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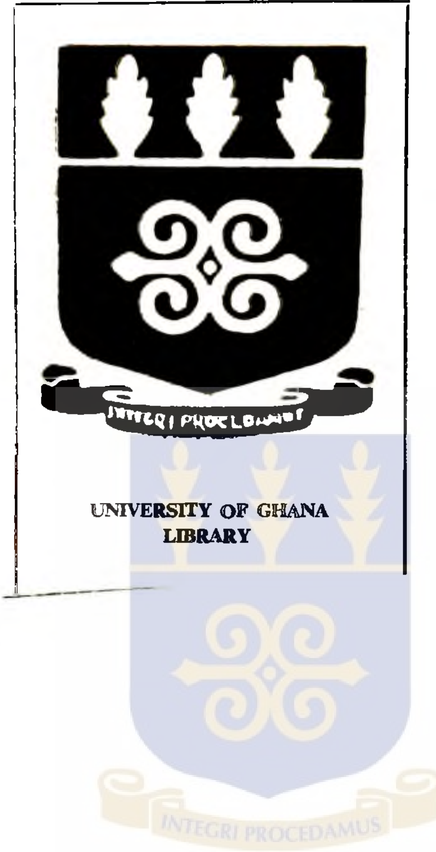

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EFFECT OF GAMMA IRRADIATION ON AGRICULTURAL WASTE-DECOMPOSING AND FERMENTATION MICROORGANISMS IN GHANA

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ABSTRACT

Maize production in Ghana between 1984 and 1990 exceeded 500,000 metric tonnes per year except in 1985 where there was a shortfall to 395,000 metric tonnes. The unfavourable years of drought between 1981-1983 were attended by a sharp decline in maize production (140,000-333,200 metric tonnes). The bulk of the national maize production was contributed by Ashanti, Brong-Ahafo, Eastern and Northern Regions. Maize husk available after removing cobs was commensurate with the total maize harvest for each Region. As a raw material for fungal protein production, maize husk has a potential for sustainable use.

Gamma irradiation (0-200 Krad) was used as a mutagen to evaluate its effect on vegetative growth, cellulolytic, pectinase and amylase activity of *Aspergillus niger*. *Rhizopus oryzae*, *Trichoderma viride* and *Lactobacillus plantarum* screened for use in the production of fungal protein on corn husk slurry by solid substrate fermentation. A dose of 20 Krad decreased vegetative growth of *A. niger* by 46.6 percent and further increases up to 50 Krads increased dry matter accumulation by the fungus. Vegetative growth of *R. oryzae* was increased by about 30 percent by 50 Krad and remained nearly the same up to 200 Krad. There was no stastitical difference ($\leq$ 0.05, Student's t-test) between dry weight of mycelium obtained with 50, 100, 200 Krad of gamma irradiation. The best vegetative growth of *T viride* was obtained when 100 or 200 Krad of gamma irradiation was applied to spores prior to culturing. The best vegetative growth of the

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bacterium (*L.plantarum*) was attained when the culture was exposed to 50 Krads prior to incubation at 28°C for 5 days.

Optimum cellulase, amylase and pectinase activity was induced by 50 Krad of gamma irradiation in *A. niger* and *L. plantarum* ; on the other hand, optimal cellulase activity in *T. viride* and *R. oryzae* was induced by a dose of 100 Krad whilst 50 Krad was optimal for maximal production of amylase and pectinase enzymes by the same fungi (*T. viride* and *R. oryzae*). Generally, the gamma irradiation dose that induced optimal vegetative growth was also attended by optimal cellulase activity.The pH of the medium containing spores of *T. viride* treated with 100 krad was between pH 5.0 - 6.5 corresponding to the best pH for cellulase activity in *T viride*. The potential for use of gamma irradiation as a mutagen for enzyme production is promising.

Gamma irradiation up to 200 Krad linearly increase acid production by *A. niger* as pH shifted from 2.9 to 2.2. The culture medium containing gamma-irradiated (≥ 50 Krad.) *R. oryzae* sporangiospores also became more acidic (pH 4.5 - 3.8) presumably indicating accumulation of acids.

Hydrolysis of corn husk into a slurry was achieved by using either one percent or five percent sodium hydroxide or hydrochloric acid and heating at 80° , 100° or 120°C for 1-3 hours. The best treatment combination for corn husk hydrolysis was heating at 100°C fot at least 1 hr. in either one or five percent sodium hydroxide or 1 percent Hydrochloric acid. This gave good

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accessibility of cellulase for microbial enzyme attack leading to high crude fungal protein (20.0 percent) produced by *T. viride* irradiated with 100 Krad of gamma irradiation prior to innoculation of the solid substrate.

Irradiation caused morphological changes in *T. viride* and *A. niger* cultures. In *T viride*, as much as the conidiophores remained irregularly branched, the bright green colour development decreased progressively with increasing gamma irradiation dose (eg. at 200 Krad there was no colour development).

Practical implications of these findings are discussed and future studies leading to commercial application of the technique suggested.

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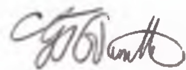
DECLARATION

I, CHARLES MAWULOM GBEDEMAH, hereby declare that, except for references to other peoples work which have been duly cited, this work is the result of my own original research and that this dissertation had neither in whole nor in part been presented for another degree elsewhere.

CHARLES M. GBEDEMAH.
Student.



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1. INTRODUCTION

Biomass, composed of wood and crop residues and manures is the largest renewable source of organic carbon in the world.It has been estimated that 2.25 billion tonnes of cereal straws, 560 million tonnes of leguminous crop residues and 234 million tonnes of sugarcane bagasse are produced every year in the world (Anon, 1988).

Lignocellulose are made up of structural polymers, cellulose, lignin and hemicelluloses; cellulose fibrils are embedded in an amorphous matrix of lignin and hemicelluloses.In the native state lignocelluloses are also associated with various non-structural components.

The non-structural components make up a considerable percentage of the weight of certain lignocelluloses,such as agricultural residues and wood of certain tropical trees. These components, particularly the organic extracts (phenolics, terpenes, alkaloids etc.) can significantly influence biodegradative ability of microorganism (Scheffer and Cowling, 1966).Table A shows cellulose, hemicellulose and lignin contents of representative lignocelluloses in nature. Plant cells are surrounded by a structural tissue,the cell wall (which is made up of a thin primary wall and secondary wall with middle lamella located between adjacent cells); Cellulose, hemicellulose, pectin and lignin are the main constituents of the plant cell wall.Of the structural components, cellulose is the most abundant, making up 35-40 percent of the dry weight of most tissues. The hemicelluloses and lignin make up 20-40 percent and 15-35 percent respectively.

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TABLE 1.

Cellulose, hemicellulose and lignin contents of lignocelluloses. (After Kirk, 1983)

	COMPOSITION (% dry wt.)			
Lignocellulose	Cellulose	Hemicellulose	Lignin	Reference
Birch Wood	42	38	19	Timell,1967
Maple	45	29	24	Timell,1967
Spruce Wood	41	31	27	Timell,1967
Hemlock Wood	41	23	33	Timell,1967
Bagasse (Sugar cane) (*Saccharum officinarum*)	41	>20	20	Dunning & Lathrop,1945
Soybean stalk (*Glycine max*)	35	>25	20	Aronovsky Nelson & Lathrop,1943
Wheat straw (*Triticum aestivum*)	40	>28	17	"
Rice straw (*Oryzae sativa*)	36	>25	12	"
Maize staw (husk) (*Zea mays*)	67	>30	10	"

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Below 18-20% lignin content, lignocelluloses are degraded to increasing extent with decreasing lignin content by cellulases and hemicellulases, and consequently by many bacteria and fungi that secrete these enzymes (Kirk, 1983). Fungi belonging to the *Aspergillus spp.* produce pectinase enzyme (Beldman *et al*, 1984) Table 2 shows major industrial fungal enzymes and their uses. Gilbertson (1980) estimated that there are about 1600-1700 North American species of wood and litter decomposing fungi. On a world-wide basis, there are certainly over 2,000 species. Some of these fungi use an apparently unique mechanisms for circumventing the lignin, pectin and other barriers to enzymatic cellulose degradation.

CELLULOSE DEGRADATION:

Degradation of crystalline cellulose by white-rot fungi, (eg. *Sporotrichum pulverulentum* Nov.) and various soil fungi result from the concerted, synergistic action of three types of hydrolyses (i) endo-1,4-β-glucanases, (C_x enzyme) which cleave the cellulose randomly; (ii) exo-1,4-β-glucanases, (cellobiohydrolases, C_1 enzymes) which release cellobiose (together with glucose in some cases) from non-reducing ends of cellulose; and (iii) β-glucosidases, which split cellobiose into glucose units or it may be oxidised to cellobionic acid and then cleaved.

The endo- and exoglucanases which actually act synergistically - perhaps as a loose complex (Wood and McCrae, 1977) are repressed by high concentrations of monosaccharides (Eriksson, 1978).

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TABLE 2.

Major industrial fungal enzymes and their uses.

Enzyme	Source	Catalytic action	Examples of use
Glucose oxidase	*Aspergillus niger*	Oxidizes glucose to D-glucono-δ-lactone with consumption of oxygen.	Blood glucose analysis Egg de-sugaring
Catalase	*Aspergillus niger*	catalyses the decomposition of hydrogen peroxide to oxygyen and water	H_2O_2 removal after milk sterilization
α-Amylase	*Aspergillus oryzae*	Breaks α-1,4-links on the interior of the starch molecule giving a mixture of maltodextrins.	Maltose syrup production
Gluco-amylase	*Aspergillus niger*	Acts from the end of the starch chains liberating glucose	Glucose syrup production
β-Gluanase	*Aspergillus niger*	Hydrolyses β-1,3-glucans which are not susceptible to hydrolysis by amylase or gluco-amylase	Filtration of beer
Cellulase	*Trichoderma sp* *Aspergillus sp* *Penicillium sp*	Catalyses the breaking of β-1,4-links in cellulose and in some cases its specificity permits it to act on other β-linked glucans	Digestive aids
Pectinase	*Aspergillus sp*	A polygalacturonase effecting hydrolysis of α-1,4-D-galactosiduronic linkages in pectin	Extration and clarification of fruit juices and wines
Lactase	*Aspergillus niger*	A β-galactosidase that hydrolyses milk sugar (lactose) to galactose and glucose	Whey processing
Fungal rennin	*Mucor pusillus*	Cleaves certain peptide bonds in casein	Cheesemaking
Lipase	*Aspergillus sp*	A triacylglycerol acylhydrolase that liberases fatty acids from glycerol esters	Cheese flavour enhancer
Fungal protease	*Aspergillus oryzae*	Has a wide spectrum of proteolytic activities	Soy sauce production Biscuit dough improvement

[illegible] Fungi. Vol. 4. Fungal Tech. [illegible] *al.* (1983).

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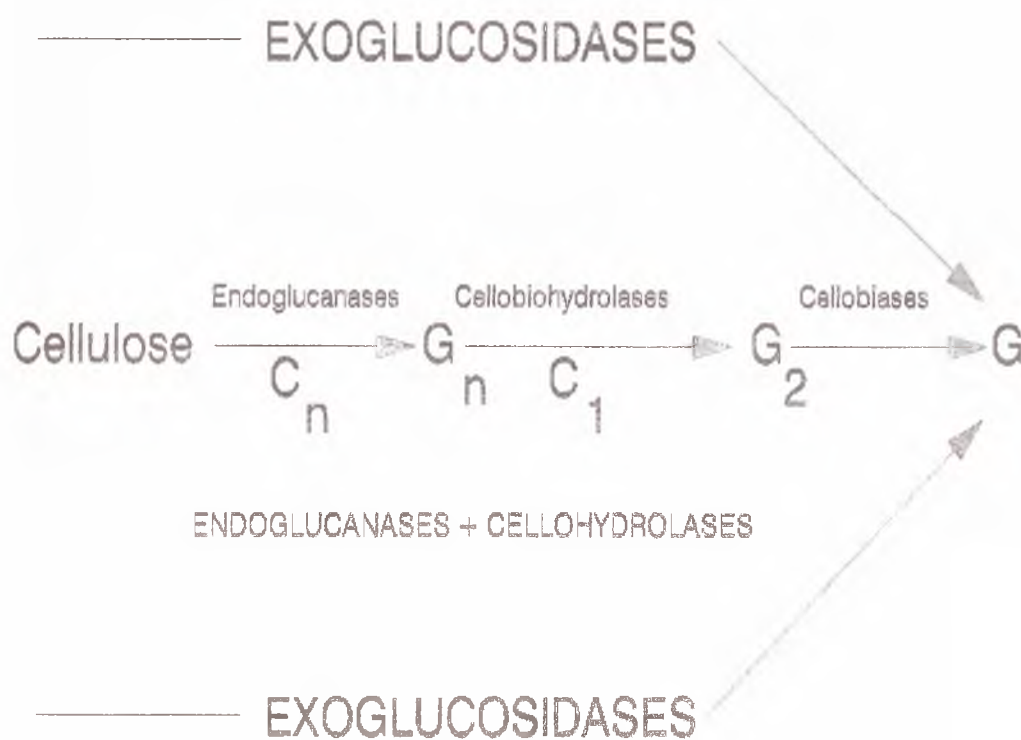
Fig. 1.
A model for the degradation of native cellulose by the action of these enzymes is shown below.



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The cellulases from *Trichoderma spp* notably, *Trichoderma reesei*, *T. koningii* and *T. viride* have been studied. (Beldman *et al.* 1984,1985; Mandels *et al*. 1981; Tangu *et al* 1981; Shin *et al* 1978). Cellulases from fungal origin also known to be powerful in cellulose hydrolysis have been investigated in *Sporotrichum pulverulentum* (Eriksson, 1978; Eriksson and Petterson, 1975) *Fusarium solani*, *Penicillium fumiculosum* and *Talaramyces emersonii* (Wood *et al* 1980, 1982)

HEMICELLULOSE DEGRADATION

Dekker and Richards (1976) reviewed microbial hemicellulases. Wood-rotting fungi produce enzymes capable of hydrolysing a variety of β-(1->4) linked glycan (mannan and xylan) substrates as well as various glycosides (Ahlgren and Eriksson, 1967; Keilich, Bailey and Liese, 1969; Highley, 1976). Endoglucanase from white-, brown- and soft-rot fungi all apparently act randomly, producing dimeric and higher oligomeric products (Ishihara and Shimizu, 1980). Informatiom regarding regulation of the synthesis of hemicellulose is somewhat contradictory (Dekker and Richards, 1976). However, multiple hemicelluase activity is found in culture filtrates of many fungi including white-, brown- and soft rot fungi after growth on a variety of substrates including simple sugars (Highley, 1976).

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LIGNIN DEGRADATION

The pertinent literature is replete with research on fungal degradation of lignin. Some of the reactions comprising degradation have been elucidated, and the unusual boichemical and physiological features are gradually begining to be described (Amer and Drew, 1980; Crawford and Crawford, 1980; Kirk *et al* 1980; Kirk, 1980,1982).

Lignin degradation is distinct from cellulose and hemicellulose degradation. Indeed, it differs from the biodegradation of all other studied biopolymers. Not a single enzyme involved in lignin degradation has been identified (Kirk, 1971; 1983). Because the lignin polymer is attacked by an extracellular non-specific oxidising agent, it is possible that enzymes may not be directly involved. Hall (1980) suggested that "diffusible species" derived from molecular oxygen may be involved. Current evidence suggests that regulation of secondary metabolism, including lignin degradation is somehow connected with glutamate metabolism (Kirk, 1981).

LIGNOCELLULOSE DEGRADATION AND CONVERSION INTO FEED OR FOOD.

Because of their abundance, lignocellulose plays a dominant role in the terrestrial carbon cycle. Lignin-degrading filamentous fungi play a prominent role, and probably predominant, role in the biodegradative part of this all-important cycle.

Recent studies have aimed at feed production by controlled cultivation of lignolytic fungi on lignocelllosic substrates. Substantial increases in crude protein have been

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reported for woods, barks and lignocellulosic waste following cultivation of various fungi (Daugalis and Bone, 1978; Ek and Eriksson, 1980; Matteau and Bone, 1980). Increases *in vitro* polysaccharide digestibility of lignocelluloses have accompanied solid substrate incubation with fungi (Kirk and Moore, 1972; Detroy *et al* 1981; Zadrazil,1980; Zadrazil and Brunnert, 1980, 1981).

Agricultural lignocellulosic waste disposal problems are the order of the day in Ghana. During the harvest season, considerable amount of maize plant debris is left to decompose in the fields as stubble or mulch. The removal of the maize cob from the husk is accompanied by the disposal of husk to waste. The local kenkey industry makes use of a small percentage of the maize husk as wrapping material for balls of kenkey during the manufacturing process. Kenkey consumers normally discard the husk after use. There is therefore a high potential for such waste being used as substrate in the production of microbial protein supplement biomass. The increased nutritive value of the husk could be used as animal feed supplement.

One of the largest single factors affecting animal production in the developing countries is poor nutrition as farmers cannot afford to buy the recommended feed from the mills (McDowell, 1968). This is because the high cost of the ingredients of the feed contribute immensely to the exorbitant price of animal feeds used in livestock production. For example, in Ghana it is estimated that feed cost alone constitute 80 percent of the total cost of animal production (Andah, 1974).

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Recent rapid increases in prices of ingredients of feedstuffs namely fish meal, wheat bran, maize, vitamin premixes etc. exacerbate the problem. If indeed microorganisms can provide, during their growth on solid waste, high levels of protein double their weight in a relatively smaller space (Dunlop, 1973), it would be worthwhile to explore the possibility of exploiting their potential to produce feeds by solid substrate "fermentation".

There are three technical problems in using lignocellulosic fungi to produce feeds by solid substrate "fermentation":

1. In scaling-up with the required careful control of humidity, aeration and temperature necessary for uniform treatment.
2. Preventing contamination of unwanted microbes.
3. The slowness of degradation of some plant residues.

The development of a low-technology process, analogous to ensiling for use on a small scale from easy-to-treat lignocellulosics should be the rule rather than the exception.

Conversion of lignocellulosic substances into economic products can be achieved through hydrolysis followed by fermentation (Ladisch *et al*, 1983) or by direct fermentation (Ng *et al*, 1981).

Hydrolysis of lignocellulose can be achieved with either chemical or enzymes or both. Because of certain disadvantages associated with acid hydrolysis, the enzymatic or biological method has received more attention (Manonmami and Sreekantiah, 1987)

Biomass in the form of maize husks, oat hulls and rice

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husks are taken through three types of pretreatment so that they can suitably be fractionated into their major components: cellulose, hemicellulose and lignin.

1. ALKALINE TREATMENT: Substrate is treated with 5-10 percent (w/w) sodium hydroxide at 80-121°C for a prescribed period depending on the nature of the substrate.
2. STEAM TREATMENT: The moist substrate is treated with steam at a temperature between 200-230°C for a prescribed period.
3. PARTIAL HYDROLYSIS: Substrates like maize husks, oat hulls and rice husks can also be partially hydrolysed by treatment with 3 percent HCl at a temperature of 90°C for four hours (Anon,

1988). This partially maize husk could contain approximately 10 percent reducing sugar content and can serve as an excellent substrate on which to grow microoganisms.

Cellulolytic, pectinolytic and amylolytic enzymes are produced by a large number of microorganisms including fungi. Highly active cellulases are often found in culture media supporting growth of *Trichoderma viride*. *T. viride* is noted for its high cellulolytic activity and its ability to utilize various substrates (oat hull, maize husk cellulose etc.) with the attendant production of high protein into the medium (Mandel and Weber, 1961).

The fungus *Aspergillus niger* produces pectinase enzymes and there is a synergistic action of cellulases from *T viride* and pectinases from *A. niger* in plant biomass conversion. In this thesis, the effect of low gamma radiation doses on the cellulolytic, pectinase and amylase enzymes activity by *T. viride*

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and *A niger* were tested. Two other microorganisms *Rhizopus oryzae* and *Lactobacillus plantarum* (bacterium) were included for purposes of comparison. Finally protein production by gamma-irradiated *T. viride* on partially digested corn husk slurry was also investigated.

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11. LITERATURE REVIEW.

The abundance of agricultural waste materials in the form of plant lignocellulose is well known. The attendant problem of utilization of lignocellulose for the purpose of producing fungal protein for direct human consumption (Spicer, 1973) or as animal feedstuff (Forss *et al.* 1972; Imrie, 1973) has engaged the attention of many researchers in the developed countries. Developing countries have only recently taken keen interest in plant biomass conversion.

The plant cell wall which consists of middle lamella, primary wall and a secondary wall has pectic substances, present as polygalacturonides with non-uronide carbohydrates covalently bound to an unbranched chain of (1,4) α-D-galacturonic acid units. The walls of soft, non-differentiated tissues are of the primary type rich in cellulose, hemicellulose and pectin; the cellulose here has a lower degree of polymerisation and crystallization than in the secondary wall (McNeil *et al.* 1984).

The degradation of cellulosic materials by fungi has been well documented. Sandhu and Sidhu (1980) showed that fungal succession during compositing process of agricultural waste material involved fungi like *Aspergillus fumigatus*, *A. niger*, *A. terreus*, *A. flavus*, *Mucor pusillus*, *Penicillium* spp., *Rhizopus microsporus*, *Trichoderma viride*, *T. longibrachiatum* and members of the order Agaricales (Basidiomycotina). Prior to this, Siu (1951) summarised research findings on cellulolytic activity of fungi and he placed *Aspergillus fumigatus and Trichoderma viride* among the strongly cellulolytic group, the moderately cellulolytic ones were

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Fusarium moniliforme, *F oxysporum* and *F solani*. The fungi *Aspergillus japonicus* and *A. ochraceus* were placed in the weak cellulolytic category whilst *A. candidus*, *A. flavus* and *Rhizopus oryzae* were classified as non-cellulolytic. Basu (1948) reported that his strain of *Aspergillus niger* on jute fibre was non-cellulolytic but *A. ustus*, *A. terreus* and *A. fumigatus* were cellulolytic. Reese and Downing (1951) classified twelve *Aspergillus* spp according to their ability to degrade cellulose and he placed *A. flavus*, *A. ochraceus* and *A. niger* in the non-cellulolytic group. On the other hand, Flannigen (1970) reported *A. fumigatus* and *A. niger* isolated from barley kernel as cellulose decomposing. Presumably, cellulolytic activity of fungi might be species and strain specific as well as depending on the type of substrate which is being metabolised by the fungus (Odamtten and Kampelmacher, 1985). Several members of the genus *Aspergillus* appear to be capable of hydrolysing the β-(1-4)-glucosidic linkage in the cellulose chain (Alexander, 1961; Raper and Fennel, 1972; Mazen, 1973; Steward and Walsh, 1972). But this does not itself determine whether they can attack cellulose or not since many non-cellulolytic microorganisms possess the C_x enzyme carrying out that reaction (Reese *et al.* 1950), (Fig. 1).

Weber (1969) found *Trichoderma viride*, *T. reesii*, and *T koningii* to have very high cellulolytic activity with the ability to utilize substrates with both reducing sugars in hydrolysed oat hull as well as cellulose. When grown on media containing maize or cotton fiber as sole carbon source, *T viride* synthesizes and releases into solution all enzymes that are

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essential for the breakdown of native cellulose to glucose (Mandels and Reese, 1964).

Beldman *et al.* (1985), and Beldman, (1986) purified the cellulases of *T. viride* and characterised and compared all detectable endoglucanases, exoglucanases and β-glucosidases. Synergism in cellulose hydrolysis by endoglucanases and exoglucanases purified from *T viride* were demonstrated by Beldman, (1986). Cellulolytic enzymes are also produced by other microorganisms including actinomycetes, gliding bacteria and bacteria (Wood, 1985). However whilst bacterial cellulases are often cell wall bound, highly active cellulases of fungi are released into the culture media.

It showed that only pre-treatment of cellulose involving either swelling or dissolution increases acessibility to attack by both cellulases and other reagents. Oat hulls contain 34 percent cellulose, and 30 percent pentosans giving a total carbohydrate content of 64 percent (Caldwell and Pomeranz, 1973; Rosenberg *et al.* , 1978). Maize husks contain about 67.3 percent cellulose, 10.3 percent lignin, 1.8 percent ash and 1.1 percent protein. The amino acid composition of four *T. viride* strains (QM 6_a, QM 913, QM 94414 and NRRL 3653) tested on hydrolysed oat hulls ammended with 12g/l glucose at 25°C for 10 days in shake culture flasks were similar. Amino acids encountered include Aspartic acid, alanine,threonine, serine, glutamic acid, proline, glycine, cystine, valine, histidine, arginine and tryptophan. Some strains exhibited higher content of alanine, valine methionine and

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histidine. The total crude protein of cultures of *T viride* QM6$_a$ on hydrolysed oat hull increased from 40.5g Kg^{-1} (10.02 percent crude protein) to a maximum of 71.0g Kg^{-1} (15.44 percent crude protein) in 8 days. This represented an overall 75.8 percent protein content enrichment of the oat hull.Growth of *T viride* on hydrolysed oat hulls therefore offer a potential for improved balanced animal feed for the livestock industry. The use of maize husk slurry as substrate for improving protein content of fungi eg. *T. viride* has not been tried to any great extent. There are limited references in the pertinent literature to the use of maize husk. Saah (1985) found an increase in the protein content of *T viride* grown on maize husk slurry. Protein content of hydrolysed maize husk was enriched by 72.7 percent in 15 days by *T viride.*

The pectinolytic enzymes of *T. viride* on the other hand has not been exhaustively investigated. The only reference to *T. viride* pectinase activity is the work of Rosenberg *et al.* (1978) who used a liquid medium of the following composition to culture *T viride*: glucose 12-18 gm/l; KH_2PO_4, 0.0250g/l; KNO_3 5.00g/l; $FeSO_4.7H_2O$, 0.013g/l; NH_4Cl, 0.750g/l . The release of protein into the medium during growth was closely and positively correlated with the appearance of cellulase and pectinolytic enzymes.

There is a synergistic action of cellulases from *T. viride* and pectinases from *A. niger* in plant biomass conversion. A combination of pectinolytic and cellulolytic enzyme was able to produce monomeric sugar solutions from beet pulp and potato fibre (Beldman *et al.* 1984). The process is optimal at pH 3.5-4.0 and

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is mainly caused by the polygalacturonase from the pectic enzyme which has its optimum at pH 6.0 (Jones *et al.*1976). In a second step, the extensive hydrolysis of the cell wall polysaccharides, a cellulose with high activity is needed (Beldman *et al.*1976).

Mutation breeding is one important tool in developing new, better-performing variety of crops. Radiation and some chemicals (mutagens) induce mutations by altering genes and creating genetic variability. Plant breeders have derived from induced mutations additional genetic resources for improving crops (Donini, 1983). Although sudden heritable changes in microorganisms have long been known to occur, it has only recently been possible for microbiologists to show that mutation in microorganisms can be induced by ultra violet light, mutagenic chemicals such as nitrogen mustard, X-rays and gamma rays.

The lethality of ultra violet radiation is most easily expressed by the fraction of irradiated spores which fail to germinate. Doses that are not lethal delay germination or induce abnormalities (Cochrane, 1958). In surveys of fungal susceptibility it is often noted that dark spores tend to be more resistant. Pigmentation alone, however, cannot explain specific differences in sensitivity (Cochrane, 1958) since ultra violet induced mutagenesis is also affected by several modifying enviromental factors.

Ionising radiations are radiations whose passage through a material cause the production of ion-pairs. The process of food irradiation involves exposing the food or microrganisms therein to ionising radiation so that a presdribed quantity (dose) is absorbed.

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Radiation sources used are the following:

A. Gamma-rays from the nuclides Co-60 or Cs-137.

B. X-rays generated from machine sources operated at or below an energy level of 5 MeV (millielectron Volts).

C. Electron beams generated from machine sources (eg. Van der Graaf generator) operated at or below an energy level of 10 MeV. The way in which the radiation dose absorbed is measured differs according to the source of radiation.

Most studies on fungi eg. *Aspergillus* spp, *Penicillium* spp and *Neurospora* spp have employed X-rays or gamma rays as mutagens. The technique has been put into practice where the time of maximum synthesis of the desired product can be accurately judged and mutagenesis carried out during this time. In the case of *Cephalosporium acremonium*, the time of maximum cephalosporin C synthesis appears to correlate with differentiation into athrospores (Nash and Haber, 1971), so that mutagenesis during the time of differentiation could increase specificity for antibiotic titre genes. Application of ionizing radiation for improvement of enzyme (amylase, pectinase, cellulase, etc.) production by *A. niger*, *T. viride R, oryzae* and *L plantarum* on the other hand has not been tried.

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111. MATERIALS AND GENERAL METHODS

1. MATERIALS:

FUNGI: Both *Aspergillus niger* Van Tiegh and *Rhizopus oryzae* Went and Prinsen-Gerling were isolated from maize stored in humid chamber at ≥ 80% R.H. for 10 days. *Trichoderma viride* was isolated from soil within the Botany Department, Univ. of Ghana, Legon.

BACTERIUM: *Lactobacillus plantarum* was isolated from fermenting cassava pulp.

Dry maize husks were obtained from the Research Farm of the Ghana Atomic Energy Commission, Kwabenya.

11. GENERAL METHODS:

A. MAINTENANCE OF STOCK CULTURE:

Stock cultures of *A. niger*, *R. oryzae* and *T viride* were maintained on slants of oxytetracycline glucose yeast extract (OGYE) agar at 10°C and subcultured every two weeks.

L. plantarum was maintained on Tryptone glucose yeast extract agar at 35°C and was subcultured every two weeks.

B. GAMMA IRRADIATION:

Dry spores of the fungi were seperated by sieving after being cultured in 100g of blended and autoclaved maize grains. The spores were then irradiated in air at pre-selected doses of 0, 20, 40, 50, 100, 200 Krads in a Cobalt-60 Irradiator (Gamma Cell 220, Atomic Energy of Canada Limited). The source dose rate was 541.44 Gy/hr. For each dose applied there were four replicates.

L. plantarum spores were cultured on Tryptone glucose Yeast Extract agar at 35°C for 3 days. The spores were then

) peptone solution (10^5 spores/ml). About

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10 ml in MaCartney tubes were irradiated with the different indicated doses as above.

C. METHODS OF INNOCULATION:

Erlenmyer flasks containing 30 ml of V-8 broth ammended with 20g/l of glucose were inoculated with 1ml aliquot of spore suspension of either control or irradiated spores of *A. niger*. *R. oryzae* or *T. viride*. Each spore suspension contained about 10^5 spores/ml. About 1ml aliquot of *L. plamtarum* was used in inoculating 30 ml nutrient broth.

D. ASSESSMENT OF GROWTH OF MICROORGANISMS:

Growth of *T. viride*, *A. niger* and *R. oryzae* in static liquid medium was assessed by estimating the dry weight of harvested mycelium at the end of the 5 days incubation. at 28-35°C. The *A. niger* and *T. viride* were incubated at 35°C (Odamtten, 1977) and *R. oryzae* at 28°C (Akushie, 1980). Mycelium collected on a previously weighed and dried Whatman No.2 filter paper was dried and weighed, after cooling in a dessicator.

Growth of *L. plantarum* was determined by measuring the optical density of the substrate at 660nm after 5 days incubation at 28°C.

E. DETERMINATION OF ENZYME ACTIVITY OF IRRADIADED MICROORGANISMS:

1. AMYLASE: Amylase activity was determined by measuring the reduction in viscosity of 0.5 per cent starch solution with Fenkse-Ostwald's viscometer at 30°C. (Plate 1) The reaction mixture contained 5ml. of starch solution and 5ml. of culture filtrate of either *A. niger*, *R. oryzae* or *T. viride*. After mixing the solution was left for 2hrs to allow enzyme digestion before measurement were taken. The control reaction mixture was heated to 100°C for 5-10min. to inactivate the enzymes.

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The reaction mixture for the determination of amylase activity for *L. plantarum* contained 5ml of starch solution and 5ml of the culture solution (ie. the solution containing the spores of *L. plantarum*).

11. CELLULASE: The cellulase activity of *A.niger*, *R. oryzae* and *T. viride* was determined by the same procedure as that of the amylase activity except that instead of starch solution, 0.5 per cent solution of carboxymethylcellulose (CMC) was used.

In the determination of the cellulase activity of *L. plantarum*, the same procedure as in amylase determination for *L. plantarum* was adopted except that CMC solution was used instead of starch solution.

111. PECTINASE: The pectinase activity of *A.niger*, *R. oryzae* and *T. viride* was determined by the same procedure as that used in the determination of amylase activity except that instead of starch solution, 0.5 per cent pectin solution was employed.

In the determination of pectinase activity of *L. plantarum*, the same procedure as in the amylase activity determination of *L. plantarum* was adopted but instead of starch solution, 0.5 per cent pectin solution was used.

The reduction in viscosity expressed as the percentage loss in viscosity was calculated by the following mathematical expression:

$$\text{Percentage loss in viscosity} = \frac{[T_o - T_1]}{[T_o - T_w]} \times 100$$

T_o = flow time of reaction mixture at 0min.

T_1 = flow time of reaction mixture at a particular time interval

T_w = flow time of distilled water.

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Plate 1. Photograph of set up used in determining changes in viscosity of different media caused by amylase, pectinase and cellulase enzymes produced by T. viride, R. oryzae, A. niger and L.plantarum. (Note the hand holding the Fenske-Ostwald Viscometer)

Plate 2. A close up of the Fenske-Ostwald Viscometer seen in Plate 1.

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F. pH DETERMINATION:

The pH of the media before and after incubation was measured by using a pH Meter (PYE Unicam Model 290 MK 2)

G. CRUDE PROTEIN DETERMINATION:

For crude protein determination, Kjeldahl's method of estimation (macroprocedure) was employed as outlined by the Association of Official Analytical Chemists.- AOAC- (1970).

H. CULTURE MEDIA:

All chemicals used in the preparation of media were either of the "Analar", B.D.H. (British Drug House) or Oxoid grade. The composition of medium varied with the experiment as stated in the appropriate places in the text. Erlenmeyer pyrex flasks (250ml-capacity) each containing 30ml media were used for all the liquid cultures. Twenty milliliter of solid agar medium were used per 9cm diameter Petri dish.

THE COMPOSITION OF THE MEDIA WAS AS FOLLOWS:

1.LIQUID MEDIUM: V-8 (Cambell Soup Company U.S.A.) Broth: 200ml of V-8 juice made up to 1,000 ml with distilled water (pH adjusted to 4.2).

RESENBERGH"S MEDIUM: (Resenbergh *et al.* 1978)

A modification of the medium used by Resenbergh *et al* (1978) was employed. The composition of the medium was as follows: 180 mg/l, glucose; KH_2PO_4 25 mg; $KNO_3.7H_2O$, 30 mg; $FeSO_4.7H_2O$, 1.3 mg; NH_4Cl, 75 mg; 1,000 ml distilled water.

11. PREPARATION OF MAIZE HUSK SLURRY:

A slurry of maize husk was prepared using different [illegible] ze husk was hydrolysed with either 1% Sodium

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hydroxide or 5% Sodium hydroxide at 80°C, 100°C or 120°C for varying periods (1, 2, 3, hours.)

In a parallel experiment, 10g of maize husk was hydrolysed with either 1% Hydrochloric acid or 5% Hydrochloric acid at 80°C, 100°C or 120°C for varying periods (1, 2, or 3 hours.).

In all instances the hydrolysed corn husk was aseptically washed with several changes of distilled water before being transferred aseptically into the sterilized media. The number of replicates in each treatment was four.

I. STUDIES ON THE MORPHOLOGY OF *T. VIRIDE* AND *A. NIGER* BEFORE AND AFTER EXPOSURE TO GAMMA IRRADIATION.

Dry conidia of *T. viride* and *A. niger* were exposed to 0-200 Krad of gamma irradiation and then used in inoculating petri plates containing 20 ml of V-8 Agar. The cultures were incubated at 35°C for 5 days to allow sporulation. Mycelia from the cultures were mounted in lactophenol cotton blue and the morphology of the cultures compared. Photomicrographs were taken to facilitate this experiment.

K. METHODS OF STERILIZATION:

All media, conical flask, McCartney tubes, Fenkse-Ostwald's Viscometer, etc. were sterilized by autoclaving at 121°C for 15 mins.(15 psi). Cotton wool plugs temporarily covered with grease proof paper were used to prevent the penetration of any condensed water during the autoclaving.

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Petri dishes (9.0 cm diameter) were sterilized by heating at 165°C for 6-8 hours in an electrically heated oven (.Gallenkamp Hotbox Oven Size 1).

The Laminar flow cabinet (Microflow®) was set going for at least 15-30 min before using the inoculating chamber. The air conditioner was put on to augment the safety of the room for inoculation.

J. EXPERIMENTAL PRECAUTIONS:

1. Glassware was kept scrupulously clean. Glassware which had already been cleaned with water and detergents was rinsed several times with tap water and three times with distilled water and then allowed to drain before use.

2. Oven-dried filter paper usually lost some weight because of heating. Filter paper used throughout these investigations was therefore heated at 75°C for 24 hours prior to use.

3. Batches of filter paper with loads of dried mycelium were always conveyed to the balance room in a closed dessicator to avoid absorption of moisture.

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1V. EXPERIMENTAL PROCEDURE

A. DATA ON MAIZE PRODUCTION IN GHANA 1981 - 1990.

The information was compiled from the 10 regions in Ghana in order to ascertain how much "waste" in terms of corn husk was available for use in supplementing feed production using microbial protein. Data from small scale farmers are not included. This connotes that data obtained could be 5-10 percent higher in the overall picture throughout the country. Results are presented in Figs. 1-4 and in Appendices 1-4.

B. EFFECT OF GAMMA IRRADIATION ON VEGETATIVE GROWTH OF *A. NIGER* *R. ORYZAE* AND *T VIRIDE*.

In this Section, vegetative growth of the listed fungi after exposure to low gamma irradiation doses was assessed. About 1ml aliquot of irradiated spores of *A. niger*, *R. oryzae* and *T viride* suspended in 0.1 percent peptone solution was used to innoculate 30ml of V-8 broth ammended with 20g/1 of glucose in Erlenmeyer flasks. There were four replicates. Each mixture was swirled around to ensure thorough mixing of the spores in the growth medium. The flasks were incubated at 28°C for 5 days.

Growth of *A. niger*. *R. oryzae* and *T. viride* was assessed by estimating the weight of harvested mycelium at the end of the incubation period following procedures outlined under Section D of the General Methods. Results are presented in Fig. 5 and Table 3A, 3B, 4.

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C. EFFECT OF GAMMA IRRADIATION ON GROWTH OF *L. PLANTARUM*.

Experiments in Section A were repeated using *L. plantarum*. About 1ml aliquot of irradiated spores of *L. plantarum* suspended in peptone solution was used in inoculating 30ml nutrient broth. The mixture was incubated at ambient temperature of 28°C for 5 days. Vegetative growth of *L. plantarum* was determined by measuring the optical density of the substrate at 660 nm. Fig. 6 and Table 5 summarise the results obtained.

D. AMYLASE ACTIVITY OF GAMMA IRRADIATED MICROORGANISMS.

Amylase production after gamma irradiation of the test microorganisms was investigated following procedures outlined under General Methods. The results are presented in Fig. 7 and Appendix 7.

E. CELLULASE ACTIVITY OF GAMMA IRRADIATED MICROORGANISMS.

The determination of cellulase activity of *A. niger*, *R. oryzae*, *T. viride* and *L. plantarum* followed the same procedure as outlined for the determination of amylase activity. The reaction mixture was made of 5 ml of culture filtrate of the appropriate microorganism and 5 ml of Carboxymethylcellulose (CMC).

F. PECTINASE ACTIVITY OF GAMMA IRRADIATED MICROORGANISMS.

Pectin degrading enzymes in microorganisms are essential for effective nutrient recycling of agricultural waste. It was

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therefore necessary to ascertain the effect of gamma irradiation on the selected organisms. The determination of pectinase activity of *A. niger*. *R. oryzae*, *T. viride* and *L. plantarum* followed the same procedure as the determination of amylase activity of the microorganisms. The reaction mixture was made up of 5 ml of the cultural filtrate of the appropriate microorganism and 5 ml of pectin. The set up was exactly as in Section D and E (Plate 1).

G. COMPARATIVE pH CHANGES DURING GROWTH OF GAMMA IRRADIATED *A. NIGER*, *R. ORYZAE*, *T. VIRIDE* AND *L. PLANTARUM*.

Vegetative gowth of microorganisms is accompanied by production of secondary metabolites that cause drift in the pH of the medium. Thus a medium may become more acidic or may drift to the basic side depending on its initial pH. The changes in the pH during growth of the irradiated spores of the indicated organisms was investigated using a pH metre (PYE Unicam Model 290 MK 2). The spores were incubated at their optimal temperatures for 5 days.

H. EFFECT OF CHEMICAL TREATMENT, HEATING TEMPERATURE, AND DURATION OF TREATMENT ON DIGESTIBILITY OF LIGNOCELLULOSE IN CORN HUSKS FOR THE PRODUCITON OF CRUDE PROTEIN NITROGEN BY *T. VIRIDE*.

Fully lignified tissues are degradable only by microbes that are able to degrade lignin, with the exception of the brown-rot fungi. Cellullose and hemicellulose are closely associated with lignin in the plant cell wall. The structural resistance of these polymers

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has made cellulosic materials generally non-nutritive and difficult to digest. For an increase in nutritive value, the material must be first converted into monomers or oligomers (Kirk, 1983).

Use of other non-lignolitic microbes to process lignocellulose requires chemical or physical disruption of the lignin barrier, to fermentable products. Extant and potential use of fungi for lignocellulose conversion can be divided into five categories:

a. conversion into feed or food; b. manufacture of mechanical pulp; c. production of microbial chemical products; d. production of chemicals from lignin; and e. treatment of lignocellulose-derived waste.

In this Section, the best combination of treatments that would facilitate the utilization of corn husk by *T. viride* was investigated. *T. viride* was selected because of its versatility in degrading lignocellulose and it is not kwown to produce any harmful toxins to human. The procedure followed is as outlined under General Methods. The results are summarised in Table 3.

I. PRODUCTION OF FUNGAL PROTEIN BY GAMMA IRRADIATED *T. VIRIDE* SPORES ON CORN HUSK SLURRY COMBINED-TREATED WITH HEAT AND CHEMICALS.

Formation of crude protein by irradiated *T. viride* was studied. If irradiation enhanced crude

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protein accumulation on hydrolysed corn husks, there would be an added advantage in using irradiation to stimulate enzymes required to effect the necessary chemical reactions. Results obtained are presented in Table 12 A-D.

J. EFFECT OF GAMMA IRRADIATION ON THE MORPHOLOGY OF *A. NIGER* AND *T. VIRIDE*.

Different strains of fungi respond differently to radiation treatment. Mutagenesis may be reflected not only in change in enzyme production patterns but also in the morphology of the test organism. In the concluding Section of this theses, the effect of low gamma irradiation on the morphology of *A. niger* and *T. viride* was studied. Temporary mounts of the mycelium were made and were stained with Lactophenol cotton blue. Plate 3 shows the results obtained.

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V. RESULTS

A. DATA ON MAIZE PRODUCION IN GHANA. (1981-1990)

Results are presented in Fig. 2-5. Maize production between 1984 and 1990 exceeded 500,000 metric tonnes except in 1985 where there was a decline to 395,000 metric tonnes (Fig. 2). The unfavourable years of drought between 1981-1983 was attented by a sharp decline in maize production. The poorest maize production of the decade (1981-1990) was in 1983 (140,800 metric tonnes) . The bulk of the national maize production was contributed by Ashanti, Brong Ahafo, Eastern and Northern Regions (Fig. 3). Maize husk production was commensurate with the total maize harvest for each region (Fig.4). Therefore as a raw material for fungal protein production, maize husk has a potential for sustainable use. The estimated cost of the maize husk (Fig.5) shows that it can serve as a source of revenue for the farmer if other uses are found for it in the feed industry.

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Fig. 2.

MAIZE PRODUCTION IN GHANA.1981-90.

(FIGURES IN .000 MT.)

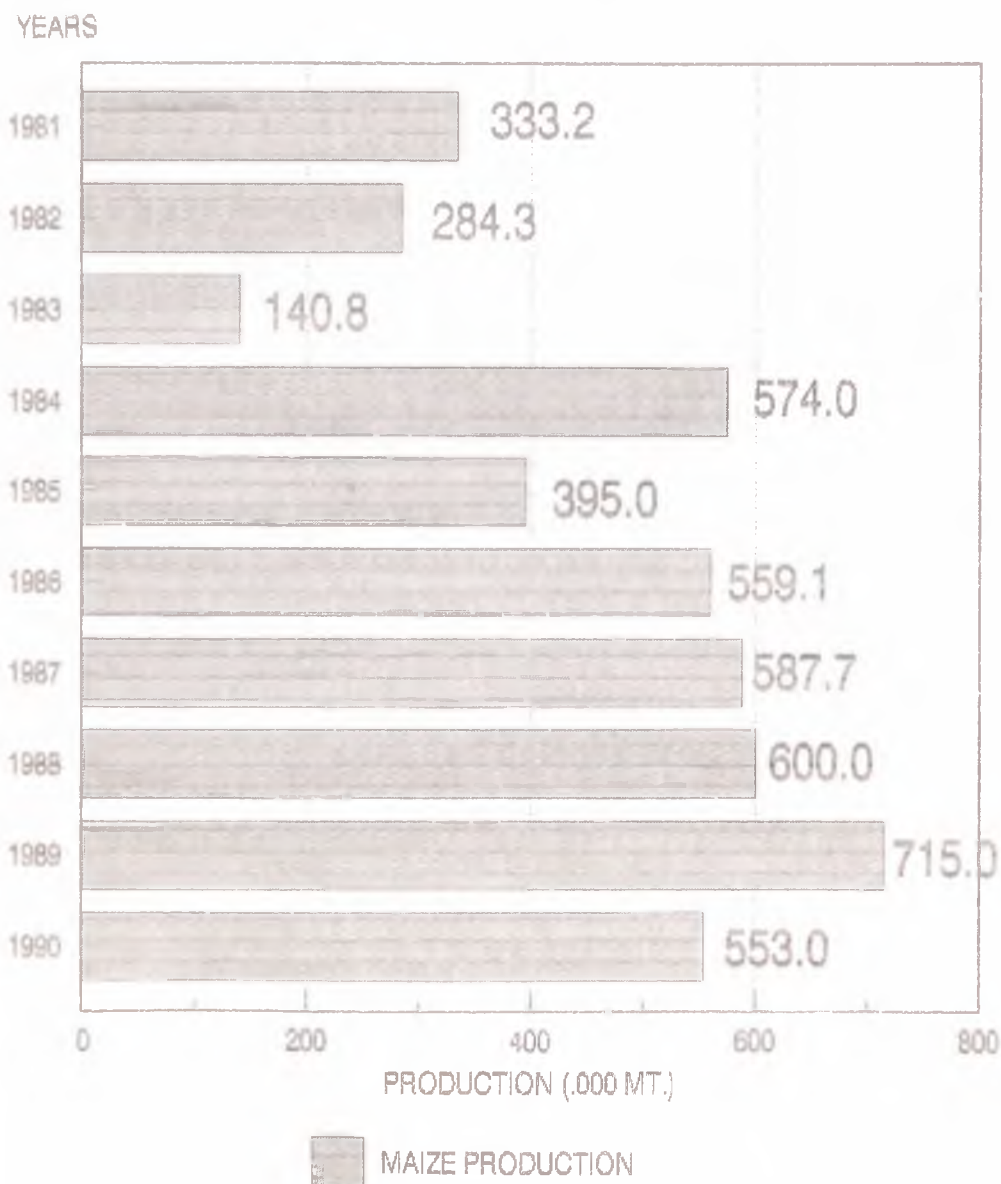

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Fig. 3.

MAIZE PRODUCTION BY REGION 1987-1990.

(FIGURES IN .000 MT)

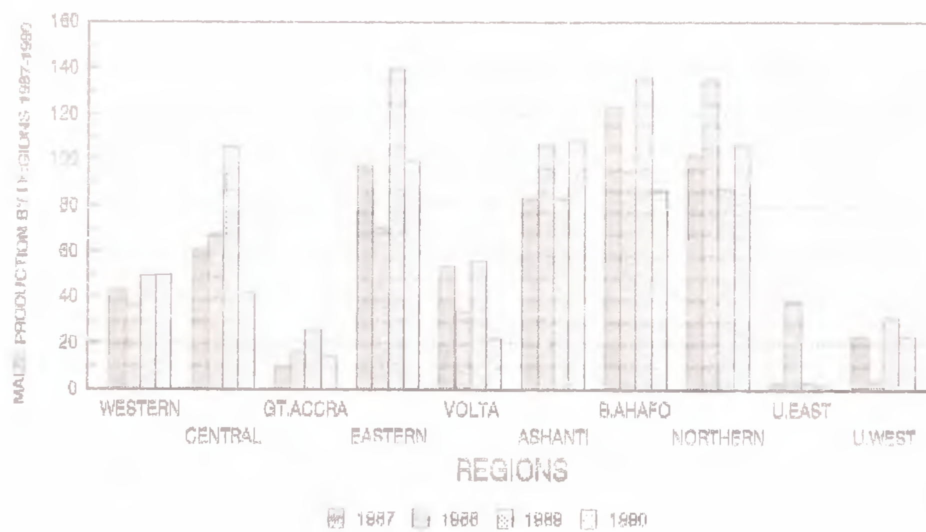

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Fig.4.

MAIZE HUSK PRODUCTION BY REGION 1987-1990.

(FIGURES IN .000 MT)

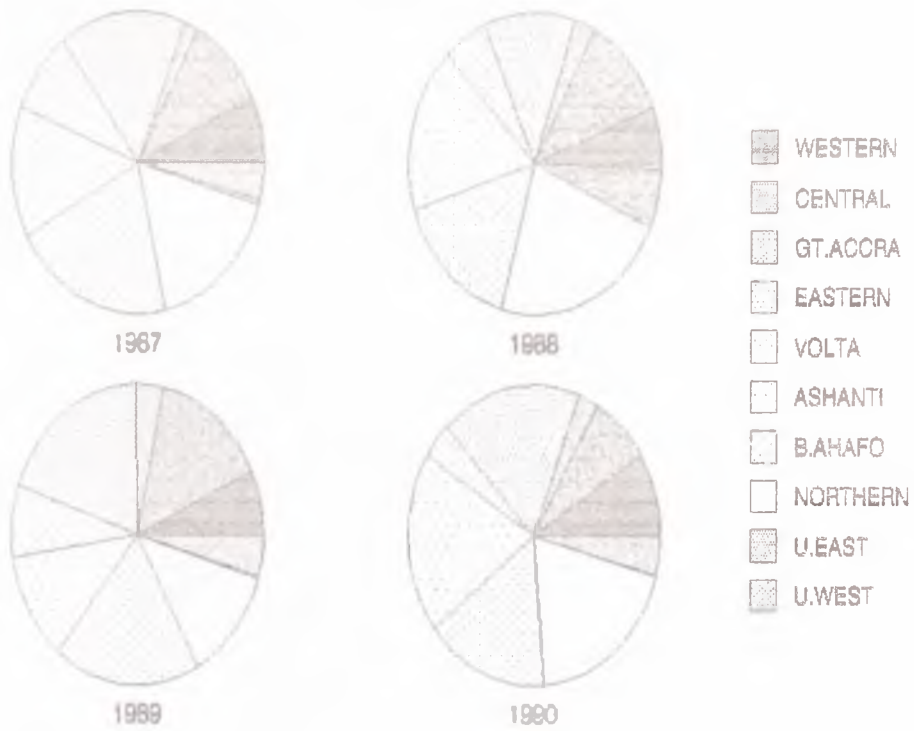

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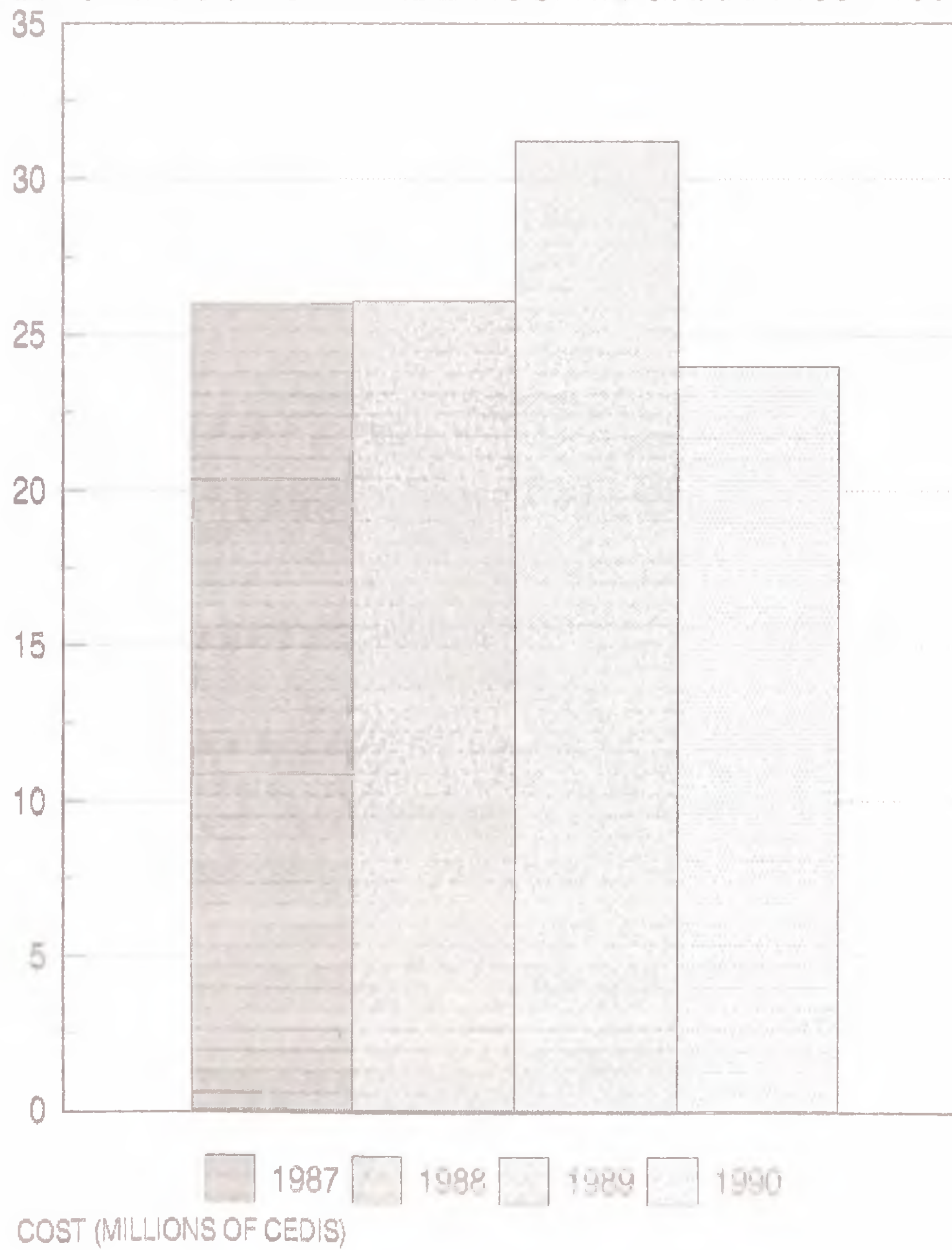
Fig. 5.

ANNUAL COST OF MAIZE HUSK IN GHANA 1987-1990.



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B. EFFECT OF GAMMA IRRADIATION ON VEGETATIVE GROWTH OF *A. NIGER*, *R. ORYZAE* AND *T. VIRIDE*.

The application of gamma irradiation to dry spores of *A. niger*, *R. oryzae* and *T. viride* prior to culturing in liquid medium had variable effects. Results are presented in Fig 6, Appendix 5, Tables 3a, 3b and 4.

A dose of 20 Krad decreased vegetative growth of *A. niger* by 46.6 percent, and thereafter further increases in dose (20-50 Krad) increased dry matter accumulation by the fungus to nearly the same level as in the control (Fig. 6).The changes in pH of medium during growth were as follows : control (pH 4.4-2.7); 20 Krad (pH 4.4-2.6); 40 Krad (pH 4.4-2.6); 50 Krad (pH 4.4- 2.5); 100 Krad (pH 4.4-2.2); 200 Krad (pH 4.4-2.2).

Vegetative growth of *R. oryzae* was increased by about 30 percent when spores were exposed to 50 Krad and remained nearly the same up to 200 Krads. (Fig 6). There was no stastistical difference ($p = 0.05$, Student t-test) between dry matter obtained at 50, 100, or 200 Krad (Fig 6, Tables 3-4).

The best vegetative growth (28-30mg) for *T. viride* was obtained when 100-200 Krad was applied to the spores.

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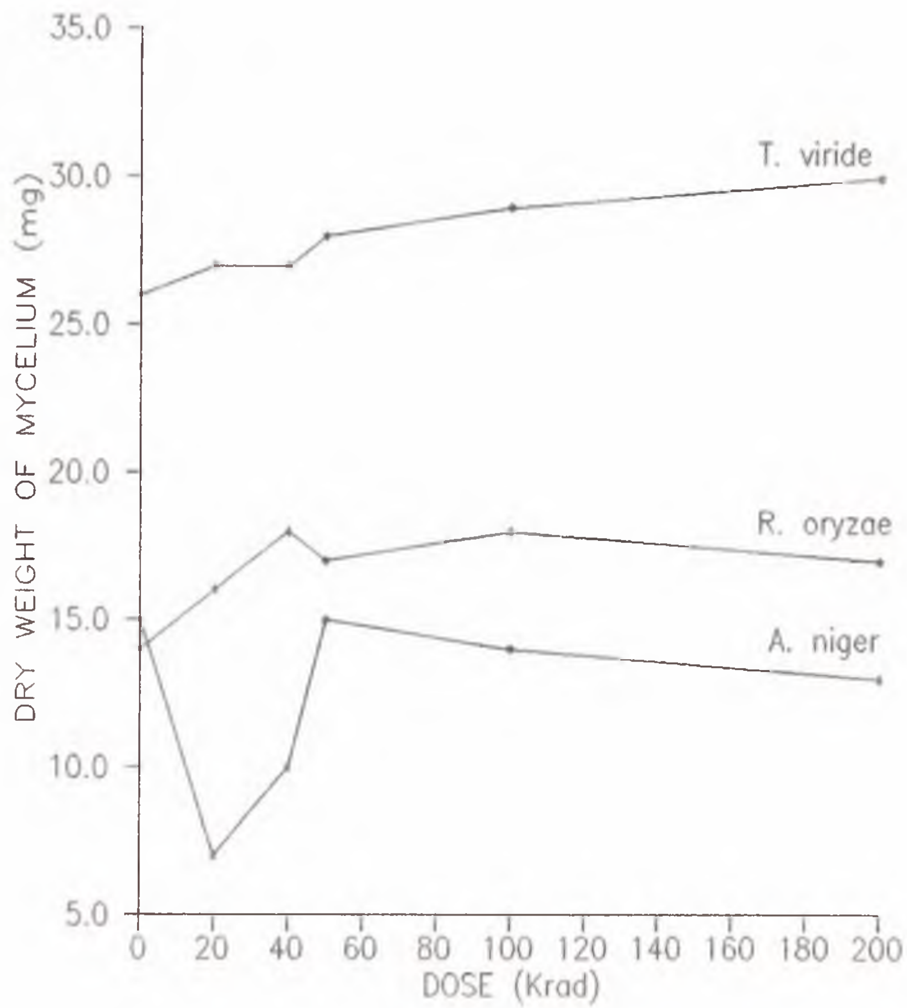

Fig. 6 Effect of gamma irradiation on vegetative growth of _A. niger_, _R. Oryzae_, and _T. viride_ for 5 days.

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Table 3a

Vegetative growth of A. niger in liquid medium at 28°C for 5days after exposure to indicated doses of gamma irradiation

Organism	Dose Applied (Krad)	Replicates	pH of Medium Initial	Final	Mean	Dry Weight of Mycelium (mg)	Mean S.E.
A. niger	0	1	4.4	2.7	2.8	13	15±1.2
		2	4.4	2.8		16	
		3	4.4	2.8		16	
		4	4.4	2.8		16	
	20	1	4.4	2.8	2.7	8	7.0±0.8
		2	4.4	2.8		7	
		3	4.4	2.6		6	
		4	4.4	2.6		6	
	40	1	4.4	2.8	2.7	12	10.0±1.2
		2	4.4	2.7		10	
		3	4.4	2.7		9	
		4	4.4	2.7		9	
	50	1	4.4	2.5	2.5	15	15±0.8
		2	4.4	2.5		14	
		3	4.4	2.5		14	
		4	4.4	2.6		16	
	100	1	4.4	2.2	2.2	15	14.0±0.7
		2	4.4	2.2		13	
		3	4.4	2.1		14	
		4	4.4	2.2		14	
	200	1	4.4	2.4	2.2	13	13±1.2
		2	4.4	2.0		15	
		3	4.4	2.3		12	
		4	4.4	2.2		12	

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Table 3B

Vegetative growth of T. viride in liquid medium at 28°C for 5 days after exposure to indicated doses of gamma irradiation (Data provided Fig 5)

Organsm	Dose Applied (Krad)	Replicates	pH of Medium			Dry Weight of Mycelium (mg)	
			Initial	Final	Mean		Mean ± S.E.
T. viride	0	1	4.4	5.4	5.4	26	26.0±1.5
		2	4.4	5.4		28	
		3	4.4	5.5		24	
		4	4.4	5.3		25	
	20	1	4.4	5.5		28	27.0±1.2
		2	4.4	5.5	5.6	28	
		3	4.4	5.8		25	
		4	4.4	5.6		27	
	40	1	4.4	5.8		27	27.0±0.7
		2	4.4	5.7		27	
		3	4.4	5.7	5.7	26	
		4	4.4	5.7		28	
	50	1	4.4	6.4		27	28.0±0.7
		2	4.4	6.3		29	
		3	4.4	6.4		28	
		4	4.4	6.5	6.4	28	
	100	1	4.4	6.3		29	29.0±0.4
		2	4.4	6.4		29	
		3	4.4	6.2		29	
		4	4.4	6.3	6.3	28	
	200	1	4.4	6.3		32	30.0±1.2
		2	4.4	6.3		30	
		3	4.4	6.1	6.2	29	
		4	4.4	6.2		29	

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Table 4

Vegetative growth of R. oryzae in liquid medium at 28°C for 5 days after exposure to indicated doses of gamma irradiation (Data provided Fig.5)

Organism	Dose Applied (Krad)	Replicates	pH of Medium			Dry Weight of Mycelium (mg)	
			Initial	Final	Mean		Mean ± S.E.
R. oryzae	0	1	4.4	4.3	4.4	13	1[illegible].0±2.0
		2	4.4	4.4		12	
		3	4.4	4.4		17	
		4	4.4	4.4		16	
	20	1	4.4	4.1	4.2	16	16.0±0.4
		2	4.4	4.3		16	
		3	4.4	4.2		15	
		4	4.4	4.2		16	
	40	1	4.4	4.0	4.1	17	18.0±0.8
		2	4.4	4.2		19	
		3	4.4	4.1		18	
		4	4.4	4.0		17	
	50	1	4.4	4.0	4.0	16	17.0±0.4
		2	4.4	3.9		17	
		3	4.4	4.1		17	
		4	4.4	4.0		17	
	100	1	4.4	4.0	4.0	17	18.0±1.2
		2	4.4	4.1		17	
		3	4.4	4.0		20	
		4	4.4	4.0		18	
	200	1	4.4	4.1	4.0	16	17.0±1.3
		2	4.4	4.0		16	
		3	4.4	3.9		18	
		4	4.4	4.0		19	

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C. EFFECT OF GAMMA IRRADIATION ON GROWTH OF *L. PLANTARUM:*

Figure 7 and Table 5 summarise results obtained. The best growth of the bacterium was obtained with a dose of 50 Krad and thereafter further increases in irradiation dose applied (100-200 Krad) depressed vegetative growth by nearly 50 percent (Fig. 7). The pH of the medium drifted from 4.4 to 5.5 in the control. There was therefore a significant difference between the bacterium and the fungi in relation to their response to irradiation.

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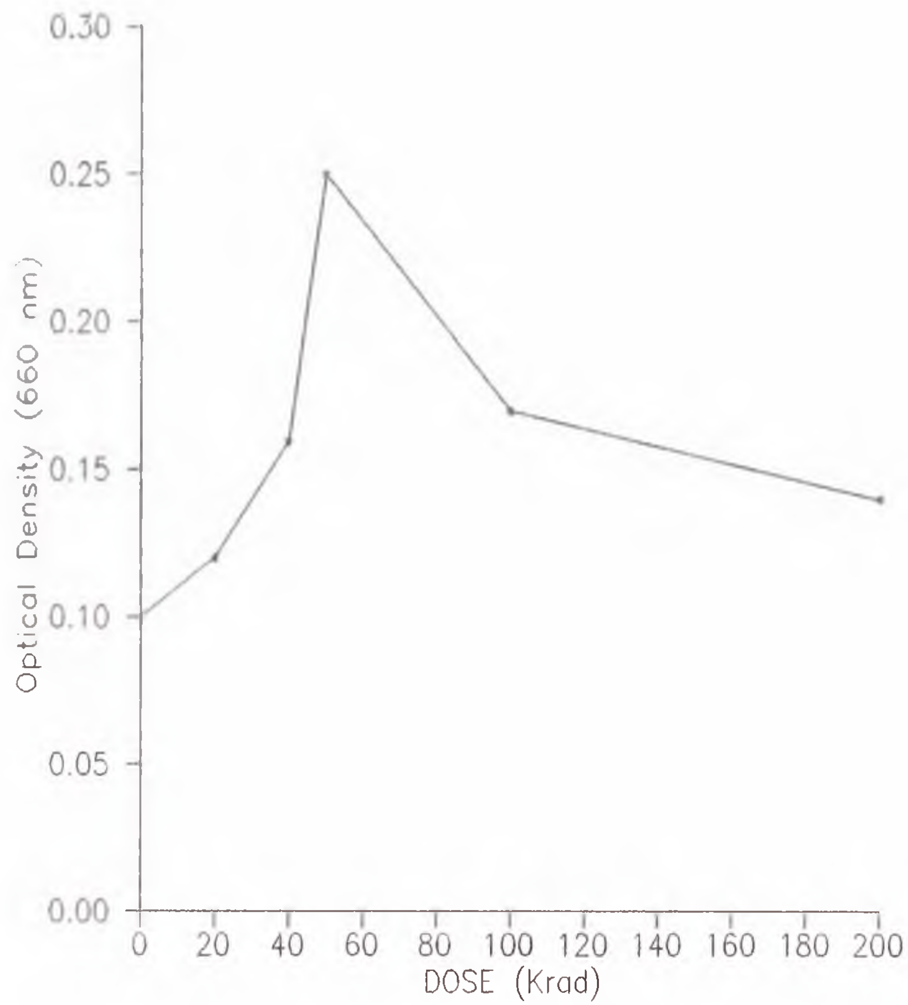

Fig. 7 Influence of gamma irradiation on vegetative growth of *L. plantarum* incubated in nutrient broth at 28° for 5 days.

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Table 5

Influence of gamma irradiation on growth of L. plantarum in nutrient broth at 28°C for 5 days. (Data provided Fig. 6)

Dose Applied (Krad)	Replicates	pH of culture medium Initial	Final	Mean	Optical Density at 660 nm.	Mean ± S.E.
0	1	7.4	5.5	5.5	0.08	0.10±0 01
	2	7.4	5.4		0.10	
	3	7.4	5.6		0.12	
	4	7.4	5.5		0.10	
20	1	7.4	5.1	5.2	0.12	0.12±0.00
	2	7.4	5.1		0.12	
	3	7.4	5.4		0.12	
	4	7.4	5 2		0.12	
40	1	7.4	4.7	4.7	0.15	0.16±0.01
	2	7.4	4.7		0.16	
	3	7.4	4.8		0.16	
	4	7.4	4.6		0.17	
50	1	7.4	4.6	4.6	0.24	0.25±0.01
	2	7.4	4.6		0.24	
	3	7.4	4.5		0.27	
	4	7.4	4.6		0.25	
100	1	7.4	4.7	4.7	0.18	0.17±0.01
	2	7.4	4.6		0.16	
	3	7.4	4.8		0.17	
	4	7.4	4 8		0.17	
200	1	7.4	4.8	4.8	0 14	0.14±0.03
	2	7.4	4 8		0.12	
	3	7.4	4 8		0.16	
	4	7.4	4.8		0.15	

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D. COMPARATIVE ACTIVITY OF AMYLASE IN GAMMA-IRRADIATED MICROORGANISMS:

Gamma irradiation doses up to 50 Krads increased amylase activity (measured as reduction in viscosity of starch) of all the microorganisms, namely *A. niger*, *R. oryzae*, *T. viride* and *L. plantarum* (Fig. 8). There was significant decline in enzyme activity ($p < 0.05$) when higher doses (100, 200 Krad) were applied. (Fig. 8).

Although the unirradiated bacteria *L. plantarum* did not show any amylase activity, its enzyme production and activity markedly increased with increasing doses (20, 40, 50 Krad) and thereafter declined.

E. CELLULASE ACTIVITY OF GAMMA-IRRADIATED MICROORGANISMS:

Optimal cellulase activity (measured as reduction in viscosity of carboxymethylcellulose CMC) of all the organisms varied depending on the dose applied prior to incubation of spores. Fig. 9 summarises the results obtained.

A dose of 100 Krads was the optimal for obtaining

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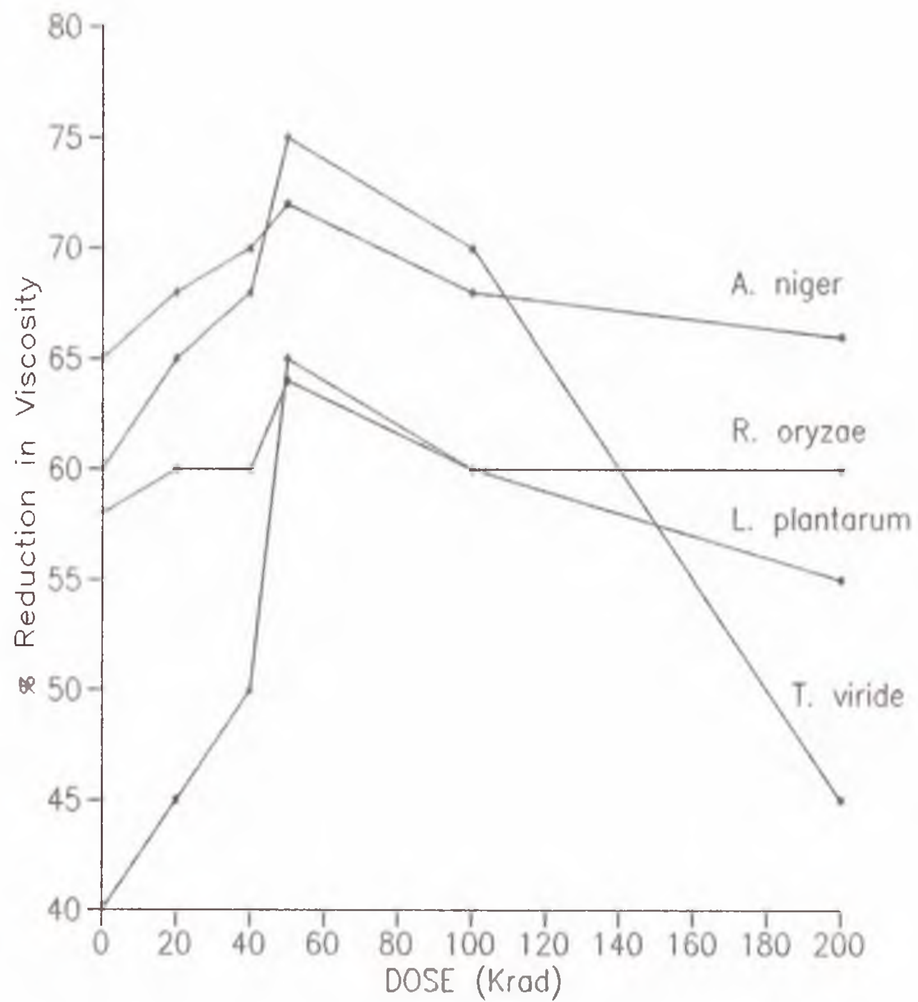

Fig. 8 Effect of gamma irradiation on amylase activity of indicated microorganisms. (Note the decline in enzyme activity after 50 Krad).

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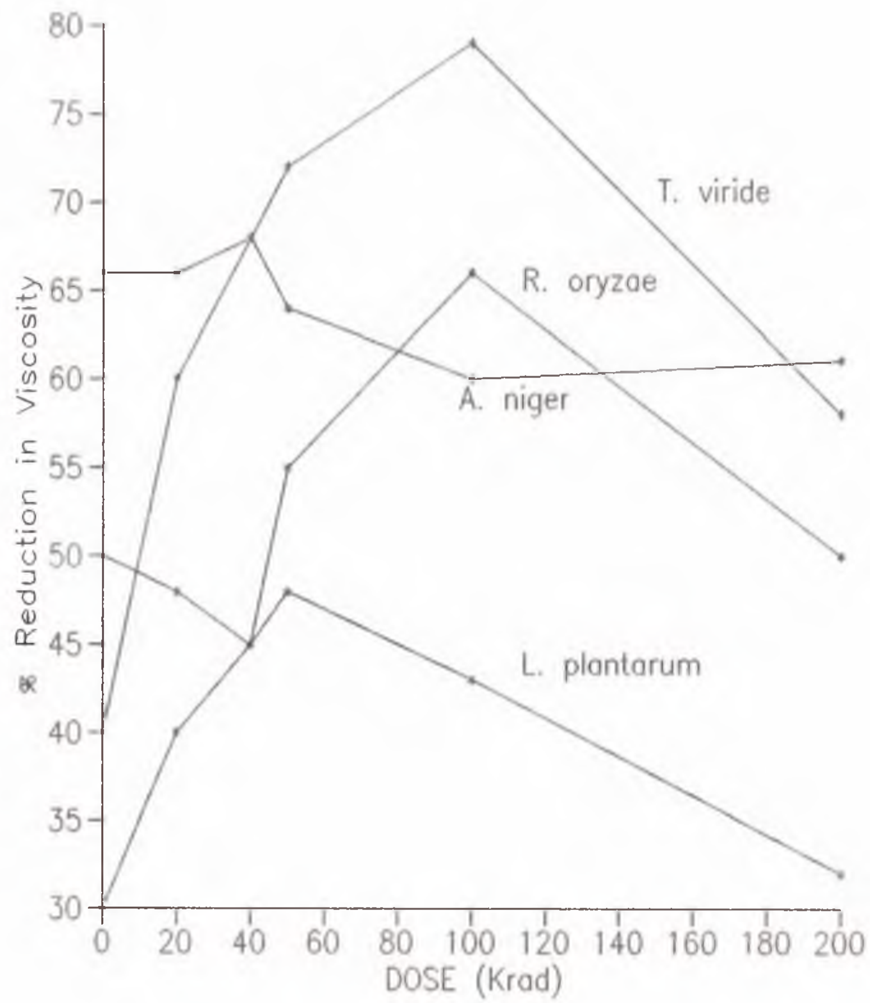

Fig. 9 Effect of gamma irradiation on cellulase activity of indicated microorganisms. (Note the variation in response to dose applied).

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maximum cellulase activity in *T. viride* and *R. oryzae*. A dose of 200 Krads was clearly unsuitable as it brought about significant ($p < 0.05$) decline in the ability of the culture filtrate in reducing the viscosity of CMC. (Fig. 9).

On the other hand 50 Krads was optimal for *A. niger* and *L plantarum* as the highest activity of cellulase enzyme was obtained at this dose (Fig. 9).

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F. PECTINASE ACTIVITY OF GAMMA-IRRADIATED MICROORGANISMS:

Fig. 10 shows results obtained when pectinase activity of the same microorganisms was tested after gamma irradiation. A dose of 50 Krad about doubled the pectinase enzyme activity in *T. viride* and increased pectinase activity of *A. niger* by about 10 percent but marginally increased the same activy in *R. oryzae* and *L. plantarum*. Clearly in this instance higher doses beyond 50 krad depressed pectinase activity.

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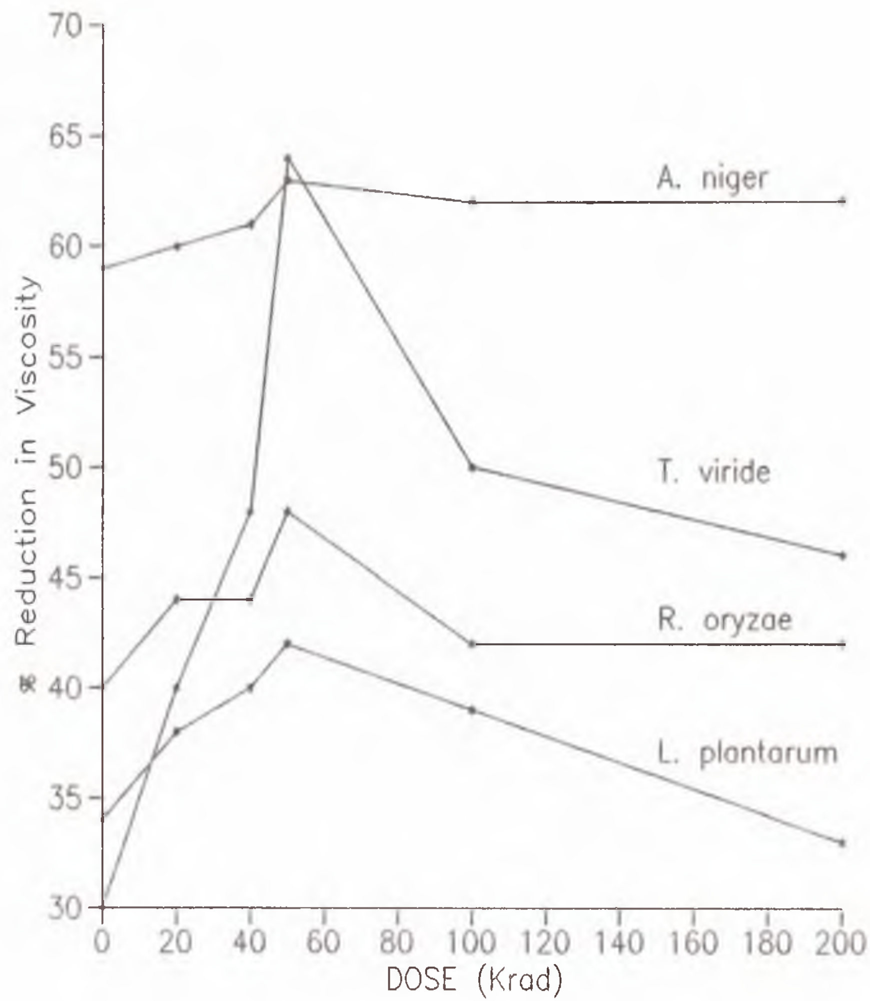

Fig. 10 Comparative pectinase activity of gamma irradiated spores of indicated microorganisms after 5 days incubation.

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G. pH CHANGES DURING GROWTH OF GAMMA-IRRADIATED *A. NIGER*, *R. ORYZAE*, *T. VIRIDE* AND *L. PLANTARUM*.:

The pH of a medium influences enzyme activity. In this Section pH of the gamma-irradiated spores of *A. niger*, *R. oryzae*, *T. viride* and *L. plantarum* during incubation was examined. Results are presented in Fig. 11. Changes in the acidity of the media varied from one microorganism to another. For example, the pH of medium in which *R. oryzae* and *L. plantarum* were cultured after gamma irradiation progressively drifted from pH 4.4 to pH 4.0 and from 5.5 to 4.5 respectively when dose applied increased from 0 to 50 Krad. Thereafter the pH of the medium remained constant or nearly so when dose applied was increased up to 200 Krad (Fig. 11).

On the other hand, the pH of the medium inoculated with *T. viride* drifted from pH 5.4 to pH 6.4 with increasing dose up to 50 Krad (Fig. 11), and thereafter declined only marginally.

Culture filtrate of *A. niger* became increasingly acidic as gamma doses applied to spores prior to incubation increased from 20 to 200 Krad. Thus whilst unirradiated spores of *A. niger* produced culture filtrate with pH 2.8, spores exposed to 100 and 200 Krad prior to incubation produced metabolites with a pH of 2.2.

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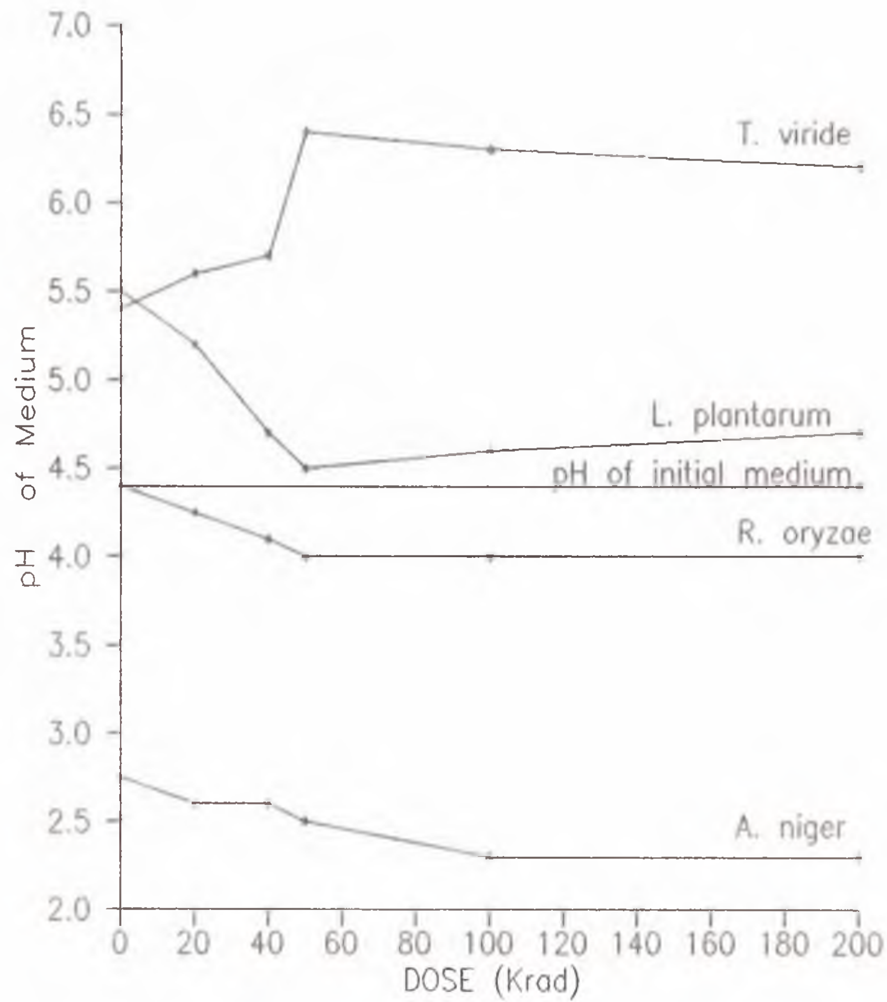

Fig. 11 Figure showing pH changes in irradiated spores of the indicated microorganisms after incubation for 5 days.

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In summary one can say that:

1. A dose of 50 Krad applied to dry spores of *R. oryzae* and *L. plantarum* was optimal for maximum acid production in the culture medium used.

2. A dose of 50 Krad applied to spores of *T. viride* prior to incubation drifted pH of the medium to the neutral side ie from pH 5.4 to 6.4.

3. *A. niger* acid production was enhanced considerably by gamma irradiation of 100 -200 Krad.

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H. EFFECT OF CHEMICAL TREATMENT, HEATING TEMPERATURE, AND DURATION OF TREATMENT ON DIGESTIBILITY OF LIGNOCELLULOSE IN CORN HUSK FOR THE PRODUCTION OF CRUDE PROTEIN NITROGEN BY *T. VIRIDE*.

Results are presented in Tables 6A-D. Analysis of variance (Table 7) showed that the structural resistance of lignocellulose in corn husk is significantly ($p = 0.01$) circumvented by chemical treatment with either 1 percent or 5 percent of Sodium Hydroxide or Hydrochloric acid.

Duncan's Multiple Range Test (Table 8) however showed that there was no significant difference between treatments with 1 percent Sodium Hydroxide, 5 percent Sodium Hydroxide or 1 percent Hydrochloric acid.

ii. PHYSICAL TREATMENT WITH HEAT:

Duncan's Multiple Range Test of the statistical analysis showed that temperature applied at (80°, 100°, 120° C) during digestion treatment significantly affect the degrative process prior to fermentation by *T. viride* for lignocellulose conversion. The optimum temperature for digestion was $100^{o}C$ which eventually yielded a product with crude protein content of 19.09 percent (Table 9).

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TABLE 6 A

Influence of temperature and duration of heating on crude protein formation by non-irradiated T. viride on corn slurry after 5 days at 28°C.

hemical Treatment With	Temperature of Digestion Temp. (°C)	Duration of Digestion (hrs)	% Crude Protein formed on slurry	Mean ± S.E.
1% NaOH	80	1	16.8	17.4±0.7
	80	1	17.0	
	80	1	18.6	
	80	1	17.2	
1% NaOH	80	2	18.6	18.3±0.2
	80	2	17.8	
	80	2	18.9	
	80	2	17.9	
1% NaOH	80	3	18.3	18.7±0.6
	80	3	18.6	
	80	3	17.9	
	80	3	19.9	
1% NaOH	100	1	18.7	18.6±0.1
	100	1	18.4	
	100	1	18.8	
	100	1	18.5	
1% NaOH	100	2	17.9	18.8±1.2
	100	2	19.8	
	100	2	18.6	
	100	2	18.9	
1% NaOH	100	3	18.9	18.9±0.8
	100	3	19.6	
	100	3	19.5	
	100	3	17.6	
1% NaOH	120	1	19.9	18.9±0.7
	120	1	18.9	
	120	1	17.9	
	120	1	18.9	
1% NaOH	120	2	19.6	19.4±0.4
	120	2	19.5	
	120	2	19.8	
	120	2	18.7	
1% NaOH	120	3	19.6	18.9±1.5
	120	3	19.8	
	120	3	16.9	
	120	3	19.2	

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Influence of temperature and duration of heating on crude protein formation by non-irradiated T. viride on corn slurry after 5 days at 28°C.

Chemical Treatment With	Temperature of Digestion Temp. (°C)	Duration of Digestion (hrs)	% Crude Protein formed on slurry	Mean ± S.E.
5% NaOH	80	1	18.8	18.8±0.4
	80	1	19.2	
	80	1	18.2	
	80	1	19.0	
5% NaOH	80	2	18.7	18.9±0.4
	80	2	19.4	
	80	2	19.2	
	80	2	18.3	
5% NaOH	80	3	18.9	19.0±0.4
	80	3	19.6	
	80	3	18.9	
	80	3	18.6	
5% NaOH	100	1	19.4	19.2±0.4
	100	1	19.0	
	100	1	19.8	
	100	1	18.6	
5% NaOH	100	2	19.6	19.4±0.1
	100	2	19.2	
	100	2	19.3	
	100	2	19.5	
5% NaOH	100	3	19.6	19.7±1.0
	100	3	19.9	
	100	3	19.4	
	100	3	20.0	
5% NaOH	120	1	19.6	18.8±0.6
	120	1	18.4	
	120	1	19.2	
	120	1	18.0	
5% NaOH	120	2	18.5	18.6±0.3
	120	2	18.9	
	120	2	18.8	
	120	2	18.2	
5% NaOH	120	3	18.2	18.0±0.3
	120	3	18.4	
	120	3	17.9	
		3	17.5	

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TABLE 6 C

Influence of temperature and duration of heating on crude protein formation by non-irradiated T. viride on corn slurry after 5 days at 28°C.

Chemical Treatment With	Temperature of Digestion Temp. (°C)	Duration of Digestion (hrs)	%Crude Protein formed on corn slurry	Mean ± S.E.
5%HCl	80	1	17.6	17.7±0.1
	80	1	17.7	
	80	1	17.7	
	80	1	17.8	
5%HCl	80	2	17.9	18.1±0.2
	80	2	17.9	
	80	2	18.0	
	80	2	18.4	
5%HCl	80	3	18.2	18.3±0.4
	80	3	18.4	
	80	3	18.4	
	80	3	18.1	
5%HCl	100	1	18.6	18.8±0.1
	100	1	18.7	
	100	1	18.9	
	100	1	19.0	
5%HCl	100	2	19.2	19.2±0.1
	100	2	19.1	
	100	2	19.3	
	100	2	19.2	
5%HCl	100	3	19.2	19.3±0.1
	100	3	19.3	
	100	3	19.3	
	100	3	19.1	
5%HCl	120	1	19.0	18.7±0.3
	120	1	18.8	
	120	1	18.2	
	120	1	18.6	
5%HCl	120	2	18.2	18.3±0.1
	120	2	18.4	
	120	2	18.4	
	120	2	18.1	
5%HCl	120	3	18.4	18.2±0.1
	120	3	18.1	
	120	3	18.1	
	120	3	18.2	

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TABLE 6D

Influence of temperature and duration of heating on crude protein formation by non-irradiated *T. viride* on corn slurry after 5 days at 28°C.

Chemical Treatment With	Temperature of Digestion Temp (°C)	Duration of Digestion (hrs)	% Crude Protein formed on corn slurry	Mean ± S.E.
1% HCl	80	1	16.2	16.2±0.9
	80	1	16.4	
	80	1	16.1	
	80	1	16.2	
1% HCl	80	2	16.6	16.7±0.2
	80	2	16.8	
	80	2	16.4	
	80	2	17.0	
1% HCl	80	3	16.6	17.1±0.3
	80	3	17.0	
	80	3	17.2	
	80	3	17.4	
1% HCl	100	1	[illegible]	17.6±0.9
	100	1	17.6	
	100	1	17.8	
	100	1	17.9	
1% HCl	100	2	17.9	17.8±0.9
	100	2	17.8	
	100	2	17.9	
	100	2	17.7	
1% HCl	100	3	17.9	18.1±0.9
	100	3	18.2	
	100	3	18.0	
	100	3	18.4	
1% HCl	120	1	18.6	18.7±0.2
	120	1	18.9	
	120	1	18.7	
	120	1	18.4	
1% HCl	120	2	18.7	18.8±0.1
	120	2	18.9	
	120	2	19.0	
	120	2	18.8	
1% HCl	120	3	18.9	19.1±0.1
	120	3	19.1	
	120	3	19.2	
	120	3	19.0	

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TABLE 7.

ANALYSIS OF VARIANCE TABLES 6 A-D.

Code	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob.
1	Rep	3	0.52	0.174	1.12	.342
2	A	3	63.91	21.304	136.84	.000
4	B	2	37.55	18.773	120.59	.000
6	AB	6	52.73	8.789	56.45	.000
8	C	2	4.67	2.334	14.99	.000
10	AC	6	3.92	0.653	4.19	.000
12	BC	4	6.48	1.620	10.40	.000
14	ABC	12	3.49	0.291	1.87	.039
16	D	1	25.03	25.028	160.77	.000
18	AD	3	4.96	1.655	10.63	.000
20	BD	2	0.37	0.184	1.18	.308
22	ABD	6	2.39	0.398	2.56	.020
24	CD	2	0.01	0.005	0.03	
26	ACD	6	0.42	0.070	0.45	
28	BCD	4	0.22	0.055	0.35	
30	ABCD	12	1.13	0.094	0.60	
-31	Error	213	33.16	0.156		

Coefficient of Variation = 2.10 %

A = Chemical Treatment (1% NaOH, 5% NaOH, 1% HCl, 5% HCl)

B = Temperature of Digestion (80, 100 120 ^{o}C)

C = Duration of Digestion (1, 2, 3, hours)

D = Non-irradiated spores.

AB, AC, BC, ABC, AD, BD, ABD, CD, ACD, BCD, ABCD are all combination treatments.

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TABLE 8

Duncan's Multiple Range Test of Chemical Treatment.

$S_{\bar{x}}$ = 4.654747-02 at alpha = 0.01

LSD value = .1710941

Original Order		Ranked Order
Mean 1 = 19.00 A	(1% NaOH)	Mean 2 = 19.13 A
Mean 2 = 19.13 A	(5% NaOH)	Mean 1 = 19.00 A
Mean 3 = 17.96 B	(1% HCl)	Mean 4 = 18.97 A
Mean 4 = 18 97 A	(5% HCl)	Mean 3 = 17.96 B

Means are the percent (%) Crude Protein produced.

TABLE 9.

Duncan's Multiple Range Test of Temperature of Digestion

$S_{\bar{x}}$ = 4.031129E-02 at alpha = 0.01

LSD value = 0.1481719

Original Order		Ranked Order
Mean 1 = 18.26 C	(80°C)	Mean 2 = 19.09 A
Mean 2 = 19.09 A	(100°C)	Mean 3 = 18.94 B
Mean 3 = 18.94 B	(120°C)	Mean 1 = 18.26 C

Figures with the same letters are not significantly different.

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TABLE 10.

Duncan's Multiple Range Test- Duration of Digestion.

$S_{\bar{x}}$ = 4.031129E-02 at alpha = 0.01

LSD value = .1481719

Original Order	Ranked Order
Mean 1 = 18.59 B (1 hour)	Mean 3 = 18.88 A
Mean 2 = 18.82 A (2 hour)	Mean 2 = 18.82 A
Mean 3 = 18.88 A (3 hour)	Mean 1 = 18.59 B

Means are the percent (%) Crude Protein produced.

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iii. DURATION OF DIGESTION OF CORN HUSK ON PROTEN NITROGEN FORMATION BY *T.VIRIDE*.

There was no statistical difference between crude protein formed by *T viride* in corn husk slurry digested for 2 and 3 hours. (18.82 and 18.88 percent respectively). Duncan's Multiple Range Test (Table 10) shows that digestion of corn husk for 1 hour prior to inoculation by *T. viride* resulted in a protein content of the fungus (18.59%) which was inferior to what obtained after 2 and 3 hours of digestion.

iv. COMBINED EFFECT OF CHEMICAL TREATMENT, HEATING TEMPERATURE AND DURATION OF DIGESTION OF CORN HUSK ON CRUDE PROTEIN PRODUCTION BY *T.VIRIDE*.

Combination of the three parameters namely, chemical treatment, heating teperature and duration of corn husk showed variable effects (Appendix 5). The best combination for optimal production of crude protein by unirradiated *T. viride* spores on digested corn husk was treatment with 5% NaOH solution at 100°C for 2 or 3 hours. In this instance nitrogen content of the product was between 19.4-19.7 percent. This was significantly ($p \leq 0.05$) different from the other treatment combinations (Appendix 5). The treatment combination which gave the lowest yield of crude protein (16.2 percent Nitrogen) was a digestion in 1 percent Hydrochloric acid solution at 80°C for 1 hour.

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I. PRODUCTION OF FUNGAL PROTEIN BY GAMMA-IRRADIATED *T. VIRIDE* SPORES ON CORN HUSK SLURRY COMBINED-TREATED WITH HEAT AND CHEMICALS.

The experiments in Section H were repeated. This time, spores of *T viride* were exposed to 100 Krad of gamma radiation prior to inoculation of the combined-treated corn husk slurry.

Results obtained are presented in Tables 11A-D. Analysis of variance of the data (Table 7) showed that irradiation significantly ($p \leq 0.05$) increased crude protein (% Nitrogen) formation by *T. viride* on corn husk slurry. Crude protein (Nitrogen Content) of the resultant culture from irradiated spores had a mean nitrogen content of 20.0 percent.

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TABLE 11A.

Influence of temperature and duration of heating on crude protein formation by irradiated spores T. viride on corn slurry after 5 days at 28°C.

Chemical Treatment With	Temperature of Digestion (°C)	Duration of Digestion (hrs)	% Crude Protein formed on slurry	Mean±S.E.
1%NaOH	80	1	18.4	18.5±0.2
	80	1	18.4	
	80	1	18.3	
	80	1	18.9	
1%NaOH	80	2	18.8	18.9±1.0
	80	2	19.3	
	80	2	18.7	
	80	2	18.9	
1%NaOH	80	3	19.0	19.2±0.3
	80	3	19.0	
	80	3	18.1	
	80	3	19.7	
1%NaOH	100	1	18.8	19.9±0.2
	100	1	19.6	
	100	1	19.3	
	100	1	19.7	
1%NaOH	100	2	19.8	19.8±0.1
	100	2	20.0	
	100	2	19.7	
	100	2	19.9	
1%NaOH	100	3	19.6	19.9±0.2
	100	3	20.2	
	100	3	19.9	
	100	3	19.9	
1%NaOH	120	1	19.6	19.2±0.4
	120	1	19.2	
	120	1	19.4	
	120	1	18.8	
1%NaOH	120	2	20.2	19.6±0.4
	120	2	19.6	
	120	2	19.4	
	120	2	19.2	
1%NaOH	120	3	19.8	19.4±0.5
	120	3	19.0	
	120	3	18.8	
	120	3	20.0	

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TABLE 11B.

Influence of temperature and duration of heating on crude protein formation by irradiated spores T. viride on corn slurry after 5 days at 28°C.

Chemical Treatment With	Temperature of Digestion (°C)	Duration of Digestion (hrs)	% Crude Protein formed on slurry	Mean±S.E.
5%NaOH	80	1	18.9	19.2±0.2
	80	1	19.6	
	80	1	18.8	
	80	1	19.5	
5%NaOH	80	2	19.6	19.5±0.2
	80	2	19.8	
	80	2	19.4	
	80	2	19.2	
5%NaOH	80	3	19.6	19.8±0.2
	80	3	19.8	
	80	3	20.2	
	80	3	19.6	
5%NaOH	100	1	19.8	19.6±0.1
	100	1	19.4	
	100	1	19.6	
	100	1	19.6	
5%NaOH	100	2	20.5	19.9 ±0.3
	100	2	20.1	
	100	2	19.6	
	100	2	19.4	
5%NaOH	100	3	20.6	20.0±0.4
	100	3	19.4	
	100	3	19.9	
	100	3	20.1	
5%NaOH	120	1	19.4	19.0±0.3
	120	1	18.6	
	120	1	19.1	
	120	1	18.9	
5%NaOH	120	2	18.5	19.1±0.4
	120	2	19.4	
	120	2	19.6	
	120	2	18.9	
5%NaOH	120	3	17.6	17.8±0.4
	120	3	18.4	
	120	3	18.0	
	120	3	17.2	

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TABLE 11C.

Influence of temperature and duration of heating on crude protein formation by irradiated spores T. viride on corn slurry after 5 days at 28°C.

Chemical Treatment With	Temperature of Digestion (°C)	Duration of Digestion (hrs)	% Crude Protein formed on slurry	Mean±S.E.
1%HCl	80	1	16.8	16.7±0.1
	60	1	16.6	
	80	1	16.7	
	80	1	16.5	
1%HCl	80	2	16.8	16.9±0.1
	80	2	16.9	
	60	2	16.8	
	60	2	16.9	
1%HCl	60	3	17.4	17.5±0.1
	80	3	17.3	
	80	3	17.5	
	80	3	17.6	
1%HCl	100	1	17.9	17.8±0.1
	100	1	17.8	
	100	1	17.6	
	100	1	17.8	
1%HCl	100	2	18.2	18.3±0.1
	100	2	18.4	
	100	2	18.2	
	100	2	18.3	
1%HCl	100	3	18.6	18.6±0.1
	100	3	18.6	
	100	3	18.7	
	100	3	18.6	
1%HCl	120	1	18.9	18.9±0.1
	120	1	18.9	
	120	1	18.9	
	120	1	18.8	
1%HCl	120	2	19.3	19.1±0.1
	120	2	18.9	
	120	2	19.1	
	120	2	19.2	
1%HCl	120	3	19.3	19.5±0.1
	120	3	19.4	
	120	3	19.5	
	120	3	19.6	

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TABLE 11D.

Influence of temperature and duration of heating on crude protein formation by irradiated spores T. viride on corn slurry after 5 days at 28°C.

Chemical Treatment With	Temperature of Digestion (°C)	Duration of Digestion (hrs)	% Crude Protein formed on slurry	Mean±S.E.
5%NaOH	80	1	18.9	18.9±0.1
	80	1	18.9	
	80	1	18.7	
	80	1	18.9	
5%NaOH	80	2	18.9	19.0±0.1
	80	2	19.1	
	80	2	19.1	
	80	2	19.0	
5%NaOH	80	3	19.3	19.2±0.1
	80	3	19.2	
	80	3	19.3	
	80	3	19.1	
5%NaOH	100	1	19.6	19.7±0.1
	100	1	19.7	
	100	1	19.6	
	100	1	19.7	
5%NaOH	100	2	19.8	19.8±0.1
	100	2	19.8	
	100	2	19.7	
	100	2	19.8	
5%NaOH	100	3	19.8	19.8±0.1
	100	3	19.9	
	100	3	19.7	
	100	3	19.8	
5%NaOH	120	1	19.8	19.7±0.1
	120	1	19.6	
	120	1	19.7	
	120	1	19.6	
5%NaOH	120	2	19.3	19.6±0.1
	120	2	19.6	
	120	2	19.7	
	120	2	19.6	
5%NaOH	120	3	19.6	19.5±0.1
	120	3	19.4	
	120	3	19.5	
	120	3	19.3	

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J. EFFECT OF GAMMA IRRADIATION ON THE MORPHOLOGY AND SPORULATION OF *A. NIGER* AND *T. VIRIDE*.

Gamma irradiation did not cause any significant changes in the morphology and sporulation of *T. viride*. In *T. viride* as much as the conidiophores remain irregularly branched, the bright green colouor development decreased progressively with incresing irradiation dose (eg. at 200 Krad there was no colour development after 8 day of incubation). Plate 3.

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Plate 3. A close up of 8 day old gamma irradiated T. viride grown on V-8 agar.

(Note the decrease in green colour formation with increasing irradiation)

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GENERAL DISCUSSION

The pertinent literature is replete with information on increasing interest in the conversion of agricultural waste, lignocellulose, effluent from chemical industries etc. by microbiological process into food and animal feed (Imrie, 1973; Schellart, 1975; Beldman *et al.* 1985). One of the resulting products, the microbial or fungal biomass itself can be used as a protein source in animal and human nutrition. It is reported that 2.3 billion metric tonnes of cereal straw, 560 million metric tonnes of leguminous crop residue and 234 million metric tonnes of sugar cane bagasse are produced throughout the year (Anon, 1988). These "waste" materials can be used as substrates for microbial protein production.

In Section A data collected showed that Ghana's annual maize production between 1984 and 1990 exceeded 500,000 metric tonnes except in 1985 when it declined to 395,000 metric tonnes (Fig. 2). The bulk of the production was contributed by Ashanti, Brong Ahafo, Eastern and Northern Regions (Fig. 3). During the harvest season, maize plant debris is left to decompose in the field as stubble. After removal of the cob, the corn husk is used as wrapping in the kenkey industry; some are discarded. The kenkey consumer normally discards the husk after eating.

In economic terms, money is being poured down the drain because maize husk from harvested maize cost 24.0 - 31.6 million cedis between 1987 - 1990. (Fig. 5). Each region has maize husk stock commensurate with its annual maize production figures (

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Therefore, as a raw material for fungal protein production, maize husk has potential for sustained feedstuff production.

In many developed countries, cellulose and lignocellulose in forestry and, agricultural waste is hydrolysed to prepare substrates for production of food and feedstuff (Bunker, 1963; Hospova, 1966) using microorganisms.

Bacteria, yeast and fungi have been investigated. Bacteria employed include: *Bacillus*, *Hydrogenomonas*, *Methamonas*, *Methylmonas*, *Lactobacillus*; yeasts: *Candida*, *Rhodoturola*, *Saccharomyces*; Filamentous fungi: *Aspergillus*, *Fusarium*, *Trichoderma*, *Rhizopus*, *Chaetomium*, *Penicillum* . (Kihlberg, 1972). The microorganisms of choice should satisfy the following conditions:

(a) Optimum utilization of waste components and simple nutritional demands; any additive required may influence the process costs adversely.

(b) High specific growth rate, and "competitive ability". This makes a non-sterile process possible.

(c) Simple and cheap separation.

(d) Non-toxic; safety in animal and human diet.

In the studies reported in this thesis, *Aspergillus niger* was chosen because Pringsheim and Lichtenstein (1920) reported the feeding of animals with this fungus and *Aspergillus fumigatus*, grown on straw supplemented with inorganic fertilizer during World War 1. During the World War 11, several industrially produced

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fungi (*Fusarium*, *Rhizopus*, *Candida*) were reported to have been incorporated into human diets with satisfactory results (Thatcher, 1954). *T. viride* is known to produce antibiotics and mycotoxins (Weindling and Emerson, 1936; Wright, 1956; Ooka *et. al.* 1966; Meyer, 1966; Pyke and Dietz, 1966) but one has to keep in mind that the production of such substances is dependent on medium composition and conditions of growth, whereas many of these substances may be destroyed by special treatments. Such feeding trials by *T. viride* gave satisfactory results (Church *et. al.*, 1972; Peitersen, 1975). On the whole *T. viride* seem to possess favourable properties for use in bioconversion of agricultural waste into animal feedstuff.

Good vegetative growth is invariably attended by high enzyme activity (Mandel and Reese, 1957). Cellulolytic, pectinolytic and amylolytic enzymes are produced during vegetative growth by a large number of microorganisms. In Section B gamma irradiation was used as a mutagen to ascertain its efficacy in improving vegetative growth of the test organisms, namely: *A. niger*, *R. oryzae*, *T. viride* and *L. plantarum*. Results obtained showed that effect of low gamma irradiation on vegetative growth is variable. A dose of 20 Krad decreased vegetative growth of *A. niger* by 46.6 percent and thereafter further increase of radiation dose up to 50 Krad increased dry matter accumulation to the same level as in the control. The general mutational effect of irradiation evidently causes damage to genetic and biochemical

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mechanisms, creating impairement of function and biological demands where non existed before (Ingram and Farkas, 1977). A dose of 20 Krad applied to *A. niger* spores presumably led to partial damage of DNA helix that altered biochemical synthetic pathways prerequisite for normal growth. However further doses up to 50 Krad was enough to unfurl the DNA helix and reform new strands to restore vegetative growth. Differences in radiosensitivity of fungal spores is well known and the best vegetative growth of *T. viride* and *R. oryzae* obtained with a dose of 100 Krad (Fig. 1) can be explained on the basis of mutations resulting from exposure to radiation. Indeed, Sadi *et. al.* (1983) stated that radiation can increase radial growth of microorganisms by up to 50 percent. However, above a certain threshold (varying from one species to another) irradiation may destroy genetic material and decrease germination capacity and metabolism of the spores (Siagian, 1983). This partly explains why the bacterium *L. plantarum* declined in vegetative growth beyond a dose of 50 Krad (Fig 2).

Gamma irradiation doses up to 50 Krad (0.5 KGy.) improved or caused a significant ($p \leq 0.05$) increase in amylase activity of *A. niger* , *R. oryzae* , *T. viride* and *L. plantarum* (Fig. 3). Many Phycomycetes (Phycomacotina) and Ascomycetes (Ascomycotina) can convert starch to sugar. Amylase activity of some *Lactobacillus* have been shown in fermentation of vegetative waste (Sibir *et. al.* 1984). *Rhizopus*, and especially *Aspergillus*

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have found practical applications in using starch for amylase, pectinase and biomass production (Moo-Young *et. al.* 1983.). In this thesis, low gamma irradiation dose of 50 Krad enhanced amylase production. This is the first record of induced enzyme activity of *A. niger*, *R. oryzae*, *T. viride* and *L. plantarum* following gamma irradiation.

Although many microorganisms produce cellulases, very few can break down lignocellulose and use it efficiently for biomass or other end product formation. The best cellulose converters include *Trichoderma reesei*, *T. lignorum*, *T. koningii*, *T. viride*, *Chaetomium cellulolyticum* and *Sporotrichum pulverulentum* (Moo-young *et. al.* 1983). In recent times, much attention has been paid to the enzymatic hydrolysis of cellulose because it is a renewable carbon source and available in large quantities. Some microorganisms produce a multicomponent cellulase enzyme system,including the 1,4-β-D-glucan glucanohydrolase (endoglucanase), 1-4-β-D-glucan cellobiose hydrolase (exoglucanase) and β-D-glucoside glucohydrolase β-glucosidase. *T. viride* produces six endoglucanases (Endo 1; 11; 111; 1V; V; V1), three exoglucanases (Exo 1; 11; and 111) and a β-glucosidase (β-gluc. 1). A conbination of these three types of enzymes is necessary for the complete hydrolysis of crystalline cellulose (Beldman *et. al.* 1985). Endoglucanase and exoglucanase are known to act synergistically in cellulose hydrolysis, while β-glucosidase is needed for removal of cellobiose (a strong

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inhibitor of both endoglucanase and exoglucanase). Although the endoglucanases and exoglucanases of gamma-irradiated spores of *T. viride* and *R. oryzae* were not determined in Section E, it is conjected that gamma irradiation at 100 Krad was presumably optimal in inducing the synergistic action in cellulose hydrolysis. Optimal cellulolytic activity of *A. niger* and *L. plantarum* was however obtained at a lower dose of 50 krad (Fig. 9) indicating differences in radiosensitivity of fungal spores used for same purpose.

Beldman *et. al.* (1985) stated that the chemical composition of plant cell wall necessitates a combination of cellulase, pectinase and hemicellulase enzyme action for efficient utilization of biomass. Results from Section F show that pectinase activity of *A. niger*, *R. oryzae* *T. viride* and *L. plantarum* was variably increased by a dose of 50 Krad and that dosebeyond 50 Krad depressed pectinase activity. Thus the overall effect of gamma irradiation on enzyme production by the test microorganisms can be summarised as follows:

TABLE 12.

Summary of dose requirements for optimal inducement of indicated physiological action

Type of activity	Dose (Krad) requirement for optimal improvement			
	A. niger	T. viride	R.oryzae	L.plantarum
Vegetative Growth	50	100	100	50
Amylase production	50	50	50	50
Cellulase "	50	100	100	50
Pectinase "	50	50	50	50

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Generally, a dose which enhanced vegetative growth of the microorganism was also optimal for cellulase activity. However the same dose (50 Krad) was optimal for inducement of amylase and pectinase activity in all the test microorganisms

In Section G, gamma irradiation of up to 200 Krad gave a linear increase in acid production by *A. niger* (Fig. 11). This fungus is used in commercial production of some acids including citric acid, oxalic acid and D-gluconic acid. It will be interesting to quantify, in future, which organic acids are formed in abundance by gamma-irradiated *A. niger* spores. Fumaric and lactic acids are produced by *Rhizopus oryzae* (Blain, 1975). The culture medium containing *R. oryzae* treated with a dose of ≥ 50 Krad became more acidic (pH 4.5 - 3.8) presumably indicating accumulation of acids. Future studies will aim at identifying the types of acids formed by this fungus after gamma irradiation treatment. The nature of the substrate (substrate composition) may also influence acid production. In subsequent experiments the medium used in culturing the microorganism will be varied to find out their effect on the quantity and type of acids formed after gamma irradiation of the spores. Cellulase activity in *T. viride* is kwown to be optimal between pH 5.0 - 6.5. This corresponds to the pH obtained by spores exposed to 100 Krad of gamma irradiation.

Some fungi are sources of industrial production of enzymes (Table 2). These include species used in these investigations namely *A. niger* (α amylase, amyloglucosidase,

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pectic enzymes, proteases, glucose oxidase, naringinase) *Rhizopus oryzae* (Amyloglucosidase, lipase, rennet proteases, pectic enzymes), *Trichoderma viride* (Cellulose). Results from this study show that gamma irradiation could improve yield of these enzymes. This information provides a springboard for future studies on quantities and qualities of enzymes produced after irradiation treatment.

Structural resistance of cellulose and hemicellulose closely associated with lignin in the plant cell wall makes cellulosic materials generally non-nutritive and difficult to digest. For an increase in nutritive value, the material must first be converted into monomers or oligomers (Kirk, 1982). This is achieved by pre-treatment hydrolysis process using either chemical or enzymes or both (Dunlap *et. al.* 1976).

In Section H of this thesis , hydrolysis of corn husk into a slurry was achieved by using either sodium hydroxide (1% and 5%) or Hydrochloric acid (1% and 5%), heating at 80°, 100° and 120° C for 1 - 3 hours (Tables 6 - 11). The best treatment combination was heating at 100°C for at least 1 hour in either 1%, 5% NaOH or 1% HCl. Alkaline treatment of cellulosic material is probably the oldest and best kwown method of enhancement of microbial degradation of cellulose. The effects of the alkaline treatment are multiple. In general, the application of alkaline results in removal of lignin, an increase in surface area by swelling, and an alteration of crystalline and amorphous

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structure. The effectiveness of this treatment was demonstrated with an increase in enzymatic digestibility (Detroy *et. al.* 1980) Acid treatment also decreased lignin content of variuos woods (More *et. al.* 1972) and pre-treatment of cellulosic waste by steam at 100-220° C for 15 min to 2 hours provides a high yield of soluble xylo-oligomers and xylose and good accessibility of cellulose for microbial and enzymatic attack (Buckholz *et. al.* 1981).

Gamma irradiation (100 Krad) applied to *T. viride* spores prior to inoculation of hydrolysed corn slurry improved crude protein formation (% N x 6.25). Crude protein content of the resultant culture from irradiated local isolate of *T. viride* spore was 20.0 percent. Rosenberg *et. al.* 1978 showed that total crude protein of cultures of *T. viride* $QM6_a$ on hydrolysed oathull was 15.4 %. (Tables 12 - 13). Rhodes *et. al.* (1961) and Gray *et. al.* (1964) screened 175 species of Imperfect fungi and determined the crude and extratable protein produced in shake flasks after 4 days. They found the highest crude protein (9 % N) in *T. viride.* Irradiation of *T. viride* spores increased considerably crude protein of cultures on hydrolysed maize husk slurry from 9 % to 20 %. (ie by 122 percent).

Several wastes have been proposed in the pertinent literature as substrate for production of fungal protein such as molasses (Gray and Abou-El-Seoud, 1966), soya beans whey (

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Church *et. al.* 1972), corn waste (Church *et. al.* 1972), waste from the coffee and rum distilling industries (Updegraff *et. al.* 1973) etc. Results from this theses show that at least under laboratory conditions, the use of corn husk slurry to produce fungal protein food for animal feed is feasible. However, a number of other ancillary studies are required before practical application.

Modern fermentations require that the industrial scale of the process takes place in a fermenter which provides an environment suitable for the growth of a pure culture on a substrate free from contamination and under controlled conditions. The design must incorporate a device for mixing the contents and air supply for aerobic processes, probes to monitor the enviroment and regulators to control it. There must also be provision for inoculation and sampling, as well as for charging and discharging the vessel. In continuous culture, (as compared to the batch culture), it is necesssary to monitor and control the flow rate of the medium as well as the culture volume and mass. These should be considered in designing an appropriate fermentor for fermentation of corn husk slurry by irradiated spores of *T. viride*. A cost-benefit analysis for the process is also important to justify any commercial application of the technique.

Only a few studies have reported the nutritional value of fungal protein for animals (Litchfield, 1968). Conclusions as to the value of fungal mycelium produced by *T viride* on corn husk

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slurry as food or feed, can only be drawn after extensive feeding studies with both domestic animals and pigs, to establish nutritional value and safety. Time limitation did not allow such studies to be carried out. However it is anticipated that future studies will examine the above-mentioned suggestions.

Many morphological as well as physiological characteristics of fungi are generated by enviromental conditions. For example, Waterhouse (1974) reported that the size of *Phytophthora palmivora* (Butl) Butl sporangia vary widely according to medium, host, age of culture, moisture and light. The way in which each influenced the sporangia was, however, not stated. Nyerges-Rogrun (1975) showed that there are morphological changes of the conidial head of *Penicillium purpurogenum* following gamma irradiation. In the concluding Section of this thesis, the effect of gamma irradiation on sporulation and morphology of *T. viride* was examined. There was no significant change in the morphology. The development of colour was delayed for 8 days when 200 Krad was applied prior to culturing. Presumably the genetic coding for colour formation is somehow impaired at this dose. The physiological basis for this cannot be established by present data. What is interesting is the colourless mutant of *T. viride* produced by 200 Krad of gamma irradiation. Future studies will elucidate the significance of the colourless mutant and its implication in the ecological niche of the fungus.

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SUMMARY

1. Microorganisms namely, *Aspergillus niger*, *Rhizopus oryzae*, *Trichoderma viride* and *L. plantarum* have been used to study the effect of gamma irradiation doses on enzyme activity (amylase, cellulase, pectinase) and on vegetative growth in liquid culture.
2. A dose of 20 Krad decreased vegetative growth of *A. niger* by 46.6 percent; further increases up to 50 Krad increased dry matter accumulation of the fungus.
3. Vegetative growth of *R. oryzae* was increased by about 30 percent by 50 Krad and remained nearly the same up to 200 Krad.
4. There was no statistical difference ($p \leq 0.05$, Student's t-test) between dry weight of mycelium of *R. oryzae* obtained in culture medium containing mycelium exposed to 50, 100 and 200 Krad of gamma irradiation.
5. The best vegetative growth of *T. viride* (28-30) was obtained in flasks containing spores irradiated with 100 or 200 Krad prior to incubation.
6. The best vegetative growth of *L. plantarum* was obtained in flasks containing vegetative cells exposed to 50 Krad prior to incubation at $28^{o}C$ for 5 days.
7. Optimal cellulase, amylase and pectinase activity was induced by 50 Krad of gamma irradiation in *A. niger* and *L. plantarum*.
8. Optimal cellulase activity in *T. viride* and *R. oryzae* was induced by a dose of 100 Krad whilst 50 Krad was optimal for maximum production of amylase and pectinase enzyme.

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9. Gamma irradiation dose that induced optimal vegetative growth was also attended by optimal cellulase activity by the fungi.
10. pH of the medium containing *T. viride* treated with 100 Krad of gamma irradiation was between pH 5.0 - pH 6.5 corresponding to the best pH for cellulase activity in the fungus.
11. Gamma irradiation doses up to 200 Krad linearly increase acid production by *A. niger* and shifted pH of medium from initial 2.9 to final pH of 2.2.
12. Culture medium containing gamma-irradiated (50 Krad) *R. oryzae* sporangiophores also became more acidic (pH 4.5 - 3.8) presumably indicating accumulation of acids.
13. Hydrolysis of corn husk for use in solid-substrate fermentation by *T. viride* was achieved by using either 1 percent or 5 percent NaOH or HCl and heating at 80°, 100° or 120°C for 1-3 hours.
14. The best treatment for corn husk hydrolysis prior to enzyme digestion of cellulose was heating at 100°C for at least 1 hr. in either 1 percent NaOH, 5 percent NaOH or 1 percent HCl.
15. The treatment gave good accessibility of cellulose for microbial enzyme attack leading to high crude protein formation by *T. viride* on the maize husk slurry.
16. Irradiation of *T. viride* spores prior to inoculation of the solid substrate produced crude fungal protein which was 20.0 percent higher than that formed by the unirradiated *T. viride* spores a highly significant difference.

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17. National maize production figures between 1981 to 1990 show a shortfall in 1983 (395,000 metric tonnes) as compared to the remaining years (in excess of 500,000 metric tonnes).

    Regional contribution to the national maize production showed that Ashanti, Brong Ahafo, Eastern and Northern Regions contributed the bulk of the national production figures between 1981 to 1990.

18. Amount of maize husk available after removing the cobs was commensurate with the total maize harvest for each region and cost about 23.0 - 32.0 million cedis.

19. As a raw material substrate for fungal protein production by *T. viride*, maize husks have a potential for sustainable use.

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APPENDIX 1.

MAIZE PRODUCTION IN GHANA 1981-1990.

(FIGURES IN THOUSANDS METRIC TONNES)

YEAR	PRODUCTION
1981	334.3
1982	284.3
1983	140.8
1984	574.0
1985	395.0
1986	559.1
1987	587.7
1988	600.0
1989	715.0
1990	553.0

Source:PPMED (Statistics Division) Min. Of Agric. MAY. 1991.

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APPENDIX 2.

MAIZE PRODUCTION BY REGION (THOUSANDS OF METRIC TONNES).

REGION	1987	1988	1989	1990
WESTERN	43.5	36.5	49.0	49.7
CENTRAL	60.3	67.0	105.4	42.2
GT.ACCRA	9.2	15.7	25.2	14.3
EASTERN	97.2	70.1	139.5	99.4
VOLTA	53.4	33.6	55.7	21.6
ASHANTI	83.1	106.6	83.0	108.3
B.AHAFO	122.7	94.6	135.6	86.5
NORTHERN	102.1	134.6	87.0	106.7
U.EAST	3.0	37.9	2.7	0.9
U.WEST	23.2	3.4	31.5	23.1

Source:PPMED (Statistics Division) Min. of Agric. MAY. 1991.

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APPENDIX 3.

MAIZE HUSK PRODUCTION ESTIMATES BY REGION IN THOUSAND METRIC TONNE

REGION	1987	1988	1989	1990
WESTERN	6.5	5.5	7.4	7.5
CENTRAL	9.0	10.1	15.8	6.3
GT.ACCRA	1.4	2.4	3.8	2.1
EASTERN	14.6	10.5	21.0	14.9
VOLTA	8.0	5.0	8.4	3.2
ASHANTI	12.5	16.0	12.5	16.2
B.AHAFO	18.4	14.2	20.3	13.0
NORTHERN	15.3	20.2	13.1	16.0
U.EAST	0.5	5.7	0.4	0.1
U.WEST	3.5	0.5	4.7	3.5

Source: PPMED (Statistics Division). Min. of Agric. MAY 1991.

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APPENDIX 4.

ESTIMATED ANNUAL COST OF MAIZE HUSK PRODUCED IN GHANA 1987-90.

(CALCULATION BASED ON HUSK BEING 15% OF MAIZE PRODUCED AND AVERAGE ANNUAL COST OF HUSK BEING 290 CEDIS PER KILO.)

YEAR	TONNAGE (METRIC)	COST (MILLIONS OF CEDIS)
1987	89.7	26.0
1988	90.1	26.1
1989	107.4	31.2
1990	82.8	24.0

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APPENDIX 5.

Duncan's Multiple Range Test Combination of Chemical Treatment, Heating Temperature And Duration of Digestion.

$S_{\bar{x}}$ = 0.1396424 at alpha = 0.01

LSD value = 0.5132824

Original Order			Treatment	Ranked Order		
Mean 1	17.95	KL	1%NaOH, 80 °C, 1hr	Mean 15	A	19.86
Mean 2 =	18.61	FGHI	1%NaOH, 80 °C, 2hr	Mean 14	AB	19.65
Mean 3 =	18.94	CDEFG	1%NaOH, 80 °C, 3hr	Mean 33	ABC	19.51
Mean 4	19.10	BCDEFG	1%NaOH,100 °C, 1hr	Mean 8	ABC	19.50
Mean 5 =	19.30	ABCDE	1%NaOH,100 °C, 2hr	Mean 32	ABC	19.49
Mean 6	19.40	ABCD	1%NaOH,100 °C, 3hr	Mean 13	ABCD	19.40
Mean 7 =	19.05	BCDEFG	1%NaOH,120 °C, 1hr	Mean 6	ABCD	19.40
Mean 8 =	19.50	ABC	1%NaOH,120 °C, 2hr	Mean 12	ABCD	19.38
Mean 9 =	19.15	BCDEF	1%NaOH,120 °C, 3hr	Mean 5	ABCDE	19.30
Mean10 =	19.00	CDEFG	5%NaOH, 80 °C, 1hr	Mean 27	BCDE	19.25
Mean11	19.20	BCDEF	5%NaOH, 80 °C, 2hr	Mean 31	BCDE	19.25
Mean12 =	19.38	ABCD	5%NaOH, 80 °C, 3hr	Mean 11	BCDEF	19.20
Mean13 =	19.40	ABCD	5%Naoh,100 °C, 1hr	Mean 34	BCDEF	19.16
Mean14 =	19.65	AB	5%NaOH,100 °C, 2hr	Mean 9	BCDEF	19.15
Mean15 =	19.86	A	5%NaOH,100 °C, 3hr	Mean 4	BCDEFG	19.10
Mean16 =	18.90	CDEFGH	5%NaOH,120 °C, 1hr	Mean 7	BCDEFG	19.05
Mean17 =	18.85	DEFGHI	5%NaOH,120 °C, 2hr	Mean 10	CDEFG	19.00
Mean18 =	17.90	KL	5%NaOH,120 °C, 3hr	Mean 26	CDEFG	18.99
Mean19 =	16.44	O	1%HCl, 80 °C, 1hr	Mean 3	CDEFG	18.94
Mean20 =	16.77	NO	1%HCl, 80 °C, 2hr	Mean 35	CDEFG	18.94
Mean21	17.25	MN	1%HCl, 80 °C, 3hr	Mean 16	CDEFGH	18.90
Mean22 =	17.75	LM	1%HCl, 100 °C, 1hr	Mean 17	DEFGHI	18.85
Mean23 =	18.05	JKL	1%HCl, 100 °C, 2hr	Mean 36	DEFGHI	18.83
Mean24	18.33	HIJK	1%HCl, 100 °C, 3hr	Mean 25	DEFGHI	18.79
Mean25 =	18.79	DEFGHI	1%HCl, 120 °C, 1hr	Mean 30	EFGHI	18.75
Mean26 =	18.99	CDEFG	1%HCl, 120 °C, 2hr	Mean 2	FGHI	18.61
Mean27 =	19.25	BCDE	1%HCl, 120 °C, 3hr	Mean 29	GHIJ	18.54
Mean28 =	18.27	IJKL	5%HCl, 80 °C, 1hr	Mean 24	HIJK	18.33
Mean29 =	18.54	GHIJ	5%HCl, 80 °C, 2hr	Mean 28	IJKL	18.27
Mean30 =	18.75	EFGHI	5%HCl, 80 °C 3hr	Mean 23	JKL	18.05
Mean31 =	19.25	BCDE	5%HCl, 100 °C 1hr	Mean 1	KL	17.95
Mean32	19.49	ABC	5%HCl, 100 °C 2hr	Mean 18	KL	17.90
Mean33 =	19.51	ABC	5%HCl, 100 °C 3hr	Mean 22	LM	17.75
Mean34	19.16	BCDEF	5%HCl, 120 °C 1hr	Mean 21	MN	17.25
Mean35 =	18.94	CDEFG	5%HCl, 120 °C 2hr	Mean 20	NO	16.77
Mean36 =	18.83	DEFGHI	5%HCl, 120 °C 3hr	Mean 19	O	16.44

The combination of letters show the significant differences among the means.

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APPENDIX 6.

Duncan's Multiple Range Test -Combination of Chemical Treatment, Heating Temperature, Duration Of Digestion and Irradiation Treatment of *T. viride*.

$s_{\bar{x}}$ 4.031129E-02 at alpha = .01

LSD value - .1481719

Original Order				Ranked Order	
Mean 1=	17.40	X	1%NaOH,80°C,1hr,*;	Mean 30=	A 20.00
Mean 2=	18.50	R	1%NaOH,80°C,1hr,#;	Mean 12=	AB 19.90
Mean 3=	18.30	S	1%NaOH,80°C,2hr,*;	Mean 28=	AB 19.90
Mean 4=	18.92	JKLM	1%NaOH,80°C,2hr,#;	Mean 10=	BC 19.80
Mean 5=	18.67	OPQ	1%NaOH,80°C,3hr,*;	Mean 66=	BC 19.80
Mean 6=	19.20	GH	1%NaOH,80°C,3hr,#;	Mean 64=	BC 19.77
Mean 7=	18.60	PQR	1%NaOH,100°C,1hr,*;	Mean 24=	BCD 19.75
Mean 8=	19.60	DE	1%NaOH,100°C,1hr,#;	Mean 29=	CD 19.73
Mean 9=	18.80	MNO	1%NaOH,100°C,2hr,*;	Mean 62=	CD 19.70
Mean 10=	19.80	BC	1%NaOH,100°C,2hr,#;	Mean 68=	CD 19.67
Mean 11=	18.90	JKLM	1%NaOH,100°C,3hr,*;	Mean 16=	DE 19.60
Mean 12=	19.90	AB	1%NaOH,100°C,3hr,#;	Mean 70=	DE 19.60
Mean 13=	18.90	JKLM	1%NaOH,120°C,1hr,*;	Mean 8=	DE 19.60
Mean 14=	19.20	GH	1%NaOH,120°C,1hr,#;	Mean 26=	DE 19.60
Mean 15=	19.40	F	1%NaOH,120°C,2hr,*;	Mean 22=	EF 19.50
Mean 16=	19.60	DE	1%NaOH,120°C,2hr,#;	Mean 54=	EF 19.45
Mean 17=	18.90	JKLM	1%NaOH,120°C,3hr,*;	Mean 72=	EF 19.45
Mean 18=	19.40	F	1%NaOH,120°C,3hr,#;	Mean 15=	F 19.40
Mean 19=	18.80	MNO	5%NaOH, 80°C,1hr,*;	Mean 27=	F 19.40
Mean 20=	19.20	GH	5%NaOH, 80°C,1hr,#;	Mean 18=	F 19.40
Mean 21=	18.90	JKLM	5%NaOH, 80°C,2hr,*;	Mean 65=	G 19.23
Mean 22=	19.50	EF	5%NaOH, 80°C,2hr,#;	Mean 60=	G 19.23
Mean 23=	19.00	IJKL	5%NaOH, 80°C,3hr,*;	Mean 25=	GH 19.20
Mean 24=	19.75	BCD	5%NaOH, 80°C,3hr,#;	Mean 14=	GH 19.20
Mean 25=	19.20	GH	5%NaOH, 80°C,1hr,*;	Mean 6=	GH 19.20
Mean 26=	19.60	DE	5%NaOH,100°C,1hr,#;	Mean 20=	GH 19.20
Mean 27=	19.40	F	5%NaOH,100°C,2hr,*;	Mean 63=	GH 19.20
Mean 28=	19.90	AB	5%NaOH,100°C,2hr,#;	Mean 52=	GHI 19.13

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APPENDIX 6 (cont'd)

Mean 29=	19.73	CD	5%NaOH,100°C,3hr,*;	Mean 34=	GHI	19.10
Mean 30=	20.00	A	5%NaOH,100°C,3hr,#;	Mean 53=	HIJ	19.05
Mean 31=	18.80	MNO	5%NaOH,120°C,1hr,*;	Mean 58=	IJK	19.02
Mean 32=	19.00	IJKL	5%NaOH,120°C,1hr,#;	Mean 32=	IJKL	19.00
Mean 33=	18.60	PQR	5%NaOH,120°C,2hr,*;	Mean 23=	IJKL	19.00
Mean 34=	19.10	GHI	5%NaOH,120°C,2hr,#;	Mean 4=	JKLM	18.92
Mean 35=	18.00	V	5%NaOH,120°C,3hr,*;	Mean 21=	JKLM	18.90
Mean 36=	17.80	W	5%NaOH,120°C,3hr,#;	Mean 17=	JKLM	18.90
Mean 37=	16.23	\	1%HCl, 80°C,1hr,*;	Mean 11=	JKLM	18.90
Mean 38=	16.65	[	1%HCl, 80°C,1hr,#;	Mean 13=	JKLM	18.90
Mean 39=	16.70	[	1%HCl, 80°C,2hr,*;	Mean 50=	KLM	18.88
Mean 40=	16.85	Z	1%HCl, 80°C,2hr,#;	Mean 56=	LMN	18.85
Mean 41=	17.05	Y	1%HCl, 80°C,3hr,*;	Mean 51=	LMN	18.85
Mean 42=	17.45	X	1%HCl, 80°C,3hr,#;	Mean 19=	MNO	18.80
Mean 43=	17.73	W	1%HCl, 100°C,1hr,*;	Mean 31=	MNO	18.80
Mean 44=	17.77	W	1%HCl, 100°C,1hr #;	Mean 9=	MNO	18.80
Mean 45=	17,83	W	1%HCl, 100°C,2hr,*;	Mean 61=	MNO	18.80
Mean 46=	18.27	ST	1%HCl, 100°C,2hr,#;	Mean 49=	NOP	18.70
Mean 47=	18.13	TUV	1%HCl, 100°C,3hr,*;	Mean 5=	OPQ	18.67
Mean 48=	18.53	QR	1%HCl, 100°C,3hr,#;	Mean 67=	OPQR	18.65
Mean 49=	18.70	NOP	1%HCl, 120°C,1hr,*;	Mean 33=	PQR	18.60
Mean 50=	18.88	KLM	1%HCl, 120°C,1hr,#;	Mean 7=	PQR	18.60
Mean 51=	18.85	LMN	1%HCl, 120°C,2hr,*;	Mean 48=	QR	18.53
Mean 52=	19.13	GHI	1%HCl, 120°C,2hr,#;	Mean 2=	R	18.50
Mean 53=	19.05	HIJ	1%HCl, 120°C,3hr,*;	Mean 3=	S	18.30
Mean 54=	19.45	EF	1%HCl, 120°C,3hr,#;	Mean 46=	ST	18.27
Mean 55=	17.70	W	5%HCl, 80°C,1hr,*;	Mean 59=	ST	18.27
Mean 56=	18.85	LMN	5%HCl, 80°C,1hr,#;	Mean 69=	ST	18.27
Mean 57=	18.05	UV	5%HCl, 80°C,2hr,*;	Mean 71=	STU	18.20
Mean 58=	19.02	IJK	5%HCl, 80°C,2hr,#;	Mean 47=	TUV	18.13
Mean 59=	18.27	ST	5%HCl, 80°C,3hr,*;	Mean 57=	UV	18.05
Mean 60=	19.23	G	5%HCl, 80°C,3hr,#;	Mean 35=	V	18.00
Mean 61=	18.80	MNO	5%HCl, 100°C,1hr,*;	Mean 45=	W	17.83
Mean 62=	19.70	CD	5%HCl, 100°C,1hr,#;	Mean 36=	W	17.80

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Mean 63=	19.20	GH	5%HCl, 100 °C,2hr,*;	Mean 44=	W	17.77
Mean 64=	19.77	BC	5%HCl, 100 °C,2hr,#;	Mean 43=	W	17.73
Mean 65=	19.23	G	5%HCl, 100 °C,3hr,*;	Mean 55=	W	17.70
Mean 66=	19.80	BG	5%HCL, 100 °C,3hr,#;	Mean 42=	X	17.45
Mean 67=	18.65	OPQR	5%HCl, 120 °C,1hr,*;	Mean 1=	X	17.40
Mean 68=	19.67	CD	5%HCl, 120 °C,1hr,#;	Mean 41=	Y	17.05
Mean. 69=	18.27	ST	5%HCl, 120 °C,2hr,*;	Mean 40=	Z	16.85
Mean 70=	19.60	DE	5%HCl, 120 °C,2hr,#;	Mean 39=	[	16.70
Mean 71=	18.20	STV	5%HCl, 120 °C,3hr,*;	Mean 38=	[	16.65
Mean 72=	19.45	EF	5%HCl, 120 °C,3hr,#;	Mean 37=	\	16.23

* = Non-irradiated *T. viride*.

= Irradiated *T. viride*.

Alphabet used to show differentiations in the means.

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Appendix 7A.

Amylase activity of microorganisms after exposure to gamma irradiation shown as percent reduction in viscosity of starch.

Dose Applied	Amylase activity (% reduction in viscosity of starch)			
(Krad)	T. viride	R.oryzae	A. niger	L.plantarum
0	60	58	65	40
20	65	60	68	45
40	68	62	70	50
50	75	64	72	65
100	70	60	68	60
200	45	60	66	55

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Appendix 7B.

Cellulase activity of microorganisms after exposure to gamma irradiation shown as percent reduction in viscosity of cellulose.

Dose Applied	Cellulase activity (% reduction in viscosity of cellulose)			
(Krad)	T. viride	R.oryzae	A. niger	L.plantarum
0	40	50	65	30
20	60	48	66	40
40	68	45	68	45
50	72	55	64	48
100	79	66	60	43
200	58	50	61	32

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Appendix 7C.

Pectinase activity of microorganisms after exposure to gamma irradiation shown as percent reduction in viscosity of pectin.

Dose Applied	Pectinase activity (% reduction in viscosity of pectin)			
(Krad)	T. viride	R.oryzae	A. niger	L.plantarum
0	30	40	59	34
20	40	44	60	38
40	48	44	61	40
50	64	48	63	42
100	50	42	62	39
200	48	42	62	33

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