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A PROGRESS REPORT
ON
UTILIZATION OF AGRICULTURAL WASTES
FOR PRODUCTION OF ETHANOL

Grant No. DE-FG05-79ER10021

October 1979 - May 1980

Principal Investigator: B. Singh, Professor
Collaborator: Jacob Savage, Associate Professor
Research Assistant: Simon Ogutu
Graduate Research Assistants: A. A. Akinsanya
Horsfall M'epba
Undergraduate Research Assistants: Willie Byrd
Kelvin Ford

Institution: Alabama A & M University
Food Science & Technology
Normal, Alabama

MASTER

DOE Review
May 1980

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MASTER

1. A Review of Progress

The project entitled, "Utilization of Agricultural Wastes for Production of Ethanol," was approved in August 1979 and was started in October 1979. In developing this project, the principal investigator has consulted various workers who are engaged in research on saccharification of cellulosic material from agricultural residues, including USDA, Northern Regional Research Center, TVA - Biomass Research Group, Louisiana State University, and a few others.

At the outset, it may be pertinent to bring about a few facts about the problems associated with utilization of cellulosic biomass for production of fermentable carbohydrates: (1) Since cellulosic materials do not easily lend itself for enzymatic digestion, it is not expected that cottonseed hulls or peanut shells will, by conventional fermentation methods, produce ethanol equivalent to corn, sugar beets, or other biomass; (2) Cottonseed hulls contain higher amounts of lignins (approximately 15%). There is a considerable intermolecular bonding between the uronic acids of hemicellulose and lignin phenolic groups. Lignin forms a three-dimensional net around the cellulose fibers. It is this fashion that the complex cellulose is rendered unavailable to subsequent enzyme degradation. It is also this area of complexity of lignin-cellulose interaction where the ultimate utility of agro-residues has its future; (3) And finally, literature on digestibility of cottonseed hulls and peanut shells is almost non-existent.

The objectives of the project include conversion of agricultural residues such as cottonseed hulls and peanut shells into ethanol by developing methods for:

- (a) Efficient conversion of non-cellulosic residues, such as lignins, pectins and soluble carbohydrates into ethanol. This will include enzymatic conversion as well as microbiological conversion.
- (b) Conversion of cellulosic materials from agricultural residues (cottonseed hulls, peanut shells, corn stalks) to ethanol. This will include a comparative study of the acid hydrolysis, enzymatic and the microbial methods.

Personnel

In conducting the experiments to accomplish these objectives, following persons have been involved: Simon Ogutu (Research Assistant), A. Akinsanya (Graduate Assistant), H. M'epba (Graduate Assistant), Willie Byrd (Undergraduate Assistant), Kelvin Ford (Undergraduate Assistant).

The interdepartmental collaboration in conducting the research objectives has been provided by Dr. Jacob Savage and his associates from the Department of Biology. Cottonseed hulls has been provided by the Southern Cotton Oil Company, Huntsville.

Major Equipment

No major equipment has been purchased yet; however, several equipment listed in the project has been ordered. Several chemicals, enzymes, and microbial cultures have been obtained from various sources.

Travel

Dr. B. Singh, Principal Investigator attended Alcohol Fuel Workshop sponsored by the Farmers Home Administration, USDA and Tennessee Valley Authority in Montgomery, Alabama on November 6, 1979. He also attended the Bio-energy '80 and presented a paper at the poster session on April 21-24 in Atlanta, Georgia.

Research Accomplishments

Methods of *Trichoderma viridae*

Culture: The culture designated as *Trichoderma* 2817, *Trichoderma* 32098, *Trichoderma* 13631, *Trichoderma* 24449, *Trichoderma* 26921, and *Tilletaria anomala* 24038, *Tilletiopsis pallascens* 24345 were obtained from the American Type Culture Collection, Rockville, Maryland. *Trichoderma* 26921 was maintained on malt extract agar. Inoculum was prepared by growing in a two-step build-up process in a liquid medium described by Kaneshiro. The first step consisted of removing about half the growth from a two-week-old, 25 x 150 mm ME slant and macerating in 5 ml water. This suspension was transferred to 300 ml Erlenmeyer flask containing 100 ml of medium and then incubated for 48 hr on a rotary shaker at 25°C. The second step consisted of transferring 20 ml of the whole broth culture from Step 1 into a second flask containing 100 ml medium. Incubation again was 48 hr on a rotary shaker at 25°C.

Substrates: The raw material was cottonseed hulls (CS) obtained from the Southern Cotton Oil Company. CS was finely ground on a Homoloid Mill using 80-mesh sieve. In one series of experiments, finely ground CS was incubated with enzymes on microbial culture. In another series of experiments, CS was treated with acid or alkali to improve the accessibility of cellulase. Chemicals used in these treatments were

0.1N NH_4OH , 0.1N HCl , or 0.1N NaOH . The chemicals were added to CS as aqueous solutions in quantities sufficient to submerge the CS; the CS-chemical mixtures were allowed to stand 24 hr or 6 days at 25°C. At the end, the chemical solutions were decanted, and the treated CS were allowed to drain. The drained samples, were used for enzymatic or microbial conversions.

Enzymatic hydrolysis

Takamine brand cellulase and developmental cellulase Ty concentrate were from Miles Labs, Research Products Division, Elkhart, Indiana. Finely ground CS was incubated with 0.1% of the enzyme for 24 hr, 48 hr and 1 week. At the end of the incubation reaction was stopped by the addition of boiling ethanol. Sugars were extracted using 80% ethanol (Loomis and Shull, 1937). The arsenomolybdate reagent was used to determine reducing sugars (Nelson, 1941) and the anthrone reagent for the total sugars (McCready *et al.*, 1950).

Electron microscopy: At the end of microbial and enzymatic digestion, residue was also analyzed using scanning electron microscopy (SCM).

Results

As is evident from the Table 1, there was a significant amount of saccharification of cellulose into sugars. This is further evidenced by the SCM results. Similarly, it is apparent that cellulose is digested by *Trichoderma*. Most importantly, the organism utilized CS as a sole source of carbon.

2. Work in Progress

Work is in progress on (1) the effects of various microorganisms on the degree of saccharification; (2) effects of higher concentrations of acids, alkali, and EDTA on efficiency of microbial degradation; and (3) effects of chemicals on enzymatic digestion.

3. Future Plan

By the end of this project period, we will be able to collect data on utilization of CS for the production of ethanol using yeast fermentation on solubilized substrates. Additionally, we should be able to establish a pilot plant for preparation and modification of CS into fermentable products.

4. Publications

1. Singh, B., A. A. Akinsanya and S. Ogutu. Saccharification of cottonseed hulls by microorganisms and cellulose. Bio-energy '80 World Congress and Exposition, April 1980, Atlanta, Georgia.

SACCHARIFICATION OF COTTONSEED HULLS (CS) BY CELLULASE TV
CONCENTRATE

Amount of CS Used (g)	Amount of Enzyme Used (g)	Percent increase in total sugars* after		
		24 hr	48 hr	1 week
2	0.02	2.9**	4.0	15.8
4	0.04	2.4	3.8	21.0
6	0.06	2.3	3.4	14.0

* Amount of total sugars in cottonseed hulls treated with acetate buffer varies from 1-4%.

** Average of 3 determinations.

FIGURE 1. Hemicellulose, cellulose, lignin and weight losses in cotton-seed hulls (CS) during a 70-day fermentation with *Trichoderma viridae*.

FIGURE 2. Scanning electron micrograph showing cellulase-hydrolyzed and microbiologically-treated cottonseed hulls.

TREATED WITH TRICHODERMA (26921) FOR
7 DAYS (x 1000)

UNTREATED (x 700)

TREATED WITH IN HAOH FOR 10 DAYS
(x 700)

UNTREATED (x 700)

TREATED WITH TRICHODERMA (26921) FOR
7 DAYS (x 400)

UNTREATED (x 700)

TREATED WITH 1 N HCL FOR 10 DAYS
(x 700)

UNTREATED (x 700)

TREATED WITH 1 N NH_4OH FOR 10 DAYS
(x 700)

UNTREATED (x 700)

TREATED WITH 1 N HCL FOR 10 DAYS
(x 1000)

UNTREATED (x 700)

TREATED WITH TRICHODERMA (26921) FOR
7 DAYS AT 10,000

UNTREATED (x 700)

TREATED WITH 1 N NaOH FOR 10
DAYS (x 400)

UNTREATED (x 700)

TREATED WITH TRICHODERMA FOR 7 DAYS
(x 7,000)

UNTREATED (x 700)

RESEARCH PROPOSAL
UTILIZATION OF AGRICULTURAL WASTES FOR PRODUCTION OF ETHANOL

SUBMITTED TO
U. S. DEPARTMENT OF ENERGY
DEVELOPMENT OF ENERGY RESEARCH AND
RELATED PROGRAMS AT TRADITIONALLY
MINORITY INSTITUTIONS

NPI: OER-79-NPI-001

SUBMITTED BY
DEPARTMENT OF FOOD SCIENCE & TECHNOLOGY
ALABAMA A & M UNIVERSITY
NORMAL, ALABAMA 35762

RESEARCH PROPOSAL
NPI: OER-79-NPI-001

Name, Address and Telephone Number of Entities Submitting the Proposal:

Department of Food Science and Technology
School of Agriculture, Environmental Science and
Home Economics
Alabama A & M University
Normal, Alabama
205 859-7433

Name and Telephone Number of Project Manager:

Dr. B. Singh, Professor
Telephone: 205 859-7454

Title of the Proposed Project:

UTILIZATION OF AGRICULTURAL WASTES FOR PRODUCTION
OF ETHANOL

Proposed Duration:

2 Years

Starting Date:

October 1, 1979

R. D. Morrison
President
Alabama A & M University
R. D. Morrison

Date 5/2/79

Bharat Singh
Project Director
B. Singh

Date 4. 30. 79

Bharat Singh
Principal Investigator,
B. Singh

Date 4. 30. 79

Jacob Savage
Co-Investigator,
Jacob Savage

Date 4-30-79

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I. ABSTRACT

The project proposes to develop methods to utilize agricultural wastes, especially cottonseed hulls and peanut shells to produce ethanol. Initial steps will involve development of methods to break down cellulose to a usable form of substrates for chemical or biological digestion.

The process of ethanol production will consist of (a) preparatory step to separate fibrous (cellulose) and non-fibrous (non-cellulosic compounds). The non-cellulosic residues which may include grains, fats or other substrates for alcoholic fermentation. The fibrous residues will be first pre-treated to digest cellulose with acid, alkali, and sulfur dioxide gas or other solvents. (b) The altered cellulose will be digested by suitable micro-organisms and cellulose enzymes before alcoholic fermentation. The digester and fermentative unit will be specially designed to develop a prototype for pilot plant for a continuous process.

The first phase of the project will be devoted toward screening of a suitable method for cellulose modification, separation of fibrous and non-fibrous residues, the micro-organism and enzyme preparations. The second phase will be devoted toward development of a continuous process for ethanol production.

The project will provide an opportunity for two graduate and two undergraduate students to work with energy-related problems. The Department of Food, Science and Technology at Alabama A & M will benefit through this project by developing capabilities in studies related to agricultural wastes utilization for production of usable forms of energy.

II. STATEMENT OF THE PROBLEM

A. INTRODUCTION

The necessary research for the development of renewable energy raw material is urgent. The potential use of ethanol as an automotive fuel derived from the fermentation of cereal grains, such as corn, has been a recurring issue in the midwest for over 40 years, as it holds promise for increasing the price of these grains. More recently, it has also been pointed out that use of ethanol will reduce our requirements for energy from fossil fuels. However, utilization of grain as a source of energy will affect the price of feed and food both. Instead, some of the agricultural wastes could be utilized as a source of fuel.

Each year vast amounts of crop residues are produced (about 60 percent of the total crop production), which have not been effectively utilized because they are bulky and lignocellulosic, thus having little fuel energy per unit volume. Recent studies, however, have revealed that these crop residues could be utilized as a source of fuel after certain chemical and biological treatments. (Stephens et. al. 1975; Edwards and Wilke 1975; Humphrey and Wilke 1975.)

Since agricultural wastes, especially cottonseed hulls and peanut shells, are abundantly available in Alabama and in other Southeastern States, it will be appropriate to evaluate the efficiency of these materials in production of usable forms of energy, especially ethanol. This project proposes to develop the method (s) for breakdown of cellulosic material in laboratory conditions and then to study the feasibility on a pilot plant scale to produce ethanol.

B. CONVERSION OF AGRICULTURAL RESIDUES TO ETHANOL

Agricultural crop residues consist mainly of cellulose, lignin, pectin and other plant carbohydrates. The nature of the constituents of these residues can be best utilized as one of the five F's: Fuel, Fiber, Fertilizer, Feed and Food (Han and Smith 1978). This utilization method could be grouped into four categories: (1) Direct uses. (2) Mechanical conversions. (3) Chemical conversions and (4) Biological conversions. The proposed project aims to utilize mechanical, chemical and biological methods of conversions of cellulosic material into alcohol.

Goldstein (1975) has recently advocated using cellulose to generate ethanol and petrochemicals, and Inman (1914) has studied the use of cellulose as a renewable source of fuel for energy generation. Basile (1973) described a chemical process to use citrus peel residues (2) addition of 1% NaOBr_2 , and treatment with Ca(OH)_2 , (3) pressing of the mixture with a Louisville-type press (4) drying in a triple press dryer (5) cooling (6) shifting and (7) packing the finished product. This could, however, be extended into ethanol production. Lipinsky et. al. (1976) has suggested to utilize anaerobic conversion of sugar crop such as sugar cane, sweet sorghum and sugar beets to produce gas. Production of fuels from municipal solid wastes has already been evaluated recently, using pyrolysis or bioconversions. (Bassham and Wilk 1975; Crane et. al. 1975; Hundemann et. al. 1976; Shatizadeh 1975; Wide et. al. 1975.) Cereal residues - straw, stalks, hulls, and forest residues are a largely untapped reservoir of cellulosic material. In many cultivated plants, residues are somewhat greater in tonnage than the harvested grain. For example, in cotton-producing areas of the South and Southeast, every year tons of hulls are discarded. Similarly, peanut shells are abundantly available.

The industrial use of agricultural residues by conversion through fermentation into ethyl alcohol is technically feasible. Alcohol is a readily usable fuel for automobile internal combustion engines, and has been demonstrated in actual tests. Ethyl alcohol has served as a fuel at various times in many countries. Basically, the carbohydrate (starch or cellulose) is consumed during fermentation and converted into ethyl alcohol and carbon dioxide. The conversion of starch to ethyl alcohol is relatively more simple than the conversion of cellulose to alcohol. However, the production of soluble carbohydrates from wood and wood wastes was practiced on a large scale in Germany during World War II.

The hydrolysis process leading to wood sugars was usually followed by a fermentation of the sugars by the yeast. Also, of early interest was the direct fermentation of native, involuble cellulose for generation of protein containing biomass. Reports by Virtanen (1939), Langwell (1932) and others described attempts to use various bacteria, fungi and rumen micro-organisms to degrade and metabolize cellulose. The early process for wood sugar production yielded rather low efficiencies in producing glucose from cellulose and ineffective chemical recovery and highly polluting waste streams also held back their success. The direct fermentation processes were slow and cellulose

utilization was relatively low, making these processes economically non-viable as well. The new processes are being designed to be more specific in their attack on cellulose and result in higher product yields at increased rates and to produce a product of controlled quality at controlled rates of production. Acids and cellulolytic enzymes have commonly been used for this purpose, as have other less conventional methods such as physical disruption, alkaline oxidation, high pressure steam treatment and application of high energy atomic particles.

Acid hydrolysis involves the use of concentrated sulfuric acid (Berguis, 1931), the dilute sulfuric acid (Scholler 1935) and hydrochloric acid (Locke and Garnum 1961). Locke and Garnum (1961) also reported that three hydrolysis processes had been developed in Japan. The Nihon-Mokuzai-Kagaku process and the Kokaido process both used strong sulfuric acid, but the Noguchi-Chisso process used anhydrous hydrogen chloride gas to hydrolyze the cellulose. In the United States, Harris and Berlinger (1946) have successfully modified the process and recently Converse and Grethlein (1973) have worked on optimization of the acid hydrolysis process, where acid type and concentration, and reaction time and temperature were closely controlled. The process used a plug-flow reactor at temperatures of around 205° C and reaction times of 10-30 seconds to obtain about 60% conversion of cellulose to glucose in concentration of about 3.5-4% in the outlet stream. Acid hydrolysis processes, however, are still hindered by the uneven physical and chemical structure of native cellulose which usually restrict penetration of acid.

Enzymatic hydrolysis of cellulose has been made possible by the use of extra-cellular cellulases. Reese (1957) reported high activities of these enzymes in cellulose hydrolysis and used them to produce and recover. Later work by Ghose (1969) and Mandels and Weber (1969) improved the microbiological and operational aspects of the process. Wilke (1974) has further improved the method of enzyme production.

Enzyme hydrolysis processes have the advantage of operating under very mild chemical and physical conditions. They are capable of very specific action on cellulose with glucose or ethanol being the final product. Problems which remain to be solved are the relative expense of the enzyme and the fact that little is known about cellulase stability under reaction conditions

or the degree of enzyme recovery after hydrolysis. Also, as with acid hydrolysis techniques, the heterogeneous structure of cellulose has held yield of end product down to 80% or less. Pretreatment techniques that improve the reactivity and accessibility of the cellulose should be most useful in improving the efficiency of the enzyme hydrolysis. A pretreatment method using organic solvent has been suggested to hydrolyzed cellulose from rice hulls by Sasaki et. al. (1978). The other method has been suggested to pretreat cellulosic materials with alkali.

III. OBJECTIVES

To convert agricultural residues such as cottonseed hulls and peanut shells into ethanol by developing methods for:

- a. efficient conversion of non-cellulosic residues, such as lignins, pectins and soluble carbohydrates into ethanol. This will include enzymatic conversion as well as microbiological conversion.
- b. conversion of cellulosic materials from agricultural residues (cottonseed, peanut shells, corn stalks) to ethanol. This will include a comparative study of the acid hydrolysis, enzymatic and the microbiological methods.

IV. APPROACH AND METHODOLOGY

Conversion of Agricultural Residues into Ethanol

First phase of the conversion will be devoted towards evaluation of various methods as follows:

1. Preparatory steps.
 - a. Reduction by chopping, blending or grinding.
 - b. Separation into cellulosic and non-cellulosic fractions after blending or grinding. This will be accomplished by the use of a suitable press. A flow diagram for separation will be shown in the diagram in Figure 1.

(A), (B) and (C), as indicated in the diagram will be utilized for production of ethanol. If it is economically feasible (A) could be utilized as a source of unconventional protein for either human nutrition or for animals.

As indicated in Step 1, non-fibrous residue will be used as a substrate for production of ethanol. A lab-model converter will be used. This converter will be designed as such that a continuous process for conversion be made feasible.

For fibrous residue, more than one approach will be made. First phase will be devoted to screening of the methods, such as:

- To increase cellulose digestion rates by seeking new or mutant organisms which simply produce more (or more active) cellulase enzymes. One possible organism could be the fungus *Trichoderma Vividae*. This was isolated by Reese (1950). Recently, one of its strains, QM9136, was isolated and was found to be capable of producing several times the cellulase of the original parent (Ghose, 1969). Another organism, a bacteria "98A" digests cellulose very efficiently, could be utilized also.
- The second approach will be a chemical or physical modification of solid cellulose in such a way that digestibility of cellulose by organisms would be improved. This will include: (i) use of acids and alkali, (ii) size reduction, (iii) use of a mixture of dry HC and sulphur dioxide gas. This has been successfully used for digestion of rice hulls (IV) treatment with re-usable solvents. This may involve the procedure of dissolving cellulose by acid as one of the solvents and the preparation of cellulose with organic solvent as a means of recovering of cellulose.

Initially, heating of water or fluids will be done by conventional sources; however, a feasibility study will be made to utilize solar energy as a source. Proven flat plate collectors such as the Reynolds Aluminum collector and an insulated storage tank will be constructed for this purpose.

The end products after chemical and biological processes will be evaluated by standard procedures. The amount of cellulose left after digestion will be determined by the method outlined in AOCS (1973) for cottonseed hulls and peanut shells. The method for determination of starch and sugars will essentially be the same as outlined by McCready et. al. (1951) and Singh and Wort (1969). Cellulases will be obtained from micro-organisms by suitable procedures (Rees et. al. 1950). Cultures of bacteria will be maintained at Alabama A & M University (Ghose 1969).

The final step in the project will be a development of a pilot plant at Alabama A & M University to utilize biomass or solid waste and

residue of plant origin to convert into ethanol.

Figure 1. Fractionation Process

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VI. SUMMARY OF RESEARCH TO BE CONDUCTED (SCHEDULES):

FIRST YEAR:

The first year of research will be devoted to develop methods for blending, mixing and separating components of cottonseed hulls and peanut shells. Also, studies on methods of culturing, storing and maintaining microbial population and enzymes will be conducted.

SECOND YEAR:

A detailed study on breakdown products of cottonseed hulls and peanut shells will be conducted. The design of the converters will be completed.

THE FOLLOWING KINDS OF DATA WILL BE COLLECTED:

1. Methods of blending, mixing and separating cottonseed hulls and peanut shells.
2. Methods of separating components of cottonseed hulls and peanut shells.
3. Methods of isolating a cell-free hemogenate from bacteria (cellulase, pectinase and other hydrolases).
4. Products of chemical and biological treatments.
5. Design and efficiency of equipment for continuous production processes from cellulosic residues to ethanol production.

VII. SIGNIFICANCE OF THE PROPOSED RESEARCH TO NATIONAL, REGIONAL AND LOCAL ENERGY CONCERNS:

The proposed project will be significant to the State of Alabama, South-eastern United States and also to the nation as a whole. Alabama produces significant amounts of cottonseed hulls and peanut shells as by-products. Most of these residues are poorly utilized. Utilization of these products as a source of energy will have a dual purpose; viz., reduction of waste and production of energy.

The project will be of benefit to the processors of cottonseed and peanuts in Alabama and in the Southeastern United States.

The project will enable the Department of Food Science to further strengthen programs in energy resource utilization. There are already programs in cereal chemistry and technology. This project will be of additional value to the faculty and students in the department to utilize plant by-products for energy production.

PROJECT SCHEDULE

Task	Quarters	<u>First Year</u>				<u>Second Year</u>			
		1	2	3	4	1	2	3	4
1. Initiation Phase									
Organization of the Project									
o Graduate Students									
o Undergraduate Students									
o Procurement of Chemicals Glassware and Raw Materials (Cottonseed hulls, peanut hulls, Micro-organisms - Culture Maintenance)									
1.a. Hold Year-end Report									
2. Method for blending, mixing and conditioning									
2.a. Year End Report									
3. Analysis of Chemical and Biological Treatments									
3.a. Eighteen-Months Report									
4. Comparative Study of Various Methods for Procurement of Ethanol									
4.a. Final Report									

VIII. SPECIFICS: Training Relevant to the Project

The principal investigator is familiar with most of the techniques proposed in the project. He has had experience with the isolation, fractionation and characterization of constituents from plants materials. He has worked on a fungus *Tilletiopsis* (a wood-digesting organism) as a post-doctoral fellow at the University of British Columbia. He has experience with most plant product chemistry and has guided research of many graduate students working on enzymes. He has published papers on hydrolytic enzymes, proteases, amylases, phosphatases and phytases. His laboratories are equipped with chemicals and materials to isolate and store enzymes. He has collaborated in research with various agencies and persons, and has published more than 50 publications in various national and international journals. He has established Cereal Chemistry and Technology Laboratory at Alabama A & M.

COLLABORATIVE ARRANGEMENTS:

This proposed project requires collaboration only inter-departmentally--not inter-institutionally. Local industries, Southern Cotton Oil Company of Huntsville and Tennessee Valley Cotton Mills of Decatur are willing to collaborate in providing cottonseed hulls.

PRINCIPAL INVESTIGATOR ASSURANCE:

The undersigned agrees to accept responsibility for the scientific and technical conduct of the research project and for provision of required progress reports if a grant is awarded as the result of this application.

Date 4.30.79

Bharat Singh,
Principal Investigator

IX. ORGANIZATIONAL CHART

X. BUDGET

	<u>1st Year*</u>	<u>2nd Year</u>
A. <u>Personnel</u>		
1. Senior Scientific Personnel		
Dr. B. Singh, Food Chemist, Principal Investigator, 20%	\$ 5,000	\$ 5,400
Dr. Jacob Savage, Microbiologist Co-Investigator, 5%	1,475	1,489
2. Graduate and Undergraduate Students		
Graduate Research Assistant (2)	10,000	10,400
Undergraduate Students (2)	<u>4,600</u>	<u>4,800</u>
	\$ 21,075	\$22,089
3. Secretarial Support		
Secretary, 25% time	1,770	1,800
B. <u>Supplies and Equipment**</u>		
- drying oven	3,500	-----
- construction of anaerobic digestion system for Biomass conversion to ethanol Laboratory scale plant	15,800	-----
- solar collection and storage system for ethanol digestion	2,500	-----
- enzymes, lab chemicals	5,800	6,800
- lab glassware and supplies	6,200	7,500
- A 'press' for expressing fluids from biomass sources	<u>5,500</u>	-----
	\$ 62,145	\$ 38,189
C. <u>Travel</u>		
- to professional meetings. Visits to the sites where similar research is done.	2,540	2,640
D. <u>Consultation</u>		
- Consultation for processing system.	1,550	1,550

E. Miscellaneous

- Office supplies, telephone and mailing	\$ 500	\$ 600
- publications, page and reprint charges	300	350
	<u>\$ 800</u>	<u>\$ 950</u>

F. Indirect Cost-Employee Benefits

- Indirect cost (58% of salary and wages)	13,250	13,856
- Employee benefits (24%) Except Students	<u>1,979</u>	<u>2,085</u>
	<u>15,229</u>	<u>15,941</u>

G. TOTAL REQUESTED FROM DOE
TOTAL

\$ 82,264	\$ 59,270
	<u>\$141,534</u>

XI. JUSTIFICATION OF BUDGET

PERSONNEL:

Dr. Singh will be devoting 20% of his time to this project. His time will be utilized in setting up experiments, procuring samples and materials, and most importantly, directing graduate and undergraduate students. He has contacts with the local industry related to peanut and cottonseed processing.

Dr. Savage is a microbiologist. His laboratory has facilities to culture micro-organisms. In addition, he has fermentors of size capable of obtaining data which can be used for scale-up to an industrial operation which will help in getting started on the project. He will be devoting 5% of his time to this project.

Two graduate students will be trained. Each one will have different assignments; however, each one will have an opportunity to participate in seminars and discussions pertaining to this research. They will also be involved in setting up their own experiments, learning new techniques and collecting various data.

Secretarial help on the project will be needed by preparing reports and various other documents.

EQUIPMENT:

We have drying ovens. However, this will be a larger size air-oven to accommodate larger samples. Cost: \$3,500.00. We have asked for \$15,800 for construction of an anaerobic digestion system. This will be designed in such a way that it could handle a variety of biomass material.

Solar collection systems have been used and are currently available. We will attempt to install and study a feasibility to utilize the system for heat generation in biomass conversion. Cost: \$2,500.00.

ENZYMES, LAB CHEMICALS, GLASSWARE AND SUPPLIES:

Enzymes, lab chemicals, glassware and supplies will be procured to conduct experiments outlined in this project.

TRAVEL:

The travel budget as prepared includes travel for obtaining samples, materials, consultations outside the campus and attendance of regional

and national meetings. It includes travel for the principal investigator, co-investigator, as well as for graduate students.

CONSULTATION:

Consultation budget includes fee for experts to consult on various aspects of the project. It also includes the cost of some analytical works, if needed.

MISCELLANEOUS:

It includes expenses for Hice supplies, telephone tolls, mailing expenses, and charges for publications.

XII. FACILITIES AVAILABLE

The Department is located in Carver Complex and has laboratory space of 5,000 square feet. It also has facilities for growing micro-organisms. The equipment available includes:

- * High Speed Centrifuge (IEC Model)
- * Beckman Ultracentrifuge
- * Bausch-Lomb UV-Visible Spectrophotometer
- * Turner Fluorometer
- * pH Meters with Expanded Scale
- * Warburg Apparatus
- * Autoclave
- * Tracer MT-222 Gas Liquid Chromatograph
- * Beckman Amino Acid Analyzer
- * Facilities for Thin-Layer Chromatography
- * Scanning Electron Microscope
- * Microtome
- * Tissue-Tek Apparatus
- * Electrophoretic Equipment
- * Light Microscopes
- * Darkroom Facilities
- * Farm Facilities to Raise Rabbits, Chickens, Turkey, Beef, Cattle, and Swine
- * Distillation Apparatus
- * Fat Extractor
- * Liquid Chromatograph
- * Small Animal Room - For Raising Rabbits, Mice and Rats
- * Farm Facilities to Grow Plants
- * Technician Autoanalysis System
- * Perkin-Elmer Model 460 Atomic Absorption Spectrometer

Some facilities available in the Department of Biology will also be available for this project.

PARTICIPATING STUDENTS:

Undergraduate Trainees - 2

Graduate Trainees - 2

PARTICIPATING FACULTY:

Participating faculty members will be Dr. Jacob Savage, an associate professor in the Department of Biology. Although the major responsibility will be carried out by the principal investigator, Dr. Savage will collaborate in developing methods for microbial culture and maintenance. He has had experience with microbiological research and has wide collection of microbial populations used in degradation of natural and synthetic products.

XIII. BIOGRAPHICAL SKETCH OF THE PRINCIPAL INVESTIGATOR

NAME: Bharat Singh

TITLE: Professor

RESEARCH EXPERIENCE RELEVANT TO AREA OF PROJECT:

General: The principal investigator has considerable experience with naturally occurring plant products, enzymes and micro-organisms. He has his Ph.D. in Plant Physiology and Plant Biochemistry. His Ph. D. thesis involved characterization of enzymes related to carbohydrate metabolism in sugar beets. Further, he had a year of post-doctoral research on culture and isolation of enzymes from *Trileptium* (a wood-digesting fungus). He worked at Utah State University where he was involved with studies on various field crops. He has experience with the isolation and purification of enzymes, proteins, natural products, pentosans, sugars and nutritional inhibitors. His current research at Alabama A & M University includes work on chemistry and biochemistry of triticale. He has guided thesis research of several graduate students and has published over 50 research papers in various journals. He teaches food chemistry and food analysis at Alabama A & M University at the undergraduate level and carbohydrates and proteins at the graduate level. (A detailed resume with list of publications is attached.)

Co-investigator Dr. Jacob Savage is an associate professor in the Department of Biology. He has considerable experience with micro-organisms and industrial microbiology. He will be collaborating on developing novel methods (Koji Fermentations) for culture and also isolating enzymes for cellulose and lignin digestion.

XIV. RESUME

Name: Bharat Singh

Biographical Status:

Born: Calcutta, India

Address: Department of Food Science and Technology
Alabama A & M University
Normal, Alabama

Marital Status: Married; Two children

College Education:

B.S., Banaras Hindu University, Varanasi, India, 1958

M.S., Ranchi University, Ranchi, India, 1961

Ph.D., University of British Columbia, 1968

Professional Experience:

1975 - Present	Professor, Dept. of Food Science & Technology, Alabama A & M University, Normal, Alabama.
1972-1975	Associate Professor, Dept. of Food Science & Technology, Alabama A & M University, Normal, AL
1970-1972	Visiting Assistant Professor, Dept. of Nutrition and Food Sciences, Utah State University, Logan, Utah
1969-1970	Post-Doctoral Research Associate, Dept. of Nutrition and Food Sciences, Utah State University, Logan, Utah
1968-1969	Post-Doctoral Fellow, Medical Research Council of Canada, University of British Columbia
1966	Teaching Assistant, Plant Physiology, Botany Department, University of British Columbia
1964-1968	Research Assistant, Botany Department, University of British Columbia
1961-1964	Lecturer, St. Columba's College, Hazaribagh, Bihar, India

Experience - Teaching and Research:

Research at Alabama A & M University: Biochemical composition including carbohydrates, protein, minerals, vitamins and enzymes in triticales; Quality characteristics of triticales grain, flour and bread; Bulgur from triticales-feasibility studies including nutritive value; Starch chemistry of banana plaintain.

Teaching courses at Alabama A & M University: Introduction to Food Science; Fruits, Vegetables and Cereal Products; Food Chemistry and Food Analysis; Advanced Topics in Cereals. Participating in the following courses to cover areas related to plant foods: Protein in Foods and Nutrition; Carbohydrates and Lipids in Foods and Nutrition; Minerals and Vitamins in Foods and Nutrition; Food Toxicology.

Thesis Advisor at Alabama A & M University:

Theses (completed):

- (1) The effect of dough conditioners and gluten on the bread-baking quality of triticales
- (2) Nitrate reductase and amylase activity in triticales
- (3) Milling and biochemical characteristics of triticales
- (4) Proteases in triticales
- (5) Bulgur from triticales
- (6) Purification and characterization of triticales phytase

Master reports

- (1) The effects of methods of processing and preservation of the nutritive value of fruit and vegetables
- (2) Aflatoxins in foods

Theses (in progress)

- (1) Starch chemistry of banana plaintain
- (2) Evaluation of cookie baking characteristics of triticales
- (3) Relationship between rheological properties and proteases of triticales.

Reports (in progress)

- (1) Effects of processing on nutritive value of cereals
- (2) A critique review of the current regulatory practices in food establishments.

Thesis Advisor of Four Graduate Students at Utah State University:

Titles of the theses completed: (1) Physiological and biochemical changes in lettuce during controlled atmosphere storage; (2) Development of controlled atmosphere storage method in lettuce; (3) β -Hydroxy- β -methylglutaric acid as a precursor of carotenoids in higher plants; (4) Uptake and metabolism of radioactive telone in higher plants.

Visiting Assistant Professor, July 1970 - May 1972, Utah State University:
Continuation of the research areas below:

Post-Doctoral Research Associate, July 1969 - June 1970, Utah State University:
 Worked on the effect on some chemicals on biochemical composition and metabolism of beans, peas, corn, and spinach. Also associated with works on post-harvest physiology and biochemistry of fruits and vegetables; biosynthesis of carotenoids; metabolic control of solanine formation in potato tubers; toxicological effects of certain plant products on seed germination; effects of ionizing radiation on micro-organisms and higher plants.

Post-Doctoral Research Fellow, 1968-1969, University of British Columbia:
 Isolation and characterization of a sesquiterpene lactone responsible for contact dermatitis in human from a moss; also on purification of an oxygenase (enzyme) from *Tilletiopsis* (a fungus).

Research Assistant in Plant Physiology, October 1964 - June 1968, University of British Columbia. Worked on chemical control of growth in sugar beet.

Teaching Assistant in Plant Physiology, 1966, University of British Columbia.

Lecturer in Botany, St. Columba's College, Hazaribagh, December 1961-October 1964.

Research Project: (Grants)

Principal Investigator: Starch Chemistry and Metabolism with a special reference to the utilization of banana plantain. NSF, RIAS Grant 1978-1979

Principal Investigator: Studies on quality and biochemical characteristics of triticale 1977 - 1979.

Principal Investigator: A comparison of the baking and nutritional value of triticale with other cereals. 1972-1976.

Principal Investigator: Metabolism and Translocation of Telone in Higher Plants, Dow Chemical Company, Residue Research Division, 1971-1973. (Utah State University)

Co-Investigator: Effects of Selected Pesticides on Nutritive Composition of Fruits and Vegetables, USDA, ARS, Human Nutrition Division, 1969 - 1972 (Utah State University)

Co-Investigator: Controlled Atmosphere Storage of Lettuce, US Army, Natick Laboratories, 1969 - 1971. (Utah State University)

Member of Professional Societies

American Association of Cereal Chemists

Institute of Food Technologists

American Association of Oil Chemists

Sigma Xi

Other Activities: (Honors, Awards and Others)

Ranchi University Gold Medal, 1961, for securing first position in the class
1961

Runner-Up, Morrison-Evans Distinguished Scientists Award 1978.

Medical Research Council of Canada, Post-Doctoral Fellowship, 1968 - 1969

Who's Who in the South and Southwest

American Men and Women in Science

Personalities in South

Chairman, Symposium on Biochemical and Functional Characteristics of Triticale,
IFT Annual Meeting, New Orleans, 1974

Listed as one of the IFT Speakers, 1976

Member Graduate Council, Alabama A & M University

Member, Research and Publications Committee, Alabama A & M University

Chairman, Graduate and Undergraduate Curriculum Committees, Department of
Food Science and Technology, Alabama A & M University

Member, Research Committee, Department of Food Science and Technology,
Alabama A & M University

Member, Pesticide Task Force Committee, Alabama A & M University

Member, MBS Committee, Alabama A & M University

Publications and Paper Presented:

Listed Separately:	Publications	-50
	Paper presented	- 37

PUBLICATIONS OF BHARAT SINGH

1. Singh, B. 1968. Chemical control of growth in sugar beet (*Beta saccharifera* L.). Ph.D. thesis, Univeristy of British Columbia, Vancouver, Canada.
2. Singh, B., and D. J. Wort. 1969. Effect of Vanadium on growth, chemical composition, and metabolic processes of mature sugar beet plants. *Plant Physiol* 44:1321-1327.
3. Mitchell, J. C., W. B. Schofield, B. Singh, and G. H. N. Towers. 1969. Allergy to Frullania. *Arch. Derm. V.* 100:45-49.
4. Mitchell, J. C., B. Fritig, B. Singh, and G. H. N. Towers. 1970. The role of sesquiterpene lactones in allergic contact dermatitis from alantactone and compositae. *J. Inv. Derm.* 54:233-239.
5. Wort, D. J., and B. Singh. 1970. Growth, composition and metabolic responses of sugar beet to preharvest application of Meleic hydrazide. *Agron. J.* 62: 57-61.
6. Singh, B., and D. J. Wort. 1970. Foliar application of pyrocatechol to prevent late growth of sugar beet and increase sucrose content. *J. Am. Soc. Sugar Beet Technol.* 16:45-51.
7. Singh, B., and D. J. Wort. 1970. Effects of pyrocatechol on some metabolic processes of mature sugar beet (*Beta vulgaris* L.) plants. *Physiologia Plant* 23:920-927.
8. Wu, M., B. Singh, M. T. Wu, and D. K. Salunkhe. 1970. Effects of certain soil fumigants on essential nutritive components and the respiratory rate of carrot (*Daucas carota* L.) roots. *HortScience* 5:221-222.
9. Wu, M. T., B. Singh, J. C. Theurer, and D. K. Salunkhe. 1970. Control of sugar loss in sugar beets during storage by chemicals and modified atmosphere and certain associated physiological changes. *J. Am. Soc. Sugar Beet Technol.* 16:117-127.
10. Singh, B., N. A. Littlefield, and D. K. Salunkhe. 1970. Effects of controlled atmosphere (CA) storage on amino acids, organic acids, sugars, and rate of respiration of "Lambert" sweet cherry fruit. *J. Am. Soc. Hort. Sci.* 95:458-461.
11. Chou, T. W., B. Singh, D. K. Salunkhe, and W. F. Campbell. 1970. Effects of gamma radiation of (*Penicillium expansum* L.). I. Some factors influencing the sensitivity of the fungus. *Radiation Botany* 10:511-516.
12. Chou, T. W., B. Singh, D. K. Salunkhe, and W. F. Campbell. 1970. Effects of gamma radiation on (*Penicillium expansum* L.). II. Some enzymatic changes in the fungus. *Radiation Botany* 10:517-520.

13. Karnik, V. V., L. E. Olson, D. K. Salunkhe, and B. Singh. 1970. Evaluation of effects of controlled atmosphere storage on roots of sugar beets grown at various levels of nitrogen fertilizer. *J. Am. Soc. Sug. Beet Technol.* 16: 225-232.
14. Singh, B. M. T. Wu, and D. K. Salunkhe. The use of chemicals to increase leaf protein of certain crops. *Technical Group Meeting of Leaf Protein, International Biological Program*. Sri Avanashilingam Home Science College, Coimbatore, India, Nov. 25, 26, 27, 1970.
15. Singh, B., and D. K. Salunkhe. 1970. Some metabolic responses of bush bean plants to a subherbicide concentration of certain s-triazine compounds. *Can. J. Bot.* 48:2213-2217.
16. Chou, T. W., D. K. Salunkhe, and B. Singh. 1971. Effects of gamma radiation on (*Penicillium expansum* L.). III. On nucleic acid metabolism. *Radiation Botany* 11:329-334.
17. Dalvi, R. R., B. Singh and D. K. Salunkhe. 1971. A study on phytotoxicity of alantolactone. *Chemico-Biological Interactions* 3:13-18.
18. Patil, B. C., D. K. Salunkhe, and B. Singh. 1971. Metabolism of solanine and chlorophyll in potato tubers as affected by light and specific chemicals. *J. Food Sci.* 36:474-476.
19. Wu, M. T., B. Singh, and D. K. Salunkhe. 1971. Influence of foliar application of simazine, igran, and GS-14254 on delta-amino levulinic acid dehydratase of pea seedlings. *Phytochem.* 10:2025-2027.
20. Salunkhe, D. K., M. T. Wu, and B. Singh. 1971. The nutritive composition of pea and sweet corn seeds as influenced by S-triazine compounds. *J. Am. Soc. Hort. Sci.* 96:489-492.
21. Singh, B., M. T. Wu, and D. K. Salunkhe. 1971. The use of chemicals to increase leaf and seed proteins of certain crops. *Plant Foods for Human Nutrition* 2:171-184.
22. Chiang, J. C., B. Singh, and D. K. Salunkhe. 1971. Effects of water quality on canned carrots, sweet cherries, and apricots. *J. Am. Soc. Hort. Sci.* 96: 353-357.
23. Wu, M. T., B. Singh, and D. K. Salunkhe. 1971. Influence of S-triazines on some enzymes of carbohydrates and nitrogen metabolism in leaves of pea (*Pisum sativum* L.) and sweet corn (*Zea mays* L.). *Plant Physiol.* 48:517-520.
24. Salunkhe, D. K., M. Wu, M. T. Wu, and B. Singh. 1971. Effects of Telone and Nemagon on essential nutritive components and the respiration rates of carrot (*Daucus carota* L.) roots and sweet corn (*Zea mays* L.) seeds. *J. Am. Soc. Hort. Sci.* 96:357-359.

25. Patil, B. C., B. Singh, and D. K. Salunkhe. 1971. Formation of chlorophyll and solanine in Irish potato (*Solanum tuberosum* L.) tubers and their control by gamma radiation and CO₂ enriched packaging. *Food Sci. and Technol.* 4:123-125.
26. Salunkhe, D. K., Jack Chiang, and B. Singh. 1971. Water quality determines equality of canned products. *Utah Science* 32:18-20.
27. Singh, B., D. K. Salunkhe, and S. H. Lipton. 1972. Growth and chemical responses of bean plants to sublethal concentrations of s-triazine compounds. *J. Hort. Sci.* 47:441-448.
28. Berry, D. L., B. Singh, and D. K. Salunkhe. 1972. Incorporation of β -hydroxy- β -methylglutaric acid into carotenoids and other ether soluble compounds in maize seedlings. *Plant and Cell Physiol.* 13:157-165.
29. Wu, M. T., B. Singh, and D. K. Salunkhe. 1972. Influence of foliar application of S-triazine compounds on dry weight, chemical composition, and enzymatic activity of pea and sweet corn seedlings. *J. Expt. Bot.* 23:793-800.
30. Singh, B., C. C. Yang, D. K. Salunkhe, and A. R. Rahman. 1972. Controlled atmosphere storage of lettuce. I. Effects on quality and the respiration rate of lettuce heads. *J. Food Sci.* 37:48-51.
31. Singh, B., D. J. Wang, D. K. Salunkhe, and A. R. Rahman. 1972. Controlled atmosphere storage of lettuce. II. Effects on biochemical composition of the leaves. *J. Food Sci.* 37:52-55.
32. Singh, B., W. F. Campbell, and D. K. Salunkhe. 1972. Effects of S-triazines on protein and ultrastructure of *Phaseolus* cotyledons. *Am. J. Bot.* 59:568-572.
33. Jadhav, S. J., B. Singh, and D. K. Salunkhe. 1972. Metabolism of unsaturated fatty acids in tomato fruit: Linoleic and linolenic acids as precursors of hexanal. *Plant and Cell Physiol.* 13:449-459.
34. Dalvi, R. R., B. Singh, and D. K. Salunkhe. 1972. Physiological and biochemical investigations on the phytotoxicity of usnic acid. *Phyton* 29:63-72.
35. Dalvi, R. R., B. Singh, and D. K. Salunkhe. 1972. Toxic influences of selected pesticides on germination and associated metabolic changes in wheat and mung bean seeds. *J. Agr. Fd. Chem.* 20:1000-1003.
36. Singh, B., O. P. Vadhawa, M. T. Wu, and D. K. Salunkhe. 1972. Effects of foliar application of S-triazines on protein, amino acids, carbohydrates and mineral composition of pea and sweet corn seeds, bush bean pods, and spinach leaves. *J. Agr. Fd. Chemistry* 20:1256-1259.
37. Wu, M. T., B. Singh, and D. K. Salunkhe. 1972. Effects of s-triazines on protein synthesis in leaves of peas (*Pisum sativum* L.) and sweet corn (*Zea mays* L.) and ultrastructure of pea cotyledons. *Experientia* 28:1002-1003.

38. Singh, B. 1972. Nitrogen metabolism of sugar beets. A recurring problem gets a fresh appraisal. *Econ. Bot.* 26:182-188.
39. Singh, B., N. A. Littlefield, and D. K. Salunkhe. 1972. The accumulation of amino acids and organic acids in the fruits of apples and pears under controlled atmosphere storage conditions and certain associated changes in metabolic processes. *Indian J. Hort. Sci.* 28:245-251.
40. Singh, B., and S. G. Reddy. 1973. Triticale utilization studies as food and feed at Alabama A & M University. Proc. International Symposium on Triticale, Lubbock, Texas. (September 1973).
41. Berry, D. L., B. Singh and D. K. Salunkhe. 1974. Effect of dichloropropenes and aliphatic compounds on synthesis and degradation of carotenoids. *J. Amer. Soc. Hort. Sci.* 99:138-141.
42. Berry, D. L., W. F. Campbell, B. Singh and D. K. Salunkhe. 1975. Biochemical and ultrastructural effects of certain organohalides on tomato, *Lycopersicon esculentum* Mill. *J. Amer. Soc. Hort. Sci.* 100:607-613.
43. Singh, B., V. T. Sapra and J. A. Patel. 1976. Nitrate reductase and its relationship to protein and yield characteristics of triticale. *Euphytica*. 24:193-199.
44. Singh, B. 1978. Triticale: A new cereal grain. In: *Encyclopedia of Food Science*. AVI Publications, Westport, Conn. 759-771.
45. Singh, B. 1976. Amylograph and farinograph studies on triticale. *Baker's Digest*. 50:26-30.
46. Singh, B., J. A. Patel and V. T. Sapra. 1977. Amylase activity in triticale, *X Triticosecale* Wittmack. *Euphytica*.
47. Singh, B. and N. R. Reddy. 1977. Phytic acid and mineral compositions of triticales. *J. Food Sci.* 42:1077-1083.
48. Singh, B. and Prisca Mugwira. 1977. Effect of dough conditioners on baking quality and nutrient composition of triticale breads. *Food Sci. & Technol.* 10:89-93.
49. Singh, B. and L. M. Dodda. 1977. Studies on preparation and nutrient composition of bulgur from triticale. *J. Food Sci.* (In press).
50. Singh, B. and H. G. Sedeh. 1978. The characteristics of phytase and its relationship to acid phosphatase and certain minerals in triticale. *Cereal Chem.* (in press).
51. Singh, B., Shah, M.O. and Hughes, J. L. 1978. Interrelationships between certain rheological and biochemical characteristics of triticale flours. Proc. 6th International Cereal and Bread Congress, Winnipeg, Canada. Sep. 16-22.
52. Zeringue, H. J., Jr., B. Singh and R. O. Fenge. 1978. The functionality of lipids in triticale breads. Proc. 6th International Cereal and Bread Congress. Winnipeg, Canada. Sept. 16-22.

Papers Presented in Scientific Meetings

1. Singh, B. Chemical control of growth in sugar beet. Northwest Scientific Association Meeting, Ellensburg, Washington, March, 1968.
2. Wort, D. J., and B. Singh. Reduction of sugar loss caused by late fall growth of beets. General Meeting, American Society of Sugar Beet Technologists. Phoenix, Arizona. February, 1968.
3. Singh, B., and D. J. Wort. Regulation of enzyme activity to curtail growth of sugar beet and induce "ripening" of its roots. Canadian Society of Plant Physiologists Proceedings, London, Ontario. June, 1968.
4. Fritig, B., B. Singh, J. C. Mitchell, and G. H. N. Towers. The role of sesquiterpene lactones in allergic contact dermatitis from *Frullania* and compositae. Phytochemical Society of North America, Symposium and Meeting, Banff, Alberta. 1969.
5. Wu, Ming-Tsung, B. Singh, and D. K. Salunkhe. Control of sugar losses in sugar beet by certain pre-and post-harvest treatment. General Meeting, American Society of Sugar Beet Technologists, Denver, Colorado. February, 1970.
6. Singh, B., and D. J. Wort. Foliar application of pyrocatechol to prevent late growth of sugar beet and increase its sucrose content. 16th General Meeting of American Society of Sugar Beet Technologists, Denver, Colorado. February, 1970.
7. Singh, B., and D. K. Salunkhe. The response of bean plants to low concentrations of simazine, atrazine, igran, and GS-14254. 62nd Annual Meeting of the American Society of Agronomy and Crop Science, Tucson, Arizona. August, 1970.
8. Salunkhe, D. K., M. Wu, B. Singh, and G. G. Dull. Effects of certain soil fumigants on essential nutritive components and the respiratory rate of carrot (*Daucus carota* L.) roots. 18th International Horticultural Congress, Tel-Aviv (Israel). March, 1970.
9. Singh, B., and D. K. Salunkhe. Metabolic and compositional changes in bean plants treated with simazine, atrazine, igran, or GS-14254. Proceedings Canadian Society of Plant Physiologists, Quebec, P. Q. June, 1970.
10. Berry, D. L., D. K. Salunkhe, and B. Singh. Biosynthesis of β -carotene: Incorporation of β -hydroxy- β -methylglutaric acid-3- ^{14}C in β -carotene and other ether soluble compounds in maize seedlings. Proceedings Canadian Society of Plant Physiologists, Quebec, P. Q. June, 1970.
11. Patil, B. C., D. K. Salunkhe, and B. Singh. Formation and control of solanine and chlorophyll in potato tubers. IFT Meetings, New York. May, 1971.
12. Singh, B., C. C. Yang, D. Wang, D. K. Salunkhe, and A. R. Rahman. Controlled atmosphere storage of lettuce. Annual meetings of American Society of Horticultural Science. Manhattan, Kansas. August, 1971.

13. Salunkhe, D. K., M. T. Wu, and B. Singh. The nutritive composition of pea and sweet corn seeds as influenced by s-triazine compounds. Annual Meetings of American Society for Horticultural Science. Manhattan, Kansas. August, 1971.
14. Campbell, W. F., B. Singh, and D. K. Salunkhe. Triazine-induced rough endoplasmic reticula in *Phaseolus* cotyledon cells. Symposium, Mountain States Society of Electron Microconists. May, 1971.
15. Wu, M. T., D. K. Salunkhe, and B. Singh. Metabolic responses of pea and sweet corn plants to sublethal concentrations of s-triazine compounds. Proc. American Society of Plant Physiologists. August, 1971.
16. Jadhav, S., B. Singh, M. T. Wu, and D. K. Salunkhe. Metabolism of unsaturated fatty acids and influence of sub-atmospheric pressures on ripening behavior of tomato fruit. IFT Meetings, Minneapolis, Minnesota. May, 1972.
17. Berry, D. K., D. K. Salunkhe, and B. Singh. Effects of Telone (a soil fumigant) and related compounds in plants. Meetings of American Society of Horticultural Science, St. Paul, Minnesota. August, 1972.
18. Singh, B., O. P. Vadhwa, and D. K. Salunkhe. Effects of S-triazines on protein, carbohydrates and mineral content of bush bean seeds. Meetings of American Society of Horticultural Science, St. Paul, Minnesota. August, 1972.
19. Berry, D. L., B. Singh and D. K. Salunkhe. Uptake and metabolism of 1, 3-dichloropropene in plants. 64th Annual Meeting, American Society of Agronomy, Miami Beach, Florida. October, 1972.
20. Dalvi, R. R., B. Singh, and D. K. Salunkhe. Toxic influences of selected pesticides on germination and associated metabolic changes in wheat and mung bean seeds. 64th Annual Meeting, American Society of Agronomy, Miami Beach, Florida. October, 1972.
21. Campbell, W. F., D. L. Berry, B. Singh, and D. K. Salunkhe. Effects of certain organo-halides on fine structures of plant leaf cells. 64th Annual Meeting, American Society of Agronomy, Miami Beach, Florida. October, 1972.
22. Singh, B., Cheryl Judd, J. L. Hughes, and S. G. Reddy. A comparative study of protein, carbohydrates and mineral composition in flour and bread of wheat and tritcale. Proc. American Association of Cereal Chemists, St. Louis, MO., November, 1973.
23. Singh, B., and S. J. Gandhi. 1974. Biochemical composition of bran and middlings of tritcale. Annual meeting Southern Association of Agricultural Scientists, Memphis, Tennessee, February, 1974.
24. Mugwira, Prisca and B. Singh. Improvement of bread baking characteristics of tritcale by certain emulsifiers. Proc. Annual Meeting Southern Association of Agricultural Scientists, Memphis, Tennessee, February, 1974.
25. Singh, B., Jay A. Patel and Prisca Mugwira: Biochemical composition of tritcale. Annual Meeting of the Institute of Food Technologists in New Orleans, Louisiana, May 1974.

26. Singh, B., and Prisca Mugwira: Milling and baking characteristics of triticale. Annual Meeting of the Institute of Food Technologists in New Orleans, Louisiana, May, 1974.
27. Singh, B., Amylograph and farinograph studies on triticale. Annual Meeting of the American Association of Cereal Chemists, Montreal, Canada, October, 1974.
28. Singh, B., P. Mugwira, N. R. Reddy and V. T. Sapra. Milling and baking characteristics of Alabama-grown triticale. Annual Meeting of the American Association of Cereal Chemists, Montreal, Canada, October, 1974.
29. Singh, B., J. A. Patel and V. T. Sapra. Relationship of nitrate reductase activity to grain protein in triticale. Annual Meeting of the American Association of Cereal Chemists, Montreal, Canada, October, 1974.
30. Singh, B., V. T. Sapra and J. A. Patel. Nitrate reductase activity and its relationship to protein and yield characteristics of triticale. Annual Meeting of the American Society of Agronomy, Chicago, Illinois, November, 1974.
31. Singh, B., and Prisca Mugwira. Effect of dough conditioners on certain bread baking quality and nutrient composition of triticale breads. Annual Meeting of the Institute of Food Technologists, Chicago, June, 1975.
32. Singh, B., J. A. Patel and V. T. Sapra. Amylase activity in triticale seed, flour and developing kernel as influenced by cultivar, location and milling conditions. Annual Meeting of the American Association of Cereal Chemists, Kansas City, MO., October, 1975.
33. Singh, B. and N. R. Reddy. Phytic acid and mineral compositions of triticales. Annual Meeting of the Institute of Food Technologists, Anaheim, Calif., June, 1976.
34. Singh, B. and R. Katragadda. The characteristics of a proteolytic enzyme from triticale. Annual Meeting of the American Association of Cereal Chemists, New Orleans, Louisiana, October, 1976.
35. Singh, B. and L. M. Dodda. Studies on the preparation and nutrient composition of bulgur from triticale. Annual meeting of the Institute of Food Technologists, Philadelphia, Pennsylvania, June, 1977.
36. Singh, B. and H. G. Sedeh. The characteristics of phytase and acid phosphatase and its relationship to certain minerals in triticale. 62nd Annual Meeting, American Association of Cereal Chemists, San Francisco, October 23-27, 1977.
37. Singh, B., H. G. Sedeh and M. O. Shah. Effect of milling and baking on certain vitamin and mineral contents of triticale and wheat. Annual Meeting of the Institute of Food Technologists, Dallas, Texas, June 1978.

XV. VITAE

Jacob Savage, Microbiologist
Associate Professor
Alabama A. and M. University
Normal, Alabama 35762
Telephone: Home (205) 883-9145 Business (205) 859-7268

Specialized Professional Competence

- . Microbial Biochemistry and Physiology; Applied Microbiology; Fermentation Technology; Enzyme Technology

Present Administrative Activities

- . Program Director of Alabama A. and M.'s Research Initiation and Support Program (NSF) - \$204,200.00.
- . Program Director - Preparation of Guinea Pig Macrophages for Electrophoretic Experiments in Space (N.A.S.A. Grant - \$27,791.00 for twelve months).
- . Co-Principal Investigator - Mode of Action of Natural Insecticides in Animal and Plant Systems (Mutagenic, Toxic, Synergistic, and Carcinogenic Effects) Minority Biomedical Support Grant (NIH) - \$327,000.00.
- . Project Director for study of Microbial Degradation of Chlorinated Hydrocarbons in Freshwater Ecosystems for the Environmental Protection Agency, \$107,343.00.
- . Chairman, Alabama A. and M. University Laboratory Animal Care Committee.

Recent Research Assignments

- . Co-investigator on developing a process for converting swine waste to single-cell protein by chemical, enzymatic and microbial hydrolysis of the solid constituents.
- . Post Doctoral Research on microbial degradation of Military Standard pesticide formulations (Stanford Research Institute, Menlo Park, California).
- . Consultant (ALZA Research, Inc., Palo Alto, California).
- . Identify research priorities on Microorganisms as source of food and energy (Lawrence Livermore Laboratories, Livermore, California).

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Other Professional Experiences

- . Use of microbial enzymes of degradation of recalcitrant molecules found in the environment.
- . Composting of swine waste as waste disposal operation.
- . Survival of intestinal pathogens in composting operation.
- . Mixed culture fermentation kinetics.
- . Microbial production of novel antineoplastic compounds (Daunomycin and Adriamycin).
- . Immobilization of enzymes as cancer chemotherapeutic agents.

Academic Background

- . B. S. in Animal Sciences, Alabama Agricultural and Mechanical University.
- . M. S. and Ph.D. in Microbial Biochemistry, Rutgers University.

Publications

- . Composting conditions and survival of intestinal pathogens in hog waste windrow compost. Bacteriological Proceedings E 47, 1972.
- . Microbial aspects of composting hog wastes, M.S. Thesis Rutgers University, (1972).
- . Growth of *Candida utilis* on chemical, microbial, and enzymatic hydrolysates of swine waste. Bacteriological Proceedings E 38, 1973.
- . Microbial conversion of swine waste to single-cell protein. Global Impacts of Applied Microbiology IV International Conference, Sao Paulo, Brazil, 1973.
- . Kinetic studies on mixed culture fermentation of swine waste. Global Impacts of Applied Microbiology IV International conference. Sao Paulo, Brazil, 1973.
- . Population changes in enteric bacteria and other microorganisms during aerobic thermophilic windrow composting. Applied Microbiology 16: 969-974.
- . Microorganisms as source of food and energy (Lawrence Livermore, NTIS, in press).

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- . The stability of immobilized microbial enzymes for medical and industrial applications. Development of Industrial Microbiology 17: 375-386.

Papers Presented at Professional Meetings

- . Enzyme kinetic of Immobilized L-Asparaginase presented at the First Annual Faculty/Student Symposium of Science, Tuscaloosa, Alabama.
- . The anaerobic microbiological degradation of Lindane in a batch culture. Second Annual Faculty/Student Symposium of Science, Montgomery, Alabama.
- . Mutagenic activity of several pesticides using the Salmonella test. Second Annual Faculty/Student Symposium of Science, Montgomery, Alabama.
- . Preparation of guinea pig macrophage for electrophoretic experiments in space. Second Annual Faculty/Student Symposium of Science. Montgomery, Alabama.
- . Detection of pesticides as mutagens. Sixth Annual MBS Symposium, Atlanta, Georgia.
- . Culturing systems for the enumeration of guinea pig macrophage. Sixth Annual MBS Symposium, Atlanta, Georgia.
- . Microbial degradation of chlordane in a batch culture.

Scientific and Professional Activities (Last Five Years)

- . American Society for Microbiology, 1972 and 1973 (Presented papers).
- . Global Impact of Applied Microbiology, 1973 (Presented two papers and served on a committee to develop international research programs for developing countries to increase the world food supply).
- . American Society for Microbiology, 1974 (Invited as symposium speaker to discuss microbial solution to waste problems).
- . Institute of Food Technology, 1975 (participant in a workshop to discuss mechanisms of increasing federal fundings to universities in food research - trip supported by Lawrence Livermore Laboratory).
- . Energy in Agriculture, 1975 (participated in a workshop to develop recommendations of research needs on reducing energy consumption in Agriculture).

- o American Society for Microbiology, 1976 (Selected as Chairman of a round table discussion on the role of micro-organisms in energy systems).

Professional Associations

- o American Society for Microbiology
- o Society for Industrial Microbiology
- o Institute of Food Technology
- o National Institute of Science

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