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BIOENZYMES PRODUCTION AND DEVELOPMENT

DP/CPR/88/001

THE PEOPLE'S REPUBLIC OF CHINA

Technical report: First visit to the Wuxi Enzyme Factory, Jiangsu Province, 19 August 1992 to 5 September 1992*

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 Mr. A. Joyeaux, chief technical adviser

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Chemical Industries Branch

United Nations Industrial Development Organization
Vienna

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* This document has not been edited.

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TABLE OF CONTENTS

Summary

DP/CPR/88/001/11-51

Improvement of Technology for Production and Development of Enzymes at Wuxi Enzyme Factory.

Objective:

Assess the state of the on going research and production situation related to the project activities and prepare a working plan for the better organization and use of the consultants joint visit.

Duration :

- 21 days in total including 1 day for administrative preparation (visa in Paris, injection ...)18 days travelling, briefing, work in Wuxi and debriefing in Vienna, 2 days for reporting.

INTRODUCTION

1- General recommendations

During this mission most of the effort was to investigate the capability of WUXI research people to receive and be able to take profit of the joint consultant's visit in next november.

Several critical parameters in BAA production (fermentation and recovery) which could be taken as models are pointed. The potential of improvement is very high (doubling the yield seems possible) even in short term (six months to one year).

But this expert visit is to short to guaranty the success of the program and even the proper utilization of the equipments afterward. A one year proper supervision by the CTA with one week's visit on the site every six months would be reasonable to follow the necessary renovation of facilities, the proper maintenance of equipments, and the development of the program.

For the longer term I invited the responsible of WUXI R&D to enhance collaboration with Institutes inside and outside China in order to master the most advanced concepts in biotechnology .

2- Original objectives:

The content of the mission remains in agreement with job's description (in appendix) completed by indication following briefing in Vienna with Mme Valdes:

- define bottle neck equipment and propose eventual additional investment.

- define with Wuxi people a second priority project.

3- Realization of objectives:

Objectives were realized except:

. assistance in the utilisation of project equipment because main of these equipments were not connected to power yet.

. assistance in installation of new pilot fermentors produced locally because these fermentors were not delivered yet.

ORGANIZATION OF WUXI ENZYME FACTORY

In the plant there are more than 700 people. Historically it was a wine factory. The enzyme production started in 1965. The first product was bacterial alfa amylase. Presently alfa amylase and amyloglucosidase represent 90% and proteinase 10% of the activity of the plant. Wuxi enzyme factory produce about 20% of the China's market needs.

The factory is divided in four divisions with the following departments

- Production division with: production department 1, 2, 3 and maintenance department (410) people.

- Technological division with: technological, quality control, pilot plant and R&D departments.

- Project division with: project and analytical chemistry departments.

- Financial division with: financial, purchase, planning, store house and dispatching departments.

A- Production department

1- Organization:

Meeting with Mr HUO, Director; Mr FENG, head of the Production division and with two of his assistants Mr ZHOU and GU.

Three workshops: fermentation, downstream for solid enzymes and downstream for liquid enzymes.

- Fermentation: 3 sets of 60 m^3 and 12 sets of 20m^3 fermentors.

- Downstream : Several downstream equipments are available on the site, evaporator system, salt precipitation equipment, plate filter, spray dryer, centrifuge

2- Main problems in fermentation:

- contaminations: check cleaning of fermentors, micro holes due to corrosion protect micro-organisms; try to replace soya flour which is difficult to sterilize ; renovate the air filtration system.

- cooling : decrease viscosity; decrease sugar at the beginning and develop a feeding continuous system; .

- pH control: develop pH regulation .

3- Main problems in recovery

- contaminations: check for efficient cleaning of equipment, add micro filtration after polishing purification, develop SIP and CIP automated systems.

- losses in BAA when heat treatment for protease inactivation: decrease protease synthesis; develop protease negative mutants, replace soya flour by yeast extract + fish meal, add ammonium salts to fermentation medium.

- develop ultra filtration methods.

B- Project department:

meeting with Mr ZHU

1- Organization

The main task of the project department is the conception of new workshops and new equipments.

From 1965 to 1975 the alfa amylase was produced in $10m^3$. Now production is performed in $20m^3$.

At the beginning fermentors were made in steel and were very soon corroded. Most recent are made of steel coated inside with a 3,5 mm thickness stainless steel layer. For the next fermentors to be built it is forecasted to make them in pure stainless steel.

2- Main problems in fermentor and recovery equipment design:

- Oxygen transfer: should be based on data coming from pilot (simulation); decrease W/H ratio from 1/2 to 1/3 .

- Heat transfer: install the cooling system outside the fermentor

- contaminations: develop SIP and CIP; check for a membrane air filtration system.

C- Research and development department

1- Organization:

Meeting with Mr SHAO Institute Director and several engineers from his department: MRS ZHENG (strain department), GE (glucoamylase), MIAO (glucoisomerase), Mr ZHENG (ultra filtration), YAG (proteinase detergent), CHO (proteinase detergent).

The objectives of R&D department are:

. Development of new strains for enzymes presently produced in Wuxi.

. Supporting factory production- optimization of process in the factory.

. Pilot production for new enzymes.

The Wuxi enzyme factory was the first in China to develop enzymes. The research for new enzymes concern: pectinase for apple (aspergillus niger); proteinase food grade (B. subtilis); glucose isomerase (streptomyces); lipase (yeast); B amylase (plant); penicillin acylase (E.coli); proteinase for reagents (B. licheniformis). These enzymes are still at the lab or at the pilot level.

2- People

The Institute was created in 1976. Twelve research people are working there: 2 senior engineers, 8 graduate engineers, 2 technical assistants.

According to UNDP program five of these engineers were trained abroad : Li De Quiang (strain improvement USA); Wang Wenjia (downstream process Germany); Guo Hongfei (strain department USA); Gu Bing (downstream Germany); Fan Zihen (fermentation USA).

Only one of them Mr Li was back in Wuxi so I was unable to evaluate the competence and skill of all the others people. During my mission in Wuxi, Mr Li was permanently available for information, translation and arrange contacts with every people I have to discuss with.

3- Facilities

Several rooms are available for research and development. General conditions are of poor quality: no air conditioning, almost no correct coating for walls and floors which keep dust. Furniture and benches are also of low quality. As in biotechnology more modern and sophisticated equipments are needed, for example the equipments granted by UNDP, these equipment should be maintain in clean conditions, mainly those with electronics. For an efficient utilization of the modern equipment it is necessary to renovate and to relocate all

the labs. In the case of equipment sensitive to humidity it should be wise to put them in air conditioned conditions.

4- Equipment

Apart equipment granted by UNDP labs are very poorly equipped.

For more efficiency in strain improvement program it is recommended to develop more automated technics to prepare petri dishes.

In the pilot workshop the equipment is much better even if it not fully intrumented with captors for the main parameters: four 500 liters fermentors (power 1,7 kw), three 3000 liters fermentors (power 11,3 kw) are equipped with pH, temperature, dissolve oxygen and rpm but not regulation. A computerized data acquisition system is also available.

For a better efficiency of development it is recommended to install every modern captors available on the market for a complete regulation of fermentation including oxygen and carbon dioxide flow balance. These equipment should be able to make simulation for production and project department. Develop also data processing based on statistical treatment of figures.

D- Quality control department

1- Organization:

Meeting with Mr FU the Head of department and with Miss HU in charge of process control and Mr MA in charge of new enzymes development.

There are 28 people in this department covering four tasks:

- Control new products including pilot production
- QC preparation from production
- QC of new production
- QC for sale department

2- Nature of controls:

- Raw materials: soya bean (nitrogen); maize flour(starch and moisture); peanuts (nitrogen and water retention); minerals: ammonium sulphate, calcium chloride, sodium sulphate, potassium hydrogenosulfate...

- Process controls: sugars, nitrogen (mineral and organic) , acidity, enzyme activity, microscope examination of quality...

- Final products QC: activity, moisture, particle size, clearness for liquid enzymes. Food grade enzymes are checked for heavy metals (lead and arsenic) and for pathogenic micro-organisms(E. coli, Salmonella).

- Shelf life control.

3- Equipment

Equipment in QC department is very poor: water bath, pH meter, spectrophotometer, clean table (laminar flow bench). In the objective of development of new strains and new products it will be necessary to invest automatic or semi- automatic method. If not the QC department will be unable to meet the needs of R&D and production (priority investment).

4- Products to be controlled

All the enzymes which are developed by the factory. Main assays are for amyloglucosidase (iodine method) , bacterial alfa amylase (iodine method) and proteinase (spectro assay).

5- Data acquisition and data processing

As most of biological assays normal dispersion of figures makes results difficult to interpret. I would advise to use computer for data processing and develop statistical methods.

MAIN CONCLUSIONS AND RECOMMENDATIONS

According to the weakness of R&D and the low level of technicality in advanced technologies of people in Wuxi I would recommend that:

- before the flash visit of the experts, in next November UNDP get confirmation that all equipments should be in order and Wuxi people involved totally available.

- The consultants should not disperse efforts on several projects, but develop methodology based mainly on bacterial alfa amylase. These methods could be apply in the future for other projects

A- Research organization:

Facilities are of low quality: try to improve as possible.

Equipment granted by UNDP is partially working. According to Mr LI it will be completely in order before the expert's arrival (including pilot fermentors bought by Wuxi enzyme factory). Glassware, reagents and ingredients should be also available.

Co-ordinators and translators will be permanently available for experts needs.

UNDP should get confirmation of these points before consultants arrive in China.

UNIDO should propose 11-52 expert (Mr Joyeaux unavailable in November).

UNDP should design a co ordinator amongst the consultants.

Mr Migne could be OK for 11-56 in complement of Mr MEADA (discussed and agreed with Mr Li)

B- Work plan for the expert visit:

Should be done according to the programs 11-52;11-53;11-54;11-55;11-56;11-57.

During the joint visit of the consultants in november most of the effort should be for developing methodology based on bacterial alfa amylase improvement (BAA).

1- Research:

Develop the strain improvement technics according to the project; adapt data acquisition and processing (computerization). In pilot develop scientific models for transfer (mass transfer-Kla) and simulation. Check for domestic raw materials improving BAA production(C and N sources, yeast extract).

2- Quality control:

Develop quantification based on statistics. Develop automatic or semi-automatic process for assay.

3- Production:

Review fermentation process in order to increase filling, improve heat transfer and pH regulation. Adapt downstream process to more efficient recovery and better shelf life .

C- Bottle neck

Equipment for QC: if additional budget could be obtained from UNDP recommendation to acquire a semi- automatic equipment for assay.

D- Second priority project:

Proteinase for detergents, to be started when technologies for BAA are routinely mastered.

APPENDIX

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION

DP/GPR/88/001/11-51

JOB DESCRIPTION

Post Title: Expert in Bioenzymes production and development - Chief Technical Adviser

Duration: 0.5 m/m

Date Required: May 1992

Duty Station: Wuxi, Jiangsu Province, People's Republic of China

Purpose of Project

Improvement of Technology for Production and Development of Enzymes at Wuxi Enzyme Factory.

Duties

In October 1992 a group of 6 experts will visit together the project site for assisting in improving the present technologies from the points of view of: strain development and maintenance, optimization of fermentation and downstream processing of various enzymes, improvement of final product quality standards, Good Manufacturing Practices and Quality Assurance Procedures.

The expert will assess the state of the on-going research and production works related to the project activities and prepare a working plan for the better organiztaion and use of the consultants' joint visit.

The expert, in cooperation with national authorities, should prepare during his first mission:

- An assessment of present research and production situation in respect to selected enzymes.
- A list of main production and quality parameters that should be considered/improved and desirable final conditions to be attained.
- Work plan for the experts visits; sequence, coordination matters, priority subjects, technical workshops.
- Assist in the utilization of the project equipment.
- Assist in selection and installation of new pilot fermentors produced locally.
- A final report with his findings and recommendations, that will serve as TORs for the experts visit and the technical workshop.
- Second priority project.
- Equipment (additional equipment if needed).

BACKSTOPPING OFFICER'S TECHNICAL COMMENTS ON THE FIRST MISSION REPORT OF MR. JOYEAUX

The expert has fulfilled his duties according to his job description and expectations. Therefore, this report qualifies to be used as Terms of Reference for the work of the group of experts scheduled to visit the Wuxi Enzyme Factory in November 1992.

The report points out the more critical parameters in the different stages of production, quality control, research and development, design and construction of equipment and systems in the factory and at the pilot level and gives ideas to improve the general performance of the Factory.

Based on this and on their particular (personal) criteria/ experience, the experts should elaborate detailed recommendations and guidelines for improving the critical parameters identified. The impact of the upgrading programme will be alternately measured by the increase in production quantity and quality.

Two main recommendations should be considered immediately:

- the adequate preparation of equipment, premises, counterpart personnel, glassware and reagents/ingredients, time in advance (before) the experts' visits.
- the decision to devote the efforts of experts and national counterpart to keep improving the bacterial alfa amylase process and establish model procedures for this product, instead of beginning the work with the second priority enzyme.

Considering the importance/weight of the alfa amylase in the Wuxi Enzymes Factory production programme and the possibility/ expectancy of doubling its production in a six month period, this recommendation seems reasonable and convenient to be followed and is supported by the backstopping officer. The national authorities should decide upon this point, prior to receiving the visits of the experts.