# PRODUCTION OF FODDER YEAST FROM BARLEY
## I. Preliminary Studies on the Use of the Waldhof Fermentor[1]

K. J. GOERING[2] AND M. J. HOULE[2]

**ABSTRACT**

A severe shortage of protein in Montana has stimulated interest in converting inexpensive carbohydrate and fertilizer-grade nitrogen into yeast protein. With the use of barley, yeast yields of over 50% dry yeast, based on initial carbohydrate in the barley used, have been obtained in a continuous fermentation by the use of a Waldhof fermentor. The effect of feeding rate, rate of aeration, nitrogen concentration, phosphate concentration, and reuse of spent beer on yield and protein content of yeast has been studied. Of the four yeasts studied, *Candida utilis* appears best adapted for use in this unit.

The intermountain area of the United States is blessed with a large surplus of low-cost carbohydrates, but at the present time it does not have an adequate source of protein. Recently there has developed a great demand for protein to feed livestock for West Coast markets. As a result, there has been increasing interest in developing a local source of protein, since high freight cost makes

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[2]Department of Chemistry, Montana Agricultural Experiment Station, Montana State College, Bozeman.

imported soybean meal a very expensive protein source. This situation led to the suggestion that it might be feasible to utilize cheap carbohydrates as an energy source for converting fertilizer-grade nitrogen into good-quality protein by means of yeast. On the basis of published reports, it was decided that a continuous fermentation would be most practical for this work.

## Materials and Methods

Betzes barley was used throughout this study. It was converted into fermentable sugars by the following procedure. Sufficient ground barley was mixed with water to constitute a mash strength of 10% by weight. Then 0.5% of bacterial amylase[3] was added and the mixture was heated to 75°–80°C. and held at this temperature for 2 hours. The mash was then cooled to 55°C. and 10% distiller's malt was added. Both enzyme percentages were based on the total weight of grain in the mash. The mash was maintained at this temperature overnight. The grain residues were then removed by filtration, and the wort was diluted to the desired sugar strength and boiled to sterilize it. Pressure cooking did not improve the conversion of barley mashes. Sugar determinations were made on the wort and spent beer by a modified dinitrosalicylic acid procedure (3). The total carbohydrate was determined by direct acid hydrolysis (2, p. 289) and the resulting sugars by the method of Dubois *et al.* (4). The nitrogen determinations on the spent beer were made by the method of Folin and Youngberg (5), except that a standard urea curve was used instead of color comparison with ammonium sulfate standard; and in the ammonia runs by a modified Kjeldahl procedure (2, p. 119). The phosphate determinations were made by wet-ashing with nitric and perchloric acids followed by colorimetric determination of phosphorus as described by Allen (1). The sugars from the wort were chromatographed on paper using the procedure described by Gordon *et al.* (6). The yeast was dried by mixing a weighed amount of yeast with approximately three to four times its weight of weighed clean dry sand. The protein analyses of the "as-is" yeast were made by the official Kjeldahl determination (2, p. 12), and corrected for moisture.

Preliminary experiments on the requirement of minerals and nitrogen for adequate yeast growth of *Candida utilis* NRRL Y-900 on barley mash media were made by growing this yeast in 1-liter shake flasks containing 110 ml. of wort. The yeast was grown for 20 hours, then removed by centrifugation, and the weight of dry yeast was used as a criterion for optimum conditions. This informa-

[3] HT-44, produced by Takamine Division of Miles Laboratories, Clifton, N.J.

tion was then used in starting the 20-liter Waldhof fermentor.

The Waldhof fermentor is an apparatus designed for continuous production of yeast. Figure 1 is a picture of this unit, which is a stand-

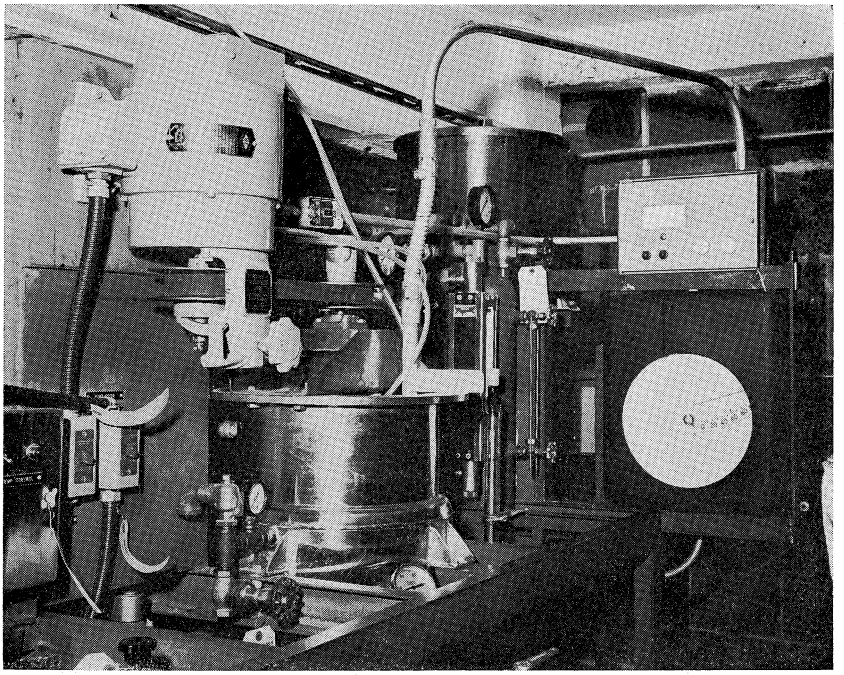

Fig. 1. Pilot-plant Waldhof fermentor.

ard manufactured item[4]. It consists of an open, jacketed, stainless-steel vessel equipped with temperature control, constant-level control, air humidifier, pH control, and a mechanical foam breaker. The wort is drawn down the interior of the draft tube shown in Fig. 2. The air passes through a rotameter and is forced down the two tubes, where it is intimately mixed with the wort by the impeller. In Fig. 3 the impeller has been removed from the draft tube to show how the air is supplied. In spite of the large size of the tube, aeration is very efficient in this unit. Figure 4 is a schematic illustrating the principal parts of the Waldhof unit. Although this unit is rated as a 20-liter fermentor, under our operating conditions it held only from 9 to 12 liters of wort.

The yeast culture was built up to a concentration of 10% (wet basis) by centrifuging out the cells and adding them back to the fer-

[4] Stainless and Steel Products Co., St. Paul, Minn.

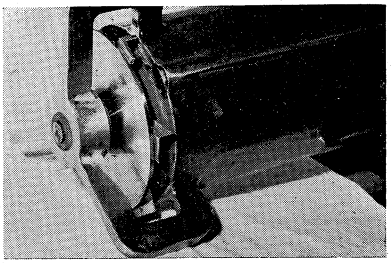

Fig. 2. Assembled draft tube, showing agitator and air outlet.

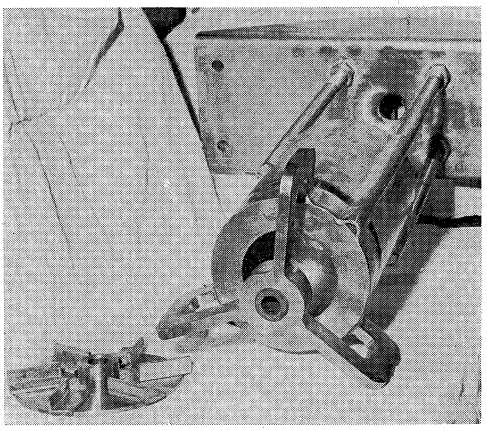

Fig. 3. Draft tube with agitator removed.

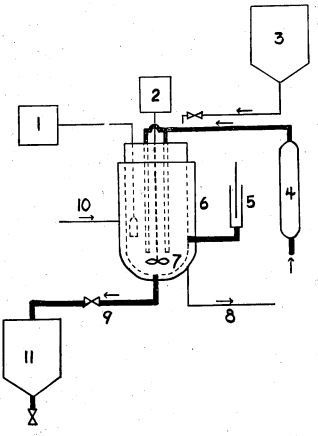


Fig. 4. 1, pH control unit; 2, variable speed impeller drive motor; 3, delivery tank and delivery line; 4, air humidifier and air injection line; 5, liquid level control; 6, water-jacketed fermentor well; 7, impeller and air injection tubes; 8, water discharge line of the temperature control system; 9, yeast cream delivery line; 10, thermostatically controlled water input for the temperature control system; 11, receiver tank.

mentor. Once a culture was obtained, it was observed that the cells could be kept active by washing several times in water, centrifuging, and storing the concentrate at pH of 3.0–3.5 in a refrigerator. This yeast cream was viable for as long as 10 days. When a new run was started, the fermentor was seeded with enough yeast cream to make 10% by weight of wet cells. After aeration for several hours, the feeding could begin. The usual practice was to feed for 6 hours so that replacement of yeast cells was nearly complete before collection of data was begun. If the yeast culture was viable, under our conditions no build-up of sugar occurred during the feeding period. After the first 6 hours, the sugar concentration in the fermentor had dropped from 1% to a negligible value. All runs were made on a continuous basis for periods of 6–8 hours or longer. An analysis was made of the fermentor at the start and completion of each run, and the yields were based on the yeast produced and materials entering fermentor during the run. Corrections were made for any differences in fermentor composition at the beginning and completion of the run. Yeast yields on the pilot-plant runs were determined by centrifuging the yeast from the beer in a Westfalia Yeast Separator, resuspending in water, and centrifuging a second time. The recovered yeast was weighed and the moisture determined on an aliquot so that the dry weight could be calculated. Although it was recognized that slightly better yeast growth could be obtained at pH 5, all pilot-plant runs were made in the pH range 4.4–4.7 as insurance against contamination.

Although *Candida utilis* was used for most of the work, several other yeasts were examined.

## Results and Discussion

*Effect of Added Nutrients on Yeast Yield from Barley Wort as Determined by Shake Flasks.* No attempt was made to study optimum production of fermentable sugars, since this investigation was concerned only with the yields of yeast to be obtained from barley wort produced under certain conditions. It was suspected that this wort would have inadequate amounts of essential minerals and nitrogen. Therefore, a series of experiments involving shake flasks was run to study these requirements. In this experiment barley wort was diluted to 1% sugar as determined by reducing power, and the effects of the concentration of urea, primary potassium phosphate, primary calcium phosphate, ammonium sulfate, and potassium chloride were studied.

Since analysis of the barley wort indicated an average phosphate

content of 1.91% based on sugars present, an additional 1% primary potassium phosphate was added as a starting point to determine optimum urea concentration. Once the optimum urea concentration was established, other combinations of minerals were determined at this nitrogen content. Figure 5 shows that yeast yields

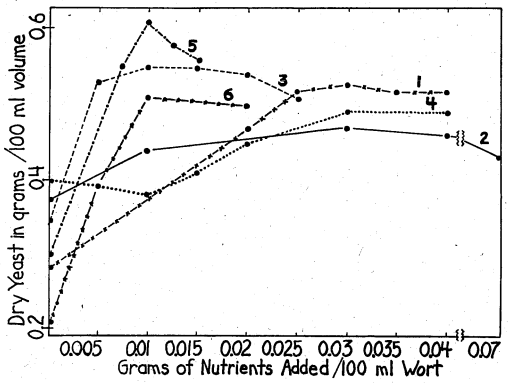


Fig. 5. 1, 100-ml. wort, 0.01 g. $KH_2PO_4$, and varying amounts of urea. 2, 100 ml. wort, 0.01 g. $KH_2PO_4$, and varying amounts of $(NH_4)_2SO_4$. 3, 100 ml. wort, 0.03 g. urea, and varying amounts of $Ca(H_2PO_4)_2$. 4, 100 ml. wort and varying amounts of $(NH_4)_2HPO_4$. 5, 100 ml. wort, 0.03 g. urea, 0.015 g. $Ca(H_2PO_4)_2$, and varying amounts of KCl. 6, 100 ml. wort, 0.03 g. urea, and varying amounts of $KH_2PO_4$.

varied in the unsupplemented worts. However, each determination was made on barley converted on different days, and it is possible that differences in soluble nitrogen content resulted from the variations in laboratory conversions. The fact that these initial differences disappeared as soon as adequate nitrogen was added would tend to verify this assumption. From the data reported in Fig. 5, it was concluded that it would be necessary to add 3% urea, 1% primary calcium phosphate, and 1% potassium chloride based on sugar present to give the most satisfactory results. These values were somewhat less than those indicated by Peterson *et al.* (8) as optimum for wood hydrolysates. This was to be expected, since barley wort contains some protein and considerable amounts of minerals.

*Effect of Flow Rate on Yeast Yields in the Waldhof Fermentor.* Although several commercial Waldhof units are in operation in the United States, no detailed information could be obtained on their operation. The only extensive information available was that of Harris *et al.* (7), and since his fermentor was somewhat different from ours, it was decided to study this effect in a unit which is a standard manufactured item. The air flow was arbitrarily set at 1.5

liters per liter of wort in fermentor per minute, approximately the optimum calculated value based on the data of Harris *et al.* (7). The effect of feeding rate on yeast production was studied, using the mineral and nitrogen concentrations as determined to be optimum in Fig. 5. This information is reported in Table I.

TABLE I

EFFECT OF FLOW RATE ON SUGAR UTILIZATION AND YIELD OF *Candida utilis*[a]

| RATE OF FEED | SUGAR USED | DRY-YEAST YIELD | | CRUDE PROTEIN CONTENT OF YEAST |
|---|---|---|---|---|
| | | Based on Sugar | Based on Carbohydrate | |
| *liters/hour* | % | % | % | % |
| 1.00 | 95 | 61 | 52 | 36.5 |
| 1.50 | 99 | 65 | 59 | 25.6 |
| 1.75 | 75 | 78 | 53 | 23.4 |
| 2.00 | 92 | 61 | 52 | 23.7 |

[a] Culture NRRL Y-900, airflow 15 liters/minute, temperature 30°C., 4% sugar wort containing 3% urea, 1.5% $Ca(H_2PO_4)_2$ and 1% KCl.

The yeast yields, based on sugar consumed, have little significance and were reported only because these results frequently occur in the literature. Since grain wort is a mixture of glucose, maltose, and higher oligosaccharides, the determination of sugars by reducing power is an arbitrary figure which varies with conversion conditions. Furthermore, the yield based on sugar consumed is a function of the sugar utilization, which in a continuous operation can be varied somewhat by the operator. For this reason, the yeast yield based on initial concentration of carbohydrate in the barley was used as a criterion in this work. It was recognized that this value could be greatly influenced if the conversion of starch into fermentable sugars was not uniform. By using dilute mash strengths and long holding times under carefully regulated conditions, this variable was rather successfully eliminated. From Table I it appeared that a flow rate of 1.5–2.0 liters per hour of 4% sugar was maximum for this unit. One very unexpected result obtained in the above experiment was the significant drop in the protein content as the more rapid propagation of yeast was induced. This was surprising, because the spent beer still had an appreciable urea content. At first it was thought that something had gone wrong, either in the culture or in subsequent steps leading to the protein determination, but a rerun verified these results. Apparently the diffusion of the urea into the yeast cells is a limiting factor when yeast is being produced at a rapid rate. This indicated that data obtained in the shake flasks (Fig. 5) for optimum conditions were not reliable for use in the Waldhof unit.

*Effect of Rate of Air Flow on Yeast Yields from the Waldhof Unit.* This fermentor has a draft tube (Figs. 2 and 3) which serves as a mechanical foam breaker. Considerable aeration was obtained without introduction of any air through the aerating system. This was especially true with grain wort, since a rather high agitator speed is required because of the tendency of this material to foam excessively and because small amounts of dextrin present may stabilize the foam (10). The original rotameter was too large for accurate measurement of air; hence, the data reported in Table II were obtained at two different times with different rotameters.

TABLE II

EFFECT OF RATE OF AIR FLOW ON YEAST YIELDS

| AIR FLOW | SUGAR USED | NITROGEN USED | YEAST YIELD | | CRUDE PROTEIN CONTENT OF YEAST |
|---|---|---|---|---|---|
| | | | Based on Sugar | Based on Carbohydrate | |
| *liters/minute* | % | % | % | % | % |
| 0[a] | 89 | .. | 61 | 49 | ... |
| 7.5[a] | 97 | .. | 61 | 54 | ... |
| 15.0[a] | 75 | .. | 78 | 53 | ... |
| 0 | 87 | 74 | 63 | 46 | 50.5 |
| 2.5 | 92 | 78 | 66 | 47 | 51.0 |
| 5.0 | 95 | 74 | 67 | 47 | 51.3 |
| 10.0 | 93 | 75 | 66 | 45 | 51.0 |

[a] These runs were made with 5.3% nitrogen as urea, and optimum salt concentration as determined by shake flask data. The other runs were made 1 year later using 5.3% nitrogen as aqueous ammonia, 3% $Ca(H_2PO_4)_2$, and 1% KCl. Culture was NRRL Y-900, temperature 30°C., and feeding rate 1.5 liters/hour of 4% sugar.

From the data in Table II, it was apparent that very little additional air was required. However, to be on the safe side 7.5 liters per minute were used for the remainder of this investigation. To obtain an exact requirement, it will be necessary to add a tachometer to this fermentor so that agitator speed can be recorded and controlled.

*Effect of Nitrogen Concentration on Yeast Yields in Waldhof Unit.* Since our shake flask information appeared unreliable in regard to optimum conditions for maximum yeast yields in the Waldhof unit, a study was made on the effect of nitrogen concentration on protein content of the yeast and yeast yields. These results are reported in Table III.

The protein content of the yeast appeared to be a function of the nitrogen concentration of the wort up to the 5.3% level. Higher nitrogen levels gave some increase in protein content, but in most cases these increases were insignificant. We were unable to repeat

TABLE III

EFFECT OF NITROGEN CONCENTRATION ON YEAST YIELDS AND PROTEIN CONTENT OF *Candida utilis*[a]

| Nitrogen Added | Nitrogen Used | Sugar Used | Dry Yeast Yield | | Crude Protein Content of Yeast |
|---|---|---|---|---|---|
| | | | Based on Sugar | Based on Carbohydrate | |
| % | % | % | % | % | % |
| 1.2[b] | 75 | 80 | 65 | 46.6 | 26.7 |
| 2.5[b] | 72 | 99 | 53 | 48.5 | 34.9 |
| 5.3[b] | 66 | 97 | 58 | 51.5 | 49.8 |
| 10.6[b] | 50 | 99 | 46 | 41.0 | 60.0 |
| 5.3[c] | 82 | 99 | 77 | 49.3 | 52.2 |
| 7.9[c] | 76 | 99 | 60 | 40.0 | 52.6 |
| 10.6[c] | 70 | 99 | 50 | 33.0 | 53.9 |
| 13.5[c] | 60 | 98 | 50 | 33.0 | 53.4 |
| 5.3 | 82 | 98 | 66 | 49.0 | 49.1 |
| 10.6 | 68 | 97 | 62 | 47.0 | 53.5 |

[a] Nitrogen based on total sugars present.

[b] These runs were made 1 year previous to others with 1.5% $Ca(H_2PO_4)_2$ and 1% KCl.

[c] Runs were made with 3% $Ca(H_2PO_4)_2$, 1% KCl, and urea as nitrogen source. Last two runs were made with aqueous ammonia, otherwise same as 5.3 and 10.6 next above. Culture NRRL Y-900, temperature 30°C., feeding rate 1.5 liters/hour of 4% sugars.

the run in which we obtained 60% protein in the yeast. It was observed that this mash was slightly sour, and therefore it was suspected that lactic souring might be involved. However, other mashes which were deliberately soured with *L. delbruckii* failed to improve our normal runs. At high protein levels, total yeast yields were always somewhat lower, and nitrogen utilization less efficient. Ammonia nitrogen was not quite as satisfactory as urea at the same nitrogen level, but more satisfactory results are obtained at higher levels, and economics would dictate its use.

*Effect of Phosphate on Nitrogen Utilization by* Candida utilis. The work reported in Fig. 5 using shake flasks indicated that the efficiency of nitrogen utilization was influenced by phosphate concentration. This accounts for the difference in efficiency of nitrogen utilization observed in the previous table, where the runs made the second year had twice the concentration of phosphate. An experiment was run to verify these data, and this information is reported in Table IV.

It was apparent that additional phosphate not only increased the efficiency of nitrogen utilization, but also increased the yeast yield and protein content of the yeast. The decrease observed at the highest level was also observed in the shake flask results and, therefore, is believed to be real although the reason for this is not clear.

*Effect of Reuse of Spent Beer on Yeast Yields.* One of the major problems confronting a yeast plant is disposal of spent beer. If a substantial amount of this liquid could be used in preparing fresh

TABLE IV

EFFECT OF PHOSPHATE ON NITROGEN UTILIZATION BY *Candida utilis*[a]

| Primary Calcium Phosphate | Phosphate Utilized | Nitrogen Utilized | Sugar Utilized | Dry Yeast Yields | | Crude Protein Content of Yeast |
|---|---|---|---|---|---|---|
| | | | | Based on Sugar | Based on Carbohydrate | |
| % | % | % | % | % | % | % |
| 1.5 | 87 | 81 | 97 | 66.0 | 43 | 50.5 |
| 3.0 | 73 | 86 | 99 | 74.5 | 48 | 54.0 |
| 4.5 | 65 | 70 | 96 | 75.0 | 47 | 53.1 |

[a] Culture NRRL Y-900, temperature 30°C., feeding rate 1.5 liters/hour of 4% sugars, airflow 7.5 liters/minute, 5.3% nitrogen as urea, 1% KCl, and indicated phosphate.

mashes, not only would disposal cost be reduced, but appreciable savings could be made in the amount of phosphate and nitrogen needed to bring the levels up to recommended optimums. To test this thesis, a 6-day continuous run was made in which one-half of the makeup water for the mash was spent beer, and the run was continued sufficiently long so that part of the spent beer was recycled a second time. These results are reported in Table V.

TABLE V

EFFECT OF RECYCLING ON YEAST YIELDS[a]

| Recycle No. | Phosphate Utilized | Nitrogen Utilized | Sugar Utilized | Dry Yeast Yields | | Crude Protein Content of Yeast |
|---|---|---|---|---|---|---|
| | | | | Based on Sugar | Based on Carbohydrate | |
| | % | % | % | % | % | % |
| 1 | 64 | 71 | 97 | 65 | 45.7 | 52.1 |
| 2 | 63 | 72 | 93 | 65 | 44.0 | 50.0 |

[a] Culture NRRL Y-900, temperature 30°C., feeding rate 1.5 liters/hour of 4% sugars, airflow 7.5 liters/minute, 5.3% nitrogen as ammonia, 1% KCl, and 3% $Ca(H_2PO_4)_2$.

Although the yields reported were slightly lower than our average values, it is doubtful if this difference is significant. An entirely unexpected result was observed; namely, these mashes had far less tendency to foam than did those mashes not containing spent beer. As a result, the agitator speed was reduced approximately one-half, and as indicated previously, it is possible that the rate of aeration was not optimum under these conditions. This problem is now under investigation in our laboratory. No apparent contamination was observed during this 6-day continuous run. The recycling of one-half the spent beer would allow a saving of approximately 20% of the phosphate cost and about 10% of the nitrogen cost over the conventional procedure.

*Utilization of Sugars in Barley Wort.* During the course of this investigation, a number of paper chromatograms were made on

the sugars present in fresh barley wort and on spent beer. A typical chromatograph is reproduced in Fig. 6.

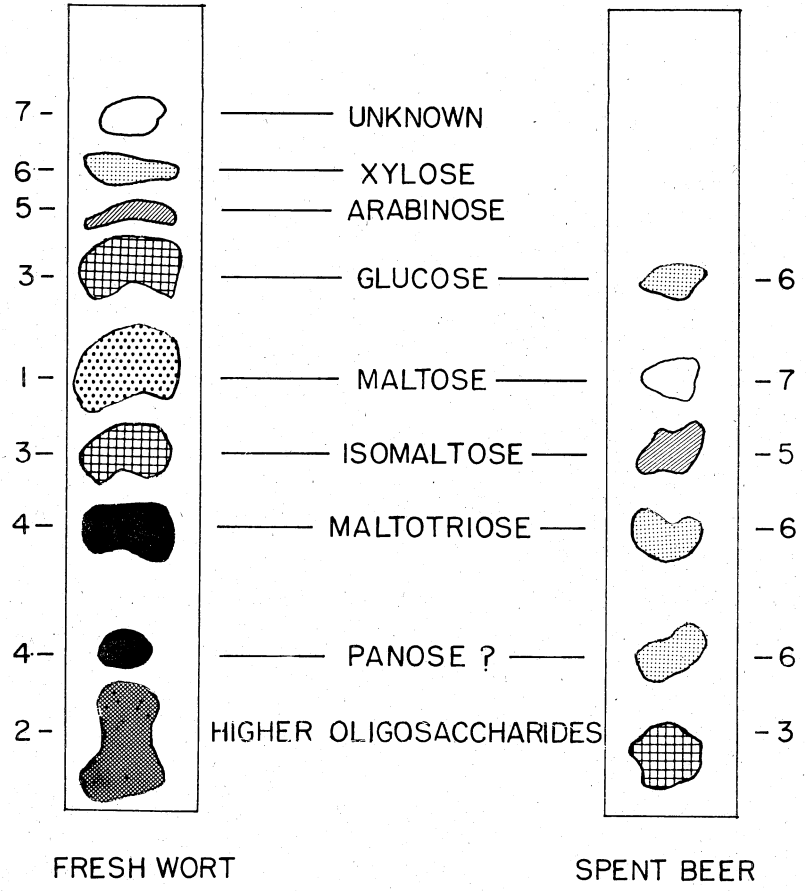


Fig. 6. Sugars present in fresh barley wort and spent beer.

It was observed that in the fresh wort, a small concentration of pentoses was present along with glucose and the expected oligosaccharides. The spent beer failed to show any pentoses, and a marked reduction in the higher oligosaccharides indicated that the pentoses were utilized and that this yeast must have some saccharifying ability. To study this problem more in detail, a quantitative study was made on the sugars present before and after fermentation (Table VI).

The results of Table VI confirmed the assumptions made as the result of our paper chromatographs, namely, that the pentoses were

used and that isomaltose and higher oligosaccharides were greatly reduced in amount during the fermentation. One surprising result was the amount of glucose remaining in the spent beer. The fact that

TABLE VI

DISTRIBUTION OF SUGARS IN STARTING WORT AND IN SPENT BEER

| CARBOHYDRATE | STARTING WORT | SPENT BEER |
|---|---|---|
| | $\gamma/ml$ | $\gamma/ml$ |
| Pentoses | 200 | 0 |
| Glucose | 3,000 | 200 |
| Maltose | 24,800 | 250 |
| Di and Tri[a] | 9,700 | 950 |
| Higher[b] | 8,200 | 1,025 |

[a] This includes isomaltose, maltotriose, panose, and isomaltotriose. No effort was made to determine each as individuals.

[b] Higher fraction contains all the oligosaccharides left. These were made up of principally 4–12 glucose units. Very few, if any, residues were larger than this.

the glucose and maltose were found at nearly the same level in the spent beer, although the maltose was originally present at approximately ten times the glucose concentration, was puzzling. Present concepts of how sugars are transported across cell membranes involve either phosphorylation or a so-called "permase." The mechanism of the latter is still uncertain. The fact that glucose is supposedly more easily phosphorylated than maltose would not fit the above data for the common phosphorylase mechanism. Likewise, it is difficult to see why maltose should be more rapidly transported across a membrane by a "permase." Therefore, it would appear to be desirable to investigate this mechanism using tagged sugars under operating conditions of the Waldhof fermentor.

*Production of Other Yeasts of Possible Industrial Significance in the Waldhof Unit.* To compare the growth of *Candida utilis* with other yeasts, a high-ergosterol yeast, *Saccharomyces cerevisiae* NRRL Y-132, *S. diastaticus* NRRL Y-2416, and two cultures of a self-agglutinating yeast, *S. kluyveri* NRRL Y-4288 Code 3 and Code 26 were run in the pilot plant. These three yeasts gave average yields of 46, 12, and 46.4% respectively based on total carbohydrate in the barley. However, it was observed that the feeding rate used for *C. utilis* had to be appreciably reduced, as they did not reproduce nearly as rapidly under our conditions. The *S. diastaticus* did show some saccharifying ability as determined by sugar analysis, but its rate of growth and handling characteristics were deemed unsatisfactory. *S. kluyveri* was grown to test the possibility of eliminating the centrifuging of yeast cells in a commercial plant. This yeast was reported to agglutinate

rapidly and to settle out when the two code strains of opposite-sexed cells were mixed (9). Although the seed culture indicated these tendencies, the pilot plant runs failed to show any signs of agglutination, even after standing several hours. Since extreme care was used in sterilizing the fermentor between runs, so that the sexes would not become mixed, it appeared as if something in the barley mash prevented the agglutination reaction. It is possible that conditions might be worked out so that this agglutination could proceed. However, from our observations on the growth characteristics of this yeast, it would appear doubtful if it could compete with *C. utilis* even if the agglutination reactions were successful.

## Literature Cited

1. ALLEN, R. J. L. The estimation of phosphorus. Biochem. J. **34:** 858–865 (1940).
2. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, Official methods of analysis. (9th ed.). The Association: Washington, D.C. (1960).
3. COLOWICK, S. P., and KAPLAN, N. O. Methods in enzymology, Vol. I; Section II, Enzymes of carbohydrate metabolism, p. 149. Academic Press: New York (1957).
4. DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A., and SMITH, F. Colorimetric method for determination of sugars and related substances. Anal. Chem. **28:** 350–356 (1956).
5. FOLIN, O., and YOUNGBURG, G. E. Note on the determination of urea in urine by direct Nesslerization. J. Biol. Chem. **38:** 111–112 (1919).
6. GORDON, H. T., THORNBURG, W., and WERUM, L. N. Rapid paper chromatography of carbohydrates and related compounds. Anal. Chem. **28:** 849–855 (1954).
7. HARRIS, E. E., SAEMAN, J. F., MARQUARDT, R. E., HANNON, M. L., and ROGERS, S. C. Fodder yeast from wood hydrolysates and still residues. Ind. Eng. Chem. **40:** 1220–1223 (1948).
8. PETERSON, W. H., SNELL, J. F., and FRAZIER, W. C. Fodder yeast from wood sugar. Ind. Eng. Chem. **37:** 30–35 (1945).
9. WICKERHAM, L. J. Sexual agglutination of heterothallic yeasts in diverse taxonomic areas. Science **128:** 1504–1505 (1958).
10. VAN DER LANDE, B. L., and VAN THIEL, E. Aeration in yeast propagation. U. S. Patent 2,119,188 (1938).