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THE PEOPLE'S REPUBLIC OF CHINA

Technical report: Second visit of the consultant in fermentation antibiotics*

Prepared for the Government
of the People's Republic of China
by the United Nations Industrial Development Organization,
acting as executing agency for the United Nations Development Programme

Based on the work of G. Hanscomb, consultant in fermentation

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* This document has not been edited.

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ABSTRACT

The Consultant in Fermentation Antibiotics Spent a further period of almost four weeks at the Research Institute.

Repairs to both fermenters had been completed together with preliminary testing.

Specific instructions were provided to achieve continued successful operation of the fermenters. However, the unreliable nature of the services poses a risk of repeated failure.

Apart from minor difficulties the fermenters were successfully operated during the period of the visit.

A short programme of fermentation investigation was performed and further recommendations made for on-going research experimentation.

INTRODUCTION

This report covers the second visit of the Consultant in Fermentation Antibiotics to the Research Institute of the Guangzhou Pharmaceutical Factory. The work involved experimental fermentations of Penicillin V, using the B. Braun 20 litre fermenters, following replacement of the double-mechanical seals of each vessel.

Prior to the visit some relevant publications were obtained together with some useful .minor items of equipment.

During the visit purchasing information was obtained on some further items considered necessary for continued operation of the fermenters.

I arrived in Guangzhou on 14 September and departed on 8 October 1993.

I. REVIEW SINCE PREVIOUS VISIT

Due to the failure of the double-mechanical seals of each vessel no work had been performed, using the two fermenters, since my previous visit.
Modifications had been made to one shaker table to allow larger (1 litre) flasks to be used for generation of greater inoculum volumes.
A channel had been cut to restrict rainwater access into the Microbiology laboratory.
The Pilot Plant was not in operation during my stay and was undergoing maintenance.

II. REPAIR OF FERMENTERS

Arrangements for my visit had been made to coincide with the visit of a B. Braun engineer from Europe to replace the double-mechanical seals of each vessel. This, plus testing, to be carried out over a three day period to allow for full discussion with the engineer on the likely causes and how to minimise recurrence of seal failure. At the very last moment the arrangements were altered by B. Braun and in the event the work was completed in only two days, prior to my arrival, such that only two hours was available to discuss the many problems. My disappointment in these alterations to the pre-arranged plans, together with subsequent difficulties (see later), was expressed to the Assistant Manager (Ms. Cheung) in Hong Kong and copied to the Service Manager in Melsungen, Germany, (Hr. Kahlert).

A. Comments by B. Braun engineer relating to successful operation of equipment.

a) A pressure differential of 0.5 - 0.8 bar between the sterile condensate reservoir and the fermenter must be maintained at all times.
b) The supply air pressure was observed to fall to 1 bar - this is not high enough during sterilisation of the fermenter as the tank pressure could exceed that in the sterile condensate reservoir.
c) The supply steam pressure was observed to increase above 3 bar. This can lead to overheating after sterilisation causing excessive pressure build-up in the fermenter.
d) The sterile condensate reservoir is best sterilised during the fermenter sterilisation holding period in order to avoid thermal shock to the seal.
e) The steam filter to the sterile condensate reservoir had become blocked with rust from mild steel pipework fitted after the main steam supply filter. - [this pipe was replaced]
f) Three steam condensate pipes were all joined into a small, common outlet pipe causing a back-pressure on each system. - [a temporary modification was made to alleviate this but a further alteration is required].
g) A regular pressure test of the fermenters was recommended to test for leakage of O-ring seals.
h) If the fermenters are not used for 2 months or more then the agitator speed should be slowly increased. This is due to the creation of pressure spots on the carbon seats of the double-mechanical seals.
i) When power failure occurs, the digital control units should be switched off for a few minutes before switching on again.
j) Harder, and longer lasting, seats for the double-mechanical seals are available. However, a good working lifetime (2 years?) can be expected with careful operation of existing seats - [details of these were obtained but cost more than double the price of those in use].

B. Testing of the fermenters

The two fermenters were tested for sterile operation by batching a soluble, nutrient medium, sterilising, then incubating at 35deg. C. with airflow and low speed agitation (200 r.p.m.) for 48 hours.

The test was satisfactory with both vessels staying sterile.

III PENICILLIN V FERMENTATIONS

A programme of fermentation investigation was proposed and agreed to cover the period of my visit. This included a comparison of inoculum preparation methods and volumes, a test of an alternative antifoaming agent and automatic control of dissolved oxygen concentration via agitator speed.

Recommendations were also made for an on-going , medium term, research programme (Annex 1).

Four penicillin V fermentation batches were completed and two were still in operation when I departed. One fermentation was aborted due to failure of the cooling water supply leading to a high temperature.

Using a 1% inoculum level, from a single stage seed prepared in shaken flasks, pen. V titres were achieved up to 21,000 u/ml by iodometric assay [13,500 u/ml by HPLC]. This compared well with similar conditions used during my visit in May.

Using a 10% inoculum level, generated from a two stage seed in shaken flasks (more similar to the 1000 litre process) only produced up to 8,000 u/ml by iodometric assay [3,000 u/ml by HPLC]. In two fermentations using these conditions adequate levels of dissolved oxygen could not be maintained. Proposals were made for further investigations.

The test of an alternative antifoaming agent, polypropylene glycol PPG2000, provided good control of foaming both during sterilisation of the medium and during fermentation. There was, however, some indication of an effect on growth rate and commencement of pen. V accretion, therefore requiring further studies.

The automatic control of dissolved oxygen concentration linked to the agitator speed was not achieved by the end of my visit. Manual control was therefore used to obtain the desired control of oxygen levels. Contact with B. Braun may be required if further reference to the operating manuals is not successful.

IV ADDITIONAL OPERATING DIFFICULTIES ENCOUNTERED

a) When one fermentation had been lost , after the cooling water had been turned off elsewhere, a system of staff coverage was instituted, 24 hours a day, to monitor correct operation.

b) After replacement of the double-mechanical seals one vessel developed a "bearing noise" when operated at 550 r.p.m. This was mainly overcome by slackening the drive belt. However, significant loss of sterile condensate was also observed from the reservoir. It was necessary, therefore, to terminate the run prematurely to avoid risk of damage to the new seal. This problem appeared to be overcome by extended sterilisation of the seal as recommended by the B. Braun engineer.

c) Subsequently some loss of sterile condensate was also noticed from the other vessel. This may have occurred during a power failure (a regular event). Careful monitoring of condensate levels is essential.

d) After sterilisation of the fermenters there is a rapid loss of pressure from the vessels when the air exhaust valves open. This is due to the lack of a pressure control valve fitted to either

vessel. It is, therefore, necessary to exercise manual control over the pressure in the vessel to provide some control at this stage It had also been noticed on some occasions, that damage occured to the dissolved oxygen probes at this time.

e) The omission of pressure control valves, together with pressure transducers, plus an automatic pressure compensation system for the sterile condensate reservoirs, does not allow for the vessels to be run adequately under a positive pressure. This poses some limitations on oxygen supply levels.

f) A nitrogen gas cylinder is used to supply the required pressure to operate the pneumatic valves. On one occasion the cylinder ran out sooner than expected causing the air outlet valve to close. This created a pressure rise in the vessel which became greater than that on the sterile condensate reservoir, again putting the seal at risk.

g) The pH rose too high in one fermentation when the sugar feed failed. The problem was identified due to particulate rust contamination of the sugar feed solution which fouled the pump tubing. It was recommended that rust was not desirable as a medium component and, in the meantime, to filter the solution.

h) When foaming is not adequately controlled the air outlet filter becomes blocked causing pressure to rise in the fermenter - again putting the seal at risk. The use of packed glass-fibre was recommended in the outlet filter since this would reduce the pressure build up. It would also be very much cheaper to buy than the cartridge filters supplied. The cartridge filters are more necessary for the inlet air filtration.

V EQUIPMENT AND LITERATURE PROVIDED

Some useful items of equipment and publications were presented to staff at the Research Institute. These are itemised in Annex 2.

ANNEX 1

Recommendations made for further fermentation work

1. In general:- Obtain good growth data routinely on biomass production in order to determine specific growth rates and specific productivity rates.

Compare such data obtained from 20 litre, 1000 litre and higher scale when possible.

Apply methods of modelling the process, as far as possible, from the references provided. This will be most important, if the process is scaled up to production scale, in order to optimise yields.

2. Complete assessment of antifoam agent PPG2000
   - test at different levels
   - test for toxicity at different levels in shaken flasks
3. Obtain and test other antifoam agents as available.
4. Compare other vegetable oils e.g. Peanut, Soybean, Cottonseed, Sunflower, Corn and any others available.
5. When consistent results are obtained test cornsteep liquor in fermenters with close monitoring of HPLC results. Note:- HPLC results are always important to check what is really happening to the pen. V yields.
6. Test inoculum, from 500 litre fermenter in Pilot Plant, in 20 litre fermenters at different concentrations (1 - 10%). If improved morphology in pellets is obtained in the final stage, together with improved titres of pen. V, then examine alternative flask preparation of two stage inoculum. Important - does the dissolved oxygen level fall below 30% of saturation when a 10% inoculum is used from the 500 litre fermenter? - i.e. when the pellets are " tight ".
7. Test effect of acetic acid added to the sugar feed. - see references.

ANNEX 2

Equipment and Literature provided

1. Four stainless steel inoculum needles were donated - similar needles are best made in-house or obtained locally to a similar pattern.
2. Two sets of hexagonal keys (metric) 1.5 - 10 mm
3. Two copies of "Practise and Theory of pH Measurement" by Ingold (manufacturers).
   Also reprints were obtained from B. Braun in Hong Kong of similar literature for pO_2 and pH probes.
4. A copy of a maintenance schedule for B. Braun fermenters was also obtained from Hong Kong
5. Copies of the following reprints -
   a) "The influence of acetic acid on penicillin production" Jensen et al. Eur. J. Appl. Microb. Biotechnol. (1981) 13 29-33
   b) "Process Engineering Investigations of Penicillin Production" Konig et al. Ibid.(1981) 12 205-211
   c) "Penicillin Production - Biotechnology at its Best" van der Beck and Roels Antonie von Leeuwenhoek (1984) 50 625-639
6. Two references were also recommended to obtain as soon as possible:-
   a) "Application of Balancing Methods in Modelling the Penicillin Fermentation" Heijnen et al. Biotechnol. Bioeng. (1979) 21 2175-2201
   b) "The Penicillins:- Properties, Biosynthesis and Fermentation" Hersbach et al. (1984) 45-140 *in* E.J Vandamme (ed) Biotechnology of Industrial Antibiotics, Drugs and the Pharmaceutical Sciences, vol. 22 - Marcel Dekker. Inc. New York.

BACKSTOPPING OFFICER'S TECHNICAL COMMENTS

In his report, Mr. G. Hanscomb gave a very accurate snapshot of the current situation at the Microbiological Laboratory of the Guangzhou Pharmaceutical Factory and Research Institute. His assessment was very important since his visit was the last international expert's trip before the terminal Tripartite Review.

He reported that apart from minor difficulties, the Braun's fermenters were successfully operated during the period of his visit. He gave a very detailed inventory of these difficulties and specific recommendations for further uninterrupted operation. These recommendations covered instructions to avoid respected failures. He performed commission and trial runs of the fermenters successfully and gave further recommendations for on-going research programmes.

The backstopping officer agrees with the Chief Technical Adviser, Mr. F.R. Batchelor that the Braun's fermenters can be used for optimization of fermentation parameters of other activities, in addition to Penicillin V. Since the antibiotics product mix of the Guangzhou Pharmaceutical Factory is becoming old, and therefore, within a couple of years, it might become obsolete even in the People's Republic of China, one of the top priorities of the Microbiological Laboratory could be to develop the fermentation technologies of new types of antibiotics.

As an alternative suggestion, in order to make the Microbiological Laboratory financially sustainable, it could be developed as a regional training centre in fermentation biotechnology. Workshops, seminars and training courses could be held regularly. The trainees should be charged a reasonable fee for their participation. This fee could be used to make further investment to keep the Laboratory technically maintained, updated or even expanded.