# Grain Alcohol Fermentations

## SUBMERGED MOLD AMYLASE AS A SACCHARIFYING AGENT

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**Experiments were conducted on a pilot plant scale by utilizing conventional distillery equipment and were designed to supply data on the following points: type and concentration of enzymes produced in submerged fermentation under varying rates of aeration; concentration and quantity of mold culture required to replace malt; alcohol yields obtainable by the continuous use of fungal amylase for the saccharification and fermentation of corn; and investment and operating costs for the production of fungal enzymes by the submerged process. Results showed that saccharification was satisfactory and alcohol yields were comparable to those obtained with malt when mold culture liquor equivalent to 6 to 10% of the final mash volume was used. The cost of the amylase is estimated to be 6.06 cents per bushel of grain processed, as compared with 12.1 cents in the case of malt.**

NUMEROUS reports (*1, 3, 8, 10*) show that the use of fungal enzymes to supplement or replace distillers' malt generally results in at least comparable yields of alcohol and a more rapid rate of fermentation. While most of these investigations have been carried out on a laboratory scale, two procedures for producing mold enzymes—i.e., the mold-bran method and the amylo method—have been utilized commercially.

Mold bran is produced by cultivating an organism of the *Aspergillus flavus-oryzae* group upon moistened cereal bran under conditions of carefully controlled humidity, temperature, and aeration (*11*). After a growth period of 30 to 40 hours, the mold bran is removed from the incubators and dried. The dried product is substituted directly for distillers' malt. Based on the total weight of grain in the mash bill, from 3 to 4% of mold bran suffices for the saccharification of the starch.

In the amylo process (*6*), special strains of *Rhizopus* or *Mucor* are cultivated submerged in preliquefied, cooked grain mashes. Within 24 to 48 hours sufficient amylase is formed to convert a large portion of the starch. Yeast is then introduced and fermentation is continued anaerobically. In some instances, the yeast is propagated aerobically with the mold.

From the standpoint of distillery operation, both the amylo and mold-bran methods possess certain disadvantages. In mold-bran production, considerable labor, processing space, and equipment, as well as a cereal bran substrate are required; while in the amylo process the entire mash volume must be preliquefied and aerated under pure-culture conditions.

As a result of a survey of a large number of fungi (*4*), a few molds have been found which are capable of appreciable synthesis of starch-hydrolyzing enzymes when cultured under submerged conditions. In laboratory studies, culture liquor from one strain, *Aspergillus niger* NRRL 337, completely replaced malt when added to corn mashes at the rate of 10 to 20% of the final mash volume.

This paper describes pilot plant experiments primarily designed to provide data on the following points: extent of enzyme production in deep tank fermentations under varying rates of aeration; the relationship between enzymatic potency of the mold culture liquor and the quantity required to replace malt completely; alcohol yields obtainable by the continuous use of fungal amylase for the saccharification and fermentation of corn; and design and cost of operation of the submerged process for production of fungal enzymes.

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### EXPERIMENTAL METHODS

Stock cultures of *Aspergillus niger* were carried on slants of nutrient agar or on thin layers of moistened wheat bran in Erlenmeyer flasks. To prepare inoculum, a loop of mold culture was transferred to 200 ml. of liquid medium contained in a 1-liter flask. After incubation at 28° to 30° C. on a reciprocating shaker for approximately 48 hours, the content of the flask was transferred to 8 liters of sterile medium in a 12-liter Pyrex bottle. This culture was aerated by introducing sterile air through a cloth sparger and was permitted to grow for 48 hours before being transferred to 250 gallons of stillage medium in a pilot plant fermentor. Every precaution was taken to maintain pure culture conditions during the propagation of the inoculum and production of the diastatic liquor. Media contained in flasks and Pyrex bottles were sterilized for 1 hour at a pressure of 20 pounds per square inch gage, and the pilot plant fermentor with medium and connecting lines was sterilized for 2 hours at a pressure of 10 to 12 pounds per square inch gage. Air was sterilized by passing it through a tower of activated carbon.

For most of the experiments described herein, the liquid medium prior to the final stage was composed of 20 grams of corn steep liquor, 10 grams of commercial glucose, 2.5 grams of calcium carbonate, and tap water to make 1 liter. It was subsequently found, however, in experiments on the continuous use of mold amylase, that more uniform culture development and higher alcohol yields were obtained when the inoculum was developed from the spore stage on stillage medium and when somewhat larger amounts of the inoculum were employed. Because this step was found to be quite critical, the preferred method is given in detail below.

Four 1-liter Erlenmeyer flasks containing 200 ml. of stillage-glucose medium of the following composition were each inoculated with a loop of spores from the stock culture:

| | |
|---|---|
| Stillage sirup (prepared by evaporating thin stillage in vacuo to 38% solids), g. | 50 |
| Commercial glucose, g. | 20 |
| $CaCO_3$, g. | 1 |
| Tap water to make 1 liter | |

After 48 hours' incubation on a reciprocating mechanical shaker, the contents of two flasks were transferred to each of two 12-liter Pyrex bottles containing 9 liters of stillage-glucose medium. Sterile air was admitted at the rate of 0.5 volume of air per volume of medium per minute and the temperature was maintained at 28° to 30° C. Two bottles of such inoculum were then used after 48 hours to seed an 800-gallon fermentor containing 25 pounds of ground corn, 15 pounds of calcium carbonate, and 250 gallons of thin stillage obtained from a previous alcoholic fermentation of corn. The thin stillage is designated either corn malt or corn mold, depending upon the saccharifying agent used. This medium was agitated by means of a large bladed propeller rotating at 100 r.p.m. and aerated with 0.25 volume of air per volume of medium per minute. After 48 to 72 hours it was ready for use.

The determination of dextrinizing enzyme and maltase as well as the laboratory evaluation of mold culture liquors as saccharifying agents in grain fermentations has been described previously (*4*). Dextrinizing enzyme was determined by the method of

TABLE I. EFFECT OF RATE OF AERATION UPON SYNTHESIS OF DEXTRINIZING AND MALTASE ENZYMES BY *Aspergillus niger* NRRL 337

| Rate of Aeration, Air Volume, % of Mash Volume per Minute | Periodic Data on Liquors at 24 hours pH | 24 hours Dextrinizing enzyme, units/ml. | 48 hours pH | 48 hours Maltase activity, % hydrolysis of maltose | 48 hours Dextrinizing enzyme, units/ml. | 72 hours pH | 72 hours Maltase activity, % hydrolysis of maltose | 72 hours Dextrinizing enzyme, units/ml. |
|---|---|---|---|---|---|---|---|---|
| None | | 0.1 | | None | 0.6 | | ... | ... |
| 12.5 | 5.5 | 0.6 | 5.4 | 29.4 | 10.8 | 5.3 | 39.8 | 15.1 |
| 25 | 5.5 | 0.1 | 5.6 | 16.5 | 7.5 | 5.2 | 30.2 | 12.5 |
| 50 | 5.4 | 1.7 | 5.2 | 30.8 | 14.4 | 5.2 | 32.4 | 16.6 |
| 75 | 5.7 | 0.6 | 5.4 | 19.4 | 9.5 | 4.6 | 33.5 | 16.8 |
| 100 | 5.6 | 1.1 | 4.3 | 35.7 | 11.5 | 4.4 | 36.5 | 12.8 |

TABLE II. CONVERSION OF MASHES WITH VARYING QUANTITIES OF MOLD CULTURE LIQUOR, *Aspergillus niger* NRRL 337

| Expt. No. | Quantity of Liquor for Conversion, % of Final Mash Volume | Dextrinizing Enzyme, Units/Ml. | Fermentation Data: pH 0 hr. | pH 24 hr. | pH 48 hr. | pH 72 hr. | Alcohol production, % by volume at 24 hr. | Alcohol production, % by volume at 48 hr. | Alcohol production, % by volume at 72 hr. | Final yield of alcohol, proof gal./bu. |
|---|---|---|---|---|---|---|---|---|---|---|
| 3 | 20 | 8.5 | 5.2 | 4.5 | 4.0 | 3.8 | 3.58 | 6.16 | 6.41 | 5.25 |
| 6 | 20 | 6.3 | 4.9 | 4.8 | 4.4 | 4.2 | 4.58 | 6.71 | 6.76 | 5.20 |
| 1 | 20 | 7.5 | 5.3 | 4.5 | 4.0 | 3.8 | ... | 4.91 | 6.04 | 4.75 |
| 2 | 20 | 9.8 | 5.0 | 3.9 | 3.7 | 3.7 | 5.44 | 6.30 | 6.41 | 4.90 |
| 1a | 15 | 7.5 | 5.0 | 4.4 | 4.2 | 4.0 | ... | 5.46 | 6.13 | 4.85 |
| 2a | 15 | 9.8 | 5.3 | 4.4 | 4.0 | 4.0 | 5.61 | 6.78 | 6.82 | 5.17 |
| 3a | 15 | 8.5 | 5.2 | 4.4 | 3.7 | 3.7 | 4.78 | 6.77 | 6.77 | 5.35 |
| 4 | 15 | 8.7 | 5.2 | 4.7 | 3.9 | 3.8 | 5.28 | 6.43 | 6.43 | 4.75 |
| 5a | 15 | 8.9 | 4.9 | 4.5 | 4.4 | 4.0 | 4.51 | 6.79 | 7.10 | 5.40 |
| 6a | 15 | 6.4 | 4.9 | 4.5 | 4.3 | 4.1 | 4.71 | 6.54 | 6.58 | 5.30 |
| 7 | 15 | 7.1 | 5.0 | 4.1 | 4.0 | 3.5 | 4.13 | 6.60 | 6.95 | 5.20 |
| 7a | 10 | 7.0 | 5.1 | 4.3 | 3.6 | 3.6 | 3.64 | 6.23 | 6.60 | 4.95 |
| 8a | 10 | 9.0 | 4.8 | 4.5 | 4.2 | 4.8 | 4.06 | ... | 6.43 | 4.80 |
| 9 | 10 | 12.2 | 5.0 | 4.6 | 4.2 | 4.2 | 2.22 | 5.52 | 6.52 | 5.01 |
| 10a | 10 | 8.0 | 4.6 | 3.9 | 3.6 | 3.5 | 2.48 | 5.79 | 7.13 | 5.45 |
| 13 | 5 | 15.5 | 5.1 | 4.2 | 4.1 | 4.0 | 3.64 | 6.19 | 6.83 | 4.88 |

Sandstedt, Kneen, and Blish (*7*) as modified by Evans and Dickson (*5*). The units reported herein are the number of grams of soluble starch previously modified by excess β-amylase which are dextrinized in 1 hour at 20° C. Maltase activity is reported as the percentage hydrolysis of 20 ml. of 1.05% maltose solution by 10 ml. of culture liquor in 2 hours at pH 4.6 and 30° C. Reducing sugars were determined by the method of Somogyi (*9*).

Pilot plant fermentations were started by weighing 2240 pounds of corn meal into a conventional horizontal cooker and adding water at the rate of 21 gallons per bushel of grain. One tenth of the total amount of malt or whole mold culture to be used was then added and the slurry was heated to 155° C. in approximately 25 minutes. The mash was held at this temperature for 5 minutes, cooled to 66° C., and the remaining mold culture or malt in water slurry was added. This lowered the mash temperature to 59° to 60° C. where it was maintained for 30 minutes. After conversion the mash was passed through a double-pipe cooler (which lowered the temperature to 26° C.) and into a fermentor. Three per cent by volume of yeast inoculum (NRRL Y-567) propagated in a medium of commercial glucose and corn steep liquor was added and the final mash volume was adjusted with water to 36 gallons per bushel of grain. Fermentation progress was followed by measuring the Balling, pH, and alcohol concentration of the mashes. Yields of alcohol were corrected for the alcohol produced in the inoculum. Control fermentations carried out by the same procedure with 10% malt as the saccharifying agent and with equivalent amounts of total grain gave an average yield of 5.10-proof gallons of alcohol per bushel of grain. The yields of alcohol reported throughout this paper are based on corn containing approximately 13% moisture.

## RESULTS

CULTURE REQUIREMENTS FOR ENZYME PRODUCTION. Results of laboratory experiments, reported in detail elsewhere (*4*), showed that a wide variety of media composed essentially of fermentable carbohydrate, protein or protein degradation products, and calcium carbonate were adequate for enzyme production by *Aspergillus niger* NRRL 337. Among the carbohydrates investigated and found satisfactory were corn meal, starch, molasses, maltose, and commercial glucose. Corn-steep liquor, stillage, soybean oil meal, and animal tankage were found to be good sources of protein. Calcium carbonate appeared to be beneficial principally because of its buffering action which maintained the pH between 5.0 and 5.5 throughout the fermentation. Calcium chloride was unsatisfactory as a replacement for the carbonate.

In laboratory trials maximum amylase production was obtained when thin stillage was supplemented with 1% carbohydrate, 0.5% calcium carbonate, and an aeration rate of about 1 volume of air per volume of medium per minute. Preliminary pilot plant experiments, in which concentrations of corn meal and calcium carbonate were varied, substantiated the laboratory trials relative to optimum levels of these supplements. The requirement for air was considerably lower than that established in laboratory equipment, as shown below.

INFLUENCE OF AERATION RATE UPON ENZYME PRODUCTION. With constant agitation, the aeration rate was varied from 0 to 1 volume of air per volume of medium per minute. It is apparent (Table I) that enzyme production was negligible in unaerated media despite agitation. However, with aeration rates ranging from 0.125 to 1 volume of air per volume of medium per minute, similar potencies of both dextrinizing and maltose-hydrolyzing enzymes were formed at all aeration levels. Although the optimum rate of aeration will probably vary with the dimensions of the fermentor, it appears that, when combined with agitation, a rate varying from one eighth to one fourth of the mash volume per minute will be adequate. It should be noted also in Table I that the more intense aeration resulted in a somewhat lower final pH, perhaps because of the production of a greater quantity of organic acids. Since dextrinizing enzyme is inactivated by acid, it is preferred to aerate at a rate which is suitable for enzyme production but below that which appreciably lowers the pH of the medium.

QUANTITY OF MOLD CULTURE REQUIRED FOR CONVERSION. It has been demonstrated frequently that the ability of fungal amylases to replace malt can be determined only by actual fermentation trials. The quantity of enzyme required for rapid and complete conversion must be determined in the same manner. With culture liquors from *Aspergillus niger* NRRL 337 grown on stillage medium and aerated by mechanical shaking, it was previously found that alcohol yields comparable to those from barley malt were obtainable when the volume of mold culture used was equivalent to 10 to 20% of the final mash volume. Usually such laboratory cultures had been incubated for 4 to 6 days before use.

In Table II are shown results of pilot plant experiments in which mold culture liquid for conversion was varied from 20 to 5% of the final mash volume. In each case the mold was cultivated for 66 to 72 hours and the rate of aeration was approximately 30% of the volume of medium per minute. Laboratory observations were confirmed in that mold culture liquors supported yields of alcohol equal to those obtained with malt when the mold liquors were employed at levels of 10 to 20% of the mash volume. When the level was reduced to 5% (experiment 13), the yield was lower than that obtained with malt despite the fact that the liquor had nearly twice the potency of dextrinizing enzyme contained in preparations which were satisfactory when

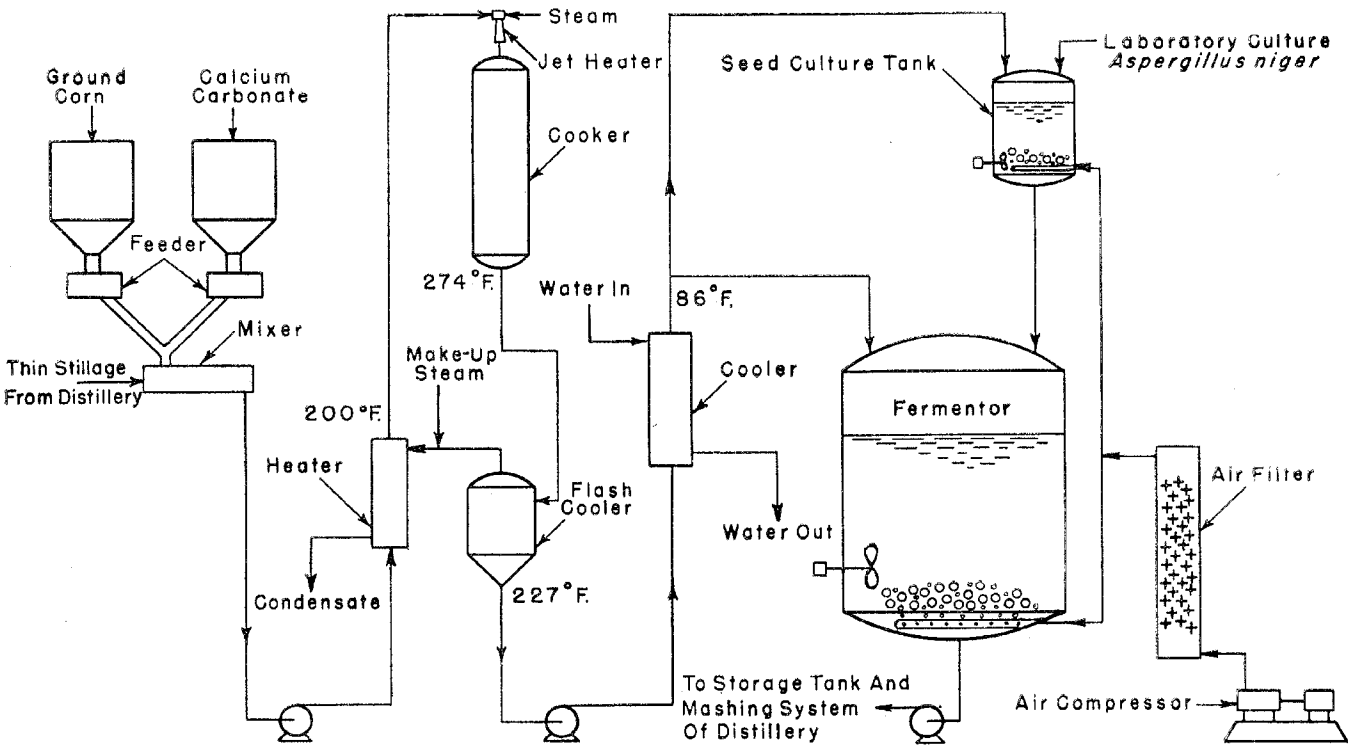


**Figure 1. Flow Sheet for the Production of Mold Amylase**

employed at a 10% level (experiment 10a). In some of the fermentations summarized in Table II (experiments 2, 3a, 7a, 10a), satisfactory yields of alcohol were obtained despite the development of acidity which generally would be deleterious in malt-saccharified mashes. This confirms previous observations (*10*) that the amylolytic systems of certain molds, when compared with malt, are more resistant to inactivation by acid.

In the next series of experiments, the quantity of mold culture liquor was varied while the level of dextrinizing enzyme remained constant. Culture liquors from five different preparations were utilized in each case to supply 0.8 unit of dextrinizing enzyme per ml. in the final mash. Results summarized in Table III show again that with the lowest level of culture liquor (5%) the yield was less than in mashes converted with large volumes of culture liquor and with malt. Since the concentration of dextrinizing enzyme was the same in all liquors, it alone does not determine the suitability of amylolytic preparations.

CONTINUOUS USE OF MOLD STILLAGE. In previous experiments *Aspergillus niger* was cultivated on malt stillage. If submerged mold amylase were to be employed in a distillery, however, it is obvious that the substrate for enzyme production would be mold stillage. To determine whether continuous operation on mold stillage is feasible, a series of fermentations was carried out in which malt stillage was utilized only in the first run.

Prior to the study on continuous operation, it was found that amylolytic preparations of high saccharifying activity were rich in maltase. Consequently, *Aspergillus niger* NRRL 330, which forms less dextrinizing enzyme but appreciably more maltase than *Aspergillus niger* NRRL 337 (*2*), was used in this series. Results shown in Table IV reveal that mold stillage is a satisfactory substrate for enzyme production. With the exception of one fermentation, all the yields were 5.0-proof gallons per bushel or higher. In the one case in which the yield was 4.82-proof gallons per bushel, the mash became contaminated with an acid-forming bacillus. The importance of maintaining aseptic techniques in so far as possible, likewise, is illustrated in this one instance.

### PLANT DESIGN AND COST CALCULATIONS

These pilot plant experiments on the production of an amylolytic liquor by submerged culture methods have demonstrated that the operation of the process on a large scale is feasible and practicable. Logically, the location of such an installation would be in or adjacent to a distillery, since the distillery both produces thin stillage, the major raw material, and uses the finished product. Such a location would obviate the problems and associated expense of concentration or other stabilization of both raw ma-

TABLE III. CONVERSION OF MASHES WITH THE SAME QUANTITY OF DEXTRINIZING ENZYME, *Aspergillus niger* NRRL 337

| | Culture Liquor Used | | Fermentation Data | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | pH | | | | Alcohol Production, % by Volume | | | |
| Expt. No. | Dextrinizing enzyme, units/ml. | Quantity for conversion, % of final mash volume | 0 hr. | 24 hr. | 48 hr. | 72 hr. | 24 hr. | 48 hr. | 72 hr. | Yield of alcohol, proof gal./bu. |
| 10 | 7.9 | 10 | 4.9 | 4.6 | 4.4 | 4.2 | 4.82 | 6.71 | 6.84 | 5.20 |
| 15a | 10.4 | 8 | 5.0 | 4.6 | 4.2 | 4.2 | 2.91 | 6.36 | 7.10 | 5.05 |
| 11 | 11.0 | 7 | 4.8 | 4.0 | 3.9 | 3.9 | 2.21 | 5.68 | 6.47 | 5.00 |
| 12 | 13.9 | 6 | 5.1 | 4.5 | 4.1 | 3.9 | 2.85 | 6.36 | 7.00 | 5.22 |
| 13 | 15.5 | 5 | 5.1 | 4.2 | 4.1 | 4.0 | 3.64 | 6.19 | 6.83 | 4.88 |

TABLE IV. CONTINUOUS USE OF MOLD AMYLASE[a]

| | | Enzyme Activity | | |
|---|---|---|---|---|
| Cycle No. | Age of Mold Culture, Hr. | Dextrinizing enzyme, units/ml. | Maltase activity, % hydrolysis of maltose | Alcohol Yield, Proof Gal./Bu. |
| 1 | 66 | 4.2 | 52.4 | 5.03 |
| 2 | 66 | 3.0 | 36.7 | 5.04 |
| 3 | 66 | 3.5 | 44.4 | 5.20 |
| 4 | 66 | 4.0 | 44.6 | 5.00 |
| 5 | 66 | 3.0 | 38.0 | 4.82 |
| 6 | 66 | 4.4 | 52.9 | 5.19 |

[a] Quantity of mold-culture liquor used for conversion was in all cases 10% of final mash volume.

TABLE V. SUMMARY OF ESTIMATED OPERATING COSTS FOR A PLANT PRODUCING 18,000 GALLONS OF MOLD AMYLASE LIQUOR PER DAY

| Item | Daily Costs, Dollars | Cost per 3.6 Gal. Liquor, Dollars |
|---|---|---|
| Raw materials | 42.00 | 0.0084 |
| Supplies | 15.00 | 0.0030 |
| Utilities | 50.95 | 0.0102 |
| Operating personnel | 90.00 | 0.0180 |
| Maintenance and fixed charges | 105.16 | 0.0210 |
| Totals | 303.11 | 0.0606 |

terial and finished product. Therefore, the investment and operating cost estimates which follow are for a plant for the production of mold amylase liquor in conjunction with a distillery.

Figure 1 is a flowsheet of the process as it might be installed and operated. Estimated costs were based on this flowsheet and on the following considerations:

1. The distillery which is to install the enzyme plant has a mashing capacity of 5000 bushels of grain per day and the concentration of the mash during fermentation is 36 gallons per bushel.
2. The quantity of liquor needed to convert the mash is 10%, based on the total volume of the mash during fermentation. It is recognized that a smaller quantity of liquor might be adequate, but the higher figure was used in making the estimates.
3. In view of considerations 1 and 2, the capacity of the enzyme plant is 18,000 gallons of amylolytic liquor per day.
4. The maximum rate at which air is supplied to the fermentors during production of the amylolytic liquor is 0.25 volume of air per volume of medium per minute. This rate of aeration is probably higher than will be needed in actual practice.
5. The enzyme plant is to prepare medium 8 hours per day; the fermentors in which the liquor is produced are to operate on a cycle based on a 60-hour fermentation period.

PLANT OPERATIONS. Corn meal and calcium carbonate are fed quantitatively by means of feeders to a mixer, along with thin stillage from the feed recovery department of the distillery. Fifteen hundred pounds of corn, 750 pounds of calcium carbonate, and 17,500 gallons of thin stillage are required per day. The fortified medium is pumped through a heater where the temperature of the liquor is raised to 200° F. The hot slurry is sterilized by passage through a steam-jet heater and a holding tank which constitute the cooker. The jet heater instantaneously raises the temperature of the medium to 274° F. (30 pounds per square inch gage) and the liquor is held at this temperature for 7 minutes. The sterile liquor is cooled to a temperature of 227° F. (5 pounds per square inch gage) in the flash cooler, then further cooled to 86° F. by means of a heat exchanger. A portion of the medium is then delivered to a seed-culture tank and the remainder to a fermentor.

Approximately 10 gallons of a culture of *Aspergillus niger* are prepared in the laboratory and used to inoculate 535 gallons of medium in the seed-culture tank. Aseptic conditions are maintained throughout the plant and especially in the preparation of the inoculum. This large quantity of inoculum is grown for 24 hours and then transferred to a fermentor containing approximately 17,500 gallons of medium. After a fermentation period of 60 hours, the contents of the fermentor are pumped to a storage tank from which the distillery draws amylolytic liquor as required.

Sterile air is supplied to both the fermentor and seed-culture tank at the rate of 0.25 volume per volume of medium per minute. The air is compressed and then sterilized by passing it through a column which is packed with activated carbon.

COSTS. The investment cost for this plant is estimated to be $158,000, which includes equipment, installation charges, and a building. No steam plant is included in the estimate, since it was assumed that the amylase would be produced in conjunction with a distillery having a steam plant of sufficient excess capacity to satisfy the requirements of the enzyme plant.

A summary of the estimated production costs for the mold-amylase plant is given in Table V. These costs include all charges except administrative and selling expenses. The third column gives the cost of 3.6 gallons of liquor, the quantity used to saccharify 1 bushel of grain. The cost for enzymes is estimated to be 6.06 cents for each bushel of grain which is converted when mold amylase is employed for saccharification.

The use of mold-amylase liquors in a distillery should be neither difficult nor inconvenient. In regular practice malt is ground and mixed with water to form a slurry which is employed for liquefaction and saccharification of the mash. The fermented material from the enzyme plant is already in the form of a liquor which may be used for the same purposes as is the malt slurry. Conversion time with amylase liquor is the same as with malt, but the temperature during conversion is slightly lower than that employed when malt is the converting agent. Because of the similarity in the use of malt and mold amylase, the utilization of the latter should not involve any additional expense in the operation of an alcohol plant; hence, the two materials may be compared directly in terms of their cost, the quantities required, and the yields of alcohol obtained.

To calculate and compare the costs of barley malt and mold amylase liquor as sources of amylolytic enzymes, a simplifying assumption must be made. The yield of alcohol per bushel of grain is practically the same when malt or mold amylase is used for conversion of a corn mash. For example, 5.6 pounds of malt and 50.4 pounds of corn will yield 5.1- to 5.2-proof gallons of alcohol, and the same yield is obtained from 56 pounds of corn and 3.6 gallons of mold-amylase liquor. The mold-amylase liquor contains practically no fermentable carbohydrate; hence, the cost of the liquor is for the enzymatic activity of the material. For purposes of calculation, it is assumed that malt has a fermentable content equivalent to that of corn. Therefore, for purposes of comparison, the cost of the enzymatic activity in malt may be assumed to be the total cost of the malt less the cost of an equal quantity of corn. When 8% and 10% of malt are employed for saccharification, the amounts of malt required per 56-pound bushel are 4.48 and 5.60 pounds, respectively. At a price of 3.2 cents per pound for corn and 6 cents per pound for malt, the net cost for enzymatic activity may be considered to be 2.8 cents per pound. Therefore, the costs of enzyme to convert 1 bushel of grain is (4.48) × (2.8) or 12.5 cents when 8% malt is used, and (5.60) × (2.8) or 15.7 cents when 10% malt is used. It is possible, therefore, for a distillery producing alcohol to save from 6.4 to 9.6 cents per bushel of grain mashed by utilizing mold amylase liquor instead of malt. This is equivalent to a saving of 2.4 to 3.6 cents per gallon of 190-proof alcohol. By using the mold preparation, a distillery mashing 5000 bushels of grain per day may reduce its cost of operation by as much as $480 per day.

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