Review of Processes and Products for Utilization of Lactose in Deproteinated Milk Serum

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ABSTRACT

Identification of the most cost effective means for the utilization of lactose in deproteinated milk serum (e.g., permeate) is of interest to most dairy companies worldwide. Typical gross composition and mineral data are provided for whole milk and skim milk permeates and Cheddar cheese and lactic casein whey permeates. A review of a diverse range of technically feasible processes and products for utilization of lactose in permeate is outlined under the categories of lactose recovery processes, enzymatic and chemical modifications, and fermentation. In addition, manufacture of crystalline lactose is reviewed extensively, and results of studies at the New Zealand Dairy Research Institute are presented. Literature reviews and experimental data also are given for production of hydrolyzed lactose syrups, acetone/butanol/ethanol fermentation, and anaerobic digestion of permeate for production of methane. Subjective comment and comparison are provided of market, technical, and economic aspects associated with fermentative production of yeast, solvents, methane, food acids, enzymes, gums, and amino acids.

INTRODUCTION

The disaccharide lactose (4-O-(β-D-galactopyranosyl)-D-glucose) (Figure 1), or "milk sugar" as it commonly is known, is the characteristic carbohydrate of cows' milk, and in whole milk is up to 40% of the total solids content. In skim milk and whey, lactose is the major solid component accounting for approximately 50% and 70 to 80%, respectively. Because of the chemical, physical, and functional properties of lactose (113), it is of major importance in the manufacture and utilization of dairy products. Moreover, because most of the lactose enters the whey during the manufacture of cheese and casein, in most countries it poses a major environmental problem.

The large mass of whey produced during the manufacture of cheese and casein (e.g., typically 7 to 9× and 25× the mass of cheese and casein, respectively), the increased capacity of modern cheese and casein plants, and the high biological oxygen demand (BOD) of whey (e.g., BOD ∿35,000 mg/liter) make it necessary for dairy companies either to process whey or to dispose of it in some environmentally acceptable manner.

Extraction of proteins from whey by ultrafiltration has become a relatively well-established process. In the future, it might be expected that ultrafiltration and other protein extraction processes (e.g., ion exchange) will be used with increasing frequency as means of improving the financial return from processing of whey. The extraction of protein, however, does little to relieve problems inherent in whey production because at best (e.g., production of 35% protein powder), the volume and BOD are reduced only by approximately 15 and 30%. Furthermore, it is possible that ultrafiltration also will find future application for concentration of whole milk and skim milk (and possibly buttermilk) for the manufacture of cheese, casein, and high-protein milk products. These processes, too, could yield copious volumes of lactose and mineral rich, protein depleted, by-product streams, (i.e., deproteinated milk serum [DPMS]). The concentration of whole milk on the farm by ultrafiltration has been suggested (5) as one method of alleviating the problem of processing DPMS at the dairy processing plant because in this case the DPMS could be left on the farm to feed stock.

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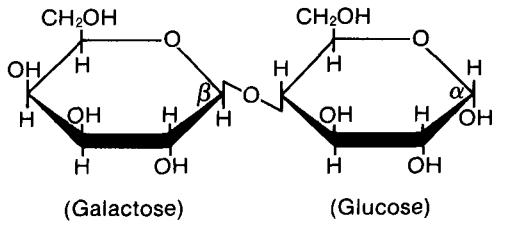

4-O-(β-D-galactopyranosyl-D-glucopyranose)

Figure 1. Lactose (milk sugar) 4-0-(β-D-galactopyranosyl-D-glucopyranose).

The challenge that confronts dairy companies worldwide is to identify suitable processes to utilize lactose in DPMS for maximum economic return. The unique political, economic, technical, and marketing factors that determine individual company policy necessitate such decisions be case by case.

Short (147) reviewed prospects for utilization of deproteinated whey in New Zealand, and Coton (29) recently examined economic aspects of a limited number of processes.

This paper presents a contemporary overview of the vast number of technically feasible processes that may have potential value for utilization of lactose in DPMS. Furthermore, a range of processes that recently have been studied in New Zealand are highlighted and evaluated critically.

Composition of Deproteinated Milk Serum

It is not possible to supply precise data on the composition of DPMS as they are influenced by many factors, including milk production, type of process, and operating conditions used to remove protein, and analytical methods. Some typical gross composition and mineral composition data for permeate derived from ultrafiltration of milk and whey are in Table 1. The gross composition data represent means and standard deviations from a range of samples manufactured by a variety of ultrafiltration equipment at the New Zealand Dairy Research Institute. No attempt has been made to close the mass balance.

Compositions of sweet (e.g., nonacid) permeates are similar particularly if considered on a dry basis. In these DPMS lactose accounts for approximately 90% of the total solids, whereas for lactic permeate lactose comprises only approximately 76% of the total solids. The remaining solids in the lactic permeate are made up by increased concentrations of nitrogen (particularly nonprotein nitrogen), minerals (particularly calcium and phosphate), and lactic acid.

LACTOSE RECOVERY PROCESSES

A wide range of processes have been developed to utilize the lactose in DPMS as the essential component. Possibly the simplest process involves using lactose as a source of energy for feeding livestock either directly (72), as a concentrated solution, solid lick-block, or in combination with other feed ingredients (55, 140).

Similarly, DPMS has been suggested as a valuable source of "crude lactose" for use as a food ingredient (56) either in solution, or as a spray-dried or roller-dried powder. The spray-dried powder may contain lactose as predominantly crystalline alpha-monohydrate (69) or amorphous "glass", whereas in the roller-dried product the lactose may be crystalline beta-anhydride (50). However, for the majority of food applications it is necessary to reduce the mineral content; for example, by electrodialysis and ion exchange processes (64, 73, 147) either prior to or during concentration.

The ability of carbohydrates to complex with alkaline-earth metals (the basis of the Steffen process developed for the recovery of sucrose from beet sugar molasses) recently has received attention for lactose recovery (80, 125) and has been suggested to have potential for commercial recovery of lactose (114). Similarly it has been suggested (89) that solubility characteristics of lactose in alcohols (e.g., decreasing solubility with increasing alcohol concentration and alcohols of longer chain) (97) might be exploited in the recovery of lactose. It is possible that DPMS could be an ideal feedstock for these processes.

CRYSTALLINE LACTOSE MANUFACTURE

Technology for manufacture of crystalline alpha-lactose monohydrate from whey is well-known (112, 168). Process operations involve concentration by evaporation, crystallization, separation, refining, drying, and

TABLE 1. Typical gross composition (% wt/vol) and mineral composition data (% wt/vol) for a range of ultrafiltration-derived deproteinated milk serum (DPMS). Gross composition data represent means (and standard deviations) from a variety of equipment.

Component	Whole milk		Skim milk		Cheddar cheese whey		Lactic casein whey	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
Total solids	5.60	.23	5.77	.19	6.41	.15	5.97	.45
Lactose (monohydrate)	5.03	.20	5.06	.31	5.80	.23	4.55	.45
Total nitrogen	.052	.02	.060	.015	.047	.009	.062	.011
Nonprotein nitrogen	.032	.008	.023	.006	.036	.005	.042	.006
Mineral (ash)	.46	.02	.47	.02	.54	.05	.74	.05
Lactate							.62	
Calcium	.03		.02		.05		.14	
Sodium	.03		.06		.06		.05	
Potassium	.12		.16		.18		.17	
Magnesium	.01		...		.01		.07	
Phosphate (total)	.11		.09		.12		.26	
Chloride	.10		.12		.15		.11	

milling. However, direct application of this technology to DPMS is not straightforward. Because DPMS virtually is saturated with calcium (115), concentration by evaporation causes precipitation of calcium (complex) salts such as phosphate and citrate and can result in rapid "fouling" or "scaling" of heat exchange surfaces. This problem is aggravated by the inverse temperature-solubility characteristic of calcium phosphate (17, 157). Furthermore, during subsequent lactose crystallization operations, the insoluble calcium salts may contaminate the lactose crystals, and because of their low solubility they are not removed readily by washing with water (115). It generally is accepted that DPMS must be pretreated either prior to or during evaporation (82, 118). Suitable processes include exchange of calcium for sodium ions by ion exchange resins (118) and demineralization by electrodialysis and/or ion exchange processes. Other suggested pretreatments include reducing pH to eliminate formation of insoluble salts (115) and addition of food-grade calcium chelating agents (e.g., sodium hexametaphosphate) to form insoluble complexes that may be removed prior to crystallization (42, 43). Nickerson (115) suggested the possibility of separating the insoluble salts from the hot, concentrated DPMS before crystallization. A later patent (121) described a process whereby DPMS was concentrated to 40 to 45% total solids, held at 82 to 93°C for 30 to 90 min, and the resulting calcium citrate precipitate was removed prior to further concentration and crystallization.

The manufacture of crystalline lactose from DPMS (permeate) derived by ultrafiltration of lactic casein whey (and possibly cottage cheese whey) is complicated further (134). The composition of this particular DPMS makes it inherently unsuitable (Table 2). A pretreatment process has been studied at the New Zealand Dairy Research Institute (NZDRI) that may permit lactose to be recovered from lactic whey permeate (66). The process involves partial removal of calcium phosphate complexes prior to evaporation by an alkali and heat treatment precipitation, followed by centrifugal clarification. A variety of alkalis have been investigated, including sodium hydroxide (Figure 2) and calcium hydroxide, either alone or in combination with each other (Figure 3). Brothersen et al. reported similar data for milk permeate (15).

Adjustment of lactic permeate pH to 6.7 and 8.0 by sodium hydroxide and holding for 8 min at 50°C removed approximately 50 and 80% of the calcium, respectively. Increasing the tem-

TABLE 2. Characteristics of lactic casein whey permeate that make it unsuitable for the manufacture of crystalline lactose.

Characteristic	Typical (12)	Effect on lactose manufacture
Low pH	4.4	May cause severe corrosion of evaporator
High titratable acidity	.5% lactic acid	
High mineral content	.71%	Precipitation of calcium phosphate complexes during concentration may foul heat exchange surface and contaminate crystals
High calcium concentration	1.24 g/kg	
High phosphate concentration	1.99 g/kg	
High lactate concentration	.64%	May interfere with lactose crystallization (134)

perature to 70°C at pH 6.7 resulted in a marked increase of the percentage of calcium removed; however, at pH 8.0 the effect was minimal. Adjustment of pH to 5.5 with sodium hydroxide, followed by addition of 6.8 mmol/liter sodium hydroxide or 16.9 mmol/liter calcium hydroxide at 80°C removed approximately 30% of the original calcium and 60 and 98% of the phosphate, respectively. Addition of 6.8 or 16.9 mmol/liter calcium hydroxide alone was relatively ineffective in removing calcium, although in the latter case approximately 80% of the phosphate was precipitated.

We also studied the efficacy of adding sodium carbonate alone and in combination with sodium hydroxide (Figure 4) and calcium hydroxide (to precipitate calcium carbonate and phosphate complexes). At 50°C, addition of 14 or 25 mmol/liter sodium carbonate resulted in removal of approximately 24 and 36% calcium, respectively. Preadjustment of pH to 5.5 with sodium hydroxide resulted in only a slight increase of the proportion of calcium removed. However, use of a pretreatment temperature of 80°C resulted in approximately a twofold increase of the percentage of calcium removed.

Pilot-scale trials were undertaken to ascertain the suitability of the pretreatment process for the manufacture of crystalline lactose from lactic permeate (Figure 5). Removal of approximately 50% calcium was sufficient to avoid difficulties during evaporation. It was not

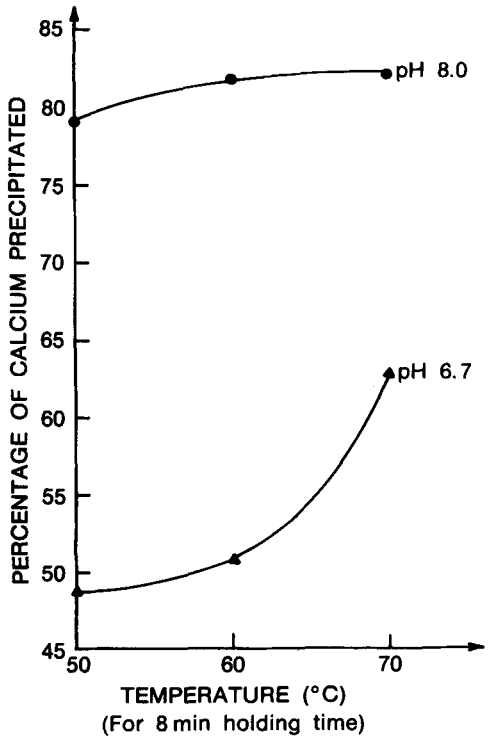

Figure 2. Percentage calcium removed versus temperature using sodium hydroxide to adjust the pH of lactic casein whey permeate to pH 6.7 (▲) and pH 8.0 (●). Holding time at each treatment (prior to centrifugation at 3000 X *g*) was 8 min.

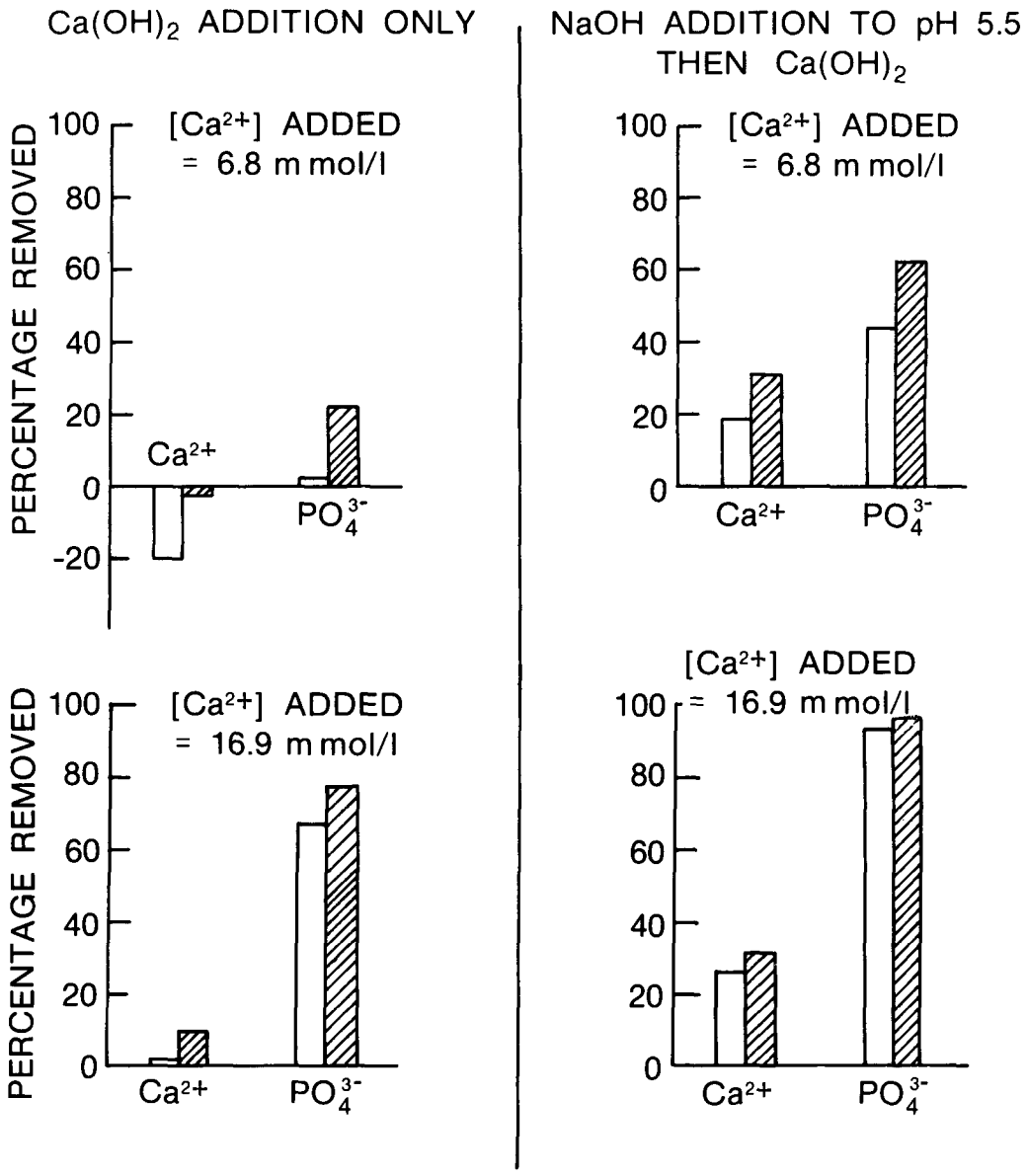

Figure 3. Percentage removal of calcium and phosphate by the addition of calcium hydroxide alone and in combination with sodium hydroxide to lactic casein whey permeate. The holding time for each treatment (before centrifugation at 3000 X *g*) was 20 min at 50°C (open) and 80°C (crosshatched). l = Liter.

possible, however, to determine an accurate yield from the trials.

In comparison with concentrated whey, absence of protein in concentrated DPMS solutions causes reduction of viscosity and thereby permits concentration to higher total solids. Additionally, the higher lactose content (as a proportion of total solids) allows the yield of crystalline lactose to be increased (13). Other potential advantages associated with absence of protein from DPMS include shorter crystallization times, continuous crystallizers

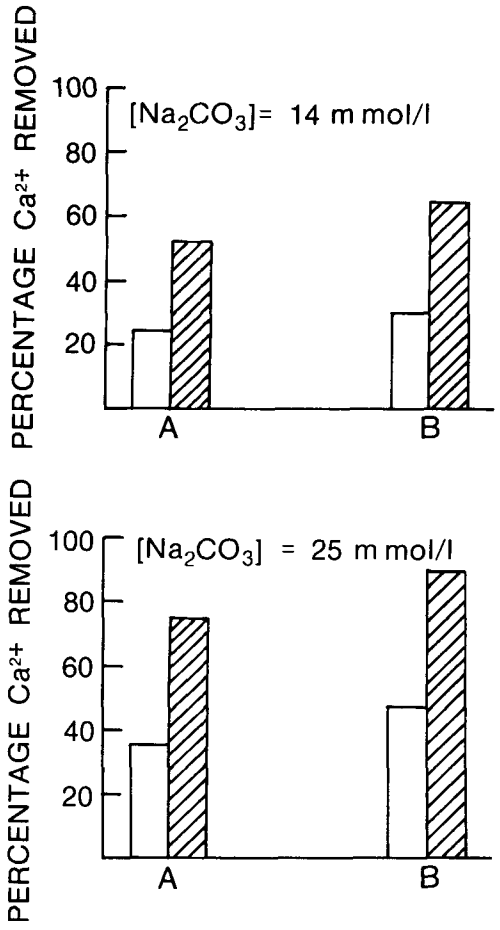

Figure 4. Percentage removal of calcium by the addition of sodium carbonate alone (A) and in combination with sodium hydroxide for pH 5.5 (B) to lactic casein whey permeate. The holding time for each treatment (before centrifugation at 3000 X *g*) was 20 min at 50°C (open) and 80°C (crosshatched). l = Liter.

(31, 114, 116), and alternative crystallization schemes such as the use of cool air either as a spray column (59) or fluidized bed (58). Additionally, the purity of the crude lactose crystals can be increased readily (e.g., >99% dry basis) by slurrying with water and re-separating.

The present world market for lactose is limited and competitive. Studies at NZDRI have indicated that the capital and operating costs of many of the pretreatment processes essential for manufacture of crystalline alpha-lactose from DPMS may be prohibitively large. Consequently, profitability of the process (particularly if considered in isolation) is extremely dependent on economies of scale and an energy and yield efficient operation.

There is opportunity for development of alternative cost-effective processes for pretreatment of DPMS to be used for manufacture of crystalline lactose. Furthermore, alternative processes (such as the Steffen process) warrant further research to ascertain their suitability for manufacture of lactose from DPMS.

ENZYMATIC AND CHEMICAL MODIFICATIONS

The lactose molecule contains a number of reactive sites (e.g., glycosidic linkage, reducing group of glucose, free hydroxyl groups, carbon-carbon bonds) that make it amenable to enzymatic or chemical modification, and in this regard it is similar to other carbohydrates. A variety of chemical and enzymatic modification processes have been investigated (Table 3) and are of potential significance commercially.

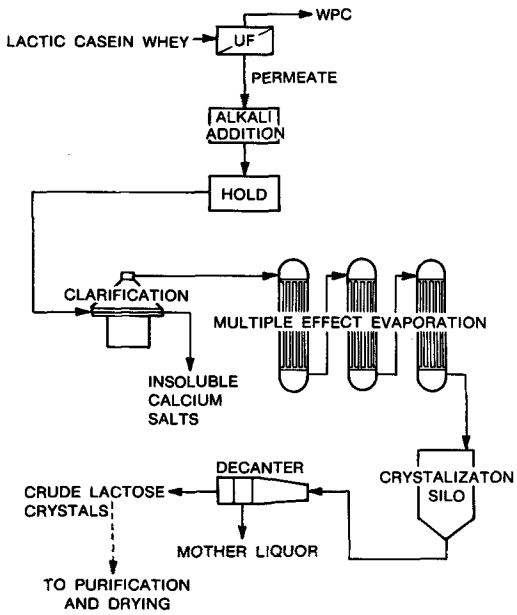

Figure 5. Flow diagram for pilot-scale process used to manufacture crystalline lactose from lactic whey permeate.

TABLE 3. List of products that have been produced by chemical or enzymatic modification of lactose.

Derivative	Process	Potential use	References
Hydrolyzed lactose syrup	Hydrolysis		
	Acid or enzyme (see text)	Food sweetner	See text
Lactulose	Isomerization		
	Alkaline solution common	Infant nutrition	101 (review)
	Reaction with boric acid in the presence of 3° or 4° – amine catalyst. High process yield (e.g., 87%)	Medical use	62, 63
	Heat treatment (e.g., sterilization)		161
Lactobionic acid	Oxidation		
	Enzyme; Lactose dehydrogenase	Food acidulant	88, 171
	Chemical		28
	Others include fermentation and electrolytic		
Lactobionamides	Reaction of lactobiono-lactone with amide	Alkaline sequesterant or chelating agent	141
Lactosylurea	Reaction of lactose with urea	Ruminant stockfood	9, 169
N-Methylol-lactosylurea	Reaction of lactosylurea with formaldehyde	Ruminant stockfood	9
Lactitol	Hydrogenation		
	Using Raney Ni catalyst	Nonnutritive sweetener	164 (review)
Lactitol palmitate	Esterification of lactitol with fatty acids of edible fats	Emulsifiers in foods or detergents	146
Polymers	Polymerization		
	Reaction with dimethyl sulfoxide	Polyurethane foam	68
Polymer precursors	Regiospecific esterification and acetylation		162
Ascorbic acid	Synthesis		
	Chemical plus enzymatic reaction	Vitamin C	32
Stearoyl-2-lactylic acid	Reaction of benzyl lactylate with stearoyl chloride	Surfactant	36

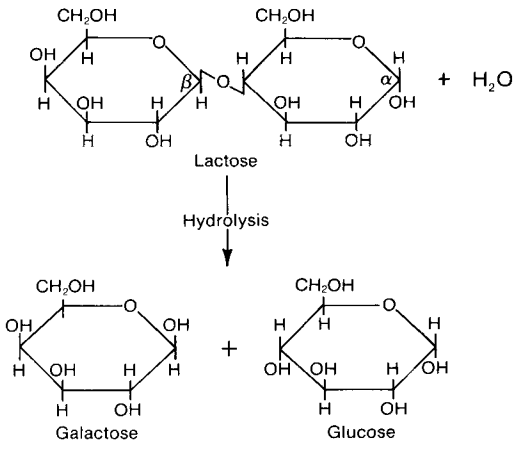

Figure 6. The lactose hydrolysis reaction.

With the exception of hydrolyzed lactose syrup and lactosylurea, markets for chemical derivatives tend to be small and costs of manufacture (by existing procedures) generally prohibitive.

HYDROLYZED LACTOSE SYRUP

If 50% of the available world lactose supply could be converted to a hydrolyzed lactose syrup (HLS), it would provide approximately 1.5 million tonnes of product, which represents less than 1% of the world's sugar production (132). Furthermore, it was considered that production of HLS would have virtually no impact on world sugar economics.

Technology

Hydrolysis of the covalent β, 1-4 glycosidic bond of lactose theoretically results in formation of equimolar concentrations of the monosaccharides, D-glucose and D-galactose (Figure 6). In practice, small amounts of oligosaccharides also may be formed. Hydrolysis can be achieved either enzymatically with β-D-galactosidase (E.C.3.2.1.23) (129, 148), commonly referred to as "lactase", or by acid catalysis. The enzyme systems of whole cells also have been investigated (104). A variety of processes have been developed to a pilot or commercial scale (Figure 7).

The basis for the "free" enzyme process is to add a known concentration of lactase directly

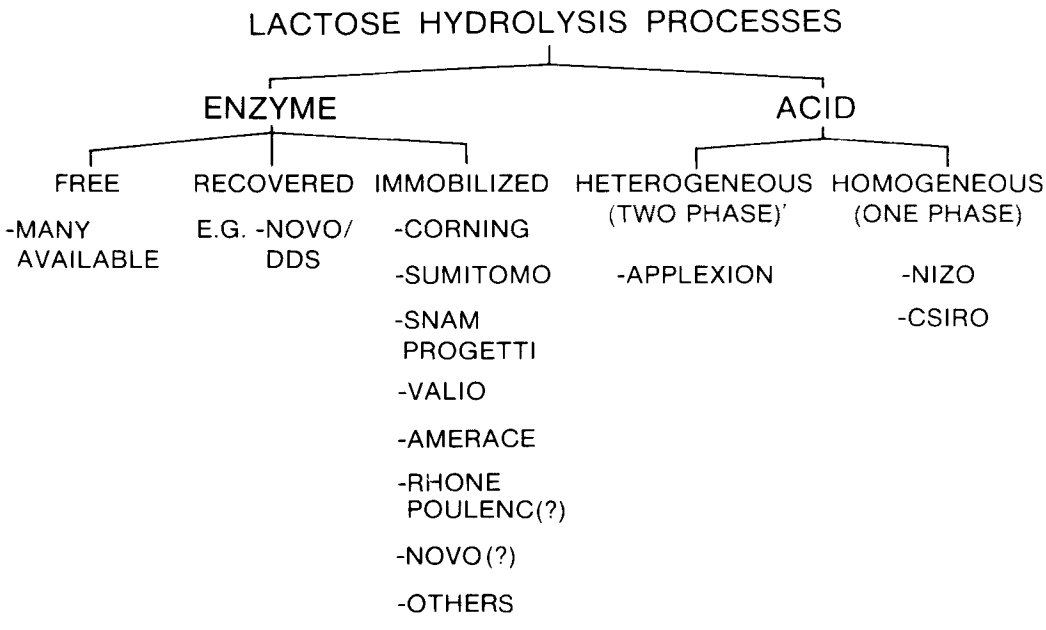

Figure 7. Lactose hydrolysis processes that have been developed to either a pilot-scale or commercial-scale and are suitable for production of hydrolyzed lactose syrup (HLS) from deproteinated milk serum (DPMS).

to DPMS and hold at a defined (optimal) temperature and pH until the desired extent of hydrolysis is attained. The DPMS then is processed further (concentrated) as required. Although the "free" enzyme process is technically straightforward, the high cost of enzyme can make it uneconomic. To overcome this problem, "recovery" of the enzyme from the DPMS (by ultrafiltration) and reuse of the enzyme have been investigated and found to be satisfactory (78, 119). Alternatively, the enzyme can be "immobilized" by either adsorption, entrapment, or covalent linkage to an insoluble support (46, 51). In these processes the DPMS is brought into intimate contact with the immobilized enzyme by a stirred tank or a column reactor until hydrolysis is complete and is separated then physically from the immobilized enzyme. Although "recovery" and "immobilized" enzyme processes may reduce the high operating costs associated with enzyme usage of the "free" process, the large capital investment required generally makes them dependent on economies of scale.

"Homogeneous" or "single phase" acid catalysis (12) uses hydrogen ions in solution (pH 1.0 to 1.5) to catalyze hydrolysis of lactose during a defined heat treatment (e.g., ranging from 60°C for 24 h to 140°C for 11 min). Hydrogen ions can be provided either by direct acidification with mineral acids or exchange of hydrogen ions for cations in solution by ion exchange resins.

"Heterogeneous" or "two-phase" processes (90) employ "insoluble" hydrogen ions bound to cation exchange resin to catalyze the reaction. In these processes DPMS is completely decationized, heated (90 to 98°C), and passed through a bed of cation exchange resin (regenerated in the hydrogen form) at a flow rate sufficient to provide the residence time (80 min) required for hydrolysis. The main advantage of the heterogeneous system is claimed to be reduced cost in catalyst per unit product, because the resin catalyst requires infrequent regeneration (and can be considered part of capital investment) (90).

A generalized flow diagram for production of HLS from DPMS by an immobilized enzyme process or a heterogeneous acid catalysis process is in Figure 8. Both of these processes have been used on a commercial scale. The immobilized enzyme process depicted involves demineralization, using electrodialysis and ion exchange, optional removal of nitrogen using adsorption resin (33, 53), adjustment of pH to the optimum required for enzyme performance, pasteurization, temperature adjustment for controlled hydrolysis, immobilized enzyme hydrolysis in a column reactor, and concentration by multiple effect evaporation to 60% total solids. Depending on the "end-use" of the HLS, it may be necessary to use an activated carbon and/or calcium hydroxide treatment process (40) to remove nitrogen and undesirable color and flavors from the HLS. Prior to storage or dispatch the HLS may be "preserved" by addition of sulfur dioxide. In the acid catalysis process the DPMS first is electrodialyzed to remove approximately 50% of minerals and then decationized completely with a strong cation exchange resin (in the hydrogen form). The demineralized, decationized DPMS (typically pH 1.0 to 1.5) then is heated to the desired hydrolysis temperature (e.g., 98°C) by a titanium heat exchanger and is passed through the hydrolysis column containing cation exchange resin (in hydrogen form). The lactose hydrolyzed DPMS then is passed through a bed of anion resin to remove further anions and effect a neutralization as well as remove color and off-flavors (formation due to Maillard browning and caramelization reactions). The solution is then concentrated to produce HLS.

Aspects of investigations into the manufacture of HLS in New Zealand were reviewed by Ennis (40). Studies have also been of storage stability of HLS, because, unlike most other countries, the seasonal milk production pattern in New Zealand could necessitate storage of HLS for up to 3 mo to provide continuity of supply to the food industry.

Experiments were undertaken to investigate storage characteristics of HLS manufactured from cheese whey permeate (41). The percentage lactose hydrolysis and concentrate total solids examined ranged from 70 to 90% and 50 to 85%, respectively. Samples were stored at temperatures ranging from 4 to 50°C. The HLS comprising 60% total solids, in which 80% of the lactose had been hydrolyzed, was stable for more than 6 wk stored at 50°C. However, at this storage temperature excessive syrup discoloration occurred, and in practice the HLS

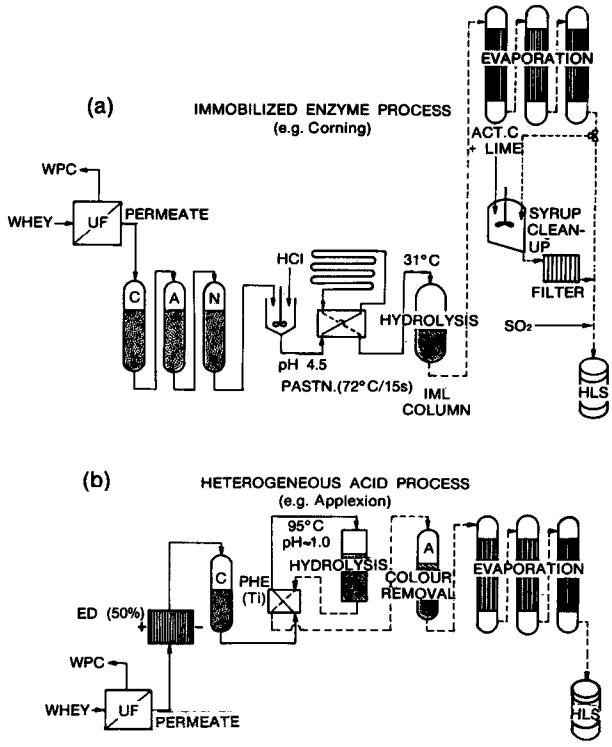

Figure 8. Flow diagram of immobilized enzyme (a) and heterogeneous acid catalysis (b) processes for the production of hydrolyzed lactose syrup (HLS) from deproteinated milk serum (DPMS). C represents cation exchange column, A represents anion exchange column, and N represents nitrogen absorption column (optional).

would require clean-up prior to dispatch. The HLS of 85% total solids and 75% hydrolysis formed a highly viscous block when cooled to 4°C and also remained stable for longer than 6 wk. Sugar crystallization during HLS storage is difficult to predict and was a function of many factors, including the extent of hydrolysis, syrup total solids content, prehydrolysis treatment of DPMS (e.g., extent of demineralization or nitrogen removal), syrup treatment at exevaporation and before storage, and storage temperature.

The profitability of manufacturing HLS for supply to the food industry as a sweetener is largely dependent on the price of competitive and traditional products, namely sucrose, dextrose syrups, and high-fructose corn syrups. Since 1974, although the world sugar price has fluctuated greatly, there has been no real price increase, and this, combined with the increasing capital investment required for a new plant, has tended to make production of HLS uneconomic (on equivalent sweetness basis).

Perhaps increasing the sweetness of HLS by enzymatic treatment with glucose isomerase (123) could improve the price competitiveness of HLS. The manufacture of a relatively impure HLS for use as a source of fermentable carbohydrate and a substitute for molasses in the production of bakers yeast (156) and xanthan gum (23) may also have future potential, as costs of demineralization, concentration, and syrup clean-up may be avoided or markedly reduced. The possibility of drying HLS by a Filtermat drier (128) either alone or in combination with other dairy-based food ingredients also may find future application.

FERMENTATION

On a global basis, the dairy industry can be considered a large fermentation or biotechnologically-based industry. During the manu-

facture of cheese alone (ignoring all other cultured milk products) the quantity of milk fermented annually exceeds approximately 90 million tonnes and perhaps, surprisingly, surpasses beer production of approximately 44 million tonnes (16). Thus, the industry superficially appears well-equipped to manufacture products by fermentation of lactose (or its component sugars glucose and galactose) in DPMS.

In US, Canada, and Brazil fermentation technology presently contributes 8% of chemical industry sales, and the market share will increase to 20% by the end of this century (48). Because the lead time required to improve fermentation ranges from 5 to 10 yr and development of new products takes 10 to 20 yr (49), it is timely that the dairy industry now seriously considers some of the fermentations of potential commercial interest if it is to be in a position to take advantage of market opportunities.

The range of fermentations of potential value is overwhelming (Table 4). Selection of a suitable process must take into account technological, market, and economic factors. A range of processes are compared on this basis in Table 5.

ACETONE, BUTANOL, ETHANOL FERMENTATION

Acetone, butanol, ethanol (ABE) fermentation was noted first by Pasteur in 1861. During the period circa 1916 to 1950 fermentation was established on a commercial scale to manufacture acetone and n-butanol from molasses and starch and at one stage was second in importance only to ethanol (124, 133, 153). Competition from petrochemical sources led to a decline in use of the fermentation process such that by 1976 only 5 and 10% of the world's production of acetone and n-butanol, respectively, was from fermentation sources (173).

Traditionally, *Clostridium acetobutylicum* or *C. butyricum* have been employed in an anaerobic batch fermentation of 30 to 36 h duration. The fermentation can be considered to occur in two phases (Figure 9). During the first phase, a logarithmic increase of cell growth occurs, and acetic and butyric acids are produced (with a concomitant increase of acidity of the fermentation broth). In the second phase cell growth ceases, organic acids are metabolized, acetone, n-butanol, and ethanol are produced along with the gases carbon dioxide and hydrogen. Because the n-butanol produced is extremely toxic to microorganism, the maximum final concentration of n-butanol is restricted to only 1.3% wt/vol (57), approximately one-seventh the limiting concentration of ethanol (52). It has been postulated (107) that the n-butanol concentration is sufficient to disrupt the cell membrane lipid bilayer and cause alteration to the membrane-bound enzyme activity (which influences transport of carbohydrates). Generally the total solvent yield is approximately 30 to 35% of the initial sugar concentration whereas the yield of carbon dioxide and hydrogen is 50% and 2%, respectively. Thus, the initial concentration of fermentable sugar is limited to 6.0 to 6.5% wt/vol. On this basis the (low) concentration of lactose in DPMS and possible low raw material cost appear to make DPMS ideally suited to ABE fermentation.

The ability of *C. acetobutylicum* to ferment lactose in whey (generally supplemented with yeast extract) to acetone, n-butanol, and ethanol is documented reasonably well (99). Maddox (93) reported that up to 1.5% wt/vol n-butanol concentration (ratio of acetone: butanol:ethanol = 1:10:1) could be achieved by fermenting sulphuric casein whey permeate (with *C. acetobutylicum* N.C.I.B. 2915) in 120-ml sealed bottles during 5-day incubation. In further work (94) using 2- and 15-liter fermentation vessels, a maximum of .9% wt/vol n-butanol concentration was achieved when the pressure of evolved gases was maintained at 105 kPa in the head-space in the vessel. It was suggested that n-butanol production was a function of head-space pressure and, more specifically, was related to the hydrogen moiety. Moreover, it was postulated that maintenance of elevated hydrogen partial pressure assisted in maintaining the required (nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide, reduced) stoichiometry for n-butanol production.

Experiments have been undertaken jointly by the New Zealand Dairy Research Institute and Biotechnology Department, Massey University (Palmerston North, New Zealand), to investigate the ABE fermentation on a 100-liter scale with sulphuric casein whey permeate and

TABLE 4. Fermentations reported to utilize lactose (or glucose/galactose).

Products	Organism	References	Notes
1. Yeast (and fungi)			
Single-cell protein (SCP)	*Kluverymyces fragilis* *Torula* spp. *Candida* spp.	44, 102, 137	Commercial
Bakers yeast	*Saccharomyces cerevisiae*	36, 105, 156	Via lactic acid or hydrolysed lactose
Derivatives			
Proteins		74	
Oils/fats	*Candida curvata*	27, 106	
Flavors		110, 138 34, 76	
Mushrooms	*Morchella* spp.		
2. Solvents			
Ethanol	Various yeasts *Zymomonas* spp.	4, 10, 25, 126	Commercial process
Acetone/butanol/ ethanol	*Clostridium* spp.	See text	Commercial (molasses feedstock)
Isopropyl Alcohol		99	
3. Stockfeeds (excluding SCP)			
Ammonium lactate	Lactic acid bacteria	127	Commercial
4. Methane	Mixed population of anaerobic bacteria	See text	Commercial (other feedstocks)
5. Food acids and derivatives			
Lactic	Lactic acid bacteria	19, 22, 92, 145, 155, 165	Commercial
Citric	*Candida* spp.	37, 139	Via pyruvate or from acid permeate. Commercial (molasses feedstock)
	Aspergillus niger	24, 150 77	
	Escherichia coli plus *Hansenula Wickerhamii* (CBS 4308)	38	
Acetic	*Acetobacter* spp.	117, 136, 143	Commercial from ethanol derived from acid permeate (ref. 136)
	Clostridium thermoaceticum	96, 166	
Lactobionic	*Pseudomonas* spp.	88, 99, 100	
Itaconic	*Aspergillus terreus*	39, 122	Commercial (other feedstocks)
Malic		47	
6. Enzymes			
β-D-Galactosidase	Various yeast and molds	71, 109	Commercial
7. Food gums (polysaccharides)		30	
Xanthan	*Xanthomonas campestris*	2, 23	Commercial (glucose feedstock)

(continued)

TABLE 4. (continued) Fermentations reported to utilize lactose (or glucose/galactose).

Products	Organism	References	Notes
Pullulan	*Aureobasidium pullulans*	35, 45	
Alginic acid	*Azotobacter vinelandii*	70, 130, 131	Under research development
Indican	*Beijerinckia indica*	83	
8. Amino acids	Various bacterial spp.	65, 75	Commercial (other feedstocks)
9. Vitamins		149	
Riboflavin	*Ashyba gossypii* *Clostridium acetobutylicum* *Candida guilleirmondi*	85, 170	
B_{12}	*Propionibacterium* spp. *Clostridium* spp.	86	
2-keto-L-gulonic acid	*Erwinia* spp.	151	Intermediate in ascorbic acid manufacture
10. Antibiotics		99	Commercial on whey
Penicillin	*Penicillium* spp.	1	in past
11. Other Biochemicals			
D(−)-3-hydroxybutyric acid	*Alcaligines eutrophus*	3, 81	
Gibberellic acid	*Fusarium moniliforme*	93	(Lactose feedstock)
2,3-Butylene glycol	*Bacillus polymyxa*	152	(From cheese whey)
Hydrogen	*Citrobacter intermedius*	14	
Diacetyl	*Streptococcus diacetylactis*	163	
Calcium gluconate	*Aspergillus niger*	79	
Propionic acid	*Propionibacterium* spp.	60	
Pyruvic acid	*Escherichia coli*	38, 108	

serum derived from heat-acid precipitation and separation of (lactalbumin) whey protein. The DPMS was supplemented with either ammonium ions or yeast extract. The pH of the media was adjusted to pH 6.5 by either ammonium hydroxide or sodium hydroxide (in the case of yeast extract supplementation). A batch fermentation was operated at 32°C, without agitation, and an (evolved) gas pressure of approximately 65 kPa. Results (Table 6) showed that supplementation of permeate with .03% wt/vol yeast extract was insufficient to obtain good fermentation. The use of 1% yeast extract or ammonium hydroxide (for pH 6.5) produced similar final butanol concentrations. In general, use of yeast extract enabled fermentation to proceed more rapidly than did ammonium hydroxide. The maximum butanol concentration achieved was .85% wt/vol in 111 h (Run 6); lactalbumin serum contained 1% yeast extract and was adjusted to pH 6.5 with ammonium hydroxide. This result is similar to that reported by Maddox et al. (94) using smaller fermentation volumes.

TABLE 5. Subjective assessment of market, technological, and economic factors associated with the manufacture of some fermentation products.

	Technology				Market				
Product	Availability	Development required	Capital expenditure	Degree of sophistication	Availability	Development required	Degree of sophistication	Potential for growth	Overall economic potential
1. Food yeast (SCP)	Good	Small	Moderate	Low	Good	Some	Moderate	Moderate	Poor to modest (29)
2. Bakers yeast	Limited to good	Moderate	Moderate	Moderate	Limited to excellent	High	High	Moderate	Variable
3. Industrial ethanol	Good	Small	Moderate	Low to moderate	Limited	Limited	Moderate	Limited	Poor (29)
4. Potable ethanol	Good	Small	Moderate	Low to moderate	Good	Some	Moderate	Moderate	Excellent (29)
5. Acetone and butanol	Restricted	Moderate	Moderate to high	Low to moderate	Limited to good	Possible	Moderate	Limited	Marginal
6. Methane	Good	Moderate to high	Low	Low to moderate	Good	None	Low	Not applicable	Variable (29)
7. Food acids	Variable	High	High	High	Good (competitive)	Possible	Moderate to high	Limited	Unknown
8. Enzymes	Restricted	High	Moderate to high	High	Variable (competitive)	Possible	Moderate to high	Limited	Unknown
9. Food gums	Restricted	Moderate	High	High	Limited	High	High	Unknown	Unknown
10. Amino acids	Restricted	High	High	High	Unknown (competitive?)	High	High	Unknown	Unknown

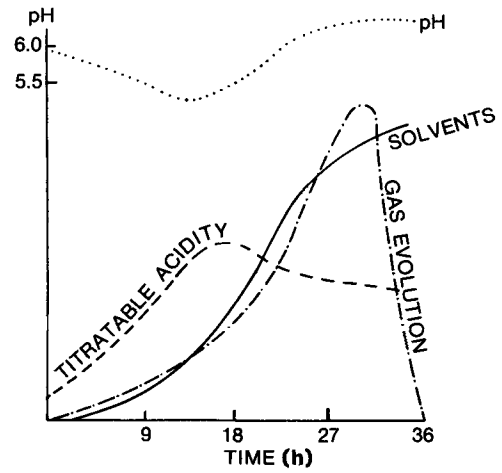

Figure 9. Progress of a typical, commercial acetone/butanol/ethanol fermentation.

It is confidently predicted that, with a minimum of further work to optimize fermentation, it should be possible to reduce the time required for completion of the fermentation from greater than 100 h to approximately 48 h. Moreover, it also would be expected that in this time a final n-butanol concentration of approximately 1.5% wt/vol could be achieved.

A cost study recently undertaken at NZDRI, based on the assumption that a total solvent concentration of 1.7% wt/vol could be achieved during a 48-h fermentation period, estimated that in New Zealand the production of n-butanol (and acetone) from DPMS, by conventional fermentation plus distillation technology, could provide a discounted cash flow rate of return of between 12 and 20% per annum (pa) (Figure 10). Lenz and Moreira (84) also considered the fermentation profitable in North America, although their costing was based on an extremely (and possibly unrealistically) large plant and was partially reliant on profit from the manufacture and sale of whey protein. In the future the economics for production of n-butanol and acetone by fermentation of lactose in DPMS could be improved markedly by: a) optimization of fermentation conditions to increase yield; b) increasing tolerance of the microorganism to butanol or final solvent concentration. (The French are reputed to have developed or isolated a microorganism resistant to 3.7% wt/vol n-butanol); c) application of continuous fermentation techniques (7, 8), immobilized cell reactors (54, 103, 167), or simultaneous extraction of solvents during fermentation (87) using membrane processes to avoid product inhibition and reduce costs associated with the fermentation operation; and d) development of alternative product recovery processes (26, 111) to reduce costs associated with conventional distillation processes [a recent US patent describes application of a fluorocarbon extractant (87)]. Improvements in one or more of these factors could make the ABE fermentation of real potential value to the dairy industry worldwide.

TABLE 6. Production of butanol from sulfuric acid casein whey permeate or serum from heat/acid precipitation of whey proteins by fermentation with *C. acetobutylcium* (N.C.I.B. 2915).

Run no.	DPMS[1] type	Supplement		Fermentation time	Butanol conc.	Lactose utilization
		Yeast	NH_4OH			
		(% w/v)		(h)	(% wt/vol)	(%)
1	Permeate	1.0	Absent	150	.65	63.5
2	Permeate	.03	Absent	70	.02	16.7
3	Permeate	Absent	pH 6.5	150	.62	45.8
4	Permeate	Absent	pH 6.5	165	.75	43.6
5	Serum	.3	pH 6.5	150	.80	45.9
6	Serum	1.0	pH 6.5	111	.84	64.0

[1] Deproteinated milk serum.

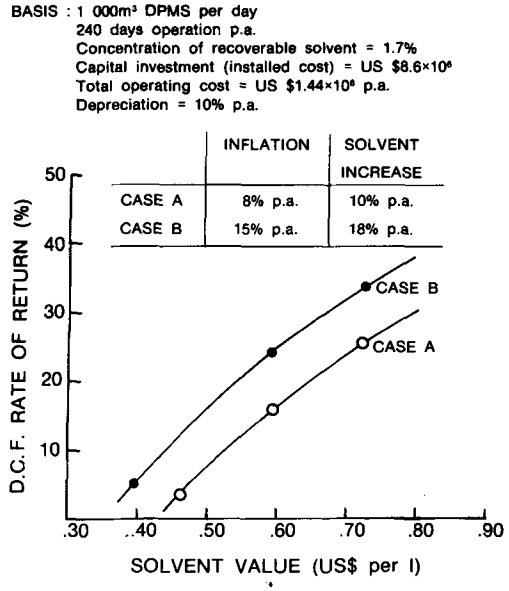

Figure 10. Summary of discounted cash flow analysis for the manufacture of butanol from deproteinated milk serum (DPMS) in New Zealand. Costs are estimates only.

Anaerobic Digestion

Anaerobic digestion is a naturally occurring process whereby a variety of bacterial species grow as a symbiotic, mixed culture under strict anaerobic conditions and degrade organic material to a mixture of gaseous by-products (referred to as "biogas") and biomass (containing C, H, O, N, S). The biogas comprises mainly carbon dioxide and methane with small amounts of other gases such as hydrogen and hydrogen sulfide.

Four trophic groups of bacteria currently are thought to be associated with the process (Figure 11) (172), which can be considered to occur in three stages: hydrolysis of polymers, fermentation, and methanogenesis.

A knowledge of the stoichiometry of the process (Equation [1]) (18) permits calculation of a theoretical mass balance between substrate composition and methane production.

$$C_nH_aO_b + (n-a/4 - b/2)H_2O \rightarrow (n/2-a/8 + b/4)CO_2 + (n/2 + a/8 - b/4)CH_4 \quad [1]$$

The stoichiometric relationship for lactose is in Equation 2:

$$C_{12}H_{22}O_{11} + H_2O \rightarrow 6CO_2 + 6CH_4 \quad [2]$$

It can be calculated that 1 kg of lactose theoretically will yield .75 m^3 of biogas (135) at standard temperature and pressure (STP) containing approximately 50% vol/vol methane. For a "typical" DPMS the theoretical yield of methane is approximately 20.7 m^3 methane/m^3 DPMS (Table 7), which has an energy equivalent of approximately 740 MJ/m^3 DPMS (equivalent to 18.6 liters of fuel oil). In practice, however, the biogas yield is typically only 80 to 90% of theoretical and usually contains 52 to 60% methane because: some of the organic matter is used to support cell growth, digestion of organic matter is usually incomplete, and carbon dioxide dissolves and reacts in solution (and thereby enriches the gas with methane).

ANAEROBIC DIGESTION PROCESSES

Anaerobic digestion has been practiced for circa 100 yr (91) to reduce the mass and

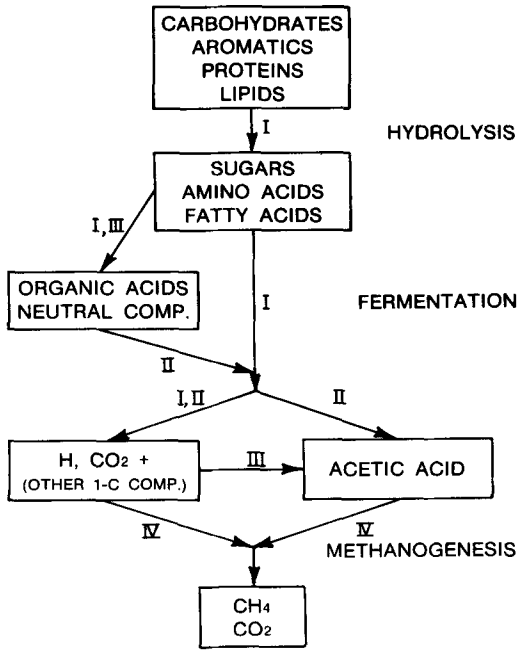

Figure 11. Current conception of the microbiology of anaerobic digestion (172). I) Digestion by hydrolytic (or acidogenic) bacteria; II) digestion by hydrogen producing or acetogenic bacteria; III) digestion by homoacetogenic bacteria; IV) digestion by methanogenic bacteria.

TABLE 7. Theoretical yield of biogas and methane from anaerobic digestion of deproteinated milk serum (DPMS).

Digestible organic material	Concentration	Gas volume (STP)[1]		Gas yield	
		Biogas	Methane	Biogas	Methane
	(%)	— (m^3/kg dry matter) —		— (m^3/m^3 DPMS) —	
Lactose (anhydrous)	4.75	.75	.37	35.6	17.6
Protein	.30	1.44	1.04	4.3	3.1
Lipid	0	.98	.49	0	0
Total				39.9	20.7

[1] Standard temperature and pressure conditions.

putrescible nature of the organic material able to settle in domestic and municipal wastewaters. Traditionally, the process involved holding the material in an unmixed tank for 30 to 40 days. During the 1950's mechanical agitation of the digester contents was introduced, because it had produced a marked increase in digestion rate (i.e., the first "high-rate" process). A second major advance also occurred and arose from recognition that the hydraulic residence time of the material being treated could be reduced greatly (e.g., a 10-fold reduction for equivalent digestion) by increasing the solids (cell) retention time by cell recycle (154). These two advances culminated in the development of the widely used contact process also suitable for the economic treatment of large volume, dilute industrial wastewaters containing a low concentration of suspended solids [e.g., meat and fermentation industry wastes (142)]. A variety of alternative processes (Figure 12) have been developed throughout the past 15 yr to increase further the solids retention time (or effective cell concentration) and improve the efficiency and economics of the anaerobic digestion process. All of these processes have relied on either cell recycle (internal or external) or cell immobilization.

Anaerobic Digestion of Deproteinated Milk Serum

The anaerobic digestion of lactose in DPMS (or whey) for the production of methane could be considered an ideal means of utilizing the lactose because: a) the process is relatively simple, b) the product does not have to be marketed and can be used "in-house" to supplement other energy sources up to 46% of the requirements of a cheese plant (160), c) almost 100% of the chemical oxygen demand (COD) in DPMS is biodegradable. There is, however, a scarcity of documented information on the use of anaerobic digestion for the production of methane from DPMS (or whey), particularly on a pilot or commercial scale. A summary of work is in Table 8.

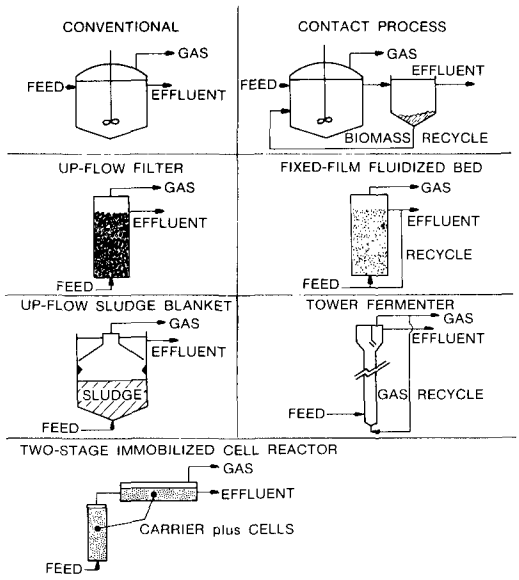

Figure 12. Anaerobic digestion processes.

TABLE 8. Summary of documented laboratory and pilot-scale investigations into anaerobic digestion of deproteinated milk serum (DPMS) (or whey).

Feed material	Feed COD[1]	System	Performance		Reference
			Loading rate	CH_4 Yield	
	(mg/liter)		(kg COD/m^3 digester vol per day)	(m^3/kg COD fed)	
Dilute sweet whey	10,000	Fluidized bed (500 μm aluminum oxide)	22 for 80% COD removal	.201	159
Dilute sweet permeate	7,000-8,000	Fluidized bed (sand)	8.5 – 12.2 for 65% COD removal	.205	158
Lactic permeate	2,000-7,000	Fluidized bed (sand)	8 for 90% COD removal	...	11
			20 for 70% COD removal	...	
Whey	69,800	Tower fermenter	12 for 99.4% COD removal	.33	20
Lactic whey	72,500	Filter	3.6 for 88% COD removal	.16	98
			4.82 for 82% COD removal		
Lactic whey	36,000 BOD[2]	Conventional/ sludge blanket (?)	...	...	120
Whey	50,000 BOD	Conventional	.5	.205	67
Acid whey	50,890		13.4 for 83.6% COD removal	.363	61
	52,260		37.6 for 72% COD removal		
Cheese whey	76,834	CSTR[3]	4.9 for 90–93% COD removal		144
		Sludge blanket	9.4 for 93% COD removal		
Cheese whey	...	Fluidized bed	...	...	160
Cheese whey	65,000	Modified sludge blanket (mix/ settle mode)	12.3 for 97% COD removal		21

[1]COD = Chemical oxygen demand.

[2]BOD = Biological oxygen demand.

[3]CSTR = Continuous stirred tank reactor.

Laboratory investigations recently have commenced in New Zealand (6) to compare the performance of an up-flow filter, an up-flow sludge blanket, and a fixed-film fluid bed process for production of methane from lactic permeate (COD ∿ 47,000 mg/liter and BOD ∿ 35,000 mg/liter). Ammonium hydroxide (approximately .2 g/liter permeate) was added to the permeate prior to feeding to the digesters. The anaerobic filter and sludge blanket digesters were operated at one steady-state organic loading rate, whereas two steady-state loading rates have been investigated for the fluidized bed. Steady-state for the sludge blanket was achieved with permeate diluted to 60% of full strength. A summary of some of the results is in

TABLE 9. Comparison of steady-state data for anaerobic digestion of lactic permeate.

Measure	Digester type			
	Filter	Sludge blanket	Fluid bed	
Loading rate, kg COD/m^3 per day	1.16	2.35	2.33	4.38
HRT,[1] days	40.5	12.4	21.0	10.7
COD removed, %	87.6	93.7	95.7	93.4
BOD removed, %	91.5	99.0	99.6	99.2
Total volatile acids, mg/liter	2350	<45	<45	<45
Methane yield, m^3/kg COD removed[2]	.371	.398	.421	.432
Methane productivity, m^3/m^3 digester volume per day[2]	.383	.877	.917	1.74

[1]HRT = Hydraulic residence time; COD = chemical oxygen demand; BOD = biological oxygen demand.

[2]Gas volumes for methane yield and methane productivity are measured at 36°C.

Table 9. The performance of the anaerobic filter was inferior to both sludge blanket and fluidized bed reactors in all respects and under the conditions of operation was overloaded (as indicated by a total volatile acids concentration of greater than 2000 mg/liter). The performance of the sludge blanket and fluidized bed digesters was similar and far superior to the filter. Increasing the loading rate of the fluidized bed digester from 2.33 to 4.38 kg COD/m^3 per day caused a slight reduction in performance. For both the sludge blanket and fluidized bed digestors the methane productivity rates were less than the rates (1.0 to 3.3 m^3 methane/m^3 digester per day) observed by other workers. However, it was considered that the low rates were a function of the low loading rates. More recently the loading rate of the sludge blanket digester has been increased by changing the feed to undiluted permeate. No problems have been experienced.

Modern high-rate anaerobic digestion processes can be used to produce methane from DPMS. On the basis of laboratory studies the upward-flow anaerobic sludge blanket process (or modifications of this process) possibly has the most potential for commercial application. Further research is required, however, particularly on a pilot scale, to compare attributes of alternative processes and to elucidate possible scale-up problems.

The economics of using anaerobic digestion to produce methane from DPMS have to be assessed case by case. In general, particularly if the costs of alternative disposal systems are accounted for, anaerobic digestion processes have the potential to provide a profitable method for utilizing lactose in DPMS (29).

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