0273-2289/96/5758—0593$6.50

Improvement of Productivity of Yeast Cell with a Novel Airlift Loop Reactor

DEHUA LIU,*,[1] SHIAN HUANG,[1] MING LI,[1] YAN SUN,[2] TIANZHONG LIU,[1] FAN OUYANG,[1] AND GEORGE T. TSAO[3]

[1] State Key Laboratory of Biochemical Engineering, Institute of Chemical Metallurgy, Chinese Academy of Sciences, Beijing 100080, P. R. China; [2] The STORE Institute of Grain Storage Technology, 13 Ma Lian Dao Road, Beijing 100055, P.R. China; and [3] Laboratory of Renewable Resources Engineering, Purdue University, West Lafayette, IN 47907

ABSTRACT

Two different strains of baker's yeast are cultivated using a fed-batch process with a novel airlift loop reactor. The reactor can be operated not only under steady-state conditions as the traditional airlift loop reactor, but also under forced periodically operational conditions in which the direction of liquid circulating flow is alternatively changed. Compared with the traditional steady-state operation, both the growth rate and yield of cells are much higher in the forced periodic operation.

Index Entries: Airlift loop reactor; forced periodic operation; baker's yeast; fed-batch culture; specific growth rate.

INTRODUCTION

The airlift loop reactor has been used in a wide range of applications in the biochemical industry, because the energy consumption and the damage of shear force to cells can be minimized as compared with the traditional stirred-tank reactor *(1–6)*. However, the traditional airlift loop reactor is generally operated in a steady-state mode, i.e., the liquid circulating flow in the reactor is always along the same direction. It leads to slow relative movement between the cells and the liquid medium because of the proximity of their density and thus the poor mass transfer characteristics. Another problem with traditional airlift loop reactor is that the concentration of dissolved oxygen is too low to meet the demand of cells in the downflow region.

When a chemical reactor is dynamically operated by periodically oscillating one or a few of its operation parameters, its behavior may be very different from that under steady-state operation conditions. This is the so-called forced periodic operation. Many research reports have been published about this field during the past few decades *(7–11)*. Based on the principle of forced periodic operation of reactor, a novel airlift loop reactor was proposed *(12,13)*. Because of its special configuration,

*Author to whom all correspondence and reprint requests should be addressed.

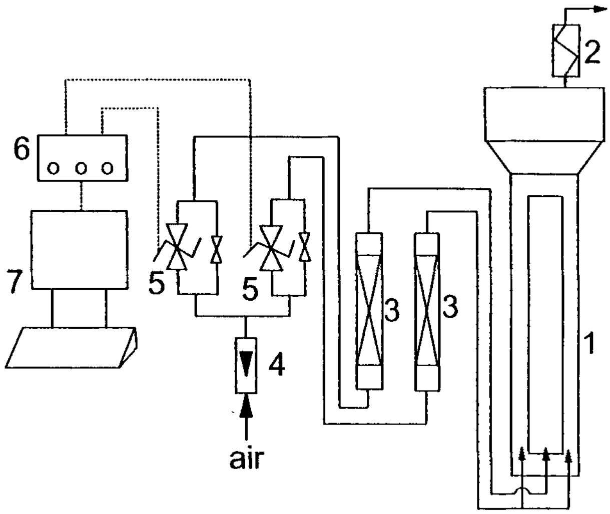

Fig. 1. The schematic diagram of experimental apparatus: 1, reactor; 2, condenser; 3, filter; 4, flow meter; 5, solenoid valve; 6, controller; 7, computer.

it can be operated not only in a steady-state mode as a traditional airlift loop reactor, but also in an oscillating mode. During the operation in an oscillating mode, the direction of circulating flow is periodically changed, so that the relative movement between the cells and the liquid medium is significantly enhanced, and the mass-transfer coefficient is increased very much. In addition, the upflow and downflow regions are alternatively changed, resulting in the change of circulation direction, so the deficiency in dissolved oxygen at the downflow region will be diminished.

In this study, two strains of baker's yeast were cultured with this new airlift loop reactor in different modes of operations. The experimental results show that both the specific growth rate and the conversion yield of cell mass are much larger in the oscillating operation than in the traditionally steady-state operation.

MATERIALS AND METHODS

Figure 1 shows the schematic representation of airlift loop reactor. The total volume of the reactor is about 15 L. At the bottom of the reactor, there is a central nozzle through which air can be delivered into the inner compartment of the draft tube. There are three nozzles that are installed around the circumference of the reactor through which air can be sparged into the annulus between the walls of the draft tube and the reactor. The two solenoid valves are alternatively switched on and off by the control of a computer so that the air can be delivered alternatively into the inner compartment of the draft tube or the annulus, and then the direction of the liquid circulating flow is reversed periodically.

When the reactor is run in a periodic operation mode, the oscillating frequency is found to have great effects on its behavior. The optimal frequency was determined by measuring the fluctuation sign of static pressure of liquid and mass-transfer coefficient at different frequencies of operation *(12,13)*. Its principle is shown in Fig. 2. During the periodically oscillating operation, each operation cycle consists of two durations during which air is delivered once into the inner compartment of the draft tube and also once into the annulus between the walls

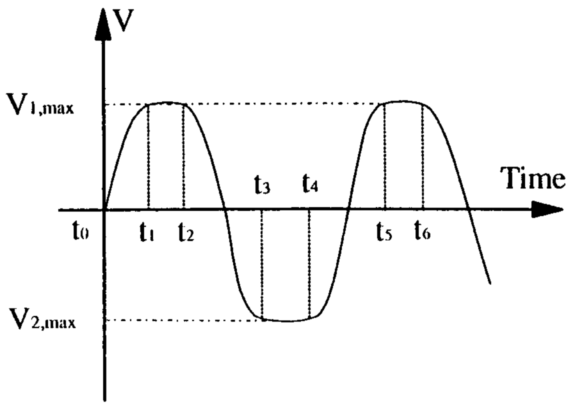

Fig. 2. The variation of circulating velocity during the periodic operation.

of the draft tube and the reactor. The liquid circulating velocity will be changed not only in its value, but also in its direction, as shown in Fig. 2. Based on the principles of forced periodic operation, the optimal frequency in which the reactor can be operated always under transient state, i.e., the switching of operating parameter, should happen just when the reactor reaches its next steady state. Therefore, the optimal length of each operation cycle should be the duration from t_2 to t_3 and one from t_4 to t_5. However, it is very difficult to measure such a circulating velocity, but it is relatively easy to measure the liquid static pressure during the change of circulating velocity. Actually, the liquid static pressure will also be periodically changed in its value as the change of circulating velocity, so it is available to determine the optimal oscillating frequency by measuring the static pressure. In this study, the optimal frequencies determined with the above method were further modified by measuring the mass-transfer coefficients.

In order to compare the different behaviors of the reactor in different operation modes, the baker's yeasts were fed-batch cultured in the steady and periodic state operations, respectively. In different operation modes, all the other parameters, such as medium composition, aeration rate, feed rate, and temperature, were kept the same. During reactor operation, glucose concentration was maintained at about 5 g/L by feeding glucose at a concentration of 200 g/L, and the temperature and pH were kept 30°C and 4.5. The initial concentrations of peptone and yeast extract were 10 and 5 g/L, respectively. The aeration rate and the feed rate of glucose were changed once every hour and increased according to exponential law.

MICROORGANISMS

Two different strains of baker's yeast were used. The first one is commercial dry baker's yeast, which is purchased from the supermarket and given representative number ICM-10 (Institute of Chemical Metallurgy) in this article. Before being inoculated into the reactor, about 30 g of dry yeast were incubated for 2–3 h with medium containing 3 g/L malt extract. The other one is a baker's yeast stored in the State Key Laboratory of Biochemical Engineering, Institute of Chemical Metallurgy, Beijing, China, and it was given the representative number ICM-11. Before being inoculated into the reactor, it was precultured for 20–24 h with medium containing 3 g/L malt extract.

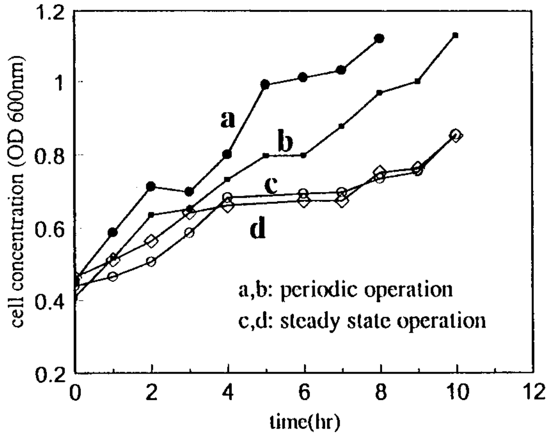

Fig. 3. Growth curves of yeast ICM-10.

RESULTS AND DISCUSSIONS

Glucose Feed Rate and Aeration Rate

Generally, cell growth and the consumption of glucose and oxygen follow the exponential law, so the glucose feed rate and aeration rate were increased in exponential law. The law of cell growth rate can be described as:

$$(dx/dt) = x_0\mu\exp \mu t \tag{1}$$

If the concentration of glucose and dissolved oxygen is to be kept as constant during the culture process, the glucose feed rate and aeration rate should be:

$$G(t) = (1/Y_{x/s})\,(dx/dt) = (x_0\mu\exp\,(\mu t)/Y_{x/s}) \tag{2}$$

$$A(t) = (1/Y_{x/0}\eta_0)\,(dx/dt) = (x_0\mu\exp\,(\mu t)/Y_{x/0}\eta_0) \tag{3}$$

where the meanings of those symbols are A=aeration rate, G=glucose feed rate, t=time, x=cell concentration, $Y_{x/s}$=yield based on glucose, $Y_{x/0}$ yield based on oxygen, η_0=consumption percentage of oxygen aerated, and μ=specific growth rate of cells. During the whole culture process, the glucose feed rate and aeration rate are changed once every hour and kept as constant during 1 h. Therefore, the G(t) and A(t) from t_1 to t_2 are calculated as:

$$G(t) = [x_0(\exp^{\mu t_2} - \exp^{\mu t_1}) \;/\; Y_{x/s}(t_2 - t_1)] \tag{4}$$

$$A(t) = [x_0(\exp^{\mu t_2} - \exp^{\mu t_1}) \;/\; Y_{x/o}\eta_0(t_2 - t_1)]^{(t_2 - t_1)} \tag{5}$$

where $Y_{x/s}$, $Y_{x/0}$, and η_0 were determined as 0.5, 1.2, and 10%. The values of aeration rate are increased from 0.85 to 4.4 vvm during the whole culture process.

Results of Comparative Culture

The yeast ICM-10 was repeatedly cultured twice in steady-state operation, and also twice in periodic operation. Figure 3 shows the growth curve of the yeast ICM-10 in different operation modes. Data describing the specific growth rate and yield based on glucose consumption in different operation modes are shown in Table 1. It is clear that both the specific growth rate and yield are much higher in a periodic operation than those in a steady-state operation.

Table 1
Comparison of Specific Growth Rate and Yield of Yeast ICM-10 in Different Operation Modes

	Growth rate μ, 1/h	Comparison of μ	Yield $Y_{x/s}$	Comparison of $Y_{x/s}$
Steady-state operation	0.079	1.00	0.241	1.00
Steady-state operation	0.074	0.94	0.230	0.95
Periodic operation	0.125	1.58	0.447	1.85
Periodic operation	0.109	1.37	0.360	1.49

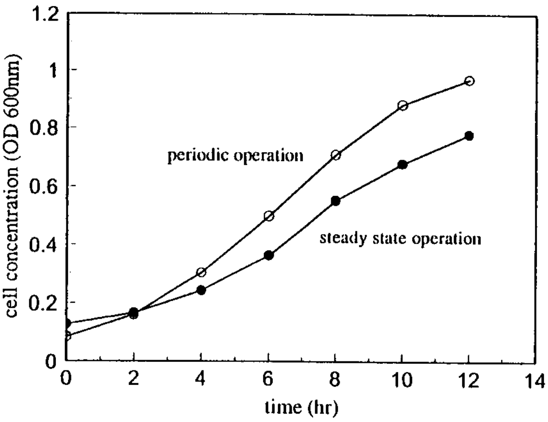

Fig. 4. Growth curves of yeast ICM-11.

Table 2
Comparison of Specific Growth Rate and Yield of Yeast ICM-11 in Different Operation Modes

	0–1 h μ, 1/h	1–8 h μ, 1/h	8–12 h μ, 1/h	0–12 h μ, 1/h	Maximum μ, 1/h	Overall yield, $Y_{x/s}$
Steady-state operation, μ_1	0.154	0.206	0.121	0.190	0.227	0.277
Periodic operation, μ_2	0.243	0.300	0.127	0.237	0.339	0.327
$(\mu_2 - \mu_1)/\mu_1$ (%)	57.2	45.6	4.4	24.7	49.3	18.0

The growth curve of yeast ICM-11 is shown in Fig. 4. The comparison of specific growth rate and yield of yeast ICM-11 in different operation modes is shown in Table 2. It is obvious that both the specific growth rate and yield of cells are much higher in a periodic operation than those in a steady-state operation, but the improvement in the specific growth rate is different during different stages of culture period. During the later stage (8–12 h), the specific growth rates are very close and small for both of the two operation modes. This is probably because, at their later stage of growth, the yeast cells are approaching stationary phase and are not as active.

CONCLUSIONS

The results of cultivation of two different strains of baker's yeast cells consistently show that both the specific growth rate and yield of yeast cell are much higher in forced periodic operations with the novel airlift loop reactor than in steady-state operations with a traditional airlift loop reactor. There is great potential of application in the biochemical industry for this novel airlift loop reactor.

REFERENCES

1. Blenke, H. (1979), *Adv. Biochem. Eng.* **13,** 121–214.
2. Kargi, F. and Cervoni, T. D. (1983), *Biotechnol. Lett.* **5(1),** 33–38.
3. Wood, L. A. and Thompson, P. W. (1986), *Applications of the airlift fermenter,* International Conference on Bioreactor Fluid Dynamics, BHRA Fluid Engineering Centre, Cambridge, England, April 15–17, pp. 157–172.
4. Smith, B. C. and Skidmore, D. R. (1990), *Biotechnol. Bioeng.* **35,** 483–491.
5. Mrtens, D. E., de Gooijier, C. D., van der velden-de Groot, C. A. M., Beuvery, E. C., and Tramper, J. (1993), *Biotechnol. Bioeng.* **41,** 429–439.
6. Byun, S. Y. and Pedersen, H. (1994), *Biotechnol. Bioeng.* **44,** 14–20.
7. Bailey, J. E., Horn, F. J. M., and Lin, R. C. (1971), *AIChE J.* **17,** 818–825.
8. Silveston, P. L. (1987), in *Reactions and Reaction Engineering,* Mashelker, R. A. and Kumar, R., eds., Indian Academy of Sciences, Bangalore 560080, India, pp. 217–264.
9. Stephens, M. and Lyberatos, G. (1987), *Biotechnol. Bioeng.* **29,** 672–678.
10. Abulesz, E. M. and Lyberatos, G. (1987), *Biotechnol. Bioeng.* **29,** 1059–1065.
11. Abulesz, E. M. and Lyberatos, G. (1989), *Biotechnol. Bioeng.* **34,** 741–749.
12. Liu, D., Ouyang, F., Yuan, N., and Ding, F. (1992), The investigation on a dynamic bioreactor, Biotechnology for 21st Century (Proceedings of Asia Pacific Biochemical Engineering Conference '92, Yokohama, Japan), pp. 421–424.
13. Liu, D. (1991), The investigations of dynamic biochemical reactors and their hydromechanical and mass transfer behavior, Ph. D. thesis, Tsinghua University, Beijing, P. R. China.