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THE PEOPLE'S REPUBLIC OF CHINA

Technical report: Fourth mission of the CTA together with report on visit to Westfalia - separator, 7-8 September 1992*

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* This document has not been edited.

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INTRODUCTION

The original objectives of this visit were to accompany Dr Cole and the new Technical Advisor Mr Bird to Guangzhou and together help set up and organise the operation of the new equipment in the new building which had been reported to be finished and ready. We would then discuss and plan the work for the next period. Mr Bird would also run seminars on quantitative methods and add to the HPLC training of the staff by dealing with the specific methods required to measure the substances of interest to the project namely - penicillin V, p-hydroxy penicillin V, 6APA, ampicillin amoxycillin and the penicilloic acids of all of them. In addition specific methods for the side-chains would be included. Just prior to the visit the CTA was asked to visit Beijing for a meeting with UNDP and CICETE to discuss and agree the future programme for the project following the unfortunate events which which occurred during the fellowship training at Braun's in Germany. Following the discovery that the laboratory building was not completed the agenda was expanded to include discussions on this matter.

Part of the first morning was taken up by introducing Mr Bird, the new TA, to the staff at GPF and also with a meeting with the newly appointed Factory Director and the new NPD. During discussions on the programme we were still given the clear impression that the building was completed. Only when it came to the installation of the HPLC equipment was there a suggestion that the room might not be ready for a few days. We were then taken to see the building. In no way could the building be described as completed and we were very surprised to find the building far from complete. The difficulties may well be partly due to a language problem and that what was meant was that the main structure was complete rather than the building was ready to be occupied.

From the point of view of the experts we must ensure that the building is ready for use and equipment can be or preferably has been installed before any future visits. One way to help avoid the problem in future would be for a more fluent English speaking person to be available in the Factory full time rather than rely on Mr Cai who is based in GPGC offices and only visits the factory when the advisers are present.

The CTA understands a futher 500,000 YUAN has now been allocated to complete the building and Mr Bird reported work had commenced when the CTA was in Beijing.

This building delay is the prime cause of all delays to the project. It was originally scheduled to be completed in week 6 of the project (Nov 1990) but the CTA revised this estimate in Dec 90 after his first two visits to week 13 (ie May 91). At best it will be almost two years behind schedule and this has had serious effects on all other activities. While is has been possible to carry out some training and make progress with some parts of the project using the old buildings, it has not been possible to start some activities at all. At the meeting with UNDP and CICETE in Beijing on July 13th 1992 it has been agreed to continue the project until the Tripartite meeting Dec 1992. Unless significant progress has been made then the CTA may have to recommend the discontinuation of the project. A separate report on the meeting in Beijing has been provided to UNIDO.

Braun visit

The problems which had arisen as a result of fellowship personnel leaving Braun before the end of the training programme to visit Westfalia were discussed. The CTA stressed how much damage this had had on the goodwill of companies with whom it was hoped to arrange fellowship training. Both Braun and Westfalia were very upset by the incident and would be unlikely to welcome further visitors arranged by UNIDO. It was essential a letter of apology was written. Apparently Mr Feng (the previous Factory Director and NPD) had indicated he was too busy in his new post and had asked Mr Zu who had got lost in Berlin to write. The CTA explained that it was not sufficient for a junior person to write such a letter, it had to be someone of Director level to be of any value in repairing the situation. The CTA expressed his own concern about the incident. The visitors not taken the advice of Braun or Dr Cole on the difficulties of travelling between the various places in Germany. While the CTA can understand the desire to have the most up-to-date equipment he is still of the opinion that the whole broth extraction should be regarded as experimental rather than a technology in general use. It still seems that Huabei are continuing to experience difficulties and there is no doubt that the technology is not universally applicable to all strains of the pennicillin producing organism.

In order to avoid additional problems with Braun the CTA and Dr Cole also took action to stop the Braun engineers arriving to install the fermenters. Although the building was not going to be ready GPF had not taken any action to postpone the installation visit scheduled for two weeks hence. Both UNDP Beijing and UNIDO Vienna were alerted to ensure the visit was postponed.

Since the visit to Guangzhou the CTA has had futher discussions with Mrs Valdes and with Westfalia representatives in both the UK and Germany and

has arranged to visit Westfalia in early September to discuss the suitability of a new small centrifuge which may allow pilot scale whole broth extraction. Since it is also capable of simple liquid/liquid separation it may well be a suitable piece of equipment for the Gaungzhou project. Meanwhile Mrs Valdes is trying to arrange the necessary funds to purchase the equipment if it seems satisfactory.

Analytical Matters

The CTA took part in both Mr. Bird's presentations on analytical matters and highlighted these parts of Mr. Bird's presentation which were of particular importance and relevance to the project in hand.

Since the new building was not completed GPF were asked to find a temporary room so that the HPLC could be installed and arrangements were then made with Waters (HongKong) to set up the equipment so that Mr. Bird would be able to begin the main objective of his visit.

There were one or two important issues which then became clear. First the Waters training provided in HongKong had not been "hands on" with the result that Mr. Xiao was not as familiar with the operation of the software as had been hoped. Mr. Bird managed to arrange some further assistance from Waters but the CTA will take up this matter with Waters Headquarters in the USA.

The CTA was given the impression that some members of GPF had expected Mr. Bird to be able to sort out the use of the software for integrating the peak area (Baseline 810) and felt it necessary to explain that though Mr. Bird was an expert in analysis he could not be expected to know the details of every software programme and on every HPLC. The other important issue was the need for some additional pieces of equipment to protect the column and possibly also equipment for producing water of adequate quality. These matters are fully dealt with in Mr. Bird's report, which the CTA fully supports. It should also be stressed that HPLC should be looked upon as a routine every day tool and this would involve a substantial running cost.

While following the results of the fermentations the CTA noticed a number of items in the use of the iodometric assay which gave him cause for concern and as a result Mr. Bird arranged to watch assays being carried out. The results of these observations are dealt with in Mr. Bird's report, and should improve both accuracy and reproductivity of these assays.

Also at the request of the CTA Mr. Bird dealt with the use of the p-dimethylamino benzaldehyde method for measuring 6-APA. The CTA has been concerned with the use of this assay which he did not believe was sufficiently specific for 6-APA - a view supported by Mr. Bird. It is not

capable of distinguishing between 6-APA and some of its degradation products and will therefore tend to overestimate 6-APA.

Fermentation

While looking at the latest assay results of the fermentation on which recovery was to be carried out the CTA became aware of a fall in titre. On obtaining the very latest results the fall was even more marked.

The CTA was concerned that these data did not appear to be routinely passed on to the person in charge of recovery. As a matter of principle when a titre falls as fast as this one was, a complete investigation should be carried out. On no account should any attempt be made to isolate the penicillin. Not only will it prove difficult, if not impossible, but if the fermentation had been contaminated with a B lactamase then all the extraction equipment would also have been contaminated. If this occurs in future the fermentation should be sterilised by steam in the fermenter and destroyed.

The problems subsequently encountered in trying to extract the penicillin clearly demonstrated the points made by the CTA. Filtration was extremely difficult and slow and was atypical throughout serving only to underline the fact.

The cause of the problem was finally tracked down by Dr. Cole and the CTA when talking with Mr. Bird over dinner, when Mr. Bird referred to a power cut of some 10 minutes which Dr. Cole and the CTA had been unaware of. This clearly co-incided with the start of the problem in the fermenter. The lack of oxygen had caused the fermentation to become anaerobic and to begin to autolyse. Penicillin production had ceased and the longer the fermentation was kept running the more penicillin was destroyed. Further autolysis had made the broth very difficult to filter.

This occurence serves to underline the need for all separate groups to work together and to transfer information quickly. It is essential to be in possession of all available data in order to solve problems, culture maintenance - fermentation - recovery and analytical groups must work together as a single team.

Penicillin V Recovery

The fermentation run, so that the CTA could follow a batch through, was atypical as described above. However, the CTA did have the opportunity to spend considerable time going through the extraction plant both before the attempted isolation and during it. There were a number of important issues which came out of this.

Firstly, the whole process of isolation of the penicillin V was taking too long. One step was finished before the next was started and as a result the isolation which should have taken no more than an hour or so was taking over 24 hours, so it was not surprising recovery yields were low.

A number of suggestions were made and these were gone through in detail with the staff concerned. The flow chart with comments was written up and given to GPF at the time, but a copy is attached to this report. If these points are dealt with, extraction yields should be very much higher.

The second important point relates to the amount of penicillin to be recovered. When the broth is filtered for assay it must be remembered that this liquid is not the fermentation broth - the latter contains the mycelium and other solids which contribute substantially to the total volume in the fermenter. The total penicillin is not the titre multiplied by the total volume of liquid in the fermenter but by a somewhat smaller figure. Ways of getting a better estimate of the total penicillin content were discussed. Perhaps the simplest is to dilute the broth by a factor of ten before centrifuging or filtering to remove the solids - in this way the volume of solids will be small enough to be ignored (though it will still contribute 2-3% of the volume). An alternative would be to filter a small vacuum filter and wash with water perhaps up to 50% of the total volume. This would lead to less dilution but would take more time.

PENICILLIN V ISOLATION

Suggestions based on Precipitation Route

PROCESS STEP (Present Timing)	COMMENTS
FERMENTATION HARVEST (30 mins) HOLDING TANK	Why not adjust pH in Fermenter. Agitation there will allow this to be done quickly (10 mins) also pH control there should be by pH meter. This will allow holding tank to become surge tank and commence filtering right away.
pH 4.5 (4.0 hrs) FILTER	Should start as soon as harvesting starts not sequentially this will minimise holding time. Use of Flocculating Agent could be investigated to see if it improves filtration time.
SURGE TANK HOLDING TANK (Not Tiled Pit) pH 2.5 (30 mins) FILTER (3.0 hrs)	Could use a small Surge Tank to adjust pH continuously to feed to larger tank which feeds filter. Do not wait till all volume filtered before taking pH down. Start filtering off the Penicillin V as soon as possible, building in short standing time. May be better to agitate vessel to to keep precipitate in uniform suspension to aid filtration. N. B. Done at ambient temp. to prevent oil formation.
REDISSOLVE IN ACETONE FILTER	Should check whether from this stage if possible to use low temperature - about 5 C. If no problem with oil formation on lab scale then cold from here on assuming no problem of condensation on product - ought to be in air conditioned room. Some charcoal (carbon) may be desirable
PRECIPITATE WITH POTASSIUM ACETATE in ETHYL ALCOHOL	Use mechanical stirrer not a stick. Adequate mixing desirable.
FILTER AND WASH BUTANOL (20 mins) DRY	This step can be carried out in any suitable equipment used for collecting crystals. Basket centrifuge probably the best but what matters is that it can be filtered and washed properly and quickly. Then dried properly.

OVERALL COMMENTS

No process of this nature can ever operate as well on a small scale as it can on plant scale. The objective should be to minimise the time taken for the overall process and particularly the time when the penicillin is in solution - whether water or solvent - and also when the material is acidic. Every attempt should be made to make the process as streamlined and continuous as possible. Operations should be carried out simultaneously not sequentially. More vessels are needed preferably of stainless steel though glass-lined or plastic is also acceptable.

The unit operations and the overall time takes far too long - shortened times will greatly increase recovery yield

Any type of filter that is readily available, suitable for the scale and allows proper washing of the product may be used for filtering the crystals.

Valid mass balances for all stages should be prepared (some may be difficult and may require discussions with experts) measuring the Penicillin V in both the rich and waste streams so on my next visit we can identify where the major losses occur and take action to minimise these losses.

PENICILLIN V ISOLATION BY SOLVENT EXTRACTION ROUTE

Whole operation can now be 5 - 10 C because no problem of oiling.

HARVEST	Cool to 5 - 10 C Heat Exchanger - just cooling coils in tank will take too long.
SURGE TANK	Acidify (pH 4.5) to aid filtration and/or use a flocculating agent.
FILTER	May still use existing filter press - on a 10,000 scale rotary drum filtration would be quicker and would certainly be used on plant.
CLARIFY	Sparkler filter may be required if emulsion problmems occur normally unnecessary.
ACIDIFY pH 2.0 - 2.2	This should be done by in line addition of H_2SO Immediately prior to mixing with solvent. On Pilot Scale may need to use small surge tank with automatic pH control - Pen V not too senstive to acid - 1/2 life is about 20 hours.
MIX WITH SOLVENT MIBK OR BUTYLACETATE 1/2 - 1/3 VOLUME	
SEPARATE USING LIQUID/LIQUID CONTINUOUS CENTRIFUGE OF SUITABLE SIZE	
COLLECT RICH SOLVENT	
(OPTIONAL CHARCOAL AND FILTER)	
BACK EXTRACT INTO BUFFER (POTASSIUM ACETATE FOR EXAMPLE) FINAL pH 7.0 - 7.5	
SEPARTE USING LIQUID/ LUQUID CENTRIFUGE OF SUITABLE SCALE	
ACIDIFY TO pH 2.0 - 2.2 TAKE BACK INTO FRESH SOLVENT USING SAY 1/2 VOLUME MIBK OR BUAC	

SEPARATE USING SUITABLE
SIZE LIQUID/LIQUID CENTRIFUGE
- AND MAYBE DEWATERED IF
NECESSARY

CRYSTALLISE BY USING
POTASSIUM ACETATE AS
PRESENT PROCESS

FILTER AS PRESENT PROCESS

DRY

The extration process would normally operate continuously on a plant perhaps taking less than one hour from broth to solid penicillin dried and bagged - on a pilot plant this will not be possible.

However, all steps should be taken to minimise the time and where the process and equipment allows you should try to operate unit processes simultaneously rather than sequentially. Again mass balances will allow us to see where material is lost and take necessary action to improve yields.

Generally the lower temperature and use of solvent would give perhaps 4 - 5 % greater yield. The benefit may well be offset by the greater volume of solvent used and problems with large columes of waste aqueous phase saturate with solvent.

THERE IS NO ONE BEST PROCESS
Virtually every Company I know operates a different process using different equipment. They all get yields between 80 and 90% using the principles described above.

MEETING WITH WESTFALIA-SEPARATOR OELDE - 7TH - 8TH SEPTEMBER 1992

Dr K H Brunner
Mr E Mesares
Dr F R Batchelor

The main purpose of this visit was to discuss in detail the suitability of certain centrifuge models for the Guangzhou project, particularly the SA I. The conclusion of the CTA following these discussions was that such a centrifuge should be recommended for purchase.

While not a whole broth extractor centrifuge such as used by Beecheam or in Huabei, the SA I can be set up to simulate whole broth extractions on a small scale. It would thus give an indication of the potential for this method of penicillin V recovery on a pilot scale. When set up for this operation it can be used for whole broth if the broth is diluted to bring the suspended solids level down to 4%. Furthermore it can easily be changed over in a matter of minutes to conventional liquid/liquid separation, so could be used for a further downstream purification or for normal solvent extraction after removal of the mycelium. In fact it would be ideal for this as any small quantities of solid coming out of solution at the extraction pH 2.2 would not pose a problem.

The capacity of the SA I in nominally 500 litres per hour total liquid ie broth and solvent but would usually be run at less than half this. If the funds were available Westfalia staff and the CTA would prefer the next largest unit, because the whole broth will need to be diluted to simulate whole broth extraction but it is not essential.

The question of training was discussed and Westfalia would accept 2 members of the Guangzhou staff for training in Germany. The CTA was concerned that such training would be for only a few days and may not justify the travelling time and cost and asked if any other educational visits could be linked in. I was told that Westfalia could arrange for perhaps a week at the GBF Institute (I believe it is in Munich). This is 90% state owned and accepts overseas people for training free of charge. The institute also holds courses every year of up to 6 weeks duration. Last years was an extraction of antibiotics - this year's was on fermentation. Mr Mesares is arranging to send the subject matter and application forms for 1993. It is suggested Mrs Valdes also follows this up through the proper Unido channels and by direct discussion with Mr Mesares.

The Westfalia staff confirmed the CTA's opinions of the suitability of the smallest whole broth extraction unit for a the Guangzhou project. The smallest centrifuge produced for whole broth extraction, two of which had been included in the orginal equipment schedule for Guangzhou prepared before the CTA had been involved, was the CA226-290. These centrifuges

have a throughput of 1 - 1.5m3 per hour of broth. Since the largest fermenter available at Guangzhou is 1m3 holding at most 800 litres of fermentation broth the maximum run would have been only about 30 minutes, too short to enable valid experiments to be carried out. It would have been very difficult to bring the system into proper balance and runs would have had to be infrequent while waiting for another fermenter to be harvested. The minimum desirable fermenter size to match the capacity of the equipment would be perhaps 5m3 and it would be desirable to have 4 or 5 of these.

The additional problem was that the CA 226 - 290 currently costs around DM300,000 and would have used up most of the UNIDO equipment budget.

The alternative solution of purchasing an SAI centrifuge not only allows a form of whole broth extraction to be tried but the decision allows the equipment to be easily altered so conventional liquid - liquid separation can also be carried out.

The CTA recommends an the purchase of SA I or (if funds are available) the next largest model for the Guangzhou project.

B.S.O. Technical Comments on the Reports of Dr. Batchelor (11-01), Dr. Cole (11-02) and Dr. Bird (11-04)

In their reports, the experts present extensive information and sound recommendations for the continuation of the experimental work in GPF.

The initial breakthrough for installation and commissioning of the HPLC and the clarification of the characteristics and problems of every analytical method used or available to be used (potentially useful), proved to be essential for the whole advance of the research and final results of the project.

Dr. Bird's clear recommendations for analytical working procedures, GLP, record-keeping and additional laboratory equipment/items required, are most valuable and should be closely followed.

The supply of reference standards and literature is highly appreciated.

The methods recommended by Dr. Cole for strain improvement and for the conduction of fermentation have to be tried from now on. As strongly recommended, it is essential to maintain a good level of communication between the project team to keep detailed records of the experiments' conditions and results, and prepare summaries of the work done, in order to get the best advantage of the experts' advice and to facilitate all the necessary analysis for the work progress. The measures suggested for the laboratory building finishing/design, especially those related to the avoidance of microbial contamination, should be faithfully followed.

The installation of pending equipment/instruments should be also finished before the next experts' visit.

Work on the Pen V recovery aspects has to be stressed after the installation is finished and fermentation conditions settled, following Dr. Batchelor's recommendations.

The possibility of purchasing the Westphalia centrifuge as pointed out by Dr. Batchelor, will require financial arrangements in the project budget which could be discussed/approved during the next experts' mission in October 1992.

The individual and team work of the experts is regarded as highly qualified and efficient; their personal interest and contribution to the project's success is also recognized.