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EFFECTS OF AERATION AND AGITATION ON THE SISOMICIN PRODUCTION BY *MICROMONOSPORA INYOENSIS*

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Abstract—Physical factors affecting the production of sisomicin by *Micromonospora inyoensis* are presented. A sisomicin productivity was affected significantly by both the degree of aeration and the type of impeller used. In addition, the value of impeller tip speed was found to be an important factor in the sisomicin production. Therefore, scale-up on the basis of impeller tip speed was attempted and satisfactory results were obtained.

INTRODUCTION

Sisomicin was first described in 1970 as being produced by a new species of *Micromonospora* [1, 2]. The sisomicin, like gentamicin, was found to be active against most species of both gram-negative and gram-positive bacteria [3]. Recently another sisomicin producer *Micromonospora rosea* was isolated from a Hungarian soil sample [4]. Although the sisomicin titre by *M. rosea* (600-700 mcg/m*l*) was three times higher than that by *M. inyoensis*, the fermentation broth contained about 15% of other accompanying antibiotics beside sisomicin.

Previous studies have shown that accumulation of carbon dioxide in the fermentation broth inhibited the sisomicin production significantly [5]. In this study, the effects of aeration and agitation on the sisomicin production by *Micromonospora inyoensis* were investigated in detail in a 30-1 jar fermentor. The aim of this study was to investigate factors responsible for scale-up.

MATERIALS AND METHODS

Microorganism and media

A strain of *Micromonospora inyoensis* ATCC 27600 was used throughout the course of this work. The strain was grown at 32°C for 3 days under 180 rpm (New Brunswick, Model G 25) in a 250-m*l* flask containing 50 m*l* of seed medium [6] as a pre-seed culture having the composition (g/*l*): potato starch 24.0; dextrose 1.0; tryptone 5.0; beef extract 3.0; yeast extract 5.0; $CaCO_3$ 2.0.

The pre-seed culture broth was then transferred to 5-1 flasks containing 700m*l* of each of the seed medium described above. After cultivation at 32°C for 2 days, they were inoculated to a 30-1 jar fermentor or a 500-1 pilot fermentor. The fermentation medium contained (g/*l*): dextrin 70; soybean flour 35; $CaCO_3$ 5; cobalt chloride 0.004; DL-methionine 2; corn steep liquor 5; $MnSO_4 \cdot 7H_2O$ 0.1. A 5% (v/v) inoculum was used in this study.

Culture conditions

The fermentation was carried out without pH control. Unless otherwise specified, the environmental conditions for this present study are illustrated in Table 1 based on the preliminary optimization studies for the sisomicin fermentation.

Fermentation systems

A 30-1 jar or 500-1 pilot fermentor with two six-bladed impellers incorporating temperature control was used for this study. A comparison of fermentor geometry is summarized in Table 2.

On-line estimation of dissolved carbon dioxide concentration was achieved by the used of a pCO_2 electrode and a monitor [7]. Dissolved oxygen tension was

Table 1. Environmental conditions for sisomicin production by *M. inyoensis*.

Condition	Period (hr)	
	0-48	49-144
Temperature (°C)	34	32
Aeration (vvm)	1.0	1.5
Agitation (rpm)	200	300
Tip speed (cm/sec)	150	225

Table 2. Geometric ratios for 30 - l and 500 - l fermentators.

Part	Ratio	30-jar	500-l pilot
Vessel	Di/Dt	0.49	0.40
	Hl /Dt	0.8	1.0
	Hb/Di	0.4	0.85
Baffle	Nb	3	4
	Wb/Dt	0.12	0.10
Impeller	Type	Vaned disc	Vaned disc
	Ni	2	2
	Li/Di	0.25	0.25
	Wi/Di	0.18	0.20

Di, diameter of impeller; Dt, diameter of vessel; Hl, total liquid height; Nb, number of baffle; Ni, number of impeller; Li, length of impeller's blade; Wi, width of impeller's blade; Wb, width of baffle; Hb, height of liquid of bottom impeller.

also monitored during the fermentation. The estimation of oxygen transfer coefficient K_{La} was performed using the dynamic method of gassing out [8].

Analytical methods

Total sugar, glucose, and sisomicin titre were estimated as described previously [5]. The amount of DNA plus RNA was measured by the method described by Herbert et al. [9]. For the measurement of dry cell weight shown in Fig. 1, soybean flour was previously extracted in water at 100°C for 30 mins and the extracted soybean flour was used.

The apparent viscosity of the fermentation broth was measured by a Brookfield Synchro-Lectric viscometer (Model RVF, USA) equipped with UL-adaptor at a shear rate of 2.45 sec^{-1}.

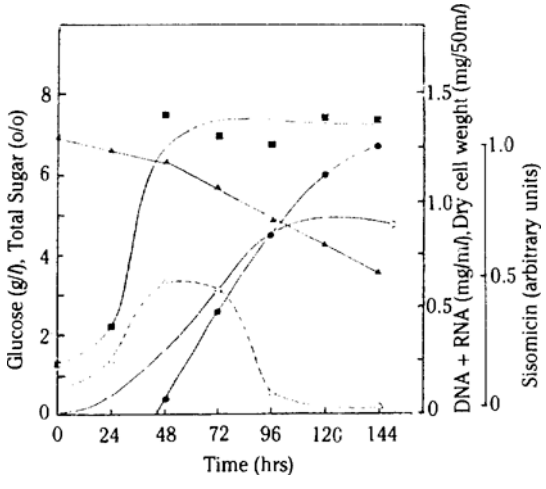

Fig. 1. A typical time course of sisomicin fermentation by *M. inyoensis*.
▲ Total sugar; △ Glucose; ■ DNA plus RNA; ○ Dry cell weight; ● Sisomicin.

RESULTS AND DISCUSSION

Batch culture kinetics of sisomicin fermentation

A typical time-course study of sisomicin fermentation by *M. inyoensis* is shown in Fig. 1. During the early period of fermentation, there was an accumulation of glucose, reaching a maximum value at 48 hours. Following this initial period, the glucose concentration declined toward the end of fermentation. As can be seen from Fig. 1, the sisomicin production by *M. inyoensis* began to occur after 48 hours.

Effect of degree of aeration on dissolved carbon dioxide concentration

The inhibitory effect of carbon dioxide on inosine [10], sisomicin [5, 14] and penicillin production [11] has been reported. In Fig. 2, results of the on-line estimations of dissolved carbon dioxide concentration during the sisomicin fermentation in relation to aeration rate are shown. It is clear that an increase in aeration rate from 1.0 vvm to 1.5 vvm during sisomicin production phase resulted in the decreased dissolved carbon dioxide concentration by about 20-40%. This was due to aeration effect in removing carbon dioxide

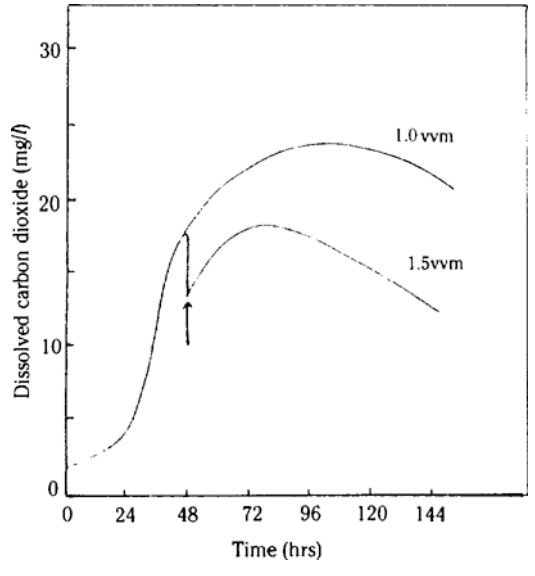

Fig. 2. Effect of increase in aeration rate from 1.0 vvm to 1.5 vvm on dissolved carbon dioxide concentration. Arrow indicates the step change of aeration rate from 1.0 vvm to 1.5 vvm.

Table 3. Effect of various types of impeller on sisomicin production.

Type	Sisomicin (arbitrary units)	Final pH	Residual Sugar (%)
Disc turbine	0.90	7.0	4.0
Vaned disc	1.00	6.9	4.2
Open turbine	0.79	7.2	4.2
Marine propeller	0.49	7.1	4.0

in fermentation broth and provided approximately 15% improvement in sisomicin productivity.

Comparison of various types of impeller used for sisomicin production

In order to find out the best type of impeller for sisomicin production, various types of impeller were tested as shown in Table 3. In fact, both the ability to break up air streams and the flow pattern produced by impeller depend on the type of impeller. It was evident that the best sisomicin productivity was achieved with vaned disc type of impeller. When marine propeller type (number of blades=3) of impeller was used, dissolved oxygen tension was decreased significantly up to zero level after 4 day cultivation. This resulted in a significant reduction in sisomicin productivity.

Effects of impeller tip speed and impeller size on sisomicin production

The effects of impeller tip speed and impeller size on sisomicin production were investigated with vaned disc or disc turbine type of impeller. The results in Fig. 3 and Fig. 4 were obtained from a series of fermentations. The impeller tip speed (Vi) is defined as follows:

$$Vi = \pi N Di / 60 \quad (1)$$

where N is the rpm and Di is the diameter of impeller. As can be seen from Fig. 3, tip speeds above 300 cm/sec caused significant reduction in both sisomicin production and apparent viscosity. Similar results have been observed for penicillin production [12]. The strain *M. inyoensis* appears to be sensitive to mechanical damage. It was found also that the culture showed pseudoplastic behavior rheologically.

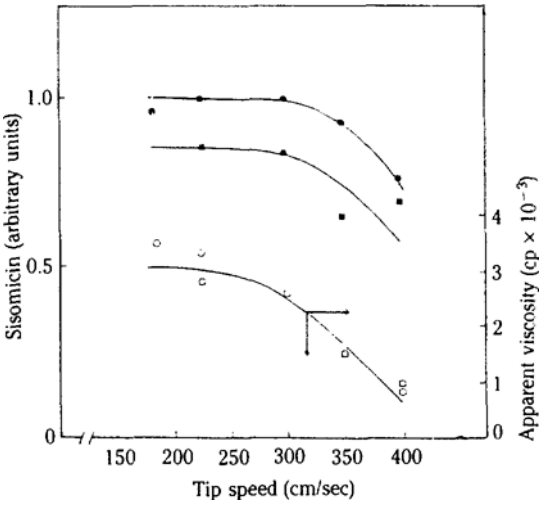

Fig. 3. Effect of impeller tip speed on sisomicin production with the impeller diameter of 14.2 cm.
■□ Disc turbine; ●○ Vaned disc.

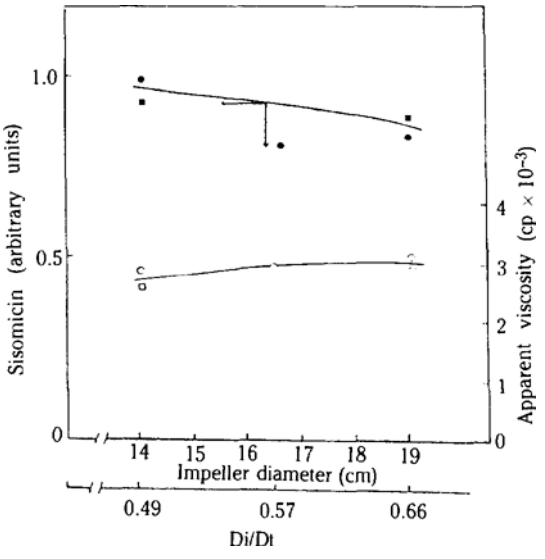

Fig. 4. Effect of impeller size on sisomicin production at the impeller tip speed of 225 cm/sec.
■□ Disc turbine: ●○ Vaned disc.

Fig. 4 shows the effect of impeller size (or Di/Dt) on sisomicin production and apparent viscosity at a fixed tip speed of 225 cm/sec. Although sisomicin biosynthesis was reduced by about 15% as the Di/Dt was increased from 0.49 to 0.66, the value of apparent viscosity was largely unchanged, indicating the importance of tip speed in the performance of sisomicin fermentation.

Relationship between apparent viscosity and sisomicin titre

As represented in Fig. 5 which shows the relationship between apparent viscosity and sisomicin formation, a linear relationship exists between the two variables. The regression equation is expressed as follows:

$$Y = 4.5 \times 10^{-4} X - 0.12 \quad (2)$$

where Y is the arbitrary sisomicin titre and X is the apparent viscosity.

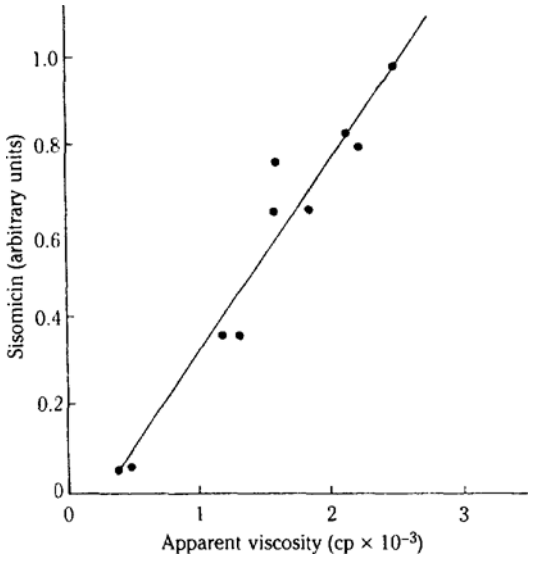

Fig. 5. Relationship between apparent viscosity and sisomicin formation.
$Y = 4.5 \times 10^{-4} X - 0.12$; $r = 0.9622$.

It is worthwhile to note that the value of K_{La} during sisomicin production phase was in the range 20-60hr^{-1}. The K_{La} value compares well with the data of 50-70hr^{-1} reported by Tuffile and Pinho [8] for viscous streptomycete fermentations.

Scale-up on the basis of constant tip speed

Scale-up on the basis of constant tip speed would be the method of choice when an organism sensitive to mechanical damage such as *M. inyoensis* was employed [13]:

$$Vi_1 = Vi_2 \quad (3)$$

where the subscripts 1 and 2 refer to small and large scales respectively. Combining eq (1) and (3) gives:

$$N_2 = N_1 (Di_1 / Di_2) \quad (4)$$

This equation was used for scale-up studies with 500-l pilot fermentor. It was found that the fermentation was complete after 130 hr cultivation and a similar level of the final sisomicin titre (110%) was obtained. This means that the sisomicin fermentation by *M. inyoensis* was successfully scaled-up on the basis of constant impeller tip speed.

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