Scale-up of Antibiotic Production by Streptomyces sulfonensis

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ABSTRACT
The present investigation is aimed to increase the diphenyl sulfone yield which was isolated from a new Streptomycte-S. sulfonensis. The fermentation studies were carried out in Bioflow IV New Brunswick Scientific Edison N.I, USA 10 litre fermenter. The fermentation was carried-out at 28°C with aeration of 3 litres/minute at 200rpm. The antibiotic production was monitored at regular intervals against B. subtilis by cup-plate method. The highest titre was (7980 µg/ml) was observed after 108 ~ 120 hours of incubation.

Key words: Diphenyl sulfone, S. sulfonensis, Fermenter, Cup-plate method, Antibiotic titre, Potency.

1. INTRODUCTION
The conversion of a laboratory procedure to an industrial process is termed as scale-up. It is well established in the field of biotechnology, that a process which works well at the laboratory scale may work poorly or not at all when first attempted at large scale. The transfer of a process from a shake-flask to a 5-litre fermenter is a scale-up step (Reisman, 1993). The scale-up problems may occur in the transfer of a research project or process through development into volume manufacturing.
Scale-up is a serious matter and involves careful planning and careful execution. The fact that scale-up is seldom simple indicates that the obviousness is not clear to everyone who should know better. Successful scale-up means that a process has been designed and built giving a predictable increase in production capacity (Reisman, 1993).

The scale-up importance consists of two words: time and money. Most emerging companies are short of both commodities. Any delay or problem in scale-up means loss of one and probably both needs. Selection of a fermenter is an early scale-up need. The appropriate scale is the 5 or 10 litre stirred, aerated vessel which has most of the characteristics of large fermenters. While simulations not perfect, such vessels have been shown to have predictive value, especially in the early years of developing a product. Five-litres is the smallest size of vessel that can be used for filamentous organisms without special problems arising (Hockenhull, 1979).

2. MATERIALS AND METHODS
The present fermentation studies were carried-out in Bioflow IV, New Brunswick Scientific, Edison, N.J., USA, 10 litre fermenter. The strain S. Sulfonensis was maintained on ATCC-172 medium (Cullen et al., 1987, Ellaiah, 1998).

i. Selection of producing culture and development of inoculum:
The inoculum for antibiotic production was prepared in a medium consists of the following composition: soybean meal 1%, corn steep solids 1%, glucose 0.5% and calcium carbonate 0.5% with pH 7.0 prior to sterilization.

A 500 ml Erlenmeyer flask contained 90 ml of inoculum medium was inoculated with 10 ml suspension of 7 day old culture of S. sulfonensis. All the flasks were incubated at 280°C on a rotary shaker for 72 hours.

ii. Production of diphenyl sulfone:
The production medium was prepared with the following ingredients: soy bean meal 1%, corn steep solids 0.5%, soluble starch 1%, dextrose 0.5%, calcium carbonate 0.7% and pH 7.2 before sterilization. The production medium was sterilized in situ by introducing steam into fermenter (10 litres) at 115°C for 20 minutes and contents were allowed to cool. The production medium was inoculated with 10% level of 72 hours old inoculum under carefully controlled conditions.

The fermentation was carried-out at 28°C with aeration of 3 litres/minute and agitation at 200 rpm. The contamination, antibiotic production, pH, foam, aeration and agitation were monitored at regular intervals of time. The antibiotic production was monitored against 16 hours old culture of B. subtilis by cup-plate method (Grove, 1955). The fermentation was run up to 168 hours and antibiotic titre was determined with structurally related pure compound. The results are presented in Table 1.

3. RESULTS
The scale-up of antibiotic production by S. sulfonensis was carried-out in 10 litre fermenter at 28°C with aeration 3 litres/minute and agitation at 200rpm. The antibiotic production was monitored at regular intervals against B. subtilis by cup-plate method. The time course of fermentation has shown in Table 1 which indicates that the optimum production was observed at 120 hours where as in shake-flask it was observed at 144 hours. The highest titre (7980 µg/ml) was observed after 108~120 hours of incubation

4. DISCUSSION
The antibiotic titre obtained in 10 litre fermenter was higher than that of the shake-flask at optimum period of fermentation. An improvement of antibiotic yield (27%) was achieved in the fermenter due to good aeration and agitation. It was also observed a remarkable reduction of optimum production period from 144 hours to 120 hours. During fermentation process it is evident that pH was raising from 7.2 to 8.5 due to metabolic reactions and it was controlled by the addition of hydrochloric acid. The excessive foam which was liberated due to agitation was also controlled by the addition of sterile mineral oil as anti-foaming agent.

5. CONCLUSIONS
Scale-up studies resulted in successful yields giving a remarkable increase in antibiotic production (27%).

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REFERENCES


Table 1
Time course of fermentation of diphenyl sulfone in Bioflow IV -10 litres fermenter

<table>
<thead>
<tr>
<th>Time course (hours)</th>
<th>Inhibition zone diameter (mm)</th>
<th>Antibiotic titre (Potency) µg/ml</th>
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<tbody>
<tr>
<td>0</td>
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<td>0</td>
</tr>
<tr>
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</tr>
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<tr>
<td>60</td>
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<tr>
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</tr>
<tr>
<td>132</td>
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</tr>
</tbody>
</table>

(*diluted broths: 1 in 5.5, 1 in 4 and 1 in 2.5 respectively)