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Review

Bioutilisation of whey for lactic acid production

Parmjit S. Panesar [a], John F. Kennedy [b,c,*], Dina N. Gandhi [d], Katarzyma Bunko [c]

[a] *Department of Food Technology, Sant Longowal Institute of Engineering and Technology, Longowal 148 106, Punjab, India*
[b] *Birmingham Carbohydrate and Protein Technology Group, School of Chemistry, University of Birmingham, Birmingham B15 2TT, UK*
[c] *Chembiotech Laboratories, University of Birmingham Research Park, Vincent Drive, Birmingham B15 2SQ, UK*
[d] *Division of Dairy Microbiology, National Dairy Research Institute, Karnal 132 001, India*

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Abstract

The disposal of whey, the liquid remaining after the separation of milk fat and casein from whole milk, is a major problem for the dairy industry, which demands simple and economical solutions. The bioconversion of lactose present in whey to valuable products has been actively explored. Since whey and whey permeates contain significant quantities of lactose, an interesting way to upgrade this effluent could be as a substrate for fermentation. Production of lactic acid through lactic acid bacteria could be a processing route for whey lactose and various attempts have been made in this direction. Immobilised cell technology has also been applied to whey fermentation processes, to improve the economics of the process. A fermentative means of lactic acid production has advantages over chemical synthesis, as desirable optically pure lactic acid could be produced, and the demand for optically pure lactic acid has increased considerably because of its use in the production of poly(lactic acid), a biodegradable polymer, and other industrial applications. This review focuses on the various biotechnological techniques that have used whey for the production of lactic acid.

Keywords: Whey; Lactose; Lactic acid bacteria; Lactic acid; Immobilisation

Contents

* Corresponding author. Address: Chembiotech Laboratories, University of Birmingham Research Park, Vincent Drive, Birmingham B15 2SQ, UK.
E-mail address: jfk@chembiotech.co.uk (J.F. Kennedy).

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1. Introduction

The dairy industry generates significant liquid waste, whose disposal requires a large amount of capital investment. Approximately 85% of the total milk used for manufacturing cheese and paneer (a sort of cheese which is an un-aged, acid-set dairy product common in India, which is similar to acid-set fresh mozzarella, except that it does not have salt added) is discarded as whey. Most milk plants do not have proper treatment systems for the disposal of whey and the dumping of whey constitutes a significant loss of potential food and energy, as whey retains about 55% of total milk nutrients. Among the most abundant of these nutrients are lactose, soluble proteins, lipids and mineral salts. Although several possibilities of cheese whey utilisation have been explored, a major portion of the world cheese whey production is discarded as effluent. Its disposal as waste poses serious pollution problems for the surrounding environment, since it affects the physical and chemical structure of soil, resulting in a decrease in crop yield and when released into water bodies, reduces aquatic life by depleting the dissolved oxygen (Gonzalez-Siso, 1996; Marwaha & Kennedy, 1988). Thus, whey poses a major threat to environmental and human health, for which an effective and permanent solution is urgently needed. Most of the industrially developed countries have stringent legislation governing the disposal of effluents. Biological wastewater treatment technologies can assist in safer disposal of whey within environmental specifications, but these are expensive. To overcome this problem, a better alternative is subjecting whey to processes through which value added products can be manufactured, and which may contribute wholly or partially to the disposal costs. Availability of the lactose carbohydrate reservoir in whey and the presence of other essential nutrients for the growth of microorganisms makes whey a potent raw material for the production of different bio-products through biotechnological means.

2. Whey types and composition

Whey may be defined broadly as the serum or watery part of milk remaining after separation of the curd, which results from the coagulation of milk proteins by acid or proteolytic enzymes. The type and composition of whey at dairy plants mainly depends upon the processing techniques used for casein removal from liquid milk. The most often encountered type of whey originates from manufacture of cheese or certain casein cheese products, where processing is based on coagulating the casein by rennet, an industrial casein-clotting preparation containing chymosin or other casein-coagulating enzymes (Fox, Guinee, Cogan, & McSweeney, 2000). Rennet-induced coagulation of casein occurs at approximately pH 6.5; this type of whey is referred to as sweet whey (Table 1). The second type of whey, acid whey (pH < 5), results from processes using fermentation or addition of organic or mineral acids to coagulate the casein, as in the manufacture of fresh cheese or most industrial casein (Jelen, 2003; Table 1). In general, whey produced from rennet-coagulated cheeses is low in acidity, while the production of fresh acid cheeses such as ricotta or cottage cheese yields medium acid or acid whey.

Table 1
Typical composition of sweet and acid whey

Components	Sweet whey (g/l)	Acid whey (g/l)
Total solids	63–70	63–70
Lactose	46–52	44–46
Protein	6–10	6–8
Calcium	0.4–0.6	1.2–1.6
Phosphate	1–3	2–4.5
Lactate	2	6.4
Chloride	1.1	1.1

(*Source*: Jelen, 2003).

The main components of both sweet and acid wheys, after water, are lactose (approximately 70–72% of the total solids), whey proteins (approximately 8–10%) and minerals (approximately 12–15%) (Jelen, 2003; Table 1). The main differences between the two whey types are in the mineral content, acidity and composition of the whey protein fraction. The acid coagulation approach results in substantially increased acidity (final pH approximately 4.5), necessary for casein precipitation. At this low pH, the colloidal calcium contained in the casein micelles in normal milk is solubilised and partitioned into the whey. Rennet clotting produces a fragment *k*-casein molecule, termed glycomacropeptide (GMP), which ends up in whey. GMP constitutes approximately 20% of the whey protein fraction of sweet, rennet-based wheys but is not present in acid wheys, unless renneting is included in the fresh cheese manufacturing process. Other technological steps used in the pretreatment of milk before the main processes may also influence the composition of whey.

3. Microorganisms involved in lactose fermentation

Most lactic acid bacteria (LAB) are facultatively anaerobic, catalase-negative, non-motile and non-spore forming. Lactic acid bacteria are recognised as 'generally regarded as safe' (GRAS) bacteria (Limsowtin, Broome, & Powell, 2003). This GRAS status underlines their increasing use in traditional foods and in an expanding range of novel foods and products designed to have specific nutritional or other health-enhancing benefits (nutriceuticals, prebiotics, probiotics, etc.).

The genera that comprise LAB are at its core *Lactobacillus* (L.), *Lactococcus* (Lc.), *Leuconostoc* (Ln.), *Pediococcus* (P.), and *Streptococcus* (S.) as well as the more peripheral *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Oenococcus*, *Teragenococcus*, *Vagococcus*, and *Weisella*. *Lactobacillus* is by far the largest genus in LAB, and more than 125 species and subspecies names are currently recognised (Axelsson, 2004; Euzeby, 1997; Limsowtin et al., 2003). The key property in defining LAB is that these bacteria produce lactic acid as the major or sole fermentation product.

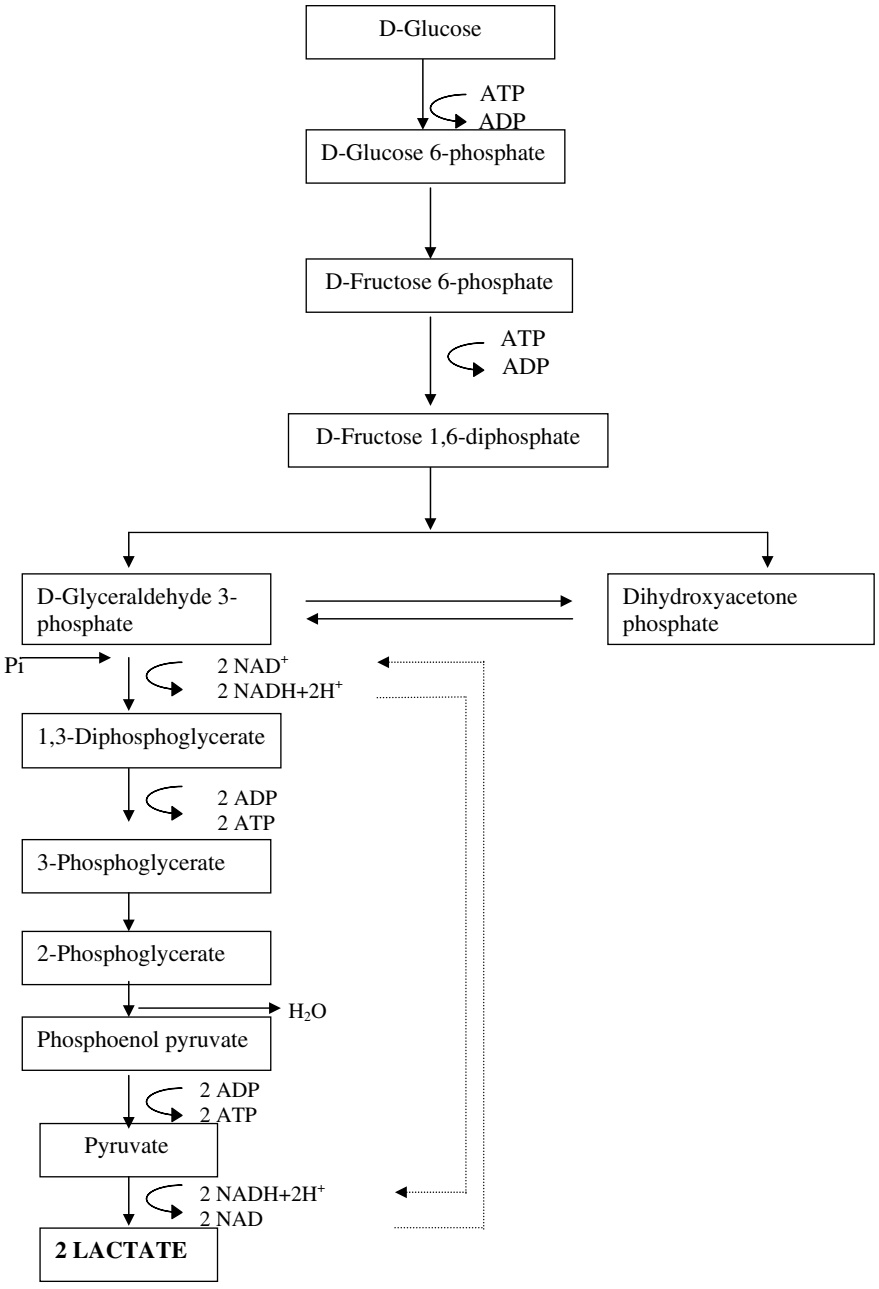

Fig. 1. Homolactic fermentation (*Source*: Axelsson, 2004).

A typical LAB bacterium can be described as Gram positive, non-spore forming, catalase-negative, devoid of cytochromes, of nonaerobic habit but aerotolerant, fastidiously acid tolerant, and strictly fermentative, with lactic acid as the major end products during sugar fermentation (Axelsson, 2004). A key feature of LAB, which must be emphasised is their inability to synthesise porphyrin groups (e.g. haem).

The essential feature of LAB metabolism is efficient carbohydrate fermentation, coupled to substrate level phosphorylation. Basically, there are two major pathways for hexose fermentation in lactic acid bacteria (Figs. 1 and 2); however, based on their fermentation characteristics, *Lactobacilli* are divided into three groups (Table 2). Transport and phosphorylation of glucose may occur through transport of free sugar and phosphorylation by an ATP-dependent glucokinase. Some species use the phosphoenolpyruvate: sugar phosphotransferase system (PTS), in which phosphoenolpyruvate is the phosphoryl donor (Parente & Cogan, 2004). In either case, a high-energy phosphate bond is required for activation of the sugars. Theoretically, one molecule of glucose, through homolactic fermentation, produces 2 molecules of lactic acid and a net gain of 2 ATP molecules per molecule of glucose. Homolactic fermentative *Lactobacilli* species metabolise sugars through the Embden–Meyerhof glycolytic pathway, and lactic acid is the only or highly dominant end product under typical fermentation conditions (Limsowtin et al., 2003). They

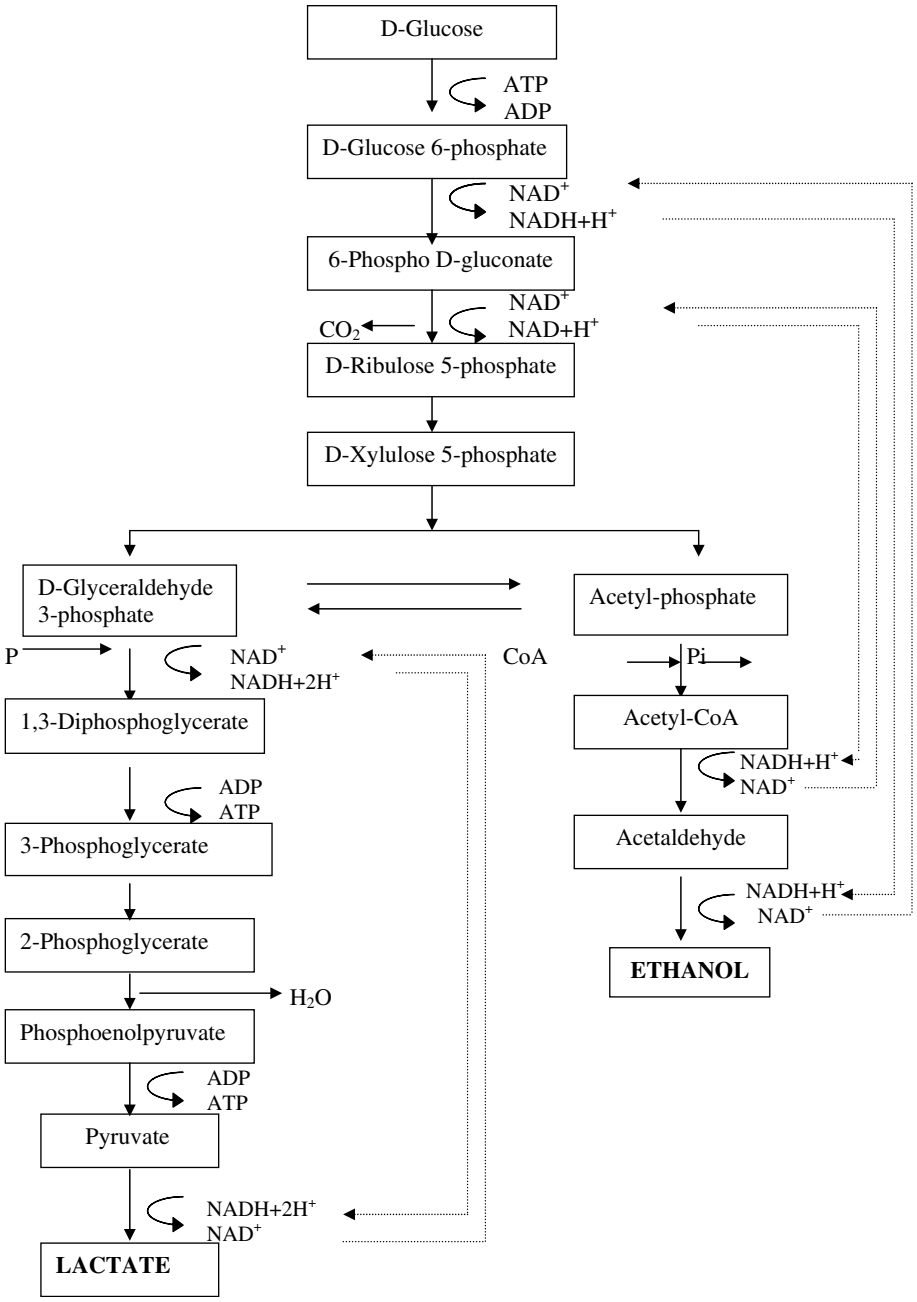

Fig. 2. Heterolactic fermentation (*Source*: Axelsson, 2004).

Table 2
Common dairy lactobacilli classified by fermentation group

Homolactic fermentative	Heterolactic fermentative	
	Facultative	Obligate
L. acidophilus	*L. plantarum*	*L. brevis*
L. helveticus	*L. rhamnosus*	*L. buchneri*
L. delbrueckii subsp. *delbrueckii*	*L. coryneformis*	*L. fermentum*
L. delbrueckii subsp. *lactis*	*L. curvatus*	*L. kefir*
L. delbrueckii subsp. bulgaricus	*L. casei*	*L. reuteri*
Lc. lactis	*L. paracasei*	*Leuconostoc* sp.
S. thermophilus		

(*Source*: Curry and Crow, 2003b).

do not ferment pentoses or gluconate. The facultatively heterolactic fermentative species metabolise hexoses through the Embden–Meyerhof glycolytic pathway, but pentoses and some other substances are metabolised *via* a phosphoketolase-dependent pathway to produce lactic acid and other products (typically acetic acid and ethanol). The obligate heterolactic fermentative species use only the phosphoketolase-dependent pathway for sugar metabolism, and so besides lactic acid, they produce significant quantities of acetic acid and/or ethanol with generation of carbon dioxide (Axelsson, 2004). The presence of oxygen may also significantly effect the metabolism (Condon,

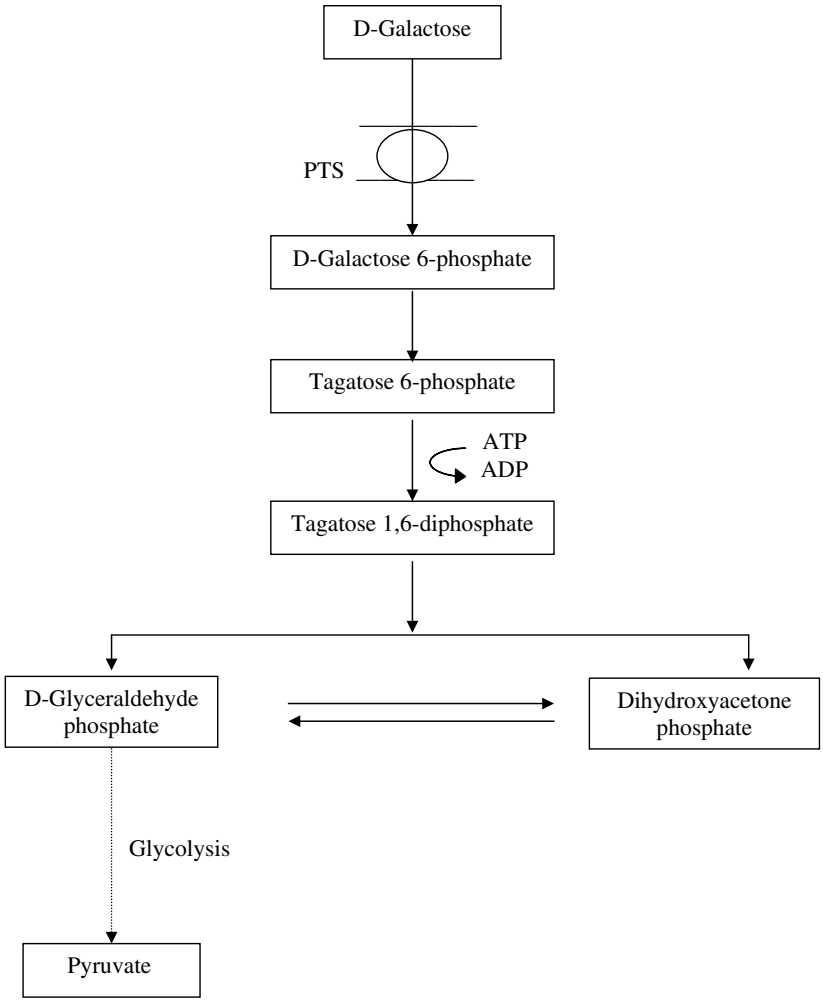

Fig. 3. Tagatose 6-phosphate pathway in lactic acid bacteria (*Source*: Axelsson, 2004).

1987). D-Galactose is metabolised either through the tagatose 6-phosphate pathway or via the Leloir pathway (Figs. 3 and 4). Some lactic acid bacteria (e.g., *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. delbrueckii* subsp. *lactis*, and *L. acidophilus*), only metabolise the glucose moiety after transport of lactose and cleavage by β-D-galactosidase, while galactose is excreted into the medium (Hickey, Hillier, & Jago, 1986; Hutkins & Morris, 1987). Galactose excretion has been attributed to a low galactokinase activity.

Different factors affect the growth of LAB in fermentation media. Besides complex nutritional requirements, temperature is one of the most important factors influencing LAB growth. There is an optimum temperature at which growth rate is highest and that depends on the characteristics of the microorganism used, as well as on the environmental conditions. When the temperature of the medium is above or below that required for optimum growth, microbial activity is substantially reduced and organisms may eventually die (Peleg, 1995; Rosso, Lobry, Bajard, & Flandrois, 1995). The optimal temperature for growth varies across the genera from 20 to 45 °C (Dicks, Dellaglio, & Collins, 1995; Wood & Holzapfel, 1995). Depending on the optimal temperatures, most lactobacilli come under the mesophilic category; however, *L. delbrueckii* subsp. *bulgaricus* (*L. bulgaricus*), *L. thermophilus*, and *L. delbruckii* can be grouped under the thermophilic category. Lactic acid production by fermentation can be carried out at comparatively high temperatures using suitable bacteria. In fermentations using *L. delbrueckii*, and *L. bulgaricus* a temperature of 45 °C or higher may be maintained (Buchta, 1983). *L. helveticus*, and *L. acidophilus* can be used in a temperature range of 37–45 °C. However, for other bacteria, such as *L. casei*, a temperature of 28–35 °C is preferred.

The hydrogen ion concentration of the environment during fermentation also affects microbial growth and product production rate. pH affects at least two aspects of microbial cells, i.e. the functioning of its enzymes and transport of nutrients into the cell. It can limit the synthesis of metabolic enzymes responsible for the synthesis of new protoplasm. pH values also affect RNA and protein synthesis (Klovrychev, Korolev, & Bulgakova, 1979). Therefore, pH is another important parameter, which has a strong effect on lactic acid production. Lactic acid produced during fermentation has to be continuously

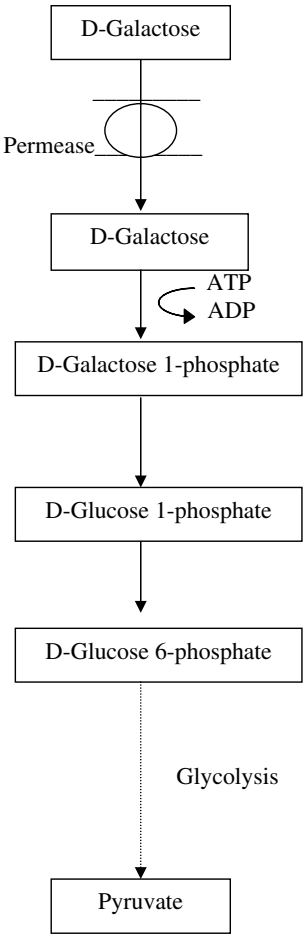

Fig. 4. Leloir pathway in lactic acid bacteria (*Source*: Axelsson, 2004).

neutralised. Generally, calcium carbonate is added as a buffering agent during batch fermentations. For rapid and complete fermentation, the optimal pH range is 5.5–6.0, in some cases 6.0–6.5, depending upon the culture used. Fermentation is strongly inhibited at lower pH and ceases at pH values below 4.5. However, LAB acid tolerance gives them a competitive advantage over many other bacteria.

Table 3
Lactic acid isomers produced by common dairy lactobacilli

Name of bacteria	D(−) Lactic acid	L(+) Lactic acid	DL (±) mixture
L. acidophilus	No	No	Yes
L. delbrueckii subsp. *lactis*	Yes	No	No
L. delbrueckii subsp. *bulgaricus*	Yes	No	No
L. helveticus	No	No	Yes
L. casei	No	Yes	No
L. paracasei subsp. *tolerans*	No	Yes	No
L. paracasei subsp. *paracasei*	No	Yes[a]	No
L. rhamnosus	No	Yes	No
L. plantarum	No	No	Yes
Lc. lactis	No	Yes	No
S. thermophilus	No	Yes	No
Leuconostoc sp.	Yes	No	No

[a] Some strains produce D(−) and L(+) lactic acid (*Source*: Curry and Crow, 2003a).

The type of fermentation and the configuration of lactate produced depends on the genera of LAB (Table 3). The configuration of lactic acid is very important from a nutritional point of view. A dietary intake higher in D-lactic or DL-lactic acid can result in an enrichment of D-lactic acid in the blood, and hyperacidity of the urine may occur. These findings caused the WHO to limit human consumption of D-lactic acid to 100 mg/kg/day (Kandler, 1982). During fermentation of sugars, different species of lactic acid bacteria produce either exclusively L-lactic acid, exclusively D-lactic acid, approximately equal amounts of both, or predominantly one form but measurable amounts of the other (Garvie, 1980; Kandler & Weiss, 1986; Schleifer, 1986). This depends on the presence of specific NAD^+-dependent lactate dehydrogenase (*n*LDH) and the respective activities of the LAB.

4. Whey utilisation

Dairy industries all over the world generate ample amounts of whey per litre of milk processed, depending upon the processes employed, products manufactured and housekeeping exercised. About 50% of total world cheese–whey production is treated and transformed into various food products, of which about 45% is used directly in liquid form, 30% in the form of powdered cheese whey, 15% as lactose and byproducts from its removal, and the rest as cheese–whey–protein concentrates (Marwaha & Kennedy, 1988). Since lactose is the major component of whey solids, in addition to water-soluble vitamins, minerals and proteins, numerous biotechnological processes have been developed to utilise whey to make useful products of industrial importance, such as lactic acid. This review is focused on LAB and their effective utilisation of whey for production of lactic acid.

Lactic acid or α-hydroxy propionic acid, as an unnamed component of soured milk has been known since the days when man first herded animals. It was discovered by the Swedish chemist Scheele in sour milk. Lactic acid and its derivatives are widely used in the food, pharmaceutical, leather, and textile industries (Buchta, 1983; VickRoy, 1985). Recently, there has been an increased interest in lactic acid production, since it can be used as a raw material for production of polylactic acid, a polymer used as a specialty medical and environmental-friendly biodegradable plastic (Datta, Tsai, Bonsignore, & Moon, 1995). Of the 80,000 tonnes of lactic acid produced worldwide every year, about 90% is made by LAB fermentation and the rest is produced synthetically by the hydrolysis of lactonitrile (Hofvendahl & Hahn-Hagerdal, 2000). Microbial fermentation has a significant advantage in that by choosing a strain of LAB producing only one enantiomer, an optically pure product can be obtained, whereas synthetic production results in a racemic mixture of DL-lactic acid. As the physical properties of polylactic acid depend on the

enantiomeric composition of lactic acid, the production of optically pure lactic acid is essential (Litchfield, 1996; Lunt, 1998). For example, optically pure L(+)-lactic acid is polymerised to a high crystal polymer suitable for fibre and oriented film production and is expected to be useful in production of liquid crystal as well (Amass, Amass, & Tighe, 1998).

4.1. Lactic acid production using free cell systems

Different *lactobacilli* cultures (*L. helveticus*, *L. delbrueckii* subsp. *bulgaricus*, *L. acidophilus*, *L. casei*, etc.) have been used for the utilisation of whey for lactic acid production. *L. heleveticus* is the generally preferred organism, as it produces almost twice the amount of lactic acid from milk, compared to other common LAB (*S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*), is homolactic fermentative and produces a racemic mixture (DL) as compared to only dextrorotatory lactic acid (D) produced by *L. delbrueckii* (Roy, Goulet, & LeDuy, 1986). Moreover, it also provides an alternative solution to the phase contamination in dairy industries, which is generally encountered during *L. delbrueckii* subsp. *bulgaricus* fermentation. Application of *S. thermophilus* has some drawbacks; only a few strains of this bacterium are able to ferment galactose, and it requires some growth factors for lactic acid production in milk-based medium (Roy et al., 1986). In recent studies other organisms such as *L. delbrueckii* and *Bifidobacterium longum* have also shown considerable promise (Li, Shahbazi, & Coulibaly, 2005; Satyanarayana & Venkateshwar, 2004). Most of the work has been carried out on the fermentation of cheese whey from cow milk, however, paneer whey and the wheys produced from other sources, such as camel milk, have also been tested for lactic acid production (Gassem & Abu-Tarboush, 2000; Kumar, Jha, & Chauhan, 2001). Sweet cow whey displayed the highest productivity and lactose conversion compared to camel whey samples (Gassem & Abu-Tarboush, 2000).

Temperature and pH are the key environmental parameters that affect the lactic acid production process. *L. helveticus* showed enhanced lactose utilisation and lactic acid production with increasing temperature (23–42 °C) displaying maximum lactic acid production at 42 °C (Tango & Ghaly, 1999). The effect of pH control range on the morphology of *L. helveticus* was studied in batch cultures, and highest lactic acid productivity was obtained at pH 5.5 (Norton, Lacroix, & Vuillemard, 1993). A temperature of 42 ° C and pH 5.8 were optimal during lactic acid production at high cell concentrations in whey ultrafiltrate by *L. helveticus* (Kulozik & Wilde, 1999). Highest lactic acid productivity values by *L. casei* were obtained at 37 °C and pH 5.5 (Büyükkileci & Harsa, 2004). Batch productivity was 1.87 g/l/h at 37 °C at flask level studies, whereas at the fermenter level a productivity of 3.97 g/l/h was obtained.

The nutritional requirements of lactic acid bacteria and especially their nitrogen sources are complex (Chopin, 1993; Desmazeaud, 1983; Pritchard & Coolbear, 1993; Stainer, Ingraham, Wheelis, & Painter, 1986; Torriani, Vescovo, & Scolari, 1994), and only part of the available peptides are metabolised. Cultivation media, which may have high protein content, are usually supplemented with yeast extract or protein lysates (peptones). In many fermentation studies, yeast extract is considered to be an essential nutrient for *lactobacilli* for efficient lactic acid production in lactobacilli (Aeschlimann & von Stockar, 1990; Amrane, 2005; Arasaratnam, Senthuran, & Balasubramaniam, 1996; El-Sabaeny, 1996; Murad, Abd El-Ghani, & Effat, 1992; Schepers, Thibault, & Lacroix, 2002). Few other nitrogen sources have proved to be as good in promoting the process (Hujanen & Linko, 1996). Lowering of nitrogen supplementation of the preculture medium can result in an increase in lag phase length of the culture, corresponding to a cellular adaptation to the new medium (Amrane, 2003).

The possibility of using hydrolysed whey for better production of lactic acid has also been explored (Amrane & Prigent, 1993; Lund, Norddahl, & Ahring, 1992). Best results were achieved using whey hydrolysed for 2–3 h. A significant lower concentration of carbohydrate in the effluent was seen with *L. delbrueckii* subsp. *bulgaricus*. Highest lactic acid production rate was obtained with *L. helveticus* precultivated on hydrolysed whey supplemented with yeast autolysate and transferred into whey permeate, with corn steep liquor as the nitrogen source (Amrane & Prigent, 1993). In some reports, hydrolysed whey protein has been shown to constitute a rich nutrient source for LAB (Krischke, Schroder, & Trosch, 1991; Leh & Charles, 1989; Lund et al., 1992; Senthuran, Senthuran, Mattiasson, & Kaul, 1997).

Besides yeast extract, supplementation using other nutrients, like molasses, corn steep molasses, lactose, vitamins, minerals, amino acids and inorganic supplements etc., to whey, has also been investigated for increasing lactic acid yield (Amrane, 2000; Borgardts, Krischke, Trosch, & Brunner, 1998; Cox & Macbean, 1977; Liu, Liu, Liao, Wen, & Chen, 2004; Mistry, Kosikowski, & Bellam, 1987). Lactic acid productivity of 9.7 g/L/h at a dilution rate of 0.352 h^{-1} using *L. helveticus* strain *milano* has been obtained during continuous fermentation of cheese–whey yeast extract permeate medium (Roy et al., 1986; Roy, Goulet, & Le Duy, 1987). However at high dilution rates, the cells were elongated to several times their normal size, as a result of excess growth of the cell wall.

Addition of molasses to whey has resulted in increases in lactic acid production (Chiarini & Mara, 1990). Size of inoculum and preculture medium play a significant role in the amount of lactic acid produced during the process. High lactose consumption (94.1%) together with good lactic acid production (26.1 g/l) and yield (0.90%) were obtained in whey ultrafiltrate supplemented with 1% beet molasses (Chiarini, Mara, & Tabacchioni, 1992). Addition of inorganic phosphate to the whey medium has also been

found beneficial and its supplementation has increased lactic acid production rate (by 40% for media supplemented with 2 g l^{-1} yeast extract), but had no effect on growth rate (Amrane, 2000). This beneficial effect was absent for high-nitrogen supplemented medium (20 g l^{-1} yeast extract), because of the lack of phosphorus limitation.

Manganese addition has a very significant beneficial effect on the fermentation of whey permeate by *L. casei*, because of its role as a constituent of lactate dehydrogenase (Borgardts et al., 1998; Senthuran et al., 1997). The fermentation time was reduced from 120 to 24 h with addition of $MnSO_4 \cdot H_2O$ during batch fermentations using *L. casei* (Fitzpatrick, Ahrens, & Smith, 2001). Moreover, addition of manganese allowed a lowering in the amount of yeast extract required, while maintaining high sugar conversion and lactic acid yield.

Using malt combing nuts (a low value byproduct from the malting industry) as an alternative to yeast extract in whey fermentation, a yield similar to yeast extract supplementation has been reported. However, malt combing nuts add much more ash to the fermentation and there is more unused nitrogen remaining at the end of fermentation, which is undesirable for the production of high purity lactic acid (Pauli & Fitzpatrick, 2002). Recent studies have shown that the supplementation of whey with yeast extract or protein lysates (peptones) can be conveniently replaced by *in situ* treatment of the cultivation medium with proteolytic enzymes or proteolytic microbes (Vasala, Panula, & Neubauer, 2005). Fastest acid production was obtained with addition of protease enzymes, however, treating of the medium with proteolytic microbes (*Bacillus megaterium*) was equally effective (Vasala, 2005).

Batch production of lactic acid by cheese whey fermentation has some disadvantages, such as a long lag period, unusually long fermentation times that require greater fermenter capacity and increased operational costs, and the requirement of ammonium or calcium ions to neutralise the lactic acid produced. In contrast, a continuous process has the advantages of high productivities and does not require high volume fermenters.

The continuous system for whey fermentation has been studied by several workers (Aeschlimann & von Stockar, 1991; Boyaval, Corre, & Terre, 1987; Roy et al., 1987; Urribarri et al., 2004). Recycling of cells in a continuous fermentation process is used to retain cells and thereby increase the biomass concentration in the fermenter, allowing an increased lactic acid production rate and decreased retention rate. Recycling also reduces substrate concentration in the effluent. In a cell recycling continuous system, volumetric lactic acid productivity was approximately doubled by increasing the dilution rate and at the same time the L-lactate fraction increased from approximately 60% to 70% (Aeschlimann & von Stockar, 1991).

Lactic acid productivity in whey fermentation can be improved using a membrane recycling bioreactor. When reconstituted whey permeate medium was used for lactic acid production, productivity was 6–18 times better in a continuous stirred tank reactor-membrane recycle bioreactor than in a batch reactor (Mehaia & Cheryan, 1986). In continuous mix batch bioreactors, maximum conversion efficiency (75.8%) was achieved with a 75 g/l initial lactose concentration. However considering the economic feasibility of the process, a lactose concentration of up to 100 g/l was recommended (Ghaly, Tango, Mahmood, & Avery, 2004). Yeast extract and/or microaeration increased specific growth rate, lactose consumption, lactic acid concentration and lactic acid yield; and reduced lag period, fermentation time and residual lactose. Combined yeast extract and microaeration produced better results than each one alone. When the dilution rate was varied (0.05 and 0.4 h^{-1}) during continuous culture of *L. helveticus* ATCC 8018 on deproteinised whey, the maximum concentration of lactic acid (11.00 kg/m^3) was obtained at a dilution rate of 0.1 h^{-1} (Urribarri et al., 2004).

In whey fermentation, there is an inhibitory effect caused by the lactic acid produced. The inhibitory effects of lactic acid have been alleviated to a certain extent by conducting fermentation in a continuous dialysis process, in a hollow fibre fermenter (VickRoy, Blanch, & Wilke, 1982) and in an electrodialysis system (Bazinet, 2004; Hongo, Nomura, & Iwahara, 1986).

Electrodialysis is an electrochemical separation process, by which electrically-charged species are transported from one solution to another, and has a great potential in downstream processing. It is a combined method of dialysis and electrolysis and can be performed with two main cell types: multi-membrane cells for dilution-concentration and water dissociation applications (membrane phenomena), and electrolysis cells for redox reactions (electrode phenomena). Electrodialysis with monopolar and dipolar membranes has been applied in the production of lactic acid during whey fermentation (Bazinet, 2004). A three-stage continuous fermentation pilot plant for the production of lactic acid has been developed, which resulted in a lactic acid productivity of 22 g/l/h (Boyaval et al., 1987). When the electrodialysis unit was coupled, the outlet concentration of lactate was stabilised at 85 g/l.

During whey fermentation with *B. longum*, cells and protein from the fermentation broth were separated using an ultrafiltration membrane (Li et al., 2005). A nanofiltration membrane was used to further separate lactic acid from lactose in the ultrafiltration permeate, 99–100% of lactose could be retained in the concentrate, with a lactic acid recovery of 40–60%. Higher initial lactic acid concentrations caused significantly higher permeate flux, lower lactose retention, and higher lactic acid recovery. The permeate flowrate decreased with time, due to fouling of the membrane (Li, Shahbazi, & Coulibaly, 2006).

4.2. Lactic acid production using immobilised cell systems

Immobilisation technology has several advantages; it permits higher cell densities in bioreactors, improves stability, makes reutilisation and continuous operation possible,

and precludes the need to separate the cells from the substrate products following processing. Adsorption, gel entrapment, and covalent attachment are the popular methods of immobilisation used in various bioprocesses. In adsorption, the biocatalysts are held to the surface of the carriers by physical forces (van der Waal's forces). The advantages of adsorption are that it is simple to carry out and has little influence on conformation of the biocatalyst (Hartmeier, 1986). However, a major disadvantage of this technique is the relative weakness of the adsorptive binding forces. The entrapment method is extremely popular for the immobilisation of whole cells. The major advantage of the entrapment technique is the simplicity by which spherical particles can be obtained, by dripping a polymer-cell suspension into a medium containing precipitate-forming counter ions or through thermal polymerisation. The major limitation of this technique is the possible slow leakage of cells during continuous long-term operation. However, improvements can be made by using suitable cross-linking procedures.

LAB have been immobilised by several methods on different supports (Table 4) and the immobilised systems have been investigated for lactic acid production from whey (Boyaval & Goulet, 1988; Mehaia & Cheryan, 1987; Roy et al., 1986; Zayed & Zahran, 1991). Generally, covalent binding is generally not preferred for immobilisation of lactic acid bacteria, due to the use of aggressive chemicals, which are harmful to the cells. In search of economical immobilisation supports, wood chips, brick particles and porous glass and egg shell have been tested for immobilisation of *L. casei*. Out of these, wood chips showed the highest adsorption (Kazemi & Baniardalan, 2002; Nabi, Gh, & Baniardalan, 2004). This immobilised preparation displayed the highest rate of production of lactic acid (16 g/L) from whey in a batch system, and a lactic acid production rate of 14.8 g/l with a dilution rate of $0.2\,h^{-1}$ was observed in a continuous system after 5 days.

In lactic acid production, entrapment is the most common technique used. Entrapment of bacterial cells in natural polysaccharide gel beads allows high cell density continuous fermentations, and may result in improved productivity. Immobilised cells have been successfully used in repeated batch fermentation. Use of *lactobacilli* immobilised in alginate beads for lactic acid production resulted in an increase in fermentative activity during the first 7 of 10 consecutive fermentations in which the beads were reused, but firmness of the beads decreased thereafter due to decalcification and resultant solubilisation (Champagne, 1992).

Among the two matrices assessed, agar was better than polyacrylamide in its effectiveness to carry out batch fermentation in whey permeate medium for up to three repeated runs (Tuli, Sethi, Khanna, Marwaha, & Kennedy, 1985). The supplementation of Mg^{2+} and agricultural by-products (mustard oil cake) in whey permeate medium further improved the acid production ability of the immobilised cells. Among different matrices (calcium alginate, κ-carrageenan, agar, and polyacrylamide gels) tested for co-immobilisation of *L. casei* and *Lc. lactis* cells, alginate proved to be the best matrix for the production of lactic acid from deproteinised whey (Roukas & Kotzekidou, 1991). The polyacrylamide was polymerised *in situ* and this could cause significant cell death, due to the toxicity of the monomer and catalyst chemicals present. The

Table 4
Different immobilisation matrices used for lactic acid production from whey

Microorganism	Immobilisation matrix	References
L. casei	Agar-agar	Tuli et al. (1985)
L. casei	Polyacrylamide	Tuli et al. (1985)
L. delbrueckii subsp.*bulgaricus*	Hollow-fibre	Mehaia and Cheryan (1987)
L. helveticus	Alginate	Boyaval and Goulet (1988)
S. thermophilus	κ-Carrageenan/locust bean gum	Arnaud et al. (1989)
S. thermophilus	κ-Carrageenan/locust bean gum	Audet et al. (1990)
L. delbrueckii subsp. *bulgaricus*	κ-Carrageenan/locust bean gum	Audet et al. (1990)
L. casei and *Lc. lactis*	Alginate	Roukas and Kotzekidou (1991)
L. casei and *Lc. lactis*	κ-Carrageenan	Roukas and Kotzekidou (1991)
L. casei and *Lc. lactis*	Agar-agar	Roukas and Kotzekidou (1991)
L. casei and *Lc. lactis*	Polyacrylamide	Roukas and Kotzekidou (1991)
L. casei	Agar-agar	Zayed and Zahran (1991)
L. casei subsp. *casei*	Porous sintered glass beads	Krischke et al. (1991)
L. delbrueckii subsp. *bulgaricus*	κ-Carrageenan	Buyukgungor (1992)
L. helveticus	κ-Carrageenan/locust bean gum	Norton et al. (1994)
L. helveticus	Alginate	Oyaas et al. (1996)
Lc. lactis subsp. *lactis*	κ-Carrageenan/locust bean gum	Lamboley et al. (1997)
Lc. lactis subsp. *lactis* biovar *diacetylactis*	κ-Carrageenan/locust bean gum	Lamboley et al. (1997)
L. casei	Poraver beads	Senthuran et al. (1999)
L. brevis	Delignified cellulosic material	Elezi et al. (2003)
L. casei	Wood chips, brick particles porous glass, and egg shell	Nabi et al. (2004)
L. casei	Apple and quince pieces	Kourkoutas et al. (2005)
L casei	Alginate–chitosan	Göksungur et al. (2005)
L. helveticus	κ-Carrageenan/locust bean gum	Schepers et al. (2006)
L. casei	Pectate gel	Panesar et al. (2007)

immobilisation process protected the cells from adverse conditions and improved the yields of lactic acid.

Rheological evaluation is an important tool for the selection and optimisation of the support. Rheological properties of various κ-carrageenan/locust bean gum mixed gel disks containing *S. thermophilus* were studied during the fermentation of supplemented whey permeate medium (Arnaud, Lacroix, & Choplin, 1989). An 8% complementation of locust bean gum gave the best rheological properties, a gain in G' (elastic modulus) brought about by KCl treatment was 175% compared to untreated gels. *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* immobilised separately in κ-carrageenan-locust bean gum gel beads were used in a batch system for fermentation of supplemented whey for lactic acid production. Beads with high initial cell density increased fermentation rates compared to low cell density beads or free cells, and smaller diameter beads showed a better stability (Audet, Paquin, & Lacroix, 1989; Audet, Paquin, & Lacroix, 1990). These immobilised cells were also used continuously in a bioreactor, yielding lactic acid concentrations in the effluent of 17.3 and 4.3 g/l for dilution rates of 0.5 and 3.0 h^{-1}, respectively (Audet, Lacroix, & Paquin, 1992). Mixed strain continuous fermentations of whey permeate medium with *Lactococcus* strains immobilised separately in κ-carrageenan-locust bean gum gel beads was also carried out (Lamboley, Lacroix, Champagne, & Vuillemard, 1997). The process showed a high biological stability and no strain became dominant, or was eliminated from the bioreactor. The beads demonstrated a high mechanical stability throughout the 53-day continuous fermentation.

In a recycle batch reactor system using *L. casei* immobilised by adsorption, the overall productivity of the recycle system was higher, in comparison with the batch process using free cells (Senthuran, Senthuran, Hatti-Kaul, & Mattiasson, 1999). The enhancement in productivity in the recycle batch reactor was also accompanied by an increase in density of suspended cells.

L. brevis cells immobilised by adsorption on delignified cellulosic material resulted in 70% yield, whereas the remaining lactose in whey was converted to alcohol by-product, leading to 90% lactose exploitation (Elezi et al., 2003). The system showed high operational stability with 10 repeated batch fermentations without any loss in cell activity. *L. casei* cells immobilised by adsorption on fruit (apple and quince) pieces have been used for 15 successive fermentation batches of whey and milk (Kourkoutas, Xolias, Kallis, Bezirtzoglou, & Kanellaki, 2005). These immobilised biocatalysts proved to be very effective and suitable for food grade lactic acid production.

L. helveticus cells immobilised in calcium alginate beads showed higher lactic acid production rates than free cells (Boyaval & Goulet, 1988). However, cell leakage was observed during continuous operation. Treatment of calcium alginate beads with polyethyleneimine to increase the stability of the gel did not reduce the cell leakage and caused severe shrinkage of the beads. After a week of operation plugging in the packed bed occurred. Similar problems were also observed by other workers during continuous fermentation using multistage packed bed columns (Roy et al., 1987). Plugging can be due to decalcification of calcium alginate by lactic acid. As a result, the beads are softened and have a tendency to compress into a solid mat, and thus block the column. The other reason for cell leakage can be overgrowth in the beads with time. Thus leaked cells accumulate in large quantities at the later stages of the column. This phenomenon was observed usually after the 5th day of operation. These workers suggested that a fluidised bed column could be more suitable than a fixed bed column.

Fluidised bed reactors with *L. casei* subsp. *casei* immobilised by adsorption showed higher productivities of lactic acid than conventional stirred tank reactors in a continuous lactic acid production (Krischke et al., 1991). A fibrous bed bioreactor has also been tested for continuous lactic acid production from unsupplemented acid whey, using adsorption biofilm immobilised cells of *L. helveticus* (Silva & Yang, 1995). Reactor performance was stable for continuous, long-term operation for both sterile and non-sterile whey feeds for a 6-month period. The chemostat system in salt whey permeate fermentation with *Lactobacillus* cells immobilised in agarose beads displayed a steady lactic acid concentration of 33.4 mg/ml (Zayed & Winter, 1995). In a packed bed bioreactor, the highest lactic acid production rate (3.90 g/l/h) was obtained with an initial lactose concentration of 100 g/l and a hydraulic retention time of 18 h (Tango & Ghaly, 2002).

Recently, a two-stage process has been used for continuous fermentation of whey permeate medium with *L. helveticus* immobilised by entrapment, which resulted in high lactic acid productivity (19–22 g/l/h) and low residual sugar (Schepers, Thibault, & Lacroix, 2006). However, after continuous culture operation with very low or no residual sugar for several days, loss of productivity was observed in the second reactor, due to loss of biomass activity, as a result of cell death by starvation.

A fermentation apparatus for the continuous production of lactic acid from whey has also been described (Prigent, 1983). A pump inserted between the fermenter and an ultrafiltration apparatus allowed for recycling of the liquid, as well as processing the filtrate by electrodialysis for lactate recovery. An electrodialysis fermentation method, in which lactic acid is continuously removed from the fermentation broth, resulted in a continuous fermentation with productivity three times higher than a non-pH controlled culture. With this method, the amount of lactic acid produced was 82.2 g/l, approximately 5.5 times that produced in non-pH controlled fermentation. However, fouling of anion exchange membranes by cells was a problem (Hongo et al., 1986). The morphology of immobilised cell preparations in continuous fermentations is much less pH-dependent than free cultures, which could be due to the long

response time of entrapped cells to daily random changes in pH set point (Norton et al., 1993; Norton, Lacroix, & Vuillemard, 1994).

Immobilised *B. longum* in sodium alginate beads and on a spiralsheet bioreactor have also been evaluated for production of lactic acid from cheese whey (Shahbazi, Salameh, & Ibrahim, 2005). *B. longum* immobilised in sodium alginate beads showed better performance in lactose utilisation and lactic acid yield than *L. helveticus*. In producing lactic acid, *L. helveticus* performed better when using the spiral sheet bioreactor and *B. longum* showed better performance with gel bead immobilisation. Response surface methodology was used to investigate the effects of initial sugar, yeast extract and calcium carbonate concentrations on lactic acid production from whey by immobilised *L. casei* NRRL B-441 (Göksungur, Gunduz, & Harsa, 2005). Higher lactic acid production and lower cell leakage was observed with *L. casei* cells immobilised in alginate–chitosan beads, compared with calcium alginate beads and these gel beads were used for five consecutive batch fermentations without any marked activity loss and deformation.

5. Conclusions

Whey is undoubtedly an excellent growth medium for various types of microorganisms. However, economical problems in transporting the whey have been posed as obstacles to adopting any process or utilisation of whey. Clearly this is because of its high water content and storage problems due to it being readily subjected to bacterial and fungal spoilage. These problems have been solved to a great extent with the development of reverse osmosis and ultrafiltration techniques used for the concentration of whey. The use of immobilisation technology in utilisation of whey is of significant importance to improve further the economics of the process. Immobilisation has been the convenient method to allow reutilisation of cells, higher cell densities in bioreactors and easier purification of the final product. Moreover, continuous operation is more easily and efficiently controlled using this technology, which has an advantage over the free cell system in the bioconversion of whey.

High lactic acid productivities, with high cell densities retained in the bioreactors, and long-term stability, have been reported for immobilised *Lactobacillus* sp. and continuous fermentation processes with yeast-extract supplemented whey. However, complete sugar conversion could not be attained in a continuous immobilised cell process with a single stage. In most cases, lactic acid productivity was limited due to factors such as non-uniform pH control and clogging of the column reactors, destabilisation of the alginate gel used for immobilisation/entrapment by calcium-chelating lactates, and loss of biocatalyst activity. Mechanical stability of the beads and diffusion limitations of substrate and product within the gel bead matrix appeared to be the main problems encountered by previous researchers, particularly during continuous fermentation. Thus, the success of these processes could rely on the optimisation of all fermentation parameters in order to achieve high stability, along with high productivity, and low operating and capital costs. A clear understanding of the effects of immobilisation on lactic acid bacteria kinetics is required to reach this objective. Moreover, suitable bioreactor design for lactic acid production is also very important to make the process successful. Innovative uses of whey through microbial fermentation, along with recent biotechnological techniques, and bioreactor design, will certainly remain topics of great interest when trying to solve the major environmental problem faced by the dairy industry.

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