African Journal of Biotechnology Vol. 9(42), pp. 7186-7191, 18 October, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1106
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Oxygen uptake rate (OUR) control strategy for improving avermectin B_{1a} production during fed-batch fermentation on industrial scale (150 m^3)

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Accepted 10 September, 2010

Glucose metabolism plays a crucial role in the process of avermectin B_{1a} biosynthesis. Controlling glucose feeding based on oxygen uptake rate (OUR) was established to improve the efficiency of avermectin B_{1a} production. The result showed that avermectin B_{1a} production was greatly enhanced by OUR control strategy. In the glucose feeding phase, OUR was maintained at approximate 12 mmol/L/h, which was conducive to avermectin B_{1a} biosynthesis. Using this OUR control strategy, an adequate supply of organic acid precursors produced avermectin B_{1a} 5228 U/mL, which was 22.8% higher than that of the control (batch fermentation, 4256 U/mL) on industrial scale.

Key words: Avermectin B1a, glucose feeding, oxygen uptake rate, industrial scale.

INTRODUCTION

Oxygen uptake rate (OUR) is one of the pivotal physiological parameters that can be correlated with other parameters like carbon dioxide evolution rate (CER), respiratory quotient (RQ), and dissolved oxygen (DO), etc. As an indicator of cellular activity, it enables the indirect measurement of substrate utilization. OUR delivers a real-time online assessment of the status of microbial enrichment or evolutionary processes and provides opportunities for feedback systems that control and optimize these processes. In the course of monoclonal antibody culture, an ''oxygen uptake rate-amino acids" (OUR-AA) strategy was established, in which amino acid feeding was controlled according to variations of OUR during controlled feeding perfusion as a means of enhancing monoclonal antibody productivity (Feng et al., 2006). Avermectin B_{1a} is an important macrocyclic polyketide produced by *Streptomyces avermitilis* and is widely used as an anthelmintic agent in the medical, veterinary, and agricultural fields (Mironov et al., 1997). Glucose was used for the enhancement of avermectin B_{1a} (Novak et al., 1992; Rajkarnikar et al., 2007), and played an important role in *S. avermitilis* metabolism. Research (Schulman et al., 1986) indicated that all the avermectin B_{1a} molecule was derived from glucose using U-^{13}C]glucose, and showed that the oleandrose units were also derived from glucose directly with ^{3}H/^{14}C at C_6, with ^{13}C at C_1. Thus, avermectin B_{1a} biosynthesis during fermentation has been suggested to be affected by glucose metabolism (Mironov et al., 2003). In this work, we aimed to study avermectin B_{1a} production by integration of glucose feeding and OUR monitoring during the fermentation process. It was found that the physiological and metabolic status of *S. avermilis* could be well controlled using OUR real-time monitoring. This study is the first report on employing online OUR

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Abbreviations: OUR, Oxygen uptake rate; **CER,** carbon dioxide evolution rate; **RQ,** respiratory quotient; **DO,** dissolved oxygen; **OUR-AA,** oxygen uptake rate-amino acids; **PMV,** packed mycelium volume; **HPLC,** high performance liquid chromatography; **MDW,** mycelial dry weight; **PKS,** polyketide synthetase.

monitoring control for the fed-batch process of avermectin B_{1a} production by *S. avermilis* on an industrial scale (150 m^3).

MATERIALS AND METHODS

Microorganism

S. avermitilis Biok No.04 was provided by Zhejiang Shenghua Biok Biology Co., Ltd. (China).

Medium

The agar slant solid medium contained (g/L): Glucose, 4.0; yeast extract, 4.0; malt extract, 10.0; and agar, 20.0, pH 7.0-7.2. The seed medium and avermectin B_{1a} production medium used in this study were the same as those described in a previous study (Gao et al., 2009).

Batch and fed-batch fermentation conditions

Batch and fed-batch fermentation were conducted as follows: 150 m^3 Fermenter was used for avermectin B_{1a} production (Biok505, Sheng Hua Group, China). The first seed culture was prepared in a 200 L fermenter under the same conditions described in a previous study (Gao et al., 2009). For the second seed culture, the first seed of 140 L was inoculated into a 20 m^3 fermenter (BioK403, Sheng Hua Group, China), containing 14 m^3 of seed medium. Then, the culture broth was cultivated for 42 h at 28°C,120 rpm and 0.5 vvm. The second seed of 14 m^3, which was obtained from a 20 m^3 seed tank, was transferred into the 150 m^3 fermenter (BioK505, Sheng Hua Group, China). Under fed-batch condition, glucose solution (30%, w/v) was added continuously for about 145 h monitored by OUR control (approximate 12 mmol/l/h).

Packed mycelium volume (PMV) was used to represent the cell mass, in the seed medium, PMV needed to reach 30% before transferr into the next fermentor.

Assay of organic acids

The method for the preparation of extracellular and intracellular organic acids was described previously (Wu et al., 2008; Chen et al., 2004). To determine extracellular and intracellular organic acids in the broth, the high performance liquid chromatography (HPLC) system (JASCO PU2080, Japan) was equipped with a AquaSep C_8 column (250 ×4.6 mm, 5 L, ES, USA) and a UV detector (210 nm). The mobile phase was delivered at a flow rate of 0.6 mL/min using 0.01 M H_3PO_4 solution. The column was operated at 30°C.

Determination of OUR as a monitoring parameter

The OUR was used as a monitoring parameter in an industrial 150 m^3 fermenter. It was kept constant with the addition of glucose. OUR was determined by an exhaust gas analyzer (H&B, Germany) and a Biostar software (East China University of Science and Technology).

Analytical methods

Glucose concentration in the fermentation broth was determined using a glucose kit (Shanghai Institute of Biological Products, Shanghai, P.R. China). Liquid cultures (10 ml) were harvested for washing three times and filtering, and then dried to constant weight at 80°C for mycelial dry weight (MDW) determination.

Assay of avermectin B_{1a} production

The concentration of avermectin B_{1a} in the broth was measured by HPLC (Waters Ltd., USA). The Novapak C_{18} column (Waters Ltd, USA: 3.9 ×150 mm) was eluted with methanol: water (85:15, v/v) at a flow rate of 0.8 mL/min in ambient conditions. An equal volume of methanol was added to the sample. The mixture was shaken for 30 min to extract avermectin B_{1a} from the mycelia. A sample (10 µL) of the methanol extract was tested. Quantity of avermectin B_{1a} was calculated based on the integration value at 246 nm. An authentic sample of avermectin B_{1a} was used as a standard (McKellar and Benchaoui, 1996).

RESULTS AND DISCUSSION

Fed-batch fermentation process of avermectin B_{1a} production based on OUR control in a 150 m^3 fermenter

In general, antibiotic production is regulated by various parameters like medium, aeration, and temperature, whereas the production of some antibiotics is suppressed by glucose, ammonium ions, and phosphate ions (Elibol, 2001). Avermectin B_{1a} biosynthesis during fermentation was affected by glucose metabolism and significantly suppressed by the addition of glucose at the early stage of fermentation (Haruo, 1988; Xu and Chen, 1999). We employed online OUR monitoring control strategy for the fed-batch process of avermectin B_{1a} production by *S. avermilis* on an industrial scale (150 m^3). OUR as a scale-up parameter, can be easily measured on line and give important information on the metabolic activity of cells in cultures (Higareda et al., 1997). OUR has been utilized for estimating online viable cell concentration at the growth phase of insect and mammalian cell cultures. In addition, OUR can be used to assess rapid metabolic shifts in cells (Dumsday et al., 2009). For example, a sudden decrease in respiration has been correlated with glutamine depletion in cultures (Wang et al., 2010). In the present work, as shown in Figure 1 and Table 1, glucose depletion caused a reduction of OUR during avermectin B_{1a} production. *S. avermitilis* utilized glucose not only for primary metabolism as an energy and carbon source but also for avermectin B_{1a} biosynthesis. OUR reached its maximum at about 36 h, then OUR rapidly declined during 36-144 h, finally, at 145 h, glucose feeding by OUR monitoring was implemented and OUR was kept at approximate 12 mmol/l/h. Results showed that the MDW reached its peak at 144 h (Table 1). Under the online OUR monitoring, the pH (5.52±0.1) remained relatively consistent and stable during 144 -264 h. The specific growth rate (µ, h^{-1}) was maintained at a low level (Table 1) in the whole feeding glucose phase, which may be conducive to the secretion of secondary products

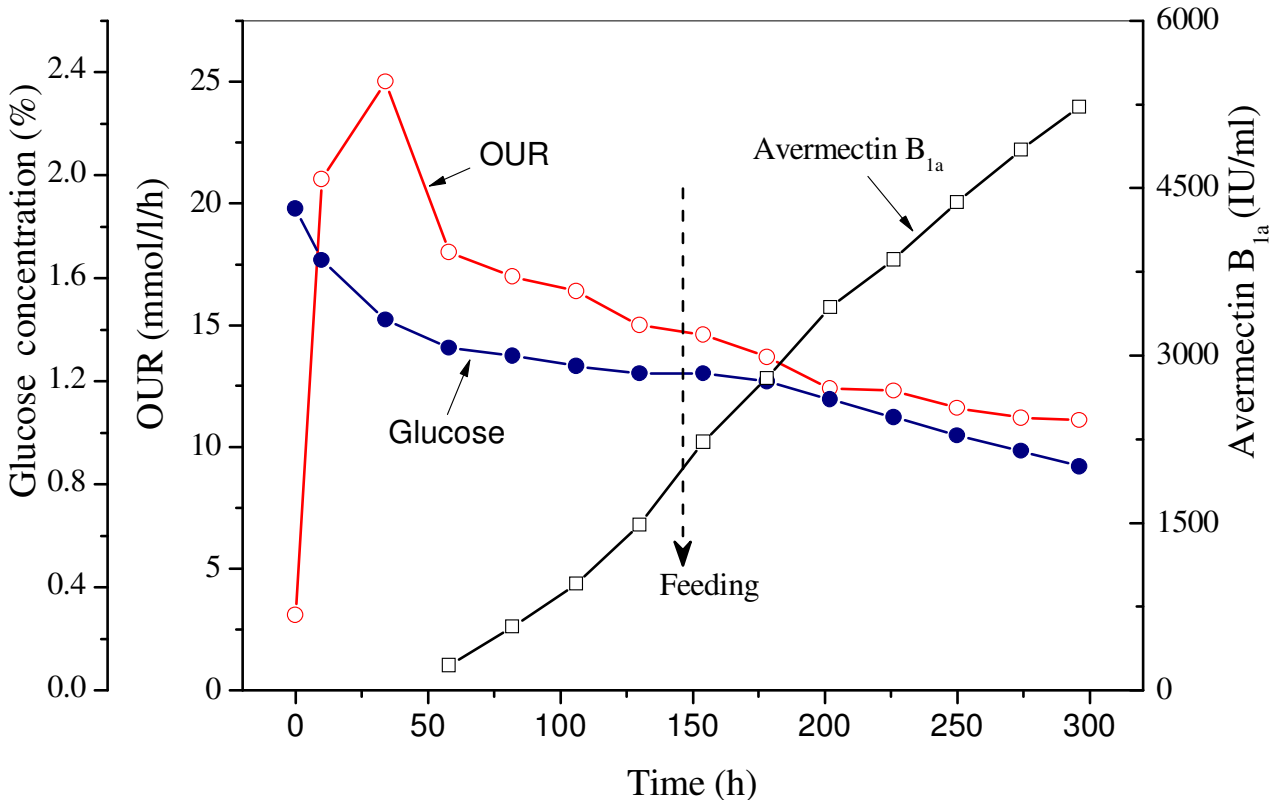

Figure 1. Avermectin B_{1a} production based on OUR control and glucose consumption in the fed-batch fermentation process.

Table 1. Changes of specific growth rate, pH and mycelial dry weight by OUR control strategy.

Time (h)	MDW (g/10 ml culture broth)	Specific growth rate (μ,)	pH
24	0.2119	0.0194	6.75
48	0.2493	0.0223	6.61
72	0.2583	0.0175	5.85
96	0.3526	0.0166	5.68
120	0.4016	0.0117	5.56
144	0.4325	0.0083	5.56
168	0.4088	-0.0089	5.54
192	0.4036	-0.0054	5.52
216	0.3813	-0.0041	5.53
240	0.3583	-0.0034	5.55
264	0.3462	-0.0027	5.61
288	0.3443	-0.0017	6.13

(avermectin B_{1a} was achieved at 5228 U/ml).

Improvement of avermectin B_{1a} production by OUR control strategy in a 150 m^3 fermenter

By using the OUR monitoring glucose feeding strategy in the middle and late stage of fermentation (144 - 264 h), glucose feeding rate by controlling OUR gradually increased from 36 to 60 L/h (144 - 264 h). During the fed-batch phase, although glucose content of culture broth gradually reduced, OUR slightly decreased (maintained about 12 mmol/l/h) after some 140 h of fermentation (Figure 2). However, under batch fermentation, OUR drastically declined from 13.3 to 5.8 mmol/L/h (144 to 288 h of fermenation) due to mycelia aging or cell death.

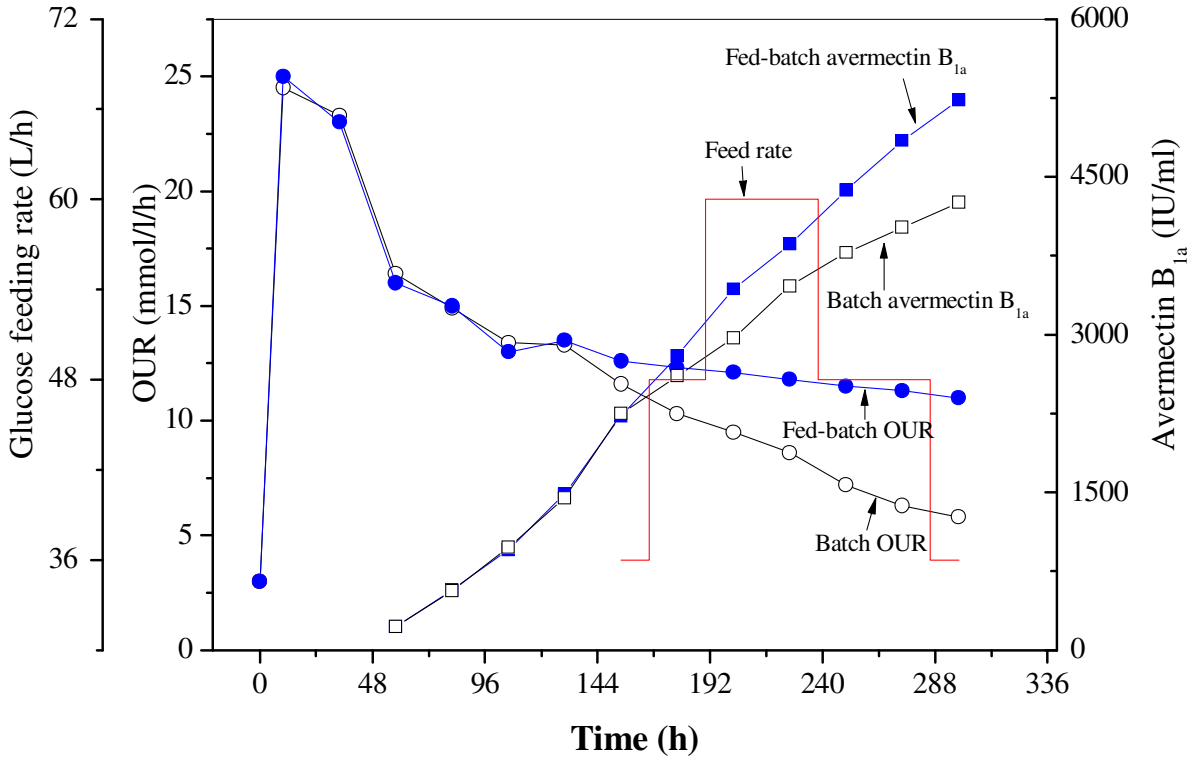

Figure 2. Comparison of batch fermentation and fed-batch fermentation based on OUR control strategy during avermectin B_{1a} production.

Compared to batch fermentation, the avermectin B_{1a} titer of 5228 U/mL was obtained using the OUR monitoring of glucose consumption rate, which was 22.8% higher than that of the control (4256 U/mL). Therefore, this strategy can be used as a good method to efficiently improve avermectin B_{1a} production.

Comparison of some organic acids changes between the batch and fed-batch fermentation

In the previous study, it was reported that the avermectin B_{1a} aglycone is composed of seven acetates, five propionates, and one 2-methylbutyrate or isobutyrate, and its biosynthesis follows polyketide synthetase (PKS) pathway (Cane et al., 1983). In this work, intercellular and extracellular organic acid concentrations (acetic, propionic, and pyruvic acids of the carbon central metabolism) of an industrial culture of *S. avermitilis* were analyzed in typical avermectin B_{1a} batch and fed-batch fermentation. To our knowledge, propionic acid and acetic acid were the main sources of precursors for avermectin B_{1a} biosynthesis, and the accumulated intracellular propionic acid and acetic acid (Figure 3B and C) were obviously higher than that of the control after glucose feeding in the process of fermentation. The accumulated extracellular pyruvic acid (Figure 3A) was higher than that of the intracellular pyruvic acid at the early phase of fermentation (before 96 h). The accumulated extracellular acetic acid (Figure 3B and C) were lower than those of the control after glucose feeding in the fermentation. The results indicated that OUR control can supply more precursors than batch mode and improve avermectin B_{1a} biosynthesis. Therefore, fed-batch fermentation by OUR control strategy can enhance the metabolic flux of avermectin B_{1a} biosynthesis by regulating glucose feeding at a suitable level.

Conclusions

Avermectin B_{1a} productivity can be enhanced by glucose feeding with OUR control on an industrial scale (150 m^3). In the present work, the OUR, pH, and glucose feeding rate showed close relationship, and organic acids (acetic, propionic and pyruvic acids) were strongly influenced by the glucose feeding condition. Avermectin B_{1a} production of 5228 U/mL was obtained using the OUR monitoring of the glucose feeding rate. Results of the present study suggested that, by adjusting the feeding rate using the OUR control strategy, it is feasible to increase avermectin B_{1a} production continuously.

ACKNOWLEDGEMENTS

This work was financially supported by a grant from the

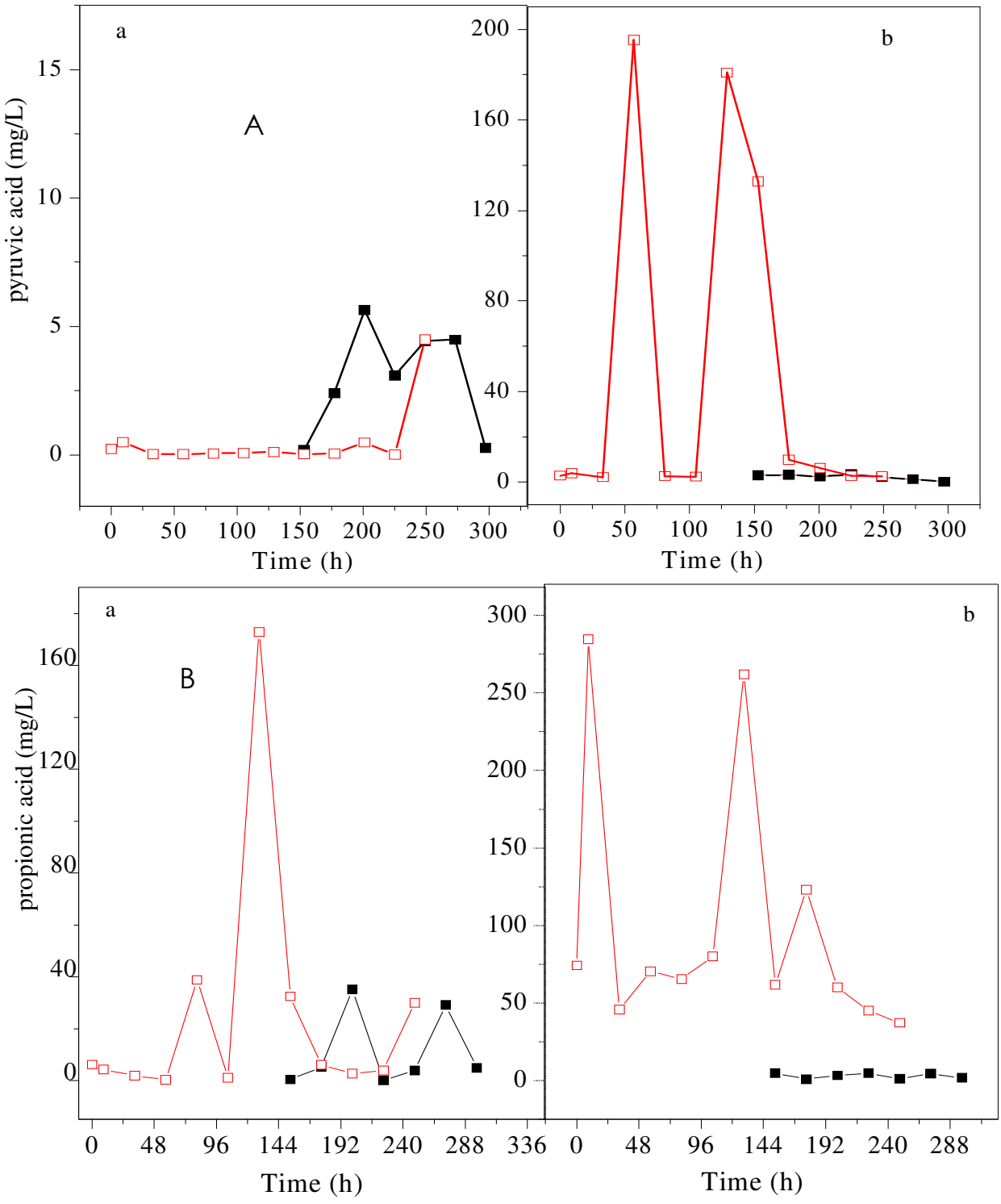

Figure 3. Changes of organic acids during batch fermentation and fed-batch fermentation in a 150 m^3 fermenter. Intracellular organic acid (a) and extracellular organic acid (b). ■, Fed-batch; □, batch.

Major State Basic Research Development Program of China (973 Program, No. 2007CB714303), the National Key Technology R&D Program (No. 2007BAI26B02 and No. 2008BAI63B01), and the National High Technology Research and Development Program of China (863 Program, No.2009AA032904), and the Shanghai Leading

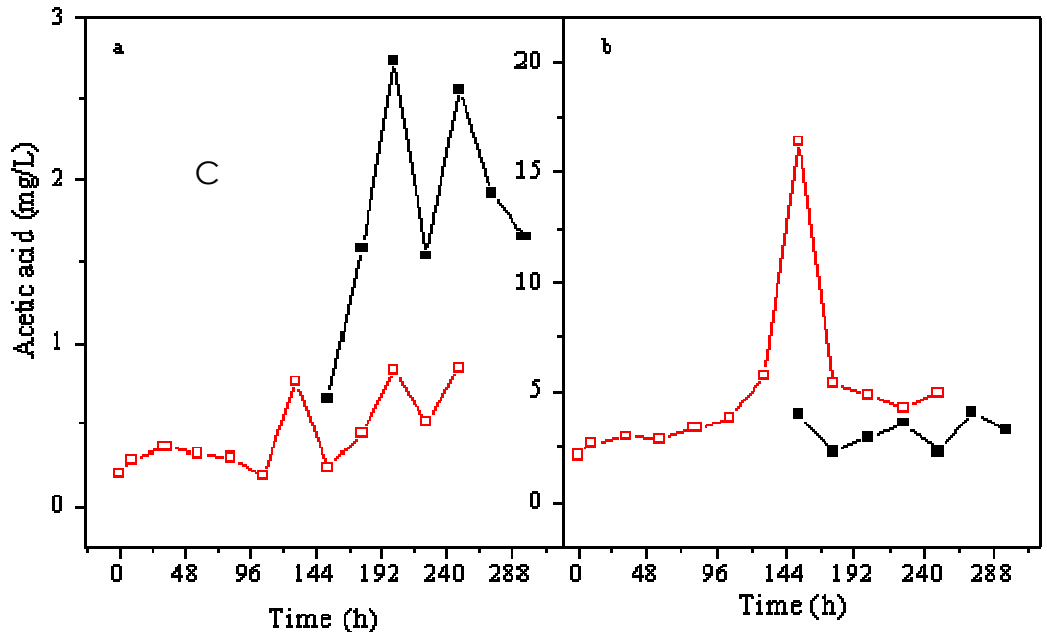

Figure 3. Contd.

Academic Discipline Project, No.B505.

REFERENCES

Cane DE, Liang TC, Kaplan LK, Nallin MK, Schulman MD, Hensens OD, Douglas AW, Albers-Schonberg G (1983). Biosynthesis origin of the carbon skeleton and oxygen atoms of the avermectins. J Am. Chem. Soc. 105: 4110-4112.

Chen J, Chu J, Zhuang YP, Zhang SL (2005). Correlation between organic acids accumulation and biosynthesis of avermectin. J. East China Univ. Sci. Technol. 31: 731-734. (in Chinese).

Dumsday GJ, Ocal G, Bridger JS, Zachariou M (2009). The use of oxygen uptake rate to monitor discovery of microbial and enzymatic biocatalysts. Biotechnol. Bioeng. 102: 673-683.

Elibol M (2001). Improvement of antibiotic production by increased oxygen solubility through the addition of perfluorodecalin. J. Chem. Technol. Biotechnol. 76: 418-422.

Feng Q, Mi L, Li L, Liu R, Xie L, Tang H, Chen Z (2006). Application of oxygen uptake rate-amino acids associated mode in controlled-fed perfusion culture. J Biotechnol. 122: 422-430.

Gao H, Liu M, Liu J, Dai H, Zhou X, Liu X, Zhuo Y, Zhang W, Zhang L (2009). Medium optimization for the production of avermectin B_{1a} by *Streptomyces avermitilis* 14-12A using response surface methodology. Bioresour. Technol. 100: 4012-4016.

Haruo IHK, Haruo T, Satoshi O (1988). Involvement of Glucose Catabolism in Avermectin B_{1a} Production by *Streptomyces avermitilis*. Antimicrob. Agents Chemother. 32: 282-284.

Higareda AE, Possani LD, Ramirez OT (1997). The use of culture redox potential and oxygen uptake rate for assessing glucose and glutamine depletion in hybridoma cultures. Biotechnol. Bioeng. 56: 555-563.

McKellar QA, Benchaoui HA (1996). Avermectin B_{1a} and milbemycins. J. Vet. Pharmacol. Ther. 19: 331-351.

Mironov V, Sergeeva A, Gavrilina A, Danilenko V (2003). Dependence of the Composition of the Avermectin B1a Complex of *Streptomyces avermitilis* on the Glucose Content in the Medium. Appl. Biochem. Micro. 39: 183-187.

Mironov VA, Sergeeva AV, Voronkova VV, Danilenko VN (1997). Biosynthesis of avermectin B_1 as: Physiological and technological aspects. Antibiot. Khimioter. 42: 31-36.

Novak J, Hajek P, Rezanka T, Vanek Z (1992). Nitrogen regulation of fatty acids and avermectin B1as biosynthesis in *Streptomyces avermitilis*. FEMS Microbiol. Lett. 72: 57-61.

Rajkarnikar A, Kwon HJ, Ryu YW, Suh JW (2007). Two threonine residues required for role of AfsKav in controlling morphogenesis and avermectin B_{1a} production in *Streptomyces avermitilis*. J. Microbiol. Biotechnol. 17: 1563-1567.

Schulman M, Valentino D, Hensens O (1986). Biosynthesis of the avermectin B_{1a} by *Streptomyces avermitilis* incorporation of labeled precursors. J. Antibiot. 39: 541-549.

Wu B, Chen CH, Zhang Q (2008). Effect of cell morphology and organic acid accumulation to lovastatin production by*Aspergillus terreus*. J. East China Univ. Sci. Technol. 34: 189-202. (in Chinese).

Wang ZJ, Wang HY, Li YL, Chu J, Huang MZ, Zhuang YP, Zhang SL (2010). Improved vitamin B(12) production by step-wise reduction of oxygen uptake. Bioresour. Technol. 101: 2845-2852.

Xu ZN, Chen PL (1999). Enhanced production of avermectin B_{1a} by medium optimization and glucose feeding with *Streptomyces avermilitis*. Bioproc. Biosyst. Eng. 20: 67-71.