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WHEY UTILIZATION. V. GROWTH OF *SACCHAROMYCES FRAGILIS* IN WHEY IN A PILOT PLANT

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SUMMARY

Saccharomyces fragilis was grown in whey medium in a pilot plant study. A flow diagram of the process is given and the equipment described. The oxygen absorption rate (OAR) of the propagator was determined by the sulfite oxidation method. Eighty per cent of the OAR found necessary in the laboratory to supply the oxygen demand of yeast growing under similar conditions could be obtained by modifying the propagator. The yeast yield was 75% of the theoretical value. The dried yeast product is a bland powder with a pleasant, cheese-like flavor.

Whey, a by-product of cheese-making, can be used as a medium for the rapid production of a yeast suitable for the supplementation of human and animal diets. Wasserman, Hopkins, and Porges (6) reported maximum yeast growth in 4 hr., using raw whey and an inoculum of *Saccharomyces fragilis* equal on a dry weight basis to 25% of the weight of the whey sugar. High yields were dependent on the supply of sufficient oxygen to satisfy the demand of the yeast. Under the given growth conditions, the yeast required about 570 mg. oxygen/gallon medium/minute (4.75 m*M* O_2/L/min) at their peak demand period (4, 5). The yeast can only utilize dissolved oxygen, and the relation between yeast yield and the oxygen absorption rate of the propagation equipment has been reported (5).

The data reported in this paper show that the growth of *S. fragilis* in whey medium in a pilot plant operation of approximately 800-gal. volume could be successfully based on the results of the 500-ml. and 15-liter laboratory propagators reported by Wasserman *et al.* (4, 6, 7).

METHODS AND MATERIALS

S. fragilis, a lactose-utilizing yeast, was grown in raw Italian cheese whey obtained through the courtesy of the M. Maggio Company, Philadelphia, Pa. The whey was supplemented with 0.5% quantities of $(NH_4)_2SO_4$, K_2HPO_4, and dried Brewers yeast. The pH of the medium after the addition of the salts was approximately 5.5, which is within the optimum pH range for maximum yeast yields. The conditions for growing the yeast were the same as those previously described for *S. fragilis* growth in the laboratory propagators (4, 6, 7).

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Propagation apparatus. A steel storage tank, 5 ft. in diameter and 12 ft. 8 in. high, was converted into a yeast propagator. The agitation-aeration unit, designed and constructed by the Walker Equipment Company,[2] was a six-blade turbine suspended from the top of the tank and powered by a 7½-h.p. motor. The turbine rotated at 300 r.p.m. In operation, the power supplied by the motor was inadequate, but a more powerful motor could not be used because of difficulties in keeping the medium in the tank under the described conditions. A Spencer turbo-compressor,[2] rated at 360 c.f.m. at 5 p.s.i. gauge, supplied air through jet orifices mounted on a sparger ring beneath the agitator. Temperature control of the medium was effected by passing cold water (40° F.) through 118 ft. of copper tubing (1½ in. o.d.) arranged in a coil within the tank.

Although the tank was rated to contain 1,600 gal., only 700-800 gal. of medium were actually used in the propagation of the yeast, leaving about 50% of the tank capacity as head space to accommodate the increased volume of the emulsion created by aerating and agitating the medium.

Analytical determinations. The oxygen absorption rate (OAR) of the propagation equipment was determined by a modification (2) of the sulfite oxidation method of Cooper, Fenstrom, and Miller (1).

The course of the yeast growth was followed by periodic determinations of the dry weight of the yeast, and the disappearance of lactose. A sample of the yeast suspension was taken from the middle of the tank and the yeast removed by centrifugation. The dry weight of the yeast was obtained after washing the cells once with distilled water, and drying overnight at 105° C. Lactose was determined by the method of Stiles, Petersen, and Fred (3) on the supernatant medium. For immediate information of the progress of the yeast growth, cell counts were made with a hemocytometer. Although it was known that cell counts were of doubtful accuracy, the rapidity of the determination made it a useful qualitative test.

RESULTS

OAR determination. Before an attempt was made to grow yeast in the propagator, the OAR of the equipment was determined to assure solution of sufficient oxygen for maximum growth. Eight hundred gallons of 1 N sulfite solution was placed in the propagator and the agitator blades were set at 25-in. diameter. The air throughput was 360 c.f.m.; lower air rates could not be used because the resistance of the solution was sufficient to stop the motor. Under the above described conditions, the OAR was 3.4 mM O_2/L/min (418 mg. O_2/g.p.m.). Since the peak O_2 demand of the yeast was 570 mg. O_2/g.p.m. (4), only 72% of the required oxygen was dissolved by the equipment. To increase the OAR the apparatus was modified by reducing the diameter of the agitator blades to 23 in. and installing four steel baffles 90 in. long and 8 in. wide on the walls of the tank. The OAR rose to 4.0 mM O_2/L/min. Although this value was still below the O_2 requirements of the yeast, the apparatus could not be

[2] It is not implied the USDA recommends the above company or its product to the possible exclusion of others in the same business.

modified further at this time, and the yeast propagations were undertaken with the realization that maximum yields might not be achieved.

Yeast propagation. The flow diagram for a simple yeast propagation operation is shown in Figure 1. The raw, unheated whey and the solid medium ingredients were mixed in the propagation tank, and the yeast inoculum—equal in dry weight to 25-30% of the weight of the whey lactose—was added. The inoculum may be grown in increasing volumes of whey or, as in this case, it may

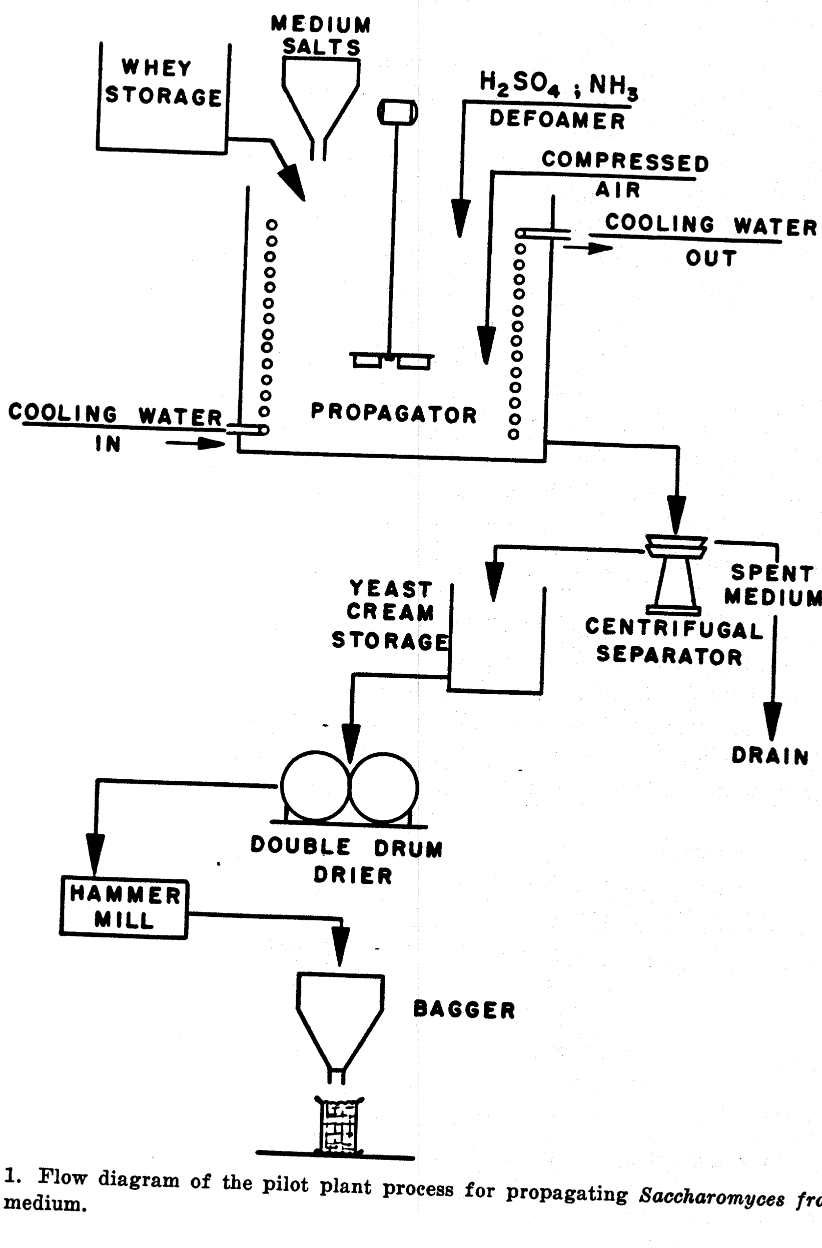

FIG. 1. Flow diagram of the pilot plant process for propagating *Saccharomyces fragilis* on whey medium.

be a concentrated yeast cream from a previous tank. The pH of the propagation was maintained at about 5.5 by the addition of concentrated H_2SO_4 or NH_4OH. At the conclusion of the experiment the yeast suspension was passed through a DeLaval separator [2] to concentrate the yeast to a 15-18% slurry. The yeast cream can be washed by suspension in water and reseparation as many times as necessary. The yeast cream was fed to a double drum dryer operated at a steam pressure of 85 p.s.i. gauge and rotating at 12 r.p.m. The dried yeast flakes were carried by a worm conveyor to a hammer mill to be pulverized, then to a bagging apparatus.

The product was a light cream-colored powder with a bland, slightly cheese-like flavor. The intensity of the flavor can be controlled by the number of washes the yeast is subjected to prior to drying.

Data for two typical batches of yeast grown as described are presented in Table 1, and the course of a representative propagation is shown in Figure 2. Although the volumes of medium used were different (530 gal. for Batch 1 and 800 gal. for Batch 2), all of the available lactose in Batch 2 had been utilized within 4 hr. and less than 0.5% still remained in Batch 1 at this time. The maximum yeast growth in both runs was achieved in 4 hr. Propagation beyond this time led to no further increase in yeast yield and could even be conducive to destruction of some of the yeast already formed.

Laboratory data indicated that 0.55 lb. of dry yeast could be obtained per pound of lactose used (6). Thus, from the 182 lb. of sugar in Batch 1, 100 lb. of dried yeast were anticipated, but 74 lb. were actually obtained. In Batch 2, the 242 lb. of lactose should yield 133 lb. of yeast, and 105 lb. were actually found. A yeast yield of approximately 75% of theory, or about 0.42 lb. per pound lactose, was obtained.

The factors involved in the decreased yeast yield were not determined. However, since laboratory-scale propagations in Italian cheese whey media resulted in yeast crops approximating the theoretical yields, the similar medium components in the 700-gal. propagator could be expected to yield an equally large crop of yeast. A direct correlation between the OAR of a propagator and the yeast yield has been demonstrated (5); therefore, if the quantity of dis-

TABLE 1

Representative balance sheet for the growth of *Saccharomyces fragilis* in whey medium in pilot plant equipment

	Batch 1	Batch 2
Volume whey *(gal.)*	450	600
Lactose (4.86% of whey) *(lb.)*	182	242
Lactose disappearing *(lb.)*	182	242
Volume seed yeast *(gal.)*	81	150
Weight seed yeast *(lb.)*	66	110
Final volume in tank *(gal.)*	530	800[a]
Gross weight of yeast yield (dry) *(lb.)*	140	215
Net weight of yeast yield (dry) *(lb.)*	74	105
Theoretical yeast yield (55% of sugar weight) *(lb.)*	100	133
% Theoretical yield	74	79

[a] Fifty gallons of water added accidentally.

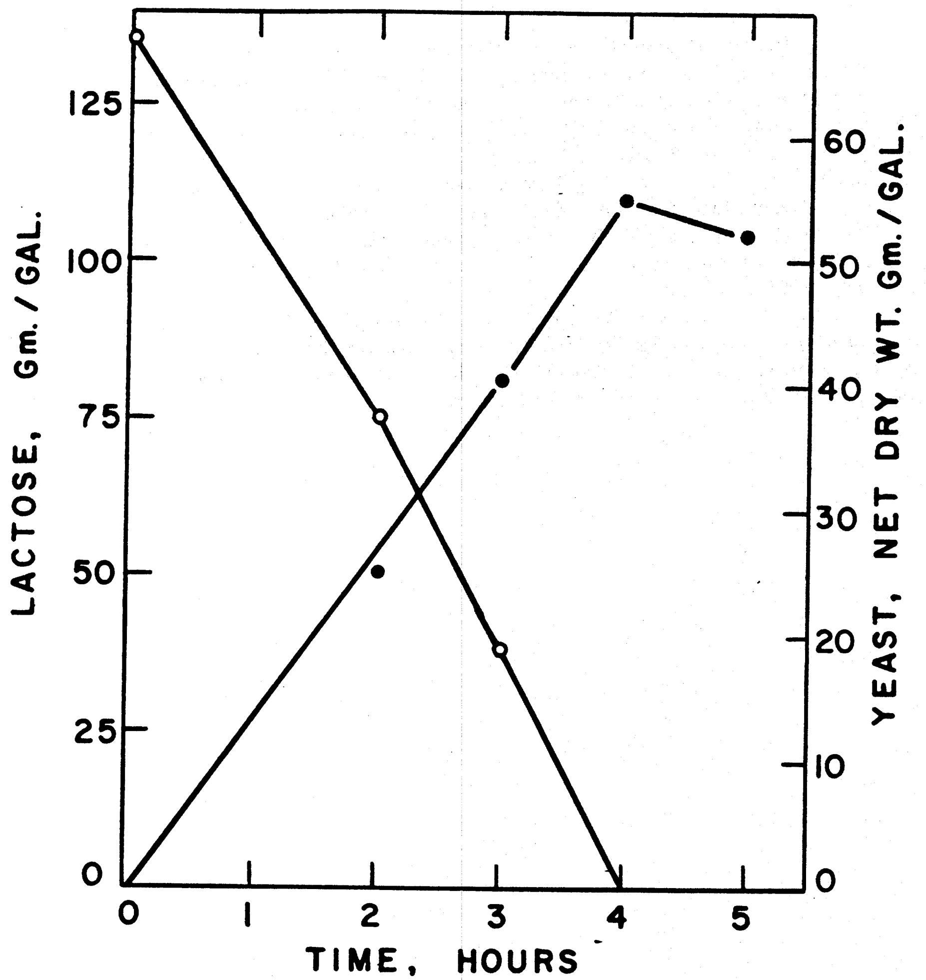

FIG. 2. Lactose utilization and *Saccharomyces fragilis* production in pilot plant Batch 2 (see text). Lactose ○———○; Yeast (net dry weight) ●———●.

solved oxygen in the 700 gal. of medium were only 80% of the yeast requirements, the observed decrease in yeast yield might be expected.

To produce 1 lb. of dried yeast, 2.45 and 2.3 lb. lactose were required in Batch 1 and Batch 2, respectively. Approximately 0.27 lb. of each of the other medium ingredients was used per pound yeast.

The data indicate that whey can be used as a medium for the batch production of yeast in good yield. Increased yields might be expected with a more efficient oxygen-dissolving system and conversion of the batch process into continuous or semicontinuous operation.

REFERENCES

(1) COOPER, C. M., FENSTROM, G. A., AND MILLER, S. A. Performance of Agitated Gas-Liquid Contactors. Ind. Eng. Chem., 36: 504. 1944.

(2) Corman, J., Tsuchiya, H. M., Koepsell, H. J., Benedict, R. G., Kelley, S. E., Feger, V. H., Dworschak, R. G., and Jackson, R. W. Oxygen Absorption Rates in Laboratory and Pilot Plant Equipment. Appl. Microbiol., 5: 313. 1957.

(3) Stiles, H. R., Peterson, H. W., and Fred, B. E. A Rapid Method for the Determination of Sugar in Bacterial Cultures. J. Bacteriol., 12: 427. 1926.

(4) Wasserman, A. E. Whey Utilization. II. Oxygen Requirements of *Saccharomyces fragilis* Growing in Whey Medium. Appl. Microbiol., 8: 291. 1960.

(5) Wasserman, A. E., and Hampson, J. W. Whey Utilization. III. Oxygen Absorption Rates and the Growth of *Saccharomyces fragilis* in Several Propagators. Appl. Microbiol., 8: 293. 1960.

(6) Wasserman, A. E., Hopkins, W. J., and Porges, N. Whey Utilization: Growth Conditions for *Saccharomyces fragilis.* Sewage and Industrial Wastes, 30: 913. 1958.

(7) Wasserman, A. E., Hopkins, W. J., and Porges, N. Rapid Conversion of Whey to Yeast. Proc. XVth Intern. Dairy Congr., 2: 1241. 1959.