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**PILOT PLANT PRODUCTION OF CITRIC ACID**

**DP/PHI/85/010 / 11-02**

**Republic of Philippines**

**Report on: Activities at the Laboratories of ITDI, Bicutan,\*  
Metro Manila The Philippines**

**Prepared for the Government of Philippines  
by the United Nations Industrial Development Organization  
acting as executing agency for the United Nations Development Programme**

**Based on the work of Professor Max Roehr  
Expert in Fermentation Technology  
Institut für Biochemisch Technologie  
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Vienna, Austria**

**Backstopping Officer: R.O. Williams, Chemical Industries Branch  
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1. Assessment of R & D at the laboratories of ITDI in Ermita and in the pilot plant in Bicutan was done by a number of discussions with Dr. Lydia M. Joson (Chief, Microbiology and Genetics Division and Program Leader, Integrated Citric Acid Project) and her Staff (see Appendix 1) as well as by studying and evaluating the Annual Report (January - December 1988) of this project and esp. the laboratory protocols covering the period from January, 28, 1987 to October, 5, 1988, i.e. our arrival.

According to these sources, research work mainly comprised

- Mutation and selection experiments to obtain mutants with altered properties with respect to improvements in fermentation performance.

- Fermentation experiments on the laboratory scale (14 Liter) to optimize the fermentation process for pilot plant operation with respect to the following parameters:

  - molasses quality and pretreatment conditions

  - performance of strains

  - all aspects of control of the fermentation process

In the course of the mutation and selection experiments several mutants had been obtained, each exhibiting altered properties regarding tolerance towards higher concentrations of citric acid and iron ions as trace element, resp.. Attempts to combine these two properties in a hybrid by protoplast fusion had failed, however, due to the fact that the induction and selection of auxotrophic mutations as marker traits had not been successful.

Fermentation experiments to obtain citric acid on the laboratory scale to evaluate the properties of the strains necessary for pilot plant operation were conducted in 14 Liter fermenters (Microferm, New Brunswick Co.), of which three units are in the laboratory. According to the protocols, the strain mainly in use was a production strain, designated JK, a gift of the consultant, Dipl.-Ing. J. Kominek, as the mutants obtained as mentioned above were found only to be suitable for the utilization of sugar as raw material.

With this strain JK only few successful experiments could be performed using local molasses as raw material, and unfortunately attempts at obtaining reproducible results had failed. There are several reasons for these failures in the opinion of the author:

- Failures of laboratory equipment, e.g. air compressors, fermenters etc.
- Frequent breakdown of electric power, unavailability of water for several important purposes during most of the time of operation
- Lack of personnel - there is not enough personnel (trained as well as untrained) to make up three shifts for work round the clock, which in citric acid fermentation is necessary through the main (critical) parts of the fermentation cycle.
- Failures of the strain to perform properly under the conditions applied, esp. with regard to the pretreatment of the sugar raw material (molasses) and the inhibition treatments following during the starting period of the fermentation.

Having assessed all the necessary details, the author (immediately as well as in the course of work in the laboratory) made the following suggestions (A to E):

#### A. Selection of auxotrophic mutants for protoplast fusion experiments

According to the author's experience, who is familiar with the problem of protoplast fusion and the concomitant difficulties of selecting proper markers, the selection of the respective mutants requires a high amount of labour esp. with respect to personnel as well as laboratory equipment (glass and plastic ware) - and probably also better experimental conditions (sterility facilities). However, the author knows an improved method of auxotrophic mutant selection, which would considerably reduce these requirements. The respective detailed descriptions of procedures were handed over to the staff - experimental work was not possible due to the fact that the lady supposed to conduct the experiments skilfully was just about to give birth to a healthy baby. Start of work was thus scheduled for January, and there is agreement that the course and success of the work will be communicated by regularly exchanging letters.

For the evaluation of other research activities the annual report 1987 as well as the laboratory protocols of 1987/88, which were prepared precisely, reliably and comprehensively as well, were studied and further informations were obtained by the permanent discussions with the laboratory staff throughout the stay in the laboratory. As already mentioned, almost no reproducible results regarding the performance of the strain with respect to the sugar raw material applied was obtainable, and it was therefore almost impossible to decide whether the raw material to be used was not suitable for the strain applied, although this strain is known as suitable for almost all similar materials, or whether the observed inconsistencies were only due to the drawbacks mentioned above. The suggestions made to meet these circumstances were:

- B. Experiments with an additional strain of *Aspergillus niger* - the author has brought two production strains from his laboratory - in order to compare this strain (designated R 150) with strain JK previously used, and to try to elaborate a standard laboratory fermentation procedure with end for these strains and the molasses available.

Evaluation of the protocols and subsequently also of the results of experiments performed during the consultants' stay revealed that the molasses provided for experimentation and subsequent use in the pilot plant apparently posed severe problems:

The specific technology of molasses fermentation involves heat treatment of the molasses with the addition of potassium hexacyanoferrate (HCF) in order to remove and complex metal and other impurities of the fermentation raw material. In the course of the fermentation the same compound is used to inhibit certain physiological (growth) functions in order to obtain effective acid production. The amount of HCF necessary for the treatment and to obtain a certain excess is determined by small scale simulation experiments and may vary considerably. Moreover, it may be necessary to add HCF additionally during the first phase of the fermentation, which is determined by trial and error during the growth period of the fungus monitoring the morphological development of the mycelial mass under the microscope.

According to Dr. Josen, experiments in the laboratory with various molasses from different local sources, which were considered as potential raw materials for commercial use, had revealed that their HCF demand was exceptionally high - in the treatment as well as in the subsequent fermentation - which apparently was one of the reasons - probably the main reason - of the scattering of results in the laboratory experiments.

The author therefore suggested a

- C. Selection strategy for the isolation of substrains (mutants) with specifically altered (increased) resistance to HCF with regard to the necessary high concentrations applied.

Such substrains were obtained by cultivation of strains JK and R150 on molasses agar media with varied HCF concentrations and isolation of spores from sporulating colonies or - on media with higher HCF concentrations, where even sporulation was already inhibited - of mycelial colonies, and subcultivation on suitable media. In this way 14 and 12 substrains resp. were obtained of which several were used for further fermentation experiments.

These strains exhibited a slightly improved fermentation behaviour and apparently proved to be more uniform for experimentation showing that the principle of this strategy would be promising especially if this procedure would be applied repeatedly. This was suggested to the staff for consideration.

During the two months stay at Bicutan 14 fermentations using strains JK and R150 as well as substrains as mentioned above were performed each requiring average operation times of 4 - 6 days. The results may be summarized as follows:

1. It is possible, although rather difficult, to obtain reproducible results in the laboratory fermentations.
2. The various drawbacks as mentioned before are considerable obstacles in the endeavour to do systematic research work. Some of these problems are expected to be solved in near future, but others seem to remain unsettled s.a. personel, laboratory equipment etc., and it appears that this may also considerably impede the impetus of the laboratory staff involved.
3. Although the strains used are potent industrial producer strains under normal conditions , fermentations with the molasses available were not satisfactory enough. Thus a further strategy was developed and this, after having commenced at the end of the consultants' stay, would be supposed to be continued as a longer term strategy in the following months:

#### D. Molasses strategy

It probably turns out that it is necessary not only to adapt the strain to the raw material as outlined in the job description but also to adapt the raw material to the capability of the strain(s). Therefore the following procedure was suggested:

1. Treatment of molasses in different ways as outlined by Dipl.-Ing. J. Kominek thereby varying especially the pH (acid or alkaline treatment), but eventually also temperature and time (other variations s.a. addition of adsorbents may also be considered).

2. Elaboration of a fractionation procedure for molasses including dialysis or Sephadex filtration followed by chromatography on Sephadex G25 - 75, and monitoring the eluted fractions by physical means (UV spectrophotometry, refractometry) to obtain characteristic patterns according to the molecular size of the fractions. By these patterns molasses may be characterized with respect to their composition prior to and after the respective treatments as well as in comparison to each other.

3. Upon performing standard fermentations the results may be compared with the fractionation patterns, which enables to

- evaluate the success of treatments with respect to their effect on citric acid formation and production
  
- evaluate the quality or suitability of a novel molasses sample
  
- characterize substances detrimental to the production organism

The author left two descriptions of such fractionation procedures and several samples of Sephadex from his laboratory for instant experimentation. Again the staff was informed that permanent contact by exchange of letters would be appreciated.

If this procedure would be successfully adapted to the local raw materials, it should be possible to consider a most promising further strategy:

#### E. Advanced molasses strategy

This strategy involves isolation of a detrimental compound (recognized as such by the above-mentioned procedures) from the molasses by a similar, preparative chromatographic procedure, of which several versions are accessible. The resulting compounds can be applied as selective agents to isolate strains (mutants) resistant to this molasses component. This procedure would yield improved strains especially adapted to extreme conditions. It should be mentioned that this approach would also be useful for other fermentations.

As may be seen from these conceptions, it might be necessary and would be recommended to extend the duration of the project by about 1/2 year - this also in the light of the fact that several of the technical problems as well as those regarding personnel may not be settled in due time.

The author of this report believes, however, that the aims of the project, i.e. to create the proper conditions for development of a useful process of citric acid fermentation on the pilot plant scale can be accomplished if the Philippine team is able and willing to conduct the necessary experimentation as outlined in the suggestions offered to and discussed with the staff.

There was only little time for the staff for further activities such as attending lectures as planned according to the job-description, since laboratory work and the preparation for a presentation of the plant to the public in a trimedia conference (see Appendix 2) absorbed all the available manpower. Nevertheless, a larger lecture on the biochemical and regulatory aspects of citric acid fermentation was given (see Appendix 3) on December 5, 1988.



INTEGRATED CITRIC ACID PROJECT  
 II. PILOT PLANT PRODUCTION OF CITRIC ACID  
 ANNUAL REPORT  
 JANUARY - DECEMBER 1987

**RESEARCH PERSONNEL:**

Research Administrator	Dr. Rufino C. Lirag Jr. Director NIST, Manila
Program Leader	Dr. Lydia M. Joson Program Coordinator Industrial Fermentation Program
Project Leader I ( Project A )	Mr. Romeo M. Cabacang Process Engineering and Design IFP
Project Leader I ( Project B )	Mrs. Natividad D. Palo Microbiological Research IFP
Study Leaders Project A1 Pilot Plant Operation	Mr. Melchor Valdecanas Food Research and Development Program NRDC
Project A2 Quality Assurance	Dr. Ernesto S. Luis Chemical Laboratory NSTC
Project A3 Feasibility Study	Mr. Erwin B. Casareno IFP NRDC
Project B1 Strain improvement	Mrs. Blanquita B. de Guzman IFP NRDC
Project B2 Fermentation technology	Ms. Cynthia P. Madrid IFP NRDC
Researchers	Lorgia L. Husmillo Josette H. Carillo Eden T. Luna Victorino M. Custodio Mario Aguinaldo Belen Mercado Mario Bigol
Consultant	Dr. Wilfredo Jose U.P. College of Engineering

## ACTIVITIES

### Highlights of Accomplishments

#### I. Summary of 1986

##### A. Process Engineering and Design

1. The design for the 200 l capacity pilot plant had been finished and modified to include the use of air lift fermenters and separate cation and anion exchangers as recommended by Dipl. Ing. Jiri Kominek, our UNDP Consultant.

2. The fabrication of the support foundation for pilot plant components was finished.

3. The construction of the pilot plant building was 25% completed.

4. The process of recovering citric acid from the fermented brew had been optimized through the use of process controllers. Citric acid crystals produced has been analysed for its purity.

##### B. Fermentation Product Development

1. Eleven good mutants of Aspergillus niger JK (given by Dipl. Ing. Kominek) were selected from more than 1,500 strains for citric acid production from molasses.

2. Parameters were optimized for citric acid production from molasses by shake-flask method.

3. The capability of mutants of A. niger 72-4 for the production of citric acid from sucrose by both shake flask and fermentor was confirmed.

#### II. For the period 1 January to 31 December 1987

##### A. Process Engineering and Design (PED)

###### 1. Construction of the Pilot Plant Building

For the first half of the year, the construction of the building was done by hired laborers under the supervision of the PED staff. This was followed by a contractor, the Abe Relleve Construction to hasten up the completion of the building. At the end of 1987, the building was almost finished, about 95% completed.

###### 2. Fabrication of Equipment

The following equipment have been completed:

- a. Liming/Acidulation Tank
- b. Process Water Holding Tank

- c. Slaking Tank
- d. Flash Tank
- e. Horizontal Crystallizer Tank
- f. Short Tube Vertical Evaporator
- g. Fermentation Mixing Tank
- h. Boiler (Steam Generator)
- i. Citric Acid Solution Holding Tanks (3)
- j. Condenser (2 units)

The following equipment are still being fabricated:

- a. Continuous Sterilizer
- b. Air lift fermentor
- c. Boiler Feed Water Tank
- d. Vacuum Pan

### 3. Product Recovery

The process of recovering citric acid from different sources was undertaken, namely: 1. simulated fermented molasses obtained by dissolving citric acid crystals in molasses medium; 2, distillery slops containing citric acid and sucrose and 3, fermented molasses medium. 4, fermented sucrose medium. The standard procedure was followed using the conductivity controller to follow-up the reaction. Pure citric acid crystals were obtained after repeated crystallization and analyzed for purity.

### 4. Characterization of Substrates and Products

Refined sugar and molasses samples (6) from different sugar centrals were analyzed. Molasses from Central Azucarera de Tarlac contained the least amount of iron (1 ppm). Other samples contained from 100 to 300 ppm iron rendering them unsuitable for citric acid production. Citric acid crystals were analyzed for purity; the fermented brews for citric acid and other ions.

## B. Microbiological Research

### I. Strain Improvement of *Aspergillus niger* for higher citric acid production.

The two good mutants of *A. niger* 72-4 N and 72-4 Y could produce citric acid with high conversion efficiency from untreated sucrose solutions but not from molasses. The JK strain is better suited for molasses medium which, however, requires high concentration of potassium ferrocyanide (K<sub>4</sub>Fe(CN)<sub>6</sub>) for sequestering the iron present in it. In both cases, optimum temperature is about 30 C to be maintained during fermentation.

Since it is more economical to use molasses as substrate especially at high sugar concentration the

two good mutants were subjected to strain improvement as follows ;

- a. Adaptation to high sugar and iron concentrations at high temperature.
- b. Selection for non-sporulating strain preliminary to protoplast fusion.
- c. Further selection of good mutants by nitrous acid treatment.

a. Adaptation to high sugar and iron concentration at high temperature.

Selections for good citric acid producing strains of the two good mutants, 72-4 N and 72-4 Y by single colony technique were done using media containing increasing concentrations of iron and of carbohydrate sources, namely sucrose and molasses (Table 1) with bromocresol green and calcium carbonate as indicators.

Zones of acid produced and acid unitage ( diameter of acid zone ) were compared among the diameter of colony size the 180 colonies (90) each. Strains of 72-4 Y appeared to be more tolerant to high sugar concentration with 79 mm acid zone, as compared with 64 mm from 72-4 N at 22 % sucrose and 0.3 mg/l Fe. At low sugar concentration of 18 % sucrose and 0.1 mg/l Fe, 72-4 N gave bigger zone of 76 mm as compared with 74 mm from 72-4 Y ( Table 2 ) confirming previous results.

+50%

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Good acid producers were transferred by single spore technique on molasses media ( Table 1 ) and incubated at 32 and 38 C. It was observed that strains incubated at 38 C showed more distinct zone of clearance.

Further screening on molasses media ( Table 3 ) was done by single spore technique. At low sugar (S10F5) the N strains could grow faster giving correspondingly higher acid zones than the Y strains while the Y strain could grow relatively faster than the N strain at 25 % sugar concentration ( S25F5) with correspondingly bigger zones ( Table 4 ).

Since the behavior of the strains on solid media could be different when propagated in liquid media, strains with big zones of clearance and high acid unitage were selected as potentially good acid producers for further screening in liquid media.

b. Selection of non-sporulating strains

Combining the good characteristics of N and Y into a single strain by protoplast fusion technique may result in a much better citric acid producer from molasses. One of the markers that could be used is the non-sporulating ability due to some block at

different stages of biosynthesis in the two good mutants.

The Shu and Johnson medium was modified by increasing the concentrations of N, P and Zn. Spores of 72-4 N and 72-4 Y were transferred by single spore method into agar plates of SJ0 and SJ1 (Table 5). After 5 days of incubation at 32 C colony growth and zone of clearance were measured and their morphological characteristics noted.

Spore formation was inhibited by the added nutrients but no acid was produced. The two mutants were shown to be genetically different by their behavior in the media. The N mutants did not grow well on SJ1 as compared with Y mutants which even caused cracking of the agar due to gas production.

### c. Further production of mutants by induced mutations.

The spores of Y mutant was subjected to 0.3 M nitrous acid at different time exposure and then plated on a Shu and Johnson molasses medium (25 % sugar concentration) and calcium carbonate. The survival curve and rate of mutation at different time exposure is given on Fig.1. Twenty-one good acid producers were obtained after repeated screening. Time exposure at 20 minutes gave the most number of high acid producers.

## II. Development of Fermentation Technology

The R & D activities were concentrated in using molasses as raw material. The organisms used were *A. niger* JK and its mutants.

### A. Shake flask method

Different parameters were studied to maximize citric acid production from molasses as follows:

1. Best JK strain for citric acid production.
2. Best propagation medium for spore production.
3. Best production medium.

Two kinds of molasses obtained from Central Azucarera de Don Pedro (CADP) (100 mg/l Fe) and Central Azucarera de Tarlac (CAT) (1 mg/l Fe) were used.

### 1. Selection of best *A. niger* JK strains

The parent strain JK and six selected mutants from over a hundred were further screened using CADP and CAT molasses treated with 6 g HCF/kg molasses of different sugar concentrations in shake flasks incubated for seven (7) days.

Two mutants, JK-j and JK-s gave an overall better

performance in the two trials as given in Table 6.

## 2. Selection of best medium for spore production.

Three different molasses media, treated with 6 g HCF/kg molasses were used, namely; a) Malt agar peptone (MAP), b) Shu and Johnson (10% sugar) and c) Shu and Johnson (25% sugar).

Six-day old spores of JK and four good mutants from different molasses agar slants were propagated in Shu and Johnson molasses medium (20% sugar) and its titratable acidity determined after 7 days. There was no significant difference among the three media but more spores were produced in MAP medium.

## 3. Selection of best medium for citric acid production from molasses.

The medium given by Dipl. Ing. Jiri Kominek contains more components and in bigger amounts than the Shu and Johnson medium. For this reason the two media were compared for citric acid production by the selected JK strain. Under the condition of the experiment with 6 g HCF/kg molasse added the Shu and Johnson medium (> 3% acid) compared favorably with Kominek medium (1% acid) (Table 7).

## B. Stirred tank fermentor

Using Dipl. Ing. Kominek's method and with his supervision several experimental trials were undertaken:

- a. Effect of molasses treatment with different HCF concentrations (5, 10, 12, 14, 15, 15.5 and 16 g HCF/kg molasses). After several trials, 15.5 g HCF/kg molasses was found to be the optimum amount for cleaning molasses before inoculation.
- b. Optimum amount of HCF treatment to inhibit the filamentous growth of the selected JK strain. Observations showed that a maximum of 400 ppm should be added if hyphal growth exceeds 7-8 mm in diameter after 8 hours.
- c. The proper germination of conidia into mycelia for good citric acid producers is shown in Figs. 2-5.

Nineteen fermentation runs were conducted using *A. niger* JK and two kinds of molasses in the 14L NBS fermentor. Results are given on Table B. The highest per cent citric acid was produced from Kominek's medium using CAT molasses treated with 15.5 g HCF/kg molasses (Fig. 6).

### III. UNIDO Expert

Dipl. Ing. Jiri Kominek worked with the staff of the project from 10 February to 7 April 1987. In addition to directing the development of fermentation technology, Mr. Kominek also reviewed the pilot plant design and equipment. His recommendations for the improvement of the design and the instrumentation controls were adapted.

### IV. Training of Personnel

Four of the regular staff were sent to different institutions abroad to undergo training on the different phases of pilot plant production of citric acid. Mr. Melchor Valdecanas studied design and fabrication of equipment at Vogelbush in Vienna, Austria from September 13 to December 13, 1987. At the same time, Mr. Erwin Casareno and Ms. Cynthia P. Madrid underwent training on recovery and purification of citric acid and citric acid fermentation technology respectively at the Institute of Biochemical Technology and Microbiology, Technische University, also in Vienna, Austria. At present, Ms. Blanquita B. de Guzman is still at the Department of Bacteriology, Royal Postgraduate Medical School in London studying the different techniques on strain improvement, having started on September 1, 1987 and will end January 1988.

### V. Sources of Molasses

Due to the high amount of HCF being utilized for pre-treatment of the CAT molasses, different sources of molasses were tapped: Balayan Sugar Mill and PASUMIL through Dr. Ruben Camurungan, President, ARCAM & Co. has been generous in giving us two batches (3 carbouys each) of molasses. Both Balayan Sugar Mill and PASUMIL molasses were found not suitable for citric acid production. It requires more than 20 g HCF/kg molasses before the iron content could be chelated.

### VI. Visit of the UNIDO Director-General to the Pilot Plant.

Dr. Domingo Siazon, Jr., together with two of his directors visited the Pilot Plant last 17 November 1987. According to them, the ICAP Project is the only one of its kind being undertaken under the UNIDO funding. They were quite impressed with the results of the project.

### VII. Meetings

1) A meeting with the Special Projects Services together with the UNDP, UNIDO and NEDA

representatives was held to evaluate the project and looked into the request for extension up to the end of 1988.

2) Several meetings were conducted with FCIERD regarding activities of the project:

- a. The schedule of activities for 1987 and 1988;
- b. The construction of the pilot plant building which will be given to a contractor to facilitate completion by December 1987.
- c. Budget requirements



## CITRIC ACID PILOT PLANT

1. CITRIC ACID: WHAT IT IS

## . Its Properties:

Anhydrous citric acid is a colorless, chemical compound composed of:

Citric Acid (anhydrous), %		99.5
Ash, %	max.	0.05
Heavy Metals (as Pb), mg/kg*	max.	10.00
Arsenic, mg/kg*	max.	3.00
Sulfate, %		trace
Oxalate		trace
Readily Carbonizable Substances		trace

## . Its Uses:

Softdrinks, beverages and pharmaceutical industries constitute the present end-users of citric acid as flavoring agent.

## . Its Imports and Value

In 1987 (See Table 1.0), the Philippines imported a total of 1,478.58 MT of citric acid worth \$2,077,505 from the USA, Brazil, Belgium, Israel, West Germany, England and Ireland. It is projected that the Philippine requirement for citric acid will be 7,100 Tons in 1998.

\* mg/kg = ppm

TABLE 1.0: Import Data of Citric Acid in the Philippines\*

Year	Quantity (ton)
1982	1218.676
1983	1488.429
1984	1830.737
1985	955.954
1986	1168.869
1987	1478.578
1988(Jan-Aug)	1337.810

Aside from the Philippines, other Asian countries like Malaysia and Taiwan are importers of citric acid. For example, in 1986 they imported 1530 MT and 610 MT citric acid respectively.

\* Source : Central Bank of the Philippines  
Bureau of Investments, Department of Trade and Industry

## II. HOW TO PRODUCE CITRIC ACID

The developed technology has two main processes namely: fermentation and the recovery and purification of citric acid.

### A. Fermentation :

The process initially prepares the fermentation wort using molasses/cane sugar as raw material. Appropriate amounts of molasses/cane sugar and other nutrients are dissolved in water. The resulting solution is sterilized prior to fermentation. Fermentation occurs within 6 - 7 days. The fermented broth contains citric acid and mycelia, which is the resulting biomass.

## B. Recovery and Purification of Citric Acid:

The mycelia is separated from the citric acid solution which then is purified by filtration, liming, acidulation, purification, evaporation, decolorization, demineralization, crystallization and drying.

The process flow chart is shown on the following page.

### III. DEVELOPMENT OF THE TECHNOLOGY BY ITDI

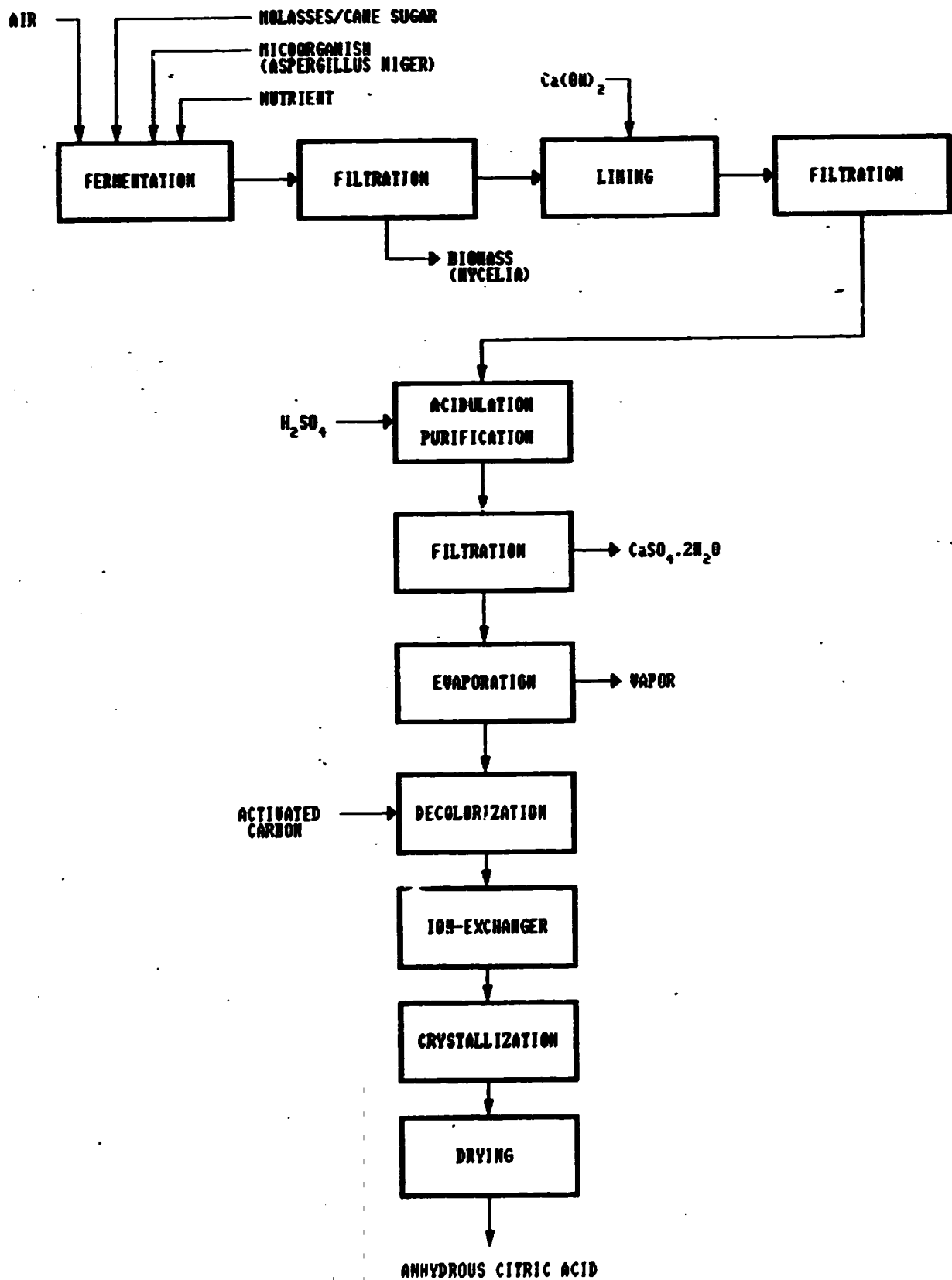
The technology is the result of the project entitled, "Integrated Citric Acid Project". The United Nations Development Program (UNDP) and the Department of Science and Technology (DOST) are financing this project while the Industrial Technology Development Institute (ITDI) is implementing the research and development. The project aims to develop and design indigenous technology for the economic production of citric acid. Using the submerged process, indigenous raw materials like molasses, sugar cane and sucrose are utilized.

The project was implemented by undertaking the following activities :

#### A. Strain Improvement of *Aspergillus niger* for Submerged Citric Acid Production.

This is a laboratory scale operation in which local strains of *Aspergillus niger* were screened and

# CITRIC ACID PROCESS FLOW CHART



improved by mutations and genetic engineering. The resulting improved strain is intended for use in pilot plant operation and commercial production. Strain improvement is an on-going activity in support of R & D and production efforts because stability of strains last for only a few years.

#### B. Development of Fermentation Technology

Done at laboratory and bench scale, optimization of fermentation conditions using indigenous raw materials was the main concern. Temperature, degree of aeration and composition of fermentation medium were determined for optimum yield. Information obtained at this stage was extensively used in designing the pilot plant.

#### C. Recovery and Purification of Citric Acid

Established separation and purification procedures were used to determine the operating conditions, i.e. temperature, pressure, concentration of reactants and concentration of in-process materials, for optimum recovery and acceptable purity level.

#### D. Characterization of Substrate and Product

As the quality assurance stage of the project, it was carried out in cooperation with the ITDI Tests and Standards Division. Testing of the raw materials and

products was done to insure that specifications for both the wet tests for in-process materials were also selected and validated.

#### E. Pilot Plant Testing

The project is now at the pilot plant testing stage. Production conditions will be simulated at pilot scale with the objective of obtaining data for commercialization of the technology.

### IV. ITDI CITRIC ACID PILOT PLANT

The designed pilot plant uses two units of 170 liter airlift fermenter with an annual capacity of 1,000 kgs citric acid. Using the expertise of ITDI technical staff, the pilot plant equipment such as mixing tanks, holding tanks, condensers, steam boiler, crystallizer, evaporator, vacuum pan dryer, filter, carbon column and two airlift fermenters have been fabricated.

The citric acid pilot plant was constructed to establish the technical feasibility and economic viability of local commercial citric acid production. It is designed to utilize various raw materials of similar properties to yield the required end product. The production output of anhydrous citric acid is 1,000 kilograms per year at 80 % recovery. The cost of setting up the plant is about ₱8,700,000.

## V. FINANCIAL HIGHLIGHTS

The estimated investment cost for a commercial production with an annual capacity of 2,000 MT citric acid is as follows :

Fixed Capital	P 97,300,000
Working Capital	P 7,303,000
Pre-operating Expenses	P 13,000
Contingencies	P 4,690,000

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**TOTAL CAPITAL INVESTMENT P109,306,000**

Based on the production experience gained from pilot plant, the above cost may be reduced by the use of locally fabricated equipment.

## VI. POTENTIAL BENEFITS OF COMMERCIAL CITRIC ACID PRODUCTION

o **IMPORT SUBSTITUTION** - Local production could allow the country to be self-sufficient in its requirements. Based on the projection of 7,100 MT by 1998 at \$1,700/MT, this means foreign exchange savings of about \$12 Million.

o **DIVERSIFICATION OF THE SUGAR INDUSTRY** - Since the developed technology utilizes raw materials such as cane sugar and molasses produced by the local sugar industry, local production of citric acid should stimulate the sugar industry particularly as sugar becomes more

difficult to export.

o MANPOWER TRAINING ON DESIGN AND OPERATION OF FERMENTATION BASED INDUSTRY - Commercialization of this developed industry will result to more trained personnel competent in the design and operation of fermentation plants.

o DEVELOPMENT OF OTHER SPECIALTY CHEMICAL INDUSTRIES SUCH AS AMINO ACIDS, ANTIBIOTICS, ENZYMES, ETC. - Beyond the present project, the citric acid pilot plant can be used to establish the technical and economic feasibility of the production of other important chemicals and spur the development of the specialty chemical industries.



App. 3

1500

FEDERAL BUREAU OF INVESTIGATION (FBI)  
 DEPARTMENT OF JUSTICE  
 FEDERAL BUREAU OF INVESTIGATION (FBI)  
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5

CONFERENCE  
ON CITRIC ACID PRODUCTION

CITRIC ACID PRODUCTION  
TECHNOLOGY

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