Conversion of Cheese Whey and Whey Permeate to Oil and Single-Cell Protein[1]

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ABSTRACT

Cheese whey and ultrafiltration permeate were used as media for producing oil and single-cell protein with two strains each of the yeasts *Candida curvata* and *Trichosporon cutaneum*. Optimum temperatures for cell multiplication and oil production by the four yeasts ranged from 28 to 33 C. Optimum pH's for cell multiplication were from 5.4 to 5.8. The optimum pH for oil production differed slightly from that for cell multiplication. Greater cell numbers were obtained if ammonium hydroxide was added to the medium, and mineral supplements also were sometimes stimulatory. Fermentation of whey by these organisms for 60 h reduced the chemical oxygen demand of the medium by a maximum of 85%, produced (depending on the organism) 6.3 to 9.3 g of oil/liter of whey, and left most of the protein unused. Fermentation of permeate for 72 h reduced chemical oxygen demand 95% and produced (depending on the organism) 4.0 to 15.6 g oil, 19.6 to 26.8 g cells, and 2.2 to 3.2 g single-cell protein/liter of permeate. The two strains of *C. curvata* were the most efficient in producing oil and reducing oxygen demand. The fat contained oleic (∿50%), palmitic (∿30%), stearic (∿15%), and linoleic (∿8%) acids. The cell protein was rich in methionine and other essential amino acids except that the two *C. curvata* strains were deficient in lysine.

Received April 21, 1978.

[1] Journal Paper No. J-9133 of the Iowa Agriculture and Home Economics Experiment Station, Ames. Project 2144.

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INTRODUCTION

The disposal of cheese whey continues to be a major problem for the industry (3). Recovery of valuable whey proteins by ultrafiltration is commercially feasible, but the resulting permeate is, like whole whey, difficult to dispose of profitably (3). Incorporation of condensed or dried whey and permeate into animal feeds has been a frequent method of disposal.

Although fermentation of whey to produce single-cell protein has been suggested (16), fermentation to produce lipid-rich microorganisms has not been considered seriously for many years. Fermentation to produce oil is attractive for several reasons. Conversion of carbohydrate to a lipid retains most of the energy of the carbohydrate and, compared with growth, requires few cofactors and minimal metabolic machinery. The fat can be harvested easily with the cells and extracted and refined by existing technologies. The residual cell mass can be incorporated into feeds or used as a source of single-cell protein. With adequate reduction of chemical oxygen demand (COD), disposal of the spent medium is no longer a serious problem.

This report describes the discovery of yeasts capable of rapid fermentation of whey and permeate to oil with effective COD reduction.

MATERIALS AND METHODS

Cultures

Fungi able to ferment lactose and produce oil were not readily available. A representative sampling of fungi reported to produce oil from other substrates (15, 16) was obtained from the USDA/ARS Northern Regional Research Center, Peoria, IL and the Iowa State University Department of Botany and Plant Pathology. Fungi were collected from environmental samples in Ames, IA. Samples were applied on potato dextrose agar acidified to pH 4.5 with tartaric acid to select for yeasts and

molds. Colonies were observed microscopically for fat production. Fat-producing isolates were purified by consecutive streaking on potato dextrose agar. These isolates and the known stock cultures were screened for their ability to produce oil from whey. Organisms that grew well at 25 C on whey agar (6.5% dried whey + 2% agar) and also produced oil as evidenced by staining with oil red O (Pfaltz and Bauer, Inc., Flushing, NY) were evaluated in fermentor trials. Oil red O was prepared as a saturated solution in isopropanol and diluted 1:1 with water before use as a wet mounting medium.

Fermentation Media

Dried sweet whey (formula 521, Associated Milk Producers) and condensed whey permeate (Mississippi Valley Milk Producers, Luana, IA) both reconstituted to 6.5% solids, were used. The permeate was prepared from Swiss cheese whey with a Dorr-Oliver ultrafilter equipped with a polysulfone membrane with a 24,000-dalton rejection limit. The permeate was condensed in a Henze double effect evaporator and frozen until used.

Fermentor-Description

A 14-L fermentor (Microferm, New Brunswick Scientific Co., New Brunswick, NJ) equipped with air flow, temperature, and aggitation controls was used. The pH was monitored with a Radiometer combined electrode (Radiometer, Copenhagen, Denmark) and controlled with a Radiometer automatic titrator (Type TTT 1) by adding concentrated HCl or 50% (wt/vol) NaOH. Dissolved oxygen was measured with a steam sterilizable probe (6). Antifoam (Medical antifoam AF emulsion, Dow Corning, Midland, MI) was added when needed.

The medium, and pH and dissolved oxygen probes were sterilized separately and placed in the sterile fermentor jar immediately before a fermentation trial. The pH and temperature were adjusted, the medium was saturated with air, and 10% inoculum was added.

Gas Measurements

When the air supply to the fermentor was stopped, the dissolved oxygen concentration fell linearly with time. The slope of this decrease represented the rate of oxygen consumption of the microorganisms. Consumption rates of O_2 were calculated by assuming the concentration of O_2 at saturation in the media to be equal to that of O_2 in air-saturated water (5.78 $cm^3/1000\ cm^3$).

Carbon dioxide in the exit gas was measured by pumping an aliquot through a gas dispersion tube into 75 ml of .5 N $BaCl_2$ maintained at pH 10.0 by an automatic titrator adding .4 N NaOH. The $BaCl_2$ solution was changed after every 50 ml NaOH. Recovery of CO_2 was not quantitative and varied with aeration rates and concentration of CO_2. The CO_2 recovery was calibrated with known gas mixtures.

Nutrient Optimization

Stimulation of growth was slight in shake flasks when yeast extract, $(NH_4)_2SO_4$, and certain metal salts were added to the medium, but the changes in growth were small, and further optimization was in the fermentor.

It was assumed that if a physical or nutritional parameter was changed during fermentation, its beneficial or detrimental effect could be judged by an increase or decrease of the rate of growth or respiration (4, 9). After a nutrient was added, observations were made of changes in cell numbers and respiratory gases for about 1.5 h. If the nutrient was stimulatory, changes were within 15 min during logarithmic growth and 30 min in stationary phase. New growth and/or respiration rates would persist until the nutrient was exhausted; then the rate would be as it was previously. The concentration of the rate-limiting nutrient required for growth was calculated from the cell number at the time of testing and the maximum cell number expected. This calculated amount was increased 5-fold over the rate-limiting concentration. A nutrient stimulating oil deposition in the stationary phase was added at 10-fold the concentration giving maximum stimulation. These amounts afforded an adequate excess, and further stimulation by these nutrients late in the fermentation could not be demonstrated. After 1.5 h of stabilization, search for another stimulatory nutrient was begun.

Optimization of Physical Conditions

The pH, temperature, air flow, and stirring rates for growth and oil production were optimized as for nutrient optimization. Small

changes in physical conditions caused an immediate change in respiration rates or phase adjustment (12) which stabilized in about 30 min. After a new rate was established, it was monitored for about 1.5 h. Optimization during the fattening (stationary growth) stage required more time for rate stabilization. Aeration rates from .5 to 14 liter air/liter medium/min (stirring rate 5000 rpm), pH's between 5.0 and 6.2, and temperatures between 25 and 37 C were explored. Those pH's outside this range were inhibitory as were pH changes greater than .4 unit. Temperature changes were in 2° increments starting from 25 C.

Analyses

Periodically, 200-ml samples were withdrawn from the fermentor, and a portion was centrifuged for 30 min at 9,000 × *g* at 5 C in a Sorvall RC2-B refrigerated centrifuge. Cells were washed once in distilled water, recentrifuged, and frozen until analyzed. A Max Levy mold-counting chamber was used for direct microscopic counts on medium diluted, usually 1:10, with water. Cells with buds and pseudomycelium were counted as two cells when the daughter cells were >.5 the size of the mother cell. For dry weight, 2- to 10-ml samples of fermentation liquor were filtered through a tared .45-μ membrane filter (Millipore Corporation, Bedford, MA), the filter was washed with 5 ml water, dried under vacuum for 12 h at 45 C, and weighed. The weight of a blank made with uninoculated fermentation media was subtracted from the dry weight determinations. Nitrogen was determined by the Kjeldahl method. Chemical oxygen demand (COD) was determined on centrifuged media by chromate oxidation (1). Glucose, galactose, and lactose were determined in the centrifuged medium by the enzymatic method of Hettinga et al. (5). Only glucose was determined in most samples, because this proved the more reliable procedure, and there was little free galactose.

A modification of Stewart's method (13) was used for animo acid analysis. About 1 g cells and 4 ml 6 *N* HCl were autoclaved at 105 C for 24 h in a sealed lyophilization vial. The vial contents were transferred with 50 ml water to a separatory funnel and extracted with 20 ml of petroleum ether to remove lipid. To remove acids and neutral substances, the water layer was passed through a 2.5 × 15 cm column of 5 g Dowex 50-X8 cation exchange resin at 5 ml/min, and the column was washed with water until the pH of the effluent was 6.0. The column was eluted with 5 ml 6 *M* NH_4OH and washed again until the pH of the effluent was 6.0. To remove bases, the eluate from the cation resin was passed through a column containing 5 g of Amberlite IRA-400 anion exchange resin (OH) at 5 ml/min. The column was eluted with 5 ml 6% $(NH_4)_2CO_3$ and washed with distilled water until the pH reached 6.0. The eluate was dried on a rotary vacuum evaporator, weighed, and amino acids were determined with a Beckman amino acid analyzer (Beckman Instruments, Fullerton, CA). The recovery of hydrolyzed bovine serum albumin, arginine, lysine, and glutamic and aspartic acids in the ion exchange clean-up was 96% or more.

In whey fermentation medium, dry-weight nitrogen, and amino acids were not determined because of interference by whey proteins.

Fatty acids were extracted with hexane from an alcoholic KOH digest of wet cells (11). The fatty acids were converted to methyl esters according to Luddy et al. (8), and the methyl esters were analyzed by gas liquid chromatography on a Beckman GC-5 chromatograph equipped with a flame ionization detector and a 180 × .3-cm stainless steel column packed with 15% EGSSX on 100/120 mesh Chromosorb P (Applied Science Lab. Inc., State College, PA). Oven temperature was 185 C, and the carrier gas was helium at 50 ml/min. Peak areas were integrated by an Autolab 1600 (Spectro Physics, Mountain View, CA). A standard ester mixture was used to calibrate the instrument.

Determinations of COD of dialyzed, spent culture medium were used to indicate slime production by yeasts during fermentation. A 10-ml aliquot of centrifuged (9,000 × *g* for 30 min) culture medium was transferred into prewashed dialysis tubing (Union Carbide Corporation, Chicago, IL) and the ends were secured. The sample was dialyzed against two changes of distilled water with a ratio of sample to dialysate of 1:500. After 18 h of dialysis at 8 C, the samples were transferred to a 25-ml graduated cylinder and brought to volume. Twenty milliliters of a 3:100 dilution were used for analysis of COD. Protein determinations

were performed on whey dialysates to correct for nondialyzable whey proteins.

RESULTS AND DISCUSSION

The search for oil-producing fungi that would grow efficiently on whey among species reported to produce oil on other media was unsuccessful because all grew poorly on whey agar even when it was supplemented with .1% yeast extract. All promising organisms were yeasts isolated from dairy floors and drains. Dairy plant environments exerted selective pressure for organisms able to use lactose; also, oil accumulation may be an advantage to organisms exposed intermittently to lactose. Four organisms that grew rapidly in whey fermentation, produced large oil droplets, and required few nutritional additions to whey were selected for further study. They were identified (7) as two strains each of *Trichosporon cutaneum* and *Candida curvata*. All require thiamine in minimal media, but the thiamine in whey was sufficient. One strain, *T. cutaneum* 24, grew slowly on NH_3 as a nitrogen source but grew well in whole whey or with amino acid supplements to minimal media.

Fermentation occurred in two phases. In the first, cell numbers increased, but only small amounts of oil accumulated. In the second phase, oil accumulated with little increase in cell numbers.

Optimization of Fermentation Conditions

The optimum physical conditions for fermentation of whey and permeate are in Table 1. The optimum temperature for growth and fattening was the same for an individual strain but varied among strains. The optimum temperature was near 30 C in all instances. This was expected because the initial screening was likely to select mesophiles. Optimum pH for growth and fattening was different for an individual strain.

During cell multiplication, aeration was maintained at a rate sufficient to maintain dissolved oxygen at 80% saturation. For this, .25 liter/min per liter medium was sufficient. The optimum aeration rates for fattening were higher than for growth. Oxygen-utilization rates during fattening were similar to the maximum rate for growth, but maximum CO_2 production was at aeration rates about 3.5 times those required for maximum O_2 utilization. To avoid inhibition of fat production, aeration rates were maintained at 1 liter/min per liter medium in subsequent fermentations. Possibly, increased aeration may stimulate oil accumulation by removal of volatile toxic products (such as CO_2). In general, for all four strains, physical optimization increased fermentation rate but had little effect on the fat or cell yields.

Yeast extract stimulated metabolic rate and final cell population of all four yeast strains in preliminary trials. This stimulation could be duplicated by metal ions and ammonium hydroxide (Table 2). Metal ions primarily affected rate of cell multiplication, but ammonium hydroxide increased maximum cell numbers. Whey contains .9% protein (14), but after autoclaving only .4% remained in solution. Seemingly this was inadequate or unavailable for growth.

TABLE 1. Optimum physical conditions for fermentation by four yeasts on whey or permeate.

	Physical condition					
	Logarithmic growth			Oil-producing phase		
	pH	Temperature[a]	Aeration[b]	pH	Temperature[a]	Aeration[b]
C. curvata R	5.8	30	.25	5.2	30	>.70
C. curvata D	5.4	28	.25	5.8	28	>.70
T. cutaneum 24	5.4	28	.25	5.8	28	>.40
T. cutaneum 40	5.7	33	.25	5.6	33	>.40

[a]Temperature = °C.

[b]Aeration = liters/min per liter medium.

TABLE 2. Amounts of nutrients added to 1 liter of whey or permeate for optimum growth rates, maximum cell numbers, and oil accumulation by four yeasts.[a]

Substance	*C. curvata*		*T. cutaneum*	
	R	D	24	40
NH_4OH (15.5% NH_3)	1.0 ml	.3 ml	1.0 ml	1.0 ml
$MnSO_4$	2.5 mg[a]	...	2.0 mg[b]	...
$MgSO_4$	...	...	3.0 mg[b]	55.0 mg[a]
$FeSO_4$	...	...	10.0 mg[a]	...
K_2HPO_4	5.3 mg[a]	...	...	...

[a]These growth stimulants were increased by a 5-fold safety factor in fermentor trials.

[b]These oil-production stimulants were increased by a 10-fold safety factor in fermentor trials.

After maximum cell populations had been reached, nutrients affected only the rate of oil production for *T. cutaneum* 24 which was stimulated by magnesium and manganese ions.

Whey Fermentations

Fermentations in whole whey under optimized conditions indicated that the two *C. curvata* strains were the most successful (Table 3). Both strains had short generation times, large cell numbers, produced oil rapidly, and attained maximum oil yields in minimum fermentation time. The *T. cutaneum* strains had oil coefficients (100 × *g* oil/g lactose consumed) comparable to that of *C. curvata* R, but their rates of oil production were undesirably slow, resulting in prolonged fermentation.

Of the two *C. curvata* strains, R had the larger population, rate of fat accumulation, and shorter fermentation time (Table 3). Strain D

TABLE 3. Fermentation of whey under optimized conditions with *C. curvata* strains R and D and *T. cutaneum* strains 24 and 40.

	C. curvata		*T. cutaneum*	
	R	D	24	40
Fermentation time (h)	60	72	60	96
Generation time (h)	1.31	1.05	1.88	1.60
Generations/h	.76	.95	.53	.63
Maximum cell no. × 10^8/ml	6.5	5.2	6.5	3.4
Lactose[a] start	49.8	50.0	47.1	50.2
Lactose[a] used	48.8	47.5	46.5	49.8
Rate oil[b] produced	.263	.179	.093	.084
Rate lactose[c] used	3.2	3.2	3.8	1.7
Soluble protein[a] start	5.5	4.4	5.1	4.1
Soluble protein[a] end	4.6	3.9	5.9	4.7
COD mg/liter start	59,000	65,000	60,000	62,000
COD mg/liter end	10,000	10,000	9,000	9,000
COD % reduction	71	85	85	85
Oil produced[a]	7.3	9.3	6.3	7.8
Oil coefficient[d]	15	20	14	16

[a]Grams/liter medium.

[b]Maximum rate (mM/h per liter) was during the fattening period.

[c]Maximum rate (g/h per liter) was usually at <10 h.

[d]Oil coefficient = 100 × *g* oil produced/g lactose used.

produced the most fat and had the greatest oil coefficient but a longer fermentation time. Comparison of the results for the two *C. curvata* strains as well as the two *T. cutaneum* strains illustrates the relation between cell numbers and oil yields. From the same amount of substrate, larger numbers of cells produce less oil; lower cell numbers result in longer fermentation times and greater oil yields. Both *T. cutaneum* strains solubilized some of the precipitated whey proteins and released soluble nitrogen into the medium. Presumably, they used some of the nitrogen during growth.

The residual COD was about the same for all strains. Lactose remaining at the end of the fermentation was significant only for *C. curvata* D. The soluble protein accounted for roughly 50% of the residual COD.

Some yeasts produce an extracellular polysaccharide, so we tested to see if slime contributed to the residual COD. All of the yeasts produce some slime (maximum about 5,000 mg/liter COD) during growth. The slime seemingly was metabolized during the oil production phase so that at the end of the fermentation, slime accounted for only 500 mg/liter COD.

Possibly, further reduction of COD was prevented by accumulation of inhibitors in the medium. To test this, logarithmically growing cells were harvested from whey, washed twice, and inoculated into spent medium. Very slight growth occurred, but no oil was produced unless additional lactose was added. Seemingly, oil production ceased in the fermentation because lactose was exhausted, and remaining components could not be used.

Fermentation of whey by these yeasts was relatively inefficient and left substantial residual COD which could not be discharged into sewers and streams without cost. Other attempts (10) at fermentation of wastes have reduced COD about 85%. Since a substantial portion of the residual COD was unused protein, additional studies were on ultrafiltered whey.

Whey Permeates

Whey permeates with a starting COD of 60,000 to 70,000 mg/liter and .2% nitrogen were fortified with 10 to 11 ml of NH_4OH (15.5% NH_3) and mineral supplements as in Table 2 and fermented under optimized conditions. Results are in Fig. 1 through 8, and Table 4.

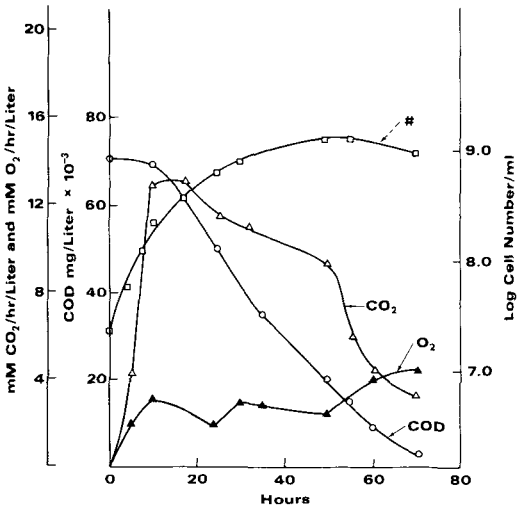

FIG. 1. Respiration rate, cell numbers, and COD during the fermentation of whey permeate by *C. curvata* R. □ Log cell number, ○ COD, △ CO_2, ▲ O_2.

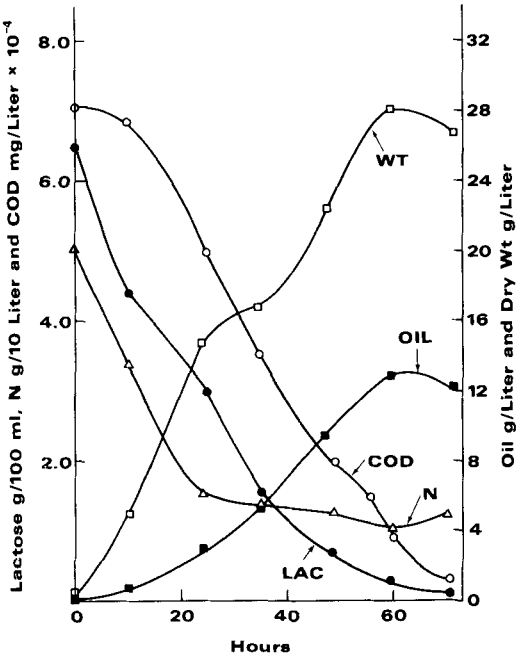

FIG. 2. Lactose concentration, COD and weight of oil, dry cells, and soluble nitrogen during fermentation of whey permeate by *C. curvata* R. ● Lactose, ○ COD, ■ oil, □ cell weight, △ nitrogen.

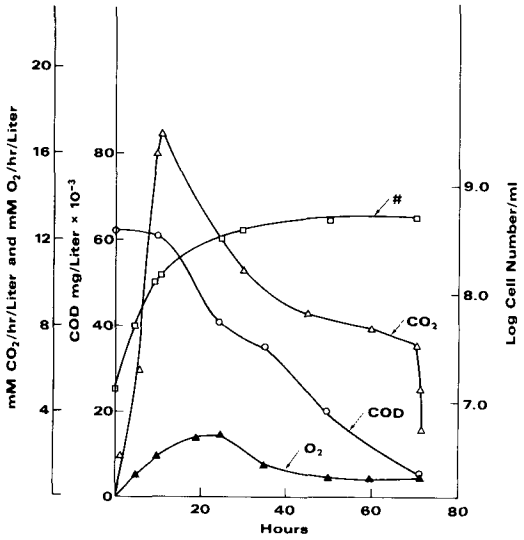

FIG. 3. Respiration rate, cell numbers, and COD during the fermentation of whey permeate by *C. curvata* D. □ Log cell number, ○ COD, △ CO_2, ▲ O_2.

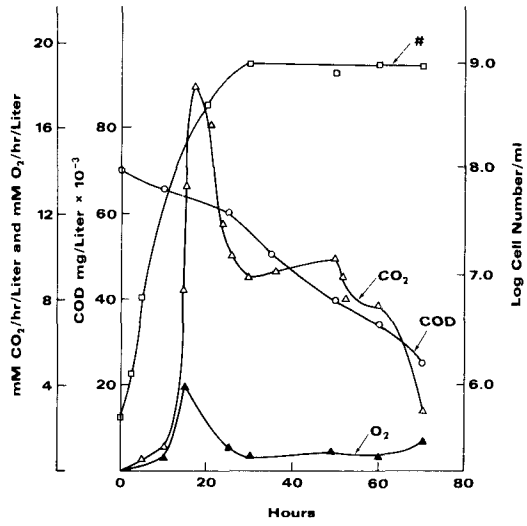

FIG. 5. Respiration rate, cell numbers, and COD during the fermentation of whey permeate by *T. cutaneum* 24. □ Log cell number, ○ COD, △ CO_2, ▲ O_2.

Rapid cell growth, lactose utilization, and oil production resulted in rapid reduction of COD and 72-h fermentation times for all four yeasts. Generation times were slightly longer for permeate than whey which indicated that some nutrients may have been obtained from the whey proteins or been removed during ultrafiltration. Maximum cell populations were similar

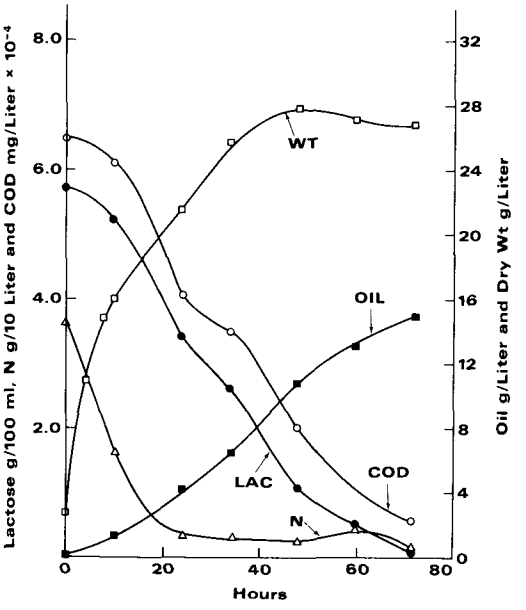

FIG. 4. Lactose concentration, COD and weight of oil, dry cells, and soluble nitrogen during fermentation of whey permeate by *C. curvata* D. ● Lactose, ○ COD, ■ fat, □ cell weight, △ nitrogen.

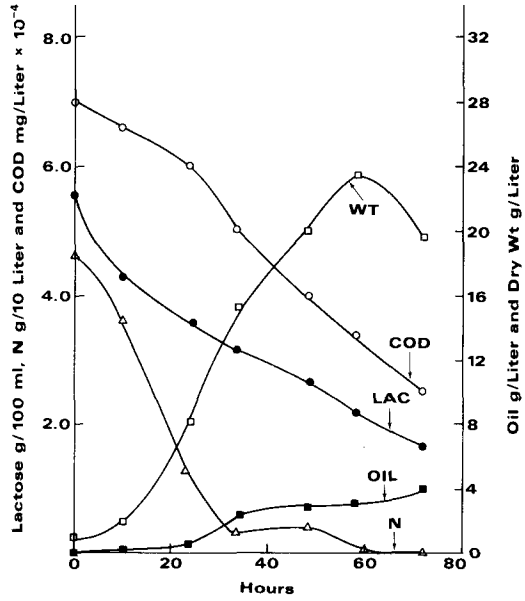

FIG. 6. Lactose concentration, COD and weight of oil, dry cells, and soluble nitrogen during fermentation of whey permeate by *T. cutaneum* 24. ● Lactose, ○ COD, ■ fat, □ cell weight, △ nitrogen.

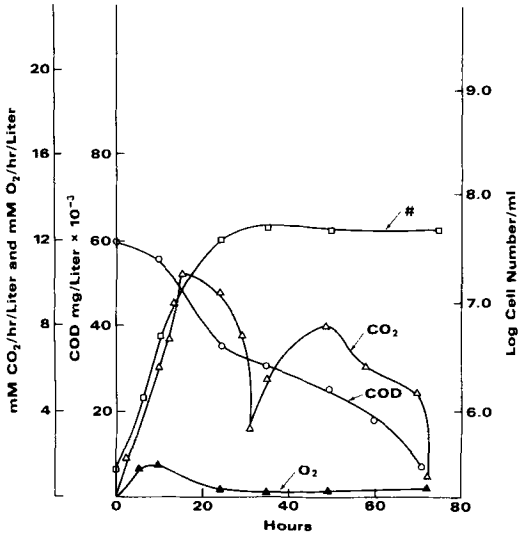

FIG. 7. Respiration rate, cell numbers, and COD during the fementation of whey permeate by *T. cutaneum* 40. □ Log cell number, ○ COD, △ CO_2, ▲ O_2.

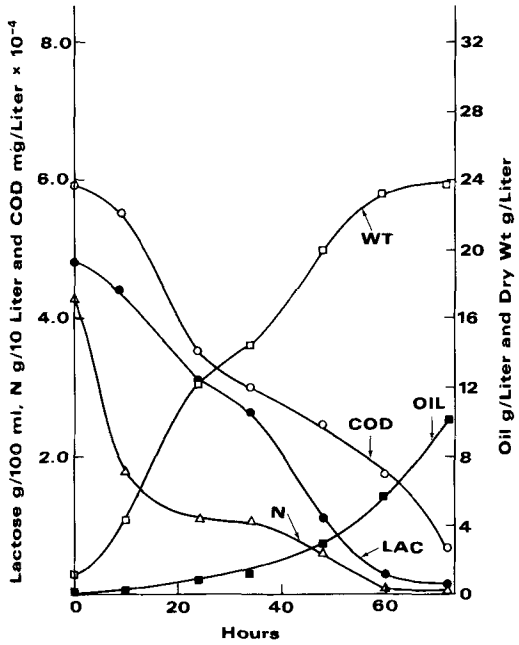

FIG. 8. Lactose concentration, COD, and weight of oil, dry cells, and soluble nitrogen during fermentation of whey permeate by *T. cutaneum* 40. ● Lactose, ○ COD, ■ fat, □ cell weight, △ nitrogen.

for whey and permeate except for greater numbers for *C. curvata* R in permeate.

For all four yeasts, the respiratory quotient (RQ = moles CO_2 produced/moles O_2 consumed) (Fig. 1, 3, 5, 7) was about 4.0 during the growth phase, rather than 1 which the complete combustion of lactose would yield. High RQ's may be caused by oil production during growth or production of an incompletely oxidized product. Oil production, although greater at later fermentation times, was continuous throughout fermentation (Fig. 2, 4, 6, 8) and could account for the high RQ because little oxygen is required for fat production. The presence of a distillable metabolic end product such as ethanol or acetaldehyde could not be demonstrated by gas chromatography. Also, the pH rose rapidly at the beginning of the fermentation indicating that lactic acid was used and acid end products were not produced.

Reduction of COD was fairly linear and followed lactose utilization for all yeast strains during the fattening phase and during the growth phase for the two *T. cutaneum* strains (Fig. 6, 8). For *C. curvata* strains there was an initial lag in reduction of COD but not in lactose utilization (Fig. 2, 4). These strains both produce a soluble, extracellular, nondialyzable slime during growth which might account partially for the initial lag in reduction of COD. This soluble slime partially disappeared during fattening and <1000 mg/liter COD were present at 60 h.

The rate of lactose utilization was greatest for *C. curvata* D (Table 2), but all four strains maintained a fairly linear utilization rate during the fermentation (Fig. 2, 4, 6, 8). When the lactose concentration fell at the end of the fermentation to 1.5 mg/ml for the *C. curvata* or 3.8 mg/ml for the *T. cutaneum*, production of CO_2 rate declined rapidly by 50% in 30 min (example, Fig. 3). If lactose was added at this time, the CO_2 rate of production increased until the lactose was consumed. If lactose was not added, production of CO_2 fell and consumption of oxygen increased, indicating consumption of oil for energy. Fermentation harvest was chosen to coincide with the first indication of decline in production of CO_2 to obtain maximum oil. Also, after production of CO_2 declined and lactose was depleted, some cell lysis occurred for all yeasts but *T. cutaneum* 40. This caused an additional loss in dry

TABLE 4. Fermentations of whey permeate under optimized conditions with *C. curvata* strains R and D and *T. cutaneum* strains 24 and 40.

	C. curvata		*T. cutaneum*	
	R	D	24	40
Fermentation time (h)	72	72	72	72
Generation time (h)	1.63	1.10	1.89	2.0
Generations/h	.61	.91	.53	.50
Maximum cell no. $\times 10^8$/ml	8.7	5.2	6.5	3.7
Rate fat[a] produced	.280	.288	.064	.322
Rate lactose[b] used	2.8	3.3	1.5	2.4
Cell mass[a] production	.470	.404	.496	.484
Lactose[c] start	65.0	57.4	57.8	47.1
Lactose[c] used	63.9	56.8	45.3	45.9
Soluble protein[c,d] start	2.91	2.29	2.79	2.87
Soluble protein[c,d] used	.625	<.24	<.015	.485
COD mg/liter start	73,000	63,000	69,000	59,000
COD mg/liter end	7,000	3,000	33,000	6,800
COD % reduction	90	95	52	88
Final cell yield[c]	25.7	26.8	19.6	23.9
Oil produced[c]	13.0	15.6	4.0	10.8
Cell protein[c]	2.25	2.47	2.92	3.22
Oil coefficient[e]	20	27	9	24
% Oil dry wt	51	57	20	45
% Protein dry wt	9	9	15	13

[a]Maximum rate (g/h per liter) was usually at 40 h.

[b]Maximum rate (mM/h per liter) was during the fattening period.

[c]Grams/liter medium.

[d]This includes the nitrogen added as NH_4OH and calculated as protein (%N $\times$ 6.25 = % protein).

[e]Oil coefficient = 100 $\times$ *g* oil produced/g lactose used.

cell weight and rise in nondialyzable COD. The nondialyzable COD was significant for the two *C. curvata* strains at the end of fermentation and accounted for about 60% of the residual COD.

The accumulation of cell mass was a fairly linear process for all strains, but *C. curvata* D (Fig. 2) and the *T. cutaneum* strains (Fig. 4 and 6) accumulated fat at the expense of cell weight at the end of the fermentation. Possibly the cells were storing some component that was metabolized to fat late in the fermentation.

Based on cell weight, COD reduction, and oil production, *C. curvata* D was the most efficient, followed in order by *C. curvata* R, *T. cutaneum* 40, and *T. cutaneum* 24. The *T.*

TABLE 5. Fatty acid composition of two strains of *C. curvata* and *T. cutaneum* grown on whey permeate.

Organism	% Fatty acid				
	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$
C. curvata R	Tr[a]	31	12	51	6
C. curvata D	Tr	32	15	44	8
T. cutaneum 24	Tr	21	33	29	10
T. cutaneum 40	Tr	30	13	46	11

[a]Tr = detected but less than 1%.

TABLE 6. Amino acid composition (g/100 g protein) of hydrolyzed cells of *C. curvata* strains R and D and *T. cutaneum* strains 24 and 40.

Amino acid	*C. curvata*		*T. cutaneum*		FAO/WHO[a]
	R	D	24	40	
Lysine	1.3	Tr[b]	5.5	5.5	5.5
Histidine	.8	Tr	5.9	2.5	
Arginine	ND	ND	ND	ND	
Aspartic acid	2.7	ND	Tr	ND	
Threonine	26.6	11.9	Tr	8.7	4.0
Serine	26.3	Tr	3.9	ND	
Glutamic acid	3.1	Tr	7.6	ND	
Proline	6.3	ND	Tr	ND	
Glycine	6.3	29.4	11.8	16.0	
Alanine	9.0	7.9	16.7	1.7	
Cysteine	ND	ND	ND	ND	
Valine	4.4	17.4	8.9	23.1	5.0
Methionine	2.5	9.3	7.7	5.7	3.0
Isoleucine	3.8	8.3	9.6	13.7	4.0
Leucine	7.0	15.7	22.4	23.0	7.0
Tyrosine	ND	ND	Tr	ND	
Phenylalanine	Tr	ND	Tr	ND	
Tryptophan	ND	ND	ND	ND	1.0
Total S	2.5	9.3	7.7	5.7	3.5
Total aromatic	Tr	Tr	Tr	Tr	6.0

[a]FAO/WHO recommended protein composition as reported by Young and Scrimshaw (17).

[b]Tr = trace amounts detected (about .5 g/100 g protein).

[c]ND = not detected (below about .5 g/100 g protein).

cutaneum 24 had similar generation times and maximum cell numbers in whey and whey permeate (Tables 3 and 4). In whey permeate, however, lactose was used slower, and oil was produced inefficiently and in small amounts. This inefficiency resulted in high residual COD's (Fig. 6 and Table 4). Since the soluble protein at the end of the whey fermentation was greater than at first (Table 3), *T. cutaneum* 24 probably obtained growth factors from hydrolyzed whey proteins. Preliminary studies indicated this strain grew poorly on minimal media without amino acids. Perhaps *T. cutaneum* 24 requires amino acids for efficient fat production in whey permeate.

Fat and Protein Composition

C. curvata D reached 57% oil (dry wt) in whey permeate and produced the greatest yield, 15.6 g/liter, with the greatest efficiency (Table 4). The oil of all four yeast strains (Table 5) was rich in oleic and palmitic acids and contained smaller percentages of stearic and linoleic acids. The oil was low in polyunsaturated acids and should be stable to oxidation. Other yeasts such as *Rhodotorula gracilis* and *Debaromyces hansenii* Y-1448 have similar fatty acid compositions (15).

The percentages of protein in the yeasts were only about 9 to 15% but on a fat free basis would be nearer 20%. The proteins were of faily high quality based on amino acid composition (Table 6). The protein produced by the *T. cutaneum* strains was similar to reported values (2) and compares favorably with FAO/WHO standards based on egg protein (17). The two *C. curvata* strains are low in lysine but are adequate in other amino acids. Three of the yeasts are high in methionine.

In summary, fermentation of whey by these four yeasts was relatively inefficient and produced low yields of oil. Fermentation of whey permeate was much more successful. Of the four yeasts, *C. curvata* D produced the most oil, reduced the COD by 95%, and required the fewest additions of nutrients. The fermentation of whey permeate to a human

food oil and protein-rich animal feed (11) is a feasible approach to a serious pollution problem in the dairy industry.

REFERENCES

1 American Public Health Association, Inc. 1967. Standard methods for the examination of water and waste water. 12th ed. Amer. Public Health Ass., Inc., New York, NY.

2 Atkin, C., L. D. Witter, and Z. J. Ordal. 1967. Continuous propagation of *Trichosporon cutaneum* in cheese whey. Appl. Microbiol. 15:1345.

3 Emmons, D. B., H. W. Modler, and J. Holme. 1974. Whey processing – some economic aspects. Page 8 *in* Proc. Whey Utilization Symp. Canada Dept. of Agr., Ottawa.

4 Herbert, D., R. Elsworth, and R. C. Telling. 1956. The continuous culture of bacteria; a theoretical and experimental study. J. Gen. Microbiol. 14:601.

5 Hettinga, D. H., A. H. Miah, E. G. Hammond, and G. W. Reinbold. 1970. Sensitive enzymatic method for determining glucose, galactose and lactose in Cheddar cheese. J. Dairy Sci. 53:1377.

6 Johnson, M. G., J. Borkowski, and C. Engblom. 1964. Steam sterilizable probes for dissolved oxygen measurement. Biotechnol. Bioeng. 6:457.

7 Lodder, J. 1970. The yeasts. North-Holland Publishing Co., Amsterdam, The Netherlands. 1385 pp.

8 Luddy, F. E., R. A. Barford, S. E. Herb, and P. Magedman. 1968. A rapid and quantitative procedure for the preparation of methyl esters of butter oil and other fats. J. Amer. Oil Chem. Soc. 45:549.

9 Mateles, R. I., and E. Battat. 1974. Continuous culture used for media optimization. Appl. Microbiol. 28:901.

10 Mickle, J. B., W. Smith, D. Halter, and S. Knight. 1974. Performance and morphology of *Kluyveromyces fragilis* and *Rhodotorula gracilis* grown in Cottage cheese whey. J. Milk Food Technol. 37:481.

11 Moon, N. J., and E. G. Hammond. 1978. Oil production by fermentation of lactose and the effect of temperature on the fatty acid composition. J. Amer. Oil Chem. Soc. Accepted.

12 Sterkin, V. E., I. M. Chirkov, and V. A. Samoylenko. 1973. Study of transitional stages in continuous culture of microorganisms. Page 53 *in* Advances in microbial engineering. Part 1. B. Sikya, A. Prokop, and M. Novak, ed. John Wiley and Sons, New York, NY.

13 Stewart, R. R. 1975. Analytical methods for yeasts. *In* Methods in cell biology. Vol. 2. Yeast cells. D. M. Prescott, ed. Academic Press, New York, NY.

14 Webb, B. H., A. H. Johnson, and J. A. Alford. 1974. Fundamentals of dairy chemistry. 2nd ed. AVI Publishing Co., Westport, CT. 929 pp.

15 Weete, J. D. 1974. Fungal lipid biochemistry. Plenum Press, New York, NY. 393 pp.

16 Woodbine, M. 1959. Microbial fat: Microorganisms as potential fat producers. Prog. Ind. Microbiol. 1:181.

17 Young, V. R., and N. S. Scrimshaw. 1975. Clinical studies on the nutritional value of single cell proteins. Page 564 *in* Single cell protein. II. S. R. Tannenbaum and D. I. C. Wang, ed. MIT Press, Cambridge, MA.