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Oxygen Transfer Characteristics for Airlift Bioreactor

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The fermentation experiment on biological fertilizer in 50 L airlift bioreactor has been studied. Oxygen mass transfer characteristic of cold and thermal model experiment and microbial growth condition in this reactor are also determined. It is found, that the biofertilizer fermentation using air lift bioreactor with static mixer can decrease fermentation period and increase facility efficiency compared with the traditional bioreactor. The mass transfer coefficient increases with the increase of gas flow rate, but decreases with the increase of liquid viscosity.

Key Words: Oxygen transfer coefficient, Airlift, Bioreactor.

INTRODUCTION

The supply of oxygen is a critical factor in all aerobic fermentations. An insufficient oxygen transfer leads to decrease of microbial growth and product formation. In order to assess if particular equipment would be able to supply oxygen at a non-limiting rate, it is essential to have estimate of the oxygen mass transfer coefficient. In this paper, an airlift bioreactor with kenics static mixer was developed and applied to produce bio-fertilizer. The oxygen transfer characteristics for airlift bioreactor and seek optimal technological condition, a fermentation experiment in 50 L airlift bioreactor was done.

EXPERIMENTAL

The experimental measurements were carried out in 50 L airlift bioreactor with kenics static mixer. The experimental apparatus is shown in Fig. 1. The bioreactor is made of stainless steel. The gas sparger was a series of spray nozzle which contains different distributed holes, the holes were respectively ϕ 7 mm, ϕ 8 mm, ϕ 9 mm, ϕ 10 mm in diameter.

Nutrient medium: The strain used in this study was *Bacillus megatherium* de Bary-ACCC10008[1]. The nutrient medium composition was: NaCl 0.03 %, KCl 0.03 %, $MgSO_4$, 0.03 %, $(NH_4)_2SO_4$ 0.05 %, $CaCO_3$ 0.2 %, $FeSO_4 \cdot 7H_2O$ 0.001 %, sucrose 1 %, pH = 7.0-8.0.

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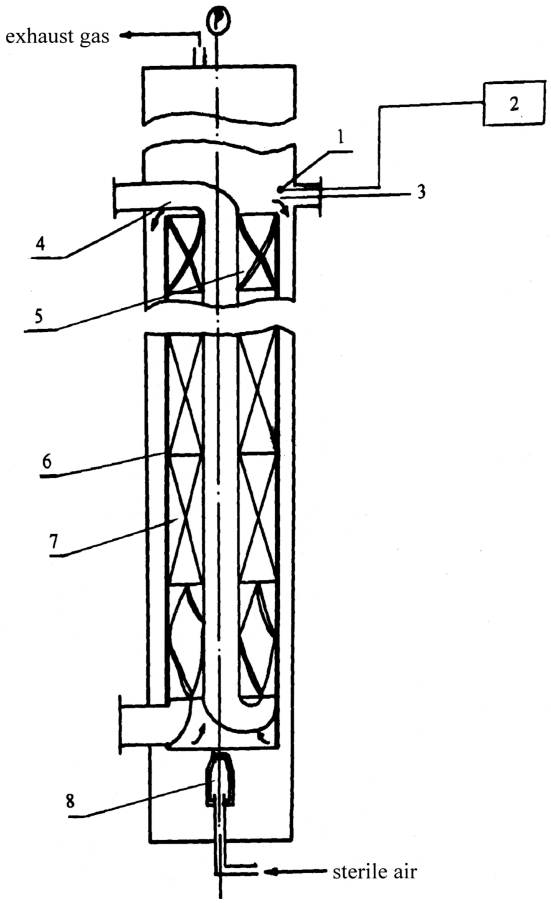

Fig. 1. Experimental apparatus for airlift bioreactor with kenics static mixer (1) dissolved oxygen sensor (2) DYS-18A dissolved oxygen instrument (3) temperature indicator (4) coil pipe for cooling water (5) right-hand screw kenics static mixer (6) flow distributor (7) left-hand screw kenics static mixer (8) air spout

Inoculation method

First-class inoculation: 50 mL culture medium was added to 250 mL triangle flask and were sterilized at 121 ºC and 200 kPa for 0.5 h, the slant cells in the tube were used as inoculation and were to flask containing 50 mL of the medium given above. The cells were incubated on a reciprocating shaker (120 rpm) at 25 ºC for 24 h before being added to the bioreactor.

Second-class inoculation: 100 mL culture medium was added to 500 mL triangle flask, after sterilization, inoculate a flask of the first-class inoculum, then shake them to culture for 24 h on a reciprocating shaker.

Third-class inoculation: Inoculate the second fermentation solution in 50 L airlift bioreactor.

Experimental condition: (i) Culture volume: the filling coefficient of nutrient medium in bioreactor is 0.8; (ii) Sterilization condition: sterilized at 121 ºC and 200 kPa for 0.5 h^2; (iii) Inoculation ratio: 2-8 %; (iv) Culture temperature: 30 ºC; (v) Aeration rate: 1:0.3-1.6; (vi) Culture method: Continuous agitation, aeration and constant temperature culture.

Analysis method: Viable count measurement: Dilution plating method; The measurement of cell optical density (OD); The OD value of bacteria was determined at 580 nm wavelength with 59Wc type spectrometer; The measurement of oxygen mass transfer coefficient.

Oxygen mass transfer coefficient (K_{La}) was measured with DYS-18A digital type dissolved oxygen instrument with dissolved oxygen sensor.

RESULTS AND DISCUSSION

Cold model experiment and result: In cold model experiment, water was used as medium of experiment. Cold model experiment is divided into 4 groups in 50 L air-lift bioreactor, the spray nozzle diameter of 4 groups is respectively ϕ 7 mm, ϕ 8 mm, ϕ 9 mm, ϕ 10 mm. The determined results of oxygen mass transfer coefficient (K_{La}) were shown in Table-1 and Fig. 2. The formula used for calculation is as follows[3]:

$$N = \frac{\Delta V \times n \times 60}{M \times t \times 4} \times 10^3$$

$$K_{La} = \frac{N}{C^* - C} = 4.8 \times 10^3 N(h^{-1})$$

in which, N-volumetric rate of dissolved oxygen, mmol h^{-1}; n-molarity of $Na_2S_2O_3$; ΔV- difference of titration volume, mL; M -sample volume, mL; t -sampling interval, *i.e.*, oxidation time, min.

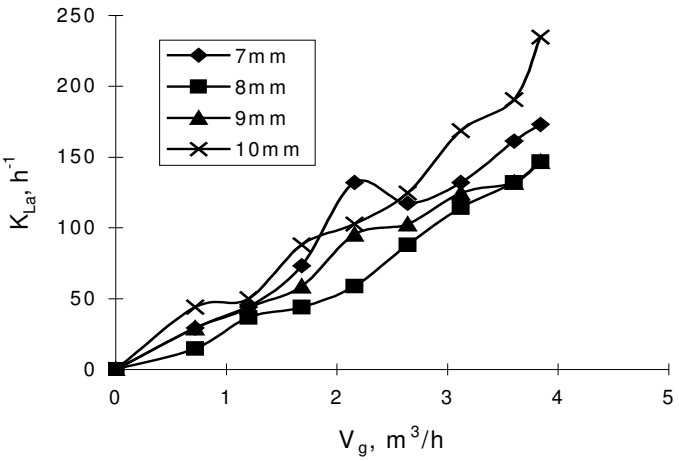

Fig. 2. Relation curve of V_g and K_{La} in different spray nozzle diameter

TABLE-1
RESULTS OF OXYGEN MASS TRANSFER COEFFICIENT (K_{La})
IN DIFFERENT SPRAY NOZZLE DIAMETERS

				ϕ = 7 mm				
Aeration rate (VVM)	1:0.3	1:0.5	1:0.7	1:0.9	1:1.1	1:1.3	1:1.5	1:1.6
Aeration volume, V_g, $m^3 h^{-1}$	0.72	1.2	1.68	2.16	2.64	3.12	3.6	3.84
V_1, mL	8.4	8.55	9.1	9.2	10.1	11.1	12.4	13.5
V_2, mL	8.6	8.85	9.6	10.1	10.9	12	13.5	14.68
$\Delta V = V_1 - V_2$, mL	0.2	0.3	0.5	0.9	0.8	0.9	1.1	1.18
M, mL	5	5	5	5	5	5	5	5
t, min	10	10	10	10	10	10	10	10
N, mmol h^{-1}	6.16	9.24	15.40	27.73	24.65	27.73	33.89	36.36
K_{La}, h^{-1}	29.33	44.00	73.33	132.05	117.38	132.05	161.38	173.14
K_{La}/V_g, m^{-3}	40.74	36.67	43.65	61.13	44.46	42.32	44.83	45.09
				ϕ = 8 mm				
Aeration rate (VVM)	1:0.3	1:0.5	1:0.7	1:0.9	1:1.1	1:1.3	1:1.5	1:1.6
Aeration volume, V_g, $m^3 h^{-1}$	0.72	1.2	1.68	2.16	2.64	3.12	3.6	3.84
V_1, mL	5.20	5.45	6.50	6.35	6.90	7.45	8.60	9.60
V_2, mL	5.30	5.70	6.80	6.75	7.50	8.23	9.50	10.60
$\Delta V = V_1 - V_2$, mL	0.1	0.25	0.3	0.40	0.60	0.78	0.90	1.00
M, mL	5	5	5	5	5	5	5	5
t, min	10	10	10	10	10	10	10	10
N, mmol h^{-1}	3.08	7.70	9.24	12.32	18.49	24.03	27.73	30.81
K_{La}, h^{-1}	14.67	36.67	44	58.67	88.05	114.44	132.05	146.71
K_{La}/V_g, m^{-3}	20.38	30.56	26.19	27.16	33.35	36.68	36.68	38.21
				ϕ = 9 mm				
Aeration rate (VVM)	1:0.3	1:0.5	1:0.7	1:0.9	1:1.1	1:1.3	1:1.5	1:1.6
Aeration volume, V_g, $m^3 h^{-1}$	0.72	1.2	1.68	2.16	2.64	3.12	3.6	3.84
V_1, mL	6.70	7.20	8.25	8.60	9.55	5.85	7.25	11.25
V_2, mL	6.90	7.50	8.65	9.25	10.25	6.70	8.15	12.25
$\Delta V = V_1 - V_2$, mL	0.2	0.3	0.4	0.65	0.7	0.85	0.90	1.00
M, mL	5	5	5	5	5	5	5	5
t, min	10	10	10	10	10	10	10	10
N, mmol h^{-1}	6.16	9.24	12.32	20.03	21.57	26.19	27.73	30.81
K_{La}, h^{-1}	29.33	44.00	58.67	95.38	102.71	124.71	132.04	146.71
K_{La}/V_g, m^{-3}	40.74	36.67	34.92	44.16	38.91	39.97	36.68	38.21
				ϕ = 10 mm				
Aeration rate (VVM)	1:0.3	1:0.5	1:0.7	1:0.9	1:1.1	1:1.3	1:1.5	1:1.6
Aeration volume, V_g, $m^3 h^{-1}$	0.72	1.20	1.68	2.16	2.64	3.12	3.60	3.84
V_1, mL	6.60	6.70	12.80	12.4	8.15	8.85	10.00	10.70
V_2, mL	6.90	7.04	13.40	13.1	9.00	10.00	11.30	12.30
$\Delta V = V_1 - V_2$, mL	0.30	0.34	0.60	0.70	0.85	1.15	1.30	1.60
M, mL	5	5	5	5	5	5	5	5
t, min	10	10	10	10	10	10	10	10
N, mmol h^{-1}	9.24	10.48	18.49	21.57	26.19	35.43	40.05	49.30
K_{La}, h^{-1}	44.00	49.90	88.05	102.71	124.71	168.71	190.71	234.76
K_{La}/V_g, m^{-3}	61.11	41.59	52.41	47.55	47.24	54.07	52.98	61.14

The result showed that the oxygen mass transfer coefficient (K_{La}) in air lift bioreactor obviously increases with the increase of aeration volume (V_g).

From the change condition of K_{La} in per unit aeration volume (K_{La}/V_g), it is found that the optimum diameter of spray nozzle is ϕ 7 mm.

Thermal model experiment and result: In thermal model experiment, the liquid nutrient medium was used as medium of experiment.

Measurement for growth curve of strain: Strains were cultured in 50 L bioreactor on the condition of continuous aeration. Optical density value of strain is determined at 580 nm wavelength with 59Wc type spectrometer for every 2 h. The results are shown in Table-2 and Fig. 3.

TABLE-2
PHOSPHORUS BACTERIA GROWTH CURVE

Culture time (h)	6	8	10	12	14	16	18	20	22	24
Optical density	0.292	0.307	0.447	0.445	0.558	0.510	0.483	0.484	0.407	0.368

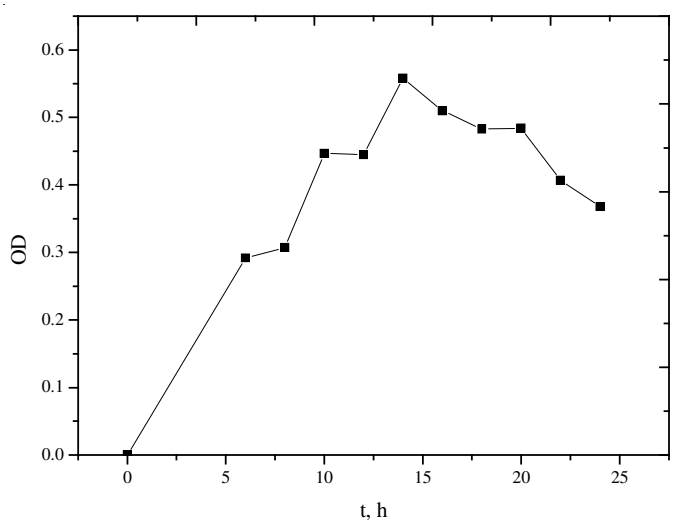

Fig. 3. Growth curve of phosphorus bacteria in 50 L bioreactor

The results showed that in the course of fermentation, phosphorus bacteria grows rapidly, the optical density of bacteria at 14 h has approached peak value, if using traditional fermentation, it will take 18-24 h, so biofertilizer fermentation using air lift bioreactor with static mixer can decrease fermentation period,increase facility efficiency compared with the traditional bioreactor.

Effect of different inoculation ratio on bacteria growth: The inoculation ratio was changed to 2, 5 and 8 %, heat preserving culture phosphorus bacteria for 8 h, 14 h, respectively to observe the effect of different inoculation rate on bacteria growth. The living bacteria number were measured and the result was shown in Table-3.

TABLE-3
EFFECT OF DIFFERENT INOCULATION RATE ON BACTERIA GROWTH

Inoculation rate (%)	Culture time (h)	The number living cell (cell/mL)
2	8	9.25×10^8
	14	1.15×10^9
5	8	7.95×10^9
	14	8.85×10^9
8	8	2.42×10^{10}
	14	3.45×10^{10}

The result showed that the more the inoculation ratio, the more the number of living bacteria, but serious foam entrainment phenomenon was found during the fermentation experiment of 8 % inoculation ratio, so the optimum inoculation ratio is in between 2-5 % in case of industrial production.

Effect of different filling coefficient of bioreactor on bacteria growth: When the filling coefficient of bioreactor is 0.7, 0.8, 0.9, respectively, the fermentation experiment was done on the condition of 2 % inoculation ratio. The result is listed in Table-4.

TABLE-4
EFFECT OF DIFFERENT FILLING COEFFICIENT OF FACILITY ON BACTERIA GROWTH

Culture time (h)	Filling coefficient (%)	The number living bacteria (cell/mL)	Microscope inspection	Optical density
14	90	6.35×10^9	Normal	0.186
14	80	8.85×10^9	Normal	0.331
14	70	1.32×10^9	Normal	0.089

The result showed that the effect of different filling volume on bacteria growth is different, in which, the optimum filling coefficient of facility is 0.8.

Oxygen transfer coefficient: The oxygen transfer coefficient K_{La} value has been measured with DYS-18A digital type oxygen measuring instrument when filling coefficient of facility is 0.8 and spray nozzle diameter is 7 mm[4-6], the result is presented in Table-5 and Fig. 4.

TABLE-5
RESULT OF OXYGEN TRANSFER COEFFICIENT

Aeration rate	Aeration volume, V_g, ($m^3 \cdot h^{-1}$)	Oxygen transfer coefficient, K_{La} (h^{-1})	K_{La}/V_g (m^{-3})
1:0.5	1.20	23.20	19.33
1:0.7	1.68	24.39	14.52
1:0.9	2.16	38.53	17.84
1:1.1	2.64	47.93	18.16
1:1.3	3.12	54.29	17.40
1:1.5	3.60	62.90	17.47
1:1.6	3.84	66.13	17.22

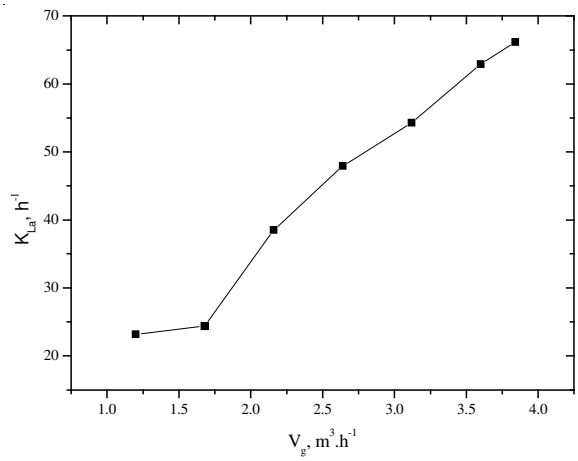

Fig. 4. Relation curve of aeration volume and oxygen transfer coefficient K_{La}

As a result of installing kenics static mixers inside elevating pipe of bioreactor, kenics static mixers in air-lift bioreactor are useful for the dispersion of gas bubbles and the absorption rate of O_2, it can increase oxygen mass transfer ratio, so K_{La} increases gradually with the increase of V_g and approximately increases in straight line, when aeration rate is 1:1.1, the value of K_{La}/V_g is optimal.

Conclusion

(a) The result of cold and thermal model experiment shows that kenics static mixers in airlift bioreactor are useful for the increase gas-liquid mass transfer characteristic of oxygen. (b) The biofertilizer fermentation using airlift bioreactor with static mixer can decrease fermentation period,increase facility efficiency compared with the traditional bioreactor. (c) The effect of different inoculation rate on bacteria growth is different, the bigger the inoculation ratio, the shorter the culture time, but during the experiment it is found that a great foam entrainment phenomenon can be brought about in 8 % inoculation ratio, it is advised to adopt 2-5 % inoculation rate in case of industrial fermentation. (d) The effect of different filling coefficient of bioreactor on bacteria growth is different,the optimum filling coefficient is 0.8, the optimum spray nozzle diameter is ϕ 7mm, the optimum aeration ratio is 1:0-9-1.1. (e) The mass transfer coefficient of oxygen K_{La} increases in straight line with the increase of gas volume.

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