Plant Scale Evaluation of a Fungal Amylase Process for Grain Alcohol

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Plant scale evaluation of the submerged fungal amylase process for grain alcohol fermentation shows that it is both practical and economically feasible. The yield of alcohol from mashes converted with fungal amylase was equal to that from mashes converted with malt, although fermentation time was increased. The yield of distiller's solubles was increased slightly by the use of fungal amylase as converting agent. Corn and wheat containing up to 50% damaged kernels could be used efficiently for production of both fungal amylase and alcohol. Detailed operating conditions and procedures are described for the use of fungal amylase in an industrial plant. Cost estimates are given for producing alcohol by the fungal amylase process and by the use of malt.

THE USE OF AMYLOLYTIC MOLDS by Calmette in 1895 was the beginning of occidental man's attempts to replace malt with microorganisms as converting agents in a grain alcohol fermentation. Since that time, three variations of the use of these organisms have been developed: (1) amylo process, (2) mold bran process, and (3) submerged culture or fungal amylase process.

The amylo process has been described by Boulard (*3*), Delemar (*6*), Foth (*9*), Grove (*10*), and Owen (*16*). In a typical operation of this process the mold culture—e.g., *Rhizopus delemar*—is grown with aeration in a mash for 24 hours at 38° C. The mash is then cooled to at least 33° C. and inoculated with a yeast. In a modification, starch hydrolysis and fermentation are carried out simultaneously by adding at the same time *Mucor boulard* No. 5 and a yeast (*19*). A modification was described by Woolner and Lassloffy (*29*) and by Erb and Hildebrandt (*7*), in which the grain mash was inoculated with about 10% by volume of a mold culture after the mash had been prepared as usual but with greatly reduced malt concentrations. This eliminated the necessity of maintaining complete asepsis in the alcohol fermentor and the large volumes of air required in the amylo process.

The mold bran process consists of growing the mold on moistened wheat bran under controlled conditions of aeration and temperature. At the end of the incubation cycle, the moldy bran is dried and used in place of malt in the alcohol fermentation. In general, improved alcohol yields are claimed, but the method of producing the mold bran is considered too cumbersome to be practical for a distiller. The process has been described by Takamine (*23*) and studied extensively by Underkofler and Fulmer and their associates (*11*, *25*). The latter investigators examined the effectiveness of various types of organisms and the conditions required for growth. Use of mold bran on an industrial scale was described by Underkofler, Severson, and Goering (*26*). Hao and Jump (*12*) compared the use of several commercial bacterial and mold amylase preparations with malt and decided all the preparations were effective and the determining factor in their use was cost.

Work on the fungal amylase process was initiated with the idea that a complete malt replacement of microbial origin could be developed, which could be produced conveniently in deep tank fermentations and would use thin stillage as substrate. The process would eliminate the contamination that is introduced by malt conversion, and would not require either the rigorous aseptic conditions in the large volume of mash or the large volumes of air needed with the amylo process. A series of papers (*5*, *13*, *14*, *24*) from the Northern Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture, described the selection of the culture, conditions for production of α-amylase and maltase, and use of the product in alcohol production from grains, both in the laboratory and in the pilot plant. Erb and Hildebrandt (*7*) developed a similar process using *Rhizopus boulard* or *R. delemar*, but it was possible to replace only 80% of the malt. The work of the Peoria group was subsequently confirmed by Adams and others (*1*) and by Erb, Wisthoff, and Jacobs (*8*).

Recently Pool and Underkofler (*18*) compared mold bran and submerged cultures of *Aspergillus niger* NRRL 330, *A. niger* NRRL 337, and *A. oryzae* ISC 38 b for α-amylase, maltase, and limit dextrinase content and the effect on yield of alcohol from corn. Bran and submerged cultures of *A. niger* NRRL 330 were highest in maltase, whereas culture *A. oryzae* ISC 38 b was highest in α-amylase. Cultures of *A. niger* NRRL 337 were highest in limit dextrinase and intermediate in both α-amylase and maltase. Highest alcohol yields were obtained with both types of culture *A. niger* NRRL 330, next with *A. niger* NRRL 337, and lowest with *A. oryzae* ISC 38 b.

In order to obtain comparative cost data with malt conversion on a plant scale, several experiments were run in the equipment of the Grain Processing Corp., Muscatine, Iowa, using *A. niger* NRRL 337 in the Northern Utilization Research Branch (NURB) process. The results of the first series of these experiments have been reported (*27*). Additional experiments and routine use of the process are reported in this paper.

Methods

Analytical Methods Alpha-amylase activity was determined by the Sandstedt, Kneen, and Blish (*21*) procedure. Maltase (*4*) was determined by the method of Tsuchiya, Corman, and Koepsell (*24*). Saccharogenic, or per cent starch conversion, values were determined by the method of Erb, Wisthoff, and Jacobs (*8*), adopted from a combination of two procedures (*2*). Titratable acidity was expressed as the number of milliliters of 0.1*N* sodium hydroxide required to titrate 10 ml. of culture filtrate to a phenolphthalein end point.

Preparation of Mold Inocula The general procedure for increasing the volume of inoculum and the details of the process are as follows:

Parent or stock culture (slant) → slant → flask → carboy (2.0 gallons) → seed tank (300 gallons) → amylase fermentor (16,000 gallons)

Several slight modifications in the media used for development of the inocula have been made; therefore, only the media employed at present are described. To initiate production of the inoculum, a few spores of a refrigerated, sporulated culture of *A. niger* NRRL 337 were transferred from the parent culture to a test tube slant. The culture medium was as follows:

	%
Dried distiller's solubles	5
Soluble starch	2
Agar	2
pH adjusted to 5.5 to 6.0 with NaOH	

After the slant had been incubated for 24 hours at 87° F., a part of the vegetative growth was transferred to a 1-liter flask containing 200 ml. of medium of the following composition:

	%
Corn steep liquor (as received)	1.0
Difco peptone	0.5
Glucose	2.0
pH adjusted to 5.5 with NaOH	

In order to aerate the medium, the flask was shaken in an 85° F. incubator. The speed of the reciprocal shaker was about 80 cycles per minute with a stroke of about 3 inches. The flask culture was incubated with agitation for 24 hours, after which the entire culture was transferred to 2.0 gallons of sterile medium in a 3.5-gallon carboy. The composition of the medium was as follows:

	%
Corn steep liquor (as received)	3.0
White dextrin	1.0
Glucose	1.0
Fleischmann malt extract	0.1
Difco yeast extract	0.1
pH adjusted to 5.5	
About 2 ml. of soybean oil antifoam added	

The inoculated medium was aerated by the passage of air into the medium through a stainless steel tube. The air was sterilized by filtration through a tube packed with cotton sterilized at the same time as the rest of the assembly. In addition to the air inlet, the carboy was equipped with an air vent which also served as the inoculum inlet and a tube through which the contents of the bottle were transferred to the seed tank. After aeration and incubation at 85° F. for 24 hours, the carboy culture was used to inoculate the seed tank in the fungal amylase plant.

Fungal Amylase Plant Operations

As none of the equipment is unusual in the line of fermentation equipment, detailed descriptions are not given.

The seed medium (300 gallons) was composed of 1.2% gum dextrin, 2.4% corn steep liquor, and 0.1% soybean oil. This medium was batch sterilized at 250° to 260° F. for 2 hours, cooled to 88° F., and inoculated with the 2.0-gallon laboratory culture of *A. niger* NRRL 337. The seed tank was aerated for 24 hours by means of a perforated pipe sparger with filter-sterilized air. About 1 volume of air per volume of medium per minute was used.

The fermentation medium (16,000 gallons) was composed of 3.5% distiller's dried solubles (although stillage containing an equivalent amount of solids may be used) and 3.2% ground corn. The pH was adjusted to 5.5 with sodium hydroxide. In the first series (*27*), calcium carbonate was used in the medium employed for propagation of the mold. In subsequent fermentations, calcium carbonate was eliminated because of its deleterious effect on yield of maltase (*24*) and the concentration of corn was raised. Continuous sterilization through cookers 12 or 5 inches in diameter or batch sterilization for 3 hours at 250° F. in the fermentor has been used, the latter being preferred at present. The fermentation was incubated at about 87° F. for about 48 hours with aeration by means of filter-sterilized air at the rate of 0.75 volume of air per volume of medium per minute through a perforated pipe sparger. Although agitation was employed in some of the earlier work it was felt that the agitators were too small to be effective, and were eliminated in the subsequent work. No further treatment was given the fungal amylase culture before it was added to the grain mash for conversion in place of malt.

Table I. Proximate Analyses of Feeds Produced from Fungal Amylase–Converted Grain Alcohol Mashes

	Distiller's Dried Grains				*Distiller's Dried Solubles*			
Run No.	*Moisture,* %	*Crude protein,* %	*Crude fat,* %	*Crude fiber,* %	*Moisture,* %	*Crude protein,* %	*Crude fat,* %	*Crude fiber,* %
9B	7.4	23.1	10.1	9.3	5.6	32.6	13.4	4.2
10B	8.0	24.1	10.2	9.2	4.3	26.7	12.1	3.8
11B	6.8	22.5	9.3	9.0	5.6	34.2	14.3	5.1
12B	9.0	27.1	10.6	8.9	4.8	30.4	14.1	5.0
13B	6.7	24.3	10.0	10.5	5.4	28.4	12.9	4.6
14B	8.2	26.4	9.6	10.3	5.2	29.6	12.6	4.7
15B	7.9	22.4	10.0	9.4	5.1	33.8	12.8	4.5
16B	8.6	24.1	10.2	8.8	4.6	27.6	14.2	4.8
17B	6.7	24.5	10.8	10.3	5.6	33.7	14.6	4.5
21B	9.4	22.8	9.8	9.4	4.3	34.6	14.2	3.9
22B	9.6	24.6	10.1	9.8	5.4	28.6	13.7	3.6
25B	8.7	26.5	10.1	10.2	5.2	30.3	12.4	4.1
26B	9.1	23.4	10.4	9.4	4.9	31.0	14.5	3.8
27B	6.4	25.1	9.6	9.2	5.1	28.7	13.7	4.2
Av.	8.0	24.4	10.1	9.5	5.1	30.7	13.5	4.3
Typical values[a]	9.4	24.9	9.5	9.9	6.0	32.0	12.5	4.5

[a] Typical values for products made with malt as converting agent.

Results

The first results obtained have been reported (*27*). The conclusions were that the operation was practical on a plant scale, provided pure culture conditions were maintained. It was found that malt could be replaced completely if sufficient conversion time was allowed. The practice of continuous mashing of grain in the Grain Processing Corp. plant meant that a conversion tank had to be installed to allow additional time for liquefaction. Alcohol quality was compared organoleptically with that made from malt-converted mashes, but no differences could be detected. Feeds were analyzed for moisture, protein, fat, and fiber with the results shown in Table I. Comparisons with typical values for feeds from malt-converted fermentations show little or no differences. The fungal amylase mashes yielded 10.6 and 5.3 pounds of distiller's solubles and dried grains, respectively, while the yields from malt-converted mashes were 9.0 and 7.0 pounds, respectively, per 56 pounds of grain processed. The lower yield of dried grains from fungal amylase mashes is due to the replacement of malt with corn. On the other hand, the increase in dried solubles is more than would be anticipated from the amount of solubles introduced with the fungal amylase liquors.

Sufficient samples of distiller's dried grains and distiller's dried solubles produced by fungal amylase conversion and by malt conversion were submitted to the Nebraska Agricultural Experiment Station at the University of Nebraska and to the Bureau of Animal Industry, U. S. Department of Agriculture, for animal feeding tests. There was no indication that feeds produced by fungal amylase conversion were inferior to malt-converted feeds (*15*). The use of stillage from fungal amylase-converted mashes for the production of fungal amylase was investigated. This recycling operation was found suitable for both enzyme and alcohol production. The only disadvantage of the process was the fact that fermentations of fungal amylase–converted mashes were slower than those of malt-converted mashes.

Table II. Summary of 14 Plant Scale Fungal Amylase Fermentations Produced with Sound Corn

Run No.	Time of Fermentation, Hours	Saccharogenic Value, Conversion, %	α-Amylase, Units (30° C.)/Ml.	Maltase, Units/Ml.	Acidity	
					Titratable, Ml.[a]	Final pH
1	62	45.9	20.0	8.9	4.3	4.29
2	52	46.6	21.2	6.7	3.4	4.85
3A	38	41.6	19.6	6.1	3.0	4.85
3B	36	39.8	21.8	6.9	3.2	4.95
4A	40	47.8	23.5	6.9	3.8	4.6
4B	40.75	44.9	23.5	7.4	3.4	4.9
5A	39	46.0	23.0	7.3	3.2	5.2
5B	43.5	40.4	21.0	6.9	3.0	4.7
6A	42.5	43.3	21.5	7.6	3.0	5.2
6B	50.5	47.7	23.4	8.3	3.2	4.8
7A	57	33.8	18.6	7.6	2.7	5.1
7B	48	34.3	18.8	6.7	1.1	5.5
8A	42.5	41.0	18.2	7.0	2.6	4.4
8B	58	47.7	19.6	6.0	3.4	5.15

[a] Ml. of 0.1*N* NaOH to titrate 10 ml. of culture filtrate to phenolphthalein end point.

Table III. Results of Use of Fungal Amylase Liquor for Mash Conversion on Production of Alcohol from Corn

Mold Liquor Produced in Run No.[a]	Mold Liquor Used, Bu. Corn as Received/Gal.	Age of Beer When Fermentor Emptied, Hr.	Final Specific Gravity, °Balling	Acidity		Alcohol in Finished Beer, %	Yield of Alcohol, 56 Lb. Dry Grain/Proof Gal.
				Titrable, ml.[b]	Final pH		
1	2.60	72	0.0	4.31	3.9	8.41	6.06
	2.62	72	−0.2	4.6	4.7	8.39	6.01
2	2.54	72	−0.1	4.2	4.22	8.42	6.08
	2.51	72	−0.4	4.2	4.29	8.51	6.27
3A	2.50	84.5	0.3	5.3	4.26	8.91	6.26
3B	2.33	83	0.3	5.1	4.5	8.85	6.31
4A	2.46	76	0.3	4.9	4.4	8.65	6.08
4B	2.62	74	0.4	4.9	4.1	8.72	5.96
5A	2.39	84	0.5	5.3	4.5	8.64	6.04
5B	2.62	92	0.5	4.7	4.49	8.28	5.83
6A	2.33	71	0.3	5.2	4.19	8.06	5.76
6B	2.30	80	0.4	4.4	4.6	8.52	6.17
7A	2.22	72.5	0.1	5.4	4.4	8.27	5.80
7B	2.37	64	0.1	3.6	4.2	8.06	5.85
8A	2.46	74	0.2	5.1	4.29	8.01	6.15
8B	2.62	92	0.0	4.5	4.6	8.78	6.20

[a] Refer to Table II for fungal amylase fermentation number.
[b] Ml. of 0.1*N* NaOH to titrate 10 ml. of filtered beer to phenolphthalein end point.

Table IV. Summary of 12 Plant Scale Fungal Amylase Fermentations Produced with 50% Damaged Grains

Run No.	Grain Used in Fungal Amylase Medium	Length of Fermentation, Hr.	α-Amylase, Units (30° C.)/Ml.	Maltase, Units/Ml.	Saccharogenic Value, Conversion, %	Acidity	
						Titratable, ml.[a]	Final pH
1	Damaged corn	48	29	10.0	46.55	3.8	4.80
2		48	25	8.8	57.80	3.8	4.90
3		57	27	9.0	51.34	3.7	4.82
4		47.5	29	8.2	46.55	3.0	4.90
5		53	28	8.5	53.66	4.0	4.80
6		49	29	9.6	44.90	3.6	4.85
7		48	32	9.4	42.67	3.2	4.61
8		51.5	30	8.8	44.35	3.3	4.67
9	Damaged wheat	47	23	8.4	47.10	2.8	4.45
10		50	28	6.0	40.42	3.4	4.80
11		50	29	6.0	39.38	4.4	4.65
12		48	23	7.7	37.08	2.7	5.10

[a] Ml. of 0.1*N* NaOH required to titrate 10 ml. of culture filtrate to phenolphthalein end point.

Experiments with No Malt for Conversion

As a small amount of malt had been used in nearly all of the fermentations in the first series of experiments (*27*), a second set of experiments was run in which no malt was used in the conversion. This necessitated the installation of a conversion tank in the distillery mashing system in addition to the conversion loop which was sufficient when malt was used for conversion. The 9119-gallon conversion tank provided a holding time of about 20 minutes. No preliquefaction step was employed in any plant operation.

The results of 14 fungal amylase fermentations are shown in Table II. The fermentations in this series were better in general than the previous ones already reported (*27*), as indicated by a 70 to 130% improvement in saccharogenic, α-amylase, and maltase values. In addition, three of the 14 fermentations were free of contamination through the entire fermentation and two were questionable at the end. Furthermore, the majority showed no evidence of contamination through 24 hours and only one was contaminated from the start.

The data on the fermentations in the distillery in which fungal amylase liquor alone was used for conversion of the mash are shown in Table III. The average fermentation time of 77 hours was shorter than that reported in the first series (*27*), and the final specific gravities were decreased.

The average amount of mold liquor here was only about 2.5 gallons per bushel of corn, as received, as compared with 3.5 gallons per bushel in the previous series and 2.7 gallons used by the Northern Utilization Research Branch workers (*5*). Beer gallonage averaged about 36 gallons per bushel (dry basis). The corn used averaged 16.2% moisture. A conversion temperature of 153° to 155° F. was used throughout, except for the last five fermentations, which were converted at 130° to 135° F., in an attempt to decrease the fermentation time. However, there appeared to be no influence on the over-all picture of the fermentation as a result of the lower conversion temperature.

The average alcohol yield of 6.05 proof gallons per dry bushel was somewhat better than the yield from 101 malt-converted fermentations, 5.88 proof gallons per dry bushel. The range of yields for the malt-converted fermentations was 5.47 to 6.287 proof gallons per dry bushel.

Use of Damaged Grains

A third series of tests was run on corn and wheat that were over 50% damaged as determined by federal or state inspectors, to determine the feasibility of the process on damaged and unsound grains.

As shown in Table IV, the use of these grains had no adverse effect on enzyme production. In this series, only runs 1, 2, and 12 were contaminated at their completion and there appeared to be no serious decrease in enzyme production as a result of contamination in these instances.

The data for alcohol production (Table V) are not conclusive with respect to yield, because of the small number of fermentations run. Although the alcohol yields were somewhat lower than

Table V. Effect of Fungal Amylase Conversion of Damaged Grains on Alcohol Production Compared with Malt Conversion

Mold Liquor Produced in Run No.[a]	Converting Agent: Fungal amylase, gal./bu.	Converting Agent: Malt, bu.	Grain in Alcohol Fermentor, Bu.: Corn	Grain in Alcohol Fermentor, Bu.: Wheat	Mash, Gal./Bu. Grain	Age of Fermented, Mash, Hr.	Final Balling, °	Final Acidity: Titratable, ml.[b]	Final Acidity: pH	Alcohol in Mash, %	Yield of Alcohol, Proof Gal./56 Lb. Dry Grain
1	3.4	...	3106	...	38.2	84	0.0	3.9	4.5	7.30	5.65
2	3.5	...	3363	...	35.5	84	0.0	3.5	4.3	8.15	5.74
3	3.3	...	2420	...	37.2	73	−1.5	4.4	4.6	8.08	6.08
4	3.3	...	3460	...	37.6	72	0.01	4.3	4.4	6.91	5.94
										Av.	5.85
	...	271	2828	...	34.9	78	0.0	3.0	4.5	8.05	5.54
	...	300	3128	...	35.2	60	0.0	3.9	4.5	8.18	5.81
										Av.	5.68
5	3.4	...	...	2916	35.9	72	1.4	7.9	4.2	6.72	5.32
6	3.4	...	...	2240	40.1	72	−0.05	6.6	4.7	8.30	6.04
7	3.5	...	...	2427	39.8	77	0.3	10.1	4.1	7.17	5.92
8	3 4	...	...	2435	37.4	82	1.7	9.9	4.2	8.12	6.18
										Av.	5.87
	...	211	...	2204	37.4	76	−1.2	8.1	4.3	7.80	5.96
	...	186	...	2096	39.5	84	1.1	7.6	4.5	8.01	6.32
										Av.	6.14
9	3.2	...	1192	1192	36.3	72	0.7	3.6	4.6	7.89	5.76
10	2.6	...	1224	1244	40.4	84	−0.2	8.4	4.1	7.09	5.66
11	3.1	...	1216	1216	36.4	72	−0.1	5.7	4.3	8.26	5.96
12	2.4	...	1220	1220	38.2	72	0.7	9.9	3.9	7.86	5.93
										Av.	5.83

[a] Refer to Table IV for fungal amylase fermentation number.
[b] Ml. of 0.1*N* NaOH required to titrate 10 ml. of filtered beer to phenolphthalein end point.

had been experienced earlier with fungal amylase, it is felt that this was due to the extent of damage to the grains. In this series, no attempt was made to keep the fermentation time at a minimum. Thus, there is very little difference between the fermentation ages with malt and those with fungal amylase.

Routine Operations with Fungal Amylase

Since July 1953 fungal amylase has been used to replace malt almost exclusively in this plant. Table VI shows that α-amylase production appeared to improve with experience and remained fairly constant after the first 2 months' operation. Difficulty with low stillage solids in January 1954 resulted in a few unproductive fermentations which reduced the average value. As seen in Table VII, operations in the distillery were very much the same with respect to alcohol yield when either converting agent was used. The fungal amylase–converted mashes required 75.5 hours' fermentation time, whereas malt-converted mashes required 58 hours. Thus, a 30% increase in fermentor capacity was required under plant conditions when the mash was converted with fungal amylase to maintain the same rate of alcohol production.

Rate of Production

As the attainment of certain levels of enzymatic activities in the culture liquor determines the fermentation time, a study was made of the rates of production of maltase, α-amylase, and saccharogenic value. The results of typical fungal amylase fermentations are shown in Figure 1. Experience indicates that minimum maltase activities of 5 units per ml. and α-amylase activities of 15 units per ml. are desirable for satisfactory alcohol fermentations under conversion and fermentation conditions employed in this study. These enzyme levels are generally obtained at 40 hours' fermentation. The saccharogenic values exceed 40% conversion at this time.

The data of Erb, Wisthoff, and Jacobs (*8*) and those from the first series of tests (*27*) indicated that 20 and 30% conversion, respectively, were required for satisfactory alcohol production. However, malt was also used in both cases. Moreover, the amounts of fungal amylase used were considerably higher than those used in the present tests, and the alcohol fermentation time of 100 hours required in the first series of tests was longer than the fermentation time of the present tests. The data from the present series of experiments were plotted against alcohol yields to see if a correlation could be established between saccharogenic value and alcohol yield. However, no correlation could be found under the

Table VI. Average Analyses of Routine Plant Scale Fungal Amylase Fermentations Conducted over a 10 Months' Period

Production Period	No. of Fermentations	Acidity, pH	Acid Rise, Ml.[a]	α-Amylase, Units (30°C.)/Ml.	Maltase, Units/Ml.
August 1953	11	4.78	1.0	25.18	8.11 (8)[b]
September	20	4.49	1.0	27.45 (18)[b]	
October	21	4.69	0.9	32.40	
November	20	4.55	1.3	30.67	
December	15	4.57	2.2	36.53	
January 1954	13	4.51	2.6	23.28	
February	10	4.70 (9)[b]	1.6	34.74	
March	22	4.49	2.2	31.05	
April	20	4.68	1.6	30.86	
May	7	4.72	1.1	34.46	9.02 (3)[b]
Av.		4.60	1.5	30.60	8.36

[a] Difference between ml. of 0.1*N* NaOH required to titrate 10 ml. of final and initial samples of culture filtrate.
[b] Values in parenthesis indicate number of values taken for average where it was not the same as the number of fermentations run during that period.

Table VII. Alcohol Production from 30 Malt- and 30 Fungal Amylase–Converted Mashes

Conversion Agent Used	Concentration[a]: Av.	Concentration[a]: Range	Age of Fermentation, Hr.	Alcohol Yield, Proof Gal./56 Lb. Dry Grain: Average	Alcohol Yield, Proof Gal./56 Lb. Dry Grain: Range
Malt	8.7	6.6–9.7	58	6.026	5.527–6.205
Fungal amylase	2.41	2.05–2.93	75.5	6.062	5.645–6.677

[a] Concentration for malt expressed as per cent, for fungal amylase as gallons per bushel of corn, as received.

conversion and fermentation conditions employed.

Discussion

This investigation of the fungal amylase process on a plant scale has demonstrated that the process is feasible and practical. At the same time it is apparent from the improvement in the results that experience in the operation of pure culture fermentations is desirable.

The process presented less difficulty than other aerobic fermentations. Two factors may be responsible for this: the acid reaction of the fermentation medium which discourages some bacterial contaminants, and the relatively short fermentation time.

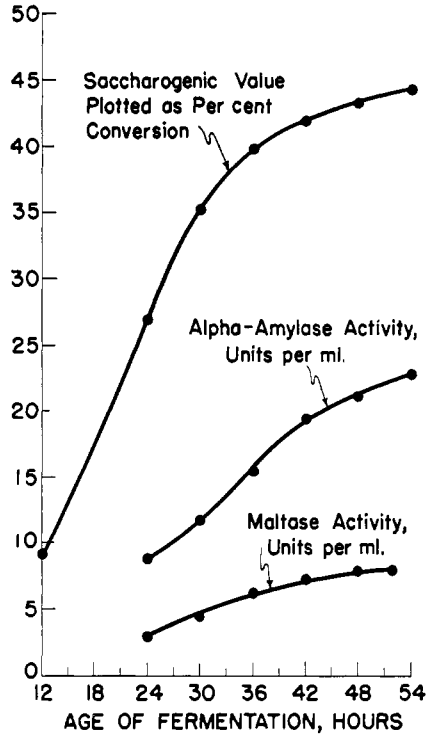

Figure 1. Comparative levels of maltase, α-amylase, and saccharogenic activity produced by *A. niger* NRRL 337 during a typical plant scale fungal amylase fermentation

An average of 2.5 gallons of fungal amylase having a potency of approximately 20 α-amylase units per ml. was used per bushel of grain for conversion. Thus, about 2.8 times as much α-amylase was used per bushel of grain compared to conversion with 9% malt having an activity of 30 α-amylase units per gram. Nevertheless, the fungal amylase-converted mashes did not liquefy as rapidly as did the malt-converted mashes. This may be due to the inherent characterisics of α-amylase from various sources, as observed by Redfern and Landis (*20*). The introduction of a holding tank in the continuous mashing system for liquefaction of the starch satisfactorily solved the difficulty encountered with thick mashes plugging the mash cooler in the initial work. This, of course, would not be a problem in a plant using batch cooking.

As much as 50% damage to the grains studied had no deleterious effect on fungal amylase production or on the alcohol yield obtained from those grains. Thus, in times of a national emergency, when sound grain is needed for food and feed, industrial alcohol could be produced, without malt, on grains that might be unfit even for feed. Furthermore, grains unfit for feed can be converted by this process to valuable, palatable feed supplements.

The increased alcohol fermentation time when fungal amylase was used for conversion represents a disadvantage that cannot be ignored when a plant is operating at maximum capacity. On the other hand, if excess capacity is available, this disadvantage obviously is no problem and the economic advantage gained by use of fungal amylase in place of malt can be fully exploited. The reason for the difference in rate of alcohol fermentation between grain mashes converted with malt and fungal amylase is not known, although the low proteolytic activity of the latter may be a factor. Van Lanen and others (*28*) showed that certain proteolytic enzymes were effective as partial replacements for malt in grain alcohol fermentations and that urea was able to stimulate the rate of alcohol production. Casein hydrolyzate produced a similar effect without the depression of yield found with urea. Ammonia has been recommended by Scott (*22*) as a supplement to a grain alcohol fermentation to increase alcohol yield and quality. Thus, if proteolytic activity is of importance to the rate of alcohol production, it may be possible to compensate for the low level of utilizable nitrogen compounds present in fungal amylase-converted mashes by the addition of simple nitrogen-containing salts. Pan, Andreasen, and Kolachov (*17*) found that the fermentation time could be reduced to 42 hours by modifying the fungal amylase medium, the fungal amylase fermentation temperature, and the temperature of alcohol secondary fermentation. Fermentation times for malt and control fungal amylase-converted fermentations were 52 and 60 hours, respectively. In their opinion, the limit dextrins formed during saccharification are not so easily converted by fungal amylase as by malt, thereby causing an increased secondary fermentation time.

It is felt that the correlation between saccharogenic value and alcohol yield

Table VIII. Estimated Cost of Fungal Amylase Plant, Equipment, and Installation for Operation in Conjunction with Alcohol Plant of Grain Processing Corp., 1954

(Capacity of plant, 32,000 gallons of fungal amylase liquor per day, operating as registered distillery, 20 days per month)

	Estimated Cost
Buildings and improvements	
One building, tile 80 × 90 × 30 with lab, office, incubator, milling, and compressor rooms	$100,000
One well, drilling, pump, motor, pumphouse, etc., 1000 gal./min.	15,000
Three transformers, 500 kva., 13,200—440 volts	9,000
Total	124,000
Equipment	
Six tanks, fermentors, working capacity 16,000 gal., with agitator, drive, and motors at $15,000	90,000
Six air compressors, 1000 cu. ft./min. at 20 lb./sq. inch gage at $9500	57,000
Four tanks, seed fermentor, working capacity 750 gal., with sparger at $1500	6,000
One bin, corn storage, with feeder	1,000
One hammer mill, with motor, complete	3,750
One tank, mixing, 2000 gal.	500
Ten air filters (1 each fermentor, 2 each pair seed tanks) at $500	5,000
Instruments and controls	15,000
Laboratory equipment	10,000
Total	188,250
Alterations in distillery	
Stillage line from dryer house	500
Conversion tank	1,000
FA liquor flow controller	1,500
Total	3,000
Installation costs	
Electric wiring and supplies	5,000
Pipe and pumps	14,500
Construction, labor exclusive of building	60,000
Total	79,500
Total	394,750
Total cost of plant ready for operation	$395,000

found in the first series of tests is not applicable to tests reported here, because small quantities of malt were used in the previous tests whereas no malt was used in the present series. With the conditions used in these tests, it appears that maltase activity or some other factor determines the length of the alcohol fermentation because the use of products listed in Tables II and IV with saccharogenic values below 40% conversion still resulted in completed alcohol fermentations in 72 hours or less, whereas about 100 hours were required in the first tests in spite of use of the small amount of malt in these tests. The greatest apparent difference between the test runs is the level of maltase activity which averaged less than 3.3 units per ml. in the first tests and exceeded 6.0 units per ml. in the tests reported here. Of course, maltase activity may merely correspond with the level of production of another factor(s) that is of greater importance to the rate of alcohol production than is maltase activity. However, of the enzymes studied, the best correlation exists between maltase concentration and secondary fermentation rates, providing adequate amounts of α-amylase are present.

Cost Estimates

In order to compare the cost of producing alcohol using fungal amylase for conversion with that using malt conversion, an estimate has been made of investment and production costs for a fungal amylase plant to operate in conjunction with the alcohol plant of the Grain Processing Corp. Because certain items of expense may be unique to this location, the estimates should be recalculated for application to any other specific area.

The estimates were based on the following assumptions.

1. The distillery is to mash 12,800 bushels (as received) of No. 2 corn per day for 20 days per month to produce 673,280 wine gallons of 190-proof or 1,280,000 proof gallons per month. The yield of alcohol is assumed to be 2.63 wine gallons of 190-proof or 5.00 proof gallons of alcohol per bushel of grain (as received).
2. Fungal amylase is used at the rate of 2.5 gallons per bushel of corn.
3. The required capacity of the fungal amylase plant to satisfy considerations 1 and 2 is 32,000 gallons per day.
4. Air is supplied at a rate of about 0.75 volume of air per volume of medium per minute.
5. The fermentation is operated on a 72-hour cycle: 48-hour fermentation period and 24-hour cleanup and sterilization. This is longer than necessary but provides time for maintenance, mishaps, and increased capacity. Furthermore, adequate cleanup and sterilization time improves the probability of sterility in the fermentation.

Table IX. Estimated Monthly Operating Costs for Production of Fungal Amylase Liquor in Full Scale Plant, 1954

(Capacity of plant, 32,000 gallons of fungal amylase liquor per day, operating as registered distillery, 20 days per month)

Fungal Amylase Liquor Production Cost	Estimated Monthly Cost	Cost, Cents/2.5 Gal. Liquor[a]
Raw materials		
Corn 170,000 lb. at $1.50 per bu.	$ 4,554	1.779
Solubles 186,000 lb. at $70 per ton	6,510	2.543
Total	11,064	4.322
Supplies	500	0.195
Utilities		
Electricity 500,000 kw.-hr. at 1 cent	5,000	1.953
Steam 6,000,000 lb., coal cost only	3,000	1.172
Total	8,000	3.125
Labor and supervision		
Two operators per shift, 1440 hr. at 1.61	2,318	0.905
One asst. operator per shift, 720 hr. at 1.51	1,087	0.425
One chemist-bacteriologist	450	0.176
One supervisor	500	0.195
	4,355	1.701
10% overhead	436	0.170
Total	4,791	1.871
Maintenance	1,500	0.586
Fixed charges		
Depreciation		
Equipment, 10% per year on $295,000	2,458	0.960
Building, 5% per year on $100,000	417	0.163
Taxes and insurance, 4% per year on $395,000	1,317	0.514
Total	4,192	1.637
Summary		
Raw materials	11,064	4.322
Supplies	500	0.195
Utilities	8,000	3.125
Labor and supervision	4,791	1.871
Maintenance	1,500	0.586
Fixed charges	4,192	1.637
Total	$ 30,047	11.736

[a] 2.5 gallons is quantity of liquor to be used for conversion of 1 bushel of grain.
[b] As yield of alcohol per bushel is assumed to be 2.63 wine gallons of 190-proof or 5.00 proof gallons of alcohol, cost for fungal converting agent is 4.46 cents per wine gallon of 190-proof or 2.35 cents per proof gallon of alcohol.

The plant operations otherwise are the same as have been discussed above.

Investment and Operating Expenses An itemized account of the investment cost for the fungal amylase plant and for the necessary distillery modifications is given in Table VIII. As the steam plant of the alcohol plant at the Grain Processing Corp. is adequate to handle the requirements of the fungal amylase plant, no charges for construction of a steam plant have been included.

The operating expenses to produce fungal amylase are shown in Table IX. The cost to produce enough fungal amylase to convert 1 bushel of grain in the distillery is estimated to be 11.74 cents, which is equivalent to 4.46 cents per wine gallon of 190-proof or 2.35 cents per proof gallon.

Comparative Costs of Producing Alcohol

The raw material cost and by-product credit for alcohol produced by malt conversion and by fungal amylase conversion are shown in Table X. As the operating costs of the alcohol plant are the same for both malt and fungal amylase operation, they are not shown. This is possible because alcohol yield is the same for both converting agents and the modifications in the distillery for the use of fungal amylase do not increase the cost of operation.

The savings from the use of fungal amylase compared with malt were 1.14 cents per wine gallon of 190-proof alcohol. On the basis of 12,800 bushels mashed per day, this amounts to a saving of about $384 per day.

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Table X. Comparative Costs of Alcohol Produced with Fungal Amylase and with Malt[a]

Item	Distillery Operation	
	Malt	FA
Bases		
Alcohol yield per bu. No. 2 corn as received, wine gal.	2.63	2.63
Distiller's dried grains recovered per bu. No. 2 corn, as received, lb.	7	5.3
Distiller's dried solubles recovered per bu. No. 2 corn, as received, lb.	9	10.6
Quantity of conversion agent used	9%	2.5 gal./bu.
Cost of corn, No. 2, per 56-lb. bu., as received	$1.50	$1.50
Cost of malt, per 34-lb. bu., as received	$1.81	
Cost of FA liquor per 2.5 gal.		11.73 cents
Value of distiller's dried grains, per ton	$50.00	$50.00
Value of distiller's dried solubles, per ton	$70.00	$70.00
Raw material cost per wine gallon 190-proof alcohol		
Corn, 19.377 and 21.293 lb.	$ 0.5190	$ 0.5703
Malt, 1.916 lb.	0.1020	
FA, 0.951 gal.		0.0447
	$ 0.6210	$ 0.6150
By-product credit per wine gallon 190-proof alcohol		
Distiller's dried grains, 2.66 and 2.02 lb.	$ 0.0665	$ 0.0505
Distiller's dried solubles, 3.42 and 4.03 lb.	0.1197	0.1411
	$ 0.1862	$ 0.1916
Savings by use of FA per wine gallon of 190-proof alcohol		
From raw materials	...	$ 0.0060
From by-products	...	0.0054
		$ 0.0114

[a] Figures based on 1954 costs and on information obtained from operation of distillery of Grain Processing Corp., Muscatine, Iowa.

Operating costs of alcohol plant would be the same per wine gallon for distillery operation with either malt or fungal amylase, so they have not been considered in comparative cost calculations.

Conclusions

The fungal amylase process using *A. niger* NRRL 337 for the complete replacement of malt is practical and economically feasible in plant scale operations.

Neutral spirits produced with this process are comparable in every respect to those produced with malt.

Distiller's dried grains and solubles produced with this process are equivalent in proximate analysis and animal feeding tests to those produced with malt, while the quantity of distiller's solubles produced is slightly increased by fungal amylase conversion.

Additional holding time is required for conversion when fungal amylase is used in a continuous mash cooking system compared with that required when malt is used.

Satisfactory conversion has been obtained with as little as 2.5 gallons of fungal amylase liquor per bushel of corn as received.

Based on recent plant operations, about 30% additional alcohol fermentor capacity was required under the plant conditions for the same production when fungal amylase was used, compared with the capacity required when malt was used as a result of an increased fermentation time in fungal amylase–converted mashes.

Wheat and corn certified as being up to 50% total damaged kernels can be used in the production of fungal amylase. When the damaged grain is used for alcohol production, this fungal amylase saccharifies the starch as efficiently as malt.

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