Full Length Research Paper

Experimental study of the production of biomass by *Sacharomyces cerevisiae* in a fed batch fermentor

M.O. Daramola[1, 2]* and L. Zampraka[1]

[1]Bioprocess Engineering Department, Wageningen University and Research Centre, The Netherlands.
[2]Department of Process Engineering, Stellenbosch University, Private Bag X1, Matieland 7602, Stellenbosch, South Africa.

The aim of this study is to investigate production of Baker's yeast using a theoretically programmed fed-batch fermentor to produce as much as possible Baker's yeast biomass using a minimal amount of substrate with low energy for stirring and aerating. Theoretical data obtained through material balance and kinetics equations were compared with experimental data and possible disagreements discussed with possible physical explanations. The result showed that the experimental biomass yield is greater than the theoretical one. An experimental yield of 92.2% was obtained for growing 191 mol of biomass with 207.8 mol of sugar with low oxygen uptake rate. Also, the obtained specific growth rate (μ) of 0.39 h^{-1} exceeds by far the maximum specific growth rate of the yeast under aerobic condition as obtained theoretically.

Key words: Fermentation, aerobic, fed-batch, yeast, Crabtree.

INTRODUCTION

Fermentation is the process of using microorganisms to produce valuable products such as antibiotics, industrial enzymes, food, and chemicals. Baker's yeast (*Saccharomyces cerevesiae*) is one of the oldest products of industrial fermentation. It is still one of the most important fermentation products based on volume of sales and its use for bread-making, a staple food for large section of

world's population. Baker's yeast, *S. cerevisiae*, is still one of the most important biotechnological products because it has several industrial applications. Baker's yeast as a commercial product has several formulations that can be grouped into two main types: compressed yeast, called fresh yeast, and dried yeast (Beudeker et al., 1990). Compressed yeast is the traditional formulation of baker's yeast, and is ready for immediate use. Dried yeast is available in two forms: active dry yeast (ADY) and instant dry yeast (IDY). Active dry yeast (ADY) is normally sold in airtight packages, vacuum seal or filled with an inert gas such as nitrogen. It is not a problem to maintain quality, but it should be rehydrated before use. Unlike ADY, instant dry yeast (IDY) does not have the cell damage during rehydration. IDY is the most expensive among the three type of baker's yeast (Prarakulsuksatid, 2000).

Metabolism of glucose by *S. cerevisiae* can be either respiratory or fermentative depending on culture conditions and availability of the limiting substrate when adequate and continuous supply of mineral supplements, nitrogen, growth factors and other physical inputs are available (Ejiofor et al., 1994a, 1994b). Baker's yeast production aims at providing aerobic conditions and eliminating or minimizing ethanol production. In practice, this is normally achieved by fed-batch operation, which

---

*Corresponding author. E-mail: daramola@sun.ac.za.

Nomenclature: M_X, Amount of biomass (mol); N, stirrer speed (rpm); F_g, gas flow rate (m^3/s); r_o, oxygen uptake (consumption) rate (mol/s); $r_{o\max}$, maximum oxygen uptake (consumption) rate (mol/s); P_S, stirrer power (J/s); p_s, pressure above the stirrer (bar); p_o, pressure at the prevailing condition in the fermenter (bar); $K_{ol}A$, oxygen transfer coefficient (m/s); v_{gs}, gas velocity (m/s); V_l, volume of the liquid (m^3); C_{ol}, oxygen concentration (mol/m^3); C_{olc}, critical oxygen concentration (mol/m^3); C_{ogo}, oxygen concentration in the off gas; C_{ogi}, oxygen concentration in the inlet gas; μ, specific growth rate (h^{-1}); and $\mu_{\max}$, maximum specific growth rate (h^{-1}).

allows for the control of a key nutrient concentration. The degree of success depends on the model used to guide the feeding. Either linear or exponential models may be used and these must anticipate the needs of the exponentially increasing cell mass concentration (Abel et al., 1994). A fed batch operation controls the specific growth rate at a value below the critical growth rate so that fermentative metabolism is suppressed. The glucose concentration is held within the desirable range of 0.1 - 0.25 gL^{-1}. Above a glucose concentration of 0.25 gL^{-1}, metabolism becomes increasingly fermentative as growth rate increases and the Crabtree effect or respiratory bottleneck sets in (Barford and Hall, 1979; Beck and Meyenburg, 1968; Rieger et al., 1981; Rieger et al., 1983; Sonnleitnerd and Kfippeli, 1986). Therefore, avoidance of the Crabtree effect is critical for the overall operation and limits the growth of the yeast below the critical value.

Under aerobic conditions, *S. cerevisiae* uses sugars such as glucose to grow cell mass rather than produce alcohol (Reed and Peppler, 1973; Orlowski and Barford, 1987; Barford, 1990). During these conditions, *S. cerevisiae* grows slowly producing large quantities of biomass. In a fed batch operation, nutrient mixture continuously flows into the fermenter without corresponding withdrawal except for sampling (Ejiofor et al., 1996; Enfors et al, 1990; Fiechter and Seghezzi, 1992). However, in a fed batch system, programmed to produce biomass, possible oxygen transfer deficiency might occur. The anaerobic conditions that are formed, favours ethanol production.

In some cases theoretical data obtained from engineering studies do not always apply to the real situation. The reasons for this might be (i) the nature of the yeast itself, (ii) the huge standard deviation that applies for the theoretical equations or mechanical malfunction during a bioprocess. Thus, this study was aimed at investigating if the production of baker's yeast using a fed-batch (semi-batch) operation theoretically programmed, will produce as much as possible Baker's yeast biomass using a minimal amount of substrate and a minimal amount of energy for stirring and aerating. Experimental data were compared with the theoretical data obtained through material balance and kinetics equations for possible disagreements. These disagreements were discussed and possible physical explanations proffered.

Equations employed to obtain the theoretical data and in the experiment

Theoretical amount of biomass

With the assumption that $\mu = \mu_{max} = 0.25 h^{-1}$, the theoretical amount of biomass produced was estimated using Eqn. (2),an analytical solution of Pirt's law (Eqn. 1).

$$r_s = -\left(\frac{\mu}{Y_{XS}} + m_s\right) * M_X \tag{1}$$

$$M_X(t) = M_X(0) * e^{\mu_{max} * t} \tag{2}$$

where M_X(t), the amount of biomass at time t in the broth during fermentation in mol; r_s , the rate of substrate consumption; u, the specific growth rate(hr-1); Y_{XS}, the yield coefficient; M_S, the maintenance coefficient; M_X(0), the initial amount of biomass in the broth at the start of the fermentation; and

$$M_X(0) = 42.20 mol$$

Oxygen uptake (consumption) rate

The oxygen uptake rate during the experiment was obtained using

$$r_o = F_g * (C_{ogo} - C_{ogi}) \tag{3}$$

Where F_g , the gas flow rate, C_{ogo}, the oxygen concentration in the off-gas; and C_{ogi}, the oxygen concentration in the in-coming gas and r_o, the oxygen uptake (consumption) rate in the broth. Theoretical oxygen uptake rate was obtained using

$$r_o = 0.0006 * e^{0.004 t} \tag{4}$$

Where t, the time for the fermentation. Here, Eqn. 4 is unsteady state equation for the oxygen uptake rate in the broth.

Oxygen transfer rate coefficient ($K_{ol}A$)

$K_{ol}A$ for coalescing was obtained from:

$$K_{ol}A = 2.6 * 10^{-2} \left(\frac{P_s}{V_l}\right)^{0.4} \left(v_{gs} \frac{p_a}{p_s}\right)^{0.5} \text{ (Wang et al., 1971) } \tag{5}$$

where $K_{ol}A$, the oxygen transfer coefficient; P_s , the stirrer power, V_l, volume of the liquid in the fermentor; v_{gs}, the gas velocity in m/s; p_o , the pressure at the prevailing condition, p_s , the pressure around the stirrer. And for non coalescing $K_{ol}A$ Model:

$$K_{ol}A = 2.0 * 10^{-3} \left(\frac{P_s}{V_l}\right)^{0.7} \left(v_{gs} \frac{p_a}{p_s}\right)^{0.2} \text{ (Wang et al., 1971) } \tag{6}$$

However, for the experiment, $K_{ol}A$ was obtained from:

Table 1. Control variables for fermentation.

z	F_g(m³/s)	F_g (l/min)	N (RPS)	N (RPM)	Substrate pump rate (ml/min)
0-0.5	0.001	60	2.837	169.6	24.6
0.5-1	0.001	60	2.827	169.6	27.9
1-1.5	0.0013	78	3.113	186.8	31.6
1.5-2	0.0013	78	3.113	186.8	35.8
2-2.5	0.0015	124	3.693	248.0	40.6
2.5-3	0.0015	124	3.693	248.0	46.0
3-3.5	0.0019	160	4.134	310.0	52.1
3.5-4	0.0019	160	4.134	310.0	59.0
4-4.5	0.0033	200	6.667	400.0	66.0
4.5-5	0.0033	200	6.667	400.0	64.0
5-5.5	0.0033	200	6.667	400.0	62.0
5.5-6	0.0033	200	6.667	400.0	60.0
6-6.5	0.0033	200	6.667	400.0	58.5
6.5-7	0.0033	200	6.667	400.0	56.9

$$K_{ol}A = \frac{N_o^{gl}}{V_l \cdot (\frac{C_{ogo}}{m} \cdot \frac{p_s}{p_a} - C_{ol})} \qquad (7)$$

Where N_o^{gi}, the oxygen flux; m, the mass transfer coefficient.

Respiration quotient (RQ) in the broth

This was calculated using the equation:

$$RQ = \frac{V_{CO_2}}{V_{O_2}} \qquad (8)$$

Fermentation control variables

Before experimentation, a properly designed strategy was carried out. The resulted experimental design uses some assumed control variables such as gas flow rate, the stirrer speed and amount of the substrate consumed. Different design strategies were considered. The consideration was based on the optimum values of the gas flow rate; the stirrer speed and amount of substrate consumed to run the fermentation for a period of 7 h (Table 1). These values were fed into Equations (1) - (8) to obtain theoretical data for the fermentation. Also, experimentation was carried out using these values and the results compared with the theoretical values.

MATERIALS AND METHOD

Preparation of substrate and inoculum

Baker's yeast, *ATCC4 111*, was used in this experiment. The inoculum was prepared by dissolving 800 g of 28% dry yeast in

Table 2. Specifications of the fermentor.

Characteristic	Value
Maximum liquid volume	0.2 m³
Diameter of the fermentor	0.594 m
Diameter of the stirrer	0.24 m

water. The substrate was made up of a solution of 6.8 kg glucose in 20 L of the solution. This solution was thoroughly mixed and kept in a substrate tank, which was linked to the fermentor through a substrate pump. Hence the name fed-batch fermentor.

Nutrients

Ammonium sulphate [($(NH_4)_2SO_4$] was used as N-source and S-source. Approximately 1.31 kg of this salt was weighed and added directly to the fermentor. As P-source potassium phosphate (K_3PO_4) was used. Approximately 93 g of it was weighed and added to the fermentor.

Fermentation system

A Bioengineering LP300 Pilot Plant (number 480625 Steinmann AG Flawil) fermentor (Figure 9) and control panel (models of 1983) was used. The specifications of the fermentor are given in Table 2. A Stepdos 08RC pump pumped the substrate into the fermentor.

Experimental procedure

The inoculums was added to the fermentor and diluted with tap water up to about 180 L. The airflow rate and the stirrer speed were adjusted periodically as presented in Table 1, every hour accordingly to the theoretical data. The substrate pump rate was adjusted every 30 min (Table 1). At every 30 min interval, some quantity of broth was withdrawn from the fermentor and analyzed for biomass.

Table 3. Absorption values obtained at various dilutions using the initial bioreactor biomass Content.

Dilution (X)	Absorption value (AV)	Concentration (g/l)
10	0.557	0.44
20	0.254	0.222
30	0.138	0.148
40	0.068	0.111

Table 4. Theoretical and experimental amount of biomass during the fermentation.

Time (t)(h)	Theoretical amount of biomass in the broth (M_X) in mol	Experimental amount of biomass in the broth (M_X) in mol
1.0	42.19	36.82
1.5	47.80	41.48
2.0	54.17	47.40
2.5	61.38	55.76
3.0	69.55	73.65
3.5	78.81	97.89
4.0	89.31	115.89
4.5	99.608	138.54
5.0	111.78	155.19
5.5	123.40	184.75
6.0	134.49	200.46
6.5	145.09	212.98
7.0	155.20	224.50

Determination of biomass

The amount of biomass in the fermentor at various time intervals was determined in a X-dilution solution (X = 5, 10, 20 or 30), using a *Pharmacia LKB-Ultrospec III spectrophotometer*. For a 5-, 10-, 20-, 30- and 40- dilution of the initial biomass concentration (0.8 kg yeast/0.18 m^3 liquid volume = 4.44 kg yeast/m^{3}liquid) in the bioreactor, absorption values (AV) were obtained. Based on the data obtained (Table 3), a regression expression (Eqn. 9 with R^2= 0.9978) with experimental amount of biomass at a time interval was obtained.

$$M_x(t) = (0.6812 * (AV) + 0.0571) * V_l(t) * X \qquad (9)$$

where $V_l(t)$, the volume of the liquid in the reactor at the end of every time step; X, the dilution of the sample; and AV, the absorption value read from the *Pharmacia LKB-Ultrospec III* spectrophotometer

Gas concentration measurements

Concentration of oxygen in the liquid near the stirrer, concentration of oxygen in the off-gas and concentration of carbon dioxide in the off-gas were measured on-line by a Servomex Oxygen Analyzer Series 500, (*model 1440 Gas Analyzer*) and processed by a *In Touch computer program*.

RESULTS

Theoretical and experimental amount of biomass

Using Eqn. 2, theoretical amount of biomass was obtained. The theoretical and experimental amount of biomass produced are shown in Table 4. Also, Figure 1 shows the plot of the theoretical and experimental amount of biomass produced. Figure 2 also shows the amount of biomass produced as a function of time in the first 4 h.

Respiration quotient and concentration of oxygen in the broth

As behaviour of the fermentation can be explained using the RQ and C_{ol}, plots of RQ and C_{ol} as functions of time were made. Figure 3 shows the plot of RQ against time while the plot of C_{ol} against time is presented in Figure 4.

Experimental $K_{ol}A$ and theoretical $K_{ol}A$

Figure 7 shows the comparison between experimental $K_{ol}A$ and the theoretical $K_{ol}A$ (obtained from the use of equations (5) and (6)). The dependence of $K_{ol}A$ on F_g and N is explained with Figure 8.
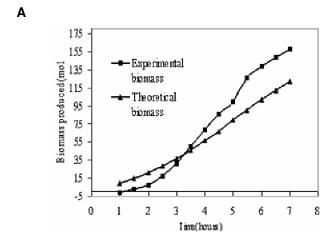
DISCUSSION

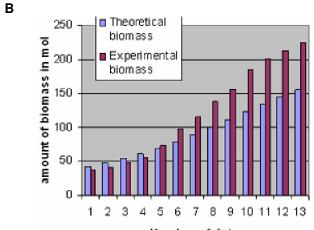### Amount of biomass produced

From Figure 1 (A, B and C) there is a clear difference between the theoretical and experimental amount of biomass. In the first 4 h (Figure 1 (A, B and C) and 2), the yield of biomass was less than expected, in contradiction with the rest of the experiment, during which the yield of biomass was more than the theoretical one.
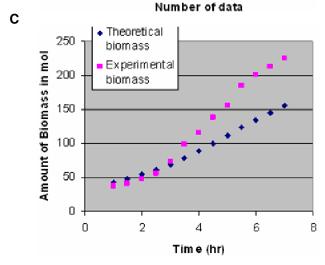
The overall yield of biomass was greater than the theoretical one. Possible reason that can lead to the disagreement between the theoretical and the experimental yield of biomass may be inaccurate measurement of the experimental biomass.

Respiration quotient (RQ) in the broth

From Figure 3, it is noticed that the values of RQ rise above 1.5 in the first hour. After the first hour (Figure 3), RQ decreased. The high RQ values observed in the beginning of the fermentation (up to 60 min) show that more CO_2 is produced than oxygen consumed (as obtained from Eqn. 7). From that observation we can conclude that the yeast is active during the first hour and

A

B

C

Figures 1A, B and C. Comparison of the experimental and theoretical biomass versus time.

that there is other source of carbon dioxide than the aerobic oxidation of the glucose during the first hours as RQ is the standard indicator when oxygen is used to metabolize the substrate (Wang et al., 1971).

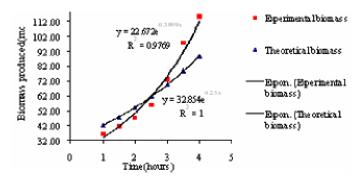In the first hour of the fermentation, there is a biomass
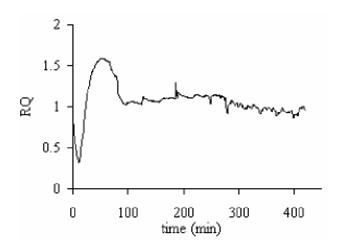

Figure 2. Biomass versus time in the first 4 h.

Figure 3. Curve of the RQ (Respiration Quotient) versus time.

yield of 3.96 mol (Table 4 and Figure 2), a value that is quite small in comparison with the overall fermentation. For example during the last hour there is biomass yield of 24 moles. Therefore, the yeast grows very slowly during the first hour. However, as mentioned before, there was much production of carbon dioxide. Furthermore, substrate conversion was observed. These observations led to the conclusion that there is a possibility that the yeast converts the substrate anaerobically.

When the yeast produces ethanol (anaerobic conversion of the substrate), the biomass yield will be less because of the utilization of part of the substrate for the formation of the product. This is corroborated by the results obtained during the first hour. Also, this validates the assumptions that the anaerobic production of alcohol is caused when the specific growth rate of the yeast exceeds a certain value (0.27 h^{-1}) and by a high substrate supply rate (Wang et al., 1971). Furthermore, this can be as a result of inefficient oxygen supply, which eventually creates anaerobic environment.

However, after the first hour RQ decreases and attains an almost constant value of 1, which indicates that almost equimolar quantities of oxygen and carbon-dioxide are consumed and produced (Eqn. 7.), respec-
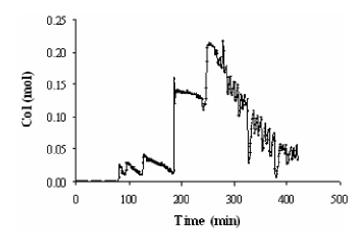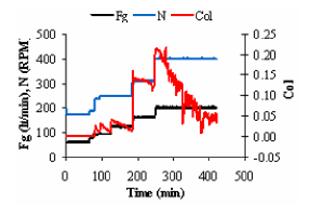
Figure 4. C_{ol} versus time.

Figure 5. Plot of the concentration of the oxygen in the liquid (C_{ol}), stirrer speed (N) and gas flow rate (F_g) versus time

Figure 6. Comparison of theoretical and experimental oxygen production rate versus time.

tively. The almost constant value of RQ indicates that a balance has been achieved between the oxygen consumption and carbon dioxide production. However, this value is normally below 1. To further investigate and explain the results obtained, a curve of C_{ol} against time was plotted (Figure 4).

Oxygen concentration (C_{ol}) during the fermentation

To further investigate and explain the results obtained, a curve of C_{ol} against time was plotted (Figure 4). In the first hours, the zero value of C_{ol} shown in Figure 4 led to the assumption that all the oxygen provided to the yeast was used. Theoretically, when C_{ol}=0, it is possible that the yeast has enough oxygen to maintain its aerobic (the yeast is provided exactly as much oxygen as it needs). However, almost in every case, a critical concentration of
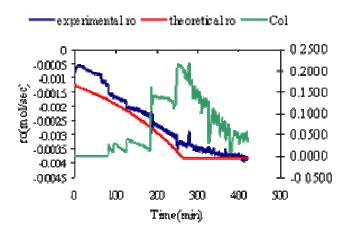
oxygen in the liquid, C_{olc}, exists so as to assure that the yeast is not limited by low oxygen supply. Our results shows exactly that the oxygen supply during the first hour have limited the growth of the yeast. Together with the results obtained from Figure 3 (RQ), the C_{ol}=0 supports the fact that there was anaerobic conversion of the substrate as a result of low oxygen supply. However, the oxygen concentration in the liquid is above zero for the rest hours of the fermentation, which indicates that the oxygen supply was not limiting the growth of the yeast.

For t=80 - 200 min, C_{ol} has a decreased value, equal to C_{olc}. However after 200 min, C_{ol} increases constantly reaching very high values. Accepting the assumption that the oxygen supply rate was limited from the beginning, these results are just the normal consequence. Better explanation for this is as follows: The oxygen supply limited the growth of the yeast during the first hour. As a consequence, the yeast started to convert the substrate anaerobically. The biomass yield during the first hour decreased, because of the limited oxygen supply. Although the production of alcohol was too low. Therefore, less oxygen than the theoretical value is needed for the growth of the yeast after the first hour. A better understanding is obtained plotting C_{ol}, N, F_g as a function of time (Figure 5).

Theoretical and experimental oxygen consumption rate (r_o)

From Figure 6, the comparison of the two r_o curves shows that the theoretical oxygen consumption rate is greater than the experimental r_o during the whole (almost) fermentation period. Except from the great deviation noticed during the first hour, the r_o curves are quite similar. This confirms our previous assumptions, that the oxygen supply in the first hour was limiting the
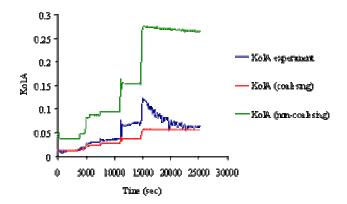
Figure 7. Experimental $K_{ol}A$ compared with coalescing and non-coalescing models.

Figure 8. The influence of F_g and N on the $K_{ol}A$.

growth of the yeast and because of that the Crabtree effect appeared.

Stirrer speed (N) and the gas flow rate (F_g)

From Figure 5, it is observed that when F_g and N increase, C_{ol} increases. At the first hour, as it was concluded earlier, the oxygen supply is limiting. After the first hour, the oxygen supply is higher than what the yeast needed. At t = 180 min, C_{ol} increases suddenly but drops gradually. From that observation it was concluded that the experimental M_X is almost equal to the theoretical M_X and the oxygen supply is exactly as much as the yeast needed. Hence, C_{ol} starts reducing gradually. This agrees with the situation in Figures 1 and 2, where the experimental biomass yield equals the theoretical biomass yield. With another change in N and F_g the same sudden increase and gradual decrease of C_{ol} was observed.

Substrate pump rate

The substrate pump rate was adjusted for the value needed in the end of each time step. Hence, for the most of the duration of each time step the substrate supply is higher than the yeast can oxidize. This allows the yeast to grow faster, but inevitably the Crabtree effect appears. If it is assumed that the yeast first uses all the oxygen provided to convert the glucose to biomass and then alters its metabolism to the production of ethanol, more biomass would be yielded than the theoretical value obtained. However, this rarely happens. It is more possible that a balance between the aerobic and anaerobic conversion of the substrate is achieved. This assumption agrees with the high C_{ol} values observed.
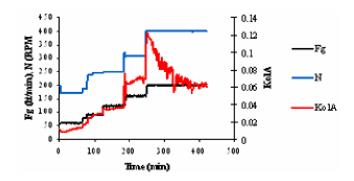
However it is good to notice that in the theoretical calculation the circulation time was neglected and the fermentor was assumed to be ideally mixed. But in practice the situation is different.

Specific growth rate (μ)

From Figure 7 the specific growth rate (μ) of the yeast for the time period between 0 and 4 hour was 0.39 h^{-1}. This value is much higher than the theoretical value assumed (μ = 0.25 h^{-1}). Also this value is greater than some values reported by some authors (Dantigny et al., 1992; Kristiansen, 1994), which were between 0.25 and 0.30 h^{-1} (Dantigny et al., 1992; Kristiansen, 1994). Theoretically, when μ>0.25 h^{-1}, the yeast produces ethanol. This confirms the fact that the Crabtree effect appeared during the first 4 hours of the fermentation.

Oxygen transfer rate coefficient ($K_{ol}A$)

In Figure 7, comparison of the experimental $K_{ol}A$ and the theoretical coalescing and non-coalescing $K_{ol}A$ is made. The difference in the theoretical and experimental r_o observed (Figure 6) could be explained by mean of the oxygen transfer coefficient. If the actual $K_{ol}A$ is made higher than the theoretical value, it will result in a higher oxygen transfer rate which would subsequently lead to the appearance of $r_{o\max}$ at later time step of the experimentation contrary to the theory (as obtained from the theoretical calculation). The yeast would stay longer in the exponential growth phase and hence more biomass would be produced. Figure 7 shows that our experiment was conducted somewhere between coalescing and non-coalescing conditions. The steep jumps in the $K_{ol}A$ values are as a result of changes in the stirring speed and/or airflow rate (Figure 8). Also, the steep fluctuation of $K_{ol}A$ in the last part may be as a result of the addition of antifoaming materials, which result in a more coalesc-

Figure 9. Pilot-scale fed-batch fermentor for the study.

ing behaviour of the broth. The oxygen bubbles coalesce, resulting in a decrease of the surface area available for transfer and hence a decrease of the oxygen transfer coefficient ($K_{ol}A$). This agrees with the situation in Figure 6, where the experimental r_o curve intersects the theoretical r_o curve at the end of the 7 h, possibly due to a decrease in the $K_{ol}A$.

Conclusions

An experimental study of yeast production in a fed-batch fermentation using pilot-scale fermentor has been done. The comparison between the experimental and the theoretical biomass yields shows that experimental biomass yield is grater than the theoretical yield. Growing 191 mol of biomass with 207.8 mol of sugar gives an experimental yield of 92.2%, an extremely high value. Also, the experimentally obtained specific growth rate (μ) of 0.39 h^{-1} exceeds by far the maximum specific growth rate of the yeast under aerobic condition. Taking into account the fact that very little growth occurred in the first hour, these facts seem ambiguous. Also the regulation of the substrate supply rate at the end of each step must have promoted the Crabtree effect observed in the fermentation. Nevertheless, we were led to the conclu-sion that there was a simultaneous anaerobic production of biomass during the fermentation which might have led to the huge experimental biomass yield (theoretical + anaerobic), higher specific growth rate and lower oxygen uptake rate observed. At the same time, we came to a conclusion that the geometry of the fermentation tank and the circulation of the liquid definitely have something to do with aerobic and anaerobic biomass production observed.

REFERENCES

Abel C, Huber U, Schugeri K (1994). Transient behavior of Baker's yeast during enforced periodical variation of dissolved oxygen concentration. J. Biotechnol. 32(1): 45-57.

Barford JP, Hall RJ (1979). An examination of the Crabtree effect in *Saccharomyces cerevisiae:* the role of respiratory adaptation. J. Gen. Microbiol. 114: 267-275.

Barford JP (1990). A general model for aerobic yeast growth: Batch growth. Biotechnol. Bioeng., 35: 907-920.

Beck C, Meyenburg. HK yon, (1968). Enzyme pattern and aerobic growth of *Saccharomyces cerevisiae* under various degrees of glucose limitation. J. Bacteriol. 96: 479-486.

Beudeker FR, van Dam HW, van der Plaat JB, Vellenga K (1990). Developments in baker' s yeast production. In *Yeast Biotechnology and Biocatalysis*, ed. Verachtert H. New York: Marcel Dekker, Inc.

Dantigny P, Ziouras K, Howell JA (1992). A structured model of baker's yeast fed-batch growth. In: Proceedings of the IFAC Symposium Series No. 10 on Modelling and Control of Biotechnical Processes (Karim MN, Stephanopoulos G, eds), Keystone, Colorado. pp. 223-226.

Ejiofor AO, Posten C, Solomon BO, Deckwer WD (1994a). A robust fed-batch feeding strategy for optimal parameter estimation for baker's yeast production. Bioproc. Eng. 11: 135-144.

Ejiofor AO, Posten C, Solomon BO, Deckwer WD (1994b). Analysis of the respiro-fermentative growth of *Saccharomyces cerevisiae* on glucose in a fed-batch fermentation strategy for accurate parameter estimation. Appl. Microbiol. Biotechnol. 41: 664-669.

Ejiofor AO, Chisti Y, Moo-Young M (1996). Fed-batch production of baker's yeast using millet *(Pennisetum typhoides)* flour hydrolysate as the carbon source, J. Ind. Microbiol. 16: 102-109.

Enfors SO, Hedenberg J, Olsson K (1990). Simulation of the dynamics in the baker's yeast process. Bioproc. Eng. 5: 191-I98.

Fiechter AW, Seghezzi W (1992). Regulation of glucose metabolism in growing yeast cells. J. Biotechnol. 27: 27-45.

Kristiansen B (1994). Integrated Design of a Fermentation Plant: The Production of Baker's Yeast, VCH Verlagsgesellschaft, Weinheim. p. 21.

Orlowski JH, Barford JP (1987). The mechanism of uptake of multiple sugars by *Saccharomyces cerevisiae* in batch culture under fully aerobic conditions. Appl. Microbiol. Biotechnol. 25: 459-463.

Prarakulsuksatid P (2000). Ultilization of microbubble dispersion to increase oxygen transfer in pilot-scale Baker's yeast fermentation units, M.Sc. Thesis, Virginia Polytechnic Institute and State Univer-sity, Virginia, USA.

Reed G, Peppler HJ (1973). Yeast technology. Westport, C. T. AVI Pub. Co.

Rieger M, Kfippeli O, Fiechter A (1981). The respiratory capacity of *Saccharomyces cerevisiae.* In: Current Developments in Yeast Research (Stewart GG, Russel I eds. Pergamon, Toronto. pp. 369-373.

Rieger M, Kfippeli O, Fiechter A (1983). The role of limited respiration in the complete oxidation of glucose by *Saccharomyces cerevisiae.* J. Gen. Microbiol. 129: 653-661.

Sonnleitnerd B, Kfippeli O (1986). Growth of *Saccharomyces cerevisiae* is controlled by its limited respiratory capacity: formulation and verification of a hypothesis. Biotechnol. Bioeng., 28: 927-937.

Wang DIC, Cooney CL, Demain AL, Dunnill P, Humphrey AE, Lilly MD (1971). Fermentation and Enzyme Technology, New York: John Wiley and sons.