pH of the fermenter.

Table 3: Measured and calculated results of steady state fermentations.

Experiment Number	Sugar Conc. In Feed (g/l)	Dilution Rate (h^{-1})	Cell Conc. (g/ l)	Residual Sugar Conc. (g/l)	Yield (g/g)	Productivity (g/l.h)
1	15	0.185	3.738	0.958	0.266	0.692
2	15	0.148	4.470	0.722	0.313	0.662
3	15	0.141	4.790	0.571	0.332	0.675
4	15	0.133	4.935	0.437	0.339	0.656
5	15	0.082	6.618	0.315	0.451	0.543
6	30	0.128	9.150	1.096	0.317	1.171

Table 4: Comparison between productivities of batch and continuous fermentations.

Experiment Number	Batch Time (h)	Cell Conc. at the end of Batch Run (g/l)	Productivity of Batch System (g/l.h)	Productivity of Continuous System (g/l.h)
1	17.5	5.17	0.295	0.662
2	18.0	4.91	0.273	0.675
3	18.0	5.03	0.279	0.656

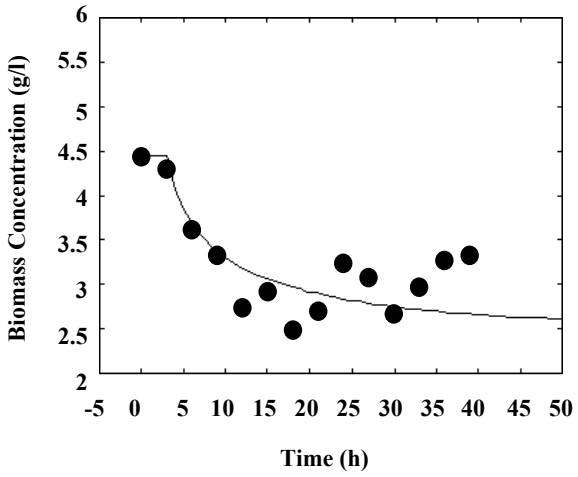

Fig. 3: Response of the system biomass concentration when subjected to a step change in dilution rate from 0.148 to 0.298 h^{-1} at the feed sugar concentration of 15 g/l.

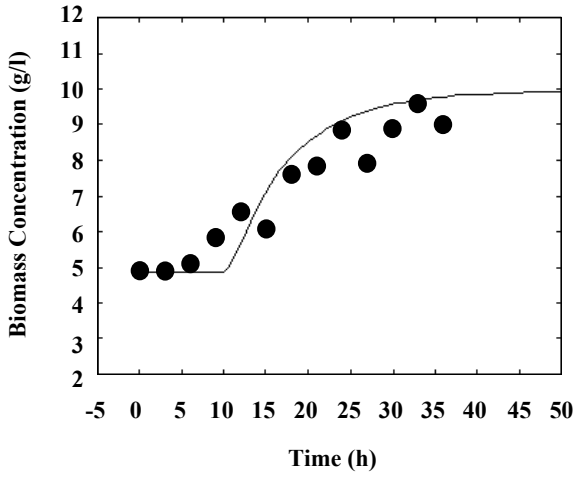

Fig. 4: Response of the system biomass concentration when subjected to a step change in feed sugar concentration from 15 to 30 g / l at dilution rate of at 0.133 /h.

CONCLUSIONS

Sugar and biomass material balances incorporating the Monod cell biokinetics were successful to develop a simple biological model to describe the behavior of the commercial strain of *Saccharomyces cerevisiae* yeast species with specific growth rate and Monod constant of 0.341 h^{-1} and 1.0 g/l respectively. Continuous process productivity was more than twice that of batch process. Model predictions of biomass concentration profile were in good agreement with experimental data when subject to step changes as large as 200% in dilution rate and feed sugar concentration. A special On-Off control strategy has been proposed to control the pH of the bioreactor and has been implemented successfully through a computer program .

Nomenclature

D	Dilution rate (h^{-1})
K_s	Parameter of Monod's growth rate equation (g/l)
Q	Flowrate of base injection (ml/sec)
S	Sugar concentration inside bioreactor (g/l)
s_f	Sugar concentration in the feed (g/l)
T_c	Time cycle (sec)
T_{inj}	Time duration of base injection (sec)
T_p	Time past from the last base injection (sec)
T	Time (h)
X	Biomass concentration inside bioreactor (g/l)
Y_{xs}	Cell yield g (cell) /g (sugar)
Y	Measured value of pH
y_r	Setpoint value for pH control
M	Specific growth rate of cell (h^{-1})
μ_{max}	Maximum specific growth rate of cell (h^{-1})

Received : 15th June 2004 ; Accepted : 30th November 2004

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