

Record Number:

Author, Monographic: Tyagi, R. D.//Centre québécois de valorisation de la biomasse (CQVB)

Author Role:

Title, Monographic: Evaluation of dairy industry waste (cheese whey) as substrate for bioconversion / Valorisation du lactosérum: survol de l'état de la situation des sous-produits et procédés

Translated Title:

Reprint Status:

Edition:

Author, Subsidiary:

Author Role:

Place of Publication: Québec

Publisher Name: INRS-Eau

Date of Publication: 1986

Original Publication Date: Août 1986

Volume Identification:

Extent of Work: 97

Packaging Method: pages

Series Editor:

Series Editor Role:

Series Title: INRS-Eau, Rapport de recherche

Series Volume ID: 211

Location/URL:

ISBN: 2-89146-209-2

Notes: Rapport annuel 1986-1987

Abstract: Rapport rédigé pour le Centre québécois de valorisation de la biomasse
15.00\$

Call Number: R000211

Keywords: rapport/ ok/ dl

VALORISATION DU LACTOSÉRUM: SURVOL DE L'ÉTAT DE LA
SITUATION DES SOUSPRODUITS ET PROCÉDÉS

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Août 1986

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EVALUATION OF DAIRY INDUSTRY WASTE (CHEESE WHEY)
AS SUBSTRATE FOR BIOCONVERSION

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SUMMARY

Dairy industry waste with special reference to cheese whey has been evaluated with respect to the quantity available in Quebec and Canada and their pollution strength. The current methods followed for the disposal (land disposal, whey powder, animal feeding, wastage) are discussed. A few industrial cases for the management of their whey in Quebec have also been included. A number of options available for recovery of protein by ultrafiltration and fermentation products (ethanol, protein, products of hydrolyzed lactose, vitamins, organic acids and solvents, oils and fats, animal feed, 2,3 - butanediol, resins, polysaccharides, etc.) from permeate thus obtained have been identified. Research carried out on the utilization of whey for these products is reviewed. Based on research data the present viability of the products is presented. An economic evaluation of these products for which sufficient data are available is made and presented.

Keywords/Descriptors:

Cheesewhey, Cheese whey, Cheese whey powder, Bioconversion,
Protein recovery, Dairy wastes, Dairy residues,
Economic evaluation, Quebec, Canada.

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1) Dairy industry wastes

Many food and dairy industry operations produce large quantities of high strength effluents that constitute a major disposal problem. Many of these streams are dilute (4 - 5 % total solids) yet have a biological oxygen demand (BOD) in the range of 20,000 ~50,000 ppm (parts per million). The BOD levels are chiefly due to sugars and starchy materials in wastewater.

Whey is a byproduct of cheese production, when fat and casein are removed, generated at a rate of about 9 kg for every 1 kg cheese or 6 kg for every 1 kg of cottage cheese. One hundred kgs of whey is equivalent to the sewage produced by 45 people (59). At this rate a medium size cheese plant discharging 50 - 150 x 10³ kg of whey/day, constitutes a polluting strength of equal to the sewage from 22,500 to 67,500 people on a daily basis (60). In many countries including Canada most of the whey is discarded as waste creating severe pollution problems because of its high BOD (35,000 ~40,000 ppm) (20, 21). This high BOD is due mainly to the lactose which is present at concentrations between 4.5 and 5 % (20-22).

Whey is very rich source of minerals, calcium, phosphorus, potassium, sodium, copper and iron. These minerals have very good digestive qualities. It is also a very good source of vitamins of B-complex group, riboflavin, pantothenic acid, etc. But their availability varies considerably. The composition of cheese whey is shown in Table 1.

Table 1
Composition of Cheese Whey (58)

	% dry weight basis
fat	0.3
protein	0.9
lactose	5.0
ash	0.5
nitrogenous compounds	1.0
minerals and vitamins	0.8

As environmental control becomes more stringent, fewer municipalities tolerate the combining of the high BOD whey with other effluents processed by local sewage disposal facilities. Similar concern are also preventing traditional land spreading practices continuing in many parts of U.S. and Canada.

The food and dairy wastes in fact should be viewed as a rich, inexpensive potential source of raw material from which valuable products can be produced. Additional advantage of these wastes is that they are available all year round at fairly constant rate.

2) Pollution control

In the past, the application of pollution control technology to the process effluents from the cheese making industry has pursued three alternatives:

- direct discharge to the municipal system
- pretreatment at the cheese plant followed by discharge to municipal system
- full treatment at the cheese plant.

The high organic content in whey coupled with a relatively balanced nutrient composition indicates that some method of biological treatment is appropriate, but experience has shown that this is not entirely without problems.

Even though whey is nutrient balanced, the lactose fraction causes the bacterial population to swing towards filamentous organisms which are unable to use the amino acids present as a nitrogen source. Protein molecules, because of their large size, are unable to pass through the bacterial cell wall. The extracellular enzymes break down the large protein molecules but the overall reaction rates are low (98). Moreover, high strength of wastes makes conventional aerobic treatment very difficult and expensive. Anaerobic treatment of waste to generate methane is possible, but in many cases has a poor return on investment.

A further consideration is the effect which whey discharge may have on an existing biological treatment plant. In many cases a cheese plant is located in a rural area and thus, the organic load from the cheese plant will be many times greater than the organic load from the community. As well because of operational nature of cheese plant, the loading rate to the pretreatment plant will not be constant unless load equalization is practiced. Experience has shown that for successful treatment of cheese plant waste the influent BOD₅ concentration to the biological system should not exceed 1,000 mg/l. At concentration higher than this, severe bulking problems have been experienced.

One method of reducing the impact on a community's waste treatment facility is to practice some degree of pretreatment at the cheese plant. Pretreatment process alternatives are many. For example, screening followed by flow equalization prior to continuous discharge to the municipal system is one possible solution.

It should also be noted that the most municipalities have a sewer discharge by-law and impose a sewer surcharge to industry if certain maximum values are exceeded. While the economics of waste treatment are site specific, it is readily apparent that opportunities for significant cost saving through pretreatment can be realized.

In any pollution control strategy study, the option of providing complete on-site treatment to achieve effluent discharge standards must be included. Experience in the cheese producing industry in this regard are many and varied. They range from activated sludge with its many process variations (extended aeration, contact stabilization, the oxidation ditch and more recently the pure oxygen injection systems and deep shaft).

The problem of whey treatment demands simple economical solutions particularly in the case of small cheese plants in which oxidative disposal methods may be too costly to install (23, 24). The large amount of whey among fewer factories can have advantages and disadvantages. If the satisfactory methods of whey disposal are not available, additional supplies of whey can mean greater economic returns. The larger volumes can make it economically feasible to adopt processing techniques not practical for smaller quantities of whey. In other words, if disposal is a problem, additional volume can increase returns.

It is in the best interest of cheese maker and specifically the small one to get rid of the whey at lowest possible cost. The quantity of whey, 91 % of the incoming milk volume, its high solids concentration (6 %) and high

biochemical oxygen demand ($\approx 30,000$ mg/l), coupled in many cases with an unfavourable environmental location of the cheese plant, make a cost-effective solution rather elusive.

3) Production of whey in Quebec and Canada^a

Annual production of whey in USA and Canada is 16 billion kg and 1.5 billion kg respectively (1). The production of cheese in the world has been increasing steadily and in 1981 reached 11.6×10^6 tons (19). The total amount of whey generated in this activity was nearly 10.4×10^7 tons. In 1985 cheddar and speciality cheese production in Canada was 178,711 metric tons and that in Quebec province 60,676 metric tons. This generated 5,746,422 metric tons and 1,697,755 metric tons of sweet whey in Canada and Quebec respectively. Muller (2) reported that of the 1.2 billion kgs of liquid whey produced in 1979 in Canada; 49 % was used for whey powder; 8 % was fed to hog; 17 % was dumped into sewers and 26 % was disposed of on land.

The production of cheese in Canada has increased to more than two times from 1965 to 1984. In Quebec the production of cheese has increased from 32,535 tons in 1965 to 76,181 tons in 1984 (Table 2). At the same time increased in cheese manufacture has mainly increased in the big plants who are equipped with drying equipment for whey. The small industries, since hike in energy price are in search of more economical solutions. With the recent decrease in the petroleum price has not really made the drying process economical. In 1978 about 35.6 % of whey was disposed in environment

Table 2

Production de fromage au Québec en tonnes métriques

Année	Fromage cheddar	Fromage cottage	Fromage de spécialité	Total	Québec/Canada
1965	27,883	1,014	3,638	32,535	34.1
1966	31,649	1,279	4,510	37,438	36.3
1967	29,204	1,389	5,078	35,671	35.0
1968	32,426	1,450	5,259	39,135	37.0
1969	31,810	1,620	6,639	40,069	35.9
1970	33,585	1,838	8,690	44,113	37.5
1971	45,524	2,029	9,726	57,279	42.3
1972	41,896	2,128	9,272	53,296	38.8
1973	45,313	2,067	9,149	56,529	40.7
1974	54,875	1,968	9,688	66,531	44.9
1975	33,445	2,171	19,940	55,556	38.0
1976	32,469	2,360	23,718	58,547	38.4
1977	35,452	2,139	23,364	60,955	37.6
1978	37,592	2,539	25,552	65,683	37.5
1979	48,060	2,385	27,645	78,090	39.9
1980	54,235	2,261	26,814	83,310	39.7
1981	46,946	2,784	28,721	78,451	37.1
1982	36,228	1,699	31,061	68,988	33.8
1983	42,400	1,907	28,762	73,069	33.6
1984	42,739	2,075	31,367	76,181	34.0
1985	53,316	2,290	-----	-----	-----

whereas in 1976 the volume of whey which was disposed in environment was 28 % of the total production. This figure amounted to 507,000 metric tons of whey in Canada and 161,000 metric tons of whey in Quebec. In 1976 only 1 % of the total volume was used in liquid form for animal feeding. In 1984 about 736×10^6 l of cheese whey was produced in Quebec, about 8 % was discharged in the environment about 16 % was consumed by the animals and about 76 % was dried. The production of whey powder in Quebec in 1985 and 1986 is shown in Table 3.

4) Current disposal methods of whey in Quebec and Canada

The most common methods of disposal and recovery other than from drying to produce whey powder are:

- returning whey to farmers for animal feeding
- dumping it as a waste or sewage
- selling it to processors
- paying processors to collect it.

The more widely used method is evaporation and drying of the whey to produce whey powder which is used in bakery and other food preparations. In 1979 it was indicated that only a little over one half of the whey produced is utilized, most of which is in the form of dried whey powder, an industry which barely recovers its production cost because of poor markets and high energy expenditure.

Table 3

Production of Whey Powder in Quebec

	<u>1985</u>	<u>1986</u>
	metric tons	
Jan.	2302	2491
Feb.	1839	2049
Mar.	2384	2182
Apr.	2510	1997
May	2655	2226
June	2196	1986
Jul.	1084	----
Aug.	3059	----
Sept.	2874	----
Oct.	3148	----
Nov.	2791	----
Dec.	2822	----
	<u>29,663</u>	<u>12,931</u>

Corresponding figure of 1985 = 18,886 m.t.

Although installations for drying and processing might be economical for large plants, their costs were reported to be prohibitive for medium and small plants (2). Because both small and medium size cheese plants are unable to afford whey drying and processing equipments. Thus only large facilities with an output $> 150 \times 10^3$ kg of whey per day are able to realize a return on investment from processed whey products (60). Also whey utilization requires adequate cooling and storage facilities.

One considers whey as a resource or a pollutant, the sheer quantity represents a significant management challenge to the cheese producer. While the large cheese producer has addressed this problem with cost effective solutions made possible through economy of scale, the smaller cheese producer has been less fortunate. Almost 50 % of Canada's cheese producing plants fall into the small scale category (Table 4). The main source of waste whey in North America is also due to smaller plants (59).

Cheese whey has been considered as a feed to the porcs. Provided there are enough hogs, the whey can be substituted in the hogs diet up to 25 ~30 % of the total. It has been estimated that the hog can consume up to 1,000 l of whey per animal annually. But due to high trucking costs, liquid whey can only be used for hog feeding within an economic radius of 30-40 kilometers. In many cases, however, the hog population is small in relation to the number of cheese plants and therefore feeding liquid whey to hogs becomes uneconomical solution (2).

Table 4
Distribution of Cheese Production Plants

Capacity (kg/day)	Number of Plants
< 11,000	90
11,001 - 24,000	28
24,001 - 45,000	38
45,000 - 91,000	14
91,001 - 227,000	7
> 227,000	5

The more recent approach for the whey utilisation is the ultrafiltration to separate the protein and so called permeate containing mostly lactose. The protein is used for food and replaces the egg white. But the permeate obtained after ultrafiltration is normally disposed without treatment or recovery. Also there are few small group of industries which are selling their whey to other industry in a radius of 80 km, to recover the protein by ultrafiltration (ultrafiltration is discussed in this report later). A few cases of cheese industry in Quebec for the management of their whey are discussed in the following pages.

5) Management of cheese whey in few Quebec industries

Case 1

This Co. has two cheese plants at Boucherville and Nicolet. The Boucherville plant produces cottage cheese and sour whey as a by product at a rate of 35,000 lb/day (17,289 kg/day) having a pH of 4 ~ 5. The protein contained in this whey is of inferior quality for human consumption and therefore this plant does not recover the protein. The whey is mixed with other plant wastewater for neutralisation before its final discharge into the municipal sewer without treatment. This way the plant is wasting about 1,750 lb/day (865 kg/day) of lactose to the sewer.

The type of cheese produced in the plant at Nicolet are mazurella, brick and cheddar type. The amount of sweet whey produced in the operation is of the order of 150,000 lb/day having a pH of about 6.2. Ultrafiltration is used

to recover the protein. The permeate, generated is partly used for pig feeding (about 50 %) and partly either for land spreading or is discharged to the municipal sewer. Apart from precessing their own whey the Co. buys whey from 6 to 7 other cheese plants, situated in 80 km radius, to recover the protein by ultrafiltration. The amount of liquid whey procured from other plants is of the order of 400,000 to 500,000 lbs/day. The whey is received either hot or cold after pasteurization. The total amount of whey processed by the plant is 550,000 to 650,000 lbs/day.

The protein concentrate obtained during the ultrafiltration has 20 ~ 50 % protein on dry weight basis. However the main product of the company is one with 35 % protein. The market price of this product is \$ 175/kg and is used to replace the milk powder, used in ice cream, bakery, sausage etc. 80 % of the dry weight of the permeate is lactose and 16 % are minerals.

The permeate from this plant some times is concentrated by reverse osmosis followed by evaporation. The concentrate thus obtained is used to replace the molasses and has a current price of 25¢ /lb. Hydrolysis of this concentrate to produce a mixture of glucose and galactose to use as table sugar has been tried. However, demineralization being costly affair limits the application. Without demineralization the powder gives a inferior taste. Moreover, the installed plant's evaporators are not of high quality leading to high cost during evaporation. For example drying cost is in the range of 13 ~ 20¢ /lb. According to this company's report demineralization is very important step without which the concentrate is difficult to use for food consumption.

Case 2

This plant produces cheese and milk powder and process 450,000 l of milk per day and produces about 255,000 l/week of whey. All this whey is evaporated and dried to produce the whey powder. The whey powder is of two types called B and N based upon denaturation criteria. The temperature used for B and N are 196°F and 168°F and market price is 58¢ /lb and 46¢ /lb respectively. The total steam consumption during this process is about 1 kg of steam per kg of dry powder produced. This whey powder is sold to Co. Prescobell and is used for bakery products.

The production and utilization of cheese whey in few industries in Quebec is shown in Table 5. As is clear from the Table 5, most of the cheese industries in Quebec utilize their whey for whey powder, protein recovery followed by disposal of permeate to sewer or agricultural land and liquid animal feed. The production of whey powder is feasible economically for large producers and the profits are marginal. Most of the industries dispose their whey as animal feed at the cost of transportation which varies from 5¢ to 10¢ per 100 lbs depending upon the location of the farm. The main problem to produce protein concentrate by ultrafiltration or to produce the whey powder by evaporation, is the high cost of equipments (\$ 250,000 for UF units). For example, the company Nutrinove used to produce whey powder before 5 years. But now they have stopped and turned towards farm disposal due to increased cost of evaporation. The transportation cost which they get from the farm is 5¢ /100 lb. This company produces 40,000 l of whey per day.

Table 5

Production and utilization of cheese whey in few plants in Quebec

	Quantity of whey produced kg/day (lb/day)	Processing
1) Agropur: Coopérative Agro-alimentaire	2×10^6 (4.4×10^6)	Whey powder, biomethanation (operational difficulties in methane bioreactor)
2) Société coopérative agricole de beurrerie	9.07×10^4 20×10^4	Sold for porc feeding at 10¢/100 lb (or 10¢/46 kg)
3) Fromagerie Princesses Inc.	2.72×10^4 (6×10^4)	Sold for porc feeding at a price of 13¢/100 lb
4) La Fromagerie d'Oka Oka, Québec	73,000 (160,000)	Till before three years used to hyperfilter. They sell to other industries and pay 50 \$ per truck transportation
5) Fromage La Chaudière	16.33×10^3 (36,000)	Partly in cream production and partly animal feeding
6) Agrinove	51,000 (112,435)	Whey powder
7) Deslile	306,000 (650,000)	Protein recovery, sewer discharge, land disposal
8) Nutrinor	40,000 (88×10^3)	Animal feeding cost 40¢/100 l

6) Global utilization of whey

On a global basis, a divergence of whey disposition practices exist (96). In Denmark no whey is wasted and in Sweeden it is illegal to discharge whey into the sewerage system. In Israel 84 % of whey produced is wasted and whey is not used for animal feed. On the other hand in Denmark 90 % of the whey is fed to animals.

Byproducts recovery for proteins and lactose specifically is accomplished using concentration technologies ranging from spray drying to ultrafiltration couples with gel filtration. Most countries practice some degree of whey conversion into useful products. The Netherlands lead the way with 93 % of their whey being processed. In Canada only 48 % is processed. In New Zealand, casein whey was used mainly for spray irrigation and pig feeding, whereas cheese whey was used for lactose manufacture and pig feeding (99). Forty-five of the plants disposed of effluent by spray irrigation, 38 by discharge into natural water ways and municipal sewers and one by means of biological treatment unit operated by the dairy.

Food uses of whey are increasing and have encompassed pharmaceutical products, dairy products, bakery products, confections and coatings, frozen and canned foods as well as powdered foods. For example production of whey powder in Canada has increased from 41×10^6 kg in 1977 to 57×10^6 kg in 1981 (97). The choice of recovery technology is a function of end product use criteria.

7) Management strategies

The successful handling of whey dictates that a management strategy embodying by-product recovery and pollution control prevention be devised. It is usually the smaller and medium cheese plant operator (< 11,000 kg cheese/day) who has difficulty in finding a cost effective solution to the whey management problems.

A correct strategy for industrial pollution control consists of minimizing feed-stock leakage, optimizing product conversion efficiency, maximizing recovery and recycle of process losses and optimizing by product generation. The objective is to reduce the amount of waste material which results from the particular industry and thus will exert the minimum economic burden to that company by way of waste treatment requirements. There are many examples in industry where by-product generation and recovery have led to economic spin-offs which more than paid for the cost of pollution control (95).

The cheese producer does have a number of options when addressing the whey problem:

- processed into edible products
- used for animal feed
- application to agricultural land
- wastage (treated to effluent discharge quality)

Recovery of sugars, proteins and minerals in the waste effluents by crystallization, evaporation and spray drying are practiced in many food and dairy plants. The energy cost especially that of natural gas (though trend in cost of petroleum product for the last few months is downward, however future situation is unpredictable), even in spite of comparatively lower cost, have made many of these operations economically unattractive.

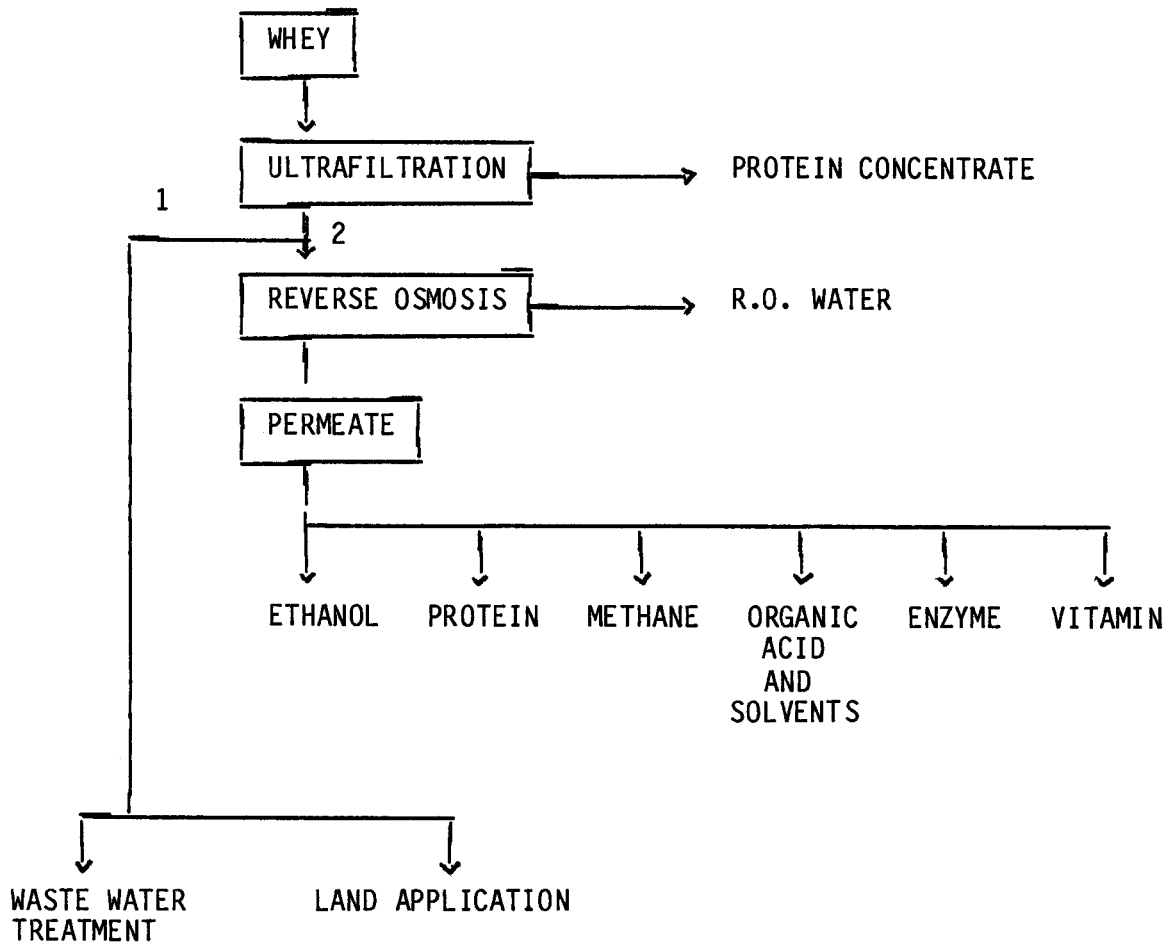
One management approach applies the principles of pollution control and resource recovery and involves application of ultrafiltration or hyperfiltration and reverse osmosis for concentrating the whey solids as shown in Figure 1.

Ultrafiltration has provided the cheese maker with a means of channeling the component of whey with the greatest value into market place. The whey protein concentrate (WPC) is an item of world wide commerce, with significant dollar value. That is the bright side. The dark side is that for each kg of WPC, 2.3 kg of permeate solids are also produced, with a lactose content of approximately 85 %.

A number of options have been proposed to convert permeate to a value added product - and other more profitable alternatives are constantly being evaluated. These alternatives includes alcohols, organic acids, solvents and protein which are discussed in the subsequent pages of this report.

Figure 1

FLOW DIAGRAM FOR POLLUTION CONTROL AND RESOURCE RECOVERY



7.1) Ultrafiltration

An ultrafiltration membrane has pores which separate the constituents of a solution on the basis of molecular shape and size. The macromolecules such as proteins, are held back by the membrane while smaller molecules, such as sugars, pass through. Ultrafiltration is a separation process rather than a concentration process, but it concentrates the retained macromolecules at the same time.

The solutions of macromolecules have very small osmotic pressures and the porous ultrafiltration membranes have very much higher water permeabilities than the non-porous RO (reverse osmosis) membranes. This means that pressure required for reasonable flow rates through the membranes are lower, and normal operating pressures are between 0.2 and 0.6 MP_a but up to 1.0 MP_a in some applications (10).

Ultrafiltration offers an alternative way of treating cheese whey. Instead of turning the whey into whey powder by concentrating and spray drying, it can be split into a much more valuable protein concentrate and a lactose stream. The process can be designed and operated in different ways to give concentrates, which are then spray dried, having 35-75 % protein in the powder. The 35 % powder is a substitute for skim milk powder and the 75 % powder can be used in place of egg white in baking. The cost of producing the powder is between (3 285 \$ can - 4 380 \$ can) per ton (10) which is considerably less than the cost of egg white (\$ 11.46/kg). The process is

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carried out at 50 to 55°C so the whey protein is not denatured and retains its functional properties.

In the ultrafiltration of whey or milk for the recovery of proteins considerable quantities of permeate (with 4-4.5 % lactose and 0.7 % ash) are produced. The permeate poses a serious disposal problem due to its high BOD and low market value. BOD levels are typically in the range of 30,000 to 40,000 ppm, and are due, in the most part, to the lactose in the permeate.

Studies on the whey solids hyperfiltration has been conducted in a small specialty cheese producing plant in the province of Quebec (La fromagerie d'Oka) (100). Hyperfiltration belongs to the membrane process family. It differs from ultrafiltration in that the membrane pore size characteristics are such that only molecules with a molecular weight less than 500 pass through the membrane. As well, the applied driving force is up to 8 times greater than for ultrafiltration. The large molecular weight proteins and caseins are retained in the concentrate whereas the permeate contains water, minerals, lactose and proteose-peptone fractions. Hyperfiltration systems are commercially available in two standard sizes, one which can operate under pressure up to 8,000 kPa, the other up to 5,000 kPa. The design is the same.

In the process of hyperfiltration studied at La Fromagerie d'Oka (100), raw whey is taken from the cheese vats and is concentrated to 24 % solids concentration from 6 % solids in whey. Economic analysis of this process for the two options considered: 1) to treat the total process effluent

(including the whey) using activated sludge technology; 2) concentrating the whey using hyperfiltration, giving concentrate away free of charge and treating the permeate and washwater again using activated sludge are very favourable (100) and cost data are summarised in the Table 6. This cost analysis shows that, the cost of complete treatment (option one) would amount to approximately 168 cents per kg of solids. Hyperfiltration and treatment of the permeate and washwater (option two) were estimated to cost 60 cents per kg solids. This was made up of 46 cents per kg for hyperfiltration and 14 cents per kg for activated sludge treatment. The net effect is that a saving of 108 cents per kg whey solids could be achieved. Application of alternative technologies must be evaluated on a case by case basis. The period of payback (the time required to repay the original investment from cost savings generated by this capital equipment) for La Fromagerie d'Oka has been calculated to be 14 months (100).

One of the assumptions in this calculations was that the concentrated whey is given away free of charge. Undoubtedly, revenues generated from the sale of whey concentrate or when its value as a feed-stock or other edible product manufacture is credited will result in an even shorter payback.

The following conclusions thus can be drawn:

- Depending on local circumstances concerning whey discharge, whey concentration and recovery can result economies to a cheese manufacturers.

Table 6

Potential cost impact of whey recovery / Treatment alternatives (Hyperfiltration / Activated sludge) (100)
 Estimated cost 1983

	Pop. equiv. (p.e.)	Dollars/capita O & M	capital	Annual O & M	Total capital	Total dollars annual	Treatment dollars (¢/kg solids)	Hyper filtration cost (¢/kg solids)	Minimum total cost (¢/kg solids)
Not recovery	14,200	9.95	224	141,300	3,180,000	936,300	168	----	168
Recovery	450	12.75	569	10,000 ^a	160,000	75,000	14	46	60

a: Considered to be a minimum O & M (operating and maintenance) cost

b: 10 % depreciation on capital, 15 % interest rate

- Hyperfiltration/ultrafiltration as a solids concentration technology for sweet whey should be considered in any economic evaluation of pollution abatement/by-product recovery schemes for cheese producers. This applies specifically to those cheese producers who are considering a change in their current whey handling procedure and should be of particular interest to the small (< 11,000 kg/day) cheese producer.

- The site specific of any cheese manufacturing operation, as well as the many variables involved in whey recovery, whey treatment and/or disposal of process effluents, do not permit one to make specific cost statements on an industry wide basis. Each cheese manufacturing operation must be evaluated on its own merits.

7.2) Concentration of whey by reverse osmosis

Reverse osmosis is used as a cheaper alternative to evaporation for removing water from, and so concentrating, dilute effluents and/or effluents from ultrafiltration unit before further processing (18). The applied pressure is usually 4 MPa but can be as high as 8 MPa (10). The process is very simple. A pump is used to raise the pressure of the solution to around 4Mpa and make it flow over the membranes. Water passes through the membrane to become the permeate stream, which is at atmospheric pressure, leaving behind a concentrate stream which flows out through a valve to control the pressure. The main commercial uses in recovering materials from effluents are in processing cheese whey and the effluent from the processing of potatoes for starch production. The pumping energy is the only energy used and

amounts to 1.1 KWh per m³ of feed (10). This is not more than is used in pumping in an evaporator system and unlike RO, evaporation requires a considerable further amount of energy in the form of steam. It is this considerably lower energy consumption of RO that has given the process a great impetus.

Due to the large energy consumption in 1974 there was no market for concentrating cheese whey by reverse osmosis. In 1975, 33 % of the water removal from whey to increase the lactose concentration from 6 to 9 % before evaporation was possible. Between then and 1978 most of the plants around the world installed 50 % water removal and increased the solids concentration to 12 %. Since then plants have covered a range of final concentrations from 12 % to 24 % and even 28 % is now possible (10). This is likely to be the limit for cheese whey because of lactose crystallization and because the osmotic pressure of a 28 % whey solution is 4 MPa. De Boer and Hiddink reviewed the literature on processing of dairy liquids by membranes (11).

According to Pepper (10) the cost of replacing the concentration of whey in two effect evaporators replaced by RO are compared in Table 7 and concluded that savings paid for the RO plant in less than a year.

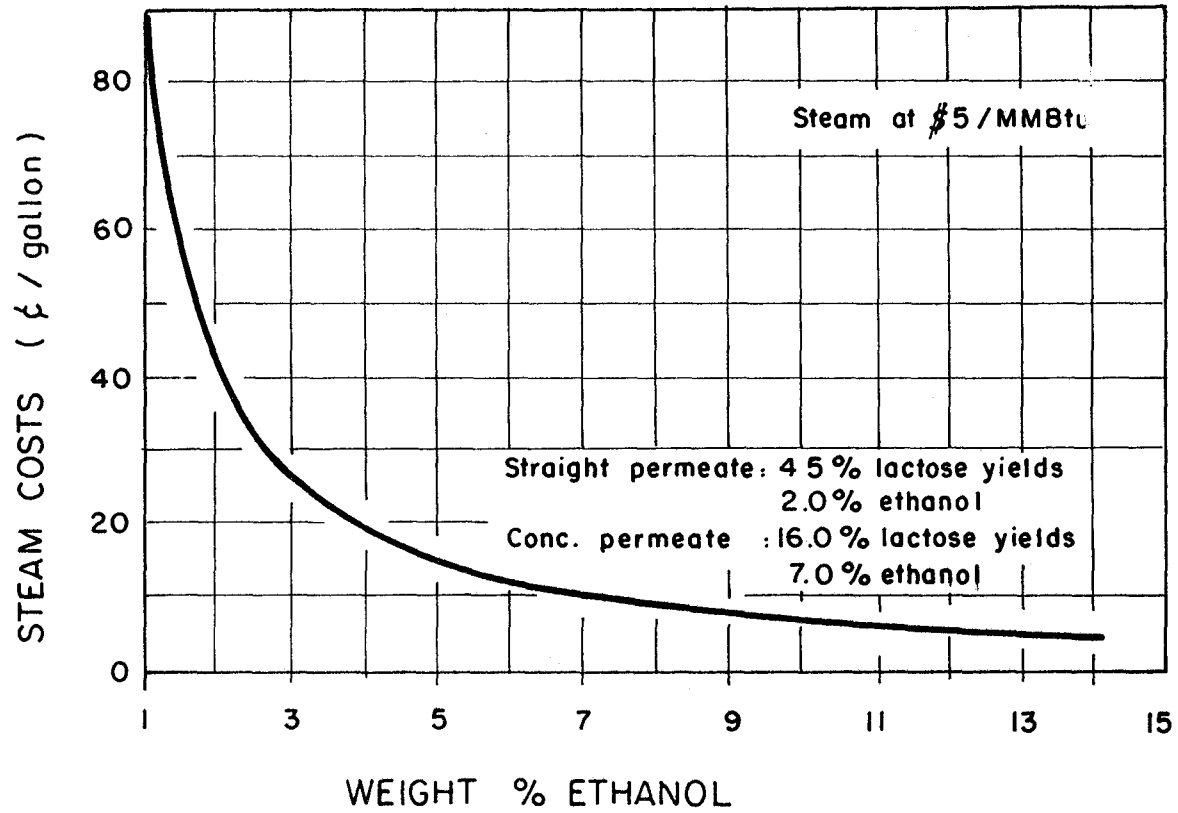
Preconcentration of the permeate prior to fermentation is one of the critical design parameters. For example, Figure 2 shows the cost of distilling broths which have different ethanol concentrations. If straight permeate at 4.5 % lactose were fermented, a 2 % ethanol broth would be produced which would cost 40¢ (US)/gal (11¢ /l) to distill. On the other hand if permeate

Table 7

Annual costs of removing 9000 liters of water per hour from cheese whey by reverse osmosis, compared with a two effect evaporator.

<u>Original Evaporator</u>	<u>\$ can</u>
Steam (2.19 \$ can/5 tons)	378,870 \$
Caustic and acid	5,475
Labour	20,367
Electricity	11,388
	<hr/>
	416,100
	<hr/>
<u>Current RO plant</u>	<u>\$ can</u>
Steam (whey reheat + CIP ^a heat)	52,560
CIP detergents	20,805
Labour	20,367
Electricity	15,330
Membranes (1 year life)	32,850
	<hr/>
	141,912
	<hr/>
<u>Annual Saving</u>	<u>274,100 \$</u>

^aCIP = cleaning in place



Distillation costs

Figure 2

is pre-concentrated to 16 % lactose and fermented, about a 7 % (W/W) ethanol broth results, which only costs 10¢ (US)/gal (\$ 2.65/l) to distill, a 4-fold reduction in steam cost. It is advantageous to pre-concentrate the permeate prior to distillation by some means more energy efficient than the still. If reverse osmosis equipment is used, it will require about 23 Btu/lb of water to be removed as compared to 200 ~ 300 Btu/lb required in, say, a multi-effect evaporator (18). Thus, using reverse osmosis prior to fermentation allows the process to produce a much higher ethanol concentration in the fermentation broth at low incremental cost thereby dramatically reducing the operating cost per unit volume of ethanol. Another advantage of pre-concentration is that the hydraulic load is reduced by as much as 65 % thus reducing the size and cost of broth fermentation and distillation equipment. Similar analysis can be applied to the products which requires high concentration in the final broth due to economical recovery.

7.3) Lactase and lactose hydrolysis

The problem of lactose in milk and whey has been very well described in the literature. Technologically lactose is easily crystallized and this sets limits to certain processes in the dairy industry. Cheese manufactured from hydrolyzed milk ripens more quickly than that made from normal milk (78, 79). The hydrolysed lactose in whey permeate can be a better substrate for further processing.

Lactase (β -D-galactosidase, EC 3.2.1.23) can be used to reduce the lactose content of milk by-products (whey). This increases their potential uses by eliminating the problems of low solubility and sweetness of the disaccharide (70, 71). Furthermore, this treatment makes milk available to a large number of adults and children intolerant to lactose fermenting yeasts are considered an excellent group for this purpose (72-76). Extraction of intracellular enzyme (up to 4.4 units per milligramme of cells) has been achieved by treating washed yeast cells with 2 % (V/V) chloroform in 0.1 M potassium phosphate buffer containing 0.5 mM Mg SO₄ and 0.1 mM Mn Cl₂, pH 6.6 or higher, for 10 hours at 30 to 37°C (72). This extraction procedure, although simple, does not seem to extract all the enzyme from the cells. Other solvent treatments, including ethanol, have been reported (77) which may improve the yields of lactose.

Lactase preparations from A. niger, A. oryzae and from saccharomyces sp (lactis or fragilis) are considered safe because those sources have already a history of safe use and have been subjected to numerous tests (91). E. Coli lactase, although the species most investigated, is not used in food processing because of its cost and the fact that it gives toxicity problems with crude extracts of coliforms (92). Fungal lactases are used for acid whey hydrolysis while yeast and bacterial lactases are suitable for milk (pH 6.6) and sweet whey (pH 6.1) hydrolysis (93). Product inhibition (namely inhibition by galactose) is the property which also depends on the source of lactose (94). Methods of hydrolysis by free and immobilized enzyme are recently reviewed (94).

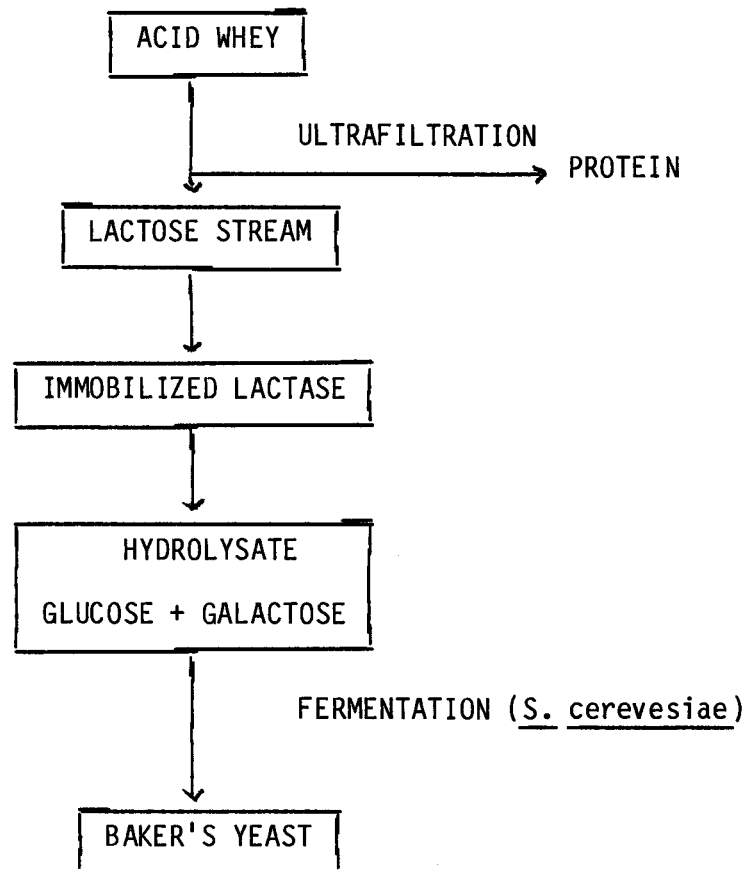
Acid hydrolysis can not be applied to protein containing substrates due to protein denaturation. It is an attractive method for deproteinized or UF-ultrafiltrated whey, because it is presently less expensive than the enzymatic methods. Immobilized enzyme systems have the possibility of continuous processing and the reusability of enzyme which leads to lower costs, a very essential factor in case of whey. However, there are no systems (up to now) commercially available which can be used in non-sterile milk and whey (94).

Lactases have been used in the processing of dairy wastes (63-67). There are two commercially plants using immobilized lactase: Nutrisearch (Winchester, Kentucky) and specialist Dairy Ingredients, SDI [Maelor, Wales, U.K.]. Both processes use whey as the raw material (Figures 3 and 4). The key step in both processes is the enzymatic hydrolysis of lactose to glucose and galactose. Both commercial processes use the corning immobilized lactase technology for this purpose.

The immobilized lactase system developed at corning has been described in the literature (63-68). Lactase is derived from Aspergillus niger. The enzyme is covalently bound to a controlled-pore silica carrier using the silane gluteraldehyde technique of Weetall and Havewala (69). The performance of the immobilized lactase at commercial scale has been good. The same column has been in place since the SDI plants began operation in May, 1985. This plant operates 16-20 hours/day at 360 liters/hour and achieves 80 % hydrolysis of lactose (63). While the system is cleaned by standard

Figure 3

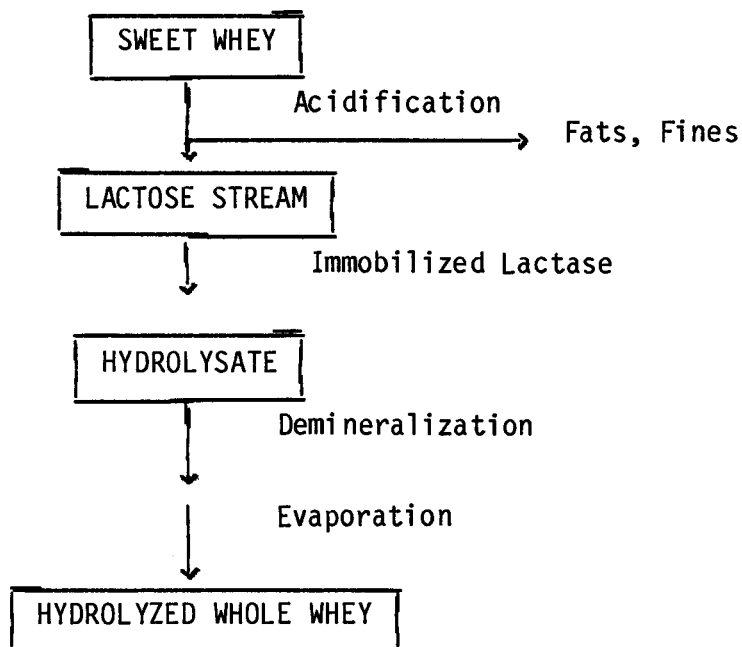
Nutri-search* Process



* Joint venture between corning glass works and Kroger Company

Figure 4

Specialist Dairy Ingredients* Process



* Joint venture between corning glass works and milk marketing board

dairy cleanliness, the lactase bed is fluidized every day for 30 minutes with dilute acetic acid.

The applexion company, France (128) have proposed utilization of ion exchange resins to:

- demineralization of whey UF permeate and lactose juice
- hydrolysis of lactose
- decolorization of lactose juice

With an applexion plant, it is claimed that all possible means of upgrading whey can be carried out. Important number of different products can be manufactured, which is particularly attractive due to the fluctuations of the whey market. The following products can be manufactured:

- partially demineralized whey powder, by demineralization of whey until the required value is reached.
- completely demineralised whey powder by a complete demineralization on ion exchange resins.
- completely demineralized protein concentrate.
- completely demineralized lactose, obtained, as is previous product, by UF and complete demineralization.
- decolorized lactose, without demineralization, by ultrafiltration and decolorization of lactose as adsorbent resin.

- completely demineralized and decolorized lactose.

- hydrolysed lactose: completely demineralised glucose galactose syrup, obtained by a combination of UF, demineralization and hydrolysis of lactose.

- hydrolysed and decolorized lactose, obtained by a combination of UF, demineralization and hydrolysis of lactose.

- hydrolysed and decolorized lactose, obtained by a combination of UF demineralization, hydrolysis and decolorization of invert sugar. This product is the one which requires the most complete installation.

The two main components of whey, protein and lactose, can be obtained by using ultrafiltration and demineralization. It is proposed that the sugar can be sold as lactose or better, still, as glucose and galactose syrup, after hydrolysis. The latter product is more important because of its higher price and the low cost of hydrolysis. The glucose galactose syrup is a liquid syrup which can be decolorized and can have a very high purity. The economic evaluation of this process showed that the capital cost is relatively small, the production cost is comparatively low and the process has been advocated as to be free of any technical difficulties (128).

7.4) Lactose hydrolysed products and their uses

- An attractive way of utilizing whey is to hydrolyse its lactose to a sweet syrup consisting of glucose and galactose. Compared with the sweetness of sucrose (taken as basis with a relative value 100) this syrup has a value of about 70, while lactose is considerably less sweet with a value of 40 (80).

- The sweet syrup prepared from whey by lactose hydrolysis can be used as a source of sugar and, in some cases, of protein in bakery products, in confectionery, in soft fruit drinks, in ice cream, in feedstuffs for cattle instead of molasses, in dairy desserts or as basis for further fermentation to alcohol (78, 81, 82, 83, 84, 85).

- Various degrees of concentration and mixing proportions with other products can be utilized. A concentrated syrup with 80 % total solids was prepared from cottage cheese whey (86) through deprotenization, hydrolysis, decolorization and concentration. The syrup had a pleasantly sweet flavour, was light yellow in colour and had an apparent viscosity of 2,050 CP. It gave to the vanilla ice cream a quality comparable to that given by maize syrup.

- Hydrolysed demineralized lactose syrup was produced by Valio Process in Finland (87). It contained glucose: 10 %; galactose: 20 %; lactose: 10 % and protein plus salts: 10 %. It was used at 12.3 % in an ice cream mix in order to substitute a part of normal sucrose and milk with no detrimental consequences to its quality various mix formulations have been tested during ice cream manufacture. The optimum formula was 11 % fat, 10 % milk (80 % hydrolysed), 16 % sugars (a part of sucrose has been replaced by corn syrup sweetness).

- An orange flavour beverage prepared from hydrolysed and deproteinized cheese whey is recommended as a shelf-stable drink for athletes (80).

- Nutrisearch Company, Kentucky, USA uses the product of whey hydrolysis to grow baker's yeast (88).

- The whey hydrolysis step can be followed by alcoholic fermentation by saccharomyces cerevesiae. Alternatively hydrolysis by co-immobilization of lactose and s. cerevesiae cells (89, 90).

7.5) Production of L-Ascorbic Acid (Vitamin C) from whey

One of the options of the utilization of cheese whey permeate is the conversion to Vitamin C. Commercial production of vitamin C presently is from D-glucose via the "Reichstein Synthesis" (7), however plants and yeasts are also capable of synthesizing L-ascorbic acid from galactose (8). This provided the impetus to investigate the route of conversion of whey into vitamin-C and has been recently studied by Cayle et al. (9).

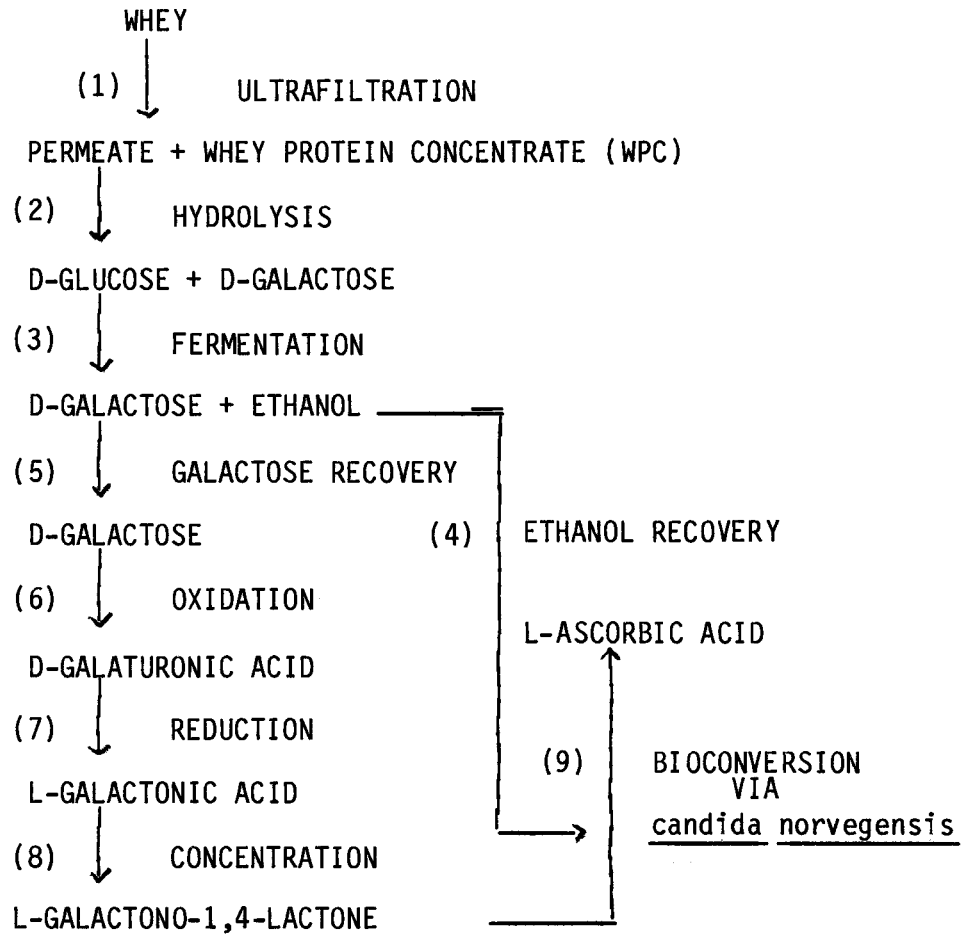
The final step in biological synthesis is the oxidation of L-galactono - 1,4 - lactone to L-ascorbic acid. In higher plants this is catalyzed by galactonolactone dehydrogenase which requires cytochrome C as the electron acceptor. However, yeast employ L-galactonolactone oxidase, an enzyme which uses oxygen as the electron acceptor. Cayle et al. (9) evaluated a yeast bioconversion as the final step in a commercially feasible synthesis of L-ascorbic acid from whey.

The following steps are involved in the total synthesis employed, for the most part using conventional catalysis to produce the lactone from whey (Figure 5).

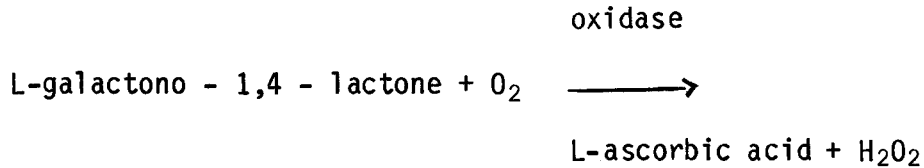
- 1) Whey is ultrafiltered and both WPC and permeate are recovered.
- 2) Permeate is continuously hydrolyzed via an immobilized lactase reactor (Danrace Hydrolysis System, Damrow Co., Madison, WI).
- 3) The D-glucose/D-galactose stream is fed to a continuous fermenter containing a mutant, flocculating strain of S. cerevesiae, where the glucose is converted to ethanol.
- 4) The beer is continuously withdrawn and the alcohol is recovered by distillation.
- 5) The galactose is recovered by ion exclusion chromatography (Dowex 1-X8, bisulfite, Dow Chemical USA, Functional Products and Systems Department Midland, MI).
- 6) The dried galactose is oxidized with conventional inorganic catalysts to D-galacturonic acid.
- 7) The D-galacturonic acid is reduced with conventional inorganic catalysts to L-galactonic acid.
- 8) The L-galactonic acid is concentrated to remove water, thereby forming L-galactono - 1,4 - lactone.
- 9) The L-galactono - 1,4 - lactone is converted to L-ascorbic acid via a strain of candida norvegensis, grown on ethanol produced in step 4.

Figure 5

SHEMATIC FOR PRODUCTION OF ASCORBIC ACID FROM WHEY



The chemical steps in synthesis of L-ascorbic acid from D-galactose are the same up to L-galactono - 1,4 - lactone followed by enzymic conversion of L-galactono - 1,4 - lactone to L-ascorbic acid.



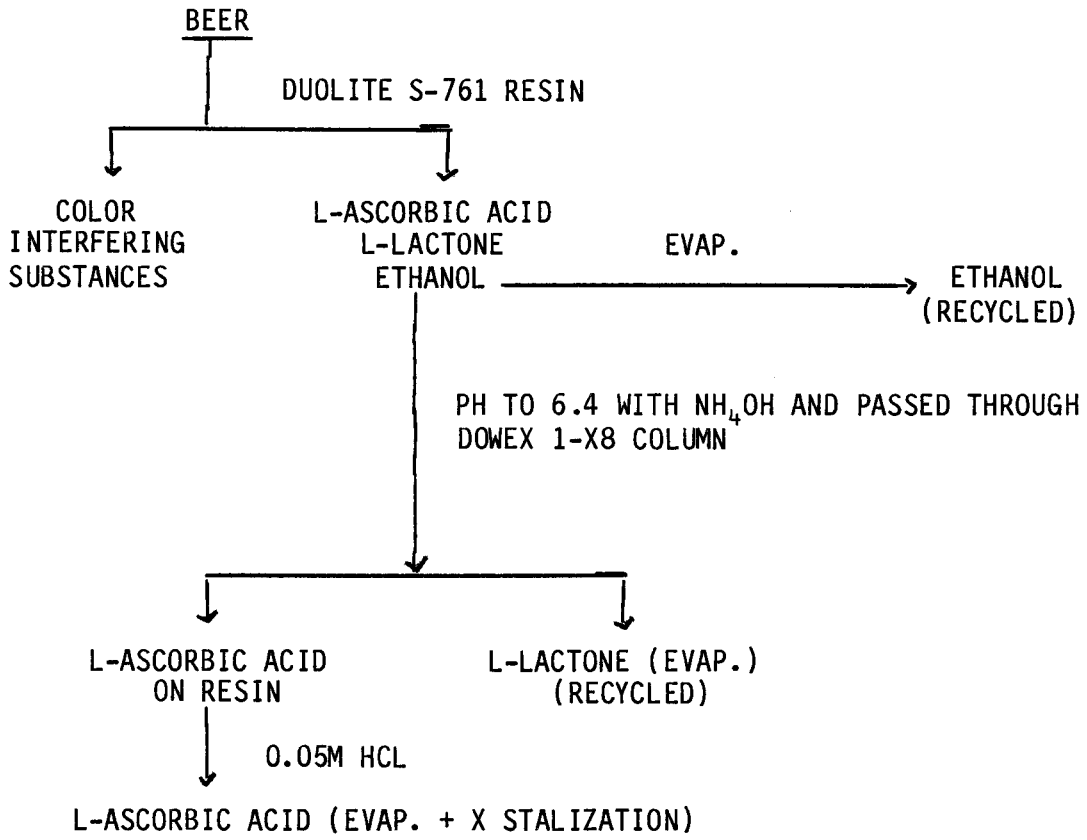
Recovery and Recycle

The clarified bioconversion mixture is passed through a Duolite S-761 resin (Diamond Shamrock Ion Exchange Functional Polymers Division, Redwood City, CA) column where color and other interfering compounds are removed. Alcohol was recovered during CONCENTRATION. The pH adjusted ascorbate-lactone mixture was passed through a Dowex 1-X8 column. The lactone was recovered with a water wash, concentrated and returned to the fermentor where it is used to maintain the lactone level during bioconversion. The L-ascorbic acid is recovered from the resin column with 0.05 M HCl, followed by concentration and crystallization from a 1:2 mixture of ethanol: ethylacetate (Figure 6).

The conditions of fermentations includes, maintenance of lactone concentration at 1.4 %, starting the ethanol concentration at 1.5 % and allowing it to fall to 0.5 % at which level it is maintained and includes the spent yeast from the alcohol recovery at a level of 0.19 % in place of the mineral mix.

Figure 6

SCHEME FOR RECOVERY/RECYCLING OF ASCORBATE, LACTONE AND ETHANOL



The stoichiometry of the bioreaction as obtained by Cayle et al. (9) is shown in Table 8.

The bioreaction is substrate driven and requires an excess of lactone. However, this component can be recycled as can excess O₂, and ethanol, with an overall recovery of 90 % of the lactone and oxygen and 95 % of the ethanol.

From a typical plant which produces a 11.3 million kg of cheese per year and if the whey from such a facility is ultrafiltered to recover WPC, 4.876 million kg of permeate solids would also be generated, with a negative market value. The procedure described here could be used to convert this whey to 1.17 million kg of L-ascorbic acid with a significant dollar values.

The amplification of L-gluconolactonolactone oxidase either within the genus candida or within another genus of microorganisms with faster generation time, and/or a faster rate of bioconversion capability, thereby, even further enhancing the value of the process, will be a breakthrough.

7.6) Vitamin B Complex

Studies on production of vitamin B₁₂ have been carried out by Marwaha and Co-Workers (155, 156, 157) using Propionibacterium shermanii and Propionibacterium arl AKU 1251 in whey permeate medium. One-day-old inoculum, 5 mg iron per liter and 4 % whey lactose were optimal for vitamin B₁₂ biosynthe-

Table 8

Stoichiometry of ascorbate production

Reactant		Product	
per liter			
Lactone	20 g	Ascorbic acid	7.5 g
Etoh	84 ml	Lactone	12.49 g
O ₂ /Air	408 1/408 1	Etoh	5 ml
		O ₂	367 l

sis in both the strains when fermentation was carried out under aerobic and anaerobic conditions at 30°C. The supplementation of whey medium with 0.5 % $(\text{NH}_4)_2 \text{HPO}_4$ enhanced the yield; however, the preference for a mixed carbon source (lactose + D-glucose or lactose + D-fructose) at different levels varied in the strains studied. P. shermanii was found to be a better strain than Propionibacterium arl AKU 1251 for vitamin B₁₂ yield (155). The yield of vitamin for P. shermanii was found to be 5.76 mg/l of whey with a fermentation time of 168 hours. The BOD of the whey was reduced by 90 %. The vitamin B₁₂ enriched whey was of use as a feed supplement to replace animal protein factor in poultry production.

7.7) Ethanol fermentation of whey permeate

Two methods for fermentation of the lactose in whey have been considered by different authors:

- One consists in the treatment of whey with lactase to hydrolyze the lactose before fermentation (25-30).
- The second approach consists of the direct fermentation of whey either with lactose utilizing yeast or with bacteria (12, 14-15, 31-46, 153, 159, 163, 166, 170).

Moulin et al. (12) claim that there is no need for any supplementations to the whey permeate, but the growth curve they showed represented an uncoupling between growth and alcohol production occurring after 20 h of cultiva-

tion. Castillo (13) found that the addition of different salts and growth factors has a negative effect on the yield of alcoholic fermentation. On the other hand, Burgess and Kelly (14) added 0.1 % yeast extract and 0.05 % urea to their 15 % lactose permeate medium, while Chen and Zall (15) determined an optimal need of 0.7 % of yeast extract to their 10 % lactose medium. Vienne and Stockar (16) found that whey permeate is in short supply of nitrogen. It was shown that whey permeate does not permit maximum growth and maximum yield of the yeast. Addition of a nitrogen source to permeate increases biomass yield almost to its maximum value but has no substantial effect on the growth rate (16). Addition of 0.1 % of yeast extract, however, has an effect on both values, and almost restores the maximum growth rate of 0.225 h^{-1} and maximum biomass yield of 0.0526 observed with the richly supplemented medium (16). It was also indicated that while whey permeate sustains growth of yeasts, this medium has two limitations to growth.

- The first is stoichiometric one due to a certain shortage of nitrogen source in the whey permeate and is demonstrated by the increase of biomass yield with the addition of 0.17 % of $(\text{NH}_4)_2 \text{SO}_4$.

- The second is a kinetic limitation. This is shown by the increase of the specific growth rate by 44 % upon addition of 0.1 % of yeast extract to the permeate. In this case, the parallel increase of biomass yield can be accounted for by the nitrogen content of yeast extract.

While it is possible to ferment the lactose in whey permeate completely to ethanol without adding any further nutrients, the fermentation kinetics may be markedly improved by formulating a well-balanced medium which can be done by adding 0.375 % of yeast extract with a lactose concentration of 4.5 %. The maximum specific growth rate in batch cultures was thus increased from 0.164 to 0.318 h⁻¹, whereas the maximum alcohol productivity was improved from 2.0 to 5.1 g/l/h.

Deproteinized concentrated whey fermentation of 20.1 % lactose was studied with candida pseudotropicalis to yield 9.7 % (W/V) ethanol concentration at pH 4.5 and 30°C (47). Using 13 g.l⁻¹ inoculum, 8.3 % ethanol was produced within 22 hours in concentrated whey containing 17.5 % lactose (47). A number of studies on direct fermentation of lactose in whey permeate by immobilized cells have also been carried out (161, 162, 165).

Because glucose and galactose are more universally fermentable sugars than is lactose, it is suggested that β-galactosidase treated whey would make a better substrate for industrial fermentation. Production of ethanol by K. fragilis and S. cerevesiae using cottage cheese whey was studied. 80-90 % of lactose had been hydrolyzed to glucose and galactose. The results show that the fermentation time was increased over that of unhydrolyzed whey (120 hours vs. 72 hours by K. fragilis at 30°C and an ethanol yield of 2 %) because of a diauxic pattern of fermentation (28).

But the authors suggested (28) that even though the hydrolyzed whey requires a longer fermentation time, it could lend itself to the preparation of

concentrates with relatively high solids contents because of the greater solubility of glucose and galactose. This may result in higher alcohol yields than is usually possible in normal whey, because high-alcohol-producing yeast strains that are unable to ferment lactose can be used for the fermentation. In a subsequent study (62) it was found that an ethanol yield of 6.5 % could be obtained using S. cerevesiae with a lactase-hydrolyzed acid whey permeate containing 30-35 % total solids.

The concentrated lactose in the whey is converted to ethanol; some of the lactose is also converted to carbondioxide and yeast. The alcohol concentration from the fermentation is about 9 % by distillation unit where the alcohol is concentrated. Yeast is produced during the fermentation and though these may be recovered for sale as feed, this is only profitable for very large plants. The still bottoms contain valuable minerals, carbohydrates and nutrients, and when concentrated are marketable as an animal feed supplement. Alternatively, still bottom contents may be anaerobically digested to produce methane gas.

Using secondary treatment, the effluent BOD from the alcohol process would be in the 30 ppm range as compared to 30,000 to 40,000 ppm for the incoming permeate.

According to studies performed by Singh et al. (18) on concentrated whey by reverse osmosis in a continuous process with cell recycle with initial lactose concentration of 16 %, a 9 % ethanol (V/V) was developed in a residence time of 12 hours. Lactose concentration at the end was less than

5 g.l⁻¹. Ethanol yield of 90 % of theoretical or 46.2 % overall is possible. The BOD was reduced by 90 % during the course of fermentation. The largest amount of water stems from the reverse osmosis unit and is water that permeates through the membranes during the concentration of whey ultrafiltrate (Figure 7). The water is clear and low in BOD, being typically 100-200 ppm BOD and can be used as washdown or dilution water. The second source of wastewater is from the still bottoms or stillage treatment unit (Figure 8).

A stillage treatment unit is necessary since stillage directly from the distillation tower has a high BOD of around 15,000 ppm. Evaporation of stillage bottoms to concentrate the valuable nutrients and minerals in the stillage for sale of animal feed supplement is recommended. This option is economically attractive if a feed blender is located in close proximity to the ethanol facility.

The second option is anaerobic digestion of the stillage to generate methane gas. This gas can be used to generate steam for the distillation unit and can produce about a third of the steam required for distillation (18).

Due to the fact that the ethanol process removes up to 90 % of the incoming BOD and that the reverse osmosis unit reduces the overall hydraulic load, the size of the stillage treatment facility can be fairly small. After primary treatment of the stillage by either evaporation or anaerobic digestion, the BOD load is reduced to around 500 to 1,000 ppm. Secondary

Figure 7: Ethanol process

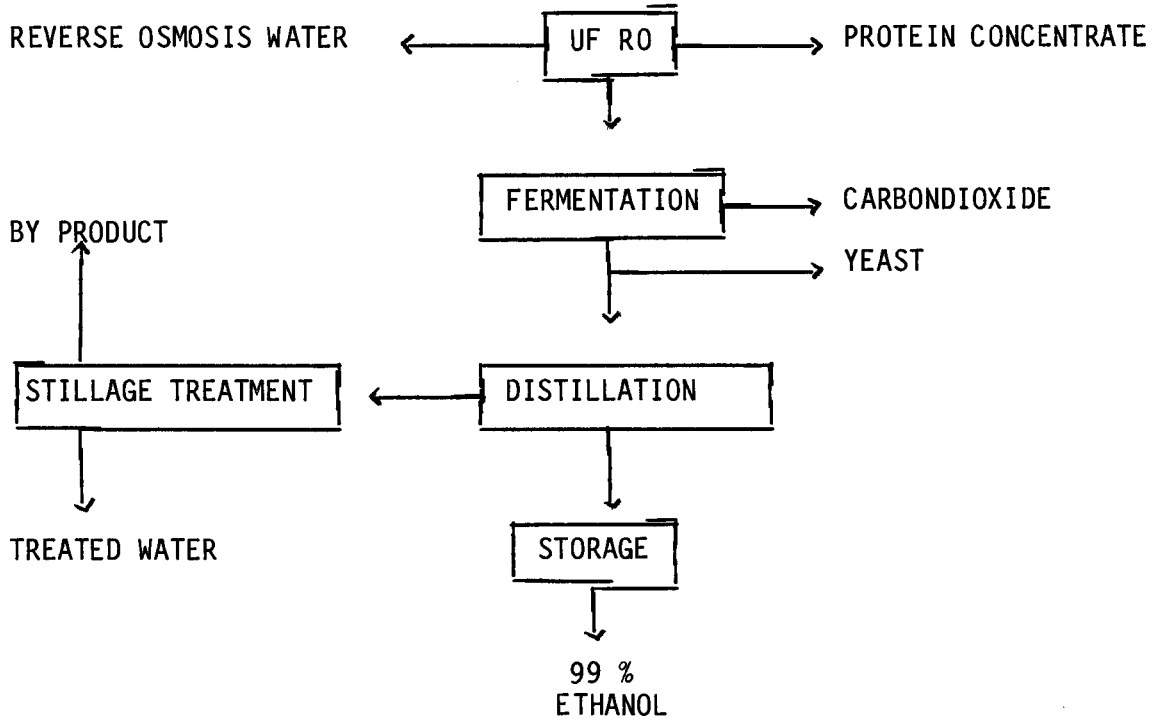
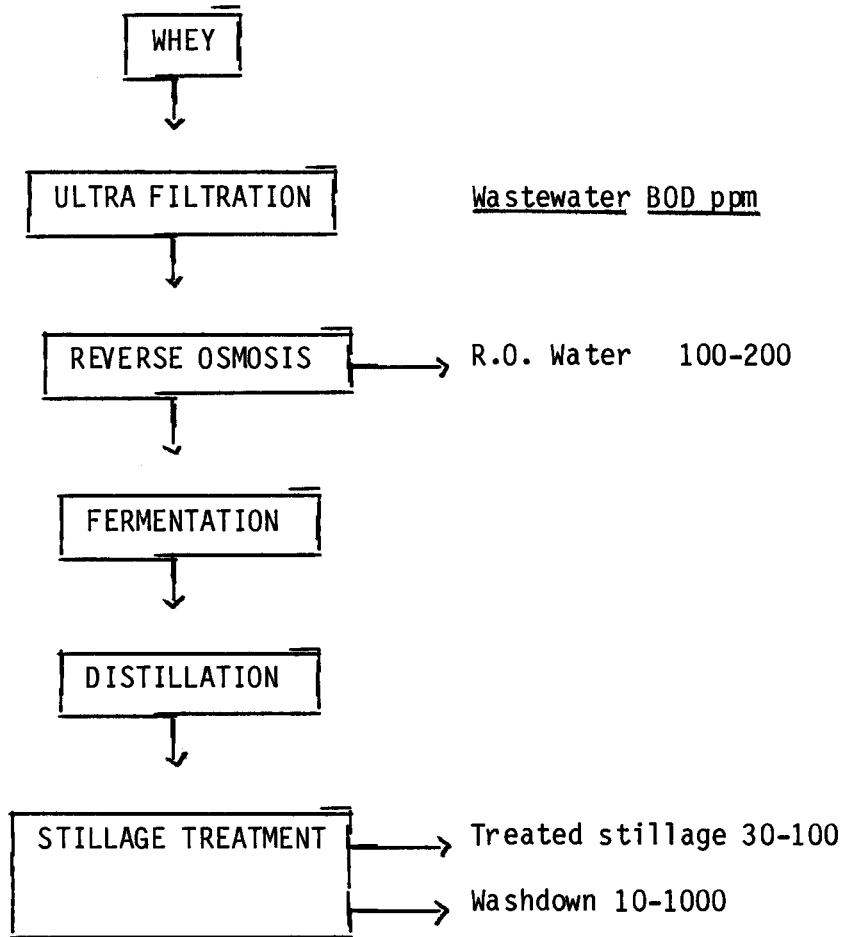


Figure 8: Ethanol plant wastewater



treatment may be required to enable discharge to a stream. Aerobic secondary treatment would bring the BOD level down to less than 30 ppm.

A third source of wastewater is due to washdown, spillage and cooling tower blowdown. This water varies both in volume and BOD and is handled in the stillage facility.

A plant is running in Denmark for the continuous fermentation of whey to ethanol for beverages. The whey is first concentrated by reverse osmosis and then by ultrafiltration. The yield of alcohol corresponds to approximately 80 % of the theoretical yield according to Gay-Lussac equation.



This corresponds to the use of approximately 42 l of whey permeate containing 4.4 % of lactose for the production of 1 litre of 100 % alcohol by using a strain of K. fragilis. The fermentation in such a process has been suggested to be continuous in two stage with yeast recycle (42). Economic analysis of this process has shown that it can compete with synthetic alcohol produced from ethylene (42). The minimum total residence time required in the system was 12 hours (42).

Wa-Hol, Inc. halves BOD of whey effluent and produces anhydrous ethanol and food-quality proteins to boot.

Associated Milk Products (AMP), the largest US dairy coop., bought exclusive rights (in 1984) to process its waste water from 660,000 lb-milk/day cheese making plant in Warsaw, Indiana. Anticipated yields: 65,000 lb protein, 1,800 lb ethanol and 400 lb yeast. Company committed five more plants (1984-1986).

Process deproteinizes concentrated whey (30 % solids) with special filters patented (Biomass Digest, January 1984), able to separate particles as small as 0.5 μ . Similar filters have been used in wine production for years, but never before with milk products. Process uses a proprietary yeast strain (Tycor, 1983) to ferment the protein-free permeate and with stands to high osmotic pressure (salt levels to 3 %). Distillation brings mash to 90 % ethanol, then standard zeolite molecular sieves dehydrated alcohol to 99.96 % pure ethanol.

Major process advantages are:

- low BOD of whey effluent
- good digestibility of protein product

Deproteinizing whey cuts effluent BOD to 140 ppm from over 300 ppm, making it safe to spread 644 liters (or 170 gallons) over an acre of farmland. Fertilizer value: potassium, nitrogen and phosphorus worth \$ 80. Protein produced cost \$ 2.2/kg (or \$ 1/lb), roughly half that of protein available today.

A 7.5 million liters/year (or 2 million gallons/year) ethenol plant using cheese-factory whey as feedstock started constructing in early 1985 in Corona, Ca. The target to sell ethanol from this plant was put at 5.3¢ per liter of ethanol (or 20¢ per gallon). The \$ 110 million Corona plant was built by the Express Dairy Group, A London-based foods organization, for Integrated Protein Technology, A California company, " Express Dairy", designed the plant and will operate it under contract. The group is using the patented whey-to-ethanol technology of a member company, Carbery Milk Products Ltd. of Ballineen, Ireland. The Carbery whey to-ethanol has been used for two plants in New Zealand as well as the carbery plant in Ireland and the California plant. One plant in New Zealand produces potable ethanol and another produces anhydrous ethanol for other purposes. These two plants based on whey as raw material have turned New Zealand from being importer of industrial alcohol to a net exporter. The carbery whey-ethanol plant in Ireland has been operating since 1978 (Source: Alcohol Week, February 8 1985).

Whey has also been used to produce beverages (146, 148). Whey containing 35 % protein (dry matter basis was used to produce a chocolate drink (pH 6.40) (146). Whey (rennet and acid-whey) with the addition of 5 or 10 % glucose was inoculated with zymomonas mobilis, which is capable of producing ethanol and lactic acid from glucose and incubated at 38°C was fermented over 72 days to obtain a fermented beverage (148).

Economics of ethanol from whey

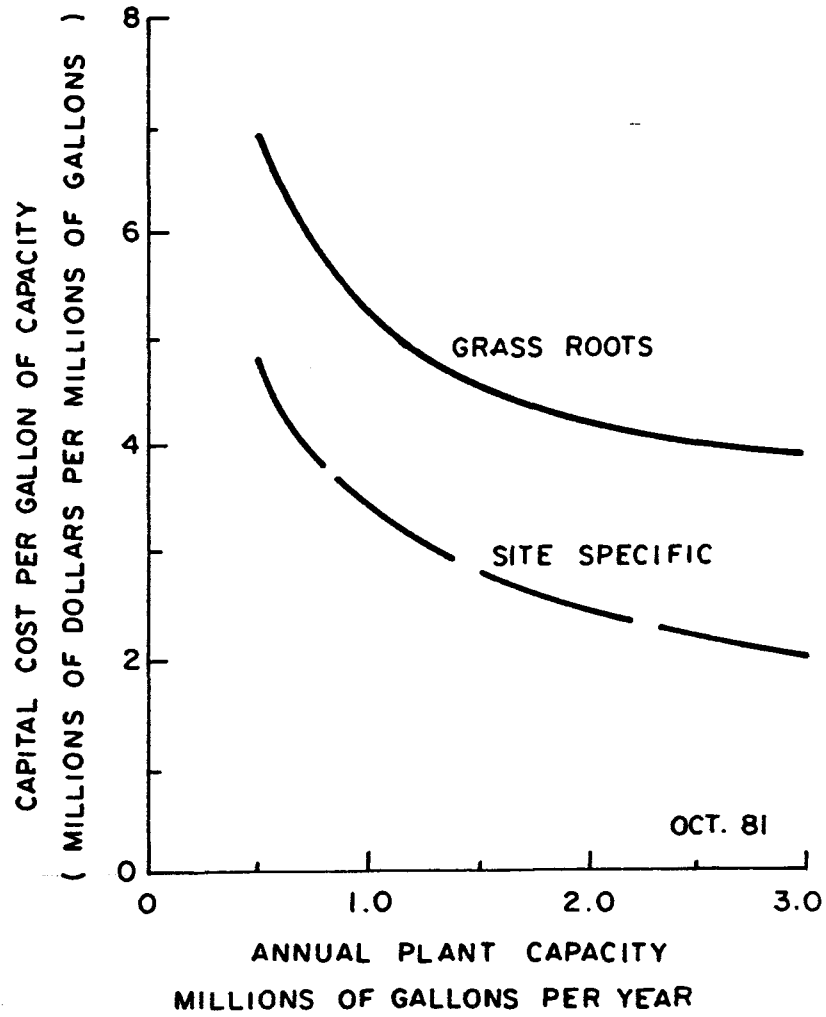
Whey as the sole carbohydrate source for ethanol production has been investigated and several processes put forth (18, 5, 61, 27). However these processes require large quantities of concentrated whey for economic operation which restricts the number of feasible plant sites. The fermentation of unconcentrated whey by lactose fermenting yeasts results in low levels of ethanol which prohibit economic recovery.

Economics of ethanol plants are dependent on capacity, cost of feed stock, site features and local price of ethanol. In all the costs presented (18), the alcohol plant is defined as starting after the ultrafiltration step down through to secondary waste treatment.

Costs of the ultrafilter and profits therefore are not accounted for in the analysis. Thus, all costs of producing the ethanol from the waste permeate are included, such as: preconcentration, fermentation, distillation, storage and waste treatment.

Whey alcohol plant as shown in Figure 9 as a function of annual ethanol production capacity. Capital costs vary from \$ 2.00 (US) to over \$ 5.00 (US) gallon⁻¹.year⁻¹ (or \$ 0.73 (US) to \$1.82 (US) l⁻¹.year⁻¹) installed capacity. The lower curve is the estimate of a site specific cost which assumes that the ethanol facility is associated with an existing cheese or whey processing plant and that certain buildings, equipment, manpower and

Figure 9



Why alcohol plant capital cost

facilities are shared with the ethanol plant. The upper curve represents the capital cost if a stand alone facility were constructed.

Operating cost number for the various size facilities are shown in Figure 10. These estimates take into account operating labour, power, chemicals and feedstock hauling costs. Operating costs range from \$ 0.80 - \$ 1.18 (US) gal⁻¹ (or 21¢ - 31.25¢ l⁻¹) ethanol for the 0.5 million gallons per year ethanol facilities to \$ 0.72 - \$ 0.75 gal⁻¹ ethanol for the 5 million gallons (or 19 million liters) per year plants. It should be noted that these figures do not include cost of capital or depreciation.

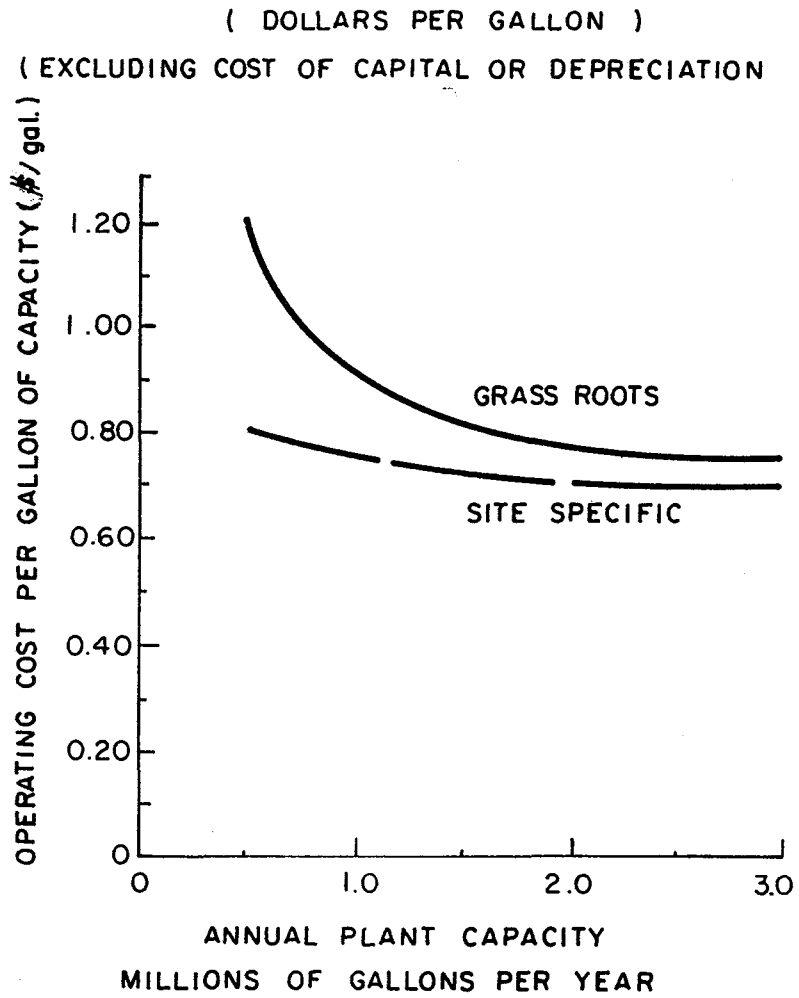
When leveraged, the ethanol facilities can require cash outlay of less than \$ 1,000,000 and can return that cash in 18 months of operation. Discounted internal rates of return to the equity holder in excess of 40 % are easily obtained.

To be economically viable, the facility must produce, typically, at least 0.5 million gallons (or 1.89 million l) of ethanol per year. The quantity of whey required to meet this capacity plant will be 80 million liters per year.

Protein

The microbes most commonly used by man in foods and feeds are Fungi: yeasts, moulds and marofungi. For centuries they have been used for food processing and for protein enrichment. More recently a yeast industry has

Figure 10



Alcohol plant operating cost.

developed who markets whole yeast and yeast products as food additives or animal feed components. Selection of strains is based on a number of criteria related to the processing requirements and properties needed in the product (Table 9). Mutant selection can be used to improve some of the characters provided a selection method is practicable (52-54).

The protein content of yeasts and moulds is however, lower than bacteria used for SCP production. Crude protein content of 40 ~ 60 % are commonly recorded, about two thirds of that being α -amino nitrogen. SCP (single cell protein) is generally inferior to the protein in eggs, fish and milk. But is considered to be an alternative to soyabean meal protein, although some bacterial products with 75-80 % crude protein contents may replace fishmeal.

Nucleic acid contents are usually in the range of 5-15 %, higher levels being found in the bacteria than in yeast and moulds. In most processes no attempt is made to remove them.

An additional feature of SCP is the presence of high content of the B group vitamins. As a result a major market for yeasts has been its use as an additive to boost vitamin contents of diets rather than as bulk protein source. As such therefore they have been incorporated into rations in low concentrations.

Kluyveromyces fragilis has been considered to be suitable as food yeast and has been grown in whey with reported satisfactory yields (47-48). Optimal

Table 9

Some important properties of fungi used for SCP

	<u>Candida</u> <u>utilis</u>	<u>Fusarium</u> <u>graminareum</u>	<u>Fusarium</u> <u>moniliforme</u>
Maximum growth rate (h ⁻¹)	0.5	0.28	0.31
Temperature optimum (°C)	30	30	35
Morphology	unicells	short hyphae	short hyphae
N x 6.25	50	54	47
Lysine g/16 N	6.7	7.2	7.0
Methionine g/16gN	1.2	6.3	1.6
Cystine + Cysteine	0.9	4.1	1.4
Toxicity in rats	none	none	none
Reference	(55)	(56)	(57)

conditions for maximum growth of this organism in deproteinized whey included supplementations with 0.3 % yeast extract and 0.6 % $(\text{NH}_4)_2 \text{SO}_4$, a temperature 40°C and initial pH 5.0 (49-51). Under these conditions, yields of 0.4 gm/gm of lactose utilized were obtained in seven hours and the yeast biomass contained 50 % protein, 9.5 % nucleic acids, 42 % carbohydrates and 1.1 % phosphorus (49). Many other studies have been carried out on production of single cell protein from cheese whey for uses as animal feed or human food supplements (1, 3, 4, 5). Although the process has proven to be technically feasible, it is still far from being economical.

Kluyveromyces lactis, K. fragilis, candida pseudotropicalis and Torulopsis candida grown in salted whey and in water diluted salted whey were analyzed for macromolecule components of cell material. Dilution of whey affected the chemical composition of the yeasts, especially the crude protein and total nucleic acid contents. The data were used to evaluate the suitability of the yeasts as a source of single cell protein (132). By using concentrated whey permeate as substrate, food grade yeast (Kluyveromyces fragilis) was grown continuously in the fermentor to very high cell densities (total broth dry weight $> 140 \text{ g/l}$) (133). Cell mass yield remained about constant at pH 3.9 - 5.1, $31-37^\circ\text{C}$ with 6-30 % whey permeate solids in the feed (133), unlike to Shay et al. (132). Dilution rate had little effect on the cell mass yield. The high cell density fermentation broth was directly dried without centrifugation and washing. Amino acid profile, vitamin content and fatty acid composition indicated a quality protein product suitable for food or feed supplementation. Ash content of the product was higher than other dried yeasts because of the high mineral content in the whey permeate. Ash

content could be reduced by the addition of a small portion of another inexpensive fermentable sugar to whey permeate feed. With this addition, cell mass yield, productivity and protein content of the product increased. Because this high cell density, direct-dry process did not require pre-concentration of the fermentor broth to dry the product economically, it did not generate a waste stream that required further treatment (133). A number of other studies for the production of single cell protein using different strains have also been carried out (144).

7.10) Organic acids

a) Gluconic acid

Production of gluconic acid by Gluconobacter oxydans from cheese whey has been studied by Vanhuynh et al. (103). The whey was hydrolyzed continuously by alginate-entrapped Kluyveromyces bulgaricus cells and more than 80 % of lactose was converted into glucose and galactose. Glucose was oxidized to gluconic acid at much faster rate than that of galactose. In order to oxidize galactose completely, it was necessary to reinoculate the medium with galactose-adapted G. oxydans cells. The main product obtained under the experimental conditions was gluconic acid.

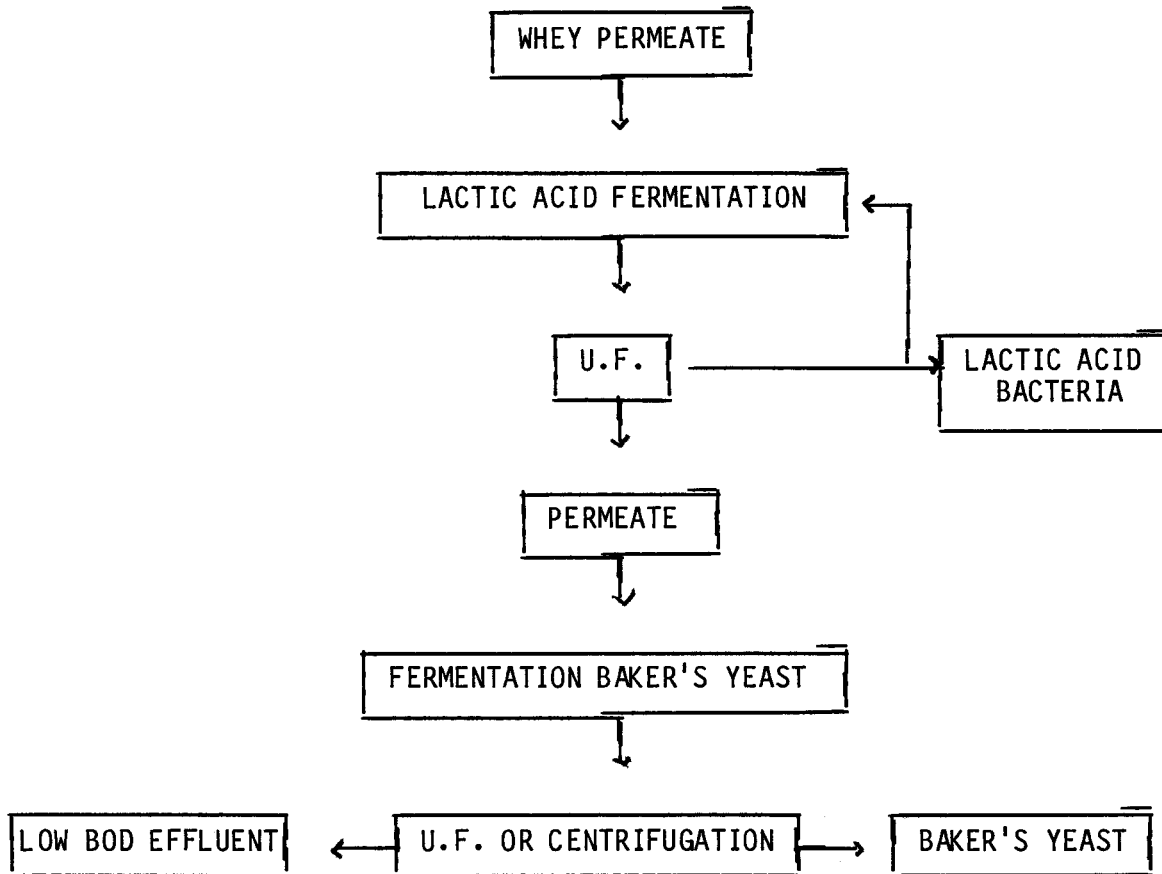
b) Lactic acid

The lactic acid fermentation of non concentrated whey permeate by thermophilic lactic acid bacteria has been studied by J. Goulet (Université Laval) at

controlled temperature of 40-45°C in batch as well as in continuous culture (Figure 11). The pH was controlled at constant level by addition of ammonia. The permeate was fortified with nitrogen source (vegetable protein, eaux de trempage de maïs, yeast extract). The lactic acid fermentation has also been studied in immobilized cell system (104-105, 154) and membrane reactor (152).

L. bulgaris, L. casei, L. fermentii and L. plantarum were screened on paneer whey for their ability to produce lactic acid (131). The acid production was efficient with 10 % inoculum of L. bulgaricus, 4.5 % whey lactose, pH 5.5 and a fermentation period of 24 h at 30°C. Addition of peptide and mustard oilseed cake to whey improved acid production. Replacement of lactose by glucose supported better conversion of lactose to lactic acid. FeCl₃ (5 ppm) increased acid fermentation. Lactic acid production was induced by glutamic acid. A fermentation apparatus for the continuous production of lactic acid from whey has been described by Prigent (169). The apparatus contains a fermentor equipped with a mechanical stirrer, a source of NH₃ or soda coupled to a pH regulator, as well as a means for continuous delivery of whey and supplements to the fermentor. A pump inserted between the fermentor and an ultrafiltration apparatus allows for recycling the liquor as well processing filtrate by electrodialysis for lactate recovery. The apparatus also allows efficient separation of lactose from lactic acid. Empirical exponential equations correlating the volume of NH₄OH solution, added in order to control the medium pH, with number of base additions during a batch lactic acid fermentations are described by Borzani and Baralle (172).

Figure 11: Bioconversion of whey permeate to lactic acid



c) Acetic and propionic acid

The annual demand for propionic and acetic acids is approximately 1.3 billion kgs (118). Acetic acid is used extensively in the manufacture of cellulose acetates, vinylacetate, acetic esters, etc. Propionic acid is used in a variety of industrial processes. Cellulose propionate is an important thermoplastic, and esters of propionic acid are used in the perfume industry.

Acetic acid and propionic acids may be produced biologically by the fermentation of sugars using a species of Propionibacterium (119). Propionibacterium acidi-propionici, a specie of Propionibacterium, produces high concentrations of propionic and acetic acids at pH values of 4.1-4.9 (a natural pH of acid whey) by utilizing sugars. Approximately 2 moles of propionic acid and 1 mole of acetic acid is produced per 1.5 mole of glucose or galactose (in whey) utilized. One mole of carbondioxide is also produced.

Fermented whey containing a high concentration of propionic acid is manufactured by 2-step fermentation, first with lactic acid-producing and second with propionic acid producing bacteria. The product is used for preserving bakery products, where propionic acid in the fermented whey acts as a mycostatic agent (147). In this process fresh whey (6.5 % solids) was pasteurized, inoculated with Lactobacillus bulgaricus and Streptococcus thermophilus for lactose fermentation (pH 4.3 - 6.0). For propionic acid, whey is adjusted to pH 7.0, sterilized and inoculated with Propionibacterium

shermanii. The final yield of propionic acid was 1.6 % (by weight) (147). Mixed culture studies for the production of propionic acid to preserve the bakery products have also been carried by Bodie et al. (164) in three stages. P. shermanii and L. casei grown together through 3 stages in the draw and fill mode produced ~ 4.5 % propionic acid in 70 h and all the lactose was consumed.

Whey fermented with Propioni-bacterium acidi-propionici resulted in a product containing more propionic acid and consequently, greater mycostatic activity, than that produced conventionally using P. shermanii (173). 25,000 gallons of sweet whey containing 7 % solids and 0.5 % yeast extract was sterilised and inoculated with 2,500 gallons solution of P. acidi-propionici and incubated for 66 h at 35° and pH 7.0 (maintained by periodic addition of NaOH). The fermented whey product containing 0.96 % propionic acid and 0.2 % acetic acid (compared with 0.8 and 0.3 %, respectively for conventionally prepared whey) was then spray-dried and packaged for use as a mycostatic agent in the manufacture of pastry, bread and other bakery products (173).

Propionibacterium acidi-propionici is a slow growing organism during fermentation, requiring fermentation times of 12 to 14 days for a 56 % conversion (120, 121). A batch fermentation of 8 days resulted in a conversion of only 30 %. In mixed culture time required is about 3 days. Consequently a very large reactor is required for the large-scale production of organic acids by fermentation.

Immobilized whole cell systems for fermentation processes, recently developed, have the benefits of a high productivity with reduced substrate and product inhibition (which is the case for organic solvents and organic acids). Recent work by Tyagi and co-workers on ethanol production in immobilized cell reactor has shown productivities 15 times the values found in a continuous stirred tank reactor (122-123). Therefore using same immobilized cell reactor for the production of organic acids and solvents anticipated time will be reduced consequently the size of bioreactor and hence the cost of overall process using cheese whey as substrate. For example, in continuous fermentation, a 90 % conversion of glucose were achieved in the continuous stirred tank reactor (CSTR) for a 72 hours retention time and a 30 g/l total sugar concentration. The fermentation in the CSTR was shown to be about four times faster than that in a batch reactor. Whereas, a 90 % conversion of glucose were found at 28 hours retention time in immobilized cell reactor (ICR). Also, about 67 % sugar can be converted in organic acids in this reactor, yielding a more than 20 g/l or organic acids in the ICR (127). For further reduction of time and hence bioreactor size systematic studies should be carried out on whey using immobilized system like investigated by Tyagi and others not only for production of organic acids and alcohol but also for organic solvents (acetone, butanol, etc.) and should be compared with other existing systems.

d) Amino acids

Whey has also been used as a carbon source for microbial synthesis of amino acids (174). Only few studies appear in the literature. This area requires further research for the economical production of essential amino acids.

7.11 Land application of cheese whey

Because of its environmental pollution potential cheese whey could be used as a valuable fertilizer and applied to the land with minimum or no damage to the environment.

The nitrogen in cheese whey is water soluble is subject to leaching. Thus, maximum whey loading rates to the land should be based on nitrogen loading that:

- a) will be most effectively used by crops
- b) will cause no damage to crops
- c) will not result in excessive nitrogen concentrations in ground water.

However, more information is needed on the environmental impact of large quantities of whey being applied onto the soil. The whey nitrogen transformation and transport in three Nova Scotia soils receiving high whey application rate and the efficiency of soil adsorption mechanism has been examined by Ghaly and Singh (6).

The whey application was tested in columns of different height filled with soil. Application of whey was made based on assumptions that:

- a) the nitrogen requirement for corn crop is 280 kg-N/ha
- b) only one half of the organic nitrogen would be available for the crop.

The total nitrogen in the cheese whey was 1,820 mg/l; most of it (96.99 %) was in the organic form.

Therefore one liter of whey was the amount required for each column.

The average monthly rainfall for period May-September was 98.5 mm. This is equivalent to 3.1 liters of water per column. The total rainfall accumulation for the five months period (15.5 liters) was applied into each column at a rate of 3.1 liters every 8 days starting on day 0 (i.e. 3.1 l on each days 0, 8, 16, 24 and 32). Leachates were collected every 4 days and analyzed for nitrogenous compounds.

The organic nitrogen concentration in the whey was 1,765 mg/l. Only concentrations of 0.6 to 1.4 mg/l of organic nitrogen were found in the leachate samples. Most of the organic nitrogen in the cheesewhey (99.9 %) was retained by the soil and will therefore be made available to the plant after conversion to ammonium. The results also showed that soil type and soil depth did not have any significant effect on the soil removal efficiency of organic nitrogen. The nitrite nitrogen concentration was reduced from

2 mg/l to 0.01 to 0.08 mg/l. Both soil type and soil depth did not have any significant effect on the nitrite nitrogen concentration in leachates.

The concentration of nitrate was reduced from 28 mg/l to 4.9 - 7.5 mg/l. Both the soil depth and soil type affected the concentration of nitrate nitrogen in the leachates. Although the concentration of nitrate nitrogen in the leachate was below the maximum allowable level (10 mg/l) in drinking water for humans, it was above the allowable concentration (5 mg/l) in drinking water for animals. Continuous application at higher rates may therefore pose a health hazard for both animals and humans.

7.12) Other products from whey

a) Isopropanol, butanol and ethanol (IBE)

The production of iso-propanol, butanol and ethanol using concentrated whey (by multiple effect evaporator) was studied with clostridium beyerinckii in a fluidized bed reactor. The products are removed from broth in two stages during the process. Firstly by evaporation of the fermentation broth in the recycle loop of the fluidized reactor continuously and simultaneously. Secondly, by conventional distillation of the effluent. A production cost of US \$ 0.19 per kg of IBE mixture has been estimated (102). The authors concluded that the combination of a concentrated feed (low transport costs), immobilized cells and continuous product removal (high reaction rates) is essential.

Continuous production of butanol from whey permeate with immobilized cells of C. beyerinckii in Ca alginate beads have been studied (160). The influence of three parameters (fermentation, temperature, dilution rate [dilution rate in continuous system is the ratio of flow rate to the bioreactor and the working volume of bioreactor] and concentration of Ca²⁺ in fermentation broth) on the butanol production was investigated. Both a fermentation temperature of 30°C and a dilution rate of $\leq 0.1 \text{ h}^{-1}$ during the start up phase are required to achieve continuous butanol production from whey permeate. 16-fold higher production of butanol was recorded than those found in batch cultures with free C. beyerinckii cell on whey medium (160). The influence of temperature on solvent production from whey by using strains of clostridium acetobutylicum and C. butylicum have been discussed by Voget et al. (167). Higher yields of solvents were observed at 37°C or at 30°C depending upon the strain. An overall reactor productivity of 0.24 g/l/h of solvents was observed during batch fermentation of whey permeate with C. acetobutylicum P262 (168). In semisynthetic media galactose was shown to be as effective as glucose.

With hydrolyzed whey permeate, preferential uptake of glucose over galactose was observed and such hydrolysis provided no advantage to the fermentation process (168). Factors that could further increase the profit margin are (102):

- further purification of the IBE mixture will lead to products (n-butanol, iso-propanol and ethanol) with a far higher market value than the full mixture.

- collection and further processing of the fermentation gas (CO₂ and H₂); use of the waste from the beer still for methane production.
- lowering salt concentration in the whey permeate; feed concentrations higher than 27.5 % (W/V) could then be used, which would lead to lower product recovery costs.
- situation of the fermentation plant next to the whey permeate producing factory; the production costs would be 20 % lower.

b) Production of oils and fats

Fats biosynthesis using whey permeate has been studied by a number of workers (149, 158). Laboratory culture of candida curvata on sugar beet molasses or deproteinized whey media for 30 h yielded < 8 and > 4 mg fat/l respectively. The effectiveness of conversion was 11.7 g fat/100 gm of lactose. The conversion by Trichosporon cutaneum was slightly less efficient. C. curvata harvested from deproteinized whey media contained 18 % solids, which contained 34 % total fat (149). C. curvata was also grown in cheddar cheese whey permeate in continuous culture at various dilution rates and yielded lipids of consistent composition except for fastest dilution rate examined (0.2 h⁻¹). The lipid produced at this dilution rate contained less oleic acid and more linoleic acid than did lipid produced at slower dilution rates and resembled the lipid found in exponential phase cells in batch culture. The continuous culture system was claimed to be more efficient than batch (158). This increased efficiency might also be seen on an industrial scale and could make the fermentation of permeate to produce oil more economically feasible. A yield of

20 g oil/100 g lactose utilized from whey was achieved by batch fermentation with candida curvata (175). The oil is similar in composition and properties to palm oil. In continuous fermentation of a whey medium with the same organism, a yield of 10 g oil/kg culture has been described by the authors (175).

c) Animal feed

Fermentation bacteria has also been used to produce fermented ammoniated condensed whey (FACW) for increased utilization of whey in place of Soyabean meal for animal feed (109). Studies on animal feed block from whey by adding a divalent cation action containing phosphate salts of Ca and Mg to concentrated whey (> 45 % solids) has been performed by Chambers et al. (134). Acidified whey was mixed with urea at 28 % of dry matter, cooled and neutralized (if necessary) to give a new feed called concentrate Ksona (135) containing lactsyl ureide (the product of reaction between whey lactose and urea). The product contains 25-39 % protein and 15-20 % lactose. The level of Ca, P and S in the product is 0.4-0.6 %. Ksona may be added to manufacturer feed concentrates (at < 17 %) to increase its biological value (135).

Whey lactose was fermented to lactic acid followed by ammonification with 20 or 25 % NH₃ water which resulted in a 3-5 fold increase in Non protein N level (171). The whey contained 30 g digestible protein per liter and 0.08 feed units per liter and was successfully fed to the fattening steers and lactating cows, permitting a savings in high-protein concentrated feeds. Another process is developed by Rachev et al. (176) for the production of a

N-enriched whey product (lactosylurea) intended as a ruminant feed. The product was made of natural whey (solids 6.0-6.7, lactose 4.5-5.0, protein 0.7-0.9, fat 0.3-4 and minerals 0.5-0.7 %), urea and catalysts. The stages of the process were: purification of whey, mixing with urea and catalysts, heating and concentration (vacuum evaporator). Lactosylurea contained: solids 58-62, N (total) 9-10, protein 2.2-4.6 %. One kg lactosylurea contained 0.45 feed units and 623 g crude protein. It can be used as a supplementary source of N for ruminants.

A full scale plant producing lacto-whey FACW containing ammonium lactate started in U.K. (136). The microorganism used is Lactobacillus bulgaricus at a 5-10 % inoculum level. FACW showed 44 % crude protein equivalent, 37 % lactic acid at the 64 % solids level with a density of 1,220 kg/m³, a viscosity of 1,320 mPa at 0°. The product is stable for six months. FACW can be fed at the rate of 0.5-1.0 kg/head daily to beef cattle and 0.5-1.8 kg/head daily to milking cows. Feeding trials showed it to be equal soyabean meal when fed to ruminant animals. Ammonium lactate is also a potential feedstock for the production of lactic acid and certain lactic acid derivatives in competition with other carbohydrate sources. A number of food and feed preparations containing whey have been prepared with or without fermentation (137-143, 145).

d) 2,3 - butanediol

2,3 - butanediol fermentation has been studied by Lee and Maddox (177). Out of the four organisms tested in semisynthetic medium, Klebsiella pneumoniae

N.C.I.B. 8017 arrived to be the most promising. When tested using rennet whey permeate as substrate, a butanediol concentration of 7.5 g.l^{-1} , representing a yield of 0.46 g/g lactose utilized, was observed after 96 hours incubation. In whey permeate where the lactose had been hydrolyzed enzymatically prior to the fermentation, a butanediol concentration 13.7 g/l, representing a yield of 0.39 g/g sugar utilized, was obtained. These results indicate that lactose utilization may be a limiting step in the fermentation process.

e) Industrial resin

Source of cheese whey as industrial resin has been examined by Chemical Process Corp. (CPC). Whey permeate from ultrafiltration is high in lactose. Separating whey after curd is salted and pressed, yields a product high in sodium chloride, making a difficult disposal problem worse. Yet CPC suspects salt content could be an advantage. Salt whey protein may contribute to cross linking in resins, resulting in stronger adhesives. By controlling pH range, investigators can direct the polymerization and crosslinking reactions (Biomass Digest, Feb. 1984).

f) A number of other products like production of aroma (107, 108), citric acid (151) and polysaccharide (106) have been tried. Xanthen campestris has been used to ferment whey-glucose medium to produce viscosities of $< 7,000$ CP at a 12-s^{-1} shear rate (150).

8) Economic evaluation

Economic analysis of dairy processing waste from a facility which process 208×10^6 lb (94.35×10^6 kg) of milk per year, 21×10^6 lb (9.53×10^6 kg) of cheese per year and 185×10^6 lb (83.9×10^6 kg) of whey per year has been studied. From this facility a whey permeate residue is produced at a rate of 62,000 gal/day (2.35×10^5 l/day). This whey permeate is highly concentrated, at approximately 5 % TS . Four options were examined for reuse/disposal of this material:

- land disposal
- anaerobic completely mixed digester
- anaerobic contact digestion
- ethanol production

On a present worth basis all the four options are within 10 % of each other (101). For each digestion and alcohol production option, no reduction in disposal cost was accounted for since this material is going to the land (Table 10).

Thus it is clear that both digestion and ethanol production are capable of producing energy at a revenue of \$ 4/MBtu for methane and \$ 2 (US)/gal (or \$ 0.53/l) of ethanol with no credit for BOD and solids reduction in the whey permeate. If disposal costs for the effluent were reduced, then compared to the raw permeate, a definite economic advantage would exist for both digestion and alcohol production.

Table 10

Dairy processing facility

Whey permeate

Item	Land disposal	Anaerobic digestion		Ethanol production
		Completely mixed	Contact digestion	
Energy produced	0	22.8 x 10 ⁶ ft ³ /CH ₄ /year	36.4 x 10 ⁶ ft ³ /CH ₄ /year	570,400 gal Etom/year
Revenue (\$/year)	0	91,200	145,600	1,140,800
Annual cash flow	-764,900	-716,700	-686,000	-286,200
Total capital investment (\$)	0	264,800	375,400	1,984,900
Present worth (\$)	-2,731,500	-2,824,100	-2,825,100	-3,006,900

Therefore, on the basis of these data it would be recommended that all energy generation options appear feasible and that further investigation is warranted to develop a more detailed economic analysis on site-specific basis to determine the most cost-effective alternative.

For small-scale cheese producers cost comparison study of whey lactose conversion has been made (47). Francisco Castillo of Engenics, Inc., working with University of California at Berkeley and Venezuelan Institute of Scientific Investigations in Caracas did detailed examination of yeast lactase and ethanol production for cheese producers. They concluded:

- all the three processes are effective waste treatment process.
- reduces BOD 90 ~ 95 %.
- only lactase production would turn profit. Even though processing costs are high, enzyme sales could net \$ 540 (US)/1,000 liters of whey treated.

The problems associated with the process are:

- market for enzyme.
- used to reduce hard-to-digest lactose content of milk and milk products.
- too small to convert this whey conversion.

The whey BOD was reduced by 90 % in each of the process and plant design were evaluated at the scale of 25,000 l whey per day, corresponding to the output of a typical cheese factory. The total treatment cost for each process are shown in Table 11.

Table 11

Cost comparisons for permeate bioconversion (47)

Process option	Treatment cost	
	Total treatment cost \$/1000 l of whey	Net treatment cost after by product credit \$/1000 l of whey (cents/kg BOD removed)
Ethanol	21.8	8.1 (26)
Biomass production	33.8	24.5 (73)
Enzyme production	65.1	-534

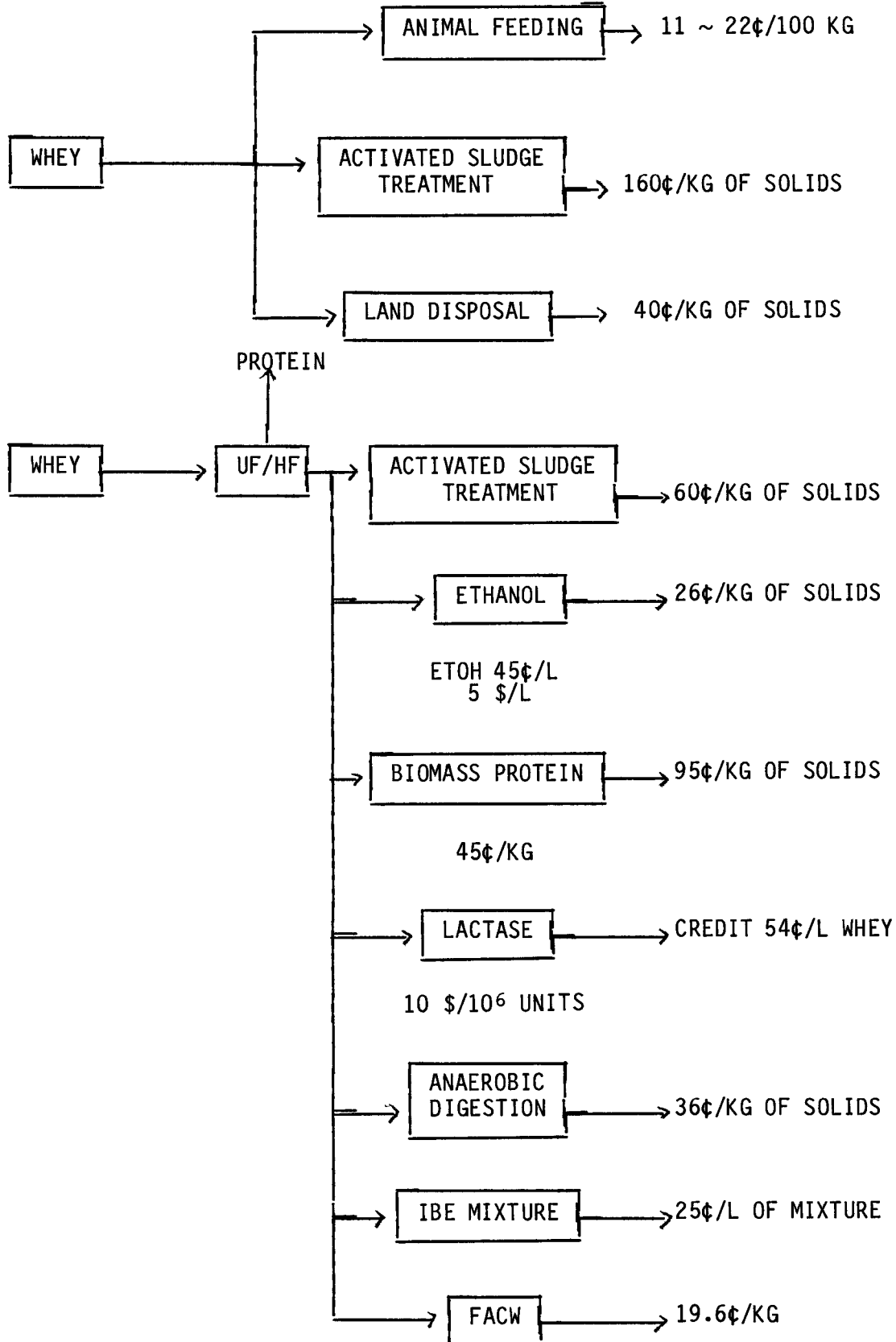
Least attractive was found to be yeast production (Kluyveromyces fragilis for single cell protein). Kluyveromyces ferments 25,000 liters of whey/day to 517 kg protein, not enough to offset high equipment costs for fermentor agitators and air compressor.

Thus ethanol production looks best. After by product credits of 45¢/liter for alcohol and 45¢/kg of for residual yeast, net treatment cost comes to only \$ 8.10/1,000 liters of whey, as compared with \$24.50/1,000 liters to produce yeast.

These figures are based on ethanol fermentation with candida pseudotropicalis ATCC 8619 which was found to be the best strain and gave 9.8 % ethanol from 28 % whey (Biomass Digest, sept. 1984).

Further a cost comparison has been made for the direct treatment with no recovery and treatment with recovery and is shown in Figure 12. Ethanol and methane production seems to be the best alternative for the first generation products. The price of FACW containing ammonium lactate worked out to be 19.6¢/kg of material produced (Fig. 12) (Dr. J. Goulet, presented at Int. Biotechnol. Symp., Quebec, August 20, 1986). Whereas the current price of molasses is 24¢/kg. Therefore, FACW has been advocated to replace molasses for fermentation products. However, the best way for cost comparison would be the cost of specific product via FACW fermentation and direct fermentation of whey permeate coupled with or without reverse osmosis and lactose hydrolysis depending upon the microbial strain used. In such a case the cost of production of FACW may be an extra burden. It requires

Figure 12: Cost comparison for different options



more data for the second generation products like organic acids and solvents, vitamins, etc. for final cost comparison.

In this respect, based on the $500 \times 10^6 \sim 591 \times 10^6$ l/year of permeate produced by one plant in Quebec will result in a plant capacity of $6.6 \times 10^6 \sim 7.88 \times 10^6$ l of 100 % ETOH/year. The cost evaluation based on such a capacity of the plant will result in an operative costs of 24¢/l (can.) for site specific and 27.4¢/l (can.) for grass root plant. These figures are comparable with the ethanol produced by a plant in Kerobert (Saskatoon) based on entirely with grain screenings collected from elevators and a plant Minnedosa (Manitoba) with 11.355 million liters of ethanol per year based on barley as feedstock (129). The cost of ethanol varies from 46¢/l to 55¢/l (can.) (130) which is almost double the cost of ethanol from whey permeate. Therefore in the present market situation ethanol can be considered as viable situation.

9) Conclusions and recommendations

The absolute dependency of canadian fermentation industries in relation with import of cane molasses and its fluctuating prices in the recent years has resulted in the search of an alternative material. The molasses, in the fermentation industries gives rise to a number of problems:

- import of cane molasses from North America
- fluctuations in the price of molasses
- chemical and microbiological qualities of molasses are variable
- clarification is an additional burdon

- storage problems
- should be sterilized before use
- being semi solid pumping of molasses is a real problem
- the residue after fermentation is high in BOD and hence creates pollution problems.

The whey can replace the molasses in a number of fermentation industries.

The current methods of whey disposal in Quebec are:

- evaporation and drying to produce whey powder
- ultrafiltration followed by land application, bimethanation, dumping to sewer
- returning whey free to farmers
- selling it to processors.

However, ethanol, biomass, enzyme production, organic acid production, polysaccharide fermentations, vitamins are all effective methods to replace molasses with whey and/or whey permeate. All these methods reduces the BOD of the whey by 90 to 95 %. The lactase enzyme process evaluation suggests the possibilities in producing high value products from whey permeate. While the market for this particular enzyme is currently inadequate to sustain the production at large cheese factory, other high value products with great market potential (antibiotics, vitamins, enzyme, organic acids and solvent, oil, 2-3 batanediol) (21, 141) can be produced from whey and might be practical in a small scale plants. For general application, ethanol production from whey permeate is most attractive even for the small quantity whey producer (25,000 l/day). Further work is required for the

improvement of process technology and then cost comparison will be necessary specially for those of small scale producers.

High equipment costs are the largest factor in the whey treatment cost and strategies for reducing equipment costs should be evaluated. Continuous fermentation processes should be developed to take better advantage of high productivities achievable with the selected yeast strains. For example, at a dilution rate of 0.2 h^{-1} a single 6,500 l continuous fermentor could replace the two 30,000 l fermentor used in the batch operated plant. Even at the scale of a small independent cheese factory, whey treatment by ethanol fermentation would add only 7.3 (US) cents per kilogramme to the cost of cheese manufacture (47). Serious consideration should be given to legislative requirements for whey waste treatment in those areas where dumping still takes place.

Further studies of both batch and continuous mode have shown that immobilize systems have better cell growth and ethanol production rates than those in a free growing systems. Productivity in a continuous immobilized system could be much higher than the free growing system due to large accumulation of cell mass in the reactor (110, 111).

The application of industrial alcohol ranges from its use as a solvent and reagent (114) in the pharmaceutical, food and chemical industries (115) to its uses as fuel (116) or fuel additive (117).

Land application of cheese whey investigated in Canada have shown that nitrate concentration in leachate was high and continuous application at high rates may result in ground water contamination and eventually may become a threat to human and animals health (6). Moreover, whey permeate should be considered as a resource rather than mere disposal problem. However land application after ultrafiltration may not pose this danger.

Among the hydrolysis methods of lactose, the acid hydrolysis can not be applied to milk or to protein containing substrates due to protein denaturation. It is an attractive method for deproteinized or UF-ultrafilter whey, because it is presently less expensive than the enzymatic methods. The free enzyme (batch) method is more attractive for the hydrolysis of lactose in milk, because its antagonistic immobilized enzyme method is related with microbial growth. One disadvantage of this method is its high cost. Immobilized enzyme systems have the possibility of continuous processing and reusability of enzyme which leads to lower costs, a very essential factor in the case of whey. However, there is no system (up to now) commercially available which can be used in non-sterile milk and whey (94).

The hydrolysis of lactose solution by using catalytic resins appears to be a very interesting approach (113), if it is considered:

- the product obtained has a very high quality
- the economics of the process are very good, compared with the high price necessary to obtain enzymatically hydrolyzed lactose.

Anaerobic digestion of the whey permeate to recover methane as energy source and to reduce the BOD by 80 ~85 % is a good approach (112). However economics and process performance observed at least in one pilot plant trial in Quebec makes it a difficult proposition of product recovery and waste treatment.

Hog feeding as is practiced by small and medium group of industries seems to be an easier way, but does not seem to be the best way of utilization. This is specially true when high valued chemicals (described in this report) can be generated via fermentation.

The drying of whey to produce whey powder is still a product of last resort that barely recovers its cost. The acidic constituents could not, however, be dried and producers are therefore obliged to demineralize their whey. Demineralization, presently is costly affair and therefore work is required to make the process feasible. This will enable to use the hydrolyzed lactose as table sugar and has a large potential for future.

The small cheese factory is still in disadvantageous position in disposal (23, 24). Its volume is too small to justify the installation of processing equipment (evaporator, dryers and for ultrafiltration unit) and therefore they are left with the option of hog feeding. It is often uneconomical to install the kind of equipment necessary to ensure that the whey is cooled and handled as required for later use for human consumption. One alternative is to join with other factories and set up some type of processing or disposal system.

Whey proteins, lactose, blended and speciality products are more profitable than dried whey. The overall market for whey products has been expanding. New uses have been developed, both as a food and for certain industrial processes. There is more interest in breaking whey down into its component parts and then using the parts in the other foods.

While a whey market as such does not exist, some relative return have been made and others can be made with the availability of more data.

Future

The production of cheese has been increasing steadily over past ten years and therefore the production of cheese whey and this trend will continue in future. In the near future pressures to use whey in an economical manner rather than treating it as waste or returning to farm will increase. Therefore, more research for new whey products (ethanol, organic acids and solvents, oil, 2-3, butanediol, etc.) is essential.

There is likely to be more regulation and standardization of whey products than there has been in the past. Better utilization will be required, both in complete products and in products that use components of whey. This should help in adjusting to changes in consumption trends and in other aspects of market.

Much of the economic incentive to use whey in food products will depend on the non fat dried milk market (NFDM). The higher the price of NFDM in

relation to dried whey, the more pressure there will be to find ways to substitute whey products for NFDM. In many bakery processes whey and whey-blended products are very satisfactory NFDM substitute.

Increasing world demand for quality protein can lead to the development of new food products. Hope fully they can be exported to food-deficient areas and still be produced for a profit.

The market for component parts and blends of whey and other products will continue to increase. While dried whey will still use a large part of total whey production, it will continue to be less profitable than selling other products. Competition for the dried whey market will continue to be tough. The firms that can develop and market unique and innovative products have the potential to make the most money.

10) Reference

- 1) Singh, R.K. and A.E. Ghaly. 1984. Single cell protein production from cheese whey. ASAE paper No 84-6528, St. Joseph, Michigan.
- 2) Muller, P.G. 1979. Economic evaluation of feeding liquid whey to livestock. Technical Report, Food Research Institute, Research Branch, Agriculture Canada, Ottawa.
- 3) Wesserman, A.E.; J.W. Hampson and N.F. Alvare. 1961. Large scale production of yeast from whey. J. Water Pollut. Control Fed. 33 (10): 1090-1094.
- 4) Atkin, C.; L.D. Witter and A.J. Ordal. 1967. Continuous propagation of Trichosporon cutaneum in cheese whey. Applied Microbiol. 15 (1); 1337-1344.
- 5) Bernstein, S.; C.H. Tzeng and D. Sission. 1977. Commercial fermentation of cheese whey for production of protein and/or alcohol. Biothechnol. Bioeng. Symp. 7, p. 1-9.
- 6) Ghaly, A.E. and R.K. Singh. Land Application of cheese whey. Agricultural Waste Utilization and Management Proc, 5th Int. Symp. on Agricultural wastes. Dec. 16-17, 1985. Chicago IL, ASAE Publication 13-85. p. 546-553.
- 7) Reichstein, T. and A. Grussner. A high yield synthesis of L-ascorbic acid [vitamin-C]. Helv. Chem. Acta. 17: 311-328 (1934).
- 8) Bleeg, H. and F. Christensen. Biosynthesis of ascorbate in yeast. Eur. J. Biochem. 127, 391-396 (1982).
- 9) Cayle, T.; J. Rolland; D. Mehnert; R. Dinwoodie; R. Larson; J. Mathers; M. Raines; W. Alm; S. Ma'ayeh; S. Kiang and R. Saunders. Production of L-Ascorbic Acid from Whey. "Biotechnology in Food Processing". S.K. Marlander and T.P. Labuza eds. Noyes Publication. p. 157-169 (1986).
- 10) Pepper, D. Recovery of materials form effluents by membrane systems. Food Industry Wastes: Disposal and Recovery. A. Herzka and R.G. Booth eds. Applied Science Publishers, 1981.
- 11) de Boer, R. and J. Hiddink. Symposium on 'Membrane Technology in the Eighties', Ystad, Sweeden, sept. 29 - oct. 1, 1980. Published in Desalination, 35, 162-192 (1980).
- 12) Moulin, G.; M. Guillaume and P. Galzy. Biotechnol. Bioeng. 22, 1277 (1980).
- 13) F.J. Castillo; M.E. Izaguirre; V. Michelena and B. Mouno. Biotechnol. Lett. 4, 567 (1982).
- 14) K.J. Burgess and J. Kelly. Ir. J. Food Sci. Technol. 3, 1 (1979).

- 15) H.C. Chen and R.R. Zall, *Process Biochem.* 17 (1), 20 (1982).
- 16) P. Vienne and V.V. Stockar. Alcohol from whey permeate: Strain selection, Temperature and Medium optimization. *Biotechnol. Bioeng. Symp.* No 13, p. 421-435 (1983).
- 17) C.E. Morris, *Food Engg.* 44, 67 (1982).
- 18) V. Singh, C.C. Hsu; D.C. Chen and C.H. Tzeng. Fermentation Process for Dilute Food and Dairy wastes. *Process Biochemistry.* p. 13, March/April 1983.
- 19) F.A.O. 'Production Yearbook' 35, p. 234 (1981).
- 20) F.V. Kosikowski. *Cheese and Fermented Milk Foods* (Ann Arbor, Michigan: Edward Brothers). 1976.
- 21) Scott, R. *Cheesemaking Practice* (London: Applied Science Publishers). (1981).
- 22) Garoutte, C; J. Lim; C.H. Amundson and B. Breslau. *Process Biochem.* 18 (2), 12 (1983).
- 23) Ripley, P. *Process Biochem.* 14 (1), 8 (1979).
- 24) Groves, F. An Economic Analysis of Whey Utilization, In: *Proceedings of the Whey Products Conference*, p. 5 (Agric. Res. Service, US Dept. of Agric., ERRL Publ. No 3779). 1972.
- 25) Bailey, R.B.; T. Benitez and A. Woodward. *Applied Environ. Microbiol.* 44, 631 (1982).
- 26) De Mott, B.J. *Cultured Dairy Products J.* 17, 17 (1982).
- 27) Gawel, J. and F.V. Kosikowski. *J. Food Sci.* 43, 1031 and 1717 (1978).
- 28) O'Leary, V.S.; R. Green; B.C. Sullivan and V.H. Holsinger. *Biotechnol. Bioeng.* 19, 1019 (1977).
- 29) O'Leary, V.S; S. Sutton; M. Bencivengo; B. Sullivan and V.H. Holsinger. *Biotechnol. Bioeng.* 19, 1689 (1977).
- 30) Roland, J.F. and W.L. Alm. *Biotechnol. Bioeng.* 17, 1443 (1975).
- 31) Bernstein, S.; C.H. Tzeng and D. Sisson. *Biotechnol. Bioeng. Symp.* No 7, 1 (1977).
- 32) De Mott, B.J.; F.A. Draughou and P.J. Herald. *J. Food Protection.* 44, 558 (1981).
- 33) Friend, B.S.; M.L. Cunningham and K.M. Shahani. *Agric. Wastes.* 4, 55 (1982).

- 34) Gawel, J. and F.V. Kosikowski. *J. Food Sci.* 43, 1717 (1978).
- 35) Janssens, J.M.; N. Burris; N.A. Woodward and R.B. Bailey. *Appl. Environ. Microbiol.* 45, 598 (1983).
- 36) Kosikowski, F.V. and W. Wzorek. *J. Dairy Sci.*, 60, 1982 (1977).
- 37) Laham-Guillaume, M.; G. Moulin and P. Galzy. *Le Lait.* 59, 489 (1979).
- 38) Linko, Y.Y.; M. Jalanka and P. Linko. *Biotechnol. Letters.* 3, 263 (1981).
- 39) Maddox, I.S. *Biotechnol. Letters.* 2, 493 (1980).
- 40) Mahmoud, M.M. and F.V. Kosikowski. *Dairy Sci.*, 65, 2082 (1982).
- 41) Moulin, G.; M. Laham-Gillaume and P. Galzy. *Ind. Alim. Agric.* 97, 471 (1980).
- 42) Reesen, L. and R. Strube. *Process Biochem.* 13 (11), 21 (1978).
- 43) Rogosa, M.; H.N. Browne and E.O. Whittier. *J. Dairy Sci.* 30, 263 (1947).
- 44) Sienkiewicz, T. and C.L. Ridel. *Lebensmittelindustrie.* 26, 306 (1979).
- 45) Vienne, P. and V. Von Stocker. 'Alcohol from Whey Permeate', In: 5th Symp. on Biotechnology for Fuels and Chemicals (Gatlinburg, Tenn.). 1983.
- 46) Yoo, B.W. *Dissertation Abstracts.* 36, 641B (1975).
- 47) Maiorella, B.L. and Castillo, F.J. Ethanol, Biomass and Enzyme Production for Whey Waste Abatement. *Process Biochem.* p. 157-161 (Aug. 1984).
- 48) Peppler, H.F. 'Food Yeasts', Rose, A.H. and J.S. Harrison eds. 'The Yeasts', 1970, Vol 3, p. 421 (London: Academic Press).
- 49) Castillo, F.J. and S.B. deSanchez. *Acta Cientif. Venez.* 29, 113 (1978).
- 50) Castillo, F.J.; S.B. deSanchez and J.A. Goncalves. *Acta Cientif. Venez.* 30, 588 (1981).
- 51) De Sanches, S.B. And F.J. Castillo. *Acta Cientif. Venez.* 31, 24 (1981).
- 52) Righelato, R.C.; J.E. Smith and D.R. Berry eds. *The Filamentous Fungi*, Vol 1, Edward Arnold, London, p. 79 (1975).
- 53) Levine, D.W. and C.L. Cooney. *Appl. Microbiol.* 26, 982 (1973).

- 54) Okanishi, M. and K.F. Gregory. *Canad. J. Microbiol.* 16, 1139 (1970).
- 55) Peppler, H.J. *J. Agr. Food. Chem.* 13, 34 (1965).
- 56) Anderson, C.; J. Longton; C. Maddix; G.W. Scammell and G.L. Solomons in S.R. Tannenbaum and D.I.C. Wang eds. *SCP II*, MIT Press, Cambridge, Mass. p. 134 (1975).
- 57) Righelato, R.C.; F.K.E. Imrie and A.J. Vlitos. *Resource, Recovery and Conservation.* 1, 257 (1976).
- 58) Nickerson, T.A. Lactose. In *Fundamentals of Dairy chemistry*, Second Edition, B.H. Webb; A.M. Johnson and J.A. Alford eds. AVI Publishing Co., Inc. Westport, C.T. p. 273-324 (1974).
- 59) Jelen, P. Industrial Whey Processing Technology - an overview. *J. Agric. Food Chem.* 27 (4): 658-661 (1979).
- 60) Modler, H.W. Wiping out our whey woes. *Cult. Dairy Prod. J.* 17 (2), 11-14 (1982).
- 61) Burgess, K.J. and J. Kelly. Alcohol production by yeast in concentrated ultrafiltration permeate from cheddar cheese whey. *Irish J. Food Sci. Technol.* 3 (1): 1-10 (1977).
- 62) Reese, E.T. *Biotechnol. Bioeng. Symp.* 5, 77-80 (1975).
- 63) Weetall, H.H. and G.P. Royer eds. In: "Enzyme Engineering", Vol 5, p. 279-291, Plenum Press, New York (1980).
- 64) Sprossler, B. and H. Plainer. Immobilized lactase for processing whey. *Food Technology.* 37: 93-95 (1983).
- 65) Jelen, P. Reprocessing of whey and other dairy wastes for use as food ingredients. *Food Technology.* 37, 81-84 (1983).
- 66) Muller, L.L. Physico-chemical Separation Processes for food wastes. *CSIRO Fd. Res. Q.* 41, 37-43 (1981).
- 67) Varna, N. and R.C. Chawla. In: "Industrial waste: Proceedings of the Thirteenth Mid-Atlantic Conference", p. 554, U. Delaware, Delaware (1981).
- 68) Pitcher, W.H. Immobilized Lactase Systems in Proceedings Whey Products Conference. *USDA ERRC Publ #996*: 104-115 (1974).
- 69) Weetal, H.H. and N.B. Havewala. In: "Enzyme Engineering" (L.B. Wingard, ed.), Vol 3, p.249, Wiley, New York (1972).
- 70) Kosikowski, F.V. 'Cheese and Fermentation Milk Foods', 1977 (Ann Arbor, Michigan: Edward Brothers).
- 71) Paige, D.M. and T.M. Bayless. 'Lactose Digestion clinical and Nutritional Implications', 1981 (Baltimore: The John Hopkins University Press).

- 72) De Bales, S. and F.J. Castillo. *Appl. Environ. Microbiol.* 37, 1201 (1979).
- 73) Sanchez, L. and F.J. Castillo. *Acta Cientif. Venez.* 31, 154 (1980).
- 74) Pedrigue, M. and F.J. Castillo. *Appl. Environ. Microbiol.* 43, 303 (1982).
- 75) Castillo, F.J. and B. Moreno. *J. Dairy Sci.* 66, 1616 (1983).
- 76) Gomez, A. and F.J. Castillo. *Biotechnol. Bioeng.* 25, 1341 (1983).
- 77) Fenton, D.M. *Enz. Microbiol. Technol.* 4, 229 (1984).
- 78) Abrahamsen, R.K. *Meieriposten, Meieriteknikk.* 71, 325 (1975).
- 79) Shukla, T. *CRC Critical Reviews in Food Technology.* 1, 325 (1975).
- 80) Coughlin, R.W. and M. Charles. W. Pitcher, ed. "Immobilized Enzymes for Food Processing" (Florida, USA: CRC Press). p. 153 (1980).
- 81) Baret, J.L. *Industries Aliment. Agric.* 97, 1051 (1980).
- 82) Bonjean, Y. *Alimentation.* 71, 47 (1979).
- 83) Dicker, R. *Food Trade Rev.* 52, 295 (1982).
- 84) Holley, W. *Int. Zeit für Lebensmittel-Technol. und Verfahrenstechnik.* 34 (5), 513 (1983).
- 85) Olson, N.F. *Dairy Ice Cream Field.* 162 (7), 55 (1979).
- 86) Hefnayw, M.M. *Dissertation Abstracts International B*, Order No 79-11680. University of Tennessee, USA. 39 (11), 5305 (1979).
- 87) Björklöf, M. and J. Nordlund. *Mejeritietellinen Aikakauskirja*, 39, 51 (1981).
- 88) Maugh, T.H. *Science.* 223, 474 (1984).
- 89) Hägerdal, B. *Acta chim. Scand.* 34, 611 (1981).
- 90) Hägerdal, B. *Livsmedelsteknik.* 5, 228 (1981).
- 91) Parizia, M.W. and F.M. Foster. *J. Food Protection.* 46 (5), 453 (1983).
- 92) Greenberg, N.A. and R.R. Mahoney. *Process Biochem.* 16 (2), 2 (1981).
- 93) Hannibal-Friedrich, O.; M. Chun and M. Sernetz. *Biotechnol. Bioeng.* 22, 157 (1980).

- 94) Vasilisgekas and Miguellopez-Leiva. Hydrolysis of lactose: A literature Review. Process Biochem. 20 (1), 2 (1985).
- 95) Campbell, M.E. and W.M. Glenn. Profit from Pollution Prevention - a Guide to Industrial Waste Reduction of Recycling. Pollution Probe Foundation Report, Toronto, Ontario, Canada (1982).
- 96) Proceeding of the whey utilization symposium, sponsored by Food Research Institute, Research Branch and Dairy Division Production and Marketing Branch, Agriculture Canada, June 27-28, 1974, Ottawa, Ontario.
- 97) Dairy Market Review, Marketing and Economics Branch, Agriculture, Canada, Ottawa (August 1982).
- 98) Riddle, M.J. and W.D. Chandler. "Waste Disposal of Whey", Proceedings of the whey utilization Symposium, June 27-28, 1974, Ottawa, Ontario.
- 99) Galpin, D.B. NZJ. Dairy Science and Technol. 16 (3), 289 (1981).
- 100) Schmidtke, N.W. and D.W. Bissett. Pollution control through by-product recovery using hyperfiltration. Case study of a specialty cheese producer. Proc. 40th Ind. Waste Conf. Purdue Univ. p. 383-398 (1985).
- 101) Lenschner, A.P.; C.E. West and E. Ashre. "Fuel Gas Systems". D.L. Wise ed. CRC Press. CRC Series in Bioenergy Systems (1983).
- 102) Schoutens, G.H. and W.J. Groot. Economic feasibility of the production of iso-propanol-butanol- ethanol fuels from whey permeate. Process Biochem. 20, 117 (1985).
- 103) Vanhuynh, V.; M. Declaire; A.M. Voets; J.C. Motte and X. Monseur. Production of Gluconic acid from whey hydrolysate by Gluconobacter oxydans. Process Biochem. 21 (1), 31 (1985).
- 104) Hamilton, K.M. and J.A. Howell. Dense culture lactate production in a hollow fibre fermenter. Advances in Fermentation 83. Chelsea College, University of London, 21-23 sept. (1983).
- 105) Stenroos, S.L.; Y.Y. Lindo and P. Lenko. Production of L-lactic acid with immobilized Lactobacillus delbrueckii. Biotechnol. Letters. 4: 159-164 (1982).
- 106) Charles, M. Production of Xanthan gum from cottage cheese whey. Paper presented at the first International Congress on Engineering and Food, Boston, Aug. 9-10 1976).
- 107) Bundus, R.H. and A.J. Luksas. Bread flavour concentrate. U.S. Patent No 3466177, Sept. 9 (1969).
- 108) Lundstedt, E. Aroma process for dairy products and the resulting product. U.S. Patent No 3048490, Aug. 7 (1962).

- 109) Henderson, H.E.; C.A. Reddy and R.G. Grickenberger. Fermented ammoniated condensed whey (FACW) as a protein source. *J. Anim. Sci.* 39, 240 (1974).
- 110) King, V.A.E. and R.R. Zall. Ethanol fermentation of whey using calcium alginate entrapped yeasts. *Process Biochem.* p. 17. Dec. (1983).
- 111) Marwaha, S.S. and J.F. Kennedy. Alcohol Production from Whey Permeate by Immobilized and Free cells of Kluyveromyces marxianus NCYC 179. *Process Biochem.* p. 79. April (1984).
- 112) Clanton, C.J.; P.R. Goodrich; M.A. Morrison and B.D. Backus. Anaerobic Digestion of Cheese Whey. *Agricultural waste utilization and management. Proc. of the 5th Int. Symp. on Agricultural Wastes.* Dec. 16-17, 1985. Chicago IL. ASAE Publication 13-85 (1985).
- 113) Demaimay, M.; Y. Le Henaff and P. Printemps. Hydrolysis of Lactose on Catalytic Resin. *Process Biochem.* p. 3-4 and 34, April (1978).
- 114) "Ethanol Chemistry - to be or not to be", Humphreys and Glasgow, ECN Petrochemicals 79 Supplement. December. p.36 (1979).
- 115) Kochar, N.K. and R.L. Marcell. *Chem. Engg.* 28th Jan., 80 (1980).
- 116) Bernhardt, W; W. Held and A. König. *Umschau.* 81 (12), 354 (1981).
- 117) Seirfert, V. and W. Held. *Chem. Ing. Techn.* 53 (2), 82 (1981).
- 118) SERI. "Fermentation chemicals and biomass". II, SERI/TR-754 (1981).
- 119) Lee, I.H.; A.G. Fredrickson and H.M. Tsuchiya. "Diauxic Growth of Propionibacterium shermanii". *Appl. Microbiol.* 28 (5), 31 (1974).
- 120) Prescott, S.C. and C.G. Dunn. "Industrial Microbiology", 2nd Ed. Chap. 21, McGraw-Hill, New York (1949).
- 121) Clausen, E.C. et al. *Biotechnol. Bioeng. Symp. Ser.* No 12, p. 67 (1982).
- 122) Tyagi, R.D. and T.K. Ghose. *Biotechnol. Bioengg.* 24, 781-795 (1982).
- 123) Ghose, T.K. and R.D. Tyagi. *J. Mol. Catalysis.* 16, 11-18 (1982).
- 124) Ghose, T.K. and R.D. Tyagi. *Recent Advances in the Engineering Analysis of chemically Reacting Systems* (L.K. Doraiswamu, ed.). John Wiley and Sons. p. 551-557 (1984).
- 125) Tyagi, R.D. "Biotechnology and India" K.S. Gopalkrishnan and Subhash Chand, eds. BERG I.I.T. Delhi. Feb. 1984.
- 126) Mukhopadhyay, A.; R.D. Tyagi and T.K. Ghose. *Proceedings of the Bioconversion Symposium* (I.I.T. Delhi) p. 399-405 (1980).

- 127) Clausen, E.C. and J.L. Gaddy. Organic Acids from Biomass by Continuous Fermentation. Chem. Engg. Prog. p. 59-63. Dec. 1984.
- 128) Demaimay, M.; Y. Le Henaff and P. Printemps. Hydrolysis of lactose on catalytic resin. Process Biochem, p. 3. April (1978).
- 129) Lyons, T.P. Ethanol Production in Developed Countries. Process Biochemistry. March/April (1983).
- 130) Murtagh, J.E. Fuel Ethanol Production - The US Experience. Process Biochem. 21 (2), 61 (1985).
- 131) Tewari, H.K.; R.P. Seth; A. Sood and L Singh. J. Res. (Punjab Agricul. Univ.), 22 (1), 89-98 (1985).
- 132) Badr-Eldin, S.M.; A.A. El-Nimr; A. Yousef and Y.B. Yousef. Salted shey utilization v. chemical composition of some yeasts grown on salted whey. Egypt J. Microbiol. 19 (2), 261-264 (1984).
- 133) Shay, L.K. and G.H. Wegner. Non polluting conversion of whey permeate to food yeast protein. J. Dairy Science. 69 (3), 876-883 (1986).
- 134) Chambers, J.V.; T.W. Perry; D.A. Lonergan; A. Dennis and S.J. Marks. U.S. Patent Appl. 583, 958, 27 Feb. (1984).
- 135) Yavorskii, M.I. and E.G. Plisko. Production of the feed concentrate Ksona. Molochn Prom-St. 6, 28-29 (1985).
- 136) Marriott, T.A. Ammonium lactate-feedstuff and feedstock. J. Soc. Dairy Technol. 38 (4), 109-116 (1985).
- 137) Semsá, K. and A. Gurdal. Study on the enrichment of whey with nitrogen through fermentation. Doga Bilim Derg. Seri A2. 9 (3), 574-581 (1985).
- 138) Erdman, M.D. and C.A. Reddy. Optimized batch fermentation of cheese whey supplemented feed waste filtrate to produce a nitrogen-rich food supplement for ruminants. Appl. Environ. Microbiol. 51 (3), 498-503 (1980).
- 139) Helbig, N.B.; S. nakai and C.Y. Ma. Modification of whey protein concentrate to stimulate whippability and gelation of egg white. Can. Inst. Food Sci. Technol. J. 18 (2), 150-157 (1985).
- 140) Reimerdes, E.M. and W. Gottschick. Biotechnological treatment of whey for improved nutritional use. GIT Fachz. Lab. 28 (1), 27-28 (1984).
- 141) Simova, B. and R. Enikova. Protease production by *S. aureus* in various nutritive substrates. Khig. Zdraveopaz. 28 (1), 31-37 (1985) (Bulg.).
- 142) Nasi, M. and M Antila. Evaluation of various whey protein preparations as protein supplement in pig diets. Kiel. Milchwirtsch. Forschungsber. 35 (3), 281-283 (1983).

- 143) Miller, A.E. Feed Supplements. U.S. 4,542,032. 17 Sept. (1985).
- 144) Grba, S. and T.V. Stehlik. Production of Single-cell protein by yeast *Kluyveromyces fragilis* on whey. Mljekarstvo. 35 (5), 138-145 (1985).
- 145) Alavivhkola, T. and M. Harju. Utilization of whey protein concentrate and hydrolyzed whey by growing pigs. Acta Agric. Scand. 35 (2), 213-216 (1985).
- 146) Vieira, S.D.A and B.S. Neves. Manufacture of acidified and chocolate beverages using ultrafiltered whey. Alimentacao. 79, 21-25 (1985) (Port.).
- 147) Ahern, W.P; L.E. Skogerson and D.F. Andrist. Europ. Pat. Appl. EP 160, 417. Nov. 1985.
- 148) Nakae, T.; K. Kataoka, T. Miyamoto, S. Iwamura, K. Endo and T. Yoneya. Production of fermented whey beverage. Rakuno Kagaku, Shokukin no Kenkyu. 34 (5), A137 - A143 (1985).
- 149) Bednarski, W. and J. Leman. Food Industry by product for fats biosynthesis by yeasts. Przem. Ferment. Owocowo-Warzywny. 29 (2), 8-11 (1985) (Pol.).
- 150) Schwartz, R.D. and E.A Bodie. Production of high-viscosity whey-glucose broths by a *Xanthomonas campestris* strain. Appl. Environ. Microbiol. 51 (1), 203-205 (1986).
- 151) Bubyrenko, T.A.; L.N. Chebotarev and V.K. Bubyrenko. Citric acid production. U.S.S.R. SU 1,211,290. 15 Feb. (1986).
- 152) Mehaia, M.A.; M. Cheryan. Lactic Acid from acid whey permeate in a membrane recycle bioreactor. Enzyme Microbiol. Technol. 8 (5), 289-292 (1986).
- 153) Cheryan, M. and M.A. Mehaia. Continuous fermentation of whey permeate of whey permeate. US Appl. 526,437. 25 Aug. (1983). PCT Int. Apl. W085 01,064. 14 Mar. (1985).
- 154) Tuli, A.; R.P. Sethi; P.K. Khanna; S.S. Marwaha and J.F. Kennedy. Lactic acid production from whey permeate by immobilized *Lactobacillus casei*. Enzyme Microbiol. Technol. 7 (4), 164-168 (1985).
- 155) Marwaha, S.S.; R.P. Sethi; J.F. Kennedy and R. Kumar. Simulation of fermentation conditions for vitamin B₁₂ biosynthesis from whey. Enzyme Microbiol. Technol. 5 (6), 449-453 (1983).
- 156) Marwaha, S.S. and R.P. Sethi. Utilization of Dairy Waste for Vitamin B₁₂ fermentation. Agric. Wastes 9 (2), 111-130 (1984).
- 157) Marwaha, S.S.; J.F. Kennedy and R.P. Sethi. Vitamin B₁₂ production from whey and simulation of optimal cultural conditions. Process Biochem. 18 (6), 24-27 (1983).

- 158) Floetenmeyer, M.D.; B.A. Glatz and E.G. Hammond. Continuous culture fermentation of whey permeate to produce microbial oil. *J. Dairy Science*. 68 (3), 633-637 (1985).
- 159) Zertuche, L. and R.R. Zall. Optimizing alcohol production from whey using computer technology. *Biotechnol. Bioeng.* 27 (4), 547-554 (1985).
- 160) Schoutens, G.H.; M.C.H. Nieuwenhuizen and N.W.F. Kossen. Continuous butanol production from whey permeate with immobilized clostridium beyerinckii LMD 27.6. *Appl. Microbiol. Biotechnol.* 21 (5), 282-286 (1985).
- 161) Marwaha, S.S. and J.F. Kennedy. Studies on structural features of sodium alginate-entrapped kluveromyces marxianus NCYC 179 cells used for alcohol production from whey permeate. *Br. Polym. J.* 17 (1), 46-50 (1985).
- 162) Marwaha, S.S. and J.F. Kennedy. Continuous alcohol production from whey permeate using immobilized cell reactor systems. *Br. Polym. J.* 17 (1), 60-63 (1985).
- 163) Vienne, P. and U.S. Von. Metabolic physiological and kinetic aspects of the alcoholic fermentation of whey permeate by Kluveromyces fragilis NRRL 665 and Kluveromyces lactic NCYC 571. *Enzyme Microbiol. Technol.* 7 (6), 287-294 (1985).
- 164) Bodie, E.A.; R.D. Schwartz and T.M. Anderson. Fermentation of whey for bakery product preservation. *Europ. Pat. Appl.* EP 141,642. 15 May 1985, US Appl. 548, 170, 02 Nov. (1983).
- 165) Dale, M.C.; M.R. Okos and P.C. Wanket. An immobilized cell reactor with simultaneous product separation. II. Experimental reactor performance. *Biotechnol. Bioeng.* 27 (7), 943-952 (1985).
- 166) Schaefer, O; P. Vienne and S.U. Von. Alcohol production from whey permeate by yeast and by a thermophile strain. *Conserv. Recycl.* 8 (1-2), 153-164 (1985).
- 167) Voget, C.E.; C.F. Mignone and R.J. Ertola. Influence of temperature on solvents production from whey. *Biotechnol. Lett.* 7 (8), 607-610 (1985).
- 168) Ennis, B.M. and I.S. Maddox. Use of clostridium acetobutylicum P262 for production of solvents from whey permeate. *Biotechnol. Lett.* 7 (8), 601-606 (1985).
- 169) Prigent, Y. Method and apparatus for the continuous preparation of lactic acid by fermentation of whey. *Fr. Demande Fr* 2,555,200. 24 May 1985, Appl. 83/18,631. 23 Nov. 1983.
- 170) Vienne, P. and U. Stockar. An investigation of ethanol inhibition occurring during the fermentation of concentrated whey permeate by Kluveromyces fragilis. *Biotechnol. Letters.* 7 (7), 521-526 (1985).
- 171) Majchrzak, R. Whey enrichment with nitrogen form nonprotein compounds. *Przem. Spozyw.* 36 (4), 128-131 (1982) (Pol.).

- 172) Borzani, W. and S.B. Baralle. Correlation between the volume added ammonium hydroxide and the number of base additions during a batch pH-controlled lactic acid fermentation of whey. *Arg. Biol. Technol.* 26 (4), 475-484 (1983).
- 173) Anderson, T.M. *Eur. Pat. Apl.* EP 95, 268 (cl. A23C21/02). 30 Nov. 1983, *US Appl.* 379,841. 20 May 1982.
- 174) Sultanova, G.T. and G. Mezina. Use of whey for the microbiological synthesis of several essential amino acids. *Mikro organizonov, Riga, 86-94* (1983) (Russ.).
- 175) Davies, J. Oil from whey. *Food Technol. N.Z.* 19 (6), 33, 35, 37 (1984).
- 176) Rachev, M.; A. Kozhev S. Aleksandrov and Stoyanov. Whey lactosylurea as a product for feeding ruminants. *Shivot novud. Nauki.* 21 (7), 86 (1984).
- 177) Lee, H.K. and I.S. Maddox. Microbial production of 2,3 - butanediol from whey permeate. *Biotechnol. Letters.* 6 (12), 815-818 (1984).