A review on processes for whey and dairy wastewater treatment and valorization

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***Abstract*—The dairy industry generates a significant amount, around 150 million tons, of losses and wastes. Particularly, whey requires treatment due to their high nutrient content and therefore, its facility to pollute. This treatment also allows its revalorization. Among the main processes that have been reported are the biochemical ones, where anaerobic fermentation is used to produce ethanol, biological processes with microalgae, for the production of fatty acids and some chemicals, and other processes, such as physical or enzymatic. If these processes are integrated, a biorefinery can be designed where the objective is to obtain a diversity of products from a biomass. Some approaches, to the use of dairy wastes in a biorefinery scheme, were reviewed. There is one biological treatment, insects, which have become of interest due to their high conversion efficiency and wide variety of products, such as protein, biofuels or chemicals, generated from waste. Specifically, black soldier fly larvae have been considered for waste degradation. Based on their advantages, the use of insects in a biorefinery scheme using dairy waste as feedstock was proposed.**

***Keywords*— waste, waste treatment, biorefinery, whey biorefinery.**

I. Introduction

According to the Food and Agriculture Organization of the United Nations (FAO), one third of the food produced worldwide for human consumption is discarded annually, which represents about 1.3 billion tons [1]. This food waste is considered a problem because, although there are strategies for its treatment, it continues to be generated in large quantities.

When this organic matter reaches landfills, it decomposes and produces greenhouse gases (GHG) such as carbon dioxide (CO_2) and methane, which contribute to global warming. In addition, from an economic point of view, its transportation, handling and disposal requires the use of considerable resources; therefore, this waste portrait a window of opportunity to be exploited [2].

Particularly, dairy waste seems attractive since approximately 750 million tons of milk were produced in 2019 [3], and according to FAO, losses and waste were up to 20 % which represent almost 150 million tons [4]. The production of cheese, which is obtained from milk, generates waste such as whey. The treatment of whey is complex, due to its important quantities of proteins, lactose, high content of organic matter, alkalinity, and it is easy to acidificate [5]. Another residue generated is dairy wastewater (DWW), which results by the washing of equipment, containers, or control analysis. This water is composed by lactose, proteins, lipids, salts and detergents [6].

These organic wastes are classified as second generation biomass [7], and can be treated through thermochemical, chemical and biological processes. Thermochemical processes include direct combustion, pyrolysis, gasification and liquefaction, which are advantageous thanks of their simple techniques to produce thermal and/or electrical energy, as well as biofuels. Moreover, biochemical processes include anaerobic fermentation and anaerobic digestion, which can produce biofuels; however, in this type of processes it is not possible to directly generate thermal and/or electrical energy. The main disadvantage of thermochemical and chemical processes is that they do not allow the further use of biomass to generate a wide range of value-added products [8]; in addition, dairy waste must be mixed with other solid waste in order to be used in thermochemical processes. On the other hand, biological conversion processes include the use of microalgae and insects, which can be cultivated using waste as food. The latter are organisms, which are contemplated as an important source of protein and have the ability to degrade biomass; moreover, as a result of their growth they generate larvae that can be processed to obtain various products [9].

As it was mentioned, these processes offer the utilization of organic waste; nevertheless, the waste biomass can also be processed through a biorefinery scheme. The International Energy Agency (IEA) defines a biorefinery as a system of sustainable technologies that transform biomass into marketable products such as food, chemicals or biofuels. Thus, this concept appears attractive because it generates profits from the diversification of the generated products, while reducing GHG emissions, generating jobs and using the resources more efficient [10]. For the treatment of whey and dairy wastewater,

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a review on the individual processes was reported [11]; however, that contribution does not contemplate the use of biological treatments neither the use of biorefineries for the revaluation of this type of residues. Therefore, this paper aims to review the processes and biorefineries reported to treat whey, their conversion efficiencies and the value added-products obtained. The use of biological treatment is highlighted.

II. Methodology

In order to find the existing diary biorefineries as well as individual process it was performed a search in the literature. The keywords "whey", "whey biorefinery", "whey treatment", "whey valorization", "dairy waste water" and "whey processing" were searched in the Science Direct, Springer, Wiley and IEEEXplore browsers within titles, abstracts and keywords. The period of time included years from 2012 to 2022. The titles and abstracts were screened in order to identify those that described whey treatment, or that obtained value-added products.

III. Biochemical Processes

Anaerobic digestion is the most frequent treatment for DWW and whey. However, this process has several problems such as reactor instability, sludge settling, low biogas production and methane yields. Thus, a study researched the feasibility of a co-digestion using cheese whey and cattle slurry [5]. A total of 628.5 L of biogas with a methane proportion of 58 % were produced with a mix of 50 % cattle slurry and 50 % whey. This mix showed good yields when compared with other substrates.

In addition to the previously mentioned problems in anaerobic digestion, another paper addressed the existence of lipids in cheese whey, which seem to be inhibitors during anaerobic systems. Therefore, a study by Vlyssides *et al.,* (2012) proposed performing a chemical oxidation before the digestion in order to reduce lipid levels. This pretreatment stage showed a reduction of 80 % of fats, observing a similar biogas production, compared with data in literature, and a methane proportion of 85 % [12].

Other obstacle that anaerobic reactors face when treating DWW is the presence of organic matter. When organic matter is degraded, the volatile fatty acids that are formed decrease the pH; it has been reported that no methane is produced when pH values go below 4.5. Therefore, a research by Karadag *et al.,* 2015 described the preference to use of biofilm reactors [6]. These reactors have showed great potential, stability and performance. However, the authors concluded that further research is needed to achieve the same efficiencies at higher and lower temperatures.

In 2019, da Silva *et al.,* reported using an anaerobic fluidized-bed reactor with different feeding rates of DWW supplemented with glucose for bacterial benefit [13]. The obtained products were biohydrogen, acetic acid and bioethanol. The results showed that biohydrogen yield decreased when the DWW feed rate increased, having the highest proportion with almost 36 % of hydrogen and 30 % of methane in the total biogas. Acetic acid was obtained in a percentage between 65 and 90 %. It was concluded that degradable substrates allow biohydrogen production.

More recently, Sakarika *et al.,* (2020) addressed the use of expired milk products such as milk, yogurt and cheese to generate products like methane and biohydrogen using an anaerobic treatment [14]. In this experiment, they compared a single-stage and two-stage co-digestion; the latter was mixed with agro-industrial wastewater. The obtained proportion of methane was of about 50-60 % in the total biogas, and the two-stage system showed a higher energy. An environmental and technical assessment is proposed as a next step.

A study made by El Achkar *et al.,* (2020) reported the use of ultrasound pretreatment for whey before anaerobic fermentation [15]. The gas production showed no change. The treated whey had a production of 0.23 cubic meters of methane per kilogram in dry matter, while the untreated had 0.13 cubic meters of methane per kilogram in dry matter. The authors concluded that the use of ultrasound stimulated biomass growth which represented an increase in methane production.

The difference between aerobic and anaerobic treatment is that aerobic treatments require oxygen and degrade biomass into CO_2 water and cellular material. This treatment also has a lower efficiency than the anaerobic, and if the effluents are rich in fat or oil, its usage is limited; therefore, anaerobic treatment is preferred [11].

IV. Biological Processes

A study by Hena *et al.,* (2015) describes the use of native microalgae to treat DWW [16]. Four strains of microalgae (*Chlorella saccharophila, Chlamydomonas pseudococcum, Scenedesmus sp* and *Neochloris oleoabundans*) were able to remove 98 % of the nutrients and chemical oxygen demand from the water within 4-5 days of culture. The obtained biomass was of 153.54 tons per hectare per year, and 29.47 thousand liters per hectare per year of oil to produce biodiesel. Another study by Daneshvar *et al.,* (2018) used freshwater and marine water microalgae (*Scenedesmus quadricauda* and *Tetraselmis suecica*, respectively) to treat DWW [17]. Both species showed removal of total nitrogen, phosphate and total organic carbon. Lipids were then extracted and characterized, showing a higher content of saturated fatty acids, such as needed for biodiesel production. In 2019, Daneshvar *et al.,* grew both microalgae in two cycles, reusing the wastewater [18]. The experiments showed that these organisms can remove pollutants, saving water and producing microalgal biomass.

Another study by Ghobrini *et al.,* (2018) proved the feasibility to grow microalgae *Chlorella vulgaris* in saline waste water obtained from demineralization of whey [19]. Their results showed that the use of this water favored microalgae growth, which reduces directly the cost of its cultivation, since traditional media are expensive. In addition, it does not require other compounds and can be used on a large scale. However, research is still needed regarding its behavior with humidity, temperature and light conditions.

The research made by Patel *et al.,* (2020) used the microalgae *Chlorella protothecoides* to treat cheese whey and obtain algal biomass [20]. The whey was pretreated with acid hydrolysis, flocculation and struvite formations with the lowest dilution. After nine days, the biomass obtained was between 4.33 and 4.54 grams per liter, which was higher than *Chlorella vulgaris*, and the lipid yields were between 0.94 and 1.80 grams per liter. Overall, although microalgae promise to be an alternative to biochemical processes, more studies are still needed to improve their efficiency and reduce the cost of the processes that are carried out before or after their cultivation.

A study by Gramegna *et al.,* (2020) reported the use of microalgae to treat DWW and obtain products such as metabolites used in cosmetics or pharmaceuticals, colorants, supplements and biofuels [21]. They used four different *Chlorella* species, known for their lipid accumulation, and a recombinant *Chlamydomonas reinhardtii* for enzymatic treatment. These species showed an important growth leaving D-Lactose, since they are not able to metabolize it. Their use not only resulted in a decrease in treatment costs for its discharge, but the obtained microalgae had a lipid content between 12 and 21 % of their weight. The recombinant species showed a stable β-glucosidase. The authors conclude that a biorefinery process could be proposed considering the observed microalgae potential.

V. Other Processes

A. Coagulation treatment.

By the process of coagulation or flocculation, suspended organic matter can be reduced, decreasing the nutrients in dairy waste. According to Ahmad *et al.,* (2019) it is important to use an adequate coagulant to make efficient the wastewater treatment [11]. Some of these coagulants could be lactic acid bacteria (which convert lactose into lactic acid, denaturing milk and thus promoting precipitation and coagulation), chitosan, tannin, polyaluminium chloride and using powdered activated charcoal after helps in removing color and odor. It is also needed an oxidation pretreatment to remove fat in almost 80 %.

B. Enzyme treatment.

Cheese whey can be used to synthesize galactooligosaccharides (GOS), which seem interesting due to their similarities to human milk oligosaccharides. In a study made by Fischer and Kleinschmidt (2015) two enzymes, lactases obtained from *Aspergillus oryzae* and *Kluyveromyces lactis*, were analyzed to estimate their suitability to produce GOS in whey [22]. This process showed that whey could be used to synthesize GOS with yields between 4.3 and 32.6 %, enabling their use in drinks or other products. Other study showed that, in addition of synthesize GOS, enzymes can be immobilized in order to reuse them, which decreases costs [23].

C. Membrane separation.

Separation membranes are used to recover whey proteins, which can be used to make products like concentrates, isolates and hydrolysates of whey and have great nutritional qualities. The stages of pre-treatment, separation and drying are followed in order to obtain these products. It is important to consider during the process the pre-treatment, since it helps to increase shelf life of whey, and the drying, so the products obtained remain valuable. It is described that the best feasible method is ultrafiltration, combined with heat and ultrasonic pretreatment of whey in order to reuse membranes and increase the feeding rate [24]. Micro and ultrafiltration can be also used to fraction whey proteins to obtain α-lactalbumin and β-lactoglobulin. α-lactalbumin is found in large quantities in breastmilk, and it is used to make infant formulas as close to breast milk as possible. On the other hand, β-lactoglobulin can produce a gel material that has the characteristics of solid fat, and it is used to replace common fat used in processed meats [25, 26]. The use of membranes requires the develop of new materials that have higher specificity and reduce fouling, while using a tangential flow and control of pore size [27].

D. Wetland treatment.

It is reported by Ahmad *et al.,* (2019) that wetlands are used in several countries such as Argentina, Italy, Canada and Ireland in order to treat DWW [11]. Wetland treatment uses the microorganisms that effluents have to remove pollutants as they serve them as food, using photosynthesis and the principle of sedimentation. They are also not deeper than two feet in order to enable the previously mentioned processes [28]. These treatment has been used for residential and municipal wastewaters. Although it requires simple construction, low operation costs and the sludge has been used to grow Rhizobium, which reduces the cost of some biofertilizers, it presents some disadvantages such as the needing of a large area, it attracts insects, and, most important, it seems a risk for surface and ground water if absorbed [11].

VI. Reported Biorefineries

This section will review the reported biorefineries for the conversion of whey and DDW, in which some of the individual processes were integrated.

In a work made by Kopsahelis *et al.,* (2018) a biorefinery approach was described for wine lees and cheese whey, both agroindustrial wastes. The proposed products were ethanol, antioxidants, tartrate salts, animal feed, oil and whey protein concentrate (WPC). The process started with wine lees being separated in liquid and solid fractions. The liquid fraction was distilled to recover ethanol, and the discarded matter was mixed with the solid fraction after antioxidants and tartrate salts extraction. Then, this mix was treated with enzymes, becoming a yeast extract. While these processes were taking place, whey was ultra-filtrated, obtaining WPC and a lactose permeate. Both, lactose permeates and yeast extract, were incorporated to carry out a fermentation process from which two products, oil and animal feed, were finally obtained. Further research is needed in order to extract, with high quality, as many products as possible, find new products and optimizing the biorefinery design [29].

Hemalatha *et al.,* (2019) proposed the treatment of DWW within a biorefinery scheme using microalgae as treatment [30].

As reviewed in the biological treatment section, microalgae have the ability to grow quickly, while being used to remove nutrients and accumulate oils. Particularly, DWW enabled growth, metabolites formation and CO_2 fixation. The biorefinery proposed by the authors included a first step where microorganisms were cultured in DWW, obtaining microalgae biomass which was then treated to extract oil. Then, on a second step, the defatted biomass was hydrolyzed with sulfuric acid to transform complex sugars into simple ones. Hydrolysis resulted in 30 % of fermentable sugars, such as glucose, fructose, galactose and mannose. These were finally fermented with *Saccharomyces cerevisiae* to generate almost 116.2 mg g^{-1} of bioethanol. It was proposed that the remaining water can be used to irrigate plants. DWW can be treated within a biorefinery scheme with microalgae obtaining diverse products. In this way, both waste water and the microalgae are exploited to the maximum, generating economic gains from the sale of the products and environmental benefits by reducing the amount of nutrients contained in DWW. The authors highlight that, in order to have a more viable process, the extraction of oil, conditions of hydrolysis and fermentation need to be optimized.

VII. Perspectives: insects as an alternative treatment

The use of insects is gaining relevance as a biological treatment of organic wastes. Specifically, black soldier fly larvae (BSFL) have attracted attention thanks to their ability to degrade a wide variety of wastes. To our knowledge, there are no reports of the use of larvae to treat the wastes mentioned here, such as whey or DWW and even less, reports of their use in biorefinery schemes. These organisms have showed conversion efficiencies of 60-70 % on a wide variety of substrates and do not approach to humans [31]. Among the products that can be obtained from these insects are protein, lipids and chitin. One study made by Cappellozza *et al.,* (2019) described a first approach to convert fruit and vegetable leftovers from markets into protein for fish feeding and oil using BSFL; the residual biomass was later used to grow earthworms that transformed it into compost [32].

Therefore, a biorefinery scheme using BSFL as biological treatment promises to offer environmental and economic benefits, thanks to their ability to generate a wide variety of products while transforming waste.

VIII. Conclusions

As reviewed in this paper, an important number of processes have been proposed in order to treat whey and dairy waste water and obtain new value-added products. However, the anaerobic fermentation process is the most widely used for the treatment of these wastes, and this can be observed in the proposed biorefineries that include it in their design. This process, although it has its advantages, also has challenges that need to be further researched. In the perspectives section, some proposals to use insects, specifically BSFL, as waste treatment were reviewed. Based on this, the use of BSFL to treat dairy waste emerges as an alternative to dairy waste disposal. This new alternative promises shorter degradation time, higher conversion efficiency as well as a greater variety of value-added products to be obtained.

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