MICROBIOLOGY DEPARTMENT

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PROGRESS REPORT NUMBER V

BEGINNING MAY 1, 1966 and ENDING OCTOBER 31, 1966

STATE COLLEGE, MISSISSIPPI

N A S A

PROGRESS REPORT NUMBER V

INFLUENCE OF METABOLIC ACCUMULATION OF PRODUCTS OF HYDROGENOMONAS CELLS ON THEIR CONTINUED GROWTH

NASA Research Grant No. NsG 650 to the Mississippi State University

For period beginning May 1, 1966 and ending October 31, 1966

Principal Investigator: Dr. Robert G. Tischer
Professor and Chairman
Department of Microbiology
Mississippi State University
State College, Mississippi 39762

DISTRIBUTION LIST OF N A S A, REPORT V

Dr. Leonard H. Bongers
Martin Marietta Company
Space Systems Division
Research Department
Baltimore 3, Maryland

Dr. Doris H. Calloway
Department of Nutritional Sciences
University of California
Berkeley, California 94720

Dr. John F. Foster
Physical Chemistry Department
Battelle Memorial Institute
505 King Avenue
Columbus, Ohio 43201

Mr. Bernard H. Goldner
Magna Corporation
Research and Development Division
1001 South East Street
Anaheim, California

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Bioscience Programs
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Office of Space Science & Applications
National Aeronautics and Space Administration
Washington, D.C. 20546

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Washington, D.C. 20546

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National Institutes of Health
Bethesda, Maryland 20014

Dr. Joseph Saunders
Chief, Environmental Biology
Bioscience Programs
Office of Space Science Applications
National Aeronautics & Space Administration
Washington, D.C. 20546

Dr. Henry M. Tsuchiya
Department of Chemical Engineering
University of Minnesota
Minneapolis, Minnesota

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SUMMARY

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Of the sugars tested, H. eutropha grew only on fructose. It produces several simple sugars as metabolic products.

The first information on the thermophilic hydrogenomonad has been accepted for publication. Work will continue in an effort to find new thermophilics and to compare these with H. eutropha.

Mutation studies have not produced useful results in terms of auxotrophic mutants which produce polysaccharides and will be both continued and expanded.

Large batch culture techniques will be strengthened if appropriate monitoring equipment is made available. At present the spray-nozzle technique is under continuous investigation.

Author

I. Extracellular Products of Hydrogenomonas eutropha

As previously reported, four sugars have been isolated from the spent medium on which Hydrogenomonas eutropha had grown. These were identified by paper chromatography as glucose, ribose, arabinose and xylose. Studies indicated that these sugars, along with galactose, lyxose, mannose, rhamnose, sedoheptulose, sorbose, and sucrose could not be oxidized by either autotrophically or heterotrophically grown cells of Hydrogenomonas eutropha, nor could these sugars support the growth of this organism. The presence of these sugars in the medium did not detectably affect the autotrophic growth of Hydrogenomonas eutropha. (See Table 1.)

Fructose which was not detected in the spent medium, was the only sugar tested which could be metabolized by Hydrogenomonas eutropha. Growth of this organism in basal medium containing 0.5% filter sterilized fructose under an atmosphere of air was abundant. In manometric experiments, autotrophically grown cells required about a twenty minute adaptive period before they could oxidize fructose, whereas fructose grown cells utilized fructose without lag.

When fructose grown cells were offered fructose, they exhibited a respiratory quotient of near the theoretical value of one indicating that fructose was converted almost quantitatively to carbon dioxide. With autotrophically grown cells, the respiratory quotient remained at zero until the cells became adapted to fructose, then the RQ value rose to slightly less than one half of the theoretical value. This may have indicated that the autotrophically grown cells were assimilating about half of the carbon dioxide produced.

Although Hydrogenomonas eutropha was unable to utilize glucose, it was found that this organism would grow abundantly in a medium containing glucose prepared by autoclaving the glucose in the buffer before the addition of the other components of the basal medium. Cells grown on the autoclaved glucose medium were shown to utilize fructose without lag in manometric experiments. Chromatographic studies of the autoclaved buffered glucose solution revealed that it contained fructose. Apparently the glucose had been changed during autoclaving in a reaction similar to the Lobry-de Bruyn-Alberda van Ekenstein transformation to yield fructose. This may explain why other workers have been able to grow Hydrogenomonas eutropha on glucose.

Autotrophically growing cells were shown to adapt to and to utilize fructose under a H_2-O_2-CO_2 atmosphere. However, the utilization of fructose did not affect the quantity of gas used or the rate at which the gas was used by this organism.

Hydrogen in the absence of carbon dioxide was found to inhibit the ability of autotrophically grown cells to adapt to fructose. This inhibition could be removed by replacing the hydrogen-oxygen atmosphere with air.

Manometric experiments indicated that the tricarboxylic acid cycle was operative in Hydrogenomonas eutropha because most of the intermediates of this cycle were utilized by both autotrophically and fructose grown resting cells without lag.

The presence of the enzymes associated with the Entner-Doudoroff metabolic pathway were demonstrated in fructose grown cells of Hydrogenomonas eutropha. These enzymes were also found in autotrophically grown cells, but to a lesser extent. Therefore, it appeared that the Entner-Doudoroff pathway was responsible (wholly or in part) for the metabolism of fructose by Hydrogenomonas eutropha.

Table 1. Growth of *Hydrogenomonas eutropha* in the presence of various sugars.

Medium	Measurements	Arabinose	Fructose	Galactose	Glucose	Lyxose	Mannose	Rhamnose	Ribose	Sedoheptulose	Sorbose	Sucrose	Xylose	Control
Basal Medium With Sugar Under Air	Final O.D.[1]	0.05	1.25	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
	Initial Sugar (mg/ml)	3.60	5.00	2.10	4.80	4.10	2.70	3.50	3.20	---[2]	4.00	---	5.10	0.00
	Final Sugar (mg/ml)	3.70	0.00	2.10	4.90	4.10	2.60	3.50	3.20	---	4.00	---	5.50	0.00
Basal Medium With Sugar Under H_2-O_2-CO_2	Final O.D.	1.45	2.65	1.45	1.50	1.45	1.45	1.60	1.50	1.50	1.40	1.45	1.40	1.50
	Initial Sugar (mg/ml)	3.70	5.10	2.20	4.90	4.20	2.70	3.00	3.30	---	4.00	---	5.10	0.00
	Final Sugar (mg/ml)	3.70	0.00	2.10	5.00	4.20	2.80	3.10	3.40	---	3.90	---	5.00	0.00
Nutrient Broth With Sugar Under Air	Final O.D.	2.30	3.00	2.35	2.25	2.30	2.30	2.25	2.25	2.35	2.35	2.25	2.30	2.35
	Initial Sugar (mg/ml)	3.20	4.90	2.30	5.00	4.10	2.70	2.90	3.50	---	4.50	---	5.00	0.00
	Final Sugar (mg/ml)	3.30	0.00	2.40	4.90	4.00	2.70	3.00	3.50	---	4.40	---	4.90	0.00

[1]Initial O.D. = 0.05 in all cases.
[2]These sugars gave no color reaction using Nelson's (1948) method.

II. A High Temperature Hydrogen Oxidizing Organism

One publication resulting from this discovery has been accepted by NATURE and will appear soon. (See Reference at end.)

Work will continue to foreward the comparison of this thermophile with H. eutropha as a possible constituent of the space ecology system.

The thermophile will also be included in the work on mutation to attempt to isolate a polysaccharide producing mutant or to establish the stability of the new strain.

III. The Isolation of Polysaccharide-Producing Hydrogen-Utilizing Micro-organisms

Considerable efforts to apply ultra violet irradiation have cost a considerable amount of time, hundreds of petri plates and medium. The net results which were sent to Dr. DeCicco were two unimpressive mutants. The sum of these occurrences suggests that H. eutropha is a fairly stable microorganism.

The efforts to produce a polysaccharide-producing strain are continuing and plans are to use several types of mutagens and to devise, if possible, better methods of detecting auxotrophs which produce polysaccharides.

IV. Large Batch Cultures

Studies with the large batch culture apparatus were continued. During the experiments, the gas mixtures, liquid volume, and growth medium constitutents were varied in an effort to increase the growth rate and total growth of Hydrogenomonas eutropha.

The results of the experiments are as follows:

The volume of growth medium used in the apparatus was 6 liters. This volume was used throughout the experiments.

The gas mixtures which proved best for growth in the large batch apparatus consisted of 67%-70% hydrogen, 20%-22% oxygen, and 10%-11% carbon dioxide.

The simplified medium as described by Bongers (1965) supported the growth of H. eutropha but yielded somewhat less growth than did the normal growth medium.

Increased concentrations of urea in the medium changed the growth rate or total growth sufficiently to merit the addition of urea in quantities above 1.0 gram per liter.

The growth experiments normally ran for a 48 hour period.

The results show that when using the growth medium described by Bongers (1963) and the 67% hydrogen, 22% oxygen, and 11% carbon dioxide mixture, the large batch culture apparatus will yield an average harvest of 2.0 to 2.5 grams dry weight of cells per liter of medium in a 48 hour growth period.

From the results of an extended growth experiment in which portions of the cells were harvested and fresh medium was added at intervals, it seems possible that this system might be modified for the continuous culture of H. eutropha.

V. Future Research

Attention will be shifted from neutral metabolic products to acid and basic products with special attention also to the separation and identification of proteins and protein break down products.

New attempts will be made to isolate thermophiles and the thermophile in hand will be tested for genetic stability.

Application of ultra-violet to H. eutropha produced few mutants and none which produced polysaccharides but this search will be continued.

Large batch cultures will be continued as in the past, in an effort to perfect the method in use. If additional monitoring equipment is made available, additional work will be undertaken to prove the course of change of pH, Eh, turbidity, temperature, dry weight, etc. during closely controlled runs in an effort to find maximum operating conditions by sequential iteration.

VI. References and Notes on Publications Forthcoming

1. Cook, D.W., Brown, L.R., and Tischer, R.G. Metabolic Pathway of Fructose in Hydrogenomonas eutropha. Submitted for publication.

2. McGee, J.M., Brown, L.R., and Tischer, R.G. Isolation and Characterization of a High Temperature, Hydrogen-Oxidizing Bacterium--Hydrogenomonas thermophilus, n.sp. Accepted for publication by NATURE.

3. Cook, David W., Tischer, Robert G., and Brown, Lewis R. Carbohydrate Metabolism in Hydrogenomonas eutropha. Accepted for publication by Canadian J. of Microbiology.

EXPENDITURES TO DATE

FISCAL YEAR 1966--1967

Equipment	$943.40	
Supplies	1834.52	
Travel	18.76	
Salaries and Wages		
Professional	1535.21	
Non-professional	280.00	
TOTAL EXPENDITURES:		$4,611.89