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To: AID Project Support Officer

Subject: Progress Report

Grant: No. 936-5542-G-SS-4003-00

Title: Optimization of Bioconversion of Liquid and Solid Residues.

Principal Investigator: António V.Xavier, Ph.D.

Applicant Organization: Centro de Química Estrutural

Period Covered: July 3 to December 31, 1984.

SIX MONTH REPORT

1. ENGINEERING

Due to unforeseen delays in receiving the new building at the Faculty os Sciences and Technology that should have been completed late last August (see enclosed letter from the Director), the engineering side of the program has not fulfilled the schedule - we are approximately two months behind, as only the construction of the reactors and buying of equipment has taken place. This is also the reason why, as per passed correspondence, we have not yet installed the gas cromatograph. Meanwhile, we have collaborated with two portuguese ethanol producing firms of Torres Novas (Vieira & Irmão and Sociedade Lusitana de Distilação) to build a pilot plant to allow us to scale up results obtained in the laboratory. This will have approximately 1 m^3, will be of fixed bed design and will operate at thermophylic temperatures, less usual than normal and thus scientifically more interesting. If all goes well this will start by late March as the design has now been finalised.

2. SCREENING OF ENZYMES AND COFACTORS

As previously indicated (Time Schedule. AID Proposal) the first year of the Project will be mainly devoted to the screening of enzymes and proteins isolated from representative microorganisms (sulfate reducing and methane forming bacteria). Two main objectives were considered:

- interspecies H_2 transfer (relevant for bacterial

hydrogenases).

- Study of cofactores, in order to control microbial digester populations, in future work.

2.1. ORGANISMS USED

Sulfate reducing bacteria: *Desulfovibrio gigas* (NCIB 9932) and *Desulfovibrio vulgaris* (Hildenborough).

Methane forming organisms: *Methanosarcina barkeri* (DSM 800 and 804) the most versatile of the mesophylic methanogens, in respect to substrate utilization.

Sulfur utilizing organisms: *Desulfuromonas acetoxidans*.

2.2. ENZYMES PURIFIED AND CHARACTERIZED

2.2.1. *D.gigas* Hydrogenase (1)

Due to a modified method of purification, requiring the use of crude cell extracts instead of extracts obtained by cell washing, The enzyme is now purified in large scale *D.gigas* hydrogenase is a [NiFe] hydrogenase. It belongs to a class of enzymes that are inactive "as isolated" (the so called "oxygen stable hydrogenases") and must go through an activation process.

The state of characterization of the enzyme "as isolated" prompted us to perform a detailed analysis of redox patterns, activation profiles, and catalytic cycle of the enzyme in the presence of hydrogen. The information available was discussed in terms of a "working hypothesis" for the mechanism of action of [NiFe] hydrogenases.

2.2.2. *M.barkeri* hydrogenase

The methanogenic bacteria are a diversified group of microorganisms that produce methane from a limited number of substracts. *M.barkeri* is one of the most versatile mesophylic methanogens. The hydrogenase of this acetoclastic organism was purified and characterized. This is the first hydrogenase obtained from methanol grown cells. The enzyme is a flavin, nickel and iron-sulfur containing protein, with a specific activity of 270 moles of H_2 produced (min.mg.of protein).

2.2.3. Sulfite reductase

Two siroheme containing proteins were purified from *Dm.acetoxidans* and *M.barkeri*. The proteins have sulfite reductase activities and are analogous to the *D.vulgaris* enzyme, also isolated (3).

A link between the sulfur and hydrogen metabolisms was established in *M.barkeri* (2) and discussed in terms of the metabolic pathways.

2.3. COBALT COFACTORS (4)

Two B_{12} proteins were isolated from *M.barkeri* (DSM 800 and 804). The Co cofactor was identified by NMR and EPR spectroscopies.

The corrinoids were also isolated and purified from cell extracts. Several corrinoids were obtained and it was shown that Factor III (the 5,6 dimethylbenzimidazole base present in Vitamin B_{12} is replaced by 5 hydroxylbenzimidazol) is the most abundant corrinoid in *M.barkeri* cells and is also

the corrinoid associated with the B_{12} protein.

3. SCIENTIFIC EXCHANGE

I.Moura, J.J.G.Moura and A.V.Xavier visited the University of Georgia at Athens, where part of the research results presented were obtained and participated in a Workshop on "Redox Proteins from Sulfate Reducing Bacteria: What do we know? Where do we go?", presenting 4 communications. Prof. J.LeGall, from the same University, visited our laboratory.

A.V.Xavier participated, under invitation from A.I.D., in a Workshop on Biogas Technology held in Boston (Dynatech).

M.J.T.Carrondo presented two communications in the Cairo Biogas Conference, under invitation from A.I.D. and is now sending advice to three african researchers (report sent to A.I.D. Lisbon Mission on January, 1985).

Following the visit to the USA last August by M.J.T.Carrondo and in relation with the Biotechnology Strategy Group at Quimigal, Prof. George Tsao of Purdue University visited us and should, in the future, tie up more and more with the cellulose bioconversion program (see attached announcement for his conference).

4. BIBLIOGRAFY

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C O N F E R Ê N C I A

CHEMICALS FROM RENEWABLE RESOURCES

Proferida pelo

Prof. George T. Tsao

Director do
Laboratory for Renewable Resources Engineering
Purdue University, U.S.A.

2ª Feira, 3 de Dezembro de 1984
17.00 horas

Ala lateral do Salão Nobre do
Instituto Superior Técnico

Com o patrocínio da U.S.A.I.D.,
Sociedade Portuguesa
de Biotecnologia e Quimigal, E.P.

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