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Full Length Research Paper

Novel feeding strategies for *Saccharomyces cerevisiae* DS2155, using glucose limited exponential fedbatch cultures with variable specific growth rates (μ)

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The dual behavior of *Saccharomyces cerevisiae* on glucose feed as function of the dilution rate near the critical specific growth rate (μ=0.25) is a bottleneck in industrial production, hence the need for more efficient feeding strategies. In this work novel feeding strategies have been generated and evaluated. For each feeding profile, the 24 h fermentation time was discretized into 7 intervals of 6 then 3 h each. Seven values of μ were selected and fed into the feed flow rate equation, at different time intervals, in a way to maintain a pseudo exponential trend. Biopro_optimizer, the monitoring and controlling software of the 4-litre bioreactor used, generated nine different feeding strategies for evaluation in fermentation processes. The software also ensured the updating of the feed flow rate every 5 min for 24 h. The control setpoints of the bioreactor were 30°C, 4.5, 200 rpm and 1 vvm for the temperature, pH, agitation and aeration, respectively. The feeding profile with a μ sequence of 0.15, 0.17,0.19 ,0.17, 0.16,0.13 and 0.11 which showed an oscillating exponential increase in feed flow rate emerged among others with a productivity of 1.80 g/h or a final concentration of 14.25 g/l from an initial of 0.3 g/l. The obtained data raise the idea of higher biomass productivity of *S. cerevisiae* in a multiphase strategy with an exponentially oscillating feeding profile.

Key words: Baker's yeast production**,** feeding profiles, Biopro_optimizer, fedbatch culture, computer control.

INTRODUCTION

Saccharomyces cerevisiae (baker's yeast), has a wide industrial importance. It is used in the production of single cell protein (SCP) for human and animal consumption, ethanol from fermentable sugars, in leavening of dough due to its ability to produce carbon (IV) oxide and alcohol from sugars present in the dough (Reed and Nagodawithana, 1991; Jorgenson et al., 2002;). *S. cerevisiae* has been reported in production of macromolecular components such as lipid, proteins including enzymes and vitamins.

Despite its various applications, the cultivation of baker's yeast is not without problems**.** *S. cerevisiae* is known to use two major metabolic pathways for glucose under aerobic culture, a fermentative and an oxidative pathway (Pronk et al., 1996). The fermentative pathway of glucose metabolism which occurs when the residual glucose concentration is high, much higher than 100 mg/L, is relatively inefficient since the energy yield is only about 2ATP per mole of glucose metabolized. This results in the production of ethanol, low biomass yield and high respiratory quotient (RQ>1). The oxidative pathway of glucose metabolism predominates at glucose concentration slightly below 100 mg/L in aerobic culture and is more efficient yielding between 16 to 28 ATP per mole of glucose oxidized and a high biomass. As the dilution rate exceeds the critical value, the residual glucose concentration rapidly increases and the burden of energy generation shifts to the fermentative pathway of glucose metabolism. These problems have made the aerobic growth of *S. cerevisiae* on glucose to continue to be of research interest (Petrik et al., 1983; Alexandra and Jeffries, 1990; Kristianson, 1994). In order to achieve a

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maximum yield at the highest possible productivity, a well designed feeding strategy for S. *cerevisiae* is needed (Ejiofor et al., 1994; Belgardt, 2000). A number of glucose feeding profiles for yeast production have been attempted using constant μ near 0.25 h-1 which is believed to be a maximum for the oxidative region. But, the exact location and amplitude of this maximum is not known. In this work, the yield and productivity of *S. cerevisiae* in glucose limited exponential fedbatch is investigated using variable specific growth rates in time discretized feeding strategies.

MATERIALS AND METHODS

Generation of feeding profiles for evaluation

The 24 h process time was dicretized into 7 intervals. The first interval was between 0- 6 h and 6 other intervals were of 3 h each. A range of specific growth rate μ values between 0.09 and 0.25 was selected. The following feed flow rate equation was used:

$$FFr(t) = \frac{X_o V_o \mu e^{\mu t}}{Y_{x/s} S_o}$$

Where, FFr is feed flow rate (l/h); X_o, initial biomass (g/l); V_o, initial Volume (l); μ, specific growth rate (h-1); t, process time (h); $Y_{x/s}$, biomass yield on glucose (0.5); and S_o, glucose concentration (g/l).

For each feeding profile, 7 values of μ were selected and fed into the above equation, at different intervals of process time. Other feed flow rate variables were keyed into the Biopro_optimizer, the monitoring and controlling software (Gueguim Kana et al., 2003a). Nine different feeding strategies were thus generated and evaluated in real fermentation processes. The software also ensured the updating of the feed flow rate every 5 min.

Inoculum preparation

The strain of S. *cerevisiae* DS2155 from German collection was obtained and resuscitated in 5% glucose concentration for 24 h at 30°C. The organisms were kept on PDA (potato dextrose agar) slants for further use. For each batch, the organism from a slant was mass-produced on six different PDA plates, incubated at 30°C for 24 h. The plates were washed with 20 ml of prepared medium, and the suspension thus obtained was used as the inoculum.

Media formulation

The composition of the medium used was a modification of Ejiofor et al. (1994), and the yeast extract was substituted with wheat bran extract.

Minerals Salt Medium (g/l): $(NH_4)_2SO_4$, 10; KH_2PO_4, 5;$CaCl_2.2H_2O$,0.5; NaCl, 0.5; $MgSO_4.7H_2O$, 0.3.

Trace Salt Solution (g/l): $FeSO_4.7H_2O$, 0.278; $ZnSO_4.7H_2O$, 0.288; $CuSO_4.5H_2O$, 0.3.

Supplement Solution (g/l): $FeSO_4.7H_2O$, 5; $ZnSO_4.7H_2O$, 5; $MnSO_4.5H_2O$, 4.

Wheat bran extraction

100 g of wheat bran was suspended in 500 ml of boiled water for 10 min and then sieved. The solution was filtered and the filtrate was used in the preparation of the complete medium. A complete mineral salt medium CMSM was prepared by adding 20 ml of trace salt solution, 10 ml of supplement solution and 10 ml of wheat bran extract into 1 litre of mineral salt medium.

Fermentation process

The bioreactor used was a 3 L bioreactor, an improved design of the model earlier implemented and discussed elsewhere (Gueguim Kana et al., 2003a). The bioreactor has five control loops namely aeration loop, pH loop, temperature loop, feed flow loop and the agitation system. These loops are monitored and control through a desktop computer using Advantech data acquisition card PCL series. All the control loops are programmable through the control panel of the monitoring and control software, the Biopro_optimizer earlier on designed and implemented in our laboratory (Gueguim Kana et al., 2003b).

For the first batch, one litre of the complete mineral salt medium (CMSM) was prepared as stated above and charged aseptically into the bioreactor. 300 g of glucose were dissolved into 500 ml of water in a separate flask to serve as feed solution. The inoculum suspension earlier obtained was fed aseptically into the bioreactor to obtain an initial biomass of 0.3 g/l. Constant control setpoints of pH, temperature and aeration of 4.5, 30°C and 2 vvm, respectively, were maintained via the Biopro_optimizer control panel (Figure 1). The feeding profile earlier generated was programmed as specified by the batch. The peristaltic pump Masterflex 77120 (Coleparmer) was used to pump the glucose in the feed stream. The feed flow rate was updated every 5min through the software while all other control parameters were kept constant. Each process ran for 24 h. Samples were taken at time 0 and 24 h, and the initial and final biomass concentration were evaluated as well as the biomass productivity.

Other batches were carried out using a similar procedure, but with the glucose being charged according to the batch feeding profile specification.

Estimation of the biomass yield and productivity

Biomass concentration were determined at the beginning and at the end of each batch by sampling 10 ml of the culture, centrifuging at 1500 rpm for 15 min, and the biomass was evaluated gravimetrically using the Baujar type 1000T (Germany).

Biomass productivity is the output of biomass per unit of time. It was estimated as a ratio of cell yield over time, using the equation below:

$$R = \frac{X\max - X_0}{t_i - t_{ii}}$$

Where R is output of biomass (g $l^{-1}h^{-1}$); X_{max}, maximum cell concentration (g/l); X_0, initial cell concentration at inoculating time (g/l); t_i, time during which the organisms grow at μmax (h); and t_{ii}, time during which the organism is not growing at μmax (h).

RESULTS AND DISCUSSION

Each process time was discretized into intervals and the specific growth rates for each of these intervals are sum-

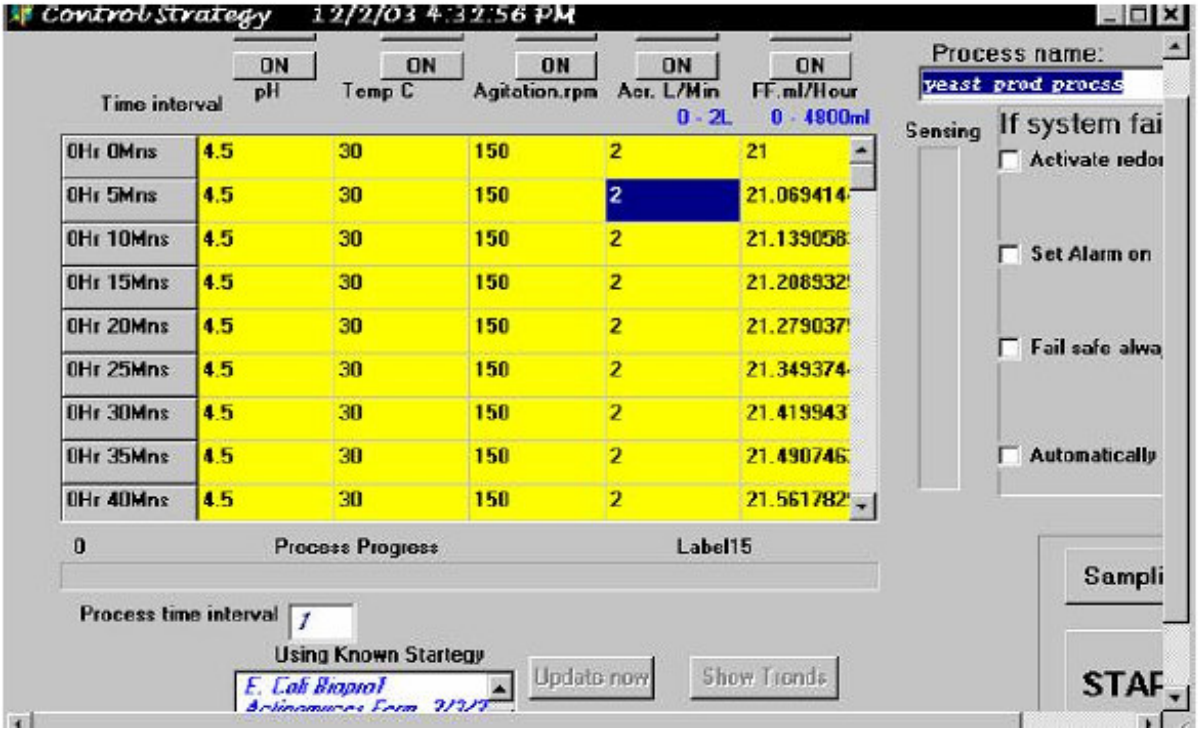

Figure 1. Control panel.

Table 1. Specific growth rate values (μ) used in the feeding profiles.

Process time(h) / Batch	0 – 6	7 – 9	10– 12	13– 15	16– 18	19–21	22 – 24
1	0.15	0.17	0.19	0.17	0.16	0.13	0.11
2	0.15	0.11	0.13	0.14	0.12	0.10	0.11
3	0.09	0.23	0.14	0.17	0.15	0.11	0.09
4	0.13	0.10	0.12	0.11	0.14	0.09	0.10
5	0.17	0.15	0.19	0.10	0.11	0.14	0.09
6	0.20	0.19	0.18	0.16	0.16	0.15	0.15
7	0.13	0.16	0.15	0.09	0.12	0.11	0.11
8	0.13	0.13	0.12	0.09	0.19	0.17	0.11
9	0.16	0.19	0.09	0.17	0.15	0.14	0.12

marized in Table 1. For each feeding profile, the values of specific growth rate maintained changed within each time interval while the initial X_0, V_0, $Y_{x/s}$ and S_0 were maintained at 0.3, 1, 0.5 and 300 respectively. Thus the feeding equation for each batch, as function of time and with variable (μ) is find in Table 2.

Using the feed flow rate equations, 9 different feeding profiles were generated (Figures 2 and 3). These profiles were imposed on the feeding pump by the monitoring and controlling software during the process.

The range of specific growth rate (μ) used (0.09 - 0.25) was chosen on the basis of a preliminary study and literature reports of possible high biomass productivities for many *S. cerevisiae* strains. The selected μ values for each interval were chosen in a way to maintain an exponential trend in a discretized profile. We were well aware of the oscillatory behavior of yeast growth associated with its kinetics at μ values between 0.09-0.25 (Satish et al., 1985), but our hopes were that some of the physiological changes might enhance the oxidative meta-

Table 2. Equations of each feeding profile in each batch.

Batch	0 to 6th hour	7 to 9th hour	10 to 12th hour	13 to 15th hour	16 to 18th hour	19 to 21st hour	22 to 24th hour
1	0.0025e0.15(t)	0.0028e0.17(t)	0.0031e0.19(t)	0.0028e0.17(t)	0.0026e0.16(t)	0.0021e0.13(t)	0.0018e0.11(t)
2	0.0025e0.15(t)	0.0018e0.11(t)	0.0021e0.13(t)	0.0023e0.14(t)	0.0020e0.12(t)	0.0016e0.10(t)	0.0018e0.11(t)
3	0.0015e0.09((t)	0.0038e0.23(t)	0.0023e0.14(t)	0.0028e0.17(t)	0.00250.15(t)	0.0018e0.11(t)	0.0015e0.09(t)
4	0.0021e0.13(t)	0.0016e0.10(t)	0.0020e0.12(t)	0.0018e0.11(t)	0.0023e0.14(t)	0.0015e0.09(t)	0.0016e0.10(t)
5	0.0028e0.17(t)	0.0025e0.15(t)	0.0031e0.19(t)	0.0016e0.10(t)	0.0018e0.11(t)	0.0023e0.14(t)	0.0015e0.09(t)
6	0.0033e0.20(t)	0.0031e0.19(t)	0.0030e0.18(t)	0.0026e0.16(t)	0.0026e0.16(t)	0.0025e0.15(t)	0.0025e0.15(t)
7	0.0021e0.13(t)	0.0026e0.16(t)	0.0025e0.15(t)	0.0015e0.09(t)	0.0020e0.12(t)	0.0018e0.11(t)	0.0018e0.11(t)
8	0.0021e0.13(t)	0.0021e0.13(t)	0.0020e0.12(t)	0.0015e0.09(t)	0.0031e0.19(t)	0.0028e0.17(t)	0.0018e0.11(t)
9	0.0026e0.16(t)	0.0031e0.19(t)	0.0015e0.09(t)	0.0028e0.17(t)	0.0025e0.15(t)	0.0023e0.14(t)	0.0020e0.12(t)

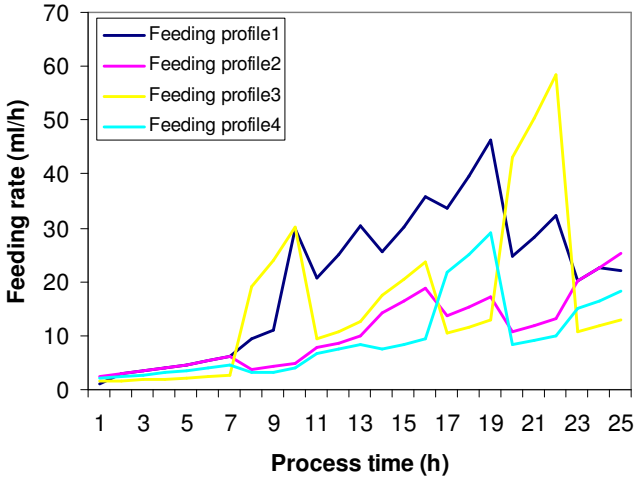

Figure 2. Four of the nine feeding profiles generated and evaluated.

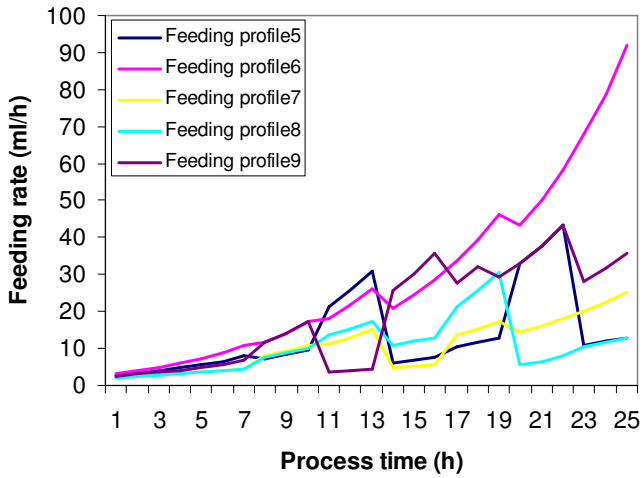

Figure 3. Five of the nine feeding profiles generated and evaluated.

bolic pathway.

The present data show that the feeding profile with μ sequence of 0.15, 0.17, 0.19, 0.17, 0.16, 0.13 and 0.11 gave a productivity of 1.80 g/h or a final concentration of 14.25 g/l (Table 3) from an initial of 0.3 g/l. In the time interval 0 to 7 h, this profile has a similar trend with the remaining eight profiles, characterized by a low feed flow rate. From 10 to 18 h, this feeding profile maintained a high, exponential and oscillatory increase of the flow rate ranging from 30 ml/h to a pick of 46.31 ml/h at 18th hour of fermentation. It eventually decreased in the same oscillatory manner to a value of 22.20 ml/h at end of the 24 h fermentation. It is unlikely that the higher yield of this profile is attributed to the high feed flow rate observed between 10 and 18th hour of fermentation, because the third feeding profile with a flow rate value of 30.11 ml/h at the 10th hour and higher flow rate values ranging from 43.21 ml/h to 58 ml/h observed between 19 to 21st hour only gave a final concentration of 12.60 g/l. This observation is again supported by the fact that the feeding profile number 6 which constantly increased exponentially in a smooth manner reaching feed flow rate values of 18.14, 28.66, 50.01 and 91.1 ml/h at the 10, 15, 20 and 24-hour of growth, respectively, gave only a biomass concentration of 5.10 g/l. Given that we operated below the critical growth rate (μ=0.25), that is in the oxidative region of metabolism, one would expect that any increase in flow rate would have a consequential increase in biomass yield.

Table 3. Final biomass concentration and productivity.

Feeding profile	Final biomass concentration (g/l)	Biomass productivity (g/h)
1	14.25	1.80
2	12.30	1.55
3	12.60	1.60
4	9.60	1.10
5	10.20	1.20
6	5.10	0.35
7	7.80	0.80
8	10.5	1.25
9	6.6	0.6

In industry, during yeast production processes, final concentration of 40 g/l can be achieved, but this is a function of the inoculum size and fermentation time. Final concentration of 59.1 g/l from an initial cell concentration of 3.8 g/l at 17 h with a yield of 0.49 g/h has been reported. For the profiles investigated above, the initial cell concentration was maintained constant at 0.3 g/l, which was quite low.

It is highly premature to advance that an industrial feeding profile with the sequence of specific growth rate as specified in the first batch can give higher biomass yield, but the present findings lay further research ground on the idea that a multiphase feeding strategy with values of specific growth rate oscillating near the critical growth rate value can be an alternative superior feeding strategy.

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