PRODUCTION OF MOLD AMYLASES IN SUBMERGED CULTURE

E. H. LE MENSE,[1] JULIAN CORMAN, J. M. VAN LANEN[2], AND A. F. LANGLYKKE

Fermentation Division, Northern Regional Research Laboratory[3] Peoria, Illinois

Received for publication April 9, 1947

During recent years many new and extended uses have been developed for fungal enzymes. Prominent among these are applications in the fields of food manufacturing, textile processing, and in the manufacture of malt beverages and industrial alcohol.

Although certain microorganisms are capable of elaborating amylases when grown under submerged conditions either aerobically (Waldmann, 1942) or anaerobically (Hockenhull and Herbert, 1945), industrial production methods generally involve cultivation on the surface of unagitated liquid or semisolid substrates. Exceptions are the "amylo" process (Owen, 1933) and a modified amylo process (Erb and Hildebrandt, 1946), in which selected strains of *Rhizopus* or *Mucor* are grown under submerged, aerobic conditions to saccharify grain mashes prior to alcoholic fermentation. More commonly, as in the production of mold bran (Underkofler, *et al.*, 1939; Boyer and Underkofler, 1945) and bacterial amylases (Beckord *et al.*, 1945, 1946), media are incubated in shallow layers in closed vessels or in open trays. Attempts to adapt these microorganisms to deep tank conditions to produce comparable yields of amylase have been unsuccessful.

The submerged culture method of producing amylases would have definite advantages when the product could be employed directly without concentration or purification as, for example, in the alcoholic fermentation of grain and in the manufacture of sugars and dextrins from starch. With these applications in mind a survey was made of a large number of molds to determine their ability to synthesize starch-hydrolyzing enzymes when cultured under submerged conditions. The present report deals with (1) the results of this survey of fungi, (2) the factors affecting the elaboration of amylases by promising strains, and (3) the substitution of mold amylase thus produced for distillers' malt. Pilot plant studies have been conducted with some of the promising strains disclosed herein, and the results of these experiments will be reported at a later date.

METHODS

Culture survey. The cultures investigated were selected from the culture collection of the Northern Regional Research Laboratory. The basal medium for the survey of cultures was thin stillage obtained from the alcoholic fermentation

[1] Present address: Mold Bran Company, Eagle Grove, Iowa.

[2] Present address: Hiram Walker and Sons, Inc., Peoria, Illinois

[3] One of the four regional laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

of corn and sorghums. It contained 4 to 5 per cent of dry substance, approximately one-third of which was protein (N × 6.25). To favor the growth of all the organisms studied, 2 per cent of glucose and 0.5 per cent of calcium carbonate were added to the stillage. This medium was sterilized with steam at 20 p.s.i. gauge for 30 minutes.

For evaluation of the selected organisms for amylase production, cultures were grown first in 50 ml of thin stillage medium contained in 200-ml flasks. After 24 hours' incubation, 10 ml of culture were transferred to 200 ml of the supplemented stillage medium contained in 1-liter flasks. All cultures were incubated at 30 C and were shaken continuously at 90 three-inch strokes per minute in a Kahn type shaker. Samples were removed periodically for the determination of amylase activity.

Culture liquors were analyzed for the presence of dextrinizing enzyme by the method of Sandstedt *et al.* (1939) as modified by Olson, Evans, and Dickson (1947). Units of dextrinizing enzyme reported herein are the grams of soluble starch (Merck, Lintner) which, in the presence of excess *beta*-amylase, are dextrinized in 1 hour at 20 C.[4]

Variations of cultural conditions. To determine the influence of different carbohydrate and protein sources on amylase production, a fungal strain which was found to possess exceptional amylolytic activity was grown under conditions identical to those obtaining in the survey except that various protein and carbohydrate materials were substituted for thin stillage and glucose.

A study of the effects of different concentrations of calcium carbonate and calcium chloride was made under the same cultural conditions used in the survey except that 2 per cent of ground corn was substituted for 2 per cent of glucose.

Aeration rate studies were conducted on a somewhat larger scale than were the aforementioned experiments; that is, 4-liter quantities of stillage medium supplemented with 2 per cent of corn meal and 0.5 per cent of calcium carbonate were dispensed in 8-liter pyrex cylinders equipped with lids of aluminum plate and with air spargers of perforated aluminum tubing. These were sterilized with steam at a pressure of 25 p.s.i. gauge for 1 hour, cooled, and inoculated with 5 per cent by volume of a 24-hour culture. Air for use in the experiments was filtered through sterile cotton before introduction into the medium.

It was observed in the course of these experiments that strains which saccharified starch rapidly, and consequently were most suitable as replacements for barley malt, formed appreciable amounts of maltase. Following this observation, both dextrinizing and maltase activities of culture liquors were determined. Maltase activity was measured by determining the increase in reducing power by the method of Somogyi (1945) after incubating 10 ml of culture filtrate with 20 ml of a 1.05 per cent solution of maltose for 2 hours at 30 C. The enzyme-substrate mixture was maintained at a pH of 4.6 by the addition of acetate buffer to the maltose solution.

[4] A recent collaborative study of the *alpha*-amylase values of experimentally produced barley malts in which this method was used showed a range of activity from 9.8 to 30.5 units per gram. Commercial distillers' malt contains in the neighborhood of 25 units per gram.

Conversion and fermentation of grain mashes. Cultures producing appreciable quantities of amylase were further evaluated by determining their ability to replace barley malt in the alcoholic fermentation of corn. Forty-nine and one-half g of ground corn and 0.5 g of ground barley malt were placed in 500-ml Erlenmeyer flasks, and 170 ml of tap water heated to 70 C were added. The flasks were placed in a 70 C water bath and the grain slurries were stirred intermittently for 30 minutes. The mashes thus premalted were then cooked in the autoclave at a steam pressure of 25 pounds for 30 minutes and cooled to 75 C; mold culture liquor was added, together with sufficient water to lower the temperature to 55 to 56 C. Conversion was continued at 55 to 56 C for 30 minutes, during which period the mashes were agitated frequently. The same procedure was followed with the control mashes saccharified with malt except that 45 g of corn and 5 g of barley malt were used, 0.5 g of malt again being used for premalting and 4.5 g for conversion. Converted mashes were cooled to 30 C and inoculated with 2 per cent by volume of a 24-hour culture of distillers' yeast, strain NRRL Y567. The final volume in each flask was approximately 250 ml. Fermentation was conducted at 30 C for 72 hours, during which time the flasks were weighed periodically. The beers were then brought to a volume of 300 ml and aliquots of 200 ml taken for the determination of alcohol. One hundred ml of distillate were collected from each aliquot, and the concentration of alcohol in the distillate was determined by measuring its refractive index.

EXPERIMENTAL RESULTS

The results of the survey of fungi for ability to produce amylase in supplemented thin stillage medium are presented in table 1. Of 80 cultures of *Penicillium*, representing 18 species, only 8 formed detectable quantities of dextrinizing enzyme. All of these were of relatively low activity, the best being a strain of *P. purpurogenum* which gave 0.6 units per ml.

Two hundred seventy-eight of the cultures that were studied belonged to the genus *Aspergillus* and represented 41 different species. Only 34 members of this group elaborated dextrinizing enzyme. The culture liquors from active organisms varied from 0.1 to 15.3 units per ml. Although there was considerable variation between strains within a species, a high percentage of strains of *A. oryzae*, *A. wentii*, and *A. niger* was active. *Aspergillus niger* NRRL 337 gave the highest potencies of any organisms tested in the survey. Under the most favorable conditions, potencies up to 22.5 units per ml were obtained with it. On a dry basis (culture liquors contained about 2 per cent of solids) such preparations would have a potency of 1,125 units per gram.

Subsequently, *Aspergillus niger* strains NRRL 326, 330, and 679 were found to elaborate an enzyme complex which rapidly saccharified starch, although the formation of dextrinizing enzyme was not so marked as with *A. niger* NRRL 337.

When strains of *Rhizopus*, *Mucor*, and *Monilia* were grown under the same conditions, little or no dextrinizing enzyme was produced, although excellent growth was obtained. Despite their apparent lack of dextrinizing enzyme, culture liquors from a strain of *Rhizopus* NRRL 1891 received under the label "Rhizopus 'Boulard,'" were capable of considerable saccharification of grain

TABLE 1

The production of amylase by various fungi grown submerged in thin stillage medium

GENUS	CULTURES TESTED	NUMBER ACTIVE*	ACTIVE CULTURES		CONCENTRATION OF DEXTRINIZING ENZYME PRODUCED
			species	*NRRL no.*	*units/ml*
Penicillium	80	8	*P. urticae*	991	0.1
			P. roseo-citreum	889	0.1
			P. spiculosporum	1027	0.2
			P. chlorophaeum	816	0.2
			P. citreo-roseum	835	0.2
			P. aurantio-griseum	972	0.2
			P. brunneo-rubrum	842	0.3
			P. purpurogenum	1064	0.6
Aspergillus	278	34	*A. versicolor*	231	0.1
			A. candidus	305	0.6
			A. alliaceus	315	1.7
			A. foetidus	341	1.3
			A. niger	622	0.1
			A. niger	624	0.1
			A. niger	606	0.1
			A. niger	617	0.1
			A. niger	607	0.1
			A. niger	605	0.1
			A. niger	614	0.1
			A. niger	354	0.4
			A. niger	679	1.1
			A. niger	326	2.2
			A. niger	330	6.0
			A. niger	337	15.3
			A. niger	363	1.6
			A. wentii	382	0.1
			A. wentii	378	0.1
			A. wentii	1207	0.2
			A. wentii	381	0.3
			A. wentii	1778	0.4
			A. wentii	377	0.4
			A. wentii	1269	0.6
			A. oryzae	480	0.1
			A. oryzae	474	0.1
			A. oryzae	464	1.2
			A. oryzae	449	2.9
			A. oryzae	694	3.1
			A. oryzae	698	3.3
			A. oryzae	454	3.0
			A. flavus	488	0.1
			A. flavus	491	0.2
			A. gymnosardae	505	0.2
Rhizopus	5	none			
Mucor	3	none			
Monilia	1	none			

* Cultures were termed active if the α-amylase activity obtained in their filtrates equaled or exceeded 0.1 unit per ml.

mashes (table 6). Other species of *Rhizopus* and *Mucor* were similar in behavior, suggesting that these organisms have amylolytic enzyme systems different from barley malt and the other molds examined in this study. In contrast to this observation, Leopold and Starbanow (1943) have reported the production of both α- and β-type amylolytic enzymes by *R. japonicus*.

TABLE 2

The production of dextrinizing enzyme by Aspergillus niger NRRL 337 cultivated in various media

PROTEIN SOURCE	CARBOHYDRATE SOURCE	CONCENTRATION OF DEXTRINIZING ENZYME
		units/ml
Corn steep liquor, 3%	None	2.2
Corn steep liquor, 3%	Glucose, 2%	8.2
Corn steep liquor, 3%	Molasses, 2%	4.6
Corn steep liquor, 3%	Corn meal, 2%	10.2
Dried tankage, 2%	None	2.1
Dried tankage, 2%	Glucose, 2%	9.3
Dried tankage, 2%	Molasses, 2%	11.5
Dried tankage, 2%	Corn meal, 2%	8.7
Soybean meal, 2%	None	7.9
Soybean meal, 2%	Glucose, 2%	7.4
Soybean meal, 2%	Molasses, 2%	8.5
Soybean meal, 2%	Corn meal, 2%	11.2
Thin stillage	None	1.7
Thin stillage	Glucose, 2%	11.5
Thin stillage	Molasses, 2%	7.9
Thin stillage	Corn meal, 2%	16.5
Thin stillage	Xylose, 2%	5.3
Thin stillage	Lactose, 2%	6.7
Thin stillage	Sucrose, 2%	11.0
Thin stillage	Maltose, 2%	14.5

Enzyme determinations were made after cultures were shaken for 5 days.

Composition of medium: Protein and carbohydrate as shown plus 0.5 per cent calcium carbonate.

Factors affecting enzyme production by Aspergillus niger NRRL 337. To determine whether nutrients other than those present in thin stillage were satisfactory for amylase production, media containing protein from several other sources were supplemented with various carbohydrates. Calcium carbonate was added to give a concentration of 0.5 per cent. After sterilization, the media were inoculated with 2 per cent by volume of a submerged culture of *Aspergillus niger* NRRL 337 and incubated, with continuous shaking, for 5 days. The results are shown in table 2.

Thin stillage, corn steep liquor, and animal tankage when not supplemented with carbohydrate gave low yields of amylase, but soybean meal appeared to be

satisfactory without added carbohydrate. When commercial glucose, molasses, or corn meal was added to the protein basal media, good amylase formation resulted except when corn steep liquor was supplemented with molasses. In this series of experiments the highest enzyme concentration (16.5 units per ml) was obtained with thin stillage to which corn meal was added. Sucrose and maltose gave good enzyme formation, whereas xylose and lactose were less effective when added to thin stillage. These results indicate that a wide variety of carbohydrates in conjunction with proteinaceous substances of animal and plant orgin can be employed for the production of amylase by this organism.

The influence of calcium carbonate on amylase production is demonstrated in the following experiment, the results of which are shown in table 3. Calcium carbonate, in varying amounts to give concentrations ranging from 0 to 1.0 per

TABLE 3

The effect of calcium carbonate and calcium chloride on the production of dextrinizing enzyme by Aspergillus niger NRRL 337

SOURCE OF CALCIUM		FINAL pH	α-AMYLASE
Salt added	Concentration		
	per cent		*units/ml*
None		4.0	1.5
$CaCO_3$	0.10	4.3	7.9
$CaCO_3$	0.25	4.9	8.9
$CaCO_3$	0.50	5.3	9.2
$CaCO_3$	1.00	5.4	8.5
$CaCl_2$	1.00	3.7	1.2

Cultures were analyzed after an incubation period of 3 days at 30 C.
Composition of base medium: Distillers' thin stillage plus 2 per cent corn.

cent, was added to thin stillage containing 2 per cent ground corn. *Aspergillus niger* NRRL 337 was cultured in these media for 3 days, after which the culture liquors were analyzed for dextrinizing potency. Whereas the enzyme activity was low in the absence of calcium carbonate, the addition of 0.1 per cent calcium carbonate gave more than a 5-fold increase in dextrinizing power, that is, from 1.5 to 7.9 units per ml. The optimum concentration of calcium carbonate appeared to be in the neighborhood of 0.25 to 0.5 per cent, resulting in potencies of 8.9 and 9.2 units per ml, respectively. The pH of the fermented liquors ranged from 4.0 in media without calcium carbonate to 4.3 to 5.4 in those in which it was used. When calcium chloride at a concentration of 1 per cent was substituted for calcium carbonate, the final pH was 3.7, and the enzyme production was lower than that in the control without added calcium salt. Since it is well known that α-amylase is readily inactivated at a pH of 4.2 or lower, it appears that the principal action of the calcium carbonate in stillage medium is to maintain the pH above this point during the fermentation. However, a specific stabilizing effect of the calcium ion upon mold dextrinizing amylase has been demonstrated (Nakamura, 1931), and this may have been a contributing factor in those instances in which the reaction was favorable to amylase stability.

The influence of aeration upon amylase production is shown in table 4. *Aspergillus niger* NRRL 337 was grown in supplemented thin stillage medium in glass cylinders, as previously described. The aeration rate was varied from 0.25 volumes to 1.0 volume of air per volume of medium per minute. Dextrinizing enzyme and pH were determined daily from the second through the seventh day. It was found that enzyme synthesis increased progressively with increased rates of aeration. With 0.25 volume of air the final potency of the liquor was 2.4 units per ml; with 0.5 volume of air, 9.0 units per ml; and with 1.0 volume of air, 22.5 units per ml. In larger fermentations in which media were both aerated and agitated, a lower rate of aeration was found to be adequate for maximum enzyme production (Le Mense *et al.*, 1947).

Substitution of mold culture liquors for malt in alcoholic fermentations. Culture liquors from the preceding experiment were investigated for their ability to replace barley malt in the saccharification of grain mashes for alcoholic fermentations. The liquors were used at levels of 8, 13, and 20 per cent of the final mash

TABLE 4

The influence of the rate of aeration on the production of dextrinizing enzyme by Aspergillus niger NRRL 337

AERATION RATE	DEXTRINIZING ENZYME AFTER					
	2 days	3 days	4 days	5 days	6 days	7 days
L air/L medium/minute	*units/ml*					
0.25	0.6	1.4	1.8	2.7	2.2	2.4
0.5	4.8	6.9	7.9	8.2	9.0	9.0
1.0	6.2	8.9	12.9	15.8	22.0	22.5

volume. One-tenth of this amount in each instance was added as premalt. Corn was the only grain used in the mashes saccharified with mold amylase, whereas control mashes contained 90 per cent corn and 10 per cent barley malt, one-tenth of the malt also being employed as premalt. The results of these experiments are shown in table 5.

Malt-converted control mashes gave an average yield of 5.15 proof gallons of alcohol per bushel of grain. When used at a level of 13 per cent of the mash volume, culture liquors produced by aerating at 0.25 volume of air per volume of medium per minute for a 7-day culture period gave only 4.24 proof gallons of alcohol per bushel of grain. Culture preparations aerated at 0.5 volume per volume of medium per minute gave yields equivalent to or better than malt when used at 13 and 20 per cent levels after 4 days of incubation and when used at 8, 13, and 20 per cent levels after 7 days of incubation. Cultures aerated at 1 volume of air per volume of medium per minute were satisfactory in all cases except those to which a liquor cultured for 2 days was added at an 8 per cent level. The highest alcohol yields, amounting to 5.50 and 5.40 proof gallons per bushel, were obtained with 20 per cent levels of culture liquor aerated at 0.5 volume of air per volume of medium per minute. When the greater quantity of

carbohydrate in the all-corn mash is taken into account and the fermentation efficiency is calculated (actual alcohol yield/theoretical alcohol yield), it is found that the best mold preparations gave more complete saccharification than did

TABLE 5

Use of culture liquors from Aspergillus niger NRRL 337 as a saccharifying agent in the alcoholic fermentation of corn

AERATION RATE	AGE OF CULTURE	AMYLASE POTENCY	ALCOHOL YIELDS WITH VARIOUS LEVELS OF MOLD CULTURE LIQUOR (PER CENT OF MASH)*		
			Proof gallons/bushel		
L air/L medium/minute	*days*	*units/ml*	*8 per cent level*	*13 per cent level*	*20 per cent level*
0.25	3	1.4		3.99	
0.25	4	1.8		4.02	
0.25	7	2.4		4.24	
0.5	2	4.8	4.06	4.52	4.86
0.5	4	7.9	4.62	5.26	5.50
0.5	7	9.0	5.00	5.37	5.40
1.0	2	6.2	4.33	5.02	5.21
1.0	4	12.9	4.97	5.10	5.24
1.0	7	22.5	5.10	5.24	5.25

*Malt control (10 per cent of mash bill) gave 5.15 proof gallons per bushel.

TABLE 6

*Dextrinizing amylase, maltase, and total saccharifying power of various mold culture liquors**

CULTURE	NRRL NO.	DEXTRINIZING ACTIVITY	MALTASE ACTIVITY	ALCOHOL YIELD
		units/ml	*per cent hydrolysis of maltose*	*proof gallons/bushel*
Aspergillus niger	330	3.9	64.5	5.11
Aspergillus niger	679	2.7	57.4	5.25
Aspergillus niger	326	5.6	57.3	5.17
Aspergillus niger	337	11.2	39.5	5.12
Aspergillus niger	363	1.8	18.9	4.70
Aspergillus oryzae	694	13.9	17.6	4.96
Rhizopus boulard	1891	0.1	17.4	4.45
Aspergillus wentii	377	0.9	8.3	3.58
Aspergillus oryzae	449	7.7	6.2	4.37
Aspergillus foetidus	341	3.2	4.7	4.45

* Mold culture liquors were employed in all cases at a 10 per cent level for the saccharification of grain mashes.

the barley malt. Mold preparations yielding 5.4 proof gallons per bushel of grain gave a fermentation efficiency of 86 per cent in contrast to 84 per cent for malt.

Maltase production and its effect upon saccharification. It may be noted from the preceding experiment that the highest yields of alcohol were obtained not

with liquors most potent in dextrinizing enzyme but rather with those of intermediate dextrinizing activity. This indicated that other enzymes capable of functioning in the hydrolysis of starch were being produced. Confirmation of this view was established by evaluating a large number of culture liquors of known dextrinizing enzyme potency for their saccharifying ability as determined by fermentation tests. The results clearly showed that saccharification of grain mashes was not well correlated with dextrinizing potency of the enzyme preparations. In view of the important role of maltase in the hydrolysis of starch (Schwimmer, 1945) the various preparations were then examined for their activity in respect to this enzyme. A summary of data on the dextrinizing potency, maltase activity, and total saccharifying power (fermentable sugar) produced by some of the cultures investigated is shown in table 6.

It is apparent that whereas dextrinizing amylase is necessary, good saccharification results with liquors relatively low in dextrinizing power if their maltase activity is high. For example, the strains *Aspergillus niger* NRRL 330, 679, and 326 gave more fermentable sugar than did *A. oryzae* NRRL 694 and *A. oryzae* NRRL 449, whereas the liquors of the last two were characterized by a much higher ratio of dextrinizing amylase to maltase. These experiments indicate, also, that the aspergilli can be evaluated as substitutes for malt by the determination of their ability to elaborate maltase and dextrinizing enzymes. However, *Rhizopus* culture liquors from NRRL 1891 contain an effective amylase complex, despite the lack of α-amylase such as is found in malt and the aspergilli, and thus may contain another type of enzyme to complement the maltase present. None of the molds studied synthesized an amylase with properties comparable to the β-amylase of cereals.

DISCUSSION

That molds when cultured under submerged, aerobic conditions vary considerably in ability to produce starch-hydrolyzing enzymes has been shown in the present study. Of more than 350 cultures examined, only a limited number were capable of elaborating a dextrinizing or α-amylase type enzyme, whereas a few of these produced an enzyme complex which both dextrinized and saccharified starch at a rapid rate. This occurrence among the molds of both dextrinizing and saccharifying enzymes has been reported similarly for certain amylolytic bacteria (Kneen and Beckord, 1946).

Although the dextrinizing enzyme formed by the aspergilli has properties in common with the α-amylase of malt, no evidence was found to indicate that actively saccharifying mold filtrates contained enzymes comparable to the β-amylase of malt. Data (to be published) from a study of several organisms confirm this conclusion. In place of β-type amylase, the dextrinizing enzyme of saccharifying types of mold filtrates is complemented by maltase.

The efficiency of conversion of grain mashes for alcoholic fermentation, likewise, was dependent upon the maltase concentration provided the converting agent contained at least 2 to 3 dextrinizing units per ml (*A. niger* strains NRRL 326, 679, 337, and 330). Much higher concentrations of dextrinizing enzyme

with limited maltase failed to increase either the rate or the degree of saccharification of grain mashes (*A. oryzae* NRRL 694). This is of special interest in view of the indicated correlation between α-amylase potency and yield of alcohol with distillers' malts (Thorne *et al.*, 1945). It must be assumed, therefore, either that malt α-amylase is capable of more complete breakdown of starch than is the corresponding enzyme from mold, or that other enzyme components of malt are more active in saccharification than generally believed. Mold amylases might also comprise other enzymes than the two demonstrated to be present, as manifested by the amylolytic activity of preparations from a strain of *Rhizopus* NRRL 1891 (labeled *Rhizopus* "Boulard" as received), which display limited dextrinizing potency.

It may be of interest to compare dry weights and dextrinizing units obtained in mashes wherein good alcohol yields resulted with fungal amylases with corresponding figures for the barley malt control mash. Thus in the best alcoholic fermentation obtained with fungal amylases as presented in table 5 a culture liquor containing about 2.5 per cent of dry solids and supplied at the level of 20 per cent of the mash volume contributed about 1.25 g of solids and 395 dextrinizing units of α-amylase and resulted in a yield of 5.50 proof gallons of alcohol per bushel of grain. In the barley malt control fermentation 5.0 g of malt with an α-amylase activity of 24 units per g (dry basis) and a mixture content of 8.05 per cent contributed 4.6 g of dry solids and 110 dextrinizing units of α-amylase and gave a yield of 5.15 proof gallons of alcohol per bushel of grain. These figures demonstrate that good preparations of fungal amylase offer higher α-amylase activity per unit of dry weight than does barley malt and that the higher yields of alcohol were associated with greater dextrinizing activity of the mold preparation employed.

The media and techniques employed in the present study might well be utilized industrially for the production of fungal amylases. By selection of the proper culture, products rich in dextrinizing or both dextrinizing and saccharifying enzymes could be obtained. A large number of substrates, now by-products or waste products of industrial grain processing, could be employed satisfactorily. Culture liquors, when feasible, could be utilized without prior treatment or the enzymes could be concentrated and recovered as dry preparations as is now done with enzymes produced by *Aspergillus oryzae* cultivated on cereal bran (mold bran). Drying of the culture liquor after the removal of mold mycelium and suspended solids would result in products having amylase potency 40- to 50-fold greater than that in the untreated culture.

ACKNOWLEDGMENT

The authors gratefully acknowledge the assistance of Dorothy Allen in making the analyses for dextrinizing amylase.

SUMMARY

The ability of more than 350 fungi to produce amylase when grown under submerged, aerobic conditions has been determined. Cultures of *Rhizopus*

Mucor, *Penicillium*, *Aspergillus*, and *Monilia* were represented. With the exception of a few species of *Aspergillus*, all of the organisms investigated elaborated only limited quantities of amylase or were incapable of its formation. Among the aspergilli, substantial amounts of dextrinizing enzyme were produced by *A. wentii*, *A. oryzae*, and *A. alliaceus*, whereas both dextrinizing and saccharifying enzymes were formed by a few strains of *A. niger*. The presence of maltase in appreciable quantities was noted among the strains which actively saccharified starch.

High amylase-producing strains of *Aspergillus niger* such as NRRL 337 were readily grown on a medium composed of thin stillage supplemented with 2 per cent of corn meal and 0.5 per cent of calcium carbonate. After incubation under continuous aeration 3 to 5 days, culture liquors were satisfactory replacements for distillers' malt in the alcoholic fermentation of corn.

REFERENCES

Beckord, L. D., Kneen, E., and Lewis, K. H. 1945 Bacterial amylases. Production of wheat bran. Ind. Eng. Chem., **37**, 692–696.

Beckord, L. D., Peltier, G. L., and Kneen, E. 1946 Bacterial amylases. Production in thin still age. Ind. Eng. Chem., **38**, 232–238.

Boyer, J. W., and Underkofler, L. A. 1945 Mold bran aids production of grain alcohol. Chem. Met. Eng., **52**, 110–111.

Erb, N. M., and Hildebrandt, F. M. 1946 Mold as an adjunct to malt in grain fermentation. Ind. Eng. Chem., **38**, 792–94.

Hockenhull, D. J. D., and Herbert, D. 1945 The amylase and maltase of *Clostridium acetobutylicum*. J. Biol. Chem., **39**, 102–106.

Kneen, E., and Beckord, L. D. 1946 Quantity and quality of amylase produced by various bacterial isolates. Arch. Biochem., **10**, 41–54.

Le Mense, E. H., Sohns, V., Blom, R. H., and Van Lanen, J. M. 1947 Substitution of distillers' malt with fungal amylases produced in submerged culture. *In preparation.*

Leopold, H., and Starbanow, M. P. 1943 Über die Natur der Amylase des *Rhizopus japonicus* (*Amylomyces* B). Biochem. Z., **314**, 232–249.

Nakamura, H. 1931 Amylase-protecting substances. VIII. Takadiastase malt amylase, pancreas amylase and calcium. J. Soc. Chem. Ind. Japan, **34**, 2 Suppl. binding 265–268.

Olson, W. J., Evans, Ruth, and Dickson, A. D. 1947 A corrected unit of alpha-amylase activity. Cereal Chem. *In press.*

Owen, L. W. 1933 Production of industrial alcohol from grain by amylo process. Ind. Eng. Chem., **25**, 87–89.

Sandstedt, R. M., Kneen, E., and Blish, M. J. 1939 A standardized Wohlgemuth procedure for *alpha*-amylase activity. Cereal Chem., **16**, 712–723.

Schwimmer, Sigmund 1945 The role of maltase in the enzymolysis of raw starch. J. Biol. Chem., **161**, 219–234.

Somogyi, M. 1945 A new reagent for the determination of sugars. J. Biol. Chem., **160**, 61–68.

Thorne, C. B., Emmerson, R. L., Olson, W. J., and Peterson, W. 1945 Evaluation of malts for production of alcohol from wheat. Ind. Eng. Chem., **37**, 1142–1144.

Underkofler, L. A., Fulmer, E. I., and Schoene, L. 1939 Saccharification of starchy grain mashes for the alcoholic fermentation industry. Use of mold amylase. Ind. Eng. Chem., **34**, 734–738.

Waldmann, R. 1942 U. S. Patent 2,302,079. Process for producing enzymes by the cultivation of microorganisms on nitrogen and carbohydrate-containing mashes.