Title: The biotechnological potential of Whey

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Abstract
Whey is a highly polluting by-product of cheese and casein powder manufacturing with worldwide production of whey is estimated at around 190×10^6 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.

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 (Panaser *et al.*, 2007, Figure 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, Figure 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

KCI, 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 40,000-60,000 mg/L and a very high Chemical Oxygen Demand (COD) of between 50,000-80,000 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 (Kosikowki, 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 70s 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 (Kosikowki, 1979). Crop kills have been reported due 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 (103 /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 (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 & 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 (Kosikowki, 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 (Jelen and Buchheim, 1976). 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 (Figure 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 Figure 2.

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 17th 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 & 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) anticancer effects (Bounous et al., 1991, Bounous, 2000), (E) help with the management of infections (Bounous et al., 1993; Playford et al., 1999; Regester & 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 (Patterson, 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 (Patterson, 2009). The amounts of lactose produced from whey have increased greatly since the 1940's (Sienkiewicz & 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 sweeter. 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 ~ 40% of the sweetness of sucrose. 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 e*t 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 & Galzy, 1984; Kosaric & 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., 2012). 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-Malinovsk et al., 2012, Golowczyc 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 & El-Taweel, 1997; Zafar & Owais, 2006), vinegar (Parrondo *et al*., 2009), antibiotics, such as the bacteriocin nisin, (Liu *et al*., 2005), yeasts for yeast extract production (de Palma 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; Ahn *et al*., 2001; Kim, 2000; Povolo & Casella, 2003; Koller *et al.,* 2007 a,b), 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 bioconversion 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 (Anupamaa and Ravindra 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 permate 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 tyupes 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 fermentations 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 fermentations. 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 ~10g/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 Guimarães 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 fermented in batches in these fermentation vessels for 12 to 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 fermentation. 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 2.5–4.2% (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 and 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 (Wongso1993). 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 and 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 $1.60-1.85 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, 2011).

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, 2011. 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 day 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 that has been authorised for used in food by the US Food and Drug Administration FDA (Jones *et al*., 2005).

Nicin 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.,* 2008). With the increasing prevalence of antibiotic resistance, bacteriocins could be a viable alternative in combatting microbial infections and whey meida 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 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 (Kuo 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 (CH_4) and carbon dioxide (CO_2) The anaerobic fermentation necessary for hydrogen creation can be carried out by both obligatory anaerobes such as *Clostridia butyricum*, *Clostridium pasteurianum* and *Clostridium beijerinkii* (Ferchichi et al., 2005) or facultative anaerobes such as *Enterobacter*, Citro*bacter* 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.

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Table Captions

Table 1: Major protein constituents of whey and their (or their peptide derivatives) functions

Table 2: Industrially useful products that can be created use whey

Figure Legends

Figure 1: Overview of the creation of whey

Figure 2: Overview of the fractionation of whey and possible uses of the products formed