

# **Biotechnological Alternatives for the Utilization of Dairy Industry Waste Products**

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### Abstract

The industrial obtainment and manufacturing of food cause high levels of pollution because of the generation of waste byproducts. Over the past few years, there has been a significantly increased interest in preserving, restoring and establishing an ecological balance during food production. Many investigators propose biotechnological solutions to the treatment of industrial wastes, especially waste from the dairy industry. The aim of this review is to present biotechnological approaches to the treatment and utilization of wastes from the dairy industry, specifically for milk whey and also discussed biotechnological methods to reduce environmental pollution and obtain chemical compounds with potential applications in the industry.

# Keywords

Bioactive Peptides, Milk Whey, Organic Acids, Biofuel, Biosurfactants

# **1. Introduction**

Traditionally, milk has been considered the most complete food provided by nature. It is an important source of essential nutrients needed for the growth and development of newborns. It is also highly recommended in the diet of adult humans due to its high nutritional value [1]. Milk is defined as a biological secretion from the mammary glands of female mammals. It is mainly composed of water, proteins, lipids, carbohydrates, vitamins and minerals (Table 1) [1] [2].

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Principal components of cow milk			
Water	78.8 (g)		
Lactose	4.7 (g)		
Proteins	3.3 (g)		
Lipids	3.3 (g)		
Minerals	0.7 (g)		
Vitamins			
Retinol	35.0 (µg)		
Carotene	16.0 (µg)		
Vitamin A	37.0 (µg RE)		
Vitamin E	0.08 (mg)		
Thiamin	0.04 (mg)		
Riboflavin	0.20 (mg)		
Niacin	0.13 (mg)		
Pantothenic acid	0.43 (mg)		
Vitamin B6	0.04 (mg)		
Folate	8.50 (µg)		
Biotin	2.0 (µg)		
Vitamin B12	0.51 (µg)		
Vitamin C	1.0 (mg)		
Vitamin D	0.20 (µg)		

Lactose is the principal carbohydrate found in milk. It has been reported that lactose can stimulate the absorption of minerals including calcium and magnesium [3]. *Maclura* are the principal lipid constituents, representing 98% of the total fat in milk. Diglycerides, monoglycerides, esters and cholesterol are found at low levels [4]. The function of the lipid materials in milk is to transport the fat-soluble vitamins A, D and E [4] [5]. Moreover, it has been demonstrated that milk not only contains nutritive compounds but also biologically active ones, like caseins and whey proteins. Caseins constitute 80% of the total protein present in milk; the other 20% is comprised of the whey proteins including  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin and immunoglobulins [2]. However, several aspects such as diet, breed, geographical conditions, illness and other factors can modify the proportions of the nutrients in milk. Mineral content is the component most affected by these factors [6]. Milk produced by many different mammalian species is consumed by humans, but cow's milk is the most commonly consumed. Milk from cows is produced in large quantities in countries including United States, India and China (Table 2) [7].

Because of its high nutritional content, milk can be used to produce a large variety of dairy products such as whole, skim, lactose-free and fat free milk; or even whole or skimmed milk powder. Additional products include condensed and evaporated milk, which can be pasteurized or ultra pasteurized. Milk is also used to produce the fermented dairy products cheese, yogurt, kefir and bulgaro. Butter, milk whey, sweet/sour cream, case inacid type, lacticorrenin, caseinates, lactose, enzymatically modified milk ingredients, cream, ice cream and other dairy beverages, are the main products we obtain from milk [9] [10]. The per capita ingestion of milk and its various derivatives has decreased worldwide, excepting the consumption of cheese. Currently, Ireland is the largest consumer of liquid milk, and France is the largest consumer of dairy products like butter and cheese (Table 3 and Table 4) [11].

The manufacturing of dairy products represents a great concern because of pollution and damage to the environment. This problem is due to the generation of waste from livestock producers and the by-products from 
 Table 2. Milk producer countries around the world in 2011 [8].

Major milk producers around the world			
Country	Production (Tons)		
United States	89,015,235		
India	57,400,000		
China	36,928,896		
Brazil	32,096,214		
Russia	31,385,732		
Germany	30,301,359		
France	24,361,095		
New Zealand	17,893,848		

 Table 3. Milk consumption in several countries around the world in 2012 [8].

Country	Liters per capita		
Ireland	135.6		
Finland	128.3		
United Kingdom	102.9		
Iceland	96		
Canada	77		
United States	74		
Uruguay	67.1		
Brazil	57.2		
Israel	53.7		
India	40		
México	34.8		
South Africa	23.1		

## Table 4. Dairy consumption in various countries around the world in 2012 [11] [12].

Country	Kg per capita	
	Cheese	Butter
France	26.3	7.4
Iceland	25.2	4.9
Finland	23.7	4.5
Israel	17.1	0.9
United States	15.2	2.5
Canada	12.1	2.8
United Kingdom	11.2	3.4
Ireland	6.7	2.4
Uruguay	6	1.6

dairy foods [13]-[15]. This kind of pollution affects the quality of water, air and soil. It even causes climatic variations [16].

Milk whey is a liquid by-product produced during the manufacturing process of cheese. It represents 85% - 90% of the original milk volume and retains 55% of the nutrients. Milk whey has proteins, lipids, soluble vitamins, minerals and carbohydrates. Of the total whey solids, 75% is lactose. Lactose is one of the most polluting by-products because of its high biochemical oxygen demand (BOD) < 35,000 ppm and its chemical oxygen demand (COD) < 60,000 ppm [17]. If this industrial effluent is discarded in the soil without prior treatment, it can modify the soil's physical and chemical composition, reducing crop yields and the availability of oxygen in water [15] [18] [19]. It has been estimated that 40,000 liters of untreated milk whey produces contamination equivalent to that produced daily by a population of 250,000 people [20]. Another by-product from the dairy industry is clarified butter sediment waste derived from the manufacturing of clarified or ghee butter. This butter waste is mainly composed of fats in the form of fatty acids and small amount of proteins [21]. It has been found that discarding milk whey in the water of rivers and lakes causes enormous pollution problems because of the large amounts of nitrogen, and phosphorus and high BOD (40 - 48,000 mg/L) and COD (89 - 95,000 mg/L) contained in this by-product [22]. The conditions mentioned above promote the process of eutrophication, causing an excessive growth of microorganisms and aquatic plants [23] [24].

The aim of this review is to present milk as a nutritious food for human consumption and describe its use as a raw material for manufacturing a variety of dairy products. However, in this present analysis it has been emphasized that the manufacturing processes of many dairy products produce large amounts of by-products that are discarded in the ecosystem, causing considerable environmental problems. Thus, scientists around the globe are looking for new biotechnological alternatives to utilize those by-products to obtain chemical compounds with potential applications in different industrial areas and to reduce the environmental damage.

## 2. Biotechnological Alternatives for the Use of By-Product Wastes from the Dairy Industry

In the last few years a social, political, scientific and technological interest in environmental protection has emerged. An international effort is underway to discover solutions to environmental problems and to create a balance between human development and care of our ecosystem. Alternatives developed by researchers for the utilization of waste of the dairy industry are discussed in the following section.

Biomass: In the process of olive oil extraction 20% of the oil is extracted and the other 80% is a waste called oil press, where 50% of oil press is composed of water. This water contains a mixture of extracts from the plant material, soft tissue of olives and water used in any stage of the extraction process [25]. Recently it was discovered that *Geotrichum candidum*, a fungus that produces lignolytic enzymes, might clear this residual wastewater from the industrial processes. The idea of using a mixture of oil press water and milk whey as a substrate to produce biomass came out because of the favorable features of this fungus [26]. The experiment was run for 5 days at 30°C under aerobic conditions using a mixture 20:80 of oil press water and milk whey respectively. The results reported high growth of the fungus, a clearance of 54.4% of the media mixture and a 55.3% reduction in phenolization. It was concluded that oil press waste and milk whey are the cheapest and most effective substrates to obtain biomass from *G. candidum*. An added benefit is that the process also reduces pollution levels. Moreover, the microbial biomass contains a large amount of protein that could be a potential option for human consumption. Yeast like *Kluyveromyces marxianus* var. *marxianus* can be grown under anaerobic conditions in batch cultures with milk whey. This fermentation process is considered a good source of unicellular protein because it shows a balanced amino acid profile according to the international standards from FAO (lysine 5.5 g/100g and methionine andcysteineare 1.73 g/100g) [27].

Bioplastics: Several studies have focused on the production of bioplastics like polyhydroxyalkanoates (PHA), which have shown to be good substitutes for plastics derived from highly polluting oil. PHAs are biodegradable polymers synthesized by bacteria in the presence of an excess of carbon. *Bacillus megaterium* SRKP-3 produces polyhydroxybutyrates (PHB), the most common type of PHA. To produce PHB, industrial dairy waste can be used as substrate. An optimal production yield of PHB (6.37 g/L) is achieved in 30 h at 30°C. The maximum production yield (11.32 g/L) was obtained in 36 h in an optimized medium [28]. Furthermore, the use of PHAs is not only helpful for the environment, but has also come to have great impact in the medical field. *Brevibacterium casei* SRKP2, which can utilize industrial waste milk as a carbon source, also produce PHB [29]. This

bacterium was shown to produce 2.940 g/L of PHB in 48 h at 37°C in a submerged culture. The products were used to produce nanoparticles of PHB to facilitate the delivery of drugs to any part of the body.

Biofuels and bioenergy: Another important contributing factor to environmental pollution is the burning of fossil fuels, which causes substantial harm to the atmosphere and contributes to climate change. A viable solution to this problem is the production of biofuels. The combustion of biofuels releases less polluting agents into the environment than burning fossil fuels [30]. Unfortunately, the production of biofuels has been more expensive than the production of conventional fuels due to the high cost of the raw materials [31]. Dairy wastes have been proposed as a solution because they can be used as substrate for fermentation by yeast like Kluyveromyces fragilis. These types of yeast are commonly used to hydrolyze lactose present in milk whey. The acquired ethanol from the hydrolyzation could be used to produce alcoholic beverages or for other industrial applications [32]. A study has also been conducted to examine how the concentration of milk whey affects the production of ethyl alcohol by Kluyveromyces fragilis. A maximum yield of 35.2 g/L of ethanol was achieved using an inoculum of 10% in a batch culture (200 rpm/30°C/24 hours) and subsequently subjected to a process of anaerobic fermentation for 34 h, utilizing 20% whey milk. However, this study also recommended the whey concentrations less than 15% (96 g/L lactose) for an efficient alcoholic fermentation with Kluyveromyces fragilis because higher concentrations cause inhibition of the process [33]. Furthermore, whey powder has also been considered as a substrate to produce ethanol using yeast species Kluyveromyces marxianus DSMZ-7239 [34]. This yeast metabolize lactose with a yield of 0.54 g ethanol/g lactose. On the other hand, thermotolerant yeasts like Candida inconspicua W16 are also able to produce ethanol from whey with higher yields. The yeast was immobilized in alginate calcium gel  $(3.03 \pm 0.02 \text{ v/v})$  for 72 h. This technique eliminates the problems of cost associated with the production of ethanol from whey [35]. In the case of the production of other biofuels such as butanol, it has been shown that the bacterium Clostridium acetobutylicum DSM 792 is capable of producing 1.5 g/L of biobutanol through the fermentation of milk whey with a lactose concentration of 4.5% to 5%; in a period of 5 days at 37°C and 150 rpm in a batch culture. The fermentation process also yields minor amounts of other biofuels such as butyric acid and ethanol (less than 10 g/L), and produces 40 g/L acetic acid [36]. The development of new alternative and renewable sources as substitutes for the use of electric energy has also been considered. For example, microbial fuel cell technology has been developed to obtain bioelectricity from milk wastes [37]. Many microorganisms that can be used as electron donors for biofuel batteries have been identified. The microorganisms are able to convert organic material into energy via redox reactions. The operation of a biofuel cell is similar to that of a conventional fuel cell with the major difference that the first one uses enzymes, bacteria and other microorganisms as catalysts under mild conditions of temperature and pressure. According to another study, bioelectricity can be obtained using milk whey and Saccharomyces cerevisiae PTCC 5269 cells. This yeast is able to use existing sugars in the substrate, generating a stable voltage for two days and an energy and a maximum current of 470  $\mu$ A and 50  $\mu$ W [38]. Prokaryotic organisms like *E. coli* are also able to use milk whey as substrate. The milk whey must first be acidified to remove excess proteins; this has to be done to facilitate the assimilation of carbohydrates. Then, bioelectricity can be generated at room temperature with a voltage of the open circuit in the absence of the mediator of 751.5 mV at stable conditions for more than 24 h. The maximum power of energy and current is 324.8 iW for riboflavin and 1194.6 iA for humic acid [39].

A promising option to reduce greenhouse gas emissions, reduce the contamination of soil and aquatic ecosystems by fossils fuels, and facilitate the disposal of dairy waste is to use the wastes to produce hydrogen as a biofuel. Hydrolysis of the substrates that are used to generate  $H_2$  is a limiting step in the anaerobic production of  $H_2$ . However, it has been reported that waste water from the dairy industry can be used to overcome this problem. This water must be pretreated with a sodium salt of sulfonic acid and 2-bromoethane at a concentration of 0.2 g/L for 24 h. Once the waste water is pretreated, the metabolic participation of an anaerobic microbial mixture is needed to allow the water to be used as a source of carbon to generate biohydrogen ( $H_2$ ). The anaerobic microbial mixture must be cultivated for 18 h for a production of 0.025 mmol and a substrate removal efficiency (chemical oxygen demand) up to 70% [40]. Photoproduction is another way to generate  $H_2$  using bacteria like *Rhodopseudomonas Cyanobacterium Anacystis* which presented a growth of 50% - 60% and produce approximately 70% - 90% of  $H_2$ . This kind of production is better than that seen with the growth of the microorganisms under typical laboratory conditions and culture media [41]. Another viable solution to produce biofuel and treat wastewater from the dairy industry is the use of *Chlorella pyrenoidosa*, a seaweed that is able to utilize the nutrients from residual water as a culture medium. The seaweed removes between 80% - 85% of the phosphorus content, and 60% - 80% of the nitrogen in the wastewater; generating a biomass of 18.8 g/L and a biofuel

production of 6.7 mL after 15 days of culture [42].

Organic acids: The manufacturing industries of food, beverages and pharmaceuticals demand a variety of chemical compounds, which are used as additives for the production of many products. These additives can act as acidifying agents, stabilizers, flavor enhancers or preservatives. Some examples of additives are the organic acids (citric, lactic, succinic and propionic acid) [43]-[47]. Numerous investigations have focused on finding new additives that satisfy consumer demand and are simultaneously compatible with care for the environment [47]. An efficient way to produce citric acid with Aspergillus niger ATCC9642 from milk whey was reported [48] The milk whey was supplemented with different types and concentrations of sugars, vitamins and minerals. The quantity of citric acid obtained from the culture media was 106.5 g/L after 16 days of culture. Other fungi, including Aspergillus carbonarius NRRL 368, Aspergillus niger NRRL 3, and Aspergillus carbonarius NRRL 67 are capable of producing citric acid from whole sweet milk serum in a culture medium under optimal conditions (30°C/180 rpm/8 days). The last strain, Aspergillus carbonarius NRRL 67, showed the highest production of 6.72 g/L [47]. The production of lactic acid has been accomplished in mixed and individual cultures of yeast (Kluyveromyces marxianus IFO 288), and two lactic acid homofermentative bacteria (Lactobacillus delbrueckii ssp. Bulgaricus and Lactobacillus helveticus ATCC-11842, ATCC-15009) using milk whey as a unique source of substrate. It was reported that the maximum production of lactic acid was 19.8 g/L with a yield of 0.47 g of lactic acid per gram lactose [49]. The use of industrial waste from yogurt production as a substrate was also reported [47]. The yogurt waste was fermented to obtain lactic acid from Lactobacillus casei ATCC393 under controlled pH conditions and yeast extract of preculture stage. The maximum production was obtained 36 h later and it was of 25.9 g/L; this demonstrates the viability and environmental friendliness of this alternative method. Another important product is succinic acid. It is mainly produced by the petrochemical industry and its production causes large amounts of pollution. An alternative approach for the reduction of environmental pollution is a biotechnological method that uses milk whey and the microorganism Actinobacillus succinogenes 130Z, to produce succinic acid. A maximum production of 27.9 g/L of succinic acid was achieved within 48 hours of fermentation using an initial whey concentration of 100 g/L with an inoculum size of 5.0% [46].

The propinobacterias have been used either as silage inoculants or to synthesize multiple functional compounds, including propionic acid. The propionic acid is primarily used as a preservative, to produce vitamin B12 or to generate biomass, as it is considered a probiotic agent [43] [44]. In a culture media enriched with pasteurized sweet milk whey at a concentration of 70 g/L for 72 h in a mesophilic temperature condition, Propionibacterium shermanii is able to grow and produce up to 10 g/L of propionic acid [43]. Propionibacterium acidipropionici can produce 32 g/L of organic solvent and 78 g/L of biomass if it is grown in filtrated sweet milk whey from swiss cheese production process. The whey can be fermented in a semi-continuous culture at a temperature of 32°C, at a neutral pH and at 200 rpm for 8 days [44]. Moreover, lactose can be chemically transformed through enzymatic and microbial processes to obtain lactobionic acid. Lactobionic acid is a high value product with potential applications as a bioactive molecule in the food and pharmaceutical industries [50]. It is also considered a functional ingredient because of its applications as a prebiotic, acidulant, antioxidant, firming agent and as a chelating agent of metal ions such as Fe [51]. The bacteria Pseudomonas sp. LS13-1 is capable to produce 175 g/L of lactobionic acid using milk whey as a substrate in a fed-batch fermentation process of 180 h. Meanwhile [52]. On the other hand, Pseudomonas taetrolens produces 1.12 g/L of lactobionic acid, using milk whey from cheese production as substrate under batch culture conditions with 30% of inoculum [53]. Moreover, the milk whey had a pH of 6.4 during the growth phase and, later the pH was maintained at 6.5. It could be concluded that production of lactobionic acid by Pseudomonas taetrolens is economically a good option. Other important organic acids are the ones used in agriculture industry, like gibberellic acid. Gibberellic acid is a phytohormone involved in the plant growth and development. Recently it has been reported that the fungus Fusarium moniliforme produces secondary metabolites, including gibberellic acid. The production of the organic acid was achieved using waste from the dairy industry, specifically milk whey supplemented with  $Mg^{+2}$ ions. The maximal growth of biomass was obtained in 6 days of culture (6 g/L) and the maximum production of gibberellic acid was with 750 mg/L after 12 days of culture [54].

Biosurfactants: Biosurfactants (BS) are amphipathic molecules due to their possession of both hydrophilic and hydrophobic chemical groups. They possess surface activity; this means that they reduce the surface tension of liquids. They are also extracellular compounds synthesized by yeasts, fungus and bacteria. The most significant characteristics displayed by them are surface activity, biodegradability, antimicrobial effects, antitumor activity, anti-adhesive, low toxicity and effectiveness at extremes of temperature, pH and salinity [55]-[58]. Due to these

features, biosurfactants have great importance in bioremediation and oil recovery processes. They are important in the pharmaceutical, agriculture, cosmetics and food industries. Yeast, including *Candida bombicola* ATCC 22,214, is able to synthesize BS as sophorolipids at concentrations of up to of 5 g/L in a synthetic medium that emulates wastewater form the dairy industry [58]. Similarly, *Bacillus licheniformis* M104 is able to grow in milk whey and produce a BS lipopeptide with antimicrobial activity against some gram-positive strains and pathogenic enterobacteria [59]. It was concluded that milk whey maybe a comparatively better medium to produce BS without the addition of specific nutrients to the culture. On the other hand, the bacteria *Pseudomonas aeruginosa* ATCC 10,145 and its recombinant strain can synthetize BS with milk whey waste from the cheese industry. The process takes 96 h at 100 rpm. The wildtype strain achieves a production of 4000 mg/L at 30°C and 7500 mg/L at 37°C. The recombinant strain generates 7000 mg/L at 30°C and 10,500 mg/L at 37°C. Finally, it can be inferred that genetic engineering could improve the yields of functional compounds from industrial waste [60].

Exopolysaccharides: Exopolysaccharides (EPS) are biopolymers composed of repeated carbohydrate units. They are commonly divided in two groups: the homopolymers, composed of a single type of sugar, and heteropolymers, made up of different types of sugars. Both types of polymers may contain organic and inorganic substituents, and the lactic and halophilic bacteria produce them extracellularly [61] [62]. These compounds allow the microorganisms to adhere to the surfaces, facilitate the formation of biofilms and protect the organisms against environmental agents. EPS show functional properties that can be applied in the food and pharmaceutical industries. These include acting as emulsifiers, viscosifiers, stabilizers, texturizers, detoxifiers, and antineoplastic agents [63] [64]. The production of EPS by a *Bulgaricus* strain using partially hydrolyzed proteins of Cheddar cheese whey were in a range from 95 to 110 mg EPS/L. The used whey was hydrolyzed with flavourzyme and then fermented at 38°C, with a pH of 5.0, using *Lactobacillus delbrueckii* ssp. [65]. In contrast, the maximum output from *Streptococcus thermophilus* SY was 152 mg/L using a culture medium containing deproteinized whey and yeast extract. The fermentation conditions for the deproteinized whey were 12 h, a pH of 6.4, and a temperature of 36°C. The obtained EPS were composed of glucose, rhamnose and galactose; their respective dispersions in water demonstrated a pseudoplastic behavior and comparable viscosity level with commercial solution sofxanthangums [66].

Bioactive peptides. In the last couple of years, compounds of protean nature with biological activity have been found in milk and in its derivatives. These compounds have been called bioactive peptides because they have a nutritional function and can exert different biological properties such as: antihypertensive activity, antimicrobial activity, vessel regulation, hormonal induction, generation of neurotransmitters, antioxidant activity, immune system regulation, antithrombotic, anti-cancer (or neoplastic), prevention of chronic diseases and facilitation of nutrient absorption. Typically, milk, colostrum and whey are the main sources of these compounds [67]-[72]. Bioactive peptides have been defined as inactive amino acid sequences found in the core of a precursor protein, which have biological activities after being released by a chemical or enzymatic hydrolysis. Depending on the nature of the precursor protein source, the types of enzymes involved and the conditions under which the active peptides are released varies [72]. The release of the bioactive peptides can occur during digestion in the stomach or during commercial fermentation processes by the metabolic activity of multiple microorganisms. During the manufacturing of yogurt or cheese, lactic acid bacteria are important for the production of bioactive peptides. The metabolic activities involve proteolytic systems that include proteinases, intracellular peptidases, endopeptidase, aminopeptidase, dipeptidase and tripeptidase. Biomolecules can also be added to regular dairy products, meat products, bakery products, confectionery, beverages and special foods specifically designed for people with high blood pressure, diabetes, high cholesterol or obesity. The bioactive peptides produced can be used as dietary supplements for human consumption because of their structure, rapid absorption, and timely elimination [73] [74].

There are several studies which showed different ways to obtain bioactive compounds using biotechnological tools. One example is a proteolytic vegetal enzyme from *Maclura pomifera*. The isolation of bioactive peptides depends on the cogulation of the milk via the addition of latex from *Maclura pomifera*. Once the milk is coagulated, peptides may be obtained from the sera. The peptide produced by this way has antioxidant properties and inhibits the angiotensin-converting enzyme [75]. The angiotensin converting enzyme is known to play a role hypertension and is a target of several anti-hypertension drugs. Furthermore, milk whey fermented by *Lactobacillus helveticus* increases bone density and osteoblast formation in comparison with normal whey. This study was based on *in-vitro* and *in-vivo* models. These properties were attributed to the peptides formed by the proteolytic activity of the bacteria or to increased bioavailability of proteins [76]. Such studies suggest the need forclinical

studies to provide a better understanding of the biological effects of the fermention products and their effects against osteoporosis. The anionic and cationic peptidic fractions obtained from hydrolyzed whey proteins showed emulsifying properties at alkaline pH. The anionic fractions demonstrated a better antioxidant activity. During this process whey hydrolyzation was achieved by incubation with vegetal cysteine proteinases such as papain and hemisphericine for a period of 360 minutes [77]. It is noteworthy that the proportion of peptides obtained either from anionic and cationic character as well as their functional properties, exhibit differences depending on the type of protease used in the hydrolysis.On the other hand, adding proteases and peptidases of *Aspergillus oryzae* to a concentrate of whey protein from goat cheese, and the subsequent incorporation of lactic acid bacteria like *Lactobacillus helveticus* and *St. Salivarius* var. *thermophilus* produce a hydrolyzed mixture that contains bioactive peptides [78]. Moreover, the bioactive peptides obtained had higher inhibitory activity against the angiotensin-converting enzyme (ACE). It could be inferred that this process could allow the use of goat whey to help prevent hypertension.

Hydrolysates. Recently enzymatic hydrolysates of proteins have been introduced into the human diet. These hydrolysates are rich in oligopeptides that improve the utilization of proteins. They have been employed in several countries as dietary supplements for middle-aged people, premature babies, athletes that control their weight, and children with diarrhea. These hydrolyzed proteins allow higher absorption of amino acids from the

Microorganisms or biological component used	Products	Potential industrial uses or applications	Ref.
Geotrichum candidum	Biomassdegradation of phenol-derived compounds	Bioremediation	[26]
Kluyveromyces marxianus var. marxianus	Unicelular protein	Food	[27]
Bacillus megaterium SRKP-3, Brevibacterium casei SRKP2	Polyhydroxyalkanoates (PHA)	Plastics	[28] [29]
Kluyveromyces fragilis, Kluyveromyces marxianus DSMZ-7239, Clostridium acetobutylicum DSM 792, E. coli, Rhodopseudomonas cyanobacterium, anacystis	Etanol, butanol, electricity, H <sub>2</sub>	Bioenergy or biofuels	[32] [33] [34] [36] [39] [41]
Propionibacterium shermanii, Propionibacterium acidipropionici, Actinobacillus succinogenes 130Z, Aspergillus carbonarius NRRL 67, Aspergillus niger ATCC9642, Kluyveromyces marxianus IFO 288, Lactobacillus delbrueckii ssp. Bulgaricus, Lactobacillus helveticus ATCC-11,842, Fusarium moniliforme	Citric acid, lactic acid, succinic acid, propionic acid, gibberellic acid	Pharmaceuticals, food industry, agriculture	[43] [44] [46] [47] [48] [49] [49] [54]
Candida bombicola ATCC 22,214, Bacillus licheniformis M104, Pseudomonas aeruginosa ATCC 10,145	Biosurfactants	Food industry, medicine, pharmaceutics	[58] [59] [60]
Lactobacillus delbrueckii ssp., Streptococcus thermophilus SY	Exo polysaccharides	Pharmaceutics, food industry, stabilizers, texturing and gelling agents.	[65] [66]
Proteolytic enzymes of <i>Maclura</i> pomifera-papain and hemisphericine	Bioactive peptides	Pharmaceutics, food industry, health	[75] [77]
Lactobacillus helveticus	Bioactive peptides		[76]
Proteases and peptidases of Aspergillus oryzae, Lactobacillus helveticus, St. Salivarius var. thermophilus	Bioactive peptides		[78]
Bacillus subtilis and proteases of Bacillus licheniformis	Hydrolyzed protein	Food and nutrition	[79] [80]

#### Table 5. Microorganisms used in the bioremediation of the dairy industry wastes.

stomach in comparison with unhydrolyzed proteins [78]. For this reason a process to obtain high yield enzymatic hydrolysates from milk whey has been designed in which milk whey was incubated for 5 hours with subtilis in enzymes of *Bacillus subtilis* and pancreatin from pork; and the obtained yields were 41.4% and 37.1% respectively [79]. A different process has been shown to generate hydrolyzed protein from milk whey in a manner that reduces the allergenic properties of the product by up to 99.97%. Proteases from *Bacillus licheniformis* were used (16 h/50°C/pH 8.5) to obtain the sehydrolysates. Moreover, the product may be suitable for the incorporation into infant food and nutritional products [78] [80]. All bioremediation processes used to treat dairy waste products have been summarized in the **Table 5**.

#### **3. Conclusion**

The byproducts of the dairy industry, like whey or sewage, are highly polluting wastes if they are deposited directly into the rivers, lakes, seas or soil. Nowdays, there are many biotechnological alternatives available that allow the use of organic wastes as substrates for useful processes. Dairy by-products can be used to obtain valuable chemical compounds that are useful in the fields of health, pharmaceuticals, food, plastics and fuels. The described strategies contribute to decreased environmental pollution and reduce the damage caused by man. In short, multiple proposals exist and others are being developed. However more studies are required to optimize the culture conditions and to develop microbial strains to obtain maximum yields at a cost that make the processes viable for scaling to industrial levels. Finally, these recent discoveries may present a path towards sustainable development in the near future; one that respects and cares for the environment.

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