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THE BIOTECHNOLOGICAL UTILIZATION OF CHEESE WHEY: A REVIEW

M. I. González Siso

Departamento de Bioloxía Celular e Molecular, Facultade de Ciencias, Universidade da Coruña, Campus da Zapateira s/n, 15071-A Coruña, Spain

Abstract

Cheese-whey utilization has been the subject of much research. BOD reductions of higher than 75%, with the concomitant production of biogas, ethanol, single cell protein or another marketable product, have been achieved and about half the whey produced nowadays is not a pollutant but a resource. However, annual world cheese-whey production is increasing and new bioproductions are being sought through biotechnology in order to get full use of the whey produced. In this paper the most representative applications of cheese whey being exploited and under research are briefly discussed. Copyright © 1996 Published by Elsevier Science Ltd.

Key words: Cheese whey, food wastes recycling, pollution reduction, bioproductions.

COMPOSITION OF CHEESE WHEY

Cheese whey is the liquid remaining following the precipitation and removal of milk casein during cheese-making. This byproduct represents about 85–95% of the milk volume and retains 55% of milk nutrients. Among the most abundant of these nutrients are lactose (4.5–5% w/v), soluble proteins (0.6–0.8% w/v), lipids (0.4–0.5% w/v) and mineral salts (8–10% of dried extract). Cheese-whey salts are comprised of NaCl and KCl (more than 50%), calcium salts (primarily phosphate) and others. Cheese whey also contains appreciable quantities of other components, such as lactic (0.05% w/v) and citric acids, non-protein nitrogen compounds (urea and uric acid), B group vitamins, and so on. (Coton, 1976; Kosikowski & Wierzbicki, 1973; Kosikowski, 1979; Yves, 1979; García Bilbao, 1981; Anon, 1983; Marwaha & Kennedy, 1988).

Two main whey varieties produced are acid (pH <5) and sweet (pH 6–7) whey, according to the procedure used for casein precipitation. Acid wheys typically have higher ash and lower protein contents than sweet wheys, their use in alimentation being more limited precisely because of their acidic flavour

and high saline content (Weetal *et al.*, 1974; Kosikowski, 1979; Mawson, 1994).

CHEESE WHEY AS A POLLUTANT

Actual market trends point to a gradual increase in cheese production that generates more than 145×10^6 t of liquid whey per year, with 6×10^6 t corresponding to lactose (Castillo, 1990). To make 1 kg of cheese, 9 kg of whey is generated (Kosikowski, 1979). Because of its low concentration of milk constituents (whey is only 6–7% dry matter), whey has commonly been considered a waste product. A dairy farm processing 100 t of milk per day produces approximately the same quantity of organic products in its effluent as would a town with 55000 residents (Sienkiewicz & Riedel, 1990).

Although several possibilities for cheese-whey exploitation have been assayed over the last 50 years, approximately half of world cheese-whey production is not treated, but is discarded as effluent.

Therefore, cheese whey represents an important environmental problem because of the high volumes produced and its high organic matter content, exhibiting a $BOD_5 = 30000–50000$ ppm and $COD = 60000–80000$ ppm (Marwaha & Kennedy, 1988; Gardner, 1989; Kemp & Quickenden, 1989; Mawson, 1994), with lactose being largely responsible for the high BOD and COD, seeing that protein recovery reduces the COD of the whey only by about 10000 ppm (Mawson, 1994). Bioconversion of whey lactose to SCP (single cell protein), ethanol or methane reduces more than 75% of the BOD while producing marketable products, but in most cases the ensuing effluent is not then ready for disposal (Mawson, 1994).

A solution to this water-pollution problem has now become urgent due to the increasing volumes of whey produced, the centralization of production plants and stricter legislative requirements regarding effluent quality (Sienkiewicz & Riedel, 1990).

CHEESE WHEY AS A RESOURCE: WHEY UTILIZATION

About 50% of total world cheese-whey production is now treated and transformed into various food products, of which, in the EEC, about 45% has been reported to be used directly in liquid form, 30% in the form of powdered cheese whey, 15% as lactose and delactosed byproducts, and the rest as cheese-whey-protein concentrates (Marwaha & Kennedy, 1988). As research in the field of whey utilization continues, a variety of new whey products are currently being developed. Some possibilities for cheese-whey utilization (Fig. 1) will be briefly described below.

Liquid whey

Without any treatment, cheese-whey surplus can be supplied into drinking waters for farm animals. In addition to high-quality proteins and lactose, whey also provides calcium, phosphorus, sulphur and water-soluble vitamins. Excessive lactose and mineral proportions can, however, limit whey use in feeding (Sienkiewicz & Riedel, 1990). Whey has also been used as an agricultural fertilizer, but with the drawback that it leaves high saline deposits. In any case, transport of liquid cheese whey is very expensive (Kosikowski, 1979).

The other alternatives for cheese-whey surplus utilization, described below, all require preliminary treatment of this byproduct.

Condensed or powdered cheese whey

In this form, the quality of fresh cheese whey is maintained for a longer period of time, facilitating manipulation and transport. Different kinds are prepared that include condensed whey, complete acid- or sweet-whey powders, demineralized whey powder, delactosed whey powder, deproteinized whey, fat-enriched whey, and so on (Kosikowski, 1979; Yves, 1979; Anon, 1983; 1990).

The principal market for these products is animal feeding, in mixtures with molasses or soya flour: smaller quantities may be used in human foods (ice creams, baked goods, cakes, sauces, milky derivatives, and so on). There are several factors that interfere with the use of cheese whey in human-food products: for instance, an excessive saline taste (a high concentration of mineral salts) constitutes a problem in using cheese whey mainly in dietetic or baby foods (therefore, several demineralization procedures have been developed (Coton, 1976; Anon, 1983; Marwaha & Kennedy, 1988)); a small protein/sugars ratio, along with a low sweetening power of lactose (only 40% when compared to sucrose) (Coughlin & Charles, 1980); the low solubility of lactose (18% in water at room temperature) (Baret, 1982). For example, ice creams are sweetened with powdered cheese whey, but only with a small proportion, because whey's reduced protein content and the high proportion that would have to be added to obtain the required sweet flavour (due to the low sweetening power of lactose) would unbalance the protein/sugar ratio required by law. Moreover, in
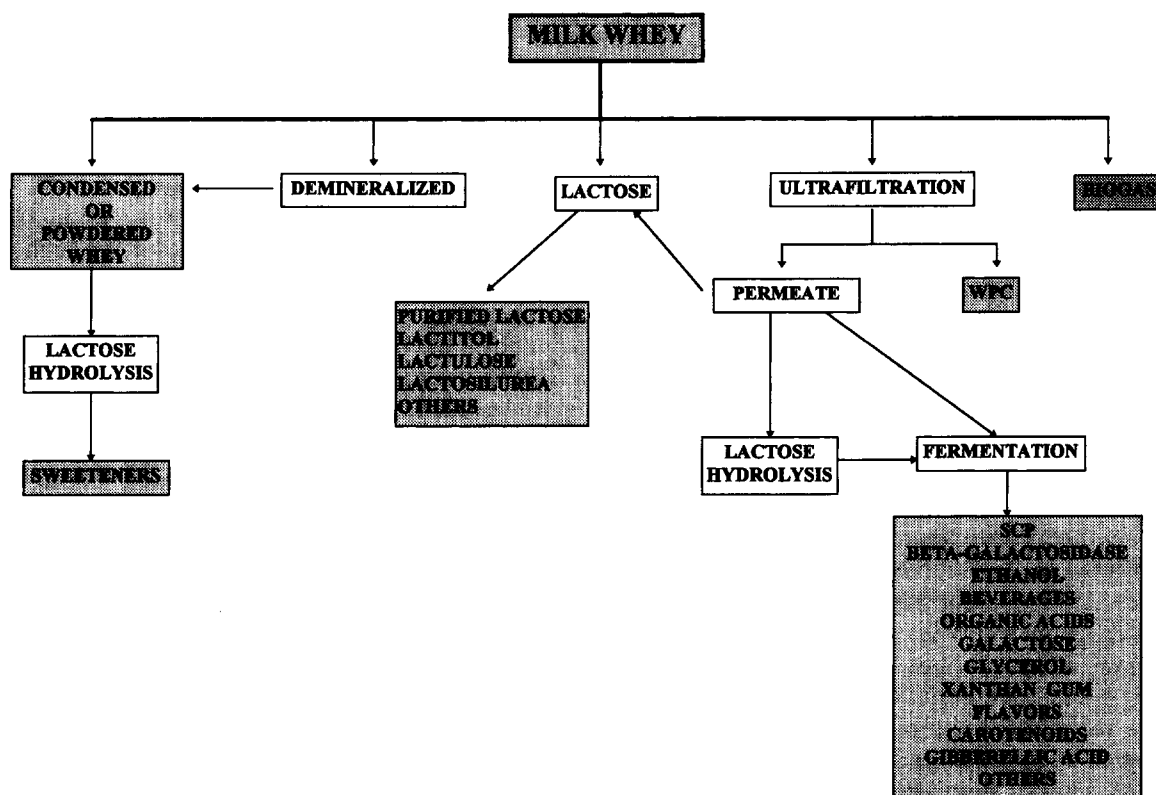

Fig. 1. Scheme for the commercial and currently-under-study utilizations of milk whey.

high concentrations lactose crystallizes and, therefore, the texture of the product is notably worsened (Yves, 1979). Both the sweetening power (up to 70% when compared to sucrose) and the solubility of lactose are increased by hydrolysis into glucose and galactose. In this way, the use of cheese whey as a sweetener is facilitated (Yves, 1979; Marwaha & Kennedy, 1988; Gekas & López-Leiva, 1985).

Whey protein concentrate (WPC)

These products derived from cheese-whey processing have a protein content of 30–60% (Kosikowski, 1979) and must be competitive with other cheap sources of protein, like soya.

Recovery of the protein proportion constitutes the first step in the majority of procedures for cheese-whey exploitation. The most commonly used methods nowadays are ultrafiltration and diafiltration because of their advantages of cost reduction, high process speed, the absence of denaturation or protein-structure modification, and the fact that the protein concentrate is free of salts, thereby making it suitable for all kinds of human foods, even dietetic- or baby-foods (Coton, 1976; Kosikowski, 1979; Gardner, 1989; Evans & Gordon, 1980).

Whey proteins represent about 20% of the milk proteins. The most abundant of these are β-lactoglobulin (50%), alpha-lactalbumin (12%), immunoglobulins (10%), serum albumin (5%) and proteose peptones (0.23%). The PER (protein efficiency ratio) value of whey proteins is high (3.4) compared to standard casein (2.8), and the proteins have a higher proportion of essential amino acids than casein (Evans & Gordon, 1980). Their biological value exceeds even that of whole egg protein (Sienkiewicz & Riedel, 1990). Heating, but only if to a high temperature (121°C, 83 min), results in precipitation of the proteins and reduces the high nutritive value of whey proteins (Sienkiewicz & Riedel, 1990; Parris *et al.*, 1993). The sulphur amino acids content of whey proteins is higher than that of whole-milk proteins (1.35% versus 0.36%) (Yves, 1979). Lysine content is also higher in whey than in total milk-proteins (10.5% versus 7.75%). Therefore, WPC has become an interesting complement to cereal-based (lysine-deficient) diets and also to baking products if Maillard reactions are required.

Whey proteins can be used not only as simple protein supplements, but may prove interesting, because of their functional and technological characteristics, for the manufacture of transformed food products (Kosikowski, 1979; Yves, 1979; Kosaric & Asher, 1985; Kinsella & Whitehead, 1989; Morr & Foegeding, 1990; Sienkiewicz & Riedel, 1990).

The production of protein hydrolysates involving several enzymatic preparations has been investigated (Moulin & Galzy, 1984; González-Tello *et al.*, 1994a, 1994b; Margot *et al.*, 1994). Whey-protein hydrolysis yields peptide mixtures, with increased solubilities

and altered foaming characteristics, that may be assimilated by microorganisms like the yeast *Kluyveromyces marxianus* (Perea *et al.*, 1993) and have also been used in the preparation of phosphopeptide complexes, which are interesting for their effect on the intestinal absorption of minerals (Moulin & Galzy, 1984).

Whey proteins have also been recently employed in the production of iron proteinate, an antianaemic preparation (Dalev, 1994).

During the manufacture of whey-protein concentrates, permeate with a high lactose content is formed as a byproduct. It is very important to take into account that protein recovery does not solve the BOD problem created by the 4–5% lactose remaining in the permeate, as lactose represents about 70% of the total solids of cheese whey (Mawson, 1994). The diverse possibilities for the utilization of this sugar have thus been, for some time now, of considerable interest (Coton, 1976; Castillo, 1990).

Lactose and its derivatives

Milk sugar lactose (4-O-β-D-galactopyranosyl-D-glucose) can be purified from cheese whey or permeate by crystallization. It is used as a supplement in baby milks and as an excipient for pharmaceutical products. Its plasticity, light flavour and reduced sweetening power make it apt for use in pill tablets (Yves, 1979). Although the production of lactose from whey has increased constantly on an international scale since 1940 (Sienkiewicz & Riedel, 1990), the amounts of purified lactose produced world-wide would require the use of only 5% of the whey available (Coton, 1980; Moulin & Galzy, 1984). Therefore, alternative uses are being sought, with most of them based on the direct fermentation of lactose or the fermentation of the glucose and galactose obtained by hydrolysis of lactose (Gekas & López-Leiva, 1985; Shukla, 1975; Friend & Shahani, 1979; Hobman, 1984; Mawson, 1988; Champagne & Goulet, 1988; Jeong *et al.*, 1991; Tin & Mawson, 1993; Nolan *et al.*, 1994). Nowadays, the processes based on microbial cultures on cheese-whey permeate are considered the most profitable alternatives for the transformation of cheese-whey surplus (Castillo, 1990). Other alternatives for lactose utilization are as follows.

Reduction to lactitol (4-β-galactopyranosyl-D-sorbitol). The sweetening power of lactitol, non-digestible for humans, is slightly higher than that of lactose. Its use as an additive in low-calorie diet foods (calorific value 2 kcal/g) shows interesting possibilities. Likewise, its ester, lactitol-palmitate, with an emulsifying effect, is also used in human nutrition (Moulin & Galzy, 1984; Sienkiewicz & Riedel, 1990).

Isomerization to lactulose (4-O-β-D-galactopyranosyl-D-fructose). Lactulose is a highly valued disaccharide with world-wide markets in pharmacol-

ogy and is normally synthesized by isomerization of lactose in an alkaline solution. Its sweetness is 48–62% that of sucrose and it is also used as a Bifidus Factor in nutrition (Sienkiewicz & Riedel, 1990; Dendene et al., 1994; Kozempel & Kurantz, 1994a, 1994b).

Production of lactosylurea (direct reaction). This is used as a non-protein nitrogen source in ruminant feeding because no toxic ammonia level is reached (Moulin & Galzy, 1984; Sienkiewicz & Riedel, 1990).

Production of galactose following lactose hydrolysis and selective glucose utilization by yeasts (Galzy & Moulin, 1976; Moulin et al., 1977; Moulin & Galzy, 1984). Galactose has been used to replace sorbitol, which is very expensive (Kosaric & Asher, 1985).

Hydrolyzed lactose solutions possess greater sweetening power than lactose and have applications in both the confectionery and ice-cream industries, replacing saccharose or starch syrup. Sweetness can be increased by the conversion of the glucose of lactose-hydrolyzed whey permeate to fructose with immobilized glucose isomerase (Moulin & Galzy, 1984; Kosaric & Asher, 1985).

Ethanol fermentation

The treatment of whey by fermenting lactose to ethanol has received wide attention to date, and various large-scale procedures have been developed. Several distilleries producing ethanol from whey are in commercial operation in Ireland, the USA and, particularly, New Zealand, where 50% of the cheese-whey production is used to produce ethanol (Mawson, 1994).

Although it is possible to find several papers related to the search for microorganisms with the capacity of producing ethanol directly from lactose in the literature (Castillo, 1990), up to now *Kluyveromyces fragilis* is the microorganism of choice for most commercial plants. In batch fermentation *K. fragilis* utilizes more than 95% of the lactose of unconcentrated whey with a conversion efficiency of 80–85% of the theoretical value of 0.538 kg ethanol/kg lactose consumed (Mawson, 1994).

In general, the production of ethanol from non-concentrated cheese whey is not economically feasible because the levels of ethanol obtained reach only about 2%, making the distillation process too expensive (Tin & Mawson, 1993). Several strains have been selected that are capable of fermenting concentrated lactose solutions and of producing ethanol with more than 90% conversion efficiency (Moulin & Galzy, 1984; Castillo, 1990). Costs are significantly reduced with the increase of lactose concentration up to about 100–120 g/l lactose (Mawson, 1994). The production of ethanol by continuous fermentation, and with cell-recycling or cell-immobilization to increase productivity, has also

been explored using both unconcentrated and concentrated whey (Friend & Shahani, 1979; Hobman, 1984; Moulin & Galzy, 1984; Sienkiewicz & Riedel, 1990; Jeong et al., 1991; Tin & Mawson, 1993; Nolan et al., 1994). Fed-batch fermentations have also been considered (Ferrari et al., 1994).

Not only is the number of microorganisms able to metabolize lactose directly to ethanol limited, but also they are inhibited by moderate sugars and ethanol concentrations (Moulin & Galzy, 1984). *Saccharomyces cerevisiae*, the yeast most utilized in wine and beer fermentations, lacks the lactose permease system (the membrane lactose carrier that controls the entry of sugar into the cells), as well as the intracellular enzyme for lactose hydrolysis, β-galactosidase, thus rendering it unable to ferment lactose directly into ethanol (Russel, 1986; Castillo, 1990). One interesting alternative consists of the hydrolysis of lactose by β-galactosidase from another microorganism and subsequent fermentation by *Saccharomyces cerevisiae* (Champagne & Goulet, 1988). This process can be developed in two steps or in only one step with mixed cultures or with the enzyme and yeast co-immobilized (Büyükgüngör, 1987; Axelsson et al., 1991). However, when *S. cerevisiae* uses the mixture of glucose and galactose as a C-source, it manifests diauxic growth and lower yields in ethanol production, even for strains previously adapted to galactose (Coughlin & Charles, 1980; Moulin & Galzy, 1984). Moreover, other disadvantages of these processes are the high price of β-galactosidase and the failure of this enzyme to hydrolyze all the lactose, thus leaving the problems associated with effluent disposal unsolved (Sienkiewicz & Riedel, 1990).

Another alternative that is currently being intensively studied consists of achieving, by recombinant DNA techniques, the expression of the genes that code for the β-galactosidase and lactose permease system of *Kluyveromyces lactis* in *Saccharomyces cerevisiae* (Russel, 1986; Sreekrishna & Dickson, 1985; Farahnak et al., 1986). In this way, *S. cerevisiae* could be developed directly on cheese whey producing high yields of ethanol or other commercially useful fermentation products (Porro et al., 1992). However, the recombinant yeasts elaborated up to this point in time are very slow growing and have reduced genetic stability, so yields are low even when these recombinant yeasts are used in specially designed bioreactors (Jeong et al., 1991).

Biogas

Anaerobic digestion producing methane that can be directly used as a source of energy *in situ* has long been employed in industrial waste treatment.

Several kinds of anaerobic digesters, some of them incorporating cell recycling or cell immobilization, have been studied (Méndez et al., 1989; Yan et al., 1989; Mawson, 1994) and several large-scale

plants have been established. Loading rates of up to 30 kg COD/m³ per day have been successfully treated, with COD removal efficiencies of greater than 95% being reported (Kemp & Quickenden, 1989; Mawson, 1994).

However, the effluents from the anaerobic processes are generally not suitable for pouring into water streams. Some secondary aerobic polishing is usually required (Mawson, 1994).

SCP (single cell protein) production

Microbial biomass has been produced commercially from whey since the 1940s. The development and operation of several pilot-scale and commercial plants in France, the USA, Germany and Austria has been reported (Sienkiewicz & Riedel, 1990; Mawson, 1994). Among the several process that have been described, the Vienna process and the Bel process stand out (Moulin & Galzy, 1984). Industrial microbial biomass production from cheese whey for use as food started in France at Fromageries Le Bel around 1958 (the patent of the process is dated 1955) (Yves, 1979; Moulin & Galzy, 1984). The process constitutes a classic example of SCP production for increasing the value of food and feed industry subproducts. Three yeast species (*Kluyveromyces lactis, K. fragilis, Torulopsis bovina*) are grown in equilibrium in cheese-whey permeate. Whole cheese whey is not used for this purpose because these microorganisms cannot metabolize its proteins; moreover, proteins promote yeast flocculation, which inhibits the fermentation. Depending on the whey used, sometimes it may be necessary to add N and P (Kosikowski, 1979).

The yeasts, in the case of Le Bel industries, are grown in continuous culture over a period of more than 1 year, without stopping, at pH 3.5 and 38°C. High temperature and low pH are recommended because they reduce the risk of contamination (Castillo, 1990). Cheese-whey permeate is pasteurized beforehand at 80°C to obtain a suitable bacteriological quality. High oxygen-transfer rates must be obtained in the fermentation tanks to avoid ethanol formation (Mawson, 1994). Even if oxygen is not the limiting factor, there is some ethanol production by *Kluyveromyces* that is utilized by *T. bovina*, which is the only yeast that does not grow directly on lactose (Moulin & Galzy, 1984).

The yield in dried yeast is 50% of the weight of the lactose used. Yeast biomass is recovered by centrifugation, plasmolyzed by heating at 85°C and, finally, it is dried in cylinders or atomization towers. The biomass obtained contains 48–52% proteins, with an equilibrated composition of essential amino acids, and it is rich in lysine and B-group vitamins. 'Protibel' was the commercial name for this product (Yves, 1979).

The biomass is primarily used as an animal-feed supplement but also in human foods (Mawson,

1994). The production by Fromageries Le Bel of about 2300 t/year of SCP has been reported, and the product has been used for more than 10 years in human dietetic nutrition. The origin of the raw material (production of foods) favours SCP's acceptability for human consumption (Olsen & Allerman, 1991).

In order to improve the nutritional characteristics of yeast biomass, methionine-enriched *Kluyveromyces lactis* mutants that grow on whey permeate have recently been isolated (Pellón & Hernández, 1986; Kitamoto & Nakahara, 1994).

In the Vienna process, a single species, *Candida intermedia*, which is characterized by exclusively oxidative lactose metabolism, is used (Meyrath & Bayer, 1979; Moulin & Galzy, 1984).

Although strains of *Kluyveromyces* and *Candida* appear for the most part to be grown commercially (Mawson, 1988, 1994) the utilization of other yeast species has been considered (Kosikowski, 1979; Yves, 1979; Friend & Shahani, 1979; Sandhu & Waraich, 1983; Pellón & Hernández, 1986). Several mixed cultures have been proposed (Carlotti *et al.*, 1991a, 1991b; Kallel-Mhiri *et al.*, 1994).

An industrial process for producing bakery yeast (*Saccharomyces cerevisiae*) from cheese whey was adopted by the Nutrisearch Company in 1983 in Kentucky. The process consists of lactose hydrolysis in cheese whey by immobilized lactase, followed by glucose–galactose fermentation (Castillo, 1990). To avoid diauxic growth, the fermentation must be performed under fed-batch conditions, and ethanol is produced from the glucose even under aerated conditions (Champagne & Goulet, 1988).

Following yeast–biomass separation, the BOD of the effluent thus obtained is reduced to an extent similar to that following anaerobic treatment and greater than that following ethanol production (Mawson, 1994).

Lactose hydrolysis

The number of microorganisms of commercial interest that are able to metabolize glucose and galactose is notably higher than the very low number of microorganisms able to directly utilize lactose as a C-source. Therefore, the prior hydrolysis of disaccharide into monosaccharides significantly increases the number of bioproducts that can be obtained from cheese whey (Van Huyn & Decleire, 1982). Conversely, both the sweetening power and solubility of lactose are increased after hydrolysis, which favours the use of the cheese whey in food products. This hydrolysis also allows for the elaboration of milk with a diminished lactose content that can be consumed by people with an intolerance for this sugar. Such a hydrolysis can be performed in two ways, the preferred way being enzymatic hydrolysis (Kosaric & Asher, 1985).

Acid hydrolysis has been investigated (Gekas & López-Leiva, 1985) both in the homogeneous phase with the acid in solution and in the heterogeneous phase with ion-exchange resins. For example, an 80% conversion is obtained at pH 1.2 and 150°C. This procedure shows some important drawbacks: very harsh operational conditions that cause protein denaturation; the necessity of previously demineralizing the cheese whey because the mineral salts inactivate the acid; the appearance of a brown colour due to Maillard reactions that requires a process of decoloration with activated carbon; and the formation of undesirable products.

Enzymatic hydrolysis is performed with the enzyme lactase, (β-galactosidase, EC. 3.2.1.32), which is found in animals, plants, bacteria, fungi and yeasts. However, the preparations that are commercially available and rated GRAS (safe for human consumption) come from only a few species of yeast and microfungi, the most important being *Kluyveromyces lactis* and *K. fragilis, Aspergillus niger* and *A. oryzae* (Coughlin & Charles, 1980; Gekas & López-Leiva, 1985; Machado & Linardi, 1990). Bacterial β-galactosidase (primarily obtained from *E. coli*) cannot be used for this purpose because it does not fulfill required hygienic standards (Gekas & López-Leiva, 1985; Moulin & Galzy, 1984), but it is commercialized for analytical uses.

Microfungi secrete this enzyme extracellularly and, thus, recovery from the culture medium is facilitated; however, in general they produce a lower quantity of enzymatic units than do yeasts. The optimum pH of microfungal β-galactosidase is acid, and activity at pH values higher than optimum is quite diminished. Microfungal β-galactosidase utilization for hydrolyzing lactose is restricted to acid wheys. In contrast, yeast β-galactosidase optimum pH is near neutral, consequently making it suitable for saccharifying milk and sweet whey. Regardless of thorough investigation, the production and industrial use of the intracellular yeast enzyme is, up to this point in time, problematic due to the high cost associated with extraction from the cells and to the low yields obtained due to enzymatic instability (Shukla, 1975; Coughlin & Charles, 1980; Fenton, 1982; Greenberg & Mahoney, 1982; Moulin & Galzy, 1984; Gekas & López-Leiva, 1985; González & Monsan, 1991; Stredánsky *et al.*, 1993). With the aim of improving yeast β-galactosidase production from cheese whey, fed-batch (Siso, 1994) and solid-state cultures (Becerra & Siso, 1996) have been proposed. The synthesis of β-galactosidase is inducible by lactose and galactose in *K. fragilis* and *K. lactis* and exhibits a catabolite repression, the intensity of which depends on the strain (Biermann & Glantz, 1968; Dickson *et al.*, 1979; Dickson & Markin, 1980; Breunig, 1989). This enzyme requires cationic co-factors such as Mn^{+2} and Mg^{+2} (Castillo, 1990).

Lactose hydrolysis in the homogeneous phase, with the enzyme free in solution in cheese whey, is uneconomical because of the lactase that cannot be re-utilized. Therefore, heterogeneous-phase processes, with the enzyme immobilized on diverse supports or insolubilized by polymerization, are recommended. These processes may be performed continuously and offer the possibility of re-utilizing the enzyme, which implies an important reduction in cost. Immobilized lactase was first prepared in 1968. Since then a great number of varieties have been developed (Weetal *et al.*, 1974; Coughlin & Charles, 1980; Trevan, 1980; Nijpels, 1981; Baret, 1982; Gekas & López-Leiva, 1985; Manjón *et al.*, 1985; Domínguez *et al.*, 1988; Dekker, 1989; Illanes *et al.*, 1990; Bódalo *et al.*, 1991a, 1991b; Narinesingh *et al.*, 1991; Ortega-López *et al.*, 1993; Piesecki *et al.*, 1993; Heng & Glatz, 1994; Irazoqui & Batista-Vieira, 1994; Siso *et al.*, 1994). Most of these call for the enzyme from Aspergilli and some are used in industrial plants. The most commonly used method is co-polymerization with glutaraldehyde, which does not preclude the ensuing use of resulting products in foods (Gekas & López-Leiva, 1985). The use of β-galactosidase in aqueous two-phase systems, which resemble immobilized systems but without the loss of activity during the immobilization step, has also been studied (Chen & Wang, 1991).

One drawback of enzymatic lactose hydrolysis is that it is sometimes followed by the polymerization of galactose or lactose, forming oligosaccharides which make it difficult to attain more than 75% hydrolysis (Guy & Bingham, 1978; Gekas & López-Leiva, 1985). Moreover, β-galactosidase may be inhibited by lactose (Shukla & Chaplin, 1993).

With the aim of avoiding the extraction of β-galactosidase from yeast cells which constitutes, in fact, one of the determining factors in the scarcity of industrially developed processes for the treatment of cheese whey based on enzymatic hydrolysis of lactose (Illanes *et al.*, 1990), several authors have proposed utilization of the whole cell as a catalytic agent (Van Huyn & Decleire, 1982; Van Huyn & Decleire, 1985; Decleire *et al.*, 1985; Joshi *et al.*, 1989; Siso & Suárez Doval, 1994).

In spite of the lactose-permease system possessed by *Kluyveromyces* yeasts (Dickson & Barr, 1983), the transport of lactose to the interior of the cells constitutes a limiting step in the rate of disaccharide hydrolysis (Joshi *et al.*, 1987, 1989). The permeability of cellular membranes to lactose may be increased by treating the cells with agents including solvents, surfactants and freezing–thawing cycles (Decleire *et al.*, 1987; Gowda *et al.*, 1991). Decleire *et al.* (1986) report that the increment of membrane permeability is related to a decrease in phospholipid content.

A comparative study on the efficacy of diverse permeabilization methods (previously described for related yeasts) for *K. lactis* cells obtained the best results with 70% ethanol as a permeabilizing agent in mild conditions (Siso *et al.*, 1992) with two advan-

tages: the high availability of ethanol at a low cost and it does not prevent the use of permeabilized cells in the food industry. Moreover, it has been demonstrated that permeabilized cells hydrolyze lactose to glucose and galactose without fermenting the monosaccharides to ethanol (Siso *et al.*, 1992; Siso & Suárez Doval, 1994). In terms of increasing the bioproduction potential of cheese whey, this characteristic is essential and has been related to two other permeabilizing agents, digitonin and CTAB (cetyltrimethylammoniumbromide) (Joshi *et al.*, 1987, 1989; Gowda *et al.*, 1991).

The procedure of ethanol permeabilization has been successfully applied to covalent immobilized cells (Siso & Suárez Doval, 1994). The immobilization of cells offers additional advantages (mainly, as in this case, when the enzyme is intracellular and unstable): not only are the processes of extraction and/or purification of the enzyme unnecessary, which leads to cost reduction, but the yields in enzymatic activity and operational stability are higher and the yeasts can be re-utilized (Karel *et al.*, 1985; Toldrá & Lequerica, 1986). A procedure for cheese-whey–lactose hydrolysis has been developed that uses whole cells of *K. lactis* immobilized on corn grits (Fig. 2) and permeabilized with ethanol; 90% hydrolysis has been attained at 37°C in laboratory-scale, packed-bed bioreactors (Siso & Suárez Doval, 1994).

Other bioproducts

In addition to the above, extensive research has been, and continues to be, conducted on profitable bioproducts that can be obtained from cheese whey. The following areas of investigation are outlined.

Several organic acids with food uses (acetic, propionic, lactic, lactobionic, citric, gluconic and itaconic), vitamins (B_{12} and B_2, or cobalamins and riboflavin, respectively), and amino acids (glutamic, lysine, threonine) can be obtained from whey by different microorganisms and processes (Hobman, 1984; Blanc & Goma, 1989; Nielsen *et al.*, 1990; Sienkiewicz & Riedel, 1990; Fairbrother *et al.*, 1991; Roukas & Kotzekidou, 1991; Zayed & Zahran, 1991; Chiarini *et al.*, 1992; Colomban *et al.*, 1993; Fournier *et al.*, 1993; Norton *et al.*, 1994).

Other whey fermentation pathways provide for the production of 2,3-butanediol with its potential uses as a basic material for the chemical industry and as an alternative energy source (Sienkiewicz & Riedel, 1990).

Glycerol production by yeast fermentation on cheese whey has been studied as an alternative to organic synthesis (Rapin *et al.*, 1994). Xanthan gum production from whey has also been accomplished. This polysaccharide has applications for oil drilling and in the textile and food industries as a thickener, emulsifier and stabilizer (Hobman, 1984; Papoutsopoulou *et al.*, 1994).

A novel anaerobic fermentation process has recently been developed to produce calcium magnesium acetate from cheese whey, which can be used as a road deicer with advantages over traditional products (Yang *et al.*, 1992).

The production of fructose-diphosphate, the salts of which are used in pharmacology, through the bioconversion of whey with genetically engineered *Saccharomyces cerevisiae* cells has been described (Compagno *et al.*, 1993).

Volatile flavouring compounds are produced by *Kluyveromyces lactis* (Jiang, 1993). Cheese whey constitutes a potential substrate for these aromas, for plant hormones, particularly gibberelic acid (Hobman, 1984; Kahlon & Malhotra, 1986), as well as for polygalacturonase (García-Garibay *et al.*, 1987) and other enzymes (Foda, 1981).

Deproteinized whey permeate has also been studied as a substrate in *Phaffia rhodozyma* production. This yeast produces large amounts of the β-carotene astaxanthin used in the colouring of eggs on farms or of salmonids in aquaculture. Growth of this yeast and astaxanthin production have been ade-
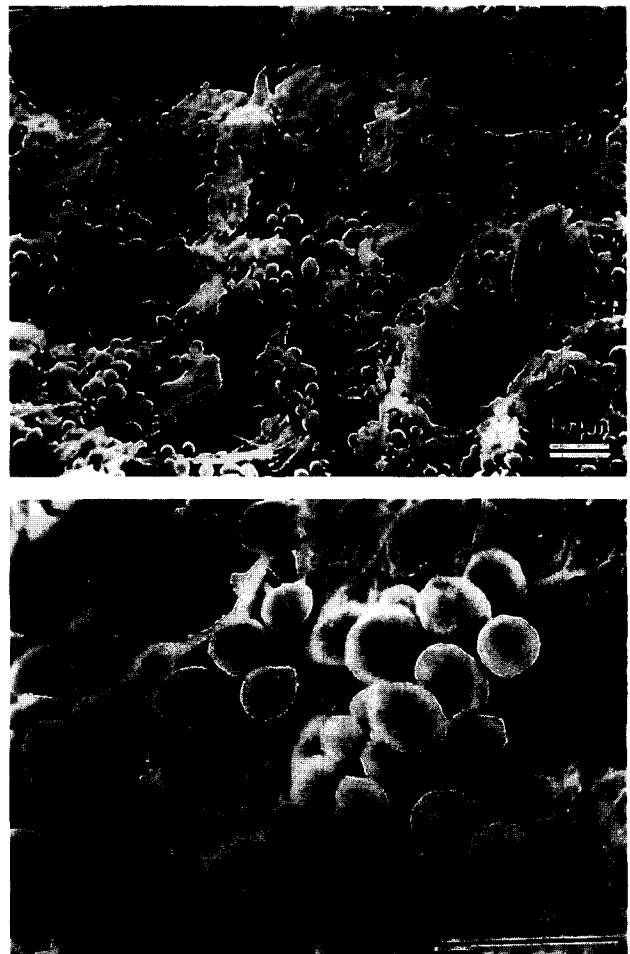

Fig. 2. Scanning electron micrographs of *Kluyveromyces lactis* cells immobilized on corn grits. Bar: (a) 10 μm, (b) 5 μm. A 90% cheese-whey-lactose hydrolysis has been attained with such immobilized cells permeabilized with ethanol (Siso & Suárez Doval, 1994).

quate, but *P. rhodozyma* can directly metabolize neither lactose nor the galactose obtained through hydrolysis. As molasses is an apt inexpensive substrate, cheese whey is not competitive by comparison (Tangeras & Slinde, 1994). More recently the utilization of *Rhodotorula glutinis* in carotenogenesis, cultivated in association with lactic acid bacteria, has been proposed (Frengova *et al.*, 1994).

REFERENCES

Anon (1983). Les produits obtenus a partir du lactosérum, *Revue Laitiere Française*, **422**, 44–47.

Anon (1990). Siero di latte, problema o risorsa? *Industri Alimentari*, **29**, 383–384.

Axelsson, A., Nilsson, M., Zacchi, G. & Hahn-Hägerdal, B. (1991). Performance of batch and continuous reactors with coimmobilized yeast and β-galactosidase. *J. Chem. Technol. Biotechnol.*, **52**, 227–241.

Baret, J.-L. (1982). Lactases. In *Les Enzymes. Production et Utilisations Industrielles*, ed. G. Durand & P. Monsan. Bordas, France, pp. 173–182.

Becerra, M. & Siso, M. I. G. (1996). Yeast β-galactosidase in solid state fermentations. *Enzyme Microb. Technol.*, **19**, 39–44.

Breunig, K. D. (1989). Glucose repression of LAC gene expression in yeast is mediated by the transcriptional activator LAC9. *Mol. Gen. Genet.*, **216**, 422–427.

Biermann, L. & Glantz, M. (1968). Isolation and characterization of β-galactosidase from *Saccharomyces lactis*. *Biochim. Biophys. Acta*, **167**, 373–377.

Blanc, P. & Goma, G. (1989). Propionic acid and biomass production using continuous ultrafiltration fermentation of whey. *Biotechnol. Lett.*, **11**, 189–194.

Bódalo, A., Gómez, E., Gómez, J. L., Bastida, J., Máximo, M. F. & Diaz, F. (1991a). A comparison of different methods of β-galactosidase immobilization. *Proc. Biochem.*, **26**, 349–353.

Bódalo, A., Gómez, E., M. F. Máximo, J. L. Gómez & J. Bastida. (1991b). Immobilization of β-galactosidase by physical adsorption on chromosorb-W. *Biotechnol. Technol.*, **5**, 393–394.

Büyükgüngör, H. (1987). Coimmobilization of yeast and β-galactosidase for ethanol formation from cheese-whey. *Proc. 4th European Congress on Biotechnology*, **2**, pp. 60–63.

Carlotti, A., Jacob, F., Perrier, J. & Poncet, S. (1991a). Yeast production from crude sweet whey by a mixed culture of *Candida kefyr* and *Candida valida* LY497. *Biotechnol. Lett.*, **13**, 437–440.

Carlotti, A., Poncet, S., Perrier, J. & Jacob, F. (1991b). Microbial interactions during the production of yeast on crude sweet whey. *J. Gen. Appl. Microbiol.*, **37**, 389–394.

Castillo, F. J. (1990). Lactose metabolism by yeasts. In *Yeast Biotechnology and Biocatalysis*, ed. H. Verachtert & R. De Mot. Marcel Dekker, New York, pp. 297–320.

Champagne, C. P. & Goulet, J. (1988). Growth of baker's yeast (*Saccharomyces cerevisiae*) in lactose-hydrolyzed cheese-whey ultrafiltrate. *Can. Inst. Food. Sci. Technol.*, **21**, 545–548.

Chen, J.-P. & Wang, C.-H. (1991). Lactose hydrolysis by β-galactosidase in aqueous two-phase systems. *J. Ferment. Bioengng*, **71**, 168–175.

Chiarini, L., Mara, L. & Tabacchioni, S. (1992). Influence of growth supplements on lactic acid production in whey ultrafiltrate by *Lactobacillus helveticus*. *Appl. Microbiol. Biotechnol.*, **36**, 461–464.

Colomban, A., Roger, L. & Boyaval, P. (1993). Production of propionic acid from whey permeate by sequential fermentation, ultrafiltration, and cell recycling. *Biotechnol. Bioengng*, **42**, 1091–1098.

Compagno, C., Tura, A., Ranzi, B. M. & Martegani, E. (1993). Bioconversion of lactose/whey to fructose diphosphate with recombinant *Saccharomyces cerevisiae* cells. *Biotechnol. Bioengng*, **42**, 398–400.

Coton, S. G. (1976). Recovery of dairy waste. In *Food from Waste*, ed. G. G. Birch, K. J. Parker & J. T. Morgan. Applied Science Publishers, UK, pp. 221–231.

Coton, S. G. (1980). Whey technology: the utilization of permeates from the ultrafiltration of whey and skim milk. *J. Soc. Dairy Technol.*, **33**, 89–94.

Coughlin, R. W. & Charles, M. (1980). Applications of lactase and immobilized lactase. In *Immobilized Enzymes for Food Processing*, ed. W. H. Pitcher Jr. CRC Press, Boca Raton, Florida, pp. 153–173.

Dalev, P. G. (1994). Utilization of waste whey as a protein source for production of iron proteinate: an antianemic preparation. *Biores. Technol.*, **48**, 75–77.

Decleire, M., De Cat, W. & Van Huyn, N. (1986). Appearance of cell-bound β-galactosidase activity in *Kluyveromyces bulgaricus* resting cells. *Appl. Microbiol. Biotechnol.*, **24**, 323–325.

Decleire, M., De Cat, W. & Van Huyn, N. (1987). Comparison of various permeabilization treatments on *Kluyveromyces* by determining *in situ* β-galactosidase activity. *Enzyme Microb. Technol.*, **9**, 300–302.

Decleire, M., van Huyn, N. & Motte, J. C. (1985). Hydrolysis of lactose solutions and wheys by whole cells of *Kluyveromyces bulgaricus*. *Appl. Microbiol. Biotechnol.*, **21**, 103–107.

Dekker, R. F. H. (1989). Immobilization of a lactase onto a magnetic support by covalent attachment to polyethylenei-mine-glutaraldehyde-activated-magnetite. *Appl. Biochem. Biotechnol.*, **22**, 289–310.

Dendene, K., Guihaard, L., Nicolas, S. & Bariou, B. (1994). Kinetics of lactose isomerisation to lactulose in an alkaline medium. *J. Chem. Technol. Biotechnol.*, **61**, 37–42.

Dickson, R. C. & Barr, K. (1983). Characterization of lactose transport in *Kluyveromyces lactis*. *J. Bacteriol.*, **154**, 1245–1251.

Dickson, R. C., Dickson, L. R. & Markin, J. S. (1979). Purification and properties of an inducible β-galactosidase isolated from the yeast *Kluyveromyces lactis*. *J. Bacteriol.*, **137**, 51–61.

Dickson, M. C. & Markin, J. S. (1980). Physiological studies of β-galactosidase induction in *Kluyveromyces lactis*. *J. Bacteriol.*, **142**, 777–785.

Domínguez, E., Nilsson, M. & Hahn-Hägerdal, B. (1988). Carbodiimide coupling of β-galactosidase from *Aspergillus oryzae* to alginate. *Enzyme Microb. Technol.*, **10**, 606–610.

Evans, M. T. A. & Gordon, J. F. (1980). Whey proteins. In *Applied Protein Chemistry*, ed. R. A. Grant. Applied Science Publishers, London, pp. 31–67.

Fairbrother, P., George, W. O. & Williams, J. M. (1991). Whey fermentation: on-line analysis of lactose and lactic acid by FTIR spectroscopy. *Appl. Microbiol. Biotechnol.*, **35**, 301–305.

Farahnak, F., Seki, T., Ryu, D. Y. & Ogrydziak, D. (1986). Construction of lactose-assimilating and high ethanol producing yeasts by protoplast fussion. *Appl. Environ. Microbiol.*, **51**, 362–367.

Fenton, D. M. (1982). Solvent treatment for β-D-galactosidase release from yeast cells. *Enzyme Microb. Technol.*, **4**, 229–232.

Ferrari, M. D., Loperena, L. & Varela, H. (1994). Ethanol production from concentrated whey permeate using a fed-batch culture of *Kluyveromyces fragilis*. *Biotechnol. Lett.*, **16**, 205–210.

Foda, M. S. (1981). Production of fungal enzymes and proteins from high salt cheese wheys. *J. Sci. Food Agric.*, **32**, 1109–1114.

Fournier, D., Schwitzguébel & Péringer, P. (1993). Effect of different heterogeneous inocula in acidogenic fermentation of whey permeate. *Biotechnol. Lett.*, **15**, 627–632.

Frengova, G., Simova, E., Pavlova, K. & Beshkova, D. (1994). Formation of carotenoids by *Rhodothorula glutinis* in whey ultrafiltrate. *Biotechnol. Bioengng*, **44**, 888–894.

Friend, B. A. & Shahani, K. M. (1979). Whey fermentation. *New Zealand J. Dairy Sci. Technol.*, **14**, 143–155.

Galzy, P. & Moulin, G. (1976). Procédé de préparation de galactose et de boisson à base de galactose à partir de solution contenant du lactose, US Patent No. 3,98,773.

García Bilbao, J. L. (1981). Aprovechamiento de suero de leche desproteinizado y de materiales nitrogenados de desecho por algunas levaduras. *Alimentaria*, **119**, 65–69.

García-Garibay, M., Gómez-Ruiz, L. & Bárzana, E. (1987). Studies on the simultaneous production of single cell protein and polygalacturonase from *Kluyveromyces fragilis*. *Biotechnol. Lett.*, **9**, 411–416.

Gardner, D. (1989). New technologies in the conversion of whey to high protein products. *Modern Dairy*, **68**, 15–17.

Gekas, V. & López-Leiva, M. (1985). Hydrolysis of lactose: a literature review. *Proc. Biochem.*, **20**, 2–12.

González, R. R. & Monsan, P. (1991). Purification and some characteristics of β-galactosidase from *Aspergillus fonsaecus*. *Enzyme Microb. Technol.*, **13**, 349–352.

González-Tello, P., Camacho, F., Jurado, E., Páez, M. P. & Guadix, E. M. (1994). Enzymatic hydrolysis of whey proteins: I. Kinetic models. *Biotechnol. Bioengng*, **44**, 523–528.

González-Tello, P., Camacho, F., Jurado, E., Páez, M. P. & Guadix, E. M. (1994b). Enzymatic hydrolysis of whey proteins: II. Molecular-weight range. *Biotechnol. Bioengng*, **44**, 529–532.

Gowda, L. R., Bachhawat, N. & Bhat, S. G. (1991). Permeabilization of baker's yeast by cetyltrimethylammonium bromide for intracellular enzyme catalysis. *Enzyme Microb. Technol.*, **13**, 154–157.

Greenberg, N. A. & Mahoney, R. R. (1982). Production and characterization of β-galactosidase from *Streptococcus thermophilus*. *J. Food Sci.*, **47**, 1824–1828.

Guy, E. J. & Bingham, E. W. (1978). Properties of β-galactosidase of *Saccharomyces lactis* in milk and milk products. *J. Dairy. Sci.*, **61**, 147–151.

Heng, M. H. & Glatz, C. E. (1994). Ion exchange immobilization of charged β-galactosidase fusions for lactose hydrolysis. *Biotechnol. Bioengng*, **44**, 745–752.

Hobman, P. G. (1984). Review of processes and products for utilization of lactose in deproteinated milk serum. *J. Dairy Sci.*, **67**, 2630–2635.

Illanes, A., Ruiz, A., Zúñiga, L., Aguirre, C., O'Reilly, S. & Curotto, E. (1990). Immobilization of lactase for the continuous hydrolysis of whey permeate. *Bioprocess Engng*, **5**, 257–262.

Irazoqui, G. & Batista-Vieira, F. (1994). Immobilization of β-galactosidase (*K. lactis*) on solid phase Ni(II)-chelate. *Biotechnol. Technol.*, **8**, 233–238.

Jeong, Y. S., Vieth, W. R. & Matsuura, T. (1991). Fermentation of lactose to ethanol with recombinant yeast in an immobilized yeast membrane bioreactor. *Biotechnol. Bioengng*, **37**, 587–590.

Jiang, J. (1993). Identification of flavour volatile compounds produced by *Kluyveromyces lactis*. *Biotechnol. Technol.*, **7**, 863–866.

Joshi, M. S., Gowda, L. R. & Bhat, S. G. (1987). Permeabilization of yeast cells (*Kluyveromyces fragilis*) to lactose by cetyltrimethylammonium bromide. *Biotechnol. Lett.*, **9**, 549–554.

Joshi, M. S., Gowda, L. R. & Bhat, S. G. (1989). Permeabilization of yeast cells (*Kluyveromyces fragilis*) to lactose by digitonin. *Enzyme Microb. Technol.*, **11**, 439–443.

Kahlon, S. S. & Malhotra, S. (1986). Production of gibberellic acid by fungal mycelium immobilized in sodium alginate. *Enzyme Microb. Technol.*, **8**, 613–616.

Kallel-Mhiri, H., Valance, C., Engasser, J. M. & Miclo, A. (1994). Yeast continuous mixed cultures on whey permeate and hydrolysed starch. *Proc. Biochem.*, **29**, 381–386.

Karel, S. F., Libicki, S. B. & Roberston, C. R. (1985). The immobilization of whole cells: engineering principles. *Chem. Engng Sci.*, **40**, 1321–1354.

Kemp, D. L. & Quickenden, J. (1989). Whey processing for profit—a worthy alternative. In *Resources and Applications of Biotechnology. The New Wave*, ed. R. Greenshields. MacMillans, London, pp. 323–331.

Kinsella, J. E. & Whitehead, D. M. (1989). Proteins in whey: chemical, physical and functional properties. *Adv. Food Nutr. Res.*, **33**, 343–438.

Kitamoto, H. K. & Nakahara, T. (1994). Isolation of an l-methionine-enriched mutant of *Kluyveromyces lactis* grown on whey permeate. *Proc. Biochem.*, **29**, 127–131.

Kosaric, N. & Asher, Y. J. (1985). The utilization of cheese whey and its components. *Adv. Biochem. Engng*, **35**, 25–60.

Kosikowski, F. V. (1979). Whey utilization and whey products. *J. Dairy Sci.*, **62**, 1149–1160.

Kosikowski, F. V. & Wierzbicki, L. E. (1973). Lactose hydrolysis of raw and pasteurized milks by *Saccharomyces lactis* lactase. *J. Dairy Sci.*, **56**, 146–150.

Kozempel, M. & Kurantz, M. (1994a). The isomerization kinetics of lactose to lactulose in the presence of borate. *J. Chem. Technol. Biotechnol.*, **59**, 25–29.

Kozempel, M. & Kurantz, M. (1994b). A continuous reactor system for production of lactulose. *J. Chem. Technol. Biotechnol.*, **59**, 265–269.

Machado, M. G. & Linardi, V. R. (1990). Production of amylase and β-galactosidase by yeasts. *Arq. Biol. Technol.*, **33**, 247–253.

Manjón, A., Llorca, F. I., Bonete, M. J., Bastida, J. & Iborra, J. I. (1985). Properties of β-galactosidase covalently immobilized to glycophase-coated porous glass. *Proc. Biochem.*, **20**, 17–22.

Margot, A., Flaschel, E. & Renken, A. (1994). Continuous monitoring of enzymatic whey protein hydrolysis. Correlation of base consumption with soluble nitrogen content. *Proc. Biochem.*, **29**, 257–262.

Marwaha, S. S. & Kennedy, J. F. (1988). Review: whey-pollution problem and potential utilization. *Int. J. Food Sci. Technol.*, **23**, 323–336.

Mawson, A. J. (1988). Yeast biomass production from acid whey permeate. *Biotechnol. Lett.*, **10**, 503–508.

Mawson, A. J. (1994). Bioconversions for whey utilization and waste abatement. *Biores. Technol.*, **47**, 195–203.

Méndez, R., Blázquez, R., Lorenzo, F. & Lema, J. M. (1989). Anaerobic treatment of cheese whey: start-up and operation. *Water Sci. Technol.*, 21, 1857–1860.

Meyrath, J. & Bayer, K. (1979). Biomass from whey. In *Economic Microbiology*, Vol. 4, ed. A. H. Rose. Academic Press, London, pp. 207–269.

Morr, C. V. & Foegeding, E. A. (1990). Composition and functionality of commercial whey and milk protein concentrates and isolates: a status report. *Food Technol.*, 4, 100–112.

Moulin, G. & Galzy, P. (1984). Whey, a potential substrate for biotechnology. *Biotechnol. Genet. Engng Rev.*, 1, 347–374.

Moulin, G., Vaarchon, P. & Galzy, P. (1977). Une nouvelle utilisation possible du lactosérum: la préparation de boisson à base de galactose. *Ind. Aliment. Agric.*, 1, 29–34.

Narinesingh, D., Stoute, V. A., Davis, G. & Ngo, T. T. (1991). Flow injection analysis of lactose using covalently immobilized β-galactosidase, mutarrotase and glucose-oxidase/peroxidase on a 2-fluoro-1-methylpyridinium salt activated fractogel support. *Anal. Biochem.*, 194, 16–24.

Nielsen, J., Nikolajsen, K., Benthin, S. & Villadsen, J. (1990). Application of flow injection analysis in the on-line monitoring of sugars, lactic acid, protein and biomass during lactic acid fermentations. *Anal. Chim. Acta*, 237, 165–175.

Nijpels, H. H. (1981). Lactases and their applications. In *Enzymes and Food Processing*, ed. G. G. Birch, N. Blakebourg & J. Parker. Applied Science, London, pp. 89–104.

Nolan, A. M., Barron, N., Brady, T., McAree, T., Smith, D., McHale, L. & McHale, A. P. (1994). Ethanol production at 45° by an alginate-immobilized, thermotolerant strain of *Kluyveromyces marxianus* following growth on glucose-containing media. *Biotechnol. Lett.*, 16, 849–852.

Norton, S., Lacroix, C. & Vuillemard, J.-C. (1994). Kinetic study of continuous whey permeate fermentation by immobilized *Lactobacillus helveticus* for lactic acid production. *Enzyme Microb. Technol.*, 16, 457–466.

Olsen, J. & Allerman, K. (1991). La biomasa microbiana como fuente de proteína. In *Biotecnología Básica*, ed. J. Bu'Lock & B. Kristiansen. Editorial Acribia S.A., Spain, pp. 285–308.

Ortega-López, J., Morales-Ramos, L. H., Montes, M. C. & Magaña-Plaza, I. (1993). Lactose hydrolysis by immobilized β-galactosidase on nylon-6: a novel spin-basket reactor. *Biotechnol. Lett.*, 7, 775–780.

Papoutsopoulou, S. V., Ekateriniadou, L. V. & Kyriakidis, D. A. (1994). Genetic construction of *Xantomonas campestris* and xanthan gum production from whey. *Biotechnol. Lett.*, 16, 1235–1240.

Parris, N., Anema, S. G., Singh, H. & Creamer, L. K. (1993). Aggregation of whey proteins in heated sweet whey. *J. Agric. Food Chem.*, 41, 460–464.

Pellón, J. R. & Hernández, P. E. (1986). Aplicaciones en el sector alimentario. In *La Ingeniería Genética y sus Aplicaciones*, ed. J. R. Pellón. Editorial Acribia S.A., Spain, pp. 145–158.

Perea, A., Ugalde, U., Rodriguez, I. & Serra, J. L. (1993). Preparation and characterization of whey protein hydrolysates: applications in industrial whey bioconversion processes. *Enzyme Microb. Technol.*, 15, 418–423.

Piesecki, S., Teng, W.-Y. & Hochuli, E. (1993). Immobilization of β-galactosidase for application in organic chemistry using a chelating peptide. *Biotechnol. Bioengng*, 42, 178–184.

Porro, D., Martegani, E., Ranza, B. M. & Alberghina, L. (1992). Development of high cell density cultures of engineered *Saccharomyces cerevisiae* cells able to grow on lactose. *Biotechnol. Lett.*, 14, 1085–1088.

Rapin, J.-D., Marison, I. W., Von Stockar, U. & Reilly, P. J. (1994). Glycerol production by yeast fermentation of whey permeate. *Enzyme Microb. Technol.*, 16, 143–150.

Roukas, T. & Kotzekidou, P. (1991). Production of lactic acid from deproteinized whey by coimmobilized *Lactobacillus casei* and *Lactococcus lactis* cells. *Enzyme Microb. Technol.*, 13, 33–38.

Russel, I. (1986). Will a recombinant DNA yeast be able to solve whey disposal problems? *Trends Biotechnol.*, 4, 107–108.

Sandhu, D. K. & Waraich, M. K. (1983). Conversion of cheese whey to single-cell protein. *Biotechnol. Bioengng*, 25, 797–808.

Shukla, T. P. (1975). β-Galactosidase technology: a solution to the lactose problem. *CRC Crit. Rev. Food Technol.*, January, 325–356.

Shukla, H. & Chaplin, M. (1993). Non-competitive inhibition of β-galactosidase (*A. oryzae*) by galactose. *Enzyme Microb. Technol.*, 15, 297–299.

Sienkiewicz, T. & Riedel, C.-L. (1990). *Whey and Whey Utilization*. Th. Mann, Germany.

Siso, M. I. G. (1994). β-Galactosidase production by *Kluyveromyces lactis* on milk whey: batch versus fed-batch cultures. *Proc. Biochem.*, 29, 565–568.

Siso, M. I. G., Cerdán, E., Freire-Picos, M. A., Ramil, E., Belmonte, E. R. & Torres, A. R. (1992). Permeabilization of *Kluyveromyces lactis* cells for milk whey saccharification: a comparison of different treatments. *Biotechnol. Lett.*, 6, 289–292.

Siso, M. I. G., Freire, M. A., Ramil, E., Belmonte, E. R., Torres, A. R. & Cerdán, E. (1994). Covalent immobilization of β-galactosidase on corn grits: a system for lactose hydrolysis without diffusional resistances. *Proc. Biochem.*, 29, 7–12.

Siso, M. I. G. & Suárez Doval, S. (1994). *Kluyveromyces lactis* immobilization on corn grits for milk whey lactose hydrolysis. *Enzyme Microb. Technol.*, 16, 303–310.

Sreekrishna, K. & Dickson, R. C. (1985). Construction of strains of *Saccharomyces cerevisiae* that grow on lactose. *Proc. Nat. Acad. Sci. USA*, 82, 7909–7913.

Stredánsky, M., Tomáska, M., Sturdik, E. & Kremnicky, R. (1993). Optimization of β-galactosidase extraction from *Kluyveromyces marxianus*. *Enzyme Microb. Technol.*, 15, 1063–1065.

Tangeras, A. & Slinde, E. (1994). Coloring of salmonids in aquaculture: the yeast *Phaffia rhodozyma* as a source of astaxantin. In *Fisheries Processing: Biotechnological Applications*, ed. A. M. Martin. Chapman and Hall, London, pp. 391–431.

Tin, C. S. F. & Mawson, A. J. (1993). Ethanol production from whey in a membrane recycle bioreactor. *Proc. Biochem.*, 28, 217–221.

Toldrá, F. & Lequerica, J. L. (1986). Reactores con microorganismos inmovilizados. *Rev. Agroquim. Technol. Aliment.*, 26, 349–364.

Trevan, M. D. (1980). *Immobilized Enzymes: an Introduction and Applications in Biotechnology*. John Wiley and Sons, Chichester, pp. 56–103.

Van Huyn, N. & Decleire, M. (1982). Les cellules entieres de *Kluyveromyces bulgaricus* comme source de β-galactosidase. Comparaison avec l'activite de l'extrait acellulaire. *Rev. Ferm. Ind. Aliment.*, 37, 153–157.

Van Huyn, N. & Decleire, M. (1985). *In situ* activation of β-galactosidase of *Kluyveromyces bulgaricus* resting cells by sodium and potassium phosphates and chlorides. *Appl. Microbiol. Biotechnol.*, 21, 390–393.

Weetal, H. H., Havewala, N. B., Pitcher, W. H., Detar, C. C., Van, W. P. & Yaverbaum, S. (1974). The prepara-

tion of immobilized lactase and its use in the enzymatic hydrolysis of acid whey. *Biotechnol. Bioengng*, **16**, 295–313.

Yan, J. Q., Lo, K. V. & Liao, P. H. (1989). Anaerobic digestion of cheese whey using up-flow anaerobic sludge blanket reactor. *Biol. Wastes*, **27**, 289–305.

Yang, S.-T., Tang, I.-C. & Zhu, H. (1992). A novel fermentation process for calcium magnesium acetate (CMA) production from cheese whey. *Appl. Biochem. Biotechnol.*, **34/35**, 569–583.

Yves, V. (1979). Le lactoserum. Matiere premiere noble pour les industries alimentaires humaines et animales. *Revue Laitiere Française*, **372**, 27–39.

Zayed, G. & Zahran, A. S. (1991). Lactic acid production from salt whey using free and agar immobilized cells. *Lett. Appl. Microbiol.*, **12**, 241–243.