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PROGRESS REPORT

BIOCHEMICAL STUDY OF MIXED CULTURE PROTOTYPE IN A CLOSED ECOLOGICAL SYSTEM

NASA Research Grant No. NsG 80-60 to the Mississippi State University

For period beginning June 1, 1960, and ending November 30, 1960.

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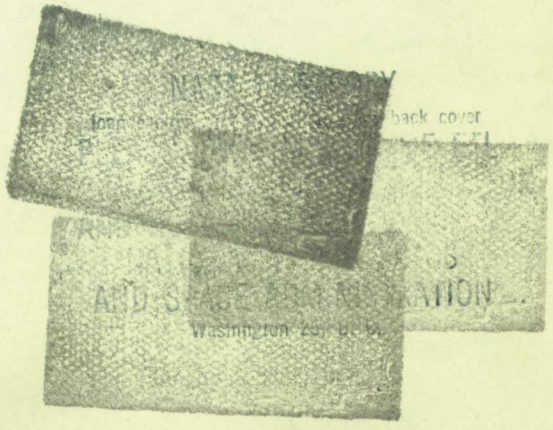

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Introduction:

Since June 1, 1960, the date of initiation of this research project, efforts have been directed toward studying cultural and fermentation patterns and the methodology of pure culture isolation of prototype microorganisms to be employed in closed ecological systems.

The research activities herein presented are divided into two major categories: research performed and research currently in progress or to be initiated in the near future.

I. Research Performed.

A. Filtration Studies on Feces.

A method designed to facilitate filtering rate of fresh feces through the enzymatic hydrolysis of intestional mucin by micro-organisms, namely members of the genus Shigella was found not to be feasible. No apparent change was observed in the filtering rate of feces inoculated with cultures of Shigella dysenteriae and Shigella flexneri as compared with the uninoculated feces.

B. Cultivation of Molds (Fungi) on Feces and Urine.

Studies were made to determine the feasibility of converting human wastes into fungal protoplasm. Experimental results of preliminary tests, using undiluted fresh feces, showed that molds were too inefficient in terms of their growth rate to be of significance.

Present studies with Fungi include a consideration of both growth rate and substrate (feces) concentration and of resolving certain metabolic manifestations applicable to a closed ecological system.

C. Cultivation and Fermentation Studies on Feces with added Glucose.

Experiments have been designed to study the feasibility of using human wastes as a substrate for bacteria and yeast for the production of carbon dioxide to be used by concommitantly grown algae cultures. Results have indicated that feces would serve as a fermentable substrate only when an external source of sugar was supplied.

These results suggest that feces are deficient in available carbohydrate material required by the yeast and bacteria employed in these trials. However, excellent carbon dioxide production was obtained on the addition of 1% to 20% concentration of sugar to the feces by Saccharomyces cerevisiae fermentation.

D. Carbon Dioxide Production with Subsequent Utilization in Algal Cultures.

Studies employing the entire carbon dioxide output from yeast fermentation reactor to supply algal cultures are still in progress.

E. Cultivation of Yeast on Solvent Extracted Feces.

Experiments were made to determine the effect of several organic solvents, namely: acetone, ethyl alcohol, ether, and chloroform on feces in terms of their ability to remove essential nutrients needed by certain microorganisms. Tests showed that solvent extraction removed nutrients needed by Saccharomyces cerevisiae.

F. Pelleting and Feeding Guinea Pigs Human Feces.

Fresh human feces solvent extracted with ethyl alcohol, acetone, ethyl ether, and subsequently dried with acetone and ground into powder form was pressed into pellet form. Twenty to thirty pellets weighing 0.8 to 1.5 grams were made available to a pen of three guinea pigs. All other feeding was restricted during the experiment.

After four days of waiting, observations revealed that the guinea pigs had not consumed any of the pellets.

These results prompted feeding experiments employing feces pellets disguised with a 1% concentration of chromium oxide (green powder). Again, guinea pigs failed to feed on the pellets.

These results suggest that before feces can be fed directly to animals a great deal more preliminary testing will be required.

II. Research Currently in Progress or Research to be Initiated in the Immediate Future.

A. Research Currently in Progress.

1. Aeration Studies.

Experiments are designed to show the effect of aeration of human wastes on the growth of bacteria. It is the purpose of this study to ascertain the effect of six different rates of aeration on the following bacteria: Escherichia coli, Aerobacter aerogenes, Bacillus subtilis, Micrococcus (spp.), and Clostridum (spp.). This is being accomplished by plates counts at various intervals for a total of six days. Changes in the bacterial counts will serve to measure the rate of oxidation of the organic matter present.

2. Screening Bacteria Capable of Growing on Human Wastes.

Experiments are being conducted to screen out bacterial species able to grow on feces ranging in concentration from 1% to 32%. Response of these organisms in terms of growth rate will in essence serve to screen out those bacteria capable of deriving their nutrients from feces. Further application is planned for these organisms in manometric studies of mixed prototype cultures.

All levels except the 5% concentration are presently being discontinued, since preliminary tests show that concentration is not very significant within the boundaries of this study.

3. Methods of Obtaining Bacteria-Free Protozoa.

Several techniques are being employed to discover a feasible method for obtaining bacteria-free and possibly pure cultures of protozoa. These methods include the use of antibiotics as an aid in obtaining bacteria-free cultures. Methods reported in the literature are presently being evaluated. Results to date indicate that simple use of penicillin and streptomycin and combinations of these and the column method (geotropic mechanism) are not as dependable as led to believe. New methods have been devised based on centrifugation and washing of cells. These are now being tested for their accurracy and applicability.

Pure culture and/or bacteria-free isolates will be studied manometrically on fecal substrates to estimate the extent of selected metabolic activities.

B. Future Research (to be initiated in the next six months).

1. Investigation of Metabolic Activities Manometrically.

Metabolic activities of algae, bacteria, and protozoa on human wastes substrate will be studied manometrically. These will be studied in terms of the relationship among one another of the species and their single and combined respiration rates in prototype culture. In addition, various derivatives and other individual fractions of feces will be investigated for their metabolic activity.

2. Infrared Analysis.

The Perkin-Elmer model 137 infracord spectrophotometer was received in August of this year. On arrival the instrument was found to have a faulty prism. The instrument was finally put back into operation in the first part of the month of December of 1960.

Currently, the instrument is being standardized for high precision work and standard curves and being determined for pure chemicals known to be fecal components.

This instrument will be employed to determine the presence and quantity of various, otherwise non-resolvable, chemical constituents of feces, urine, and derivatives of these as a part of manometric and other metabolic studies.

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

Grant No. NsG 80-60

Statement of Expenditures

Equipment:		
Perkin-Elmer Infracord Spectrophotometer	$5,151.06	
Total Equipment		$5,151.06
Supplies:		
Total Supplies		520.15
Travel:		
Robert G. Tischer	$ 385.30	
Barbara P. Tischer	198.72	
R. M. Cody	55.08	
Total Travel		$ 639.10
Salaries and Wages:		
Salaries for professional workers for period June 1, 1960--November 30, 1960	$4,333.31	
Wages for non-professional and student workers for period June 1, 1960--November 30, 1960	120.45	
Total Salaries and Wages		$4,453.76
Retirement		41.67
Total Expenditures		$10,805.74