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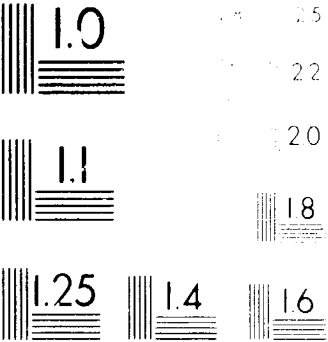
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ENGLISH

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

ST/PHI/81/001

PHILIPPINES. Dextran and fructose production.

Technical Report*

Mission 15 March - 14 April 1984

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

Based on the work of John F. Kennedy,
Consultant in Dextran and Fructose Production

United Nations Industrial Development Organization
Vienna

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GENERAL INFORMATION

General Project Title:	Sucro-based Chemicals
Specific Project Title:	Production of Dextran and Fructose from Sucrose
Project Location:	NIST, Bicutan, Taguig, Metro Manilla, Philippines
Project Leader:	Mr. Vicente C. Borlaza, M.Sc.
	Science Research Specialist III
Project Supported by:	UNIDO and UNFSSTD
Date Project initiated:	1 May 1981

PROJECT OBJECTIVES

General

The purpose of the "sucro-based chemicals" project is to develop the production of chemicals from indigenous carbohydrate raw materials with particular reference to by-products and wastes of the cane sugars industry and to other fermentable raw materials.

Specific

The purpose of the "production of dextran and fructose from sucrose" project is to develop a biological route to the production of pharmaceutical grade dextran and thence a chemical route to the complexation of the dextran with iron. The production of fructose occurs as a side reaction to the biological production of dextran, and the purpose of the project here is to provide food grade fructose from the raw fructose by-product.

INTRODUCTION

The aid to developing countries program entitled "Industrial Chemicals from Indigenous Carbohydrate Raw Materials (Sucro-based Chemicals)" was initiated in the Philippines in 1981 at the request of the Philippine Government. It was supported by UNIDO and UNFSSTD in collaboration with the Philippines National Science and Technology Authority (NSTA).

This report covers a consultancy visit by Professor J.F. Kennedy, Director of the Research Laboratory for the Chemistry of Bioactive Carbohydrates and Proteins, Department of Chemistry, University of Birmingham, Birmingham, B15 2TT, England, U.K whose brief was to direct his attention to the project of production of dextran and fructose from sucrose, one of the specific projects within the overall sucro-based chemicals project. This specific priority project is being carried out at the National Institute of Science and Technology (NIST), a major division of NSTA.

According to the briefing given, the object of the consultancy was: (i) to aid the "production of dextran and fructose from sucrose" project, hereinafter referred to as "the project", in specific detail by bringing in expertise particularly in the following areas: chemistry, biochemistry and fermentation technology of dextran production, analysis and modification, and related carbohydrates, (ii) to evaluate the work done already, and (iii) to evaluate the utilization efficiency of the equipment purchased on UNIDO funds.

The consultancy, the duration of which was 1 month (5th March - 14th April 1984), involved briefing and debriefing at UNIDO headquarters in Vienna, Austria, and detailed consultancy field work at NIST, Bicutan, Manilla, Philippines. The state of the project, in terms of the achievements and experimental results presented; the validity of the theoretical approach and experimental design and the innovative and technical competence of the scientists; the staffing structure; general attitudes of the staff towards scientific progress, professional conduct including safety measures; and the overall leadership were all actively investigated as thoroughly as possible in the time available in what turned out to be a very intensive scientific program.

ACTIVITIES UNDERTAKEN BY THE CONSULTANT

Vienna, Austria

Briefing and debriefing with Dr. M. Maung, Chemical Industries Branch, Division of Industrial Operations, and with staff of the Project Personnel Recruitment Section, Experts Administration and Briefing Unit at UNIDO headquarters.

Metro Manilla, Philippines

On the first day following general discussions with Mr. I.E. Pluhar, SIDFA, at UNDP, Metro Manilla, a brief introductory meeting with the Project Leader, Mr. V.C. Borlaza outlining the general situation led into a tour of the NIST (Bicutan) departments in which work on the project was situated. All staff working on the project were met individually and arrangements were made to interview them. Other important meetings included one as a matter of protocol with Mrs. V.P. Arida, Program Coordinator, head of the department in which the project, Mr. Borlaza and his team are based (Mrs. Arida is not specifically involved in the project other than the fact that the project leader is on her staff).

Discussions also took place at a very early stage with Ms. L.G. Tansinsin, Staff Director, Special Projects Services Staff, NSTA, to discuss the project from the NSTA's viewpoint. At this meeting, which was also attended by Mrs. Tansinsin's immediate support staff and the project leader, note was taken of the financial situation and economy of the project and predictions for the ultimate industrial production of iron-dextrans were considered.

On the second day, all staff irrespective of the seniority or juniority of their level were interviewed so as to give them full opportunity to talk freely, but confidentially, about their particular roles in the project. Each person's work was assessed in detail, and further new experimentation was immediately set up under the consultant's direction. Throughout the visit a high pressure was maintained on experimental work. However, meetings were held with individual members of staff, or groups of staff, as appropriate, on a regular daily basis. The purpose of these meetings was: (a) assessment of work done by staff, (b) correct interpretation of data, (c) design of new experiments to be set up immediately by staff, (d) encouragement of staff to be personally involved in the project as scientists rather than as technicians, (e) promotion of interaction on a scientific level between the staff, (f) instruction in data assessment, in experimental design, in general background knowledge to the project and in specific knowledge in the areas of carbohydrate chemistry, biochemistry, analysis, fermentation and general technology appertaining to the project, and (g) use of the scientific literature. Staff were also given instruction on how to present the results of their investigations in the best possible way, not only in written form, but orally.

Throughout the visit, the consultant constantly interacted with all staff and the theoretical and experimental levels, the execution of each experiment was supervised in depth, and demonstrations in laboratory techniques were given frequently. The consultant placed considerable emphasis on the importance of every member of the team to the success of the project.

Full staff project meetings were held from time to time as deemed appropriate by the consultant, and at these meetings staff were required to give orally properly descriptive presentations of the objectives and reasons for their work and their achievements.

Lectures were given to the staff on various aspects of carbohydrate chemistry and biochemistry, also on the correct professional approach to research and development, and on safety in chemistry, biochemistry and fermentation laboratories.

During the course of the consultancy, two meetings were also held with Dr. F.A. Uriate, Director of the NIST. at which time there was opportunity to discuss the project and its context in the overall scientific development programme of the Government of the Republic of the Philippines.

A public lecture entitled "Advances in Carbohydrate Technology" was given to an audience of about 100 drawn from various departments of NIST, NSTA, and the University of the Philippines.

Visits were made to other NIST establishments, National Standard and Testing Centre, National Engineering Centre, and to various sections of the University of the Philippines - College of Engineering, UP Industrial Research Centre, UP Los Baños Chemistry Department, UP National Institutes of Biotechnology and Applied Microbiology (BIOTECH), and The Philippine Analytical Laboratories Inc, Metro Manilla, and in each case advantage was taken of the opportunities to discuss existing work and to propose new concepts in the areas of carbohydrate and protein production and utilisation against the background of waste materials prevalent in the Philippines.

A concluding meeting with the Staff Director, Mrs. Tansinsin, was utilised by the consultant to present his report in verbal form and to describe the plan of action for the remainder of 1984/5 agreed in principle with the project leader and which would be the subject of the consultant's report and recommendations on his return to Vienna/UNIDO.

PROJECT STAFF

A list of the staff involved in the "Production of Dextran and Fructose from Sucrose" project at NIST, Bicutan is given in Appendix 1. In addition to those named on the list, some other NIST staff gave practical assistance from time to time. The staff in general are young and therefore have a lot of opportunity to gain from experience.

Right from the beginning the staff made an effort to receive the consultant hospitably, and it was quickly possible to develop a team spirit based on the friendly attitude of the staff. The staff responded well to requests, giving a helping hand wherever necessary and generally showing willing to undertake their assignments.

Upon arrival of the consultant, the scientific technical competance of the staff was found to be average and there was room for improvement in the display of leadership. From the viewpoint of their theoretical ability and background and understanding, the staff appeared to have a good group of the knowledge immediately available to them in their immediate area within the project. However the staff were sometimes lacking in knowledge as to why they were doing the work and were generally unaware of the relevance of their work to the overall project and of the need for integration amongst all of those working on the project. The lack of incorporation of adequate controls into experimental design was serious, and the staff were given intensive advice throughout the consultancy to rectify this. It appeared that the literature was rarely consulted for up to date methodology - one of the reasons for this was the lack of library facilities at NIST Bicutan and the considerable distance of NIST from any of the University of the Philippines departments where some library facilities exist.

From the viewpoint of their practical ability and background, although the staff were in control of many techniques in carbohydrate analysis, chemistry and biochemistry and fermentation technology, they were not generally aware of methodology which was more accurate and up to date and which would give improved experimental results.

Generally the staff were competant to use the capital equipment, the cost of which had been met out of the project grant, and in no instance was any equipment out of order due to incorrect or careless use. However the later (1983) rather than earlier (1981) arrival of such equipment has meant that the staff still have to gain considerable experience in the use of the equipment so that they are more competant to deal with experimental trouble shooting. However the intense practical program, mounted at the commencement of the visit and continued through its duration, exposed the staff to a number of experimental techniques, and promoted improved recording of experimental work.

Thus in addition to the general advance of the project through experimentation, the consultant placed emphasis on instruction in theoretical aspects, in experimental design, and in new and improved practical techniques. The staff responded well to the teaching and encouragement given them and to the temporary leadership. They were not afraid to admit their errors and were happy to aim for a new best at every opportunity even if this meant working several hours overtime, and every member of staff deserved the praise they were given. Each one, from the leader downwards, assured the consultant that they would be acting further on the advice given during the consultancy. Mrs. Areda also offered to help the project leader in ensuring the good leadership and success in the work of the project team.

Apart from the program coordinator and the project leader, all staff are involved in conducting laboratory experimental work. It is therefore noteworthy that 50% of the staff are contractual, i.e. 50% of the staff have no guaranteed position beyond the first six months of 1984. This situation clearly caused concern to the project leader, and also to the staff concerned. Undoubtedly in the case of the latter, morale and enthusiasm was dampened at times because of this.

Futhermore, some of the permanent staff, including senior staff, are only designated to work on the project part-time. Whereas these staff graciously devoted most of their time to the project during the consultancy visit, it was clear that they would not be able to do so for much longer.

The part-time position of some staff and the temporary contractual position of others, undoubtedly were sources of considerable concern to the project leader.

By the end of the visit the staff had learnt much that was new to them and it was particularly pleasing to see them applying, of their own accord, what they had learnt in one practical situation to another.

PROJECT DESCRIPTION

Sucrose is one of the largest production commodities in the Republic of the Philippines. High value, low volume derivatives of sucrose are of interest in the development of the economy. The pig farming industry in the Philippines is also of major importance. The mortality rate of infant pigs is considerably reduced by injection shortly after birth with an iron-dextran complex. Currently this material is in short supply, having to be imported and at high cost.

Accordingly UNIDO has chosen to support the project of production of iron-dextran using sucrose as a starter,

Chemically/biologically, sucrose is polymerised into dextran (a (1 $\rightarrow$ 6)-α-D-glucan) either by using microbial cells containing dextransucrase enzyme or by using isolated dextransucrase enzyme. For the former the product dextran must be separated from the cells, for the latter the product dextran must be separated from the enzyme - a simpler case.

In both of these enzymic routes, only half the sucrose molecule is utilised as monomer in the polymerisation reaction, the other half being ejected as a side product - fructose.

The raw dextran so produced must be purified and assessed for its molecular size and extraneous protein content. Since it is produced by the enzyme reaction in a molecular size too large for successful veterinary use, it must be reduced in molecular size by acid and/or enzymic degradation. Furthermore, its chemical characteristics in terms of inter-monosaccharide linkages must be defined, since side reactions in the biosynthesis cause the production of branched chains in the dextran, whereas for product efficacy, the number of side chains is best kept minimal.

When the molecular-size modified dextran has been proved to be of the required standard, it is then to be complexed with iron to produce the iron-dextran. Purification will again be necessary to bring the material to veterinary grade.

The fructose by-product is a sweetening agent, used extensively in the confectionary industry. Thus this by-product could be recovered, and purified to food grade to provide a further marketable product.

Throughout the project, analytical monitoring in various forms will be a major necessity. Various reaction conditions will have to be evaluated and optimised, and the quality of the final product will have to be proven via a variety of routes. Finally there will be the necessity to bring the project from the bench scale through to pilot scale and thence to industrial scale.

It is intended that after pilot scale trials the process will be taken over by Philippine industry, to manufacture iron-dextran for use in the Philippines, i.e. the manufacture of iron-dextran will be conducted outside NIST with perhaps a licence from NIST. The fructose, since it is found in small amounts compared with the overall amounts of sucrose available, would have to find a high value market to warrant its isolation. However, since the fructose would not be expected to be contaminated with glucose, it would rank as having a higher value than standard high-fructose syrups which do contain glucose in large amounts.

The economic viability of the whole project is partially proven, and is worthwhile, but in-depth economic studies need to be conducted in parallel with progress of the project.

STAGE OF THE PROJECT AT CONSULTANCY COMMENCEMENT

In the first two days of the visit all aspects of the work done to date were identified. In view of the time elapsed since the initiation of the project, it did not appear that the work was particularly well advanced. Whereas it is not the function of the consultant to even attempt to apportion blame for this, it is clear that training has taken much of the time in the first two years. This training (including overseas training) has been time well spent both from the project and individuals viewpoint. Furthermore the later rather than earlier arrival of the centrifuge has delayed successful experiments in fermentation where cell removal is essential - a key stage in the biological production of dextran.

However the various disciplines of expertise among the staff (chemist, biochemist, analyst, microbiologist, engineer) and the equipment available provided a very good basis for the consultant to start work. Experimentation had been put in hand by the project leader and his staff in readiness for the consultant's visit, and therefore in no sense was the consultant presented with the difficult task of trying to initiate the project using totally unmotivated and inexperienced workers.

Upon arrival, the project leader presented the consultant with the draft program of activities of the various stages of the project as he had seen fit to schedule for 1984/5. This program is given in Appendix 2. The project leader furnished the consultant with recent quarterly reports, which described some of the experiments conducted thus far. These reports provided useful information, but in certain instances the reports did not present the data in either a conventional or easily readable fashion and this had led staff to come to the wrong conclusion about some of their data (this was soon rectified during the consultancy visit).

Various sub-projects were being actively worked or theoretically considered at the time of arrival of the consultant. These were delineated by the consultant and are listed as sub-projects in Appendix 3.

LABORATORIES OF THE PROJECT AND SAFETY

The laboratory is large open plan with work going on on a number of NIST projects in addition to the project which is the subject of this report. Small, air conditioned rooms off the main laboratory house specialist equipment.

Various aspects of safety in laboratories were lectured upon, and specific matters are listed in Appendix 4, sections A and B. Whereas this list is not certified as being exhaustive, staff would do well to ensure that the relevant authorities do at least comply with the points of these lists.

The consultant was critical of the standards of safety in the laboratory. There was a complete lack of fire fighting equipment in the laboratory, (in spite of one of the other projects being based on petroleum oils), and no alternative means of escape was available. Both these aspects require immediate remedial action (see Appendix 4, Section B).

The consultant was also critical of some of the standards of working in the laboratory, and he drew the attention of all staff to various matters requiring urgent remedial action (see Appendix, section C). Considerable positive response was made by the staff to the matters of this section, but a safety advisor needs to be appointed to ensure that bad habits are not resumed, and that the remedial action initiated is pursued to adequate completion.

EQUIPMENT AND CONSUMABLES

The consultant was given to understand that all the equipment budgeted for in the grant had been supplied and installed. Since this was complete prior to the visit and since the consultant had no equipment list against which to make checks, no equipment inventory and equipment application table is provided in this report.

It is sufficient to mention that the success of the project is totally dependent upon the high speed centifuge, fermenter, spectrophotometer, and hplc equipment already in use. These provide a very valuable array of excellent equipment. A laboratory spray dryer was installed during the course of the consultancy and this will be of use in the production of the dextran and modified dextran.

The non-availability of funds in hard currency for consumables does present problems, and staff are not usually able to change their experimentation quickly if new reagents are required, due to the long delays experienced in ordering and delivery. Similarly, some essential glass-ware items are not available.

However an integrator and the appropriate conversion circuit board are needed for the hplc equipment. Provision of these items would enhance considerably the application of the hplc equipment.

Other equipment urgently required are auto-pipettes (cost approximately $35 each - 15 are required) to facilitate accurate analysis. The only equipment available currently in the laboratory for accurate volume measurement is a poor array of old-fashioned glass pipettes which are not only in a poor state of repair but constitute a safety hazard.

Other equipment also urgently required are accurately thermostatted water baths (mercury contact thermometer $\pm$ 0.1°C (cost approximately $1750 each - 5 are required) to facilitate proper maintenance of controlled reaction conditions. The only equipment currently available in the department as well as the laboratory for heating reaction vessels are general simmerstat water baths without thermostats and can only be maintained to an accuracy of $\pm$ 5°C with constant monitoring by the operator. This situation frequently requires staff to work through the night to watch the baths where reaction times in excess of eight hours are required.

Various other minor pieces of equipment are also required. The summary of equipment required but not necessarily proposed to be funded by UNIDO is shown in the section on recommendations, Appendix 6.

Expenditure on such equipment would be a sound investment. Along with the equipment already provided, these items will be of use for long beyond the completion of the current project.

THEORETICAL AND EXPERIMENTAL WORK CONDUCTED DURING THE CONSULTANCY

Upon arrival and assessment of the project, stages reached to date, equipment and the staff capabilities, the consultant deemed that the future work associated with the project, would fall into the following categories:-

a. Production of dextran from cells
b. Production of dextran from dextransucrase
c. Analytical assessment of raw dextran
d. Hydrolysis of raw dextran
e. Purification of fructose and analytical assessment
f. Chemical modification of hydrolysed dextran
g. Purification of modified dextran
h. Complexation of dextran with iron
i. Testing of iron-dextran
j. Scale up of all sections a-i to pilot plant scale.

For each of the sections, considerable ground work has to be done, since although general and specific guidelines can be obtained from the literature, local conditions, strains of dextran-producing organisms, the absolute structure of the dextran actually produced etc., all require fundamental research and development work. Ground work had been pursued to some extent prior to the consultants arrival in that work within each of the sub-project areas listed in Appendix 3 had been attempted, although in many of these the sub-projects had not advanced to a definitive stage. It was therefore the consultant's opinion that the work to date had given experience to the staff and provided a useful background on which to start work.

Nevertheless, because of the lack of progress in some areas, and the wrong conclusions having been drawn from previous data, it was best to put in hand immediately both experiments which either confirmed, denied or improved previous work, and experiments which would take the project forward.

The consultant's early decision was that categories a-e inc. should be pursued immediately so that sufficient ground work and model studies would have been covered by the end of the consultancy so as to enable the staff to progress markedly in these areas over the following months of 1984. The consultant also decided that in addition to there being no suitable product raw dextran for the studies, current working on categories f-j would dissipate staff effort on a-e, cause overload, and general lack of progress. It was not considered that the staff were yet adequately experienced with the project to tackle realistically categories f-j. Therefore the project was seen to require three phases of work:

Phase 1	Categories a-e
Phase 2	Categories f-i
Phase 3	Category j

Theoretical and experimental work during the consultancy visit was therefore confined to categories a-e.

To facilitate the work, the staff were divided into sub-groups according to their training, qualifications and expertise, so that groups of staff could identify with particular sub-projects and be vested with responsibility for same and reporting to the project leader. Minor adjustments were made to these groupings as work proceeded, and the final structure is incorporated in the recommendations.

Prior to departure from England the consultant had set up telephone communication channels with his senior research fellow back in England, and this enabled the consultant to order a steady stream of literature, samples and standards from the UK, in addition to those he had bought with him, for use on the project during the course of the visit. These essential materials would otherwise have been unavailable to the NIST staff.

The following stage of work had been achieved by the end of the Consultancy visit:

(i) Raw dextran has been successfully produced by the cell fermentation route.

(ii) Fermentations to produce dextransucrase have been run, with partial success.

(iii) An assay for dextransucrase has been established.

(iv) Assays for total carbohydrate and protein contents of raw dextrans have been established and applied.

(v) Linkage analysis for (1 $\rightarrow$ 6)- and other linkages in dextrans have been established, and applied with partial success.

(vi) Hplc and gpc analysis conditions have been devised whereby the molecular size distributions of dextrans can be defined very successfully.

(vii) A range of acid hydrolysis conditions has been investigated for the degradation of macromolecular raw dextran to molecular sizes suitable for conversion to veterinary iron-dextran.

(viii) Small scale model ion-exchange/ion-exclusion chromatographic columns potentially suitable for the partial purification of fructose have been set up.

ASSESSMENT OF THE PROJECT AND FUTURE PROGRAM

The consultancy visit provided a successful opportunity for rationalisation, rejuvenation, and acceleration of the work of the team. The team is well structured in that it has a number of expertises because of the different training backgrounds of personnel. Its team now has the opportunity to develop into an important and successful research and development team in NIST, majoring in carbohydrates. The project leader should seize this opportunity so that the progress of the team can be seen beyond the conclusion of the iron-dextran project, when attention could then be profitably turned to other carbohydrate projects of commercial importance, based on expertise gained during the duration of the iron-dextran project. This in turn will depend upon the project leader's careful attention to forward planning for grant applications, staff, equipment renewal and enlargement, and consumables.

The groupings of staff as in Appendix 5 should be maintained as the principle arrangement. It should be noted that Group 4 could find itself understaffed for the demanding program it must undertake if at least 2 members cannot work full-time on the project. The project leader should continue in his position and should work at integration of all members of his team, holding regular research meetings at which all staff should contribute so that all may feel they are an important member of the team. Further teaching of how to design experiments so that they are properly controlled and yield the maximum amount of reliable information should continue. The project leader should encourage his staff to widen their visions as to the ultimate goal of the project, and to appreciate that the project is providing them with expertise in carbohydrate chemistry and biochemistry and fermentation which will be of value to them in the general context of carbohydrate research and development in the Republic of the Philippines. Where relevant the staff should be exposed to new techniques within the field, and all staff should be taught the reasons, rationale and principles of what their colleagues within the team are doing. They should also be encouraged in original thought and innovation. Staff should be allowed to publish their development research where this would be of interest to other workers in the field.

Any residual staffing problems for the project should be resolved as a matter of urgency so that as far as possible a stable project team can be identified. This would be of great assistance to the morale of the team and the advantage of the project leader, but the project leader might ease the situation for the future planning by stating his case very clearly to his seniors.

The lack of certain pieces of equipment should be remedied swiftly - it is surprising that requests for some have not been made previously. Clearly some of these are outside the scope of funding by UNIDO but should be funded from within the Republic of the Philippines. A list of equipment etc. needed is given in Appendix 6.

Two or three further short-term (2-4 months) overseas training periods would benefit the team considerably. These should be directed at analysis techniques, carbohydrate modifications, and transitions from bench to pilot plant.

The project should be advanced by work on Phase 1 in the areas shown in the research and development program (Appendix 8).

In the meantime the project leader should present reports, based on work in Phase 1, bimonthly for consideration by UNIDO and their appointed expert consultant, so that UNIDO may be assured that satisfactory progress is being made. It is recommended that progress reports be forwarded not later than:

30 June 1984
30 August 1984
30 October 1984

Concurrent with this, economic studies of the overall process in the Republic of the Philippines should be conducted and the results made available. During this time the consultant will assess these reports, and will when possible, provide assistance in the form of correspondence, and supplies of special standards and of suitable literature. The consultant should then make a further visit at the end of November 1984 to deal with promotions of the work on Phase 2, i.e. on chemical modification of the dextran and its complexation with iron, and to deal with any remaining problems outstanding from completion of Phase 1. At the return consultancy visit, a series of lectures on carbohydrate chemistry and biochemistry should be given. It would be advisable for the consultant to spend 5 weeks in the field, in addition to briefing and debriefing in Vienna on this occasion.

RECOMMENDATIONS

The project has now been brought to a stage where it is progressing satisfactorily and warrants the further interest and support of UNIDO. The project should be seen to go through Phase 1 (Appendix 7) in 1984, and Phase 2 in early 1985, Phase 3 in late 1985 (Appendix 8), and should be further aided by provision of equipment (Appendix 6) and short-term overseas training sessions. Reports for the remainder of Phase 1 should be provided bi-monthly (Appendix 8) and the consultant should maintain advisory correspondent contact throughout the period covered by these reports culminating in a five week consultancy visit to NIST at the end of 1984, during which further practical and technical advice will be given together with a teaching-lecture program.

The consultant expresses his willingness to participate in this way and wishes to draw the attention of UNIDO and NIST to other viable projects worthy of promotion and support at NSTA/NIST, particularly in the areas of (i) utilisations of carbohydrate wastes, (ii) fermentative production of the industrially important polysaccharide xanthan, and (iii) production of sucrose esters as detergents/surfactants and of other sucrose derivatives.

APPENDIX 1

Staff
involved in the Sucro-based Chemicals Project
on
Dextran and Fructose Production from Sucrose
at
National Institute of Science and Technology

1. Mrs. Violeta P. Arida - Program Coordinator (Supervising Science Res. Specialist)
2. Mr. Vicente C. Borlaza - Project Leader (Senior Science Res. Specialist)
3. Mrs. Daisy L. Binlayo - Study Leader (Science Res. Specialist II)
4. Mrs. Araceli P. Lozano - Study Leader (Science Res. Specialist II)
5. Mrs. Malen P. Usita - Science Res. Specialist II
6. Miss Portia G. Jimenez - Science Res. Specialist II
7. Mrs. Socorro B. Tan - Science Res. Specialist III (contractual)
8. Mr. Bernardo G. Senica - Science Res. Specialist I (contractual)
9. Miss Josette H. Hiñola - Science Res. Assistant II (contractual)
10. Mr. Dominador C. Aguino - Science Res. Assistant II (contractual)
11. Miss Ursela P. Guce - Science Res. Assistant I (contractual)
12. Mr. Josejch Sebud - Emergency Employee (on-training)

APPENDIX 2

Project Leader's Original Planned Activities for 1984/5

on

Production of Dextran and Fructose from Science

at

National Institute of Science and Technology

1984

1. Verification/validation work on the production of cell-free raw dextran by the enzymatic process.

2. Hydrolysis/fractionation studies on raw dextran.

3. Purification of the dextran fractions into its commercial grade.

4. Further purification of the commercial grade fractions into pharmaceutical grade.

5. Toxicity test of the desired dextran fractions.

6. Recovery of by-product fructose.

7. Economic feasibility studies on the production of dextran fractions of commercial value.

8. Initial studies on iron-dextran.

1985

1. Studies on the production of iron-dextran.

2. Bench-scale studies on the production of raw dextran based on the established laboratory scale conditions.

3. Bench-scale studies on the hydrolysis and fractionation of dextran based on the established laboratory scale-conditions.

4. Bench-scale studies on the production of iron-dextran.

5. Economic feasibility studies on the production of iron-dextran.

APPENDIX 3

Sub-Project Areas under Action at Commencement of Consultancy
in
Production of Dextran and Fructose from Sucrose
at
National Institute of Science and Technology

1. Fermentative production of dextran.
2. Isolation of raw dextran from fermentation by precipitation.
3. Molecular size estimation of raw dextran by GPC.
4. Carbohydrate and protein compositional analysis of raw dextran.
5. Linkage analysis of dextran.
6. Acidic and enzymic degradation of dextran.
7. Fermentative production of dextransucrase.
8. Separation of fructose by column chromatography.

APPENDIX 4

Safety Aspects of Laboratory Work
for
Production of Dextran and Fructose from Sucrose
at
National Institute of Science and Technology

A. Knowledge of fire and emergency regulations

1. What to do in case of a fire

2. Fire drill

3. Permanent notices describing fire precautions, evacuation procedures, assembly points.

4. Notices dealing with how to raise the alarm.

5. Fire alarms.

6. Fire fighting equipment.

7. Knowledge of location of nearest fire fighting equipment.

8. Availability of an emergency telephone.

9. Information on the location of the control centre from which expert help can be obtained in a case of emergency.

10. Arrangement of fire officers and floor sweepers.

11. Fully equipped first aid cabinet.

B. Types of extinguishers

1. Carbon dioxide - for solvent fires and electrical fires.

2. Foam - for more-serious solvent fires.

3. Fire blankets.

C. Particular Safety Aspects of the Laboratories

1. Use of safety spectacles - at least for dispensing of acids, alkalis and other hazardous chemicals, and for handling of equipment likely to be under increased or decreased pressure.

2. Correct labelling of all containers, both temporary and permanent.

3. Proper securing of all compressed gas cylinders by chaining to benches or pillars.

4. Planning of use of electrical equipment so that no equipment has flexes trailing across walkways.

5. Reduced pressure glass equipment - plastic netting safety covering against implosion to be madatory.

6. General bench tidiness and organisation.

7. Designation of clean areas including sinks with clear notices for eating and drinking.

8. Chemical disposals system for solvents not to be put down drains.

9. Listing as a public record of all cultures of organisms kept in the laboratory.

10. Provision of a locked "poisons cupboard" for retention of all hazardous chemicals.

11. Adequate segregation of correct storage facilities for different classes of chemicals, e.g. solvents and acids.

APPENDIX 5

Staff Grouping Organisation
for
Production of Dextran and Fructose from Sucrose
at
National Institute of Science and Technology

Project Leader: V.C. Borlaza

Group 1

A.P. Lozano
D.C. Aquino
U.P. Guce
J Sebud

Group 2

A.P. Lozano
D.C. Aquino
U.P. Guce
J. Sebud

Group 3

S.B. Tan
B.G. Senica
P.G. Jimenez

Group 4

S.B. Tan
J.H. Hiñola
M.P. Usita

Group 5

D.L. Binlayo
P.C. Jimenez
M.P. Usita

APPENDIX 6

Equipment and Consumables Required
for
Production of Dextran and Fructose from Sucrose
at
National Institute of Science and Technology

Integrator for Waters hplc equipment	1 off
Circuit board for interfacing integrator with hplc equipment	1 off
CO_2 fire extinguishers, 10lb size	10 off
Fire blankets	5 off
Foam fire extinguishers	2 off
Thermostatted water baths	5 off
Autopipettes - range of sizes	15 off
Hydrolysis tubes and general glassware	
Special chemicals for standard carbohydrate analyses	

APPENDIX 7

Research and Development Program to Complete Phase 1

on

Production of Dextran and Fructose from Sucrose

at

National Institute of Science and Technology

All staff currently engaged in the project will participate actively in this program. Staff are to work in the group as per Appendix 5.

Group 1 Dextran Production

1a Identify other strains of organisms including local ones which yield dextran.

1b Define optimum fermentation conditions for maximum yield of dextran. Modify conditions in light of data on product dextran structure provided by Group 4.

1c Define best centrifugative method for removal of cells from raw dextran solution.

1d Define conditions under which amount of propan-2-ol required to precipitate raw dextran is minimal.

1e Study spray drying of dextran solutions.

1f Study storage stability of precipitated dextran.

Group 2 Dextransucrase Production

2a Identify other strains of organisms including local ones which yield dextransucrase.

2b Define optimum fermentation conditions for maximum yield of dextransucrase. Modify conditions in light of data on dextransucrase yield and potency provided by Group 3.

2c Demonstrate the cell-free biosynthesis of dextran using the dextransucrase generated. Modify conditions in light of data on product structure provided by Group 4.

2d Investigate the optimum method of storing dextransucrase.

2e Optimise the conditions of use of dextransucrase to maximise the yield of dextran.

2f Utilise information from Group 1, section 1d, 1e, 1f above to optimise the recovery, storage stability and spray-drying of dextran.

2g Initiate studies on the immobilisation of dextransucrase.

Group 3 Dextransucrase Assays

3a Use existing reducing sugar assay to provide instant monitoring of products from Group 2, sections 2a and 2b.

3b Use hplc equipment to assay utilization of sucrose in fermentations by Group 2 in section 2b.

3c Develop simpler assay for dextransucrase using dinitrosalicylic acid assay for reducing sugars.

3d Apply improved dextransucrase assay to work of Group 2 all sections.

Group 4 Dextran Analysis and Hydrolysis

4a Formalise method of determination of total carbohydrate in dextran samples from Group 1, section 1b.

4b Formalise method of determination of total protein in dextran samples from Group 1, section 1b.

4c Using hplc gpc, and model dextrans as standards, define the chromatographic conditions under which dextran can be identified as a function of molecular size independent of its structure, dispersity, and solution concentration.

4d Study the molecular sizes of dextrans produced by Group 1, section 1b, and Group 2, section 2c.

4e Study the linkage spectrum in dextrans produced by Group 1, section 1b, and Group 2, section 2c.

4f Study the conditions of hydrolysis of dextran to yield molecular sizes relevant to production of veterinary iron-dextran. Use model dextrans and hplc gpc initially for this purpose. Complete the work using dextrans produced by Groups 1 and 2.

<u>Group 5 Fructose Recovery</u>

5a Using standards define the chromatographic conditions for 100% recovery of fructose from ion-exclusion chromatographic columns at maximum fructose concentration.

5b Extrapolate use of ion-exclusion columns to purification of fructose from fermentation media produced by Group 1, sections 1b and 1c, and Group 2, sections 2b and 2c.

5c Consider the economic feasibility of fructose recovery.

5d Monitor chromatographic columns using hplc.

5e Investigate the degree of separation of fructose from impurities on the chromatographic column, and if necessary change columns to ion-exchange and carbon as appropriate, following existing work on production of fructose syrups from glucose.

5f Investigate the concentration of fructose solutions to fructose syrups.

APPENDIX 8

Program of Project Phases and Reports
on
Production of Dextran and Fructose from Sucrose
at
National Institute of Science and Technology

Phase 1

To be completed by 31 December 1984.

Further equipment to be provided.

Overseas training to be arranged for 2 to 3 staff.

Reports to be furnished to UNIDO:

30 June 1984

30 August 1984

30 October 1984

and to be immediately reviewed by consultant.

Consultant to provide correspondent advice and then to make consultancy visit to the field November 1984 to conclude Phase 1.

Phase 2

To be initiated during consultant's visit at end of 1984.

To be continued with a view to completion by mid 1985.

Reports to be provided to UNIDO.

Consultant to provide correspondent advice.

Phase 3

To be initiated late 1985.

Further decisions to be taken during consultant's Nov./Dec. 1984 field visit.

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