## OCCASION

This publication has been made available to the public on the occasion of the 50th anniversary of the United Nations Industrial Development Organisation.

## DISCLAIMER



## FAIR USE POLICY

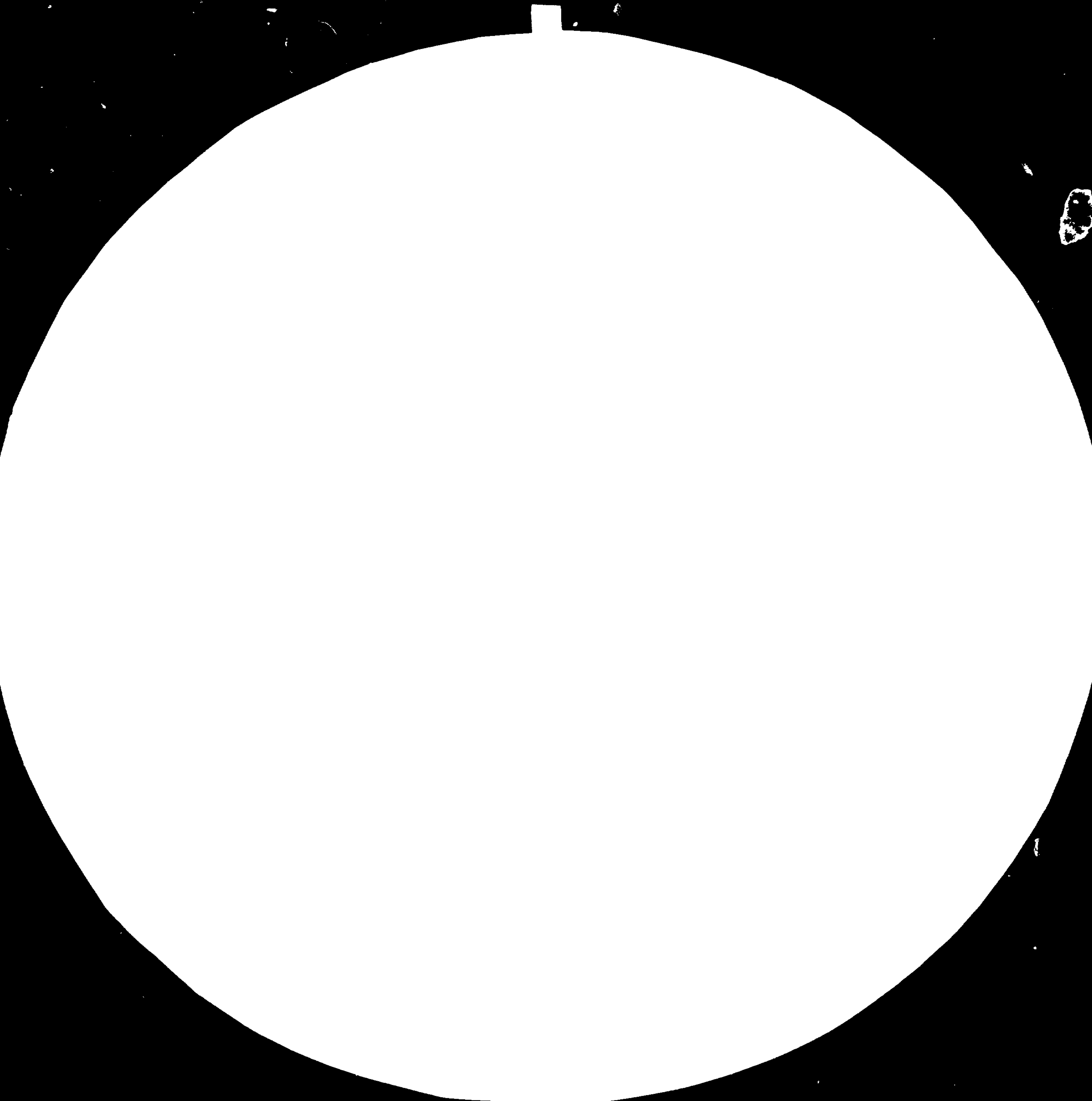


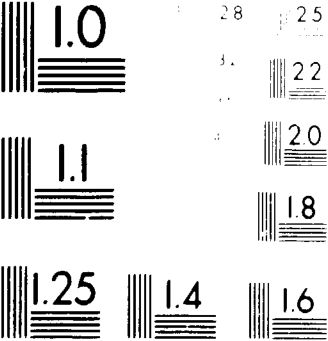
## CONTACT

Please contact publications@unido.org for further information concerning UNIDO publications.

For more information about UNIDO, please visit us at www.unido.org

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION
Vienna International Centre, P.O. Box 300, 1400 Vienna, Austria
Tel: (+43-1) 26026-0 · www.unido.org · unido@unido.org






MICROCOPY RESOLUTION TEST CHART

Distr.
RESTRICTED

UNIDO
22 November 1983

ENGLISH

UNITED NATIONS
INDUSTRIAL DEVELOPMENT ORGANIZATION

13418

# INDUSTRIAL CHEMICALS FROM INDIGENOUS CARBOHYDRATE RAW MATERIALS (SUCRO-BASED CHEMICALS)

ST/PHI/81/001

PHILIPPINES.

(Sub-project: Citric Acid Production).

Technical Report*

Prepared for the Government of the Philippines
by the United Nations Industrial Development Organization

Based on the work of Bjorn B. Kristiansen
consultant in fermentation technology

* This document has been reproduced without formal editing.

**ABSTRACT**

A consultancy was carried out for UNIDO on a project on citric acid production at the National Institute of Science and Technology, Philippines, supported by UNFSSTD and UNIDO in collaboration with the Philippines National Science and Technology Authority and the University of Philippines. The object was to assist in directing the project towards pilot scale operation providing this was justified by the results obtained so far.

A good group of researchers are associated with the project and promising results have been produced. The work has been handicapped by not being able to provide a constant temperature for the fermentations and the shortage of fermenter capacity.

An intensive R & D program on the production of citric acid in submerged culture and product recovery has been proposed. This will provide the required information for proceeding to pilot scale. The successful outcome of the program relies on rectifying the shortcomings described above, concentrating on one method of producing citric acid and adhere to the proposed program.

It is recommended that the support for the citric acid project is continued. It is advisable to use consultants to provide help if and when required and to ensure the project is progressing.

## TABLE OF CONTENTS

## INTRODUCTION

A programme entitled "Industrial Chemicals from Indigenous Carbohydrate Raw Materials (Sucro-based Chemicals)" was started in the Philippines in 1981. It was supported by UNFSSTD and UNIDO in collaboration with the Philippines National Science and Technology Authority and the University of Philippines at Los Banos. One of the priority projects, production of citric acid, has been carried out at the National Institute of Science and Technology (NIST). This report concerns an assessment of the citric acid project in the form of a consultancy carried out on behalf of UNIDO. According to the briefing given, the object of the consultancy was to evaluate the work being done and to aid the program towards pilot-scale if this could be justified.

The consultancy involved briefing and debriefing at UNIDO headquarters in Vienna and field work at NIST in Manila. The state of the citric acid project, in terms of results obtained and the attitude and technical competence of the people involved, was assessed as thoroughly as possible in the time provided. Instructions in specific topics related to citric acid production and fermentation technology in general were given.

The duration of the consultancy was one month.

## PROGRAM OF ACTIVITES

**Vienna:**

Briefing and debriefing with Mr M Maung, Industrial Operations Division, and administrative staff at UNIDO headquarters.

**Manila:**

On the first day, a meeting between Ms L G Tansinsin, Director of the Sucro-based Project, Dr C Calam, UNIDO Consultant in fermentation technology and the leaders of priority projects, was attended. During this meeting, an extensive review of the citric acid project was given by Dr Calam.

The first activities at NIST was to interview the staff. This included all staff involved in the production of citric, gluconic and acetic acids. It turned out that all these three acids were studied at NIST. Apparently, the citric and gluconic acid work is funded from the Sucro-based Chemicals project; the acetic acid is an internal NIST project. Some of the results obtained and a feasibility study on large scale citric acid production were presented.

From the second day onwards, the staff was given a range of experiments and tasks to carry out, all associated with the production of citric acid. The object of this was two-fold. Firstly, it would be used for an assessment of the technical capability of the researchers and secondly it exposed them to the range of techniques and experiments required for a successful research program on the production of citric acid. The execution of each experiment was carefully supervised and talks on practical and theoretical aspects of citric acid production were given on an individual basis while the work was going on.

A number of lectures on citric acid and fermentation technology were given to the whole Sucro-based group. All staff at NIST involved in fermentation research were also given a general lecture on Fermenter Design and Operation.

Project appraisal and discussion on the proposed restructuring and scale-up of the Industrial Fermentation Program at NIST with Dr Mauriarti, Director of NIST, and Dr L Joson, Industrial Fermentation Program Co-ordinator, took place on a number of occasions.

## PROJECT STAFF

A list of the staff involved in the Sucro-based Chemicals Project at NIST is given in Appendix I.

All staff, with the exception of Ms Palo, are doing bench work. This represents a credible utilisation of staff for practical work.

The staff were technically competent. They were all very tidy workers and not afraid of high workloads. It may be argued that on the whole the lab was lacking in microbiological expertise. However, this may not necessarily be a significant factor for a successful outcome of the project.

There was a very friendly atmosphere in the lab, with people always willing to offer a hand wherever needed. Technically, they are capable of developing a successful citric acid fermentation on the scale they are currently operating (shake flasks).

A severe shortage of fermenter experience was demonstrated. It appeared that there were few opportunities to work with the fermenters available at NIST which were shared between all projects in the Industrial Fermentation Program. The internal politics aside (on which no comment can be made with any competence), a more agressive attitude towards studying the citric acid fermentation in one of the available fermenters could have been displayed prior to my arrival. However, three researches were given practicals with the NIST fermenters which were all set aside for the citric acid project during the consultancy. In addition, a leading Science Specialist (Ms Daan) is currently on a three month study leave to gain experience with fermenters. Hence, the group will eventually have the required knowledge of fermenter operations.

It appeared that the literature was rarely consulted. Apart from searching for analytical techniques, few people had actually read papers on citric acid. This was a short-coming and should be rectified. A reading list will be provided as a starting point.

## PREVIOUS WORK

The organic acids studied within the Sucro-based Chemicals project were citric and gluconic acids (itaconic had been considered briefly). The acids were produced in both surface and submerged culture (for comments on the two processes, see below). The most promising results were obtained with citric acid. A number of wild strains (all known producers) had been tested, yielding two superior producers, strains CP-3 and 72-4. (The latter is well known for its capacity to produce citric acid). The bulk of the work had been carried out using these two strains (for comments on the strains, see below). Some of the nutritional requirements had been worked out, leading to media which favoured acid production (for comments on the media, see below).

In both surface and submerged culture, high levels of citric acid had been produced. The results showed that acid concentrations between 7% and 8% were obtained regularly. In terms of scale of operation (100mls of medium in 500ml flasks), these concentrations are very acceptable (assuming more than 90% of total acidity was due to citric acid). However, there are often no linear relationship between shake-flask and lab-scale fermenter processes. As the latter will be the starting point for an eventual scale-up of the process, it is essential that the group has full time access to a fermenter. Only then will it be possible to assess the shake-flask work completely.

The work on the recovery on citric acid has achieved some success but is severely hampered by the shortage of the required equipment. It has already been pointed out (C.T. Calam, Technical Report on Sucro-based Chemicals, submitted to UNIDO in August 1983) that the recoveery of citric acid from the fermentation broth employs standard chemical engineering techniques. These are to some extent dependent on the equipment and they are difficult to carry out realistically on a lab scale. In spite of these draw-backs, the people in product recovery were struggling on and some citric acid crystals had been recovered. The techniques applied were so unrealistic, however, that it is difficult to assess the progress. It was felt that a greater awareness of product losses could be displayed, but the researchers in general showed great ingenuity and dedication.

## EQUIPMENT

A list of the equipment bought for the Sucro-based Chemicals project is given in Appendix II. Most of this was in continuous use, as indicated in the appendix. Apart from fermenters, specific analytical and product recovery apparatus, the laboratory could be considered well equipped (access to specific analytical equipment such as atomic adsorption spectrophotometer and Kjeldahl digester was available at other research centres).

Most important of all, however, was the lack of facilities for temperature control during the fermentations. All the experiments were carried out at ambient temperature, which resulted in fluctuations of up to ± 4°C, sometimes reaching temperatures of 38 - 40°C. As temperature is known to affect the citric acid fermentation this problem must be tackled if the project is seriously going to be considered for scale up to pilot and production scale.

Another problem was the frequent power-failures experienced. If this lasts for more than a couple of minutes, irreversible damage to the citric acid producing organisms may occur. The researchers in the citric acid project appreciated these shortcomings but were probably not sufficiently aware of how seriously their work was affected.

## SPECIAL NOTES

Some decision regarding the citric acid project had been taken prior to my arrival. It is beyond the scope of the consultancy to question previous decisions. However, it is felt opportune to offer the following comment.

### Choice of producer organisms

Citric acid producing strains tend to be unstable and work in my own laboratory has indicated that the strain CP-3 is a prime example. It is also very difficult to translate shake flask results to fermenter scale with this strain. Good results had been obtained, however, and it had been decided to concentrate on CP-3.

The other strain used in the citric acid project, 72-4, is probaby the best known of all producers. It has been studied extensively in laboratories throughout the world. More important, techniques to improve the productivity of this strain are well documented. If this project is to be scaled up to production stage, high producing strains must be developed. It was decided, therefore, in consultation with the project leader and staff, to switch the attention to strain 72-4.

**Fermentation medium**

The best results had been obtained with a fermentation medium containing 2% corn oil. According to the feasibility study, corn oil represented about 32% of the raw material costs. It is well known that vegetable oils can increase the yield of citric acid. However this increase must be reached by other means (strain improvement and medium optimisation) to reduce operating costs.

It may be possible to use corn oil as an antifoam agent (not included in the feasibility study). In this case much smaller amounts will be required but it may be possible to benefit from the citric acid enhancing effect of the oil. (It should be noted that the selection of antifoam will, to a considerable extent, be influenced by the product recovery stage. The antifoam must not interfere with the acid extraction).

The major raw material for the citric acid fermentation is the carbon source. It had been decided to use sugar cane juice as the raw material for the scale-up work on citric acid. This appeared to be backed by socio/political as well as scientific reasons. Molasses is by far the most common raw material for industrial production of citric acid. It is a technically more difficult raw material than sugar cane juice, but is normally much cheaper.

Accepting that sugar cane juice will be the raw material it is essential that this will be used for all the work carried out in the citric acid project in the future. (To some extent, this had already been done). A detailed breakdown of the composition of sugar cane juice must be carried out to determine the need for addition (removal) of other nutrients. This is already in progress.

**Fermentation process**

Citric acid is produced industrially in both surface and submerged culture and both processes had been given attention in the citric acid project with no clear indications that one was superior. It had been decided, however, to concentrate on the submerged process.

The choice between surface and submerged production is influenced by two major factors. The first is scale of operation. Surface production is normally the most economical for small scale operation. The break-even point between the two processes is normally 5-8 tonnes per day. As the projected production for the proposed plant is 5 tonnes per day, scale may not be an important factor.

Surface operation is also the preferred choice when the raw material is particularly impure. Sugar cane juice is considered as a relatively pure raw material, so on these two criteria it appears that the correct choice had been made.

It must be noted, however, that the submerged process has a much higher power demand. Hopefully this was borne in mind when opting for this process. The surface process is labour intensive, but on the information given, it appeared that labour is cheap in the Philippines.

Having decided on the submerged process, research on the surface method should stop. This was discussed with the staff who willingly accepted to concentrate on submerged production.

**Feasibility study**

A comprehensive feasibility study on a production plant for citric acid was presented. This is a very impressive piece of work and shortcomings appeared to be from lack of knowledge of the large scale production of citric acid and this information is not readily available. The study was discussed in detail with the principal author. In the light of these discussions and the improvements which can be expected from the pilot plant studies, the citric acid group will be in a position to present an impressive plan for the proposed plant.

## ASSESSMENT

The citric acid project has been given considerable attention, in terms of funding and manpower. Overall, the standard of work is good, which is reflected in some of the results obtained. The major shortcomings are caused by lack of equipment rather than the researchers themselves. However, to proceed to a pilot plant may have been taken for granted, particularly if high concentrations of citric acid could be obtained in the fermentation. Thus, some of the other process parameters, such as biomass production, consumption of sugar, chemical environment effects, rates of fermentation etc., have been forgotten, with the result that inspite of having good yields, little information on the fermentation is available. It may be argued that the only important parameter is the conversion of sugar to citric acid. In this case the state of the citric acid project is quite promising. In a research program, however, it is important to study all parameters, providing basic information as well as data for process optimisation. It must be said that this is much easier, and more informative, to carry out in a fermenter than in shake flasks.

The lack of temperature control, fermenters and persistent power failures makes it necessary to carry out further studies (under controlled conditions) before deciding on translation to pilot stage. (No power failures were experienced during the consultancy, but according to the staff, this was unusual). The next stage for the citric acid project will be to carry out an intensive program which includes strain improvement, medium optimisation (nutritional studies) in shake flask culture, evaluating results in a lab fermenter and research into the major product recovery parameters. A detailed program is listed in Appendix III. The program was presented to and discussed with the people associated with the citric acid project. It involves a complete restructuring of manpower (given in Appendix IV) which was accepted by the staff.

Providing the proposed program is followed the people involved should be in a position to to produce data, relying on previous results and experience, which will support the installation of a pilot plant. The conditions for a successful outcome will, however, rely on the following :

a) submerged citric acid production adopted as the only research topic;

b) all fermentations carried out at constant temperature;

c) full-time use of a lab fermenter;

d) drastic reduction in the number and duration of power failures.

The time scale will depend on how soon a) to d) can be implemented. The work itself should not take longer than 12 months (providing the enthusiasms displayed during my stay are kept up).

## RECOMMENDATIONS

The support to the citric acid project should continue. Funds should be made available to carry out the fermentations under constant temperature e.g. placing all the shakers in a constant temperature room (where cooling below ambient temperature is possible). A fermenter must be provided. (It appears that this is on its way. In this case a constant supply of cooling water is required).

The proposed program should be followed closely. Outside expertise should be made available to aid the project whenever required, ie. help in assessing results and to ensure the project is progressing.

# APPENDIX I

**Staff involved in the Sucro based Chemicals project on citric acid at the National Institute of Science and Technoogy**

| | | |
|---|---|---|
| N D Palo | Project Leader | |
| L F Cunanan | Study Leader | |
| C T Daan | Science Research Specialist III | |
| R T Cabacang | -ditto- | |
| C P Madrid | -ditto- | II |
| E Casareno | Science Research Assistant II | |
| L de Guzman | -ditto- | |
| C Ramos | -ditto- | I |
| E T Luna | -ditto- | I |
| L Villenueva | -ditto- | I |
| E Reyes | -ditto- | I |
| P Bantugan | -ditto- | I |
| M Malabanan | Science Aide | |
| C Mamaril | -ditto- | |

## APPENDIX II

### Equipment provided for the citric acid project

| | |
|---|---|
| [1] Balance 0.002-160 g (Sartorius) | 2 off |
| [1] Balance top loading 0-3 kg (Sartorius) | 2 off |
| [1] Spectrophotometer (Baush & Lomb, Spectronic 21) | 1 off |
| [2] Waring blender | 1 off |
| [1] Whirl mixers | 2 off |
| [2] $O_2$ analyser (Beckman, Aldex 0260), no probe | 1 off |
| [1] pH meter (Corning, 125) | 2 off |
| [1] Moisture determination balance (Ohaus) | 1 off |
| [2] Titrafration unit (Fisher, titrimeter 393) | 1 off |
| [1] Microscope (Olympus, BH2) | 2 off |
| [2] Microscope camera and exposure control unit | 1 off |
| [1] Incubator shakers (New Brunswick) | 3 off |
| [1] Autoclave, small electric (Market-Forge, Sterimatic) | 1 off |
| [2] Freeze drier (Virtis, freezemobile 24) | 1 off |
| [1] Fridge/freezer (Hitachi, large) | 2 off |
| [1] Incubators (Lab-line, Imperial II) | 1 off |
| [1] Demineraliser/still (Corning, megapure) | 1 off |
| [2] Vacuum oven (Fisher, 281) | 1 off |
| [1] Evaporator (Buchi, rotavapor RE 120) | 2 off |
| [1] Vacuum pumps (Edwards) | 3 off |
| [1] Laminar flow cabinet (Laminaire) | 1 off |
| [3] Liquid chromatograph, including data module, solvent delivery system, fluoresence detector, solvent programmer and radial compression separation system (Waters) | 1 off |
| [3] Flat bed recorder (Ommiscribe) | 1 off |
| [1] Autoclave, large (Kreca, 559) | 1 off |
| [1] Orbital shaker (New Brunswick, 3 bed) | 1 off |
| [1] Oven (Labec) | 1 off |

**Code for Appendix II**

1 This equipment is in constant use.

2 Not in use during my stay, but is useful for citric acid studies.

3 Useful equipment for microbial studies in general, strictly not required for producing the necessary data on citric acid.

This list does not necessarily include all the equipment provided by Sucro-based Chemical funds.

## APPENDIX III

## Program for R & D on citric acid production

The staff currently engaged in the production of citric acid (surface and submerged method) and gluconic acid (surface method) will all take part in this programme. The staff will be divided into four groups, each working in a specific area.

**Group 1 Strain selection and maintenance**

Improvement of existing strains by mutation and natural selection. This will involve spore production, serial dilutions, growth on agar, paper media and in shake flask. The important part of the work will be to produce large amounts of spores and maintaining them with no loss of activity.

**Group 2 Medium optimisation (shake flask)**

2a) evaluation of new strains

This will involve primary evaluation of new strains, obtaining special nutritional characteristics of new isolates and mutants.

2b) evalution of existing strains

A large number of experiments will be required to determine the effect of medium components, at different concentrations, on the production of citric acid. Single and multivariable experiments are required. Standard procedures must be developed as early as possible.

**Group 3 Fermenter studies**

This group will work in close association with group 2, in particular 2b. The object is to successfully scale-up results from medium optimisation experiments. Successful or unsuccessful scale-up must be proven.

**Group 4 Product recovery**

It is very difficult to study product recovery on a lab-scale. This group will concentrate on the individual stages of product recovery and optimise these individually. A succesful product recovery process can only be developed on a pilot scale when all the required equipment can be made available. The responsibility of this group will be to obtain the parameters for successful operation of the individual product recovery stages.

Close contact with the other groups are important, in particular with group 1. This can avoid developing strains with, e.g. high pigment production which will interfere with recovery of citric acid.

## Research Programme

**Group 1 Strain selection and maintenance**

1a. Produce large number of spores of present strains on PDA (unless results have shown otherwise). This will be the supply for <u>all</u> work.

1b. Determine best maintenance method, using PDA spores from 1a and a fixed medium which is <u>known</u> to result in citric acid production.

1c. Determine best sporulation medium, using spores from stock solution 1a. Investigate effect of successive transfers. (NB. Retain sufficient spore for production of stock solution).

1d. Production of new isolates by natural selection, using agar and paper media. Test promising strains in shake flask cultures. Select for high sugar and citric acid tolerance, increased production capacity and low pigment production.

1e. Use standard mutation technique, UV; X-ray and mutating agents, proceed as in d.

**NB. Necessity to wash all spores must be determined.**

**Group2 Medium optimisation in shake flasks**

2a. Determine composition of sugar cane juice, in particular the concentrations of N, P, $Fe^{3+}$, $Cu^{2+}$, and $Mn^{2+}$. Design basic medium as a reference point for future work.

2b. Effect of medium constituents :

a) Optimisation of sugar concentration, searching for highest yield of citric acid on sugar.

b) Determination of best nitrogen source (if required, using constant amounts of nitrogen).

c) Optimisation of nitrogen concentration.

d) Optimisation of $KH_2PO_4$ concentration.

e) Optimisation of $MgSO_4.7H_2O$ concentration.

f) Determination of best source for supply of $Fe^{3+}$.

**NB. $Fe^{3+}$ may already be present in the cane juice**

Optimisation of $Fe^{3+}$ concentration.

g) Optimisation of $Cu^{2+}$ concentration.

h) Optimisation of $Zn^{2+}$ concentration.

i) Factorial experiments with trace metals, determining the best combination of metals.

j) Factorial experiments using data from a) to i).

k) Determination of best initial pH for citric acid production.

l) Determination of optimum spore concentration in the inoculum.

m) Production of complete batch curves using shake flasks (whole flask samples).

n) Studies on the effect of temperature on the rate of production and final yield of citric acid.

**Each data point must be a minimum of four whole flasks**

The initial conditions, medium composition, temperature, shaker speed, inoculum level and pH will be standard for **ALL** experiments. Variations are acceptable only when it has been proven conclusively that the new conditions will lead to increased citric acid production. (It may be necessary to confirm old conclusions in the light of the new initial conditions).

Data required from each experiment are :

final sugar concentration (initial value given)

citric acid concentration

biomass

final pH (initial value given)

other acids (if present)

**Group 3 Fermenter studies**

3a. Production of a reference fermentation based on previous results, using an inoculum from 1a (24 hr seed cultures in fermentation medium) and data from 2a.

3b. Determination of best inoculation procedure using a) vegetative, and b) spore inocula. This involves optimising the inoculum level.

3c. Evaluation of data from 2b as they are produced.

3d. Determine effect of temperature, concentrating on citric acid yield on sugar (and fermentation time).

3e. Optimisation of inlet sugar concentration with the aim to operate at the highest sugar concentration feasible. (3d and 3e are also carried out by group 2, but different results may be expected in the fermenter).

Data required : Initial conditions (chemical and physical); profiles of sugar, citric acid, biomass, nitrogen, dissolved oxygen concentration and pH for the course of the fermentations (other acids if present).

**Group 4 Product recovery**

4a. Precipitation of calcium citrate.

This must produce calcium citrate cystals of the best crystalline structure for easy handling and minimal entrapment of impurities. The parameters to consider are :

a) temperature;

b) concentration of calcium in slurry, and

c) rate of addition of calcium.

b) and c) should be carried out using a range of citric acid solutions.

4b. Removal of calcium ions

Optimise rate and amount of sulphuric acid addition using different concentrations (all concentrated solutions). Operating temperature.

4c. Purification of citric acid solution.

Determine to which extent decolorisation and demineralisation are required.

4d. Crystallisation.

Determination of the optimum temperature (pressure) of crystallisation to produce crystals which are easy to handle. The recirculation of mother liquor should be kept to a minimum (without affecting crystal formation). (This part may be difficult to carry out satisfactorily on lab-scale).

The object of 4a to 4d is to minimise loss of citric acid.

4e. Drying.

Studies on drying temperature and procoss.

A copy of Appendix III has been provided to the group involved on the citric acid project.

## APPENDIX IV

### Group Organisation

**Project Leader : N. O. Palo**

**Group 1:**

P. Bantugan

B. Blanco

**Group 2:**

L. F. Cunanan (2b)

C. P. Madrid (2a)

L. de Guzman (2a)

C. Ramos (2b)

L. Villanueva (2a)

**Group 3:**

C. T. Daan

E. Reyes

**Group 4:**

R. T. Cabacang

E. Casareno

E. T. Luna

**Aides:**

M. Malabanan

C. Mamaril