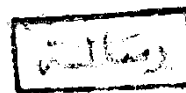


FUNGAL PROTEIN PRODUCTION FROM STARCHY WASTES

BY

Said Mohamed Mansour Mohamed



THESIS

SUBMITTED IN PARTIAL FULFILMENT

OF THE REQUIREMENTS

FOR THE DEGREE OF

MASTER OF SCIENCE

IN

Agriculture (Agric. Microbiology)



Agric. Microbiology Department

FACULTY OF AGRICULTURE

AIN SHAMS UNIVERSITY

Cairo, A.R.E

1988

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قالوا سبحانك لا علم لنا إلا ما علمتنا
إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ

IN THE NAME OF ALLAH, THE BENE FICENT,
THE MERCIFUL

They Said : " Be glorified we have no Knowledge
except that which you have taught us.
Indeed you are the Knower, the wise"



(Committee in Charge)

Prof. Dr. H. M. W. H.

Title of Thesis :

Name of Candidate :

APPROVAL SHEET

ACKNOWLEDGEMENT

This work has been carried out under the supervision of Professor, Dr. Wagdy Mashhoor, Professor of Agricultural Microbiology, Faculty of Agricultural Ain Shams University, Professor Dr. Rawia F. Gamal, Professor of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University and Dr. Sami H. Ahmed, Assistant Professor of Industrial Fermentation, Agriculture Research Center of Egypt.

The author wishes to express his thanks and gratitude to them for suggestion the problem, constant supervision, valuable suggestion, constructive criticism throughout the whole work and preparation of the thesis.

Thanks are also due to all members of the Department of Agriculture Microbiology, Soils and Water Research Institute, Agricultural Research Center as well as to all members of Agric. Microbiology Dept., Fac. of Agric. , Ain Shams University .

CONTENTS

	<u>Page</u>
1- INTRODUCTION	1
2- REVIEW OF LITERATURE	3
- Fungal Strains Used in Production of Single Cell Protein.....	3
- Starchy Wastes	6
- Hydrolysis of Starchy Wastes	10
- Factors Affecting Production of Single Cell Protein	13
* Nutritional factors	14
. Media used	14
. Effect of carbon sources	18
. Effect of nitrogen sources	23
* Environmental factors	25
. Aeration and agitation	25
. pH.....	28
. Temperature	30
. Incubation period	32
3- MATERIAL AND METHODS	34
- Materials :.....	34
* Microorganisms used	34
* Raw materials(starchy wastes).....	34
* Media used	37
- Methods:.....	39
* Fungal propagation	39
* Fungal maintenance	39
* Standard inoculum	39
* Growth of fungal strains in shake flasks as a batch culture	39
* Growth in fermentor as a batch culture	40
* Chemical determinations :.....	41

	<u>Page</u>
. Hydrolysis of crushed rice, rice bran and wheat bran.....	41
. Reducing sugars	41
. Total carbohydrates	42
. Moisture content	42
. pH values	43
. Amino acids of the crude fungal protein....	43
. Tryptophan	44
. Metals	45
. Oxygen absorption rate (OAR).....	45
. Nucleic acids extraction and determination.	45
. Fat (<i>Ether extract</i>).....	46
. Organic carbon	46
. Phosphorus	47
. Calcium and magnesium	47
. Sodium and potassium.....	48
. Total nitrogen	48
. Calculation	48
4- RESULTS AND DISCUSSION	51
- Isolation and Screening of Single Cell Protein..	51
- Growth of Fungal Isolates in Shake Flasks as a Batch Culture	51
- Effect of Medium.....	54
* Synthetic media	54
. Synthetic media	54
.. Growth curves of the best three strains on Sekeri-Potaryas medium.....	58
. Semi- synthetic media.....	61
.. Hydrolysis of starchy raw materials.....	62
- Effect of Nutritional Requirements :.....	64
* Effect of carbon sources	64
* Effect of different concentration . glucose	69

	<u>Page</u>
* Effect of starchy hydrolysate media.....	60
* Effect of different nitrogen sources	75
* Effect of ingredints elimination of modified wheat bran hydrolysate medium.....	80
* Effect of liquid volume/flasks on oxygen absorption rates (OAR).....	83
* Effect of flask ^{type} closure on oxygen absorption rate (OAR).....	85
- Effect of ^{some} Environmental Factors on Fungal Biomass Production	87
* Initial pH	87
* Temperature	89
- Factors Affecting Fungal Biomass Production by <u>Aspergillus terreus</u> Using Fermentor as a Batch Culture.....	94
* Incubation period	94
* Agitation rate	98
* Aeration rate	102
- Chemical Composition and Trace Elements Content of (<u>Aspergillus terreus</u>).....	106
* Amino acids content	108
5- SUMMARY AND CONCLUSIONS	113
6- REFERENCES	119
- ARABIC SUMMARY .	

INTRODUCTION

The continuing increase in world population has a direct and immediate effect on the consumption of food, particularly in the developing countries.

One fifth of the people in the developing countries are undernourished and between a quarter to one third are protein malnourished.

Since the efficiency of converting the raw materials into cellular constituents is much higher for microorganisms than for any farm animals, the exploitation of the microorganisms, for increasing the quantity and improving the quality of human food, was tried by several investigators. A great deal of work has been given to produce single cell protein, i.e., crude or refined protein derived from unicellular or simple multicellular organisms.

The possibility of using fungal mycelium as a source of protein for chronic food shortage areas of the world has been considered.

The idea of mass cultivation of fungal mycelium as source of human food and for animal feeding was reported around 1920 .

In Germany, several strains of molds were used in human feeding during World War II .

The present work is a trial to produce fungal protein using local starchy waste materials. Several fungal isolates were tested for their efficiency in protein production to explore the best one. Rice bran, wheat bran and crushed rice were used as carbon sources, after being hydrolyzed. Nutritional and environmental factors affecting the fungal protein production were studied. The produced mycelial protein was chemically analyzed and compared with FAO reference protein. The mycelicum yield efficiency ratio, protein efficiency ratio , substrate utilization efficiency, economic coefficient, amino acid score and biological value were calculated.

REVIEW OF LITERATURE

Fungal Strains Used in Production of Single Cell Protein (SCP) :

Fungi might be of considerable interest for their potent enzymatic capabilities, ease of harvesting from fermentation media. The protein content ranged between 45-50 % (NPU 70-50) and the essential amino acids such as methionine and cystine can be in greater concentration, up to 4 % of the total protein. Fungi at the right cyclical growth have a filamentous structure which permits their manufacture as textured foodstuffs, many Asian fermented food products are produced by mold fermentation such as wheat tempeh (Wang & Hesseltine, 1966; Spicer , 1971 and Rusmin & Ko, 1974).

Many investigators studied suitable different fungal strains to use in the production of SCP. Among these investigators Gray & Karve (1967) and Brook et al. (1969) tested cultures of Rhizopus oligosporus NRRL 2710 and it gave the most satisfactory wheat tempeh, Whereas such as R. oryzae NRRL A. 9847 and R. arrhizus NRRL 1526, known to make a good soy-bean tempeh. Other strains were used for SCP production such as Trichoderma spp. (I- 23 and I- 193), Gliocladium delignescens sopp. (I- 13), Pullularia pullulans (de Bary) Berkhout (I- 80), Dactylium dendroides (Bull.) Fr. (I- 108), Mucor racemosus, Heterocephalum aurantiacum M 1020, Cladosporium cladosporioides, Rhizopus stolonifer, R. echinatus and Cunninghamella elegans. (Brook et al. 1969).

Mitrakos et al. (1970), Spicer (1971), Nordstrom (1974), Rusmin and Ko (1974) used Aspergillus niger, Penicillium notatum, Aspergillus fumigatus and Rhizopus oligosporus for fungal protein production .

Anderson et al. (1975), Christias et al. (1975), Han & Anderson (1975), Hang et al. (1975), Imrie & Vliots (1975), Reade & Gregory (1975) and Rolz (1975) stated that Fusarium graminearum, Fusarium oxysporum, F. moniliform, Aspergillus niger , A. niger mutant 70, Candida tropicalis, Aureobasidium pullulans, Trichoderma viride, Candida utilis , A. niger A. fumigatus and Aspergillus oryzae were rich in protein content and contained high amounts of all essential amino acids.

Drouliscos et al. (1976), Moo- Young et al. (1977) and (1978) used Fusarium moniliform , Chaetomium cellulolyticum for SCP production .

Abou Zeid et al. (1979), Bajracharya & Mudgett (1979), Blain et al. (1979) and Pamment et al. (1979) reported that several yeast strains suitable for the production of fodder yeast i.e. Saccharomyces cerevisiae , S. carlsbergensis , S. fragilis , Candida lypolytica , C. tropicalis and C. utilis. In addition, Aspergillus sp. QM 994, A. niger QM877, Rhizopus nigricans QM 387, R. arrhizus were used for fungal protein production but a new cellulolytic fungus Chaetomium

cellulolyticum gave 50- 100 % faster growth rates and over 80 % more final biomass protein formation than Trichoderma viride.

El- Ashwah et al. (1980), Langar et al. (1980), Lemmel et al. (1980), El- Ashwah et al. (1981) and Elias et al. (1981) used Spicaria elegans (I- 134), Cladosporium sp. de vries (I- 175), Cladosporium cladosporioides (NRRL- 3182), Dactylium denderoides (NRRL- 2574), Linderina penni- spora (NRRL- 2237), Heterocephalum aurantiacum (NRRL- 2238), Agaricus bispours, Volvariella diplasia (edible mushrooms), Saccharomycopsis fibuligera, Spicaria elegans I- 134, Cladosporium sp. devries I- 75, Cladosporium cladosporioides NRRL 3182, Saccharomyces cerevisiae and Candida tropicalis 60- 31 for SCP production .

Ivarson & Morita (1982), Karapinar & Okuyan (1982), Wheatley et al. (1982) studied the growth and protein synthesis of Scytalidium acidophilum , Sporotrichum pulverulentum , Fusarium sp. and Geotrichum sp.. They found that the crude protein ranged from 31.9 - 46 % .

El- Masry et al. (1983), Gomez & Castillo (1983) , Hecht et al. (1983), Karapinar & Worgan (1983), Miller & Srinivasan (1983), Osman et al. (1983) utilized Candida tropicalis IF 0006, C. pseudotropicalis NCYC 744, Chaetomium cellulolyticum (ATCC 3219), Aspergillus oryzae, A. niger ,

Sporotrichum pulverulentum, Trichoderma viride, Aspergillus terreus, Candida humicola 6 for microbial protein production .

Bahar & Azuaje (1984), Monib et al. (1984), Towersey et al. (1984) studied and selected strains from non toxic fungal mycellium such as Fusarium graminearum, F. solani and Fusarium oxysporium for fungal protein production.

Martin & Bailey (1985) studied the production of pellets of Agaricus campestris NRRL 2334 using fermentor as a batch culture technique .

— Starchy wastes :

Szuecs (1958) used cereal particles and corn syrups for the production of biomass by Morchella esculenta. He added that the cereal particles served as a support of the mycelial nuclei in colony formation .

Gray (1966) used potatoes and ground corn as substrate for fungal protein production. He found that it was possible to increase the total quantity of protein by a factor of 2.019 and 2.07 for the two crops, respectively.

Gray and Abou- El- Seoud (1966 b) used the fleshy roots of manioc tropical starch - producing tuber cultivated extensively in parts of Africa, Asia, and South America as

a staple foodstuff- as a source of carbohydrates in growth medium of fungus namely Cladosporium No. 83. This fungus was grown well experimentally on manioc which contains 32% total carbohydrates and 0.7 % protein and was able to synthesize one pound of protein from 8 pounds of carbohydrates. It was possible to increase the protein content to 5.7 folds of the amount initially present in the manioc.

According to Gray & Abou- El- Seoud (1966 b), Spicer (1971), Anderson et al. (1975), Moreton (1978), Shipman & Fan (1978), Lemmel et al. (1980) and Ueda et al. (1981) the cheap abundant carbohydrate sources were sweet potatoes, hydrolyzed potato waste, corn, rice, cassava root, cassava flour, citrus molasses, cane molasses, beet molasses, whole sugar beets, beet shreds, wood flour, paper pulp and husks. These materials were investigated for producing fungal protein, the best results were obtained with sweet potatoes and beet molasses .

Gray and Karve (1967) used rice containing 70 % carbohydrate and 7.5 % protein as a substrate in growth media of some fungi for the synthesis of fungal protein .

Brook et al. (1969) used raw cassava starch as a substrate for fungal biomass production under solid -substrate and liquid fermentation methods .