The biotechnological potential of whey

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Published online: 19 August 2016
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Abstract  Whey is a highly polluting by-product of cheese and casein powder manufacture with worldwide production of whey estimated at around $190 \times 10^6$ ton/year and growing. Historically whey was considered a burdensome, environmentally damaging by-product. In the last decades however, much research has gone into finding viable alternatives for whey rather than just disposing of it. Multiple biotechnological avenues have been explored and in some cases exploited to turn this waste product into a valuable commodity. Avenues explored include traditional uses of whey as both an animal and human food to the more advanced uses such as the use of whey protein as health promoters and the potential of whey to be used as a feed stock to manufacture a whole range of useful substances e.g. ethanol.

Keywords  Whey · Biotechnology · Ethanol · Bioconversion

1 What is whey?

Whey is a yellow-green liquid by-product from the manufacture of cheese or casein from milk. The yellowish colour of whey is due to the presence of riboflavin (Vitamin B2) in the whey (De Wit 2001). The type of whey depends upon the processing technique used to remove casein from the milk, with the two main types being sweet whey and acid whey. The first type is sweet whey. Sweet whey has a pH of approximately 5.6 and comes from the production of most types of cheese or certain casein products. The first step in making cheese (and sweet whey) is the addition of rennet, a mixture of enzymes containing the protease chymosin, to milk. The rennet works by curdling the casein protein in the milk leading to the formation of curds. The curds are then strained from the remaining liquid. This is called whey. The rennet induced coagulation of casein occurs at pH 6.5 (Panesar et al. 2007; Fig. 1). The other type of whey is acid whey, which has a pH of approximately 4.5. This type of whey is created by either the activity of lactobacilli or the addition of organic (lactic acid) or mineral acids (hydrochloric or sulphuric acid) to coagulate the casein for the manufacture of most types of industrial caseins (Jelen 2003; Fig. 1).

Whey represents 85–95 % of the milk volume and retains about 55 % of the milk nutrients (Siso 1996). Approximately 20 % of the total protein content of the milk is retained in the whey (Walsh 2014). The main constituents of both sweet and acid whey are water (~93 % of the total whey volume), lactose ($C_{12}H_{22}O_{11}$, 70–72 % of the total solids), whey proteins (8–10 % of the total solids, these will be discussed below) and minerals (12–15 % of the total solids). Calcium, potassium, sodium and magnesium...
salts make up the bulk of these minerals (of >50% NaCl and KCl, calcium salts) with trace amounts of metals such as zinc and copper (Venetsaneas et al. 2009). The two whey types differ in mineral content, the pH and in the composition of the whey protein fraction (Jelen 2003). Whey also contains small quantities of other components like lactic and citric acids, non-protein nitrogen compounds (urea and uric acid) and B group vitamins (Kosikowski 1979; Marwaha and Kennedy 1988). The chemical make-up of whey can vary depending on which species the milk came from. The composition of whey proteins can be seen in Table 1.

2 Environmental issues associated with whey

The main problem associated with whey comes from its potential to damage the environment. It has a very high Biochemical Oxygen Demand (BOD) that can vary from 40,000 to 60,000 mg/L and a very high Chemical Oxygen Demand (COD) of between 50,000 and 80,000 mg/L (Chatzipaschali and Stamatis 2012). The waste load of whey is equivalent to that of 100–175 times that of a similar volume of domestic waste water (Mockaitis et al. 2006; Smithers 2008). This high polluting potential makes disposal of surplus whey expensive. Lactose, the largest constituent of whey (70–72% of the total solids), is the main component causing these high values for BOD and COD (Jelen 2003; Patel and Murthy 2011).

Whey is created in near equal volumes to the processed milk used during cheese manufacture. Worldwide production of whey is estimated at around 190 × 10^6 ton/year (Baldasso et al. 2011). It has been shown that, for every 1 kg of cheese made approximately 9 L of whey is produced (Kosikowski 1979). On average across the world, volumes of whey are growing at about the same rate as milk volumes (>2% per year; Smithers 2008).

From the 1960s and 1970s onwards, community action groups, environmental regulatory agencies and dairy processors alike came to recognise and highlight the environmental damage being triggered by the disposal of untreated whey (Smithers 2015). Disposal of whey by dumping in water bodies is now prohibited in most dairy producing nations by strict environmental legislation. Whey can have highly deleterious effects on aquatic life [in 2008 a spillage of acid whey in a waterbody in Ohio in the US killed more than 5400 wild animals, mostly fish (Hirsch 2015)] within the water body due to the depletion of the dissolved oxygen leading to eutrophication. This can also cause bad odours.

Land spreading for the disposal of whey can lead to the build-up of compounds (salts) in the soil that can damage the soil and effect the growth of plant life (Kosikowski 1979). Crop kills have been reported due
| Protein                  | % of whey protein\(^a\) | Peptide derivatives | Potential functions that are beneficial to human health                                                                 | References                                      |
|-------------------------|-------------------------|---------------------|------------------------------------------------------------------------------------------------------------------------|------------------------------------------------|
| β-Lactoglobulin         | 50–55                   | β-Lactophorin, β-Lactotensin | Angiotensin I converting enzyme (ACE) inhibition to lower blood pressure, Appetite enhancer, Appetite suppressant | Welderufael et al. (2012)                      |
| α-Lactalbumin           | 20–25                   | α-Lactophorin       | Used in infant formula, Opioid agonist, Increase serum levels of tryptophan to improve sleep quality cognitive performance under stress, and mood under stress | Sandström et al. (2008), Antila et al. (1991), Markus et al. (2000, 2002, 2005), Nurminen et al. (2000) |
| Immunoglobulins         | 10                      |                     | Lowers blood pressure, Help protect against illness such as oral or intestinal microbial infections | Hurley and Theil (2011)                       |
| Serum albumin           | 5–10                    | Albutensin, Serophorin | Prevention of cancer, Appetite suppressant, Opioid agonist                                                            | Lausen et al. (1989), Ohinata et al. (2002), Tani et al. (1994) |
| Proteose peptone 3      | 12                      |                     | Anti-microbial and anti-viral properties, Bone growth promoter, Anti-Cancer Activity                                     | Pihlanto (2011), Cornish et al. (2004), Iigo et al. (1999), Kozu et al. (2009) |
| Lactoferrin             | ~1–2                    | Lactoferricin       | Anti-microbial and anti-viral properties, Bone growth promoter, Anti-Cancer Activity                                     | Pihlanto (2011)                                |
| Lactoperoxidase         | ~0.5                    |                     | Protection against microbial infection, Prevention of dental caries, Preservation of raw milk                          |                                               |
| Glycomacropeptide (a casein peptide) | 10–15 (in renneted whey only) | N/A                | Anti-thrombotic activities, Nutritional additive in infant formula, Used in formulating foods for suffers of Phenylketonuria, Appetite suppressant | Requena et al. (2010), Lim et al. (2007), Pihlanto (2011) |

\(^a\) Depends on the type of whey
to the high application of cheese whey leading to rapid consumption of oxygen in the soil from the breakdown of milk sugars and proteins present in whey. Application of whey can also reduce soil redox potential. This can lead to the solubilisation of Fe and Mn present in the soil potentially contaminating ground water supplies. It has also been reported that for each mm (10^-3 ha) of whey applied to the soil, about 400–600 kg of total salt per hectare was added which resulted in high soil salinity and reduced crop yield (Ghaly et al. 2007). Acid whey is polluting not only the whey itself but also due to the low pH (~4.5) levels which can damage the soil.

Biological wastewater treatment technologies both aerobic (including trickling filters, aerobic lagoons, activated sludge systems, membrane bioreactors and jet loop membrane bioreactor) and anaerobic can assist in safe disposal of whey within the environmental specifications, but these treatments can be expensive. Older aerobic systems have difficulty dealing with the high polluting load of the whey [requiring high dilution ratios and long retention times (Rivas et al. 2010)] and large amounts of waste sludge are created which must also be disposed of (Prazeres et al. 2012). Anaerobic systems have the benefit of being able to take very high COD loads and of producing biogas that can be used for heating or powering plant process (Chatzipaschali and Stamatis 2012). However these systems are not suitable for all climates, can be difficult to run and maintain and may need the addition of other feed stocks.

### 3 Whey as biotechnological resource

Whey is an excellent source of functional proteins and peptides, lipids, vitamins, minerals, and lactose that could be exploited by the agri-food, biotechnology, medical, and related industries and in the last several decades major research efforts have seen whey and whey proteins transformed from ‘gutter-to-gold’ (Smithers 2008).

#### 3.1 Traditional uses of whey

Traditionally whey (in an unmodified form) was used as an animal feed (pigs, sheep, cattle) or was land spread as a fertiliser (Schingoethe 1976, Watson et al. 1977). As a direct animal feed whey (usually diluted with drinking water) provides high-quality proteins and lactose as energy sources and also provides calcium, phosphorus, sulphur and water-soluble vitamins. Excessive lactose and minerals however can cause issues for farm animals that necessitate a limit in untreated whey use as an animal feed (Sienkiewicz and Riedel 1990). There are also issues with land spreading as the application of large quantities of whey leaves high saline deposits in the soil, damaging fertility (Kosikowski 1979). Both uses have difficulties concerning volumes and high transportation costs that make these solutions impractical for the amounts of whey being created today.

#### 3.2 Whey as a human food?

Whey can be used to make human food products such as whey cheese and beverages. The most common whey beverages are fruit juices that have been mixed with whey (the brands Djoez and Taksi from the Netherlands and Nature’s Wander from Sweden) (Kosikowski 1968; Holsinger et al. 1974; Jelen 2009). An example of a carbonated soft drink is Rivella which has been manufactured and sold in Switzerland since the early 1950’s. This is made from carbonated whey permeate (discussed below) flavoured with the extract of various herbs (Pesta et al. 2007). These products however have so far failed to spread outside their home markets (Jelen 2009).

Whey can also be used to create alcoholic drinks such as a low alcoholic beverage (<1 % alcohol content) (Sienkiewicz and Riedel 1990), whey beer (Sienkiewicz and Riedel 1990), whey wine (Jeličić et al. 2008) and whey champagne (Sienkiewicz and Riedel 1990). Creation of these products involves addition of certain additives including sucrose and malt and the fermentation of the mixture with yeasts such as Kluyveromyces fragilis or Saccharomyces lactis (Holsinger et al. 1974).

Whey can also be used to create whey cheeses with there being two main types: Ricotta or Mysost. To make Ricotta cheese the whey is heated to >80 °C and the whey protein is denatured. This denatured protein then clumps (often aided by citric acid addition) forming whey protein curds that are then processed into cheese (Pintado et al. 2001). Mysost type cheeses are based on the condensation of the whey under controlled conditions. The collected whey condensate is then heated to 95 °C and processed into cheese.
Whey butter is another potential food product that can be created from whey. Whey cream is removed from whey after cheese making and before it is processed for spray drying or protein concentration. Whey butter has been found to be slightly softer than normal butter but has a saltier flavour (Jinjarak et al. 2006). These products however all have limited commercial appeal and do not present a way to treat large qualities of whey waste.

Whey proteins also have uses in the food industry as their physical properties allow them to act as emulsifiers, gelling agents/water binders and foaming/whipping agents in food systems. They are in used in many different foods including soups, salad dressings, processed meat, dairy and bakery products (Walsh 2014).

3.3 Creation of whey powders and whey permeate

To create whey powders, the whey is spray dried (Kosikowski 1979; Yang and Silva 1995). This allows the quality of the whey to be preserved for a longer period of time for transportation or further manipulation (Siso 1996). This type of whey powder is mostly used for animal feeding (mixed with molasses or soya flour) in the form of dairy nuts. Smaller quantities are used in human foods like ice-creams, baked goods, cakes, sauces, etc. (Siso 1996).

For the manufacture of higher grade whey protein powders, the whey undergoes membrane separation by ultrafiltration or diafiltration. Whey can be treated to give three different whey protein powder types: whey protein concentrate, whey protein isolate, and the third type whey hydrolysate (Fig. 2). Whey Protein Concentrate (WPC) contains levels of protein of approximately 35–80 % with low, medium and high protein powders being available. Whey Protein isolate (which can also be created by ion-exchange) has a high level of protein (>90 % protein) and is almost totally devoid of lactose and fats. Whey hydrolysate is created by the enzymatic hydrolysis of WPC or WPI. The creation of WPC/WPI leaves a secondary liquid stream, whey permeate, as a residue (Mollea et al. 2013). Whey permeate has nearly as high a BOD as whole raw whey fluid, and therefore poses a troublesome disposal problem. A generalised overview of this process can be seen in Fig. 2.

![Fig. 2 Overview of the fractionation of whey and possible uses of the products formed](image-url)
3.4 Whey as health promoter

Whey and whey derived bioactive compounds (Whey Protein Concentrate/isolate, peptides, etc.) have undergone research for their ability to enhance general health and well-being (Shah 2000; Cross and Gill 2000; Beaulieu et al. 2006; Krissansen 2007). The potential health benefits of whey have long been recognised. Whey has been used since at least the seventeenth century to treat a variety of conditions such as sepsis, wound healing, and ‘stomach disease’ (Hoffmann 1961). Whey proteins have shown a variety of positive effects both nutritional and physiological. These include (A) improvements in physical performance, better recovery following exercise, and to aid in the prevention of muscular atrophy (Ha and Zemel 2003; Ohr 2004; Tipton et al. 2004), (B) improved weight management and appetite control (Ohr 2004; Zemel 2004; Schaafsma 2006a, b), (C) improvements in cardiovascular health (Murray and FitzGerald 2007), (D) anti-cancer effects (Bounous et al. 1991; Bounous 2000), (E) help with the management of infections (Bounous et al. 1993; Playford et al. 1999; Regester and Belford 1999), (F) improve infant nutrition (Jost et al. 1999), and (G) healthy aging (Yang et al. 2012). Some of these effects are reputed but a number have undergone substantial scientific examination and they have been corroborated in numerous laboratories globally.

3.4.1 Whey proteins/peptides

Whey protein is considered a high quality protein sources as it contains high levels of all essential amino acids, and has a biological value (measure of the proportion of absorbed protein from a food which becomes incorporated into the proteins of the organism’s body) that is 15 % greater than the former benchmark egg protein (Ismaila and Gub 2010). Whey proteins are heat labile (Fox and McSweeney 1998; Walstra et al. 2005). The main types of whey proteins are β-lactoglobulin (β-Lg), α-lactalbumin (α-La), bovine serum albumin (BSA) and immunoglobulins (IGs) (Table 1). BSA is found in low levels in bovine milk and this is thought that the protein leak through from blood serum. IGs are antibodies that are created in response to specific antigens and their purpose is to provide immunological protection to the young mammal. IGs are large glycoproteins (proteins with sugar moieties attached) and are heat labile in the presence of other whey proteins (Walstra et al. 2005).

Miscellaneous whey proteins include lactoferrin and several different enzymes including lysozyme, oxidoreductases, phosphatases, lactoperoxidase, lipolytic enzymes and proteinases. (Table 1; Walstra et al. 2005).

Creation of casein curds using rennet produces a fragment k-casein molecule that is called glycomacropeptide (GMP) and this peptide ends up in the whey. The GMP constitutes approximately 15 % of the whey protein fraction of sweet, rennet-based wheys, but is not present in the acid whey (Lim et al. 2007; Neelima et al. 2013).

Bioactive peptides are derived from whey proteins by enzymatic hydrolysis using pancreatic enzymes such as trypsin and chymotrypsin or the stomach enzyme pepsin these enzymes usually come from bovine or porcine sources (Madureira et al. 2010) or from bacterial, fungal or yeast proteases (Morais et al. 2014). Some bioactive peptides can also be created through microbial fermentation of whey protein. For example Lactobacillus helveticus fermentation of whey proteins creates an angiotensin I-converting enzyme inhibitory (ACE) peptide (Yamamoto et al. 1999). Different whey derived peptides are listed in Table 1.

Whey has also been found to contain several growth factors including insulin-like growth factor, platelet-derived growth factor, transforming growth factor, and, fibroblast growth factor (Pouliot, and Gauthier 2006). These growth factors could be potentially used as a reliable replacement for, or as a supplement to fetal bovine serum (Smithers et al. 1996). Fetal bovine serum is used in mammalian cell culture, used in the manufacture of vaccines and biopharmaceuticals. The use of a whey derived growth factor media would provide a cheaper and safer alternative to fetal bovine serum that would not be at risk of contamination from mycoplasma, viruses and Bovine Spongiform Encephalopathy (BSE) (Keenan et al. 2006).

3.5 Lactose

Milk sugar lactose (4-O-β-D-galactopyranosyl-D-glucose) can be recovered from cheese whey or more likely from whey permeate by crystallization (Patonson 2009).

Lactose is used widely within the food and confectionery industries due to its low sweetness
[16 % that of sucrose (Joesten et al. 2006)]. It is used in the baking industry to promote crust browning through the Maillard reaction. It is also added to cow’s milk (4.4–5.2 % in bovine milk compared to 7 % in human milk) in the preparation of infant formula. Lactose is also used in the pharmaceutical industry as an excipient (Paterson 2009). The amounts of lactose produced from whey have increased greatly since the 1940’s (Sienkiewicz and Riedel 1990).

Lactose can also be used for the direct production of various other compounds. Lactulose (4-O-β-D-galactopyranosyl-D-fructose) is a lactose derivative that has several potential uses. It can also be used as a sweetener. Lactulose has an advantage in this use as it is more soluble than lactose which makes it easier to use in food applications and it also has a greater sweetness value [48–62 % of sucrose (Parrish et al. 1979; Mizota et al. 1987)]. It can be used as a sweetener for diabetics, as a substitute for sucrose in confectionery products, as an additive in milk/dairy applications, and in various foods (liquid or dry) that are made for the elderly (Mayer et al. 2004). It can also be used as a laxative in the treatment of acute and chronic constipation (Tramonte et al. 1997), and also in the treatment of hyperammonemia (excess of ammonia in the blood) and chronic hepatic encephalopathy (impairment of brain function due to liver issues) (Blanc et al. 1992). More recently, it has been considered for use as a prebiotic (De Souza Oliveira et al. 2011). It is created via an alkaline isomerization of lactose however research has been undertaken on enzymatic synthesis of the compound (Aider and Halleux 2007; Tang et al. 2011).

Lactitol (4-O-(β-galactopyranosyl)-D-sorbitol) is a sugar alcohol used as a replacement bulk sweetener for low calorie foods, slimming products and in foods specially formulated for diabetics with the addition of sugar alcohol used as a replacement bulk sweetener for low calorie foods. It has been investigated as a potential prebiotic due to the fact that it can be metabolised to short chain fatty acids by the colonic microbiota (Dills 1989). Lactitol, like lactulose, can also be used to treat constipation and chronic hepatic encephalopathy (Patil et al. 1987; Faruqui and Joshi 2012). Lactitol is created by the chemical hydrogenation of lactose (Zacharis 2012).

Lactosylurea (4-O-β-D-galactopyranosyl-1-N-β-o-glucopyranosyl) urea) is another potential lactose derivative. This can be used as a non-protein nitrogen source in ruminant feeding. It has major advantages over other non-protein nitrogen sources such as urea, as due to the slow breakdown of the product no toxic ammonia level is reached (Yang and Silva 1995). No product of yet is commercially available.

Another potential use of the lactose present in whey permeate is to hydrolyse the lactose into glucose and galactose. This can be achieved by immobilised β-Galactosidase. Hydrolysed lactose solutions have greater sweetening power than lactose [glucose has 80 % and galactose 60 % of the relative sweetness of sucrose (Joesten et al. 2006)] and have applications in the confectionery, ice-cream and soft drink industries, potentially replacing saccharose or corn starch syrup (Gänzle et al. 2008). The sweetness of the hydrolysed lactose solution can be increased by the conversion of the glucose present after hydrolysation of the lactose to fructose (110 % of the relative sweetness of sucrose) with glucose isomerase (Moulin and Galzy 1984; Kosaric and Asher 1985).

Lactose from whey can also be used in the creation of Galactooligosaccharides (GOS). These are prebiotics that can have a positive effect on human health by encouraging the growth of probiotic bacteria in the gut. They are frequently produced from lactose in a reaction catalysed by β-galactosidase, termed transglycosylation or they can be produced chemically (Torres et al. 2010). GOS’s are made from a variable number (2–8) of galactose units linked to a terminal glucose. They can be created from the lactose present in whey (Jovanovic-Malinovska et al. 2012; Golowczyk et al. 2013).

3.6 Bioconversion of whey into useful products

Significant efforts are being undertaken worldwide to find ways to upgrade surplus whey to a feed stock for bioconversion towards various value-added products. Lactose (present in whey permeate), as the major carbohydrate component of whey, can act as a carbon source for growth and product formation in numerous biotechnological processes. In the literature, the production of bioethanol (Ghaly and El-Taweel 1997; Zafar and Owais 2006), vinegar (Parrondo et al. 2009), antibiotics, such as the bacteriocin nisin, (Liu et al. 2005), yeasts for yeast extract production (de Palm Revillion et al. 2003) and baker’s yeast (Champagne and Goulet 1988), surface active compounds (surfactants) like sophorolipids (Daniel et al. 1999), single-cell protein (Schultz et al. 2006), “green bioplastics” like Polyhydroxyalkanoates, PHAs, (Ahn
et al. 2000, 2001; Kim 2000; Povolo and Casella 2003; Koller et al. 2007), and lactic acid as both a “green bioplastic” in the form of polylactic acid (PLA) and as an important as food additive (E270) and in pharmaceutical use (Wee et al. 2006) are all due to the biocconversion of lactose/whey.

3.6.1 Single cell protein/yeast

Single-cell protein (SCP) refers to sources of mixed protein that are extracted from either pure or mixed cultures of algae, yeasts, fungi or bacteria. These are grown on agricultural wastes. They are used as a substitute for protein-rich foods, in human and animal feeds (Anupama 2000). The production of SCP can be carried out by the fermentation of whole whey or whey permeate via direct use of lactose by microorganisms or of hydrolysed lactose (hydrolysed by enzymatic or chemical means) (Boze et al. 1995). Lactose can be converted directly into biomass by numerous microorganisms. Much of these studies have been done with lactose utilizing yeasts, mostly K. fragilis or Kluyveromyces marxianus strains, which offer advantages of good growth yields and are GRAS (Generally Regarded As Safe) microorganisms (Mahmoud and Kosikowski 1982; Willetts and Ugalde 1987; Ghaly et al. 2005; Anvari and Khayati 2011).

3.6.2 Ethanol

Whey permeate is an attractive as a feed source for the creation of ethanol as it is a polluting by-product that can be used instead of food resources (such as corn) and does not require the extensive preprocessing (high temperature acid treatments to break apart the different tyypes of cellulose) that is required for the production of ethanol from cellulose.

Over the last three decades, many authors have researched the production of ethanol from lactose carried out using fermentionations with yeasts such as K. fragilis, K. marxianus and Candida kefyr [formally Candida pseudotropicalis] (Rogosa et al. 1947). Gabardo et al. (2014) reported that cells of K. marxianus immobilised in Ca-alginate improved ethanol yield in continuous culture fermentionations. The maximum value achieved was a productivity of 6.97 g/L/h; one of the highest values reported to date.

The use of S. cerevisiae for lactose fermentation has also attracted much research. Intially use of S. cerevisiae involved the fermentation of pre-hydrolysed (enzymatically) lactose solutions (mixtures of glucose and galactose). However since of the advent of advance genetic manipulation techniques attempts have been made to create lactose-consuming S. cerevisiae strains. These include protoplast fusion, expression of heterologous ß-galactosidases that are then secreted to the extracellular medium or the simultaneous expression of the permease and ß-galactosidase of K. marxianus. This system operating under continuous bioreactor resulted in ethanol productivity of ~ 10 g/L/h (Domingues et al. 2001). However to date none of these S. cerevisiae systems have been used on an industrial basis. An excellent review covering this subject can be found in Guimara ˜es et al. (2010).

A few commercial scale processes to manufacture ethanol using whey as a fermentation feed stock have been established, with plants in Ireland, the United States, New Zealand, Denmark and Germany (Lyons and Cunningham 1980; Pesta et al. 2007; Siso 1996; Muller 2015).

Carbery Group (Cork, Ireland) was the first company in the world to operate a whey to ethanol process on an industrial scale. This plant first opened in 1978. Until 2005 the plant produced potable ethanol (the main uses of this was for beverages, pharmaceuticals and industrial products (printing inks, etc.) but since 2005 it has supplied fuel ethanol to the Maxol oil company for E85 [85 % ethanol] (since withdrawn from the market [www.rte.ie/news/business/2010/1210/295386-maxol/]) and E5 [5 % ethanol] blends (Doyle 2005; Ling 2008). As of 2008, the plant operates with eleven fermentation vessels that use compressed air for mixing and aeration. The whey permeate is fermentionated in batches in these fermentation vessels for 12–20 h, depending on the initial concentration and yeast (thought to be K. marxianus) activity. The whey permeate is concentrated by reverse osmosis (from 4 to 8 %) to attain higher lactose content. This is done in order to ensure a more efficient fermentionation. The yeast is recovered at the end of the fermentation process. The yeast can be potentially reused a number of times before it is discarded. Ethanol levels at the end of fermentation range from 2.5 to 4.2 % (v/v). After the fermentation is completed a continuous distillation process is used to extract the ethanol and create a product that is usable (Pesta et al. 2007; Ling 2008). Once completed, the yeast is separated from the fermented substrate, and the remaining
liquid (which is called beer) is sent to a distillation process to extract ethanol. This ethanol is dehydrated by use of a rectifier. Petrol is added to the ethanol if it is going to be used for fuel (to prevent any misuse). The effluent (the remaining liquid after ethanol has been removed from the beer) is sent to a waste treatment system. The yeast can be reused in the process, directly sold as animal feed, or undergo further processing to create a higher quality animal feed (Ling 2008). Carbery produces about 10.5 million litres of ethanol per year (Irish Bioenergy Association 2012).

In New Zealand, Anchor Ethanol, a subsidiary of the dairy processor the Fonterra Cooperative Group (New Zealand’s largest company), operates 3 plants that convert whey to ethanol (at Reporoa, Edgecumbe and Tirau). These produce around 15 million litres of ethanol per year (Anchor Ethanol 2009). These plants use two different processes with the Reporoa plant using the Carbery process and the Tirau plant uses a continuous fermentation process (Wongso 1993). The type of plant operated at Edgecumbe is not publically disclosed. These plants produce different ethanol grades, from potable ethanol for beverages to anhydrous alcohol for fuels (Thiele 2005). The main markets for the whey derived ethanol has been pharmaceutical, cosmetics, industrial solvents (including inks) as well as the food and beverage industry (Hamilton 1998; Thiele 2005). Since 2007, fuel ethanol has also been supplied to a petrol company in New Zealand for an E10 [10 % ethanol] blend (Ling 2008).

The process of ethanol production varies between plants, but they all share common basic principles and steps. After whey protein has been harvested from whey by ultrafiltration, yeast can be added the whey permeate. Lactose in whey permeate is fermented by specially adapted strains of yeast (thought to be K. marxianus in the Carbery process, Streptococcus fragilis in the Dansk Gaerings process, K. fragilis in the Milbrew process) that are efficient in fermenting lactose. The yeast is added to the fermenting substrate and pumped to the fermentation vessels.

The fermentation of whey permeate to create ethanol is a highly attractive prospect but as things stand it is not economically competitive when compared to ethanol production from other sources such as sugarcane, corn or lignocellulose biomass. Whey permeate to ethanol production is estimated to cost between $1.60 and 1.85 per gallon compared with $1.14 per gallon for ethanol from corn and $0.83 per gallon for ethanol from sugar cane [all currency U.S dollar] (Budny and Sotero 2007; Ling 2008).

Biobutanol production from whey has also been investigated. Biobutanol has advantages over ethanol such as the fact that it can be used directly in petrol engines whereas the use of ethanol requires engine modifications. Limited research using Clostridia species to transform whey into butanol has been carried out (Raganati et al. 2013; Qureshi et al. 2014).

### 3.6.3 Bio-plastics

Using whey as a raw material to create bioplastics has also become an area of investigation. Compounds such as polyhydroxyalkanoates (PHA’s) and Polylactic Acid (PLA) can be made into bioplastics through the bioconversion of the lactose present in whey permeate. These materials have many advantages when compared to traditional plastics in that they come from renewable biomass sources instead of finite petroleum oil and most, but not all, are designed to biodegrade. Common uses of bioplastics are packaging materials for food and other materials, disposable cutlery and as insulation (Chen and Patel 2012).

PHAs are polyesters (polymers that contain ester functional groups in their main chain) of various hydroxyalkanoates which are created by numerous microorganisms (Solaiman et al. 2006) where they act as a carbon and energy reserve material. PHAs are synthesised when an essential nutrient such as nitrogen or phosphorus is limited but when there is an excess carbon source (Lee 1996). Further reading about PHAs can be found in Sudesh et al. (2000). PHA’s have many potential applications in medicine such as the material for sutures, rivets, tacks, staples, screws and surgical mesh (Chen and Wu 2005).

The microbial conversion of whey lactose to PHAs can follow three possible strategies. The easiest way is through the direct conversion of lactose to PHA however only a limited number of microorganisms, such as Hydrogenophaga pseudoflava and recombinant Escherichia coli can carry out this task (both have β-galactosidase activity). Another possible way is the fermentation of the monomers glucose and galactose after the enzymatic or chemical hydrolysis of lactose. The resulting monomers, glucose and galactose, will be used by microorganisms (such as Pseudomonas hydrogenovora or Haloferax mediterranei) to produce PHAs (for strains that do not have β-galactosidase
activity). The third way involves the conversion of lactose to lactic acid (via Lactobacilli) and the latter used for PHAs production (by all common PHA producers such as Alcaligenes latus) (Koller et al. 2007).

Lactic Acid is created as the major metabolic end-product of carbohydrate fermentation of the Lactic Acid Bacteria (LAB) (Todar 2014). This lactic acid can be converted through condensation reactions to PLA (Södergård and Stolt 2010). Further reading about PLAs can be found in Chen and Patel (2012). The conversion of whey lactose to lactic acid is carried out by lactic acid bacteria.

In May 2014 Cellulac became the first company worldwide to carry out a continuous industrial level production of lactic acid from deproteinised lactose whey. A 10 days production run delivered pure D-lactic acid that was suitable for conversion to bioplastics and other industrial chemicals. The system uses a whole cell non-GMO (Genetically Modified Organism) lactobacilli to transform the lactose present in the deproteinised lactose whey in D-lactic acid that was used to create polylactic acid (Cellulac 2014).

PLA (as with PHA) can be used in medical materials such as in the form of screws, plates, pins, rods, and meshes for surgery. It has also been used for producing loose-fill packaging, compost bags, compostable food packaging, and compostable, disposable tableware (cups, plates, etc.). In fibers and non-woven textiles, PLA can be used for disposable garments, awnings, feminine hygiene products, and disposable diapers (Auras et al. 2011).

Other potential types of bioplastics from whey are binary bioplastics created using whey protein. Whey protein has been explored for packaging applications due to its strong oxygen barrier properties (Markus Schmid et al. 2012). However, whey protein based bioplastics have inherent stiffness/brittleness that makes them difficult to use. To overcome this problem binary based bioplastics have been investigated. These are created by blending the whey protein with other plentiful biopolymers such as natural latex and egg white albumin (Sharma and Luzinov 2013). This type of bioplastic can be used as recyclable food packaging (Wheylayer Project 2014). They can also be used to form edible films or coatings to improve food appearance or for preservation. They have better mechanical and barrier properties when compared with most other protein/carbohydrate based products especially when a plasticizer such as glycerol or sorbitol are added to reduce brittleness and improve the moisture resistance of the film/coating (Walsh 2014).

3.6.4 Bacteriocins

Bacteriocins are antimicrobial peptides that are created by both Gram positive and Gram negative bacteria that inhibit bacterial growth. They are considered to be narrow spectrum antibiotics with the spectrum of activity of the bacteriocin depending on the producing species (Chen and Hoover 2003; Cotter et al. 2013). The positive effect of bacteriocins in different types of food including dairy, meat and fish products, fruit and vegetables has been well defined (O’Sullivan et al. 2002). Further applications are possible in the medical, pharmaceutical and veterinary fields (Jones et al. 2005). LAB’s (Lactic Acid Bacteria) are prolific in bacteriocins and are able to synthesize different classes of bacteriocins (Beshkova and Frengova 2012). The LAB Lactococcus lactis produces nisin which is the only bacteriocin that is industrially synthesised and has been authorised for used in food by the US Food and Drug Administration FDA (Jones et al. 2005).

Nisin can be produced using (supplemented) whey as a growth media. Other bacteriocins such as pediocin (form Pediococcus acidilactici), enterocin (from Enterococcus faecalis) and a bacteriocin created by Bacillus licheniformis have also been created using a whey media (Flores and Alegre 2001; Pérez et al. 2010; Goulhen et al. 1999; Ananou et al. 2008; Cladera-Olivera et al. 2004). With the increasing prevalence of antibiotic resistance, bacteriocins could be a viable alternative in combatting microbial infections and whey media could be a cheap and effective means to create these (Cotter et al. 2013).

3.6.5 Enzymes

Several different bacteria and yeast species have been grown on whey/whey permeate for the purpose of harvesting enzymes for possible use in industrial processes or in detergents. Enzymes include proteases, amylases, polygalacturonases (plant enzymes that are involved in the ripening process and bacterial and fungal enzymes that are involved in the rotting process) (Table 2); however most research has been directed at β-Galactosidase production. In order to
| Products from whey | Use | Production | Commercially available products | Organism/process used for bioconversion | References |
|-------------------|-----|------------|---------------------------------|----------------------------------------|------------|
| Bioethanol        | Fuel, alcoholic beverages, chemical industry | Fermentation of whey permeate | Yes | Kluyveromyces lactis, Kluyveromyces marxianus, recombinant Saccharomyces cerevisiae (direct conversion of lactose to ethanol) Lactococcus lactis (direct conversion of lactose to ethanol) S. cerevisiae (conversion of hydrolysed lactose to ethanol) | Guimarães et al. (2010) Zafar and Owais (2006) Silva et al. (2010) |
| Biobutanol        | Fuel, chemical industry | Fermentation of whey | | Clostridium acetobutylicum DSM 792, Clostridium saccharobutylicum (conversion of whey to butanol) | Raganati et al. (2013) Qureshi et al. (2014) |
| Antibiotics (bacteriocins) | Food industry, health industry | Fermentation of whey permeate | Lactococcus lactis [conversion of lactose (supplemented with Yeast extract and casein hydrolysate) to bacteriocin-nisin] | Pediococcus acidilactici (conversion of lactose [supplemented with Yeast extract] to bacteriocin-pediocin) Enterococcus faecalis (conversion of lactose to bacteriocin-enterocin) Bacillus licheniformis (conversion of whey to bacteriocin) K. marxianus, K. fragilis (conversion of lactose/ hydrolysed lactose to biomass) Kefir (conversion of lactose to biomass) Pseudomonas hydrogenovora, Halofex mediterranei (conversion of hydrolysed lactose to PHA) | Pérez Guerra et al. (2005) Goulhen et al. 1999 Ananou et al. (2008) Cladera-Olivera et al. (2004) Schultz et al. (2006) Mansour et al. (1993) Panaskevopoulos et al. (2003) Koller et al. (2007) Ahn et al. (2000, 2001) Povolo et al. (2010) |
| Single cell protein | Animal feed, food industry | Fermentation of whey permeate | Yes | Hydrogenophaga pseudoflava, recombinant Escherichia coli and Cupriavidus nector (direct conversion of lactose to PHA) Lactobacilli (conversion of lactose to Lactic acid) followed by C. nector (conversion of Lactic acid to PHA) | Koller et al. (2007) Ahn et al. (2000, 2001) Povolo et al. (2010) |
| PHA              | Bioplastics | Fermentation (can be direct or 2 step) of whey permeate | | | |
| Products from whey | Use | Production | Commercially available products | Organism/process used for bioconversion | References |
|-------------------|-----|------------|---------------------------------|----------------------------------------|------------|
| Lactic acid/PLA   | Bioplastics, food industry | Fermentation (and chemical polymerisation of PLA) of whey permeate | Pilot plant is operational | *Lactobacilli* (conversion of lactose to Lactic acid) | Panesar et al. (2007, 2010) |
|                   |                               |                               |                                 | Lactic acid then converted to polylactic acid by chemical polymerisation | Cellulac (2014) |
| Glycerol          | Fuel, chemical industry       | Fermentation of whey permeate |                                   | *K. fragilis* [direct conversion of lactose (supplemented with sodium sulfite) to glycerol] | Jenq et al. (1989) |
|                   |                               |                               |                                 | *K. marxianus* (direct conversion of lactose to glycerol) | Rapin et al. (1994) |
|                   |                               |                               |                                 | *Aspergillus melleus, Aspergillus flavus, Aspergillus sclerotiorum* (direct conversion of lactose to glycerol) | Zohni (2000) |
| Sophorolipids     | Pharmaceutical, cosmetic, food, petrochemical industries | 2 step fermentation           |                                 | *Cryptococcus curvatus* (conversion of lactose to single cell oil) *Candida bombicola* (conversion of single cell oil to sophorolipids) | Daniel et al. (1999) |
|                   |                               |                               |                                 |                                                      | Otto et al. (1999) |
| Single Cell Oil   | Food and pharmaceutical industries | Fermentation of whey           |                                 | *Mortierella isabellina, Thamnidium elegans, Mucor sp* (conversion of lactose to single cell oil) *Fusarium spp.* (conversion of lactose to single cell oil) | Vamvakaki et al. (2010) |
| Citric Acid       | Chemical industry             | Fermentation of whey           |                                 | *Candida lipolytica* (conversion of whey [supplemented with date seed hydrolysate] to citric acid) *Aspergillus niger* (conversion of whey [supplemented with sucrose] to citric acid) | Abbou-Zeid et al. (1983) |
|                   |                               |                               |                                 |                                                      | Hosan (1983) |
|                   |                               |                               |                                 |                                                      | El-Holi and Al-Delaimy (2004) |
| Propionic Acid    | Chemical, food, pharmaceutical industry | Fermentation of whey           |                                 | *Propionibacterium sp.* conversion of lactose to Propionic Acid | Colomban et al. (1993) |
|                   |                               |                               |                                 | *Propionibacterium freudenreichii* ssp. Shemani (conversion of whey [supplemented with crude glycerol] to propionic acid) | Kosmider et al. (2010) |
|                   |                               |                               |                                 | *Propionibacterium acidipropionici* (conversion of lactose to propionic acid) | Morales et al. (2006) |
| Gibberellic acid  | Agricultural uses             | Fermentation of whey permeate  |                                 | *Fusarium moniliforme* (conversion of lactose to Gibberellie acid) *Aspergillus niger* (conversion of lactose to Gibberellie acid) | Maddox and Richert (1977) |
|                   |                               |                               |                                 |                                                      | Gohlwar et al. (1984) |
|                   |                               |                               |                                 |                                                      | Changir and Aksöza (1997) |
| Products from whey | Use | Production | Commercially available products | Organism/process used for bioconversion | References |
|-------------------|-----|------------|---------------------------------|----------------------------------------|------------|
| Gluconic acid     | Food, pharmaceutical industry | Fermentation of whey/whey permeate | Gluconobacter oxydans (conversion of Kluyveromyces bulgaricus hydrolysed lactose to ethanol) Aspergillus niger (conversion of lactose to gluconic acid) | Van Huynh et al. 1989 Chaturvedi et al. (1999) Mukhopadhyay et al. (2005) |
| 2,3-butanediol    | Manufacture of synthetic rubber and solvents | Fermentation of whey/whey permeate | Bacillus polymyxa (conversion of whey to 2,3-butanediol) Klebsiella pneumoniae (conversion of lactose to 2,3-butanediol) Enterobacter aerogenes (conversion of whey to 2,3-butanediol) | Speckman and Collins (1982) Lee and Maddox (1984, 1986) Perego et al. (2000) |
| Succinic acid     | Chemical industry | Fermentation of whey | Anaerobiospirillum succiniciproducens (conversion of whey to Succinic acid) Actinobacillus succinogenes (conversion of whey to Succinic acid) | Lee et al. (2000) Wan et al. (2008) |
| Lactobionic acid  | Cosmetics, pharmaceutical industry, medical industry | Fermentation of whey | Pseudomonas taetrolens (conversion of whey to Lactobionic acid) | Alonso et al. (2013) |
| Vinegar (acetic acid) | Food industry | 2 step fermentation of whey permeate | K. marxianus (conversion of lactose to ethanol) Acetobacter pasteurianus/Gluconoacetobacter liquefaciens (conversion of ethanol to acetic acid) | Parrondo et al. (2009) de Bales and Castillo (1979) Vasiljevic and Jelen (2001) Kaur et al. (2015) Bansal et al. (2008) Bajpai et al. (1991, 1992) El-Shora, and Metwally (2008) |
| Enzymes | Washing detergents, industrial processes | Fermentation of whey | K. marxianus, Candida pseudotropicalis, Lactobacillus delbrueckii subsp. Bulgaricus, Streptococcus thermophiles, Aureobasidium pullulans (β-galactosidase) Bacillus sp. (α-amylase) Aspergillus niger, Aspergillus terreus, Serratia marcescens (proteases) K. fragilis (endo-polygalacturonase) | Rao and Dutta (1977) de Bales and Castillo (1979) Vasiljevic and Jelen (2001) Kaur et al. (2015) Bansal et al. (2008) Bajpai et al. (1991, 1992) El-Shora, and Metwally (2008) Romero et al. (2001) Gomez-Ruiz et al. (1988) Donaghy and McKay (1994) Kaur et al. (2015) |
achieve useable enzyme levels in the case of proteases or amylases additional nutrient supplementation is usually required. This supplement could come in the form of yeast extract, starch, amino acids or a protein such as gelatin, casein or albumin (El-Shora and Metwally 2008). This supplementation differs with different species and even different strains of the same species.

3.6.6 Organic chemicals

Whey and whey permeate can be used to create several organic chemicals that can have applications in the food, pharmaceutical and chemical industries through fermentations. These fermentations are carried out by microorganisms (see Table 2) with complex biosynthesis pathways that can bio-transform the whey into a whole range of useful organic chemicals. Examples include citric acid, acetic acid (vinegar), propionic acid, glycerol, etc. (Table 2).

3.6.7 Bio-hydrogen

Hydrogen is a clean energy source which does not create greenhouse gases or contribute to acid rain. The use of various carbohydrate-rich wastewaters such as agricultural waste or waste cheese whey can be a feasible option for the production of bio-hydrogen (Kapdan and Kargi 2006; Yang et al. 2007). Whey could be particularly useful as the fermentation of lactose could lead to a theoretical yield of 8 mol of hydrogen for every mol of lactose (Lee et al. 2014), however to date the highest yield that has been obtained is 3 mol of hydrogen for every mol of lactose (Collet et al. 2004). The maximum amounts of hydrogen created from whole whey were 2.9 L of hydrogen per litre of cheese whey (Venetsaneas et al. 2009). The biogas mixture formed in hydrogen production also contains methane (CH₄) and carbon dioxide (CO₂). The anaerobic fermentation necessary for hydrogen creation can be carried out by both obligate anaerobes such as Clostridia butyricum, Clostridium pasteurianum and Clostridium beijerinckii (Ferchichi et al. 2005) or facultative anaerobes such as Enterobacter, Citrobacter sp. or E. coli (Rosales-Colunga et al. 2010). The reactions can be carried out at both mesophilic (Yang et al. 2007) and thermophilic temperatures (Azbar et al. 2009).

4 Conclusions

With milk production rising globally each year by 2 % (Smithers 2008) and cheese production growing by 3 % annually (Anon 2014) evidence suggests that the volume of whey produced will continue to increase in the coming years. Developing sustainable methods of dealing with the whey produced is of great necessity. Outside of the enormous growth in the use of whey proteins however much work is needed to truly exploit the potential of whey. New, more efficient and economic processes and systems must be developed especially in the emerging fields of bioplastic and bioethanol production where whey can be utilized as a building block in the replacement of fossil fuels.

Acknowledgments This work was funded by the EPA under the Science, Technology, Research and Innovation for the Environment (STRIVE) Programme 2007–2013. 2012-WRM-MS-9.

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