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THE PEOPLE'S REPUBLIC OF CHINA

Technical report: Visit to B. Braun Biotech International 11th-15th May, 1992 during the Training Programme for the Chinese team from Guangzhou Pharmaceutical Factory*

Prepared for the Government
of the People's Republic of China
by the United Nations Industrial Development Organization,
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Based on the work of M. Call, expert in industrial microbiology

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United Nations Industrial Development Organization
Vienna

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* This document has not been edited.

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Background information to B.Braun and the training programme:

B. Braun has several sites in Melsungen. Their Biotech International research, development and manufacturing facilities are based at Schwarzenberger Weg 73-79, Post fach/POB 120, D.3508, Melsungen, Germany. Melsungen is about 2½ hours by rail (3 trains) north of Frankfurt airport. My visit was arranged with Mr. Gerlach (Tel: from UK 010 49 5661 71 - 3880, secretary Mrs. Seitz ex. 3766) who was responsible for the training programme for the Chinese visitors.

A copy of the provisional programme was received from UNIDO Headquarters via Dr. Batchelor, before the visit. I was given a somewhat revised programme when I arrived, a copy of which is attached. I was unable to see Mr. Gerlach as he was visiting China for a trade exhibition and conference in Beijing. However, I understand the Chinese visitors did meet him when they arrived the previous week.

I was met by Dr. Elizabeth Fraune, Dipl Ing, from Research & Development, who conducted a large part of the training and also looked after all the general arrangements which were very satisfactory. Dr. Fraune has extensive experience of culturing cells in bio reactors and has published on the production of monoclonal antibodies in such vessels with on-line monitoring. She was proficient in English and had attended a course on mammalian cell culture run by Prof. Spear at the University of Surrey, U.K.

The Chinese Team:

The Chinese team from the Guangzhou Pharmaceutical research laboratories consisted of Mr.Feng Yong Hong (director), Mr. Yang Jian Hua (electronics/maintenance) and Mr. Zu Ding Xing (fermentation technology). They had arrived the previous week and had been introduced to the facilities and products of the Company and had started training on the mechanical design of the bio reactors, setting them up for a run, calibrating electrodes, etc.

Only Mr. Zu had an understanding of English; his pronounciation improved much while we were there and his vocabulary seemed quite extensive. He translated for the other two members of the team, whose knowledge of English was minimal. In spite of this, Dr. Fraune thought that they were understanding things, particularly the mechanical and electronic aspects. Mr. Zu showed particular interest in the control systems; I think he will be running the two computer controlled 20 l fermenters. These have been delivered but not yet installed.

Computer Management Systems for Fermenters:

On Tuesday, 12th May, Mr. Ralf Brune, Dipl. Ing., who is responsible for automation, spent the morning describing the computer management systems (called the Micro MFCS - multi fermenter control system) and the afternoon providing demonstrations in the laboratory with hands-on experience for the Chinese team. A copy of the applications manual for the Micro MFCS has been given to me. Also copies of all manuals have been given to the Chinese. The Micro MFCS appears to be a very comprehensive system for the automatic management of the fermenter vessels according to preset parameters such as the sterilisation cycle, dissolved oxygen and pH.

The computer controls addition of acid or base (for pH), stirring speed and airflow (for dissolved O_2) and antifoam addition (via a foam detection probe). With all such automated systems there is a need for visual inspection and manual checks. This was well illustrated when, during the sterilisation cycle, the temperature would not quite reach 121° C which had been set for the start of the 30 minute sterilization cycle. It turned out that a cooling water valve had stuck slightly open (quick adjustment fixed it) and was cooling the fermenter at the same time as steam was trying to heat it - a salutory lesson I hope for the Chinese. The Micro MFCS system also records all fermentation parameters, will process the data (time averaging, ratios, etc.) and when called for will present the data in a variety of forms (tables, histograms or graphs).

Design and Production Facilities at B.Braun:

During part of the afternoon Dr. Fraune showed me around the design and production facilities, which are concerned not only with laboratory and pilot plant fermenters for mammalian cells and micro organisms, but also with a range of other equipment such as laboratory shakers, intravenous infusion pumps and kidney dialysis machines. (Intravenous infusion fluids are manufactured in a separate factory in Melsungen). Many pieces of equipment were seen undergoing their pre-delivery tests, including fermenters. I was impressed by the excellent design and manufacturing facilities. The various buildings, including laboratories, were clean, tidy and modern, and should have created some kind of reference standard for which the Chinese could aim. The staff restaurant was

Practical Work in Laboratory & Pilot Plant:

Wednesday, 13th May was spent on practical laboratory and pilot plant scale work with fermenters. One two litre MCD fermenter containing foetal calf serum was inoculated with hybridoma cells (ATCC HB 124) making I_gG The sterile transfer technique was demonstrated. Cell counts (dead and alive) in the inoculum and in the culture vessel after inoculation, were determined using a vital stain with a haemocytometer slide and microscope. The automated control systems on the culture vessel were set up and samples taken for automated electrode analysis of glucose and lactate (YSI equipment). Entry of this off line data into the computer was demonstrated. The use of mammalian cells and the special bio reactor design (air diffusion via permeable PTFE tubing on a rotating mount for gentle agitation) was of limited direct value to the Chinese but it did provide experience in handling the control systems such as the Micro MFCS.

The rest of this day was spent on the practical work of setting up a Biostat^R UD fermenter (20 l). This involved every aspect from preparation of the culture medium (glucose 4%, yeast extract 3.5%, NaCl 0.6%, $MgSO_4$ $7H_2O$ 0.2% w/v, pH adjusted to 8.0 with caustic soda solution) to sterilisation in the fermenter using the computer controlled system. Addition bottles were prepared for acid (4N HCl), alkali (4N NaOH) and antifoam (Desmophen 3600 - a polyether based on propylene oxide, mol. wt. approx 2000). The inoculum of E.coli ATCC 11229 was prepared by bulking the overnight growth of cells in shaken flask culture. Several sterile techniques were practised, for example, inoculation of flasks in a laminar flow cabinet, using a gas torch for making connections to the bio reactor (the Chinese nearly set themselves on fire!) and sampling cells via a septum in a loop tube (mammalian cells) or via a sample port (E.coli cells).

Relevance of Training Programme:

The work with the E.coli fermentation (growth of cells) seemed more relevant to the requirements of the Chinese but I urged Dr. Fraune to consider adding a fungus such as Penicillium chrysogenum to their range of organisms, as its growth characteristics (e.g. forming pellets or lumps) require different handling procedures. Use of such an organism would have been highly relevant to the Guangzhou penicillin V project. Also, this fermentation requires the addition of a side chain precursor (phenoxyacetate) which in turn means that a fourth addition vessel and pump are needed; a fourth pump was said to have been ordered. Apparently B. Braun has no experience of handling fungi, although their fermenters have been used for these organisms by Beecham.

I spent some time talking to Dr. Fraune about the details of air filtration and antifoams, as these could be important issues in China. PALL filters are used on the air supply from the compressor. They are fairly expensive but last a long time. The Desmophen antifoam mentioned above may not be suitable for use in a penicillin fermentation.

Equipment Available:

There was a full range of equipment in the fermentation pilot plant, which had a mezzanine floor, i.e. fermenters up to 300 l with associated centrifuges. All service pipework was neatly laid out around the walls and there were trolleys loaded with all the connectors, seals, plugs, septa, sterile components, etc. that would be required. Apparently, because of the capital locked up in the 300 l fermenter it was going to be sold off.

Drawings & Spares:

I have obtained drawings of the fermenters and pipework layout being supplied to the Guangzhou Pharmaceutical laboratories. I understand that spares sufficient for two years of operation are being made available.

Meeting with Managing Director:

On Thursday, 14th May I met the Managing Director of B.Braun Biotech International, Dr. W. Kuhlmann. We had a general discussion about developments in the biotechnology field, the UNIDO Project at the Guangzhou Pharmaceutical Factory, recent organisational changes in B. Braun and about their product range. The company is a relatively recently established joint venture arrangement between B. Braun Melsungen AG, Kraftanlagen Aktiengesellschaft and Diessel GmbH & Co. However, in future the company is to be known as B. Braun Medical.

B. Braun's Product Range & Research:

Dr. Fraune took me through the product literature of the company and I have copies of brochures which are relevant to microbial fermentation and enzyme processes. Their recent research into on-line assay of antibody formation and glucose utilisation was demonstrated using a filtration unit in a loop from a bio reactor (presented with copy of publication).

Training on the 20 l Fermenters of the design being supplied to China:

During Thursday the Chinese visitors were given training in the inoculation of the 20 l fermenter, calibration of the addition pumps and control of the fermentation via on-line automatic control. The dissolved oxygen level was set at 40% of saturation and the agitator speed observed to gradually increase to compensate for depletion of oxygen. Using the Micro MFCS, plots were obtained of the progress of the various measured parameters during the growth of the culture.

Remainder of Training Programme: Visit to Westphalia Co:

For the rest of the training programme (for which I was not going to be present) it was planned to cover servicing of bio reactors, study of a continuous culture biostat, demonstration of freeze drying, sterilization and cleaning of equipment.

Mr. Feng expressed interest in visiting the Westphalia centrifuge company as he said he had questions he wanted to ask them. Dr. Fraune made arrangements for them to visit the firm on Monday, 18th May. I travelled back to England on Friday, 15th May.

Letter to Dr. Fraune:

I have written to Dr. Fraune, thanking her for her efforts and hospitality (copy attached).

Dr.Martin Cole

25th May, 1992

ANNEX 1

Training Program

Duration	:	04.05. - 30.05.1992
Participants		
Guangzhou Pharmaceutical Factory	:	Mr. Feng, Yong-llong Mr. Yang, Jian-Hua Mr. Zu, Ding-Xing
Unido Representative	:	Dr. Cole (11.05.-15.05.1992)

Coordinator of Training Program :
Dr. Fraune - head of cell culture research laboratory

04.05.92	:	Arrival at Melsungen	
05.05.92	:	Welcome of the participants	Mr. Gerlach Dr. Fraune
		Introduction of the training program	Dr. Fraune
		Introduction to B. Braun Biotech product range	Mr. Majer Mr. Grebe Dr. Asche Mr. Kappel Dr. Fenge
		Tour to B. Braun Biotech facilities	Mr. Gerlach
06.05.92	:	Mechanical design of bioreactors	Mr. Rietschel
07.05.92	:	Set up, sterilization, media filtration of Biostat MCD for mammalian cells	Dr. Fraune
08.05.92	:	- Calibration of electrodes MCD Inoculum preparation MCD off-line data analysis - Set-up Biostat UD	Dr. Fraune
09./10.05.92		Weekend	

Training Program

11.05.92	:	Electronics Measurement and control systems Digital Control Unit (DCU)	Mr. Kappel
12.05.92	:	Micro MFCS system	Mr. Kappel
13.05.92	:	- Sterilization, calibration of electrodes, medium preparation, inoculum preparation, sterilization of double mechanical seal Biostat UD	Dr. Fraune
		- inoculum and sampling Biostat MCD	
14.05.92	:	- E-coli fermentation Biostat UD including sampling and process control	Dr. Fraune
		- sampling and process control Biostat MCD	Dr. Fraune
15.05.92	:	- set-up continuous culture Biostat MCD including sampling	Dr. Fraune
		- demonstration freeze drying equipment	
		- sampling, sterilization, cleaning procedure Biostat UD	
16./17.05.92		Weekend	
18.05.92	:	Technical service of bioreactors	
19.05.92	:	Departure to Frankfurt	

COPY

MARTIN COLE CONSULTANCY

ANNEX 2

Martin Cole, B.Sc., Ph.D., D.Sc., C.Biol, F.I.Biol.
CONSULTANT IN MICROBIOLOGY & BIOCHEMISTRY
(PHARMACEUTICAL INDUSTRY)

18th May, 1992

Dr. E. Fraune,
B. Braun Biotech International GmbH,
Schwarzenberger Weg 73-79,
Postfach/P.O.B. 120,
D. 3508 Melsungen,
Germany.

Dear Dr. Fraune,

I am writing to thank you and your colleagues, Ralf Brune and Sabrina Saltzmann for looking after our Chinese visitors and myself so well. Given the language problems you did an excellent job.

I found the visit most informative and thoroughly enjoyed it; I am sure the Chinese team benefitted enormously. I was impressed by the high quality of your equipment and laboratories, and look forward to seeing the 20 l Biostats installed in China. Thank you for the full range of product literature and for taking me on a tour of the production facilities.

As I mentioned to you, I think it would be good for you to obtain some experience of handling the cultivation of a fungus, such as the pencillin-producing strain of Penicillium chrysogenum, in one of your Biostat fermenters. For such a fermentation a satisfactory way of removing the inoculum line would have to be worked out.

Thank you for introducing me to Dr. Kuhlmann, and also for the most enjoyable dinner in the square at Melsungen. You will be pleased to hear that my journey home went very smoothly.

Best wishes for the future,

Yours sincerely,

Dr. Martin Cole

ANNEX 3

Backstopping Officer's Technical Comments on Dr. Cole's Report on his Visit to B. Braun

The expert's mission was beneficial to the trainees and served to improve the planning of the activities concerning the fermentors and computer systems in Guangzhou.

In addition, the findings related to fermentor quality, the training programme and the whole production system organization at B. Braun are satisfactory.

Solutions should be prepared in advance for the precursor feeding and for the quality of the sterile air supply to the fermentors. Practice with Penicillin should be given during commissioning trials in Guangzhou.