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Design of a Spray-Cycle Bioreactor and its Application for Riboflavin Production

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ABSTRACT

A highly efficient spray-cycle reactor for oxygen supply was developed. A typical arrangement of the reactor consists of a spray column fitted with a nozzle and a coaxal tube, and a reservoir vessel. The culture broth was circulated between the column and vessel by a peristaltic pump. The volumetric oxygen-transfer coefficient, $k_l a$ was evaluated as a parameter for oxygen supply. The liquid circulation rate in the spray-cycle reactor was represented in terms of the number of circulations. The $k_l a$ value increased as the number of circulations increased, reaching 208 h^{-1} at 4.4 min^{-1} of circulation numbers. This value was 1.8 times higher than that in a 1500-mL stirred-tank reactor under the agitation of 20.7*g* and the aeration of 1.0 volume per min.

The spray-cycle reactor was applied to riboflavin production by an aerobic microorganism. The riboflavin production increased as $k_l a$ values increased and the maximal riboflavin production was 161 mg/L at 208 h^{-1} of $k_l a$. These results suggest that the spray-cycle reactor is useful to oxygen-demanding fermentation because of the high $k_l a$ value in comparison with the stirred-tank reactor.

Index Entries: Spray-cycle reactor; riboflavin production; volumetric oxygen-transfer coefficient; gas holdup.

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INTRODUCTION

For aerobic fermentation, it is important to supply sufficient oxygen to culture broth. The oxygen supply to culture broth is raised with an increase in gas-liquid interfacial area. In the traditional stirred-tank reactor (STR) usually used for fermentation, the air is introduced through a sparger and dispersed by stirring the culture broth in order to increase gas-liquid interfacial area. A large amount of oxygen is especially required for a logarithmic cell-growth phase, but extremely sufficient oxygen could not be supplied to the cells because of the limitation in the stirring. In an attempt to overcome the disadvantages of the STR, many types of tower reactors have been investigated (*1–4*). However, little research has been done on spray reactors, and little information has been published on the effects of important operating variables to achieve high k_la values. Hence, attention was focused on how oxygen is supplied to culture broth and how to increase the length of time the oxygen is held in the culture. We have designed a spray-cycle reactor (SCR) which is an attractive type of bioreactor because the gas-liquid interfacial area could be increased by atomizing the culture broth from a nozzle and the oxygen forcefully dissolved to the broth could not be released into the atmosphere at the interface. Therefore, SCR is different from the jet reactor basically, although both have a nozzle.

This paper presents the development of the SCR and its application to the riboflavin production by 5-fluorouracil-resistant mutant of *Arthrobacter* (*5*).

MATERIALS AND METHODS

Microorganisms and Medium

A 5-fluorouracil-resistant mutant of *Arthrobacter* No. 28–35 isolated in our laboratory was used for the production of riboflavin (*5*). Fermentation for the production of riboflavin was carried out in a culture medium containing 1% L-glutamic acid, 1% glucose, 0.5% KH_2PO_4, 0.1% $MgSO_4 \cdot 7H_2O$, and 0.1% yeast extract.

Bioreactor

A typical 2.0-L jar fermentor (1.5 L working volume) was used under aeration (1.5 L · min^{-1} [1vvm]) and agitation 1.3–26.3*g*.

A SCR system is schematically shown in Fig. 1. The glass SCR (100 mL working volume) consisted of a spray column with a nozzle, an air-inlet and an outlet, a reservoir of culture broth, and a peristaltic pump for circulation of culture broth. The peristaltic pump was connected to the nozzle with the neoprene tube (5 mm diameter). Culture broth was sprayed

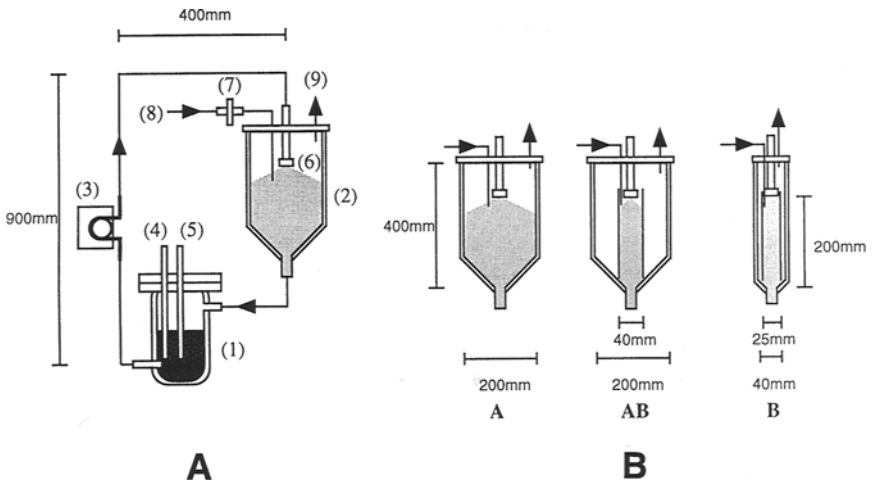

Fig. 1. Schematic diagrams of SCR **(A)** and spray columns **(B)**: 300-mL reservoir of culture broth, spray column, peristaltic pump, pH electrode, DO electrode, nozzles, air filter, air-inlet, and air-outlet.

from a nozzle into the spray column and the sprayed broth was collected in the reservoir and circulated again. The number of circulation was calculated as follows:

$$\text{number of circulation} = (\text{volume of sprayed broth / a minute}) / (\text{total volume of broth})$$

Figure 1B illustrates three different types of spray columns. Diameters of spray columns A, AB, and B were 200, 40, and 25 mm, respectively. Filtered air was supplied to the spray column at the rate of 1.5 L/min. The spray columns and the reservoir with the water jacket were used to keep the temperature of culture broth at 30°C.

Measurement of Volumetric Oxygen Transfer Coefficient, k_la

The dissolved oxygen (DO) concentration was measured by an oxygen electrode. The volumetric oxygen-transfer coefficient (k_la) was determined by the dynamic method *(6–8)*, as follows: aeration and agitation were stopped several hours after the initiation of fermentation. When the DO concentration reached near zero, aeration and agitation were started again. The k_la value was calculated from the DO concentration.

Methods of Analysis

In order to determine the cell growth and riboflavin concentration, a culture broth was diluted with a 10-fold volume of saline. The absorbance of the diluted broth was measured at 660 nm by a spectrophotometer

(SPECTRONIC 21D, Milton Roy, New York). The cell weight was calculated from a calibration curve. The specific growth rate was calculated from the cell concentration. The riboflavin concentration was determined from the absorbance at 450 nm of the supernatant from a diluted broth sample.

RESULTS AND DISCUSSION

Comparison of k_la Values Between the SCR and the STR

The efficiency of oxygen supply rendered by the SCR with an A-type spray column (Fig. 1B) and the STR was compared using k_la values. In the STR, the maximum k_la value was 120 h^{-1} at 20.7*g* of agitation (Fig. 2). To the contrary, when the liquid was sprayed at the top of spray column above its surface, the k_la value reached 130 h^{-1} at 4.4 min^{-1} of the circulation number. The k_la value increased in the SCR because broth was changed to particles by spraying and the gas-liquid interfacial area increased. This result indicates that the SCR is superior to the STR in terms of the amount of oxygen supplied. Furthermore, in the SCR the k_la was dependent on a number of circulations, indicating that the oxygen supply to the broth increased as the number of circulations increased. An intensity of circulation may be advantageous to maximize oxygen transfer. Although the energy consumption for both systems was not measured because of the small reactors, Faust and Sitting have demonstrated that a loop reactor is less energy-wasting than a STR *(9)*. Hence SCR must generate a high k_la value with a higher energy efficiency as compared to STR.

Effect of Column Diameter and Nozzle Height on the k_la Value

The extremely high oxygen content of the gaseous phase in the upper part of the bed exhibited by the SCR system was mainly because of the spraying gas holdup. The k_la value appeared to be affected by a time maintaining broth particles. The time maintaining the particles is considered to depend on a column diameter. In order to vary a diameter of spray column, a slim coaxal tube was installed in the spray column as shown in Fig. 1B. Figure 2 shows the effect of diameter of spray column on the k_la value in SCR. The maximum k_la value in an AB-type was approx 1.5 times higher than that in an A-type, suggesting that the k_la value is higher when the column diameter is shorter. Unexpectedly, the spraying bed was independent of the diameter, although the reasons are unclear. Based on this information, a highly efficient spray column with the coaxal tube was investigated and a type-B reactor with a shorter diameter tube was compared to the type-AB reactor as shown in Fig. 1B. The k_la value reached a maximum of 207.7 h^{-1} at 4.4 min^{-1} of the circulation number in the type-B reactor (Fig. 2).

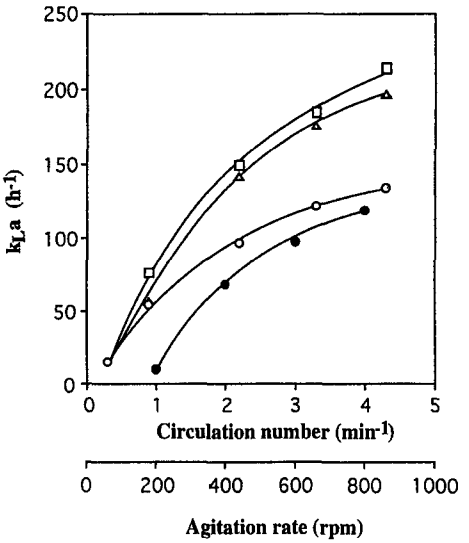

Fig. 2. Dependence of k_la value on the difference of oxygen supply systems among A-, AB-, and B-type SCRs, and STR. The k_la values were obtained in STR (●), and in each type of spray column (○: A-type, △:AB-type, □:B-type).

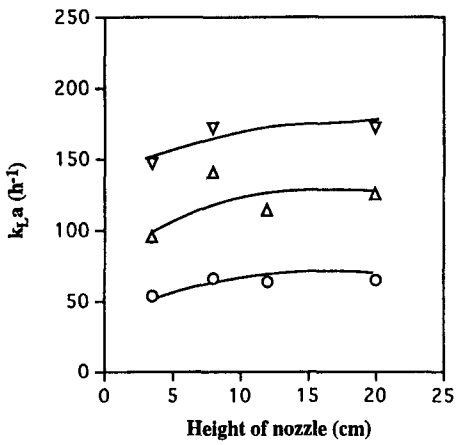

Fig. 3. Effect of the nozzle height of spray column on k_la value was examined at 0.9 (○), 2.2 (△), and 3.3 (▽) min^{-1} of circulation number in the B-type SCR.

The foggy broth particles collided with the tube walls and returned to the liquid broth. Because the liquid flows down along the tube walls, the effect of the tube length on the k_la value was investigated by varying nozzle height. The k_la values were not influenced by the length of the tube in each of the number of circulations, though it was slightly reduced with shorter height (Fig. 3). The results elucidated that the spray and the circulation number were important to supply oxygen to the cells, whereas the reduction of tube diameter appears to be a negligible parameter in the achievement of high k_la values.

Comparison of Riboflavin Production by the STR and SCRs

The above data from this work revealed that SCR is suitable for an aerobic fermentation. Then the SCR was applied to riboflavin production by an aerobic bacterium, *Arthrobacter* mutant resistant to 5-fluorouracil isolated in our laboratory *(5)*. The effect of the k_la value on riboflavin production was investigated in A- and B-type SCRs and compared to the STR. Although in the STR, specific growth rates were independent of agitation rates, the k_la values and riboflavin production increased with increasing agitation rate (Table 1). At an agitation rate of 800 rpm, the k_la value and the riboflavin production were attained 118.1 h^{-1} and 142 mg · L^{-1}, respectively. On the other hand, in the A-type SCR the k_la value and riboflavin production at 3.3 min^{-1} of the circulation numbers were 121.4 h^{-1} and 148 mg · L^{-1}, respectively. These results suggest that the oxygen supplies at 20.7*g* of agitation in STR and at 3.3 min^{-1} of the circulation numbers in A-type SCR were comparable, in spite of the difference in the oxygen-supply system. In the B-type SCR, riboflavin production increased as the circulation number increased. At the highest k_la value, the riboflavin concentration reached maximum 161 mg · L^{-1} at 4.4 min^{-1} of the circulation numbers regardless of the specific growth rate. However, a slight difference between the riboflavin concentrations at 3.3 min^{-1} of the circulation numbers in A-type and B-type SCRs suggests that this aerobic fermentation dose not extremely require oxygen for riboflavin production. Accordingly SCR should be applied to a fermentation demanding more oxygen (Fig. 4).

Time Courses of DO Concentration in SCR and STR

From the operating point of view, attention was given to how the DO concentration changes during the fermentation. Time courses of DO were investigated in SCR and STR. Both the DO levels in SCR and STR decreased with the exponential cell growth, but were restored to an initial level of DO after 20 and 50 h, respectively. In STR the DO level fell by 6.2 ppm, whereas in SCR it did not fall below 6.7 ppm. A difference in the oxygen supplies seems to result in a distinction of riboflavin productions. However, riboflavin production after approx 40 h results from releases of riboflavin by autolysis of cells; therefore, the production does not depend on DO level. Yellow substances formed in the later half of culture are already confirmed by HPLC with riboflavin as described in the previous paper *(5)*.

The results of this study have led to a better understanding of the various parameters that can be used to establish the optimum design and operation of the SCR. Although the SCR was favorable for the production of riboflavin, other fermentation processes with a high demand for oxygen remain to be investigated.

Table 1
Comparison of Riboflavin Productions in STR and SCR

Reactor	Agitation rate or circulation number (rpm or min^{-1})	kLa (h^{-1})	Maximum specific growth rate (h^{-1})	Maximum riboflavin concentration ($mg \cdot L^{-1}$)
STR	300	22	0.10	89
	400	67	0.16	111
	600	97	0.18	126
	800	118	0.18	142
SCR A-type	3.3	121	0.18	148 ± 4.0 [a]
SCR B-type	3.3	190	0.18	156 ± 4.4
SCR B-type	4.4	208	0.18	161 ± 2.6 **

[a]Values mean ± SD) represent data derived from samples in three experiments.
**$0.001 < p \leqq 0.01$ compared to A-type.

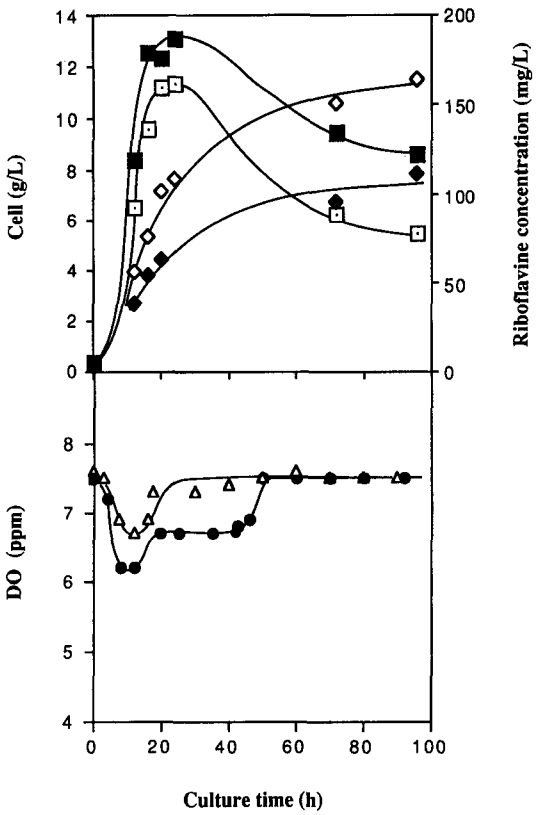

Fig. 4. Time courses during fermentation of riboflavin were explored at 20.7g of agitation in STR and at 4.4 min^{-1} of circulation number in B-type SCR. Symbols: ◆; and ▼; riboflavin, ●; and △; DO, ■; and □; cells in STR, and in SCR, respectively.

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