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Review

Cheese Whey as a Potential Feedstock for Producing Renewable Biofuels: A Review

Carlos S. Osorio-González [1], Natali Gómez-Falcon [2], Satinder K. Brar [1,*] and Antonio Avalos Ramírez [3,*]

1 Department of Civil Engineering, Lassonde School of Engineering, York University, North York, Toronto, ON M3J 1P3, Canada
2 Biotechnology Department, Scientific Research Center of Yucatan, Mérida 97205, Mexico
3 Centre National en Électrochimie et en Technologies Environnementales, 2263, Avenue du Collège, Shawinigan, QC G9N 6V8, Canada
* Correspondence: satinder.brar@lassonde.yorku.ca (S.K.B.); aaramirez@cnete.qc.ca (A.A.R.)

Abstract: Agro-industrial residues such as bagasse, pomace, municipal residues, vinasse and cheese whey are an environmental problem around the world, mainly due to the huge volumes that are generated because of the food production to satisfy the nutritional needs of the growing world population. Among the above residues, cheese whey has gained special attention because of its high production with a worldwide production of 160 million tons per year. Most of it is discarded in water bodies and land causing damage to the environment due to the high biological oxygen demand caused by its organic matter load. The environmental regulations in developing countries have motivated the development of new processes to treat transform cheese whey into added-value products such as food supplements, cattle feed and food additives. In addition, during the last decade, several processes and technologies have been developed to produce bioenergy through the biotechnological process using cheese whey as a potential feedstock. This review discusses the production of bioethanol, biohydrogen, biomethane and microbial lipid-biodiesel production using cheese whey as a potential substrate.

Keywords: bioenergy; cheese whey; bioethanol; biohydrogen; biomethane; biodiesel

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1. Introduction

Dairy is a worldwide industry and the main waste generated by the milk transformation is whey. The world production of this waste in 2020 was 183 million tons [1]. Since governments in various jurisdictions around the world acted, except for some developing countries, it is currently illegal to dispose of untreated cheese whey in water bodies [2]. In the second half of the 20th century, community action groups, environmental agencies and processors equally recognized and highlighted the environmental damage caused by the release of untreated cheese whey. Essentially, when cheese whey is released into water bodies or directly into the soil, contributes to eutrophication in the water body and increases the acidity (depending on the discarded cheese whey type) in the soil [3]. The principal compound of cheese whey is lactose (44–46%), leading to a high biological and chemical oxygen demand (30 to 50 mg/L, and 60 to 80 mg/L, respectively) that contributes to the eutrophication [4]. The increase in acidity in the soil is highly dependent on cheese making process due to factors such as type of cheese (fresh, mozzarella, cottage), curd process and milk source have an effect. For instance, cheese whey obtained from the curding process utilizing organic acids (acid cheese whey) hold pH values between 3.5 to 4.5, and higher than 5.6 when curt-enzymes processes are used. Likewise, it has been reported that secondary cheese whey holds pH values ≤ 3 [1]. The high untreated amounts that are discarded and the pollution caused by this dairy residue have led governments from all around the world to demand industries focus on the clean production of goods and services. Likewise, secondary cheese whey resulting mainly from cottage cheese production has been used directly as feedstock to produce biofuels. However, it has been reported that the substrate has some limitations to be used in the microbial process. Some of these limitations is the increase in acidity (≥3), high dissolved oxygen (80 gL^{-1}), high biological demand (30 gL^{-1}) and low solid content (8 gL^{-1}). The above nutrient limitations and the relatively small production in comparison with the other cheese whey sources are the main barriers to complete exploitation and better approach to this residue [5,6]. This situation has obligated cheese companies to create solutions to decrease their cheese whey loads by re-designing their processes and/or valorizing their by-products [7].

The exploitation of cheese whey to produce different goods has increased in recent decades. Some of the conventional goods and products are lactose, organic acids and protein fractions. During the past years, the research on the use of this residue has been intensified to produce chemical compounds for industrial sectors such as pharmaceutical, cosmetic and bioenergy [8–10]. Figure 1 shows some of the products as well as the sectors in which cheese whey is currently used as feedstock to produce added-value compounds.

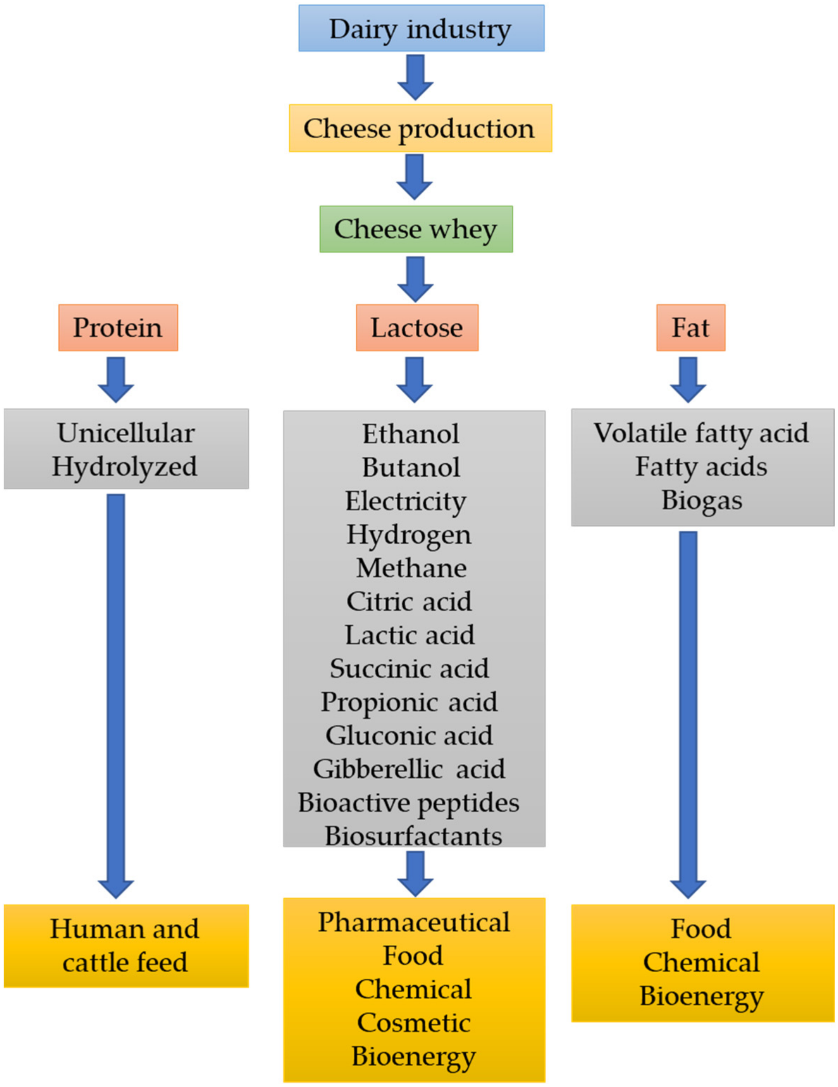

Figure 1. Biorefinery concept of cheese whey.

Several technologies have been studied to treat cheese whey with the main purpose to decrease the organic load. Figure 2 shows conventional processes to treat cheese whey. Physical treatments for cheese whey have been mainly focused on membrane technologies such as diafiltration, microfiltration, ultrafiltration, nanofiltration, electrodialysis and reverse osmosis. These technologies are particularly used to recover and remove specifically lactose and proteins. Cheese whey treatment through chemical processes consists of the use of chemical catalyzers to convert lactose into different sugar isomers such as galactose, glucose, or D-tagatose. One of the most common chemical reactions to treat cheese whey is the Lobry de Bruyn Alberda van Ekenstein (basic or acid). The isomerization is carried out using soluble catalysis with sodium hydroxide (NaOH), potassium hydroxide (KOH) or calcium hydroxide ($Ca(OH)_2$), among others [11]. Physicochemical processes are mainly focused on the use of coagulants and flocculants such as aluminum sulfate ($Al_2(SO_4)_3$, ferric chloride ($FeCl_3$) and ferrous sulfate ($FeSO_4$) [12]. Finally, biological processes to treat cheese whey mainly through aerobic and anaerobic fermentation. The use of these alternatives is the most reliable biological-base process in terms of the cheese whey transformation/elimination [13–15].

Currently, the academic community has increased its effort in renewable energy production/generation using residues from different industries as substrates. This review aims to show the recent advances in the valorization of cheese whey to produce biofuels such as bioethanol, biohydrogen, biomethane and microbial lipids to produce biodiesel. In addition, this revision provides a general overview of the main microorganisms and technologies used during the last decade in the production of biofuels mentioned above.

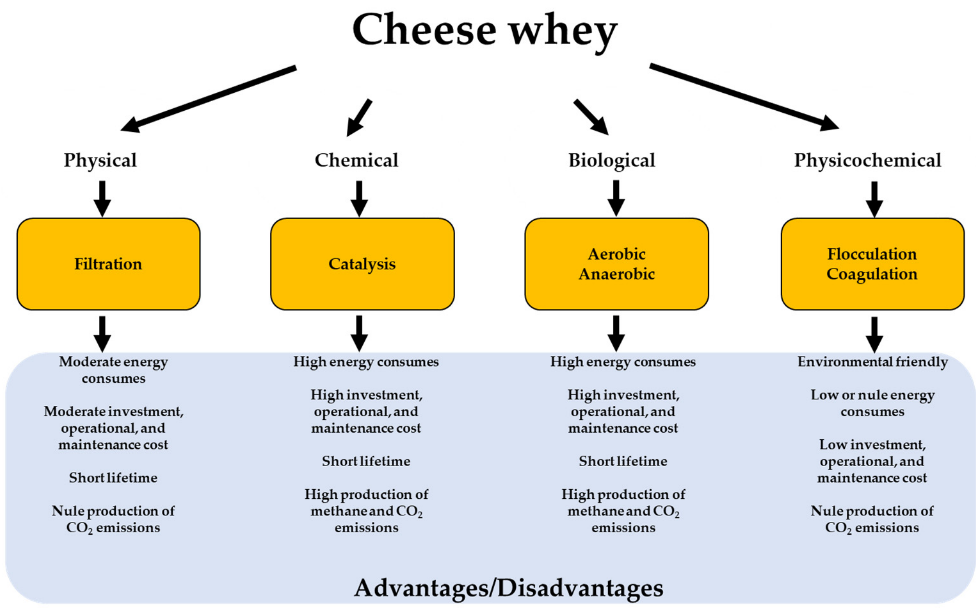

Figure 2. Conventional process to treat cheese whey.

2. Cheese Whey Properties and World Production Status

According to the Food and Agriculture Organization of the United Nations [16], cheese world production is mainly generated from four types of milk (buffalo, goat, sheep and cow). The amount of cheese whey that is generated annually in the world is about 183 million tons. Commonly, there are two types of whey: acid and sweet. Acid whey is obtained by the direct use of organic acids or by the addition of lactic cultures to produce cheese. On the other hand, sweet whey is mainly obtained by coagulation of proteins with animal or microbial enzymes (ex. chymosin complex). In summary, the physicochemical composition and cheese whey type depends specifically on the process used in the cheese-making production [17,18]. Table 1 shows the main differences between cheese whey obtained from cheese making from different animal sources.

Table 1. Chemical composition of cheese whey from different milk sources as well as acid and sweet whey.

	Type of Milk			
Component	**Goat** [19]	**Sheep** [20]	**Cow** [21]	**Cow** [22]
Moisture (%)	93.5	92.3	94.9	93.6
Total solids (%)	6.4	7.6	5.0	6.4
Lactose (%)	4.1	5.7	4.6	6.3
Protein (g/L)	1.2	1.0	2.5	6.8
Fat (g/L)	0.4	nd	4.9	1.8
Ash (%)	0.6	nd	0.3	0.6
pH	4.5	6.16	4.6	6.2

nd: not determined.

Small and medium cheese-making industries are not able to transform their residues into added value products because the technology is expensive. This situation has become an environmental, health and economic problem that leads to two solutions in which some of the cheese factories can process approximately 50% of their produced cheese whey into powder cheese whey and condensed cheese whey (Figure 3). Currently, several types of research have been carried out on technologies to use cheese whey as a substrate to produce different products for specific sectors (food, pharmacy, health, cosmetics and bioenergy) [23]. When cheese whey is valorized, one of the main products obtained is lactose, which can be used as an ingredient in the production of infant formula, bread, sweets, meats, etc. However, when this residue is used as a carbon source for microorganisms in biological processes, it is possible to obtain a wide variety of secondary metabolites such as enzymes, bacteriocins, organic acids, proteins and even biofuels or feedstock to produce it. Some biofuels that can be produced through the biochemical process using cheese whey as a substrate are bioethanol, biogas, methane, biohydrogen butanol and microbial lipids as a feedstock to produce biodiesel [24–27]. In addition, the use of cheese whey as a substrate to produce biofuels contributes firstly to improving the income of cheese producers, and secondly to decreasing the environmental impact [28].

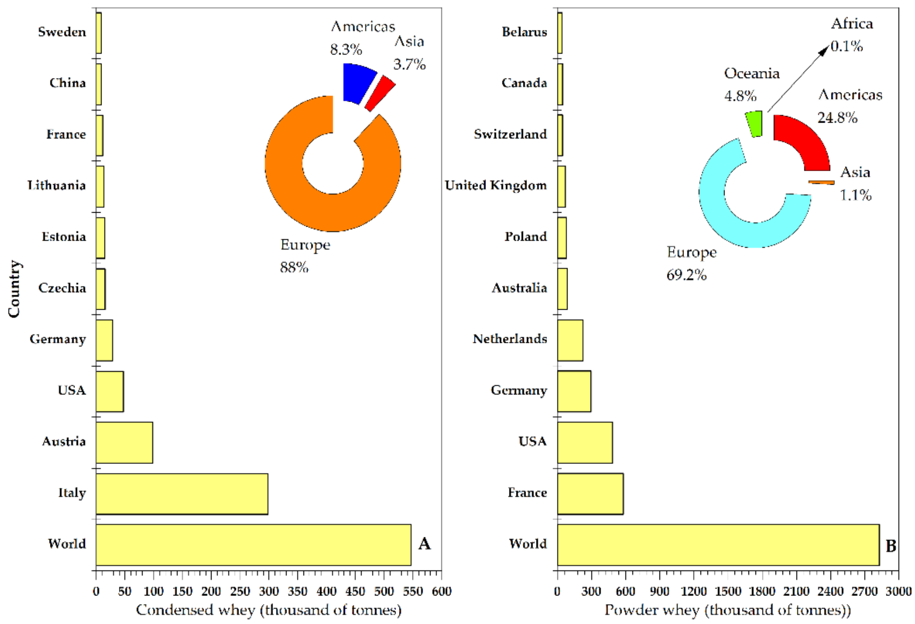

Figure 3. World cheese whey production: (**A**) Top ten condensed cheese whey producer countries and production distribution per region, (**B**) Top ten powder cheese whey producer countries and production distribution per region.

3. Bioethanol

Bioethanol production through fermentation has emerged as a potential alternative to replace fossil fuels such as gasoline. This renewable biofuel not only has application in the energy industry but is widely used as a replacement for chemical or grain-based ethanol in the cosmetic, pharmaceutical, food and beverage industries [29]. It has been reported that bioethanol production from corn and sugarcane has been produced extensively by the United States and Brazil, respectively. Nevertheless, the use of the above two feedstocks increases the total production cost and compromises food security due to the high land use for these crops [30]. In this sense, different feedstocks such as different lignocellulosic biomass, starches, food wastes and agri-food residues have been used for bioethanol production. The use of cheese whey as a substrate to produce bioethanol through fermentation is economically competitive in comparison with substrates such as sugarcane, corn and lignocellulosic biomass. In addition, it is a residue, and its valorization represents several advantages in terms of sustainable development, such as a decrease in waste, and organic carbon recycling [31].

One of the most important parameters during bioethanol production is the strain, which must present physiological characteristics to reach a high ethanol yield (>80%) from cheese whey. Many researchers have used common wild yeasts to produce ethanol from lactose, for example, *Kluyveromyces* sp. (fragilis, marxianus and lactis). However, the *Kluyveromyces* genre is overly sensitive to high ethanol concentrations in the culture media, causing its inhibition, as well as low conversion rate (30 to 40%). An alternative to solve this problem is the use of *Saccharomyces cerevisiae* and *Candida pseudotropicalis* with even 4-fold more tolerance to ethanol concentrations and an increase in conversion rate in comparison with *K. marxianus*. Nevertheless, *S. cerevisiae* and *C. tropicalis* in a wild state cannot be able to metabolize lactose as a carbon source. In this sense, advances to design strains of yeast and bacteria through metabolic engineering with the main objective to use lactose as a carbon source to produce bioethanol have been performed [32–34]. Table 2 shows wild and engineered microorganisms to produce ethanol using cheese whey as substrate. For instance, Jensen et al. [35], patented the production of bioethanol from cheese whey using an engineered *Lactococcus lactis*. The invention is related to block enzymes such as lactate dehydrogenase (LDH), phosphate acetyltransferase (PTA), aldehyde-alcohol dehydrogenase (ADHE) and overexpress operon genes (lacABCD), lactose binding precursors (LacEF) and genes to hydrolyze lactose (6-phospho-beta-galactosidase, lacG). Particularly, two transgenes are overexpressed (pyruvate decarboxylase and alcohol dehydrogenase) to improve the catalysis of pyruvate to ethanol. The designed strain showed approximately 99% lactose consumed at 55 h of fermentation time with an ethanol concentration of 30 g/L, 6-fold higher in comparison with a wild strain.

Table 2. Bioethanol production using different types of cheese whey as a substrate.

Substrate	Strain	Ethanol Concentration (g/L^{-1})	Ethanol Yield (g g^{-1})	Volumetric Productivity (g L^{-1} h^{-1})	Theoretical Yield * (%)	COD Removal (%)	Reference
Cheese whey (permeate)	*E. coli* DSM 1116	43.77	nd	0.82	63%	75.00	[36]
	K. marxianus URM 7404	8.90	0.24	0.66	44.37	86.02	[23]
	K. lactis CBS2359	22.2	0.34	0.31	31.00	nd	[37]
	K. marxianus DSM 5422	52.9	0.41	1.1	nd	nd	[38]
	S. cerevisiae Ethanol Red	45.63	0.34	0.70	nd	nd	[38]
	K. marxianus MTCC 138	7.90	0.40	1.66	nd	nd	[30]
Cheese whey (powder)	*S. cerevisiae*	23.80	nd	nd	nd	nd	[39]
	Neolentinus lepideus	33.0	0.32	0.17	nd	nd	[40]
Fresh cheese whey	*K. marxianus* URM 7404	25.81	0.50	2.57	95.80	78.94	[23]
	K. marxianus PTCC 5194	23.60	0.49	0.73	91.7	nd	[41]

nd: not determined. COD: Chemical oxygen demand. * Theoretical yield represents the percentage calculated with base in the reaction stoichiometry in which one mol of glucose produce two moles of ethanol.

Notwithstanding, several strategies that involve parameters such as lactose content, microorganisms with high ethanol tolerance, dissolved oxygen, temperature, pH, aeration (aerobic or anaerobic) and fermentation modes (batch, fed-batch or continuous), co-culture strategies, bioreactor type (membrane, fluidized bed) to produce bioethanol using cheese whey as the substrate has been tested [33,36]. An example of one of these strategies is the research performed by Sampaio et al. [37]. They tested bioethanol production using a co-culture of *Kluyveromyces marxianus* Y00963 and *Saccharomyces cerevisiae* Levulina Fb using cheese whey permeate as substrate. The author obtained a maximum substrate conversion of 82.64% using a 1:1 inoculum ratio of strains (*K. marxianus*: *S. cerevisiae*). Furthermore, they observed that when the inoculum strain ratio was modified to 3:1, the conversion rate decreased to 72.33% but an increase in 1.8% the ethanol production.

In the world, 95% of the ethanol produced is via fermentation. According to the United States, Energy Department [42] world bioethanol production until 2017 was 27,050 million gallons, being United States, Brazil, European Union, China and Canada the most important bioethanol producers. Figure 4 shows the bioethanol production in the world during the last decade. Regarding price and economic analysis of bioethanol production from cheese whey, few studies and data are available. So far, the most complete economical analysis was performed by [43]. They calculated the economical feasibility of an ethanol production process using cheese whey as a substrate. The total initial investment was US$12,781.56 to treat 6000 L of cheese whey per week, with a variable cost in ethanol production per month of US$2180.80, and a cheese whey permeates to ethanol bioconversion cost of US$4299.32. The margin ratio and contribution margin were US$0.47/L, and US$1.42/L, respectively. Furthermore, the authors calculate ethanol price per liter at US$3.02 with a hypothetical market price of US$2.21/L and a cost per unit after split-off of US$0.81/L. Finally, they calculate a total benefit of US$3816.96/month.

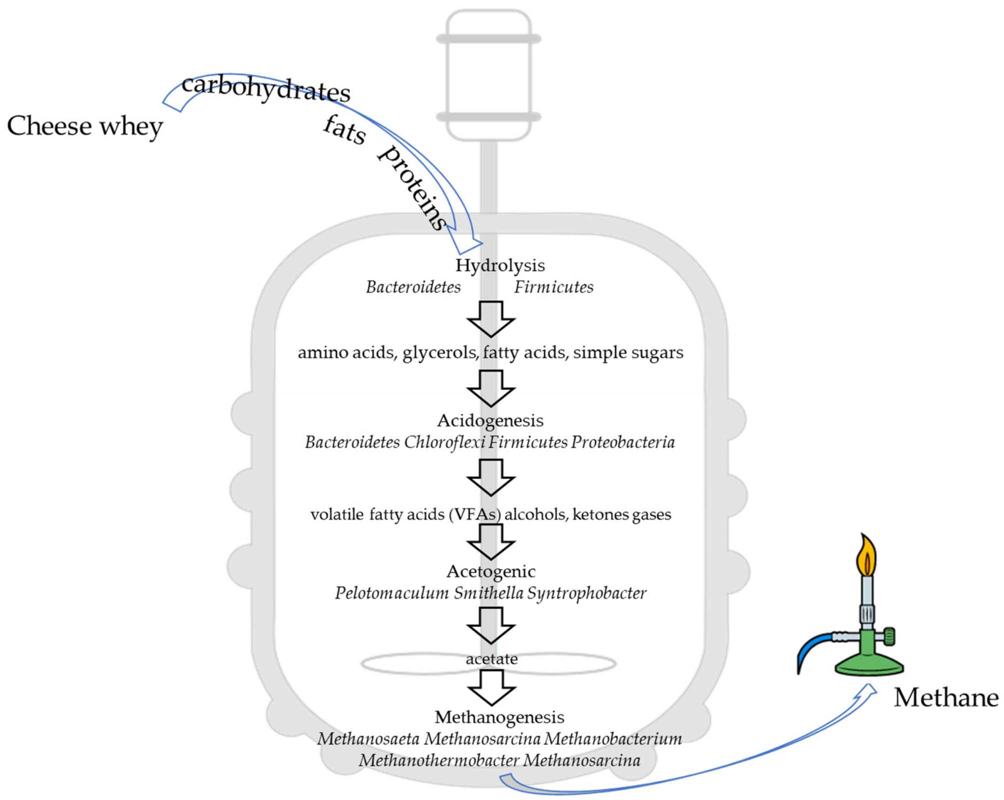

Figure 4. Main steps involved in anaerobic digestion processes.

To achieve competitive prices for bioethanol commercialization, several components (type of bioreactor, microorganism), parameters (aeration, immobilization, cultivation type) and substrate limitations (type of cheese whey, lactose, nitrogen and protein content) must be considered to design the bioethanol production process. One of the most important tools that has been used to optimized biotechnological process are trough mathematical model generated from surface response methodologies or simulations using data from engineering runs [44,45].

4. Biomethane

Biogas is produced by the anaerobic digestion of organic wastes. The carbon is transformed into methane and carbon dioxide. This biofuel can be used to produce electricity, heat and, if it is upgraded, renewable natural gas [46]. The biological pathway to produce biogas is carried out by a microbial consortium composed by hydrolytic, acidogenic, acetogenic and methanogenic bacteria. During hydrolysis, hydrolytic bacteria use complex molecules such as proteins, sugars, amino acids and fats, among others as a substrate to produce intermediates such as organic acids, alcohols, acetate, hydrogen, or carbon dioxide. During the second step (acidogenesis) the by-products obtained during the hydrolysis are used as a substrate in this step to produce volatile fatty acids, alcohols, or ketone gases that at the same time can be used in the acidogenesis (next step) to produce acetate. Finally, methanogenic bacteria use the compounds obtained previously as a substrate to produce biogas, preferably methane (methanogenesis) [47].

Methane is the one of most abundant biogas fractions produced by anaerobic digestion of organic residues, including cheese whey. As mentioned above, anaerobic digestion is a well know technology to produce methane. However, several challenges come with each specific feedstock that is used as a carbon source. These challenges can be classified into three main categories, microbiological, chemical and operational, making anaerobic digestion one of the most complicated biological processes. Moreover, this technology is highly recommended to treat wastewater and residues with high biological oxygen demand, such as cheese whey [48]. Additionally, requirements related to the installation and operation of anaerobic biodigesters such as technology, energy consumption and space are relatively low. Nevertheless, depending on the reactor type and feedstock the total cost can vary considerably. Likewise, the reactor type plays a key role during biogas production and classified the anaerobic digestion process into two different systems: low-rate system and high-rate system. The first one is characterized mainly by the liquid displacement in the digester in equal amounts of the liquid that flows out. In addition, it has relatively long hydraulic and sludge retention times (20 to 30 days), and the digester can be intermittently or continuously mixed. The second one has a shorter hydraulic retention time in comparison with low-rate systems, and the biomass can be immobilized or recycled into the digester, improving the microbial growth [49]. Figure 4 shows a general scheme of different stages involved during cheese whey anaerobic digestion to produce methane.

Anaerobic digestion of cheese whey to produce methane has been studied using expanded granular sludge bed reactor (EGSB) [50], anaerobic sequencing batch biofilm reactor (AnSBBR) [51–53], anaerobic membrane reactor (AnMBR) [54], continuous stirred tank reactors (CSTRs) [55,56] and sequencing batch reactor (SBR) [57]. During anaerobic digestion of cheese whey is preferable to add a pH stabilizer to increase the methane productivity and yield [58]. Several compounds can be used to buffer the digestate, among the most important are calcium carbonate ($CaCO_3$) and dipotassium phosphate (K_2HPO_4). Nevertheless, "biodegradable buffers" can be used, such as cattle manure and other agro-industrial residues rich in proteins and biomolecules with pKa around 7 and 8 [28,49,59]. Table 3 shows recent works to produce methane using cheese whey as a substrate and in co-digestion with agro-industrial residues. During the last decade, several works have been performed to produce biogas, and specifically, methane from cheese whey using sludge from several sources as inoculum, as well as agro-industrial residues (vinasse, dairy manure and sugarcane stillage) as a buffer. Furthermore, four factors (land use, type of feedstock, type of process and utilized energy) are considered the most important during biofuel production due to promoting food security and sustainability. Likewise, these factors are crucial to achieving the economical feasibility of the bioenergy process because the final cost of biofuels depends directly on them. In this sense, the use of agro-residues such as cheese whey is a viable option for feedstock, because no requires agricultural land use and, in most cases, has a low cost.

Table 3. Biomethane production using cheese whey as the only substrate and in co-digestion mode using some agro-industrial residues.

Substrate	Inoculum	Bioreactor	Methane Yield	COD Removal	Reference
Cheese whey powder	Sludge from poultry house wastewater treatment	EGSB	9.8 mL CH_4 g COD_{feed}	85%	[50]
Cheese whey powder + vinasse	Sludge from a poultry slaughterhouse	AnSBBR	11.5 molCH_4 kg COD^{-1}	87%	[51]
Cheese whey + sugarcane vinasse	Sludge from up-flow anaerobic sludge blanket reactor	AnSBBR	15.3 mmol CH_4 g COD^{-1}	72%	[52]
Cheese whey permeate	Granular sludge from expanded granular sludge bed reactor	AnMBR	0.28 m^3 kg^{-1} $COD_{removed}$	98%	[54]
Cheese whey + Sugarcane stillage	Sludge from poultry house wastewater treatment	AnSBBR	15.76 mmol CH_4 g COD^{-1}	89%	[53]
Cheese whey powder	Sludge from the wastewater treatment plant	Anaerobic batch reactors	0.266 L CH_4 g $COD_{consumed}$	74%	[59]
Cheese whey + Glycerin	Sludge from a poultry slaughterhouse	AnSBBR	13.3 mol CH_4 kg COD^{-1}	89%	[60]
Cheese whey + Sea lettuce	Sludge from the sewage treatment plant	CSTRs	0.30 L g COD_{feed}	68%	[56]
Fresh cheese whey	Sludge from the wastewater treatment plant	SBR	340.4 L CH_4 kg^{-1} COD_{feed}	87%	[57]
Cheese whey + Dairy manure	Dairy manure anaerobically digested	CSTR	nd	70%	[55]

nd: not determined. COD: Chemical oxygen demand.

It has been reported that when methane is used as biofuel, it fulfills partially the required energy to operate a small or medium-sized dairy waste treatment plant. Moreover, in processes where high methane yields are obtained, the surplus of energy can be transferred to the cheese-making plant to operate the units of the sort, pasteurization and coagulation, to mention some. Furthermore, the establishment of a cogenerating unit of electrical energy using biomethane as biofuel, can provide economic benefits through a decrease in conventional energy consumption or derived for its sale [58]. For instance, Pasini et al. [61], performed a technical and economic analysis of two methane production systems (liquefied biomethane and gas biomethane for grid injection). They compared both processes regarding production, connections, electricity consumption, as well as market prices. They observed that the presence of a distribution network near the biogas plant could decrease the total process costs because the pressurization of biomethane gas is better than liquefaction to transport methane long distances. Some works have proved the feasibility of biomethane production using cheese whey as substrate. For example, a successful case in Colombia consists of a tubular digester of 42 m^3 with cow manure as co-substrate. This installation produces 8.7 m^3 per day of biogas and as a by-product 2.3 m^3 per day of liquid fertilizer, offering an extra economic benefit. In addition to all the aspects mentioned before, biomethane processes optimization is required to improve yields as well as a decrease the cost of the production and purification process. Several research studies have been performed with the purpose of utilizing mathematical and statistical tools [62,63].

5. Biohydrogen

Biohydrogen is considered a promissory and environmentally friendly source of clean energy. At present, hydrogen is mainly produced from steam methane and coal gasification (90%), as well as oxide electrolyzer technologies (>10%) [64]. During the last decades, several technologies for biohydrogen production have been studied with low economic feasibility due to the high-cost production of current technologies. Biological processes such as oxygenic and anoxic photosynthesis, aerobic and anaerobic fermentation and photo-synthesis for biohydrogen production are promising options to solve this issue [65,66]. The price of raw material, the carbohydrate content and availability are factors that determine the use of organic wastes to produce biohydrogen. The production of biohydrogen from renewable sources has a positive impact on the environment. The production of greenhouse gasses generated is low during its combustion. Among the most used feedstock for hydrogen production are residues from agro-industry, such as cheese whey and liquid bovine manure [67–69]. The production of hydrogen from biological processes can be divided into three types, fermentation (dark fermentation), biophotolysis (direct and indirect) and bioelectrochemical (microbial electro-cells) [70]. Table 4 shows several works to produce biohydrogen with wild and engineered microorganisms using cheese whey as a carbon source. One of the main benefits of the biohydrogen production process using cheese whey as a carbon source is the concomitant decrease in environmental pollution. Nevertheless, biohydrogen production has several challenges and bottlenecks during its production, some of them are related to biocatalysis and its industrial scale-up, storage, compression, as well as the lack of networks for its distribution and commercialization [67,68,70]. Furthermore, research has been performed using mathematical, statistical and simulation tools to characterize, optimize and improve biohydrogen production using cheese whey as a substrate [71,72]. For instance, regarding biocatalysis, several works have been performed in strains from the *Clostridium* genre to increase the biohydrogen yield [73–76]. Likewise, research has been focused on technology development to improve biohydrogen production. As an example [77], tested microbial-chamber-electrolysis-cells to produce biohydrogen using cheese whey as a substrate. They conclude that the pH in this production method plays a critical role during the bioelectrohydrogenesis, because with high pH variation the bioanode activity is highly affected and sometimes lost, a situation that conveys a considerable decrease or a total rescinded of biohydrogen production.

Table 4. Biohydrogen production using cheese whey as a substrate.

Substrate	Strain	Hydrogen Yield	Hydrogen Productivity	Reference
Cheese whey (powder)	*Lactobacillus acidophilus*	1.00 mol H_2/mol of lactose	nd	[78]
Cheese whey (permeate)	Microbial consortium	3.60 mol H_2/mol of lactose	140.02 mmol H_2/L day	[67]
Hydrolysed cheese whey	Microbial consortium	1.93 mol H_2 mol^{-1} of sugars	5.07 L H_2 L^{-1} day^{-1}	[79]
Cheese whey (powder)	*Ethanoligenens* sp. and *Megasphaera* sp.	5.40 mol H_2 kg COD^{-1}	129.00 mol H_2 m^{-3} d^{-1}	[80]
Acid cheese whey (Mozzarella cheese)	Activated sludge consortia	371.00 L H_2/kg TOC_{whey}		[81]
Cheese whey (supplemented with buffalo manure)	Anaerobic sludge consortia	152.20 mL H_2/g of substrate	215.40 mL H2/L/d	[82]
Cheese whey (powder)	Anaerobic sludge consortia	3.67 mol H_2 mol $lactose^{-1}$		[83]
Fresh cheese whey	*Clostridium* sp.	6.35 mol H_2/mol lactose	139 mL/g/h	[65]
Cheese whey (powder)	Microbial consortium	1.12 mol H_2 mol $lactose^{-1}$	1080 mL H_2 L^{-1} d^{-1}	[84]

nd: not determined. COD: Chemical oxygen demand.

6. Lipids for Biodiesel Production

Biodiesel is one of the most popular biofuels produced due to is environmentally friendly and its net greenhouse emissions are lower in comparison with the produced from fossil fuels. Microbial lipid-base biodiesel production is one of the most promising biofuels due to its advantages (non-toxic, biodegradable, renewable, no sulfur content, high lubricity) in comparison with fossil diesel [79]. Microbial lipid-base biodiesel production is a potential alternative using low-cost residues such as cheese whey with high carbon content as a feedstock [83]. In this sense, there are certain microorganisms with the ability to accumulate a high amount of lipids, commonly called oleaginous microorganisms such as yeasts, fungi, algae and some bacteria [85,86]. Several microorganisms can accumulate a greater amount of lipids than some vegetable oleaginous crops and, unlike them, they do not require large use of land to be cultivated, they can be produced in a short time, and they are not affected by the climate conditions. One of the main problems of microbial lipid production is the feedstock that should be available, cheap and renewable. A wide variety of renewable feedstock such as lignocellulosic biomass, starch and agro-industrial residues has been tested for microbial lipid production [87]. In this sense, cheese whey has been recognized as a renewable substrate to produce microbial lipids using oleaginous yeast such as *Lipomyces* sp., *Cryptococcus* sp., *Yarrowia* sp. and *Rhodosporidium* sp, among others. Typically, biotechnological lipid production through fermentation is triggered under nitrogen limitation and an excess of carbon [88]. Table 5 shows the microbial lipid production using cheese whey as substrate.

Table 5. Main lipid-producing microorganisms use cheese whey as a substrate.

Microorganism	Substrate	Total Lipid (g/L^{-1})	Lipid Accumulation (%)	Process Conditions	Monounsaturated Fatty Acids (%)	Reference
M. circinelloides URM 4182	Fresh cheese whey	1.06	22.5	pH = 4.5 T° = 26 °C 250 rpm 120 h	80	[89]
C. oligophagum JRC1	Deproteinized cheese whey	5.64	44.12	pH = 6.6 T° = 28 °C 150 rpm 168 h	71	[90]
M. isabelline 1757	Ricotta cheese whey	4.49	37	pH= 5.8 T° = 30 °C 185 rpm 72 h	90	[91]
W. anomalus	Deproteinized cheese whey	0.65	24	pH = 6.0 T° = 28 °C 180 rpm 96 h	80	[92]
C. curvatus Y-1511	Ricotta cheese whey	6.83	63	pH = 5.8 T° = 30 °C 185 rpm 72 h	52	[93]
R. opacus MR22	Fresh cheese whey	3.00	48	pH = 7 T° = 28 °C nd rpm 120 h	46	[94]
Y. lipolytica B9	Deproteinized cheese whey	4.29	58	pH= 5.5 T° = 15 °C 150 rpm 120 h	80	[95]
C.consortia	Second cheese whey wastewater	1.2	13	pH = 7 T° = 27 °C Fluorescent illumination	79	[5]
C. oleaginosus ATCC 20509	Whey permeates	1.8	68	pH= 6.5 T° = 28 °C 150 h	50	[96]
Chlorella sorokiniana	Cheese whey	2.7	39	pH = 7 T° = 24 °C 10 days 100µmol photons $m^{-2}s^{-1}$	nd	[97]

nd: not determined.

7. Conclusions

Cheese whey is a by-product generated by the dairy industry and is highly polluting if is directly released into water bodies and soils. Due to its high nutrient content, cheese whey is a potential substrate in the biological process to produce several biofuels. The harnessing of this resource contributes to decreasing the pollution caused in water bodies and soil, due to its high biological and chemical oxygen demand. Although different technological alternatives have been developed for its transformation in biofuels, specifically bioethanol, biohydrogen, biomethane and biodiesel. According to the discussion, bioethanol production can be enhanced using wild ethanologenic strains capable to metabolize lactose. Regarding biohydrogen, the process which presents the highest yield is dark fermentation. However, one of the main challenges during biohydrogen production is the low yields obtained, so it is recommended the optimization of processes and the development of new strains that can achieve the best feedstock transformation. In summary, cheese whey is an alternative feedstock to produce liquid and gaseous biofuels that can contribute to decreasing the use of fossil fuels and consequently the environmental pollution caused by them.

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