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Production of Validamycins from Crude Substrates by *Streptomyces hygroscopicus* in an External-loop Airlift Bioreactor with a Low Height-to-Diameter Ratio*

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Abstract Fermentation experiments to produce validamycins from crude substrates by *Streptomyces hygroscopicus* were carried out in an external-loop airlift bioreactor (0.0115 m^3) with a low ratio of height to diameter of the riser of 2.9 and a ratio of riser to downcomer diameter of 6.6. The influences of gas flow rate and liquid volume on fermentation of validamycins were investigated. Comparisons of validamycin fermentation were made among the external-loop airlift bioreactor, a mechanically stirred tank bioreactor (0.010 m^3) and shaking flasks. Under the same operation conditions including fermentation medium composition, inoculum ratio and culture temperature, the fermentation time in the external-loop airlift bioreactor (45 h) was shorter than that in the shaking flasks (100 h) and the same as that in the mechanically stirred tank bioreactor. After a total fermentation time of 45 h under optimized operation conditions, average validamycin concentration obtained in the external-loop airlift bioreactor was close to 19630 $\mu g \cdot ml^{-1}$ validamycin concentration in the mechanically stirred tank bioreactor. It was demonstrated that the external-loop airlift bioreactor could substitute for the mechanically stirred tank bioreactor in production of validamycins from crude substrates with dregs by *Streptomyces hygroscopicus*.
Keywords airlift bioreactor, validamycins, *Streptomyces hygroscopicus*

1 INTRODUCTION

Airlift bioreactors are a class of bioreactors where a region of gassed liquid is connected to a region of ungassed liquid, the difference in hydrostatic pressure between the two regions resulting in circulation of the liquid phase. External-loop airlift bioreactors are those loop reactors in which the injection of air into the bottom of one of the risers aerates the broth and causes liquid circulation between riser and downcomer. This geometry gives several advantages over the traditional mechanically stirred tank bioreactor, including: (1) absence of high shear regions such as near the impeller; (2) simple construction; (3) low energy consumption; (4) few chances of media contamination; (5) ease of operation; (6) versatility. Therefore, the structures, properties and applications of the airlift bioreactor were widely investigated. However, lots of the airlift bioreactors were studied in the literature with height-to-diameter ratio (s) greater than 5.0. Woragidbumrung *et al.*[1] carried out an experimental study on production of ginseng polysaccharide in an airlift bioreactor with $s = 5.7$. Huang *et al.*[2] used an airlift bioreactor with $s > 15.0$ for thuringiensin production. Gavrilescu and Tudose[3] investigated hydrodynamic characteristics of the external loop airlift bioreactors with $s > 20$. Galindez-Mayer *et al.*[4] pointed out that most airlift bioreactors had a high geometric liquid height/column diameter ratio $s \geqslant 5.0$. The gas holdup and oxygen transfer was investigated by Bello *et al.*[5] in an airlift bioreactor with $s > 10.0$. Sajc *et al.*[6] examined the characteristics of hydrodynamics and mass transfer in an external-loop airlift bioreactor with $s = 10.0$. Hydrodynamics and oxygen transfer characteristics in external-loop airlift bioreactors with $s > 5.0$ were studied by Kawase *et al.*[7,8]. Fraser *et al.*[9,10] dealt with the hydrodynamic characteristics in an external-loop airlift bioreactor with $s > 18.0$. The liquid circulation velocity was studied by Gavrilescu and Tudose[11] in concentric-tube airlift bioreactors with $s > 7.0$. And as summarized by Bello *et al.*[5] the height-to-diameter ratios (s) of the airlift bioreactors mentioned in the papers were all greater than 6.0. There is no published information, including the hydrodynamics, oxygen-transfer characteristics and application to chemical and biochemical processes, about external-loop airlift bioreactors with $s < 3.0$ except our previous report about 2-amylase fermentation in an external-loop airlift bioreactor with $s = 2.9$[12]. In contrast, the height-to-diameter ratios of mechanically stirred tank bioreactors are mostly less than 3.0, especially in China. It may be possible to develop a novel airlift bioreactor with $s < 3.0$ to

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reform the traditional mechanically stirred tank bioreactors.

There are some reports on airlift bioreactors utilized for the productions of chemicals and biochemicals[12−19], but the airlift bioreactors with lower height-to-diameter ratio have not been used for the production of secondary metabolites, such as antibiotics, and for the fermentation from crude substrates. Validamycins are curative fungicides that are particularly useful for the control of plant diseases caused by *Basidiomycetes fungi*. They are effective against the sheath blight of rice plant and wheat plants caused by *Pellicularia sasakii*[20−25]. Validamycins produced by *Streptomyces hygroscopicus* are compounds consisting of validamycin A, B, C, D, and perhaps others. Validamycins are favored by farmers because of their long effectiveness, resistance to being washed away by rainwater and low toxicity to human beings and domestic animals. To date, the mechanically stirred tank bioreactor (MSTB) has been the sole type of bioreactor used for the submerged fermentation of validamycins. There is no report of the fermentation of validamycins with an airlift bioreactor.

In this study, an external-loop airlift bioreactor with low ratio, $s = 2.9$, is developed and used in validamycin production to investigate its suitability for the validamycin fermentation from crude substrates and the possibility of fitting a mechanically stirred tank bioreactor with external loops, then to determine whether this type of airlift bioreactor can be used in other chemical and biological technologies, especially in fermentation with dregs and fermentation of the secondary metabolites.

2 MATERIALS AND METHODS

2.1 Experimental apparatuses

The details of the 0.0115 m^3 total volume airlift bioreactor with external-loop used in this work are listed in Table 1 and the structure was given previously[12]. The ratio of height to diameter of the riser of the external-loop airlift bioreactor (ELAB) was 2.9, and the ratio of riser to downcomer diameter was 6.6. A gas distributor was equipped at the bottom of the riser in ELAB. A horizontal pipe with a diameter of 0.025 m connected the riser and downcomer. The ELAB could be measured and controlled automatically by pumping temperature-controlled water through the jacket on the riser.

The mechanically stirred tank bioreactor (MSTB) had a total volume of 0.010 m^3, a ratio of height to diameter of 2.0 and three flat-bladed disc turbine impellers stacked vertically. The rate of agitation and gas flow could be measured and controlled, and the operation temperature was controlled by pumping the water with controlled temperature through the jacket on the vessel.

Table 1 Bioreactor dimensions

Bioreactor	D_r, m	D_d, m	A_r/A_d	H, m	V_T, m^3
ELAB	0.165	0.025	43.56	0.479	0.0115
MSTB	0.185	—	—	0.370	0.0100

The above two bioreactors were fed with the medium and added with the antifoam agents, together with the steam-generation system and the filtered gas system.

2.2 Microorganism

Streptomyces hygroscopicus var. Jinggangensis yen z-10 was maintained on potato-glucose agar slants in our laboratory.

2.3 Media and cultivation

The microorganism, *Streptomyces hygroscopicus var. Jinggangensis yen* z-10, was cultured at 30 ℃ for 3—4 days in an agar slant medium whose composition was (per liter): 10 g glucose, 0.5 g L-asparagine, 0.5 g KH_2PO_4, and 20 g agar. Then it was transferred to 500 ml conical flasks containing 50 ml of sterilized inoculum medium with a composition of (per liter): 30 g rice meal, 25 g soybean meal, 10 g yeast extract and 0.8 g K_2HPO_4. After the inoculation, the cultures was held at 38—40℃ on an orbital shaker at 200 $r \cdot min^{-1}$ for 25 h. Fermentation medium for validamycin production contained (per liter) 90 g corn meal, 40 g soybean meal, 5 g yeast extract and 0.1 g K_2HPO_4. The ELAB and MSTB were each filled with 8.0—9.5 L and 6.0—8.5 L fermentation media, in separating trials, to study the effect of liquid volume on validamycin fermentation. The fermentation medium was sterilized at 121℃ for 20 min, then cooled to (39 ± 1)℃ and inoculated with 8% (by volume) inoculum medium. The culture conditions in the ELAB, were 0.08 MPa pressure, 1.0—1.3 vvm gas flow rate and at (39±1)℃. The culture in the MSTB was carried out at 0.08 MPa, 0.7—1.0 vvm gas flow rate and 300 $r \cdot min^{-1}$ agitation rate.

2.4 Analysis methods

The concentration of validamycins was measured every 5 hours using the colorimeteric method. Validamycins belong to the family of aminocyclitol antibiotics, all of which include a molecule of glucose. If the glucose is reacted with vitriol oil, it is dehydrolized and forms a hydroxy-glycuronate, which can be condensed with phenol to produce a red compound. The concentration of the red compound can be determined by colorimetery[26]. The final concentration of validamycins at the end of fermentation was determined by high performance liquid chromatography (HPLC)[26]. Chromatographic separations were performed by HPLC (LC-10AS, Shimdzu, Japan) in an ODS of 250 mm×4.6 mm column (Shimdzu, Japan).

The eluting solvent was composed of disodium hydrogen phosphate buffer solution (pH=7.0) added with 2.5% (by volume) methanol. The flow rate was 1.0 ml·min^{-1}. The elutant was monitored at 210 nm. Total sugar and reducing sugar were analyzed by the 3,5-dinitroscolycilic (DNS) acid method as described by Jia *et al.*[27].

3 RESULTS AND DISCUSSION

3.1 Effect of gas flow rate on validamycin fermentation

In aerobic fermentation process of an airlift bioreactor, gas flow rate plays an important role on improving oxygen transfer rate, turbulence and broth circulation velocity, which in turn affects metabolite productivity of the microorganisms. To optimize gas flow rate for production of validamycins by *Streptomyces hygroscopicus* in the ELAB, validamycin fermentation experiments were performed in the ELAB at different gas flow rates (0.9, 1.0, 1.1, 1.2, 1.3, 1.4 vvm) with a constant initial volume of 8.50 L fermentation medium. The results are shown in Fig. 1. It is seen that concentration of validamycins increases as gas flow rate increased up to 1.1 vvm, and at higher gas flow rates, the validamycin concentration decreases slightly. Many previous researchers have proved that the transfer rate of oxygen into solution and through the medium in the bioreactor is strongly influenced by gas flow rate, especially in the airlift bioreactors in which gas flow rate influences the fermentation results[6,28,29]. When dissolved oxygen was supplied insufficiently or excessively, the validamycin synthesis would be inhibited. Our experiments showed that there was an optimum gas flow rate (1.1 vvm) for the synthesis of validamycins in the ELAB.

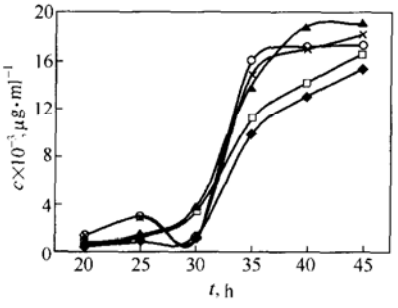

Figure 1 Validamycin fermentation at different air flow rates in the ELAB
[V_L = 8.50 L, T = (39 ± 1)℃]
V, vvm: —◆— 0.90; —□— 1.00; —▲— 1.10; —×— 1.20; —○— 1.30

3.2 Effect of liquid volume on fermentation of validamycins

It has been proved that the liquid depth may influence mixing and particle circulation time in airlift bioreactors[30,31]. The depth of the liquid in a bioreactor also affects the gas holdups, and the greater the depth of liquid in the bioreactor, the lower the gas holdup. As a result, liquid depth may affects the volumetric oxygen transfer, which affects the validamycin fermentation. Because the depth of liquid was directly proportional to the liquid volume in the given bioreactor, fermentation trials were carried out in the external-loop bioreactor with initial liquid volume from 8.00 L to 9.25 L, corresponding to liquid depth from 0.346 m to 0.400 m, in our investigation of impact of liquid height on the fermentation results. The results are shown in Fig. 2. The highest validamycin accumulation was obtained with a liquid volume of 8.75 L. Raising the liquid depth causes an increase of hydrostatic pressure in a bioreactor, which makes air bubbles compressed and become smaller, so that transfer of oxygen is decreased. However, the residence time of air bubbles in the broth increases with the depth of liquid, which improves the dissolution of oxygen. Our experimental results show that a liquid volume of 8.75 L results in a hydrostatic pressure at which the concentration of validamycins is maximized. The compression of air bullbes in the liquid is offset by increased residence time and oxygen solubility.

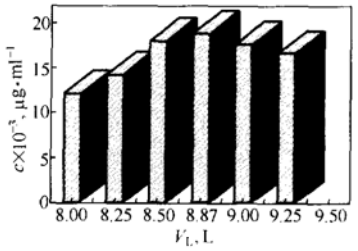

Figure 2 Effect of liquid volume on fermentation of validamycins in the ELAB
[V = 1.10 vvm, t = 45 h, T = (39 ± 1)℃]

3.3 Difference between the validamycin fermentation in the shaking flasks and in the ELAB

The optimum operating conditions for the 500 ml shaking flasks were obtained by determining the ratio of carbon source to nitrogen source, pH, liquid volume, and the speed of the orbital shaker, which resulted in the highest concentration of validamycins from fermentation by *Streptomyces hygroscopicus* from crude substrate. The process of validamycin fermentation in the shaking flasks under optimized operation conditions and in the ELAB under a gas flow rate of 1.10 vvm and a liquid volume of 8.75 L are shown in Fig. 3. Under the same operation conditions, including fermentation medium composition, inoculum ratio and culture temperature, the fermentation time in the ELAB was reduced as compared with that in the shaking flasks. Validamycins in the shaking flasks could only be detected after 45 h of fermentation and reached their maximum within 98 h. while

in the ELAB, validamycins could be detected after only 20 h of fermentation and reached its maximum at 45 h. The possible reason is that the oxygen dissolution in the ELAB is better than that in the shaking flasks so that the growth of microorganism is increased, which makes the microorganism secrete validamycins early. The maximum validamycin concentration in the ELAB was close to that in the shaking flasks.

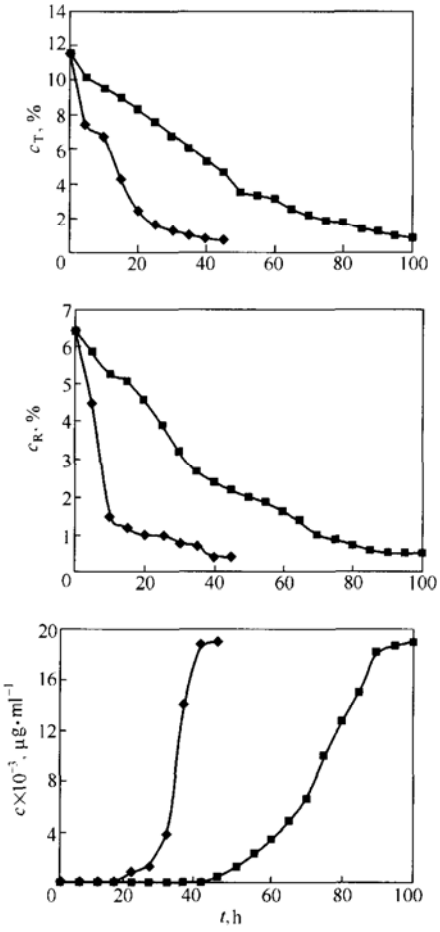

Figure 3 Validamycin fermentation in the shaking flasks and the ELAB
[ELAB: $V = 1.10$ vvm, $V_L = 8.75$ L, $T = (39 \pm 1)$℃; Shaking flasks: $V_L = 50$ ml per 500 ml, $n = 200$ r·min^{-1}, $T = (39 \pm 1)$℃]
—◆— ELAB; —■— shaking flasks

3.4 Difference of the validamycin fermentation in the ELAB and in the MSTB

The MSTB with a ratio of height-to-diameter <3.0 is with high oxygen transfer, good mixing and dispersion throughout the fermentation medium, so it has been used for the submerged fermentation of validamycins since the advent of validamycin production by *Streptomyces hygroscopicus*. To investigate the possibility of substituting the ELAB for the MSTB, it is necessary to make a comparison of validamycin production between both bioreactors. If the same or close results of fermentation are obtained in both bioreactors, it is possible for the airlift bioreactor to be employed in validamycin fermentation. Through examining the effects of gas flow rate, agitation rate and initial liquid volume on validamycin fermentation in the MSTB, the optimized operation conditions (0.8 vvm gas flow rate, 300 r·min^{-1} agitation and 7.00 L initial liquid volume) under which validamycin concentration (19630 μg·ml^{-1}) reached the maximum were obtained. Fig. 4 shows the fermentation results in the ELAB and the MSTB at the lower gas flow rates from 0.7 vvm to 0.9 vvm. It is seen that validamycin titre in the MSTB is higher than that in the ELAB in the range of gas flow rate from 0.7 vvm to 0.9 vvm., which indicates that a higher gas flow rate is needed in the ELAB as compared with the MSTB. However, in the ELAB the optimized operation conditions were the gas flow rate of 1.1 vvm and the initial liquid volume of 8.75 L as shown in Figs. 2 and 3. The highest concentration of validamycins (19975 μg·ml^{-1}) was obtained under the optimized operation conditions. Therefore, the highest validamycin concentration in the ELAB was slightly higher than that in the MSTB under the optimized operation conditions. A comparison of the optimized conditions showed that gas flow rate in the ELAB should be 37.5% higher than that in the MSTB. In the ELAB, however, there is no agitation system and power in not required for agitation. The reason for higher gas flow rate in the ELAB is that the residue time of gas bubbles in the broth in the ELAB is shorter than that in the MSTB. Taking these factors including aeration and agitation into account, the total consumed energy of the ELAB was about 25% less than that of the MSTB. Because the construction of the ELAB was much simpler than that of the MSTB, the manufacture cost of ELAB was about two thirds of that of MSTB. Terefore, it is possible to retrofit existing MSTB with an ELAB to produce validamycins from crude substrates economically.

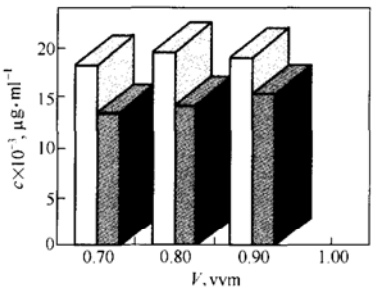

Figure 4 Comparison of validamycin fermentation at different air flow rates in the ELAB and the MSTB
[ELAB: $V_L = 10.00$ L, $t = 45$ h, $T = (39 \pm 1)$℃; MSTB: $V_L = 7.00$L, $n = 300$ r·min^{-1}, $t = 45$ h, $T = (39 \pm 1)$℃]
□ MSTB; ■ ELAB

Many researchers have found that oxygen transfer, hydrodynamic characteristics and fermentation

characteristics are affected by the sparger design. Merchuk[32] reported that there was no difference in gas holdup and liquid velocity obtained in a two-dimensional airlift reactor using a sparger of 0.3, 0.5, or 1.0 mm hole diameter and a porous plate. A difference in both gas holdup and liquid velocity was obtained between airlift bioreactors sparged by a single 9 mm orifice and fourteen 2.5 mm orifices[33]. There were reports that the design of gas sparger had a considerable influence on gas holdup because it affected both the bubble sizes and the characteristic gas velocity, a result of the fractional aerated area[30,34]. We conclude from these reports that the hole diameter of a sparger may influence fermentation of validamycins. Figs. 5—7 show the process of fermentation of validamycins in the MSTB, ELAB with a sparger hole diameter of 1.5 mm and 3.0 mm, respectively. After a total fermentation time of 45 h, a maximum concentration of validamycins in the MSTB, 19630 $\mu g \cdot ml^{-1}$, was obtained under optimized operation conditions (agitation rate of 300 $r \cdot min^{-1}$, gas flow rate of 0.8 vvm and liquid volume of 7.00 L). Under optimized operation conditions in the ELAB (gas flow rate of 1.10 vvm and initial liquid volume of 8.75 L) the maximum concentration of validamycins, 18950 $\mu g \cdot ml^{-1}$ and 19975 $\mu g \cdot ml^{-1}$, with sparger hole diameters of 3.0 mm and 1.5 mm, respectively. A higher concentration of validamycins was obtained with smaller sparger hole diameter, where smaller gas bubbles were obtained. Changes of total sugar and reducing sugar are illustrated in Figs. 5—7. After the inoculation, the total sugar and the reducing sugar decrease rapidly in the early period (0—15 h). After 20 h of fermentation, the rate of decrease becomes lowed. The trends in concentration of total sugar and reducing sugar in the ELAB and in the MSTB are similar.

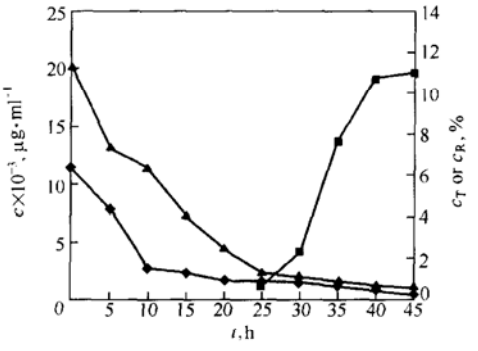

Figure 5 Validamycin fermentation in MSTB
[V = 0.80 vvm, V_L = 7.00, n = 300 $r \cdot min^{-1}$, T = (39 ± 1)℃]
—■— validamycin; —▲— total sugar; —◆— reducing sugar

4 CONCLUSIONS

For the consideration of substituting an airlift bioreactor for a MSTB, an ELAB with a low ratio

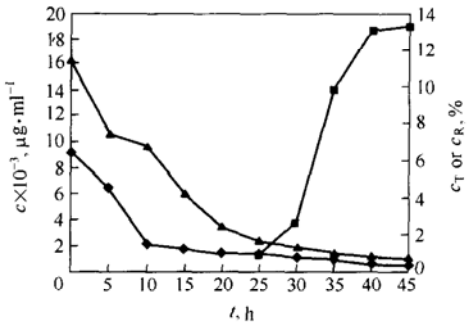

Figure 6 Validamycin fermentation in ELAB with a sparger diameter of 3.0 mm
[V = 1.10 vvm, V_L = 8.75 L, T = (39 ± 1)℃]
—■— validamycin; —▲— total sugar; —◆— reducing sugar

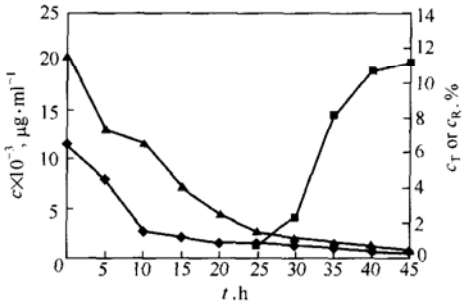

Figure 7 Validamycin fermentation in ELAB with a sparger diameter of 1.5 mm
[V = 1.10 vvm, V_L = 8.75 L, T = (39 ± 1)℃]
—■— validamycin; —▲— total sugar; —◆— reducing sugar

of height to diameter of riser of 2.9 and a ratio of riser to downcomer diameter of 6.6 was developed, and used in the fermentation of validamycins by *Streptomyces hygroscopicus* from crude substrates. Gas flow rate, liquid volume and sparger hole diameter affected validamycin fermentation. The highest concentration of validamycins, 19975 $\mu g \cdot ml^{-1}$, was obtained in the ELAB with sparger hole diameter of 1.5 mm under the optimized condition in which gas flow rate was 1.10 vvm and an initial liquid volume was 8.75 L. The fermentation cycle in the ELAB was almost the same as that in the MSTB, and much shorter than that in the shaking flasks under the same operation conditions including fermentation medium composition, inoculum ratio and culture temperature. Although under the optimal fermentation condition, gas flow rate in the ELAB was needed 37.5% higher than that in the MSTB, there was no agitation system and power was not required for agitation. Taking these factors into acount, the total consumed energy of the ELAB was about 25% less than that of the MSTB. Furthermore, the manufacture cost of ELAB was about two thirds of that of MSTB without agitation system. Therefore, it is possible to retrofit existing MSTB with an ELAB to produce validamycins from crude substrates economically.

NOMENCLATURE

A_d	cross-sectional area of downcomer, m^2
A_r	cross-sectional area of riser, m^2
c	concentration of validamycins, $\mu g \cdot ml^{-1}$
c_R	concentration of reduced sugar, %
c_T	concentration of total sugar, %
D_d	diameter of downcomer, m
D_r	diameter of riser, m
H	height of bioreactor, m
n	agitating rate, $r \cdot min^{-1}$
s	ratio of height-to-diameter
T	temperature, ℃
t	time, h
V	gas flow rate, vvm
V_L	liquid volume, m^3
V_T	total volume of the bioreactor, m^3
vvm	gas volume per liquid volume per minute

REFERENCES

1 Woragidbumrung, K., Sae-Tang, P., Yao, H., Han, J., Chaivatcharin, S., Zhong, J.J., "Impact of conditioned medium on cell cultures of *Panax notoginseng* in an airlift bioreactor", *Process Biochem.*, **37**, 209—213 (2001).

2 Huang,T.K., Wang, P.M., Wu, W.T., "Cultivation of *Bacillus thuringiensins* in airlift reactor with wire mesh draft tubes", *Biochem. Eng. J.*, **7**, 35—39 (2001).

3 Gavrilescu, M., Tudose, R.Z., "Hydrodynamics of non-Newtonian liquids in external-loop airlift bioreactors. Part I: Study of the gas holdup", *Bioproc. Eng.*, **18**, 17—26 (1998).

4 Galindez-Mayer, J., Sanchez-Teja, O., Cristiani-Urbina, E., Ruiz-Ordaz, N., "A novel split-cylinder airlift reactor for fed-batch cultures", *Bioproc. Biosys. Eng.*, **24**, 171—177 (2001)

5 Bello, R.A., Robinson, C.W., Moo-young, M., "Gas holdup and overall volumetric oxygen transfer coefficient in airlift contactors", *Biotechnol. Bioeng.*, **27**, 369—381 (1985).

6 Sajc, L., Oberadovic, B., Vakovic, D., Bugarki, B., "Hydrodynamics and mass transfer in a four-phase external-loop airlift bioreactor", *Biotechnol. Prog.*, **11**, 420—428 (1995).

7 Kawase, Y., Hashimoto, N., "Gas hold-up and oxygen transfer in three-phase external-loop airlift bioreactors: Non-Newtonian fermentation broths", *J. Chem. Tech. Biotechnol.*, **65**, 325—334 (1996).

8 Kawase, Y., Omori, N., Tsujimura, M., "Liquid-phase mixing in external-loop airlift bioreactors", *J. Chem. Tech. Biotechnol.*, **61**, 49—55 (1994).

9 Fraser, R.D., Itiel, G.A., "Hydrodynamic characteristic of a spinning sparger, external-loop airlift bioreactor", *Can. J. Chem. Eng.*, **71**, 419—425 (1993).

10 Fraser, R.D., Ritchie, B.J., Hill, G.A., "Dynamic mixing and oxygen transfer in a small airlift loop bioreactors: Model and experimental verification", *Biotechnol. Prog.*, **10**, 543—547 (1994).

11 Gavrilescu, M., Tudose, R.Z., "Modeling of liquid circulation velocity in concentric tube airlift reactors", *Chem. Eng. J.*, **69**, 85—91 (1998).

12 Zheng, Y.G., Wang, Z., Chen, X.L., "α-Amylase production by *Bacillus subtilis* with dregs in an external-loop airlift bioreactor", *Biochem. Eng. J.*, **5**, 115—121 (2000).

13 Schorr-Galindo, S., Ghomnidh, C., Guiraud, J.P., "Insulin enrichment by fermentation in a flocculating yeast reactor", *Biotechnol. Lett.*, **17**, 1305—1310 (1995).

14 Chung, I.S., Kang, Y.M., Oh, J.H., "Continuous suspended cell culture of *Mentha piperita* in cell-recycled airlift bioreactor", *Biotechnol. Tech.*, **8**, 789—792 (1994).

15 Kessler, W.R., Popovic, M.K., Robinson, C.W., "Xanthan production in an external–loop airlift fermenter", *Can. J. Chem. Eng.*, **71**, 101—106 (1993).

16 Brandes, L., Cvu, X., Maschke, H.S., "Production of fusion protein SPA ECORI in batch culture in a 60L airlift tower loop reactor", *Biotechnol. Prog.*, **9**, 122—127 (1993).

17 Al-Qodah, Z., Lsfi, W., "Modeling of antibiotics production in magneto three-phase airlift fermenter", *Biochem. Eng. J.*, **7**, 7—16 (2001).

18 Xie, D., Liu, D., Liu, T., "Modeling of glycerol production by fermentation in different reactor states", *Process Biochem.*, **36**, 1225—1232 (2001).

19 Mekchuk, J.C., Siegel, M.H., "Airlift reactors in chemical and biological technology", *J. Chem. Tech. Biotechnol.*, **41**, 105—120 (1988).

20 Agricultural Antibiotic Group in Shanghai Pesticide Institute, "Identification of validamycins producing organism", *Ac. Microb.*, **15**, 110—114 (1975). (in Chinese)

21 Iwasa, T., Higashide, E., Yamanoto, H., Shibata, M., "Studies on validamycins, a new antibiotic II. Production and biological properties of validamycins A and B", *J. Antibiot.*, **24**, 107—113 (1971).

22 Dong, H., Mahmud, T., Tornus, I., Lee, S., Floss, H., "Biosynthesis of the validamycins: Identification of intermediates in the biosynthesis of validamycin A by *Streptomyces hygroscopicus*", *J. Am. Chem. Soc.*, **123**, 2733—2742 (2001).

23 Iwasa, T., Yamamoto, H., Shibata, M., "Studies on validamycins new antibiotics I. *Streptomyces hygroscopicus* var. *Limoneusnov.* var., validamycin producing organism", *J. Antibiot.*, **23**, 595—602 (1970).

24 Kameda, Y., Asano, N., Matsui, K., "Structures of minor components of the validamycin complex", *J. Antibiot.*, **41**, 1488—1492 (1988).

25 Asano, N., Yamaguchi, T., Kameda, Y., Matsui, K., "Effect of validamycins on glycohydrolases of *Rhizoctionia Solani*", *J. Antibiot.*, **40**, 526—532 (1987).

26 Chen, T., Zhang, Y., Sun, S., Determination of the Biological Pesticides and Their Principles, Agriculture Press, Beijing (1993). (in Chinese)

27 Jia, S., Mu, G., Ren, S., "A rapid method for determination of reducing sugar, glucose and starch in fruits and vegetables with 3,5-diatrosalycylic acid", *Food Ferment. Ind.*, **2**, 30—34 (1983). (in Chinese)

28 Kamp, F., Wase, D.A.J., McManamey, W.J., Mendoza, O., Thayamithy, K., "A comparison of some methods of estimating volumetric mass-transfer coefficients in an external-loop airlift fermenter", *Biotechnol. Bioeng.*, **30**, 179—186 (1987).

29 Stejskal, J., Potucek, F., "Oxygen transfer in liquids", *Biotechnol. Bioeng.*, **27**, 503—508 (1985).

30 Snape, J.B., Fialova, M., Zahradnik, J., "Hydrodynamic studies in an external-loop airlift reactor containing aqueous electrolyte and sugar solutions", *Chem. Eng. Sci.*, **47**, 3387—3394 (1992).

31 Snape, J.B., Zahradnik, J., Fialova, M., Thomas, N.H., "Liquid-phase properties and sparger design effects in an external-loop airlift reactor", *Chem. Eng. Sci.*, **50**, 3175—3186 (1995).

32 Merchuk, J.C., "Gas holdup and liquid velocity in a two-dimensional airlift reactor", *Chem. Eng. Sci.*, **41**, 11—16 (1986).

33 Mekchuk, J.C., Stein, Y., "Local holdup and liquid velocity in airlift reactors", *AIChE J.*, **27**, 377—388 (1981).

34 McManamecy, W.J., Wase, D.A.J., Rayrnahasay, S., Thayanithy, K., "The influence of gas inlet design on gas holdup values for water and various solutions in a loop-type airlift fermenter", *J. Chem. Technol. Biotechnol.*, **34B**, 151—164 (1984).