

RAPID CONVERSION OF WHEY TO YEAST

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Received 2 September 1958

Liquid whey is a by-product of increasing importance to the dairy industry. In certain circumstances the utilization or disposal of about 9 lb of whey remaining after making each pound of cheese becomes a burden to many dairies. The simplest manner of utilization and disposal is the return of the whey to the farm, but this is feasible only in limited areas. Some of the whey is dried or further processed (1). Considerable portions are still discarded as waste because of the impracticability of economic utilization. Unfortunately, such discarded waste cannot be dumped into waterways or small sewage systems because of its very high oxygen demand. The rapid process developed for the treatment of dairy waste (2) can handle nominal amounts of whey provided aeration conditions are maintained.

Cheese whey, besides being a possible source of stream pollution, is a potential source of carbohydrate and protein for processing. Conversion of whey to yeast protein for animal nutrition has been considered in the past, and references have been cited (1, 3). The choice of yeast is limited to those forms capable of utilizing lactose. *Saccharomyces fragilis*, as well as some *Torula* species, grows well on this carbohydrate. Patents have been issued for the growth of yeast in whey, primarily as a whole protein supplement (4) or for the vitamin and enzyme content (5). Large-scale production is not carried out extensively at present primarily for economic reasons.

Reduction in processing costs could make yeast production an attractive method for the utilization of whey. Some earlier studies on the bio-oxidation of dairy waste indicated that such reduction in costs may be attained by reducing the time necessary for yeast propagation and by supplying the oxygen needed for growth.

EXPERIMENTAL

Preliminary tests

Tests were made on 500-ml quantities of whey vigorously agitated by means of a turbine-type agitator with air supplied directly under the agitator (6). Aeration and agitation were in excess to avoid anaerobic conditions.

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The whey was seeded to contain 500×10^6 cells of *S. fragilis*/ml. Maximum growth and sugar utilization were obtained within 6 h in whey supplemented with 0.5% $(\text{NH}_4)_2\text{SO}_4$, 0.5% K_2HPO_4 and 0.1% Difco yeast extract. The temperature was maintained at 30°–32°C. The best growth was at an initial pH of 5.7.

Effect of seeding rate

Studies on dairy wastes showed that the rate of removal of soluble matter from an aerated solution was directly related to the quantity of cells present (7). Therefore, the size of yeast inoculum was varied from 300×10^6 /ml to $2,000 \times 10^6$ /ml. Again, increasing the size of inoculum decreased the time necessary to reach the maximum yield. The highest quantity of seed used ($2,000 \times 10^6$ /ml) resulted in a maximum yield of 23.5 mg/ml in 3 h, with a final cell count of $4,560 \times 10^6$ /ml. The smaller seedings eventually reached about the same net yield of dry cells, but required longer time. As the size of the inoculum increased, the time required for removal of lactose from the whey decreased; sugar was completely removed in only 3–4 h with the maximum amount of seed. The dry weight of $2,000 \times 10^6$ yeast cells/ml is 25–30% of the dry weight of the sugar present.

Heat treatment

Although heat treatment of whey and removal of precipitated protein has been recommended for better yeast growth (8), our results showed that such deproteinization was unnecessary in this rapid propagation process. Yields of *S. fragilis* were the same from raw and heat-treated whey.

Nitrogen in the cell

A study of the distribution of the nitrogen between the cell and the medium showed the necessity of supplementing with nitrogen to obtain a yeast of high nitrogen content. Even without nitrogen supplementation, all the nitrogen present in whey was not available to the yeast. Thus, of 129 mg present per 100 ml whey, there remained a resistant portion amounting to about 95 mg. Table 1 shows that this available whey

TABLE 1

Nitrogen content of whey medium and its effect on nitrogen content of S. fragilis

Growth time h	Nitrogen in whey media			Nitrogen in yeast		
	Whey mg/100 ml	$(\text{NH}_4)_2\text{SO}_4$ added		Whey %	$(\text{NH}_4)_2\text{SO}_4$ added	
		0.5%	1.0%		0.5%	1.0%
0	129	252	397	7.38	7.24	7.37
1	97	194	326	6.50	7.77	7.88
2	96	116	273	5.46	7.74	7.51
3	95	99	228	4.93	6.72	8.12
4	95	99	193	4.93	6.62	8.17

nitrogen was used within the first hour. The addition of 0.5% $(\text{NH}_4)_2\text{SO}_4$ supplied enough nitrogen to last 2–3 h, while a 1% addition supplied sufficient to satisfy over 4 h of propagation.

The effect of the concentration of nitrogen in the medium is reflected in the nitrogen content of the yeast. Thus, in yeast grown on whey medium containing no added nitrogen, the nitrogen content of the harvested yeast was only about 66% of original value of the seed yeast. This would tend to show that a yeast of high nitrogen content can take up about half its own weight of carbohydrate material without nitrogen addition to yield a low-nitrogen yeast. Similar trends had been observed when mixed bacterial cultures acted upon dilute skim milk (9).

In the whey containing 0.5% $(\text{NH}_4)_2\text{SO}_4$, the nitrogen content of the cells increased at first, but then as the available nitrogen in the medium diminished (after 2 h), the nitrogen content of the yeast decreased to about 85% of its original value. The addition of 1.0% $(\text{NH}_4)_2\text{SO}_4$ resulted in maintaining or enhancing the high nitrogen content of the yeast during the 4 h of propagation. The total yields of yeast grown in the presence of both concentrations of $(\text{NH}_4)_2\text{SO}_4$ were almost the same. The later stages of growth in the presence of lower concentrations of nitrogen occurred at the expense of the nitrogen converted to cell material in the early part of the yeast propagation.

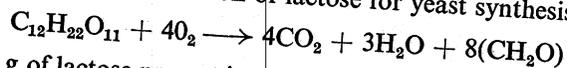
Oxygen requirements

Oxygen utilization by the growing yeast was determined by measuring the oxygen remaining in the spent air with a Beckman Oxygen Analyser (10).* Initially, oxygen consumption was low, increasing as the lactose was utilized and as the yeast population increased. At the peak of the oxygen demand the yeast consumed 100–120 ml oxygen/l. of yeast whey suspension per min under the conditions of these experiments. At no time was there a deficiency of oxygen. The average oxygen uptake per hour from a representative experiment is shown in Table 2.

TABLE 2
Oxygen utilized for propagation of *S. fragilis* per litre of whey

Time interval h	Oxygen used per litre	
	ml	g
0-1	2,580	3.69
1-2	5,760	8.23
2-3	3,720	5.31
3-4	2,280	3.26
Total	14,340	20.49

The generally accepted dissimilation of lactose for yeast synthesis proceeds thus:



Therefore the 40 g of lactose present in each l. of whey medium would require 14.5 g or 10.5 l. of oxygen for its conversion to yeast. A total of 14.3 l. were actually used. Apparently the extra 3.8 l. were required for the oxidation of the lactic acid and protein as well as for endogenous cell respiration.

Variations occur in the course of oxygen uptake during a propagation owing to factors such as the size, age and physiological condition of the inoculum. Conditions

* It is not implied the U.S.D.A. recommends the above company or its products to the possible exclusion of others in the same business.

existing in the fermenter also vary the course of oxygen consumption. Nevertheless in all cases the total oxygen uptake, regardless of the consumption pattern, approximated to 14 l. of oxygen per l. of whey medium.

Oxygen supply and 15-l. experiments

Supplying these high quantities of oxygen required a proper design of aeration equipment. At the peak oxygen requirement of 100–120 ml/min approximately 5 mM of oxygen must be transferred per min to each l. of whey. The oxygenating properties of various devices were compared by a modification (11) of the sulphite oxidation method (12). By a proper choice of agitator and air supply, the yeast propagation was scaled up from 500 ml to 15 l.

Successful propagations were obtained immediately in the large aerators. Oxygen consumption was of the same order of magnitude, in relation to the volume, as was observed with the small aerators. The yeast yields in the larger propagations expressed as net dry weight formed was 52% of the lactose and lactic acid present in the whey. Since two-thirds of the carbon of lactose and lactic acid are available for assimilation, 27 g of yeast containing 45% carbon could be expected from each l. of whey. Our results showed an average yield of 23 g of yeast or approximately 85% of the theoretical yield.

Experiments have been carried out on whey from cheddar, Ricotta and Italian curds. The yields were essentially the same. But because of differences in pH and variations in the composition of the whey from different sources, it was necessary to adjust the composition of the added salts. For example, when using Ricotta cheese whey, KH_2PO_4 was used instead of K_2HPO_4 to aid in adjusting the initial pH.

Removal of organic matter from whey

All of the organic matter as measured by a rapid chromate oxidation method (13) was not removed from the medium by the yeast. In terms of the chemical oxygen demand (COD), the whey contained about 55,000 mg COD/l. (which was increased to 68,000 mg by addition of the yeast seed). At the end of 4 h the soluble COD was

TABLE 3

Changes in organic matter as measured by COD during yeast propagation

<i>Medium and time</i>	<i>Total COD mg/l.</i>	<i>Soluble COD mg/l.</i>	<i>Yeast COD mg/l.</i>
Whey alone	55,000	55,000	0
Whey + yeast	68,000	58,400	9,600
1 h	64,600	43,200	21,000
2 h	62,000	26,000	36,000
3 h	53,800	17,000	36,800
4 h	46,400	9,000	37,400

reduced to 9,000 mg showing about 84% removal of the original soluble oxygen-demanding material.

As shown in Table 3, the total organic matter decreased as the yeast increased. Of the soluble COD, almost 60% was recovered later as yeast cell COD, the remainder

was apparently oxidized to carbon dioxide. The residual COD in solution may represent products of excretion or dissimulation or materials which cannot be assimilated.

DISCUSSION

Supplying adequate oxygen by properly designed equipment leads to a reconsideration of the utilization of whey for the rapid propagation of yeast. The amount of air needed for the aerobic treatment of whey as a waste may be used to recover a marketable product that may help to defray the cost of disposal.

The rapid conversion of the lactose to the yeast, *S. fragilis*, through mass seeding obviates the necessity of sterilizing the whey. This yeast gave a high solids recovery in the presence or absence of the whey proteins, but supplementation with nitrogen, phosphorus and yeast extract for growth factors was necessary. Seeding with yeast, whose dry weight was equal to one-fourth of the weight of the sugar in the whey, completed the propagation in 3-4 h. An appropriate amount of yeast harvest may be retained for seeding the subsequent charge of whey.

The high oxygen requirement of the growing yeast must be satisfied to maintain aerobic conditions so essential to rapid growth. Turbine-type agitators that have the capacity to dissolve oxygen at high rates were used in these studies. Translation of the propagation from one type of aerator to another has been accomplished without difficulty once the oxygen-transfer efficiency of the equipment was known. Foaming was not too severe a problem in these tests.

Plans are under way to propagate *S. fragilis* in 1,000-gal batches of whey at a local yeast plant applying the information reported in this paper.

REFERENCES

- (1) Whittier, E. O. & Webb, B. H. (1950) *By-Products from Milk*, Reinhold Publishing Corp., New York
- (2) Porges, N. (1958) *Food Technology* 12 78
- (3) Porges, N., Pepinsky, J. B. & Jasewicz, L. (1951) *J. Dairy Sci.* 34 615
- (4) Simpson, E. G. & Young, H. (1957) "Increasing the Protein Content of Milk Products". *U.S. Pat.* 2,809,113
- (5) Myers, R. P. & Weisberg, S. M. (1938) "Treatment of Milk Products". *U.S. Pat.* 2,128,845
- (6) Wasserman, A. E., Hopkins, W. J. & Porges, N. (1958) *Sewage and Ind. Wastes* 30 913
- (7) Porges, N., Jasewicz, L. & Hoover, S. R. (1955) *Proc. 10th Ind. Waste Conf.*, Purdue Univ. 135
- (8) Hanson, A. M., Rodgers, N. E. & Meade, R. E. (1949) "Method of Enhancing the Yield of Yeast in a Whey Medium". *U.S. Pat.* 2,465,870
- (9) Hoover, S. R., Jasewicz, L. & Porges, N. (1954) *Proc. 9th Ind. Waste Conf.*, Purdue Univ. 71
- (10) Hoover, S. R., Jasewicz, L. & Porges, N. (1954) *Instruments and Automation* 27 774
- (11) Corman, J., Tsuchiya, H. M., Koepsell, H. J., Benedict, R. G., Kelley, S. E., Feger, V. H., Dworschack, R. G. & Jackson, R. W. (1957) *Appl. Microbiol.* 5 313
- (12) Cooper, C. M., Fernstrom, G. A. & Miller, S. A. (1944) *Ind. Eng. Chem.* 36 504
- (13) Porges, N., Pepinsky, J. B., Hendler, N. C. & Hoover, S. R. (1950) *Sewage and Ind. Wastes* 22 318

SUMMARY

A strain of *Saccharomyces fragilis* is capable of rapidly converting whey constituents to yeast protein. Cottage cheese whey must be fortified with 0.5% $(\text{NH}_4)_2\text{SO}_4$, 0.5% K_2HPO_4 and 0.1% yeast extract to give maximal yeast growth. Heat deproteinization of whey is unnecessary. Complete lactose removal from the whey is observed in 3-4 h.

Nitrogen assimilation is completed within the first 3 h and, unless extra nitrogen is added, further growth occurs at the expense of cell N.

Rapid growth and sugar utilization is accomplished by seeding with a quantity of yeast equal in dry weight to approximately 30% of the dry weight of the sugar in the whey and most important by supplying sufficient oxygen. Carbon dissimilation and cell respiration require approximately 14 l. of O₂/l. of medium. On the basis of: $C_{12}H_{22}O_{11} + 4O_2 \longrightarrow 4CO_2 + 3H_2O + 8(CH_2O)$, the 40 g lactose in 1 l. whey should require 10.5 l. O₂. The remaining 3.5 l. O₂ is apparently required for cell maintenance and oxidation of intermediary products formed from dissimilation of nitrogenous substances.

Oxygen consumption curves show that under the conditions described, approximately 4.5 mM of oxygen/min are required per l. yeast whey suspension to satisfy the peak oxygen demand. Equipment design, therefore, must take into consideration high rates of oxygen transfer.

In 15-l. propagation studies net dry weight of yeast is 52% of the weight of lactose and lactic acid present in the cheese whey.

Similar yields are obtained from cheddar, Ricotta and Italian curd whey, but minor changes in salt supplementation may be necessary.

CONVERSION RAPIDE DU PETIT LAIT EN LEVURE

RESUME

Une race de *Saccharomyces fragilis* est à même de convertir rapidement les éléments de petit lait en protéines de levure. Le petit lait de fromage blanc doit être fortifié avec 0,5% de (NH₄)₂SO₄, 0,5% de K₂HPO₄ et 0,1% d'extrait de levure afin de donner la croissance maximum à la levure. Il ne faut pas procéder à la déprotéinisation de la levure par la chaleur. En 3-4 h, on constate que le petit lait perd tout son contenu en lactose. L'assimilation de l'azote est achevée endéans les premières 3 h et, à moins d'ajouter de l'azote supplémentaire, la croissance s'effectue au dépend de la cellule N.

On obtient une croissance rapide et l'utilisation du sucre en ajoutant une quantité de levure égale en poids à sec à environ 30% du poids à sec du sucre dans le petit lait, et, surtout, en fournissant une quantité suffisante d'oxygène. La transformation du carbone et la respiration des cellules demande environ 14 l. d'O₂ par l. de la substance en question. En appliquant la formule $C_{12}H_{22}O_{11} + 4O_2 \longrightarrow 4CO_2 + 3H_2O + 8(CH_2O)$, on trouve que 40 g de lactose dans 1 l. de petit lait doivent demander 10,5 l. d'O₂. Les 3,5 l. d'oxygène qui restent sont apparamment nécessités pour la préservation des cellules et pour l'oxidation des produits intermédiaires formés au cours de la transformation des substances azotées.

La courbe de la consommation d'oxygène indique que, dans les conditions ici décrites, un litre liquide de levure de petit lait demande environ 4,5 mM d'oxygène par min afin de pourvoir à la plus haute consommation d'oxygène. Le projet d'installation doit donc tenir compte du taux élevé du transfert d'oxygène.

Dans les études de reproduction de 15 l., le poids à sec de la levure correspond à 52% du poids de lactose et de l'acide lactique présent dans le petit lait de fromage.

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Le petit lait de Cheddar, de Ricotta et du caillé Italien donne un rendement similaire, mais il est possible que la quantité de sels ajoutés doive être modifiée légèrement.

SCHNELLE UMWANDLUNG VON MOLKE ZU HEFE

ZUSAMMENFASSUNG

Ein Stamm von *Saccharomyces fragilis* besitzt die Fähigkeit Molke Bestandteile schnell in Hefeprotein unzuformen. Quarkkäsemolke muss mit 0.5% $(\text{NH}_4)_2\text{SO}_4$, 0.5% K_2HPO_4 und 0.1% Hefeextrakt verstärkt werden, damit Maximalwachstum der Hefe stattfinden kann. Hitze Deproteinierung der Molke ist unnötig. Völlige Entfernung des Milchzuckers von der Molke kann nach 3–4 Std. beobachtet werden. Assimilation von Stickstoff ist nach Ablauf der ersten 3 Stunden vollzogen und—ausser wenn Stickstoff hinzugefügt wird—weiteres Wachstum vollzieht sich auf Kosten der Zelle N.

Schnelles Wachstum und Zuckerverwertung erzielt man mit einer Hefequantität die im Trockengewicht ungefähr 30% des Trockengewichts vom Zucker in der Molke entspricht und am wichtigsten dabei ist die Zufuhr von genügend Sauerstoff. Dissimilation von Kohlenstoff und Zellatmung brauchen ungefähr 14 l. von O_2 pro l. Nährboden. Auf der Basis von: $\text{C}_{12}\text{H}_{22}\text{O}_{11} + 4\text{O}_2 \longrightarrow 4\text{CO}_2 + 3\text{H}_2\text{O} + 8(\text{CH}_2\text{O})$, 40 Gramm Milchzucker in einem Liter Molke sollten 10.5 l. O_2 benötigen. Der Rest von 3.5 l. Sauerstoff wird scheinbar für Aufrechterhalten der Zelle und Oxidation der Zwischenprodukte, die durch Dissimilation stickstoffhaltiger Stoffe geformt werden, benötigt.

Sauerstoffsverbrauchskurven zeigen an, dass unter diesen beschriebenen Umständen, ungefähr 4,5 mM.-Gew. von Sauerstoff per Minute notwendig sind, damit 1 l. Hefemolke Suspension die Maximumsauerstoff Anforderung befriedigt. Daher muss bei Entwurf der Anlagen der hohe Grad der Sauerstoffübertragung mit in Betracht gezogen werden.

In 15 l. Fortpflanzungskulturen belüftet sich das Net Trockengewicht von Hefe auf 52% des Milchzuckers und der anwesenden Milchzuckersäuren in der Käsemolke.

Man erhält ähnliche Erträge von Cheddarkäse, Ricottakäse und italienischer Quarkmolke, jedoch müsste man eventuell kleine Änderungen in der Salzergänzung vornehmen.