# FUNGI AS A NUTRIENT SOURCE

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## INTRODUCTION

To those who have been aware of the rapidity of increase of world population and have been concerned with the problem of how to feed many additional millions of people it is rather encouraging to note that there now appears to be developing a more widespread awareness of the existence of the problem. Unfortunately all save a very small percentage of the current crop of writers and lecturers have failed to pinpoint the major issue.

We are almost constantly subjected to a barrage of statements all of which may be resolved to the single, simple statement that if the human population continues to increase it will not be able to provide all of its members with sufficient food. Since such statements are half-truths they may serve to do more harm than good in the long run because they may serve to instill in certain segments of our society a measure of confidence which cannot be justified by available data.

Human food production can be doubled or tripled quite easily in a single season on many lands in the state of Ohio merely by planting corn instead of soybeans and can be increased by an even greater factor by substituting potatoes for corn. In general it appears rather foolish to speak of an impending food shortage when it easily can be calculated and just as easily demonstrated that the annual calorific requirements of 3 billion people can be supplied from the potatoes which can be produced on a mere 2.5 per cent of the earth's arable land.

Unfortunately, although the highly optimistic opinions expressed above are quite true, they deal with food only in terms of quantity, and food quality is completely ignored. Quite typical of such opinions is that of Jacques Theodore, a Belgian writer recently converted to Castro's unusual type of democracy, who, in referring to Cuba's food shortage problems stated "Well, it may be true that there is a certain shortage of food in Cuba, but there's no shortage of calories." Such statements are no more dangerous because of their inherent potential for creating false confidence than are general statements to the effect that the world is approaching a food

shortage problem, since all such statements are rightly subject to criticism by the non-critical agriculturalist who has experienced at least a century of success in his attempts to squeeze greater and greater quantities of food from the same unit area of land. Therefore, it is highly unlikely that substantial efforts will be made to forestall the coming situation which is intensified by rapid population increases until it is more generally recognized that the approaching problem does not involve a general food shortage but actually a shortage of one food type -- protein.

Like that of all other living organisms, man's food can be classified conveniently into three well-defined categories -- carbohydrates, fats, and proteins, each of which constitutes an important part of the human diet. All three food types may serve as sources of energy, so in this sense all are of importance. The average individual probably gets the major part of his calories of energy from his carbohydrate foodstuffs; however, it has been demonstrated that if an individual obtains sufficient amounts of fat and protein he can fare very well without any carbohydrate in his diet. Such is not true for the other two food types, since if he is to exist in a state of reasonable well-being man must include in his diet adequate amounts of (1) fats, which are the sources of certain essential fatty acids which cannot be synthesized in sufficient amounts in the human body, and (2) proteins, which are the sources of most of the amino acids from which man's body synthesizes the proteins necessary for growth of the young and maintenance of the adult. Proteins are required in considerably greater amounts than fats, it usually being assumed that each individual should ingest 65 grams per day on the average (52.2 pounds per year).

A very conservative estimate of current world carbohydrate production and a few calculations of carbohydrate production potential reveals that this food type is produced far in excess of the needs of a human population many times greater than the present one. Fat is produced in more than adequate amounts, and even if it should become in short supply there need be no cause for alarm, since well-conceived methods for the production of microbial fat from carbohydrates have been part of our store of knowledge for many years. However, such optimistic views cannot be taken with respect to protein since the real issue involved in the problem of providing food for an increasing population is that of providing sufficient amounts of this particular food type. Protein shortage is not an approaching possibility but is already a stark reality in vast areas of the world and is becoming a distinct probability in many others. By way of example Africa may be cited, since it has been estimated that at least 90 per cent of the people inhabiting this vast continent are suffering from protein deficiency. Closer to home one may point to Guatemala, where a careful investigation of four rural towns by Scrimshaw and Behar (ref. 4) revealed that nearly two-fifths of the deaths occurring in the 1- to 4-year age group were due to the protein deficiency disease, kwashiorkor. There is no sound reason for the belief that similar situations might not ultimately arise in the United States if population continues to increase at its present rate of one new citizen every 10.5 seconds. It is too early to determine whether or not a trend is represented but the fact that 178 pounds of meat were available per person in 1959 whereas only 160.5 pounds were available per person in 1961 may have some significance and should give pause for thought. No great sense of security should be derived even from the higher figure, since 178 pounds of meat (assuming that a variety of cuts are represented)

will supply only about 75 per cent of the annual protein requirements of one individual.

Ironically enough considerably greater quantities of protein than are required by the present human population (approximately 2.8 billion) are produced annually on this earth. However, much of this protein is in such dilute concentration that it is of little use to man. For example, it is possible to produce more protein per acre by planting a low protein crop plant like sugar cane than it is by planting a high protein crop plant like soybeans. However undesirable it may be to the individual a man may satisfy his protein requirements by eating 186 grams of soybeans daily -- a perfectly feasible gastronomic operation. On the other hand to satisfy his requirements with sugar cane an individual would have to ingest 32 pounds of fresh cane daily -- a performance which would probably be impossible for most people.

Barring a major breakthrough by the chemist in the area of protein synthesis, the problem of supplying ever-increasing amounts of protein can be solved only in one of two ways: (1) the development of a process whereby present supplies of dilute protein can be concentrated in a palatable form or (2) the exploitation of organisms of high protein content which are not presently exploited. Pirie (ref. 3) has expressed optimism concerning the first solution, while in the Mycology Laboratory of the Ohio State University we are concerned largely with an investigation of the potential of the second solution.

In the final analysis a solution to the problem of protein deficiency can be provided only by producing increased amounts of utilizable protein per unit area of land. It would be well also to recognize that as world population increases some of the earth's present 21 billion acres of arable land will be used for purposes other than agricultural. Increasing the yield per acre of some high protein plant such as soybean cannot provide the answer since there are definite limits to the amount of plant protein which can be produced by such means. Reference to Mill's "Law of diminishing increments" and Willcox's "Inverse yield--nitrogen law" have been made earlier by Gray (ref. 1) and need not be repeated here. It is sufficient to say that traditional agriculture has already demonstrated its inability to provide a solution to the protein problem in many world areas and therefore if we are to provide any reasonable solution to the problem we must institute a radical change in our views in such manner that we are no longer handicapped by thinking along traditional agricultural lines. Rather than attempting to make pitiful contributions to the solution of a serious problem by obtaining small yield increments of low-yielding, high-protein plants it would seem far more logical to produce large quantities of high-yielding, low-protein plants and extract the protein as advocated by Pirie (ref. 3) or to convert the carbohydrate (which usually occurs in large amounts in such plants) to protein using an organism capable of performing a total protein synthesis.

## FUNGAL SYNTHESIS OF PROTEIN

Many non-green organisms have the capacity to perform a total synthesis of protein from carbohydrate and inorganic nitrogen salts; however, because

of their typically greater efficiency in the conversion of substrate carbon to tissue carbon and their greater ease of harvest, we have chosen to concentrate our efforts on filamentous fungi rather than bacteria. Furthermore, from among the several classes of fungi we have chosen the mold-like Fungi Imperfecti because of their ubiquity and the rapidity with which many will grow. Almost limitless choices of test organisms may be made from this group, since a conservative estimate of the number of form species is 20,000 which are distributed through more than 1300 form genera.

The first step in the present program was to establish criteria of acceptability on the basis of which it could be determined whether or not a particular test organism could be judged to have the potential which would warrant its further more thorough investigation. These criteria are as follows:

1. The organism must be capable of growing on medium with an inorganic nitrogen salt as the sole source of nitrogen.

2. It must be able to grow in submerged aerated culture.

3. It must efficiently convert substrate carbon to tissue carbon and at the same time accomplish a near theoretical conversion of inorganic nitrogen to organic nitrogen.

4. It must grow rapidly. Four days have been set arbitrarily as the maximum permissible length of time for the growth period.

In initial screening experiments designed to test the capacity of different fungi to utilize inorganic nitrogen, thirty-six out of thirty-eight organisms were found to be able to use ammonium nitrogen, nitrate nitrogen, or both. If this sampling of the Fungi Imperfecti is at all representative it would appear that this group will offer no especial problems with respect to the utilization of inorganic nitrogen. In later more quantitative experiments the optimum salt (sodium nitrate, ammonium chloride, or ammonium nitrate) for use with each fungus was determined so that in subsequent experiments each fungus could be supplied with the particular nitrogen salt upon which it grows most readily. Yields obtained with ten different fungi when cultured in 50 ml. portions of medium (pH 6.0) each of which contained 1 gram of glucose and equivalent amounts of nitrogen as one of the above salts are presented in Table 1. It will be noted that five of the ten test fungi grew best on medium containing ammonium nitrate and that two grew best on medium containing sodium nitrate; however, it should not be concluded in such instances that nitrate nitrogen can be utilized as readily as ammonium nitrogen. The nitrogen salt which appears to be optimum for each fungus is probably the one whose utilization does not result in pH change to a value too far from the optimum pH for that particular organism. Medium reaction could undoubtedly be controlled through use of suitable buffers; however, with the possibility of large scale production in mind it seems best not to attempt the costly buffering of large volumes of medium but to start with medium of pH value near optimum and partially control changes in pH by selection of proper nitrogen salt.

Although at this stage of the investigation a variety of fungi have been employed in experiments involving thousands of pure cultures, the

Table 1

Relation of Inorganic Nitrogen Source to Growth of Fungi Imperfecti*

| Culture No. | Form Genus | Average Yield (mg./50 ml. flask) Sodium nitrate | Ammonium nitrate | Ammonium chloride |
|---|---|---|---|---|
| I-99 | Sepedonium | 490 | 498 | 454 |
| I-30 | Gliocladium | 414 | 423 | 249 |
| I-36 | Cephalothecium | 415 | 420 | 183 |
| I-11 | Myriothecium | 214 | 299 | 284 |
| I-104 | Tritirachium | 96 | 152 | 122 |
| I-75 | Cladosporium | 399 | 417 | 461 |
| I-80 | Pullularia | 331 | 339 | 449 |
| I-41 | Bispora | 53 | 53 | 55 |
| I-73 | Trichurus | 277 | 233 | 109 |
| I-19 | Speggazinia | 203 | 169 | 149 |

*Still cultures in 250 ml. Erlenmeyer flasks incubated at 25° C. for 6 days.

fungus which has been investigated most thoroughly is I-9 (Heterocephalum aurantiacum). Wherever it has been possible to do so the work has been conducted in small shake flask cultures (50 ml. of medium in 250 ml. Erlenmeyer flasks), but in many instances 5- or 6-liter cultures in large carboys (Figure 1) have also been used. Judgment as to whether or not an organism meets the second criterion is based only on its behavior in large carboy culture.

When this project was first instituted a medium was arbitrarily selected and inoculated with spores of I-9. In this first medium 11.1 grams of glucose were required for the production of 1 gram of mycelium (dry weight) in a 4-day incubation period. By subsequently altering the various constituents a medium was finally prepared which permitted the formation of 1 gram of mycelium from 1.81 grams of glucose. The initial reaction of this medium was adjusted to pH 6.0 and a 4-day growth period at room temperature was allowed. Protein content of mycelium so produced was often as high as 35 per cent. The constituents of this medium are as follows:

| | | |
|---|---|---|
| dextrose | 100g. | |
| $NH_4NO_3$ | 3g. | |
| $KH_2PO_4$ | 25g. | adjust to pH 6.0 |
| *vitamin solution | 5ml. | and make to final |
| folic acid solution (1.3mg./l.) | 1ml. | volume of 5 or 6 |
| $FeCl_3$ solution (1.92g./l.) | 5ml. | liters. |
| **trace element solution | 5ml. | |
| $ZnSO_4$ solution (44g./l.) | 5ml. | |
| corn steep | 10ml. | |

*thiamin HCl -- 99.9mg., pyridoxin -- 50.2mg., Ca pantothenate -- 200.2mg., p-aminobenzoic acid -- 50.3mg., niacinamide -- 200.1mg., inositol -- 400mg., riboflavin -- 50mg.; make to 1 liter.

**$H_3BO_3$ -- 0.114g., $(NH_4)_6Mo_7O_{24}.4H_2O$ -- 0.484g., $CuSO_4.5H_2O$ -- 0.780g., $MnCl_2.4H_2O$ -- 0.144g., $ZnSO_4.7H_2O$ -- 16.720g.; make to 1 liter.

The above medium (varying only with respect to inorganic nitrogen source) has been used for the culture of fungi other than Heterocephalum, and, as might be anticipated when dealing with large numbers of different fungi, some have been found to be more suitable than others for the present purpose. Data obtained from large carboy cultures of eleven different fungi are listed in Table 2, and as may be seen from these data, the test organisms vary widely with respect to both crude protein content and efficiency of conversion of substrate carbon to tissue carbon. However, it should be recalled that all fungi were cultured in a medium developed specifically for Heterocephalum, and in all probability the yield of each could be improved by further alterations of the medium. This does not necessarily imply that all of the fungi listed in Table 2 can be used for the efficient synthesis of fungus protein, but experiences encountered in the development of the most suitable medium for growth of Heterocephalum certainly suggest that yields of other organisms can also be improved.

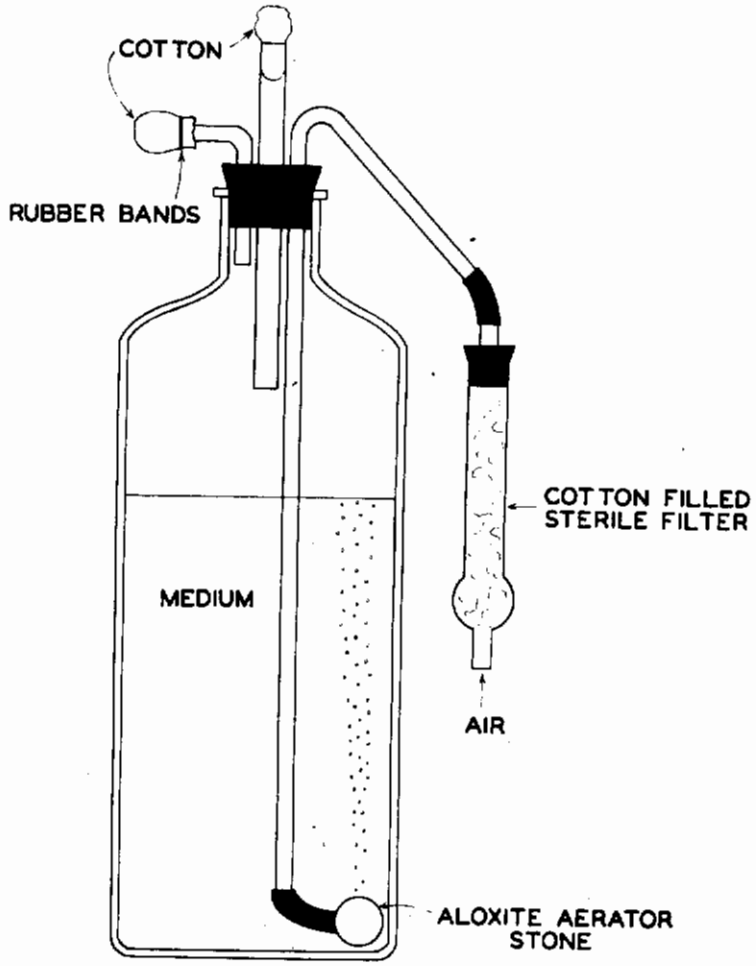


Figure 1

Apparatus for large scale laboratory cultures.

Table 2

Growth of Fungi Imperfecti in Large Carboy Culture*

| Culture No. | Form Genus | Nitrogen source | Mycelium dry wt./ bottle | Gms. glucose required to produce 1 gm. | Per cent crude** protein |
|---|---|---|---|---|---|
| I-159 | Epicoccum | $NH_4NO_3$ | 81.7g. | 1.21 | 6.00 |
| I-9 | Heterocephalum | $NH_4NO_3$ | 55.2 | 1.81 | 35.00 |
| I-58 | Colletotrichum | $NH_4NO_3$ | 55.2 | 1.81 | 11.56 |
| I-80 | Pullularia | $NH_4Cl$ | 51.8 | 1.93 | ----- |
| I-83 | Cladosporium | $NH_4Cl$ | 45.7 | 2.18 | 9.11 |
| I-134 | Spicaria | $NH_4NO_3$ | 41.1 | 2.43 | 25.25 |
| I-30 | Gliocladium | $NH_4NO_3$ | 35.7 | 2.80 | 18.92 |
| I-73 | Trichurus | $NH_4NO_3$ | 34.3 | 2.91 | 18.91 |
| I-99 | Sepedonium | $NH_4NO_3$ | 34.3 | 2.91 | 16.80 |
| I-114 | Geomyces | $NH_4NO_3$ | 22.4 | 4.46 | 25.92 |
| I-29 | Helminthosporium | $NH_4NO_3$ | 9.9 | 10.10 | 29.44 |

*Four-day incubation period at room temperature.

**Kjeldahl nitrogen X 6.25.

Optimum initial medium pH and optimum temperature for growth have been determined for most of the test fungi. Although many show different pH and/or temperature optima in general it has been found that a temperature of 25° to 30° C. and an initial pH of 5.0 to 6.0 are suitable for most forms.

Optimum sugar concentration cannot be stated at this time because in large scale production this value will have to be determined by the cost accountant. In laboratory experimentation efficiency of conversion of substrate carbon to tissue carbon varies inversely with sugar concentration, but in a large scale operation the use of dilute sugar solutions may not be economically feasible.

## FEEDING EXPERIMENTS

Most of the research to date has been concerned with the development of most efficient production methods, but it is obvious that extensive feeding trials will have to be conducted. Most trials to date have been mice feeding experiments designed primarily to determine if the materials currently being produced are toxic. To date none have been found to be toxic, but in view of the known toxicity of certain species in other classes of fungi we may expect to encounter certain poisonous species among the Fungi Imperfecti. Mice have been maintained satisfactorily with dried Heterocephalum mycelium as their sole source of food.

Like green plants different fungi differ markedly with respect to protein content and there is no reason to believe that different imperfect fungus proteins will not also differ as to quality. Hughes* has already demonstrated that the proteins of Basidiomycetes differ in quality from species to species and it would be most remarkable if the proteins of Fungi Imperfecti did not exhibit similar differences. In the event that proteins which are deficient in one or more essential amino acids are found, the results of Stokes and Gunness (ref. 5) provide hope that such proteins may be altered, since these workers have demonstrated that protein quality can be altered by varying the environmental conditions.

## POTENTIAL OF THE PROCESS

In their 1961 paper Scrimshaw and Behar (loc. cit.) have mapped the geographical distribution of kwashiorkor, and from their map it is evident that this protein deficiency disease occurs in the southern part of the northern hemisphere and throughout most of the southern hemisphere -- the great carbohydrate-producing area of the world. That protein deficiency is unnecessary in such areas is illustrated in Figure 2 in which it is shown that one acre of land devoted to POJ2878, the Peruvian hybrid sugar cane cited by Willcox (ref. 6), will produce 110 tons of harvested cane

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*Hughes, S. B. 1956. Amino acids in acid hydrolysates of some Basidiomycete sporocarps. PhD dissertation, The Ohio State University (unpublished).

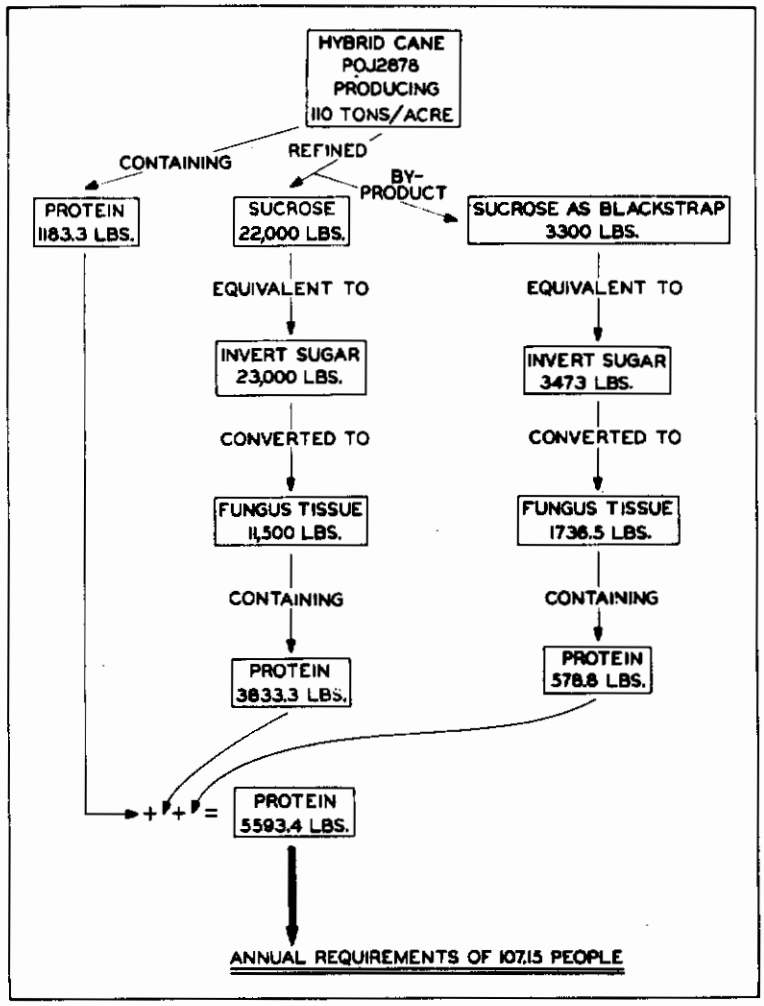


Figure 2

Potential of fungus conversion process if used with Peruvian hybrid sugar cane, POJ2878

containing 1183.3 pounds of crude protein and 25,300 pounds of sucrose. Reference to this figure shows that by using a fungus such as I-9, which is capable of synthesizing one pound of protein from six pounds of hexose sugar, sufficient protein can be produced from one acre to supply the annual protein requirements of 107.15 people. From this it may be calculated that Peru (population -- 8,405,000) could supply its entire annual protein requirements from about 79,000 acres -- 0.024 per cent of its total land area of 513,000 square miles.

Equally illuminating are the data of Figure 3 in which it is calculated that the annual sugar cane crop of Puerto Rico (another kwashiorkor area) could be used for the fungal synthesis of protein in an amount sufficient to supply the annual requirements of a population over four times as great as that presently existing in Puerto Rico. Thus it would appear that there is no valid reason for the existence of protein deficiencies in this country.

Recognizing the fact that in more temperate regions of the earth the cultivation of sugar cane is impossible, some consideration has been given to the possibility of using other high-yielding, high-carbohydrate plant materials as the sources of carbohydrate for a fungal conversion process. For example it has been calculated that protein production per acre of corn can be increased by a factor of approximately 2.5 if the corn starch is converted to fungal protein, and 75 per cent of this theoretical amount has already been attained experimentally. More recently Abou El Seoud* has successfully used whole ground sweet potatoes (protein content -- 1.8 per cent) as a crude raw material for the production, with good yields, of a material containing as much as 38.8 per cent protein. This investigator has also demonstrated that it is unnecessary to use a 4-day growth period, since after the second day no additional protein is synthesized (Figure 4). This finding makes possible either a reduction in length of growth period by one-half with subsequent saving in equipment, or the production of a series of dried products of varying protein content.

Since tremendous gallonages of water will be needed for the production of large amounts of fungus protein, the findings of Gray and Pathak (ref. 2) are of some significance. Small scale experiments with 23 different fungi revealed that the substitution of untreated sea water for distilled water in the preparation of growth media resulted in yield increases by 21 fungi. With those fungi which showed increased yields the average yield increase was approximately 50 per cent. Data obtained with ten of the test fungi are presented in Table 3. If our sampling is at all representative it seems possible that most of the Fungi Imperfecti may grow better in sea water medium than in fresh water medium. Thus, in coastal areas where large quantities of carbohydrates are produced, a paucity of fresh water need not be a deterrent factor in the establishment of a process such as that presently advocated.

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*Abou El Seoud, Mohamed O. 1962. Production of microbial protein from sweetpotato by Fungi Imperfecti. MSc thesis. The Ohio State University (unpublished).

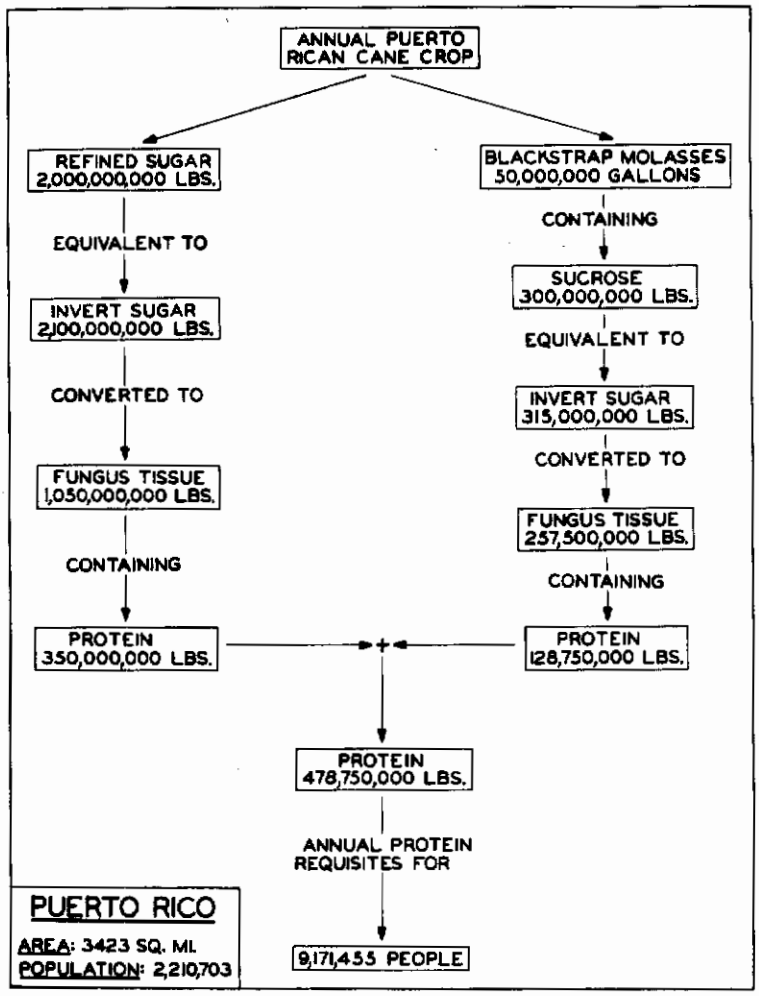


Figure 3

Potential of fungus conversion process if used with the annual Puerto Rican sugar cane crop

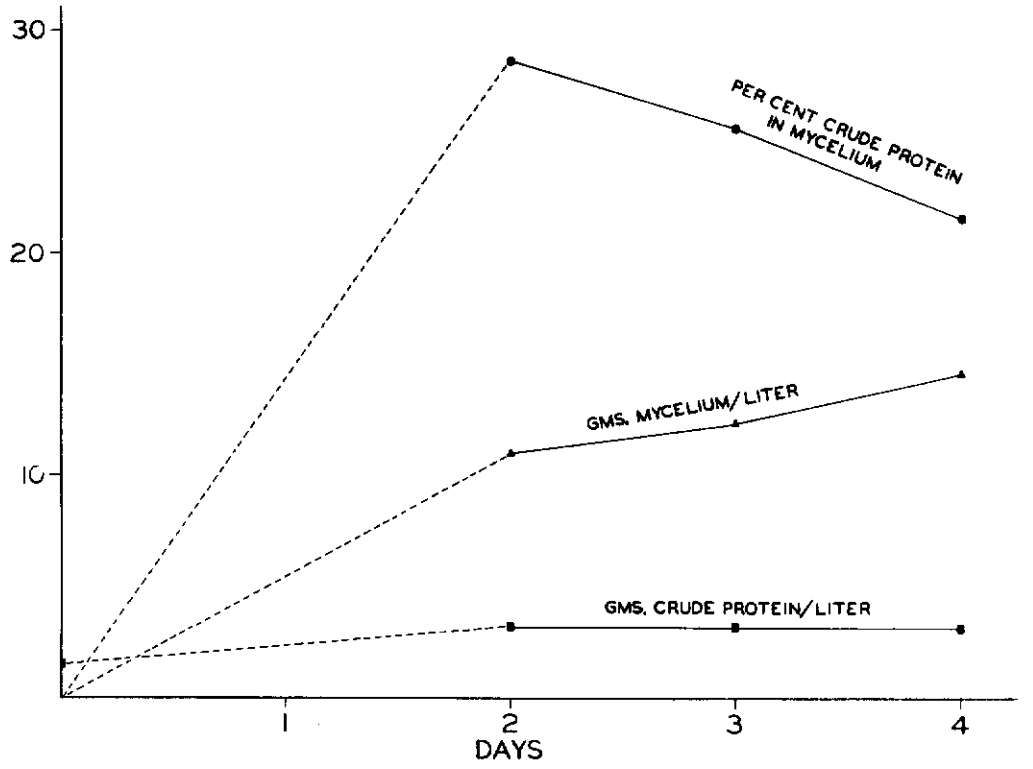


Figure 4

Rate of growth and rate of protein synthesis of Cladosporium (I-75) in medium containing ground sweetpotatoes as the sole source of carbohydrate.

Table 3

Effect of Sea Water on Mycelium Yields of Fungi Imperfecti*

| Culture No. | Form Genus | Yield (mg.) Sea Water | Dist. Water | Per cent Increase |
|---|---|---|---|---|
| I-14 | Phoma | 868 | 484 | 71.9 |
| I-36 | Cephalothecium | 576 | 438 | 31.5 |
| I-100 | Linderina | 396 | 256 | 56.0 |
| I-134 | Spicaria | 564 | 482 | 18.0 |
| I-104 | Tritirachium | 242 | 84 | 178.5 |
| I-41 | Bispora | 210 | 146 | 43.8 |
| I-81 | Brachysporium | 611 | 431 | 29.4 |
| I-83 | Cladosporium | 701 | 377 | 85.9 |
| I-80 | Pullularia | 621 | 442 | 40.5 |
| I-11 | Myriothecium | 450 | 417 | 7.6 |

*Yields are expressed as average weights of dry mycelia obtained per flask from shake flask cultures (6 replicates) containing 50 ml. of medium and incubated for 4 days at 25° C.

## LARGE SCALE PRODUCTION

No especial difficulties need be anticipated relative to the establishment of this process on a large scale. The brewing, wine-making, and distilling industries have been conducting large-scale fungus cultures for several centuries, and in more recent times the citric acid and antibiotics industries have added greatly to our knowledge of how to handle fungi on a vast scale. In Figure 5 is presented a diagrammatic flow-sheet of a small plant adapted for the production of fungal protein. All of the basic plant equipment necessary for such a process is already in use in industry, and with a few alterations most modern distilleries could be converted for use in a process of this type. While the present products of a rum distillery can probably help the protein deficient individual endure his miserable state with more fortitude, it is here suggested that by using the same raw material and the same plant for the process of protein synthesis the miserable state could be altogether eliminated.

## CHARACTERISTICS OF DRY FUNGUS TISSUE

Some comment concerning the nature of the materials whose production is briefly discussed above seems desirable. In submerged aerated culture most of the test organisms form more or less spherical mycelial pellets which vary in size and degree of roughness of surface from organism to organism. However, with some forms pellet formation is never observed and the mature cultures resemble large masses of cotton submerged in liquid. Harvest is easily accomplished by filtering the fungus tissue on a gauze filter. In a large scale operation harvesting could be readily performed by filtration on small mesh metal screens, thus eliminating the more complicated and expensive process of centrifugation which is practiced in the recovery of yeast.

Moist, freshly-harvested mycelia are typically odorless, but one form yields pellets which have the appearance of cooked tapioca and the distinctive odor of fresh sweet corn. The appearance and odor of dried material varies with the drying process. When dried at ordinary pressure at 50° C. dried mycelia are typically white to very light buff in color and are odorless; however, when dried at 70° C. the material becomes dark brown in color and has a faint molasses odor. Lyophiled mycelia are typically odorless and nearly white in color.

Because it is tasteless and odorless no especial problems should arise from its use as a protein supplement in a variety of human foods. For the United States at this time it is recommended that such material be used as a protein supplement for livestock feeding purposes. The amount of meat protein which animals can produce depends to a large extent upon the amount of protein supplied in their feed. Therefore, if larger amounts of protein can be produced per acre by converting the carbohydrate produced on that acre to protein, the amount of meat protein produced per acre can be increased. Since at current rate of population increase in the United States (3,200,000 per year) the national beef cattle herd (or its hog and/or sheep equivalent) must be increased by approximately one million head

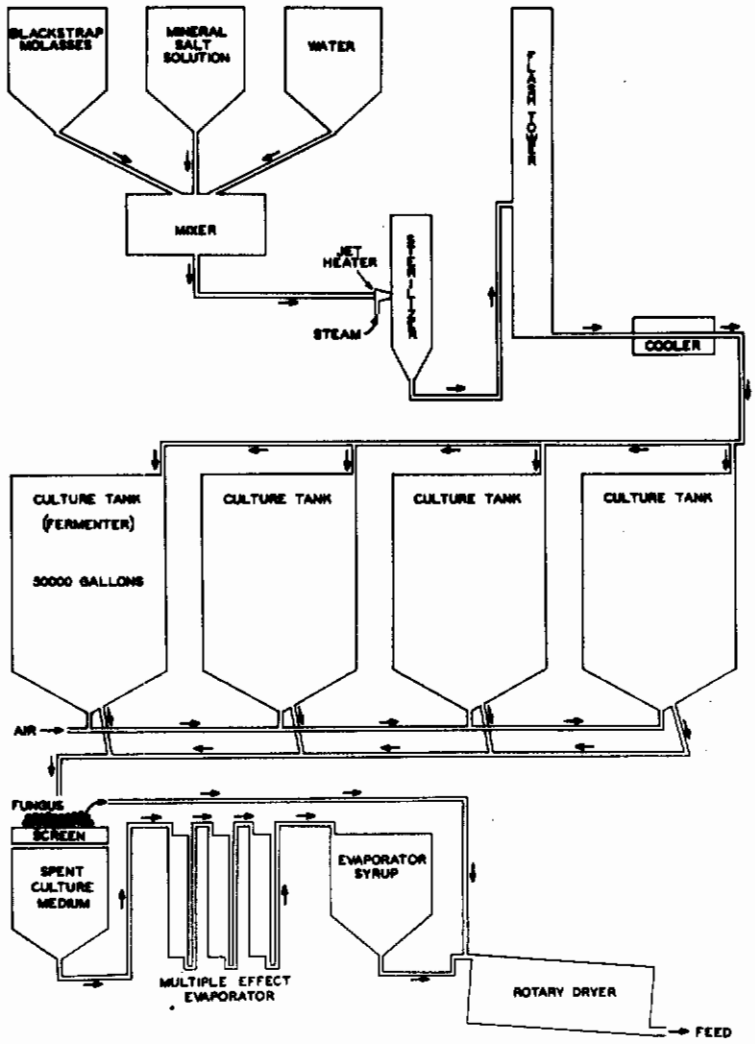


Figure 5

Flow sheet diagram of small plant capable of producing 350,000 to 500,000 pounds of protein annually by a fungus conversion process.

annually in order to maintain per capita meat availability at the 1959 level, it seems obvious that additional sources of protein for livestock feeding must be sought.

## SUMMARY

The problem of a rapidly increasing world population is briefly discussed, and the thesis that a general food shortage is imminent is denied. Instead it is pointed out that with increasing population the world faces an even greater shortage of one food type: protein. Two possible solutions to the problem have been suggested: (1) an extraction and concentration of protein from materials in which it is quite dilute, and (2) the fungal synthesis of protein from excess carbohydrates and inorganic salts of nitrogen.

The fungal synthesis of protein is briefly discussed and the high potential of such a process is pointed out. The recommendation is made that in the United States such material be used as a protein supplement for livestock feeding in order that greater amounts of meat protein may be produced to keep pace with increasing population.

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