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Review

From waste to resource: A review on advancing whey permeate valorization through anaerobic digestion

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ABSTRACT

The increasing demand for whey protein has led to a significant production of whey permeate as the byproduct. It is characterized by its high organic load, mostly in the form of lactose, but low in other nutrients. This represents both environmental risk and resource with high valorization potential. Despite of this, valorization of whey permeate is still less common when compared with other forms of wastewaters from the dairy industry. Among several methods to valorize the byproduct, anaerobic digestion (AD) has emerged as an attractive solution by offering simultaneous bioresource recovery and organic load removal. This review synthesizes current knowledge on whey permeate valorization through AD. First, whey permeate production, composition and current findings on direct and indirect utilizations of whey permeate are outlined. It is highlighted that while current utilization methods offer add value, many of whey permeate utilizations remain constrained by limitations (e.g., consumer acceptance, lactose crystallization, limited processing capacity and complexities for large volumes, and generation of secondary waste) that can be tackled through AD. The theoretical foundation of AD is then presented, with focus on process stages and key factors influencing AD performance. Published studies on whey permeate AD are critically reviewed, highlighting experimental designs, AD performance, and methodological limitations. This review identifies strengths in current approaches while underscoring persisting challenges such in effective optimization strategies. Finally, future research perspectives are discussed, pointing towards standardization of terminology to enhance reproducibility, process optimization, and viable route for advancing whey permeate AD.

1. Introduction

Cheese manufacture generates nutrient-rich byproducts originating from milk known as cheese whey. In cheese manufacturing process, cheese whey accumulates for 70–90 % of the total milk used (Panesar and Kennedy, 2012; Walstra et al., 2005). The high nutrient availability in cheese whey enable the opportunity for further processing of the product (Ahmad et al., 2019). Among several strategies, protein recovery of cheese whey by membrane filtration has been widely applied (Ganju and Gogate, 2017). The membrane filtration generates two streams: (a) whey permeate which consists of lactose and remaining constituents that pass through the filter, and (b) retentate which consists of protein and other constituents that is too large to pass through the filter. This process allows the production of protein-rich whey concentrate and lactose-rich whey permeate (Banaszewska et al., 2014). While whey protein concentrate is widely utilized in food industries for their nutritional value and versatile functionality, whey permeate as the byproduct has limited applications. Typically, whey permeate is used as raw material for lactose production due to its high lactose content. However, processes required for this approach, such as drying, demineralization, and purification are costly, especially considering the relatively low commercial value of the final product. Furthermore, lactose utilization in food applications is restricted due to lactose intolerance in certain individuals (O'Donoghue and Murphy, 2023).

Currently, the manufacture of whey protein products has reached 2.8 million tonnes in 2020 and estimated to reach 3.1 million tonnes by

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2029 (OECD/FAO, 2023). A technology and economic feasibility study of whey processing alternatives has concluded that the transformation of cheese whey into whey protein generates a large stream of whey permeate and requires further processing due to large lactose fraction (Peters, 2005). While industries have explored different strategies to enhance the economic feasibility and to minimize disposal, the commercial value of whey permeate remains low due to the complexity and high costs associated with production. On the other hand, a direct disposal of whey permeate leads to environmental pollution due to the high organic load of whey permeate (Bella and Rao, 2023; O'Donoghue and Murphy, 2023). In order to resolve this problem, physicochemical treatments have been applied to remove the organic load of the dairy waste (Arvanitoyannis and Giakoundis, 2006). However, the effectiveness of these treatments is limited due to the high reagent costs and inadequate removal of soluble organic matter (Ahmad et al., 2019). A cheaper and better option for organic load removal is biological treatment. Several biological methods commonly used for treating dairy wastewater include aerobic treatments (e.g. pond systems and activated sludge processes), and anaerobic digestion (Ahmad et al., 2019). Between these options, anaerobic digestion has shown its superior practicality due to the lack of requirement for aeration, minimal excess sludge production, and low land area demand. (Demirel et al., 2005).

Anaerobic digestion (AD) technology has become widely adopted for the treatment and recycling of organic waste (Saravanakumar et al., 2023). This technology offers a sustainable solution for managing the growing amounts of food waste while also producing valuable by-products throughout the process (Gottardo et al., 2017; Guimarães et al., 2018; Li et al., 2018). During the AD process, organic matter is removed by microbial consortia, including bacteria and archaea, in the absence of oxygen (Angelidaki et al., 2018). The primary product of AD is biogas in the form of methane (CH_4), which can be utilized for the production of renewable energy. In addition, the process also produces a nutrient-rich digestate that can be converted into biofertilizer (Chen et al., 2020), which can serve as a substitute for chemical fertilizers (Grigatti et al., 2020; Zeng et al., 2016). Furthermore, AD also generates other valuable intermediary compounds, such as short-chain volatile fatty acids (VFAs) and hydrogen gas (H_2), which can be used as an efficient carbon source in wastewater treatment and as biofuel, respectively (Lackner et al., 2018; Tampio et al., 2019; Tian et al., 2018). Most existing reviews primarily focus on the AD of whey and whey-derived products with high protein content. There has been limited attention given to recent and emerging applications of whey permeate as AD feedstock. In this paper, current findings on whey permeate production, characterization and utilization, as well as operational parameters that affect AD performance, and current findings on AD of whey permeate are reviewed. This review aims to provide information for future works to further optimize the whey permeate AD with potentially reproducible and high-quality data.

2. Whey permeate production and composition

Whey permeate is produced from the filtrate generated during membrane filtration of whey. Typically, whey permeate has 2–7 % protein (primarily non-protein nitrogen or NPN), 76–86 % lactose, 0–1 % fat, and 8–11 % ash (ADPI, 2022; Bosco et al., 2018; Jelen, 2009), with significant variation depending on the different types of permeate, suppliers and processing conditions. Research by Tsermoula et al. (2023) has further described that the NPN composition of whey permeate can vary significantly due to factors such as whey type and processing conditions. Similarly, Smith et al. (2016) has shown that whey permeate produced from lactic acid processed whey tends to have higher NPN content due to increased proteolysis by lactic acid cultures at prolonged fermentation period. Other researchers have added that the whey permeate has high biological (±67,000 mg/L) and chemical oxygen demand (±76,000 mg/L) levels (Cox and MacBean, 1977; Domingues et al., 2001). A study performed by Macedo et al. (2002) has shown that whey permeate powder contains low concentrations of protein (3.5 %), lactic acid (2 %), and ash (8 %), and high lactose concentration (83 %) as the major compound in the product. Many studies examining the mineral composition of whey permeate have identified potassium (K) as the most abundant mineral, followed by sodium (Na) or calcium (Ca) (Cervantes et al., 2020; Frankowski et al., 2014; Jiang, 2011; Majore and Ciprovica, 2022). However, it is important to note that mineral composition of whey permeate can also be varied depending on certain processes leading to its production. For example, acid-precipitated whey permeate typically contains higher Ca levels than sweet whey permeate, as ionized Ca remains in whey fraction during acid precipitation. In contrast, Ca binds to casein under rennet precipitation by forming calcium caseinates and ultimately remains within the curd structure. Several studies have shown that different sources of whey permeate have different physicochemical properties (Table 1). As mentioned before, these differences are expected as whey permeate can be produced from different sources and undergoes different processes for various purposes. It is therefore important for whey permeate to be characterized prior to being further utilized to produce a more consistent and reproducible results.

Moreover, it is found in the published literature that different terminologies have been used to identify whey permeate as a product. The difference typically depends on the source of whey and filtration methods used (Table 2). This could raise concerns for a clearer definition of whey permeate due to potential cause for confusion and poor research reproducibility. For example, Murad and Foda (1992), use the term milk permeate to address what is supposed to be whey permeate as the substrate in their research. In contrast to whey permeate, milk permeate is produced through ultrafiltration of milk, resulting in a cleaner byproduct that is free from various additives, such as rennet, enzyme, or starter culture (Byylund, 1995; Oliveira et al., 2019). Furthermore, whey permeate might not only be sourced from cheese production. A report by Bentahar et al. (2019) used the term "acid whey permeate" for Greek yogurt ultrafiltrate. In this scenario, both whey permeate from cheese and yogurt production were exposed to fermentation process prior to filtration. It is also found that parallel works from the same authors have used different terminologies to identify the same whey permeate product, where the term "whey ultrafiltrate" was initially used (Roy et al., 1986), but changed to "whey permeate" in later publications (Roy et al., 1987).

In addition, the usage of term "deproteinized whey" to refer to whey permeate, as reported by Schultz et al. (2006), may lead to confusion and should better be avoided. The reason to this is because "deproteinized whey" can be produced through thermal centrifugation or precipitation instead of membrane filtration, in which whey permeate is produced (Arslan et al., 2016; Coelho Sampaio et al., 2016). In contrast to membrane filtration, thermal precipitation of cheese whey produces secondary cheese whey, also known as scotta (Tirloni et al., 2020). In order to avoid further confusions, O'Donoghue and Murphy (2023) have explained a specific terminology to better describe the whey permeate type based on the production process (Fig. 1). By following this approach, a specific type of whey permeate could be defined accurately and improve reproducibility of research focused on whey permeate utilization.

3. Current applications of whey permeate

The high lactose content with low concentration of other nutrients in whey permeate is a major challenge in utilizing this byproduct. There are currently two applied pathways for whey permeate utilization. The first is through direct utilization of whey permeate by changing its physicochemical properties together with other materials, and the second is through indirect utilization by using whey permeate as substrate to generate novel products. In the following subsections, both direct and indirect utilization of whey permeate are briefly discussed. In general, although the direct and indirect applications discussed offer promising

Table 1
Physicochemical properties of whey permeate.

Composition	References							
	1	2	3	4	5	6	7	8
Dry matter	15.97 %	23.26–24.0 %	n/a	4.87 %	n/a	n/a	6.41 %	5.9 %
Crude protein	1.35 %	2.6–2.75 %	n/a	0.17 %	3.5 %	4.2 g/L	n/a	n/a
Total Kjeldahl Nitrogen	0.6 %	n/a	4.78 %	n/a	n/a	n/a	0.047 %	0.06 %
Nonprotein Nitrogen	n/a	0.36–0.37 %	n/a	n/a	n/a	n/a	0.036 %	0.04 %
Mineral (ash)	1.5 %	7.63–8.67 %	9.42 %	n/a	8.5 %	8.5 g/L	0.54 %	0.74 %
Total carbon	41.98 %	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Crude fat	n/a	0.03–0.07 %	0	n/a	n/a	1	n/a	n/a
Lipid	n/a	n/a	n/a	0.1 %	n/a	n/a	n/a	n/a
Lactose	11.23 %	81.6–88.1 %	84 %	n/a	83 %	48 g/L	5.8 %	4.55 %
Total sugars	n/a	82.5–88.7 %	n/a	n/a	n/a	n/a	n/a	n/a
Organic acids	n/a	4.1–6.18 %	n/a	n/a	n/a	n/a	n/a	n/a
Calcium	n/a	0.67–0.68 %	n/a	n/a	n/a	3.3	0.05 %	0.14 %
Phosphorus	n/a	0.72–1.12 %	0.683 %	n/a	n/a	n/a	0.12 %	0.26 %
Magnesium	n/a	0.13–0.17 %	n/a	n/a	n/a	n/a	0.01 %	0.07 %
Potassium	n/a	1.60–2.00 %	n/a	n/a	n/a	n/a	0.18 %	0.17 %
Chloride	n/a	1.12–2.04 %	n/a	n/a	n/a	n/a	0.15 %	0.11 %
Sodium	n/a	0.67–1.24 %	n/a	n/a	n/a	n/a	0.06 %	0.05 %
Iron	n/a	5.31–45.81 mg/kg	n/a	n/a	n/a	n/a	n/a	n/a
Copper	n/a	4.16–5.32 mg/kg	n/a	n/a	n/a	n/a	n/a	n/a
Zinc	n/a	9.82–20.82 mg/kg	n/a	n/a	n/a	n/a	n/a	n/a
pH	5.14	5.54–5.76	5.7–6.0	6.5	n/a	n/a	n/a	4.4
Density	n/a	1.10–1.11 kg/L	1.023 kg/L	n/a	n/a	n/a	n/a	n/a
Viscosity	n/a	2.2–3.5 cP	n/a	n/a	n/a	n/a	n/a	n/a
Lactic acid	n/a	n/a	3.3 %	n/a	2 %	n/a	n/a	n/a

n/a means not available. (1) Source: acid whey obtained from creamery plant in Surrey, UK, analyzed by the authors at the University of Reading; (2) Source: Canadian cheese, from Parashar et al. (2016); (3) Source: Sweet cheese whey, from González et al. (2007); (4) Source: Gorgonzola cheese whey, from Barile et al. (2009); (5) Source: whey permeate powder, from Macedo et al. (2002); (6) Source: cheese whey, from Atra et al. (2005); (7) Source: cheddar cheese, from Hobman (1984); (8) Source: lactic acid casein, from Hobman (1984). Composition for reference 1, 2, and 3 was based on dry weight basis; composition for reference 4, 5, 6, 7, and 8 was based on wet basis.

valorization opportunities for manufacturers, they come with certain challenges. Many of these applications have low economic value and may face challenges such as limited consumer acceptance, difficulties in handling due to crystallization, process complexities, and processing constraints that restrict the quantities of whey permeate that can be utilized. In addition, whey permeate also contains a variety of other components aside from lactose, such as minerals and NPN that can potentially hinder the bioconversion process even at low concentrations. Considering these challenges and limitations, indirect whey permeate utilization through anaerobic digestion (AD) technology presents as an attractive option. This is due to the versatility of AD in processing a wide range of feedstocks to generate biogas, along with valuable byproducts such as volatile fatty acids (VFAs), while simultaneously reducing the organic load. As a result, AD offers a complete circular economy loop by minimizing the need for any further waste disposal. The operational parameters of AD and recent findings on the use of whey permeate as AD feedstock will be presented in more detail later in this review.

3.1. Direct application of whey permeate

The direct application of whey permeate generally aims to improve the byproduct versatility for downstream process and to improve nutrient qualities in the finished product, whether through incorporation with other ingredients or utilizing whey permeate as salt or sugar replacement. The common direct utilization of whey permeate is to change its physical properties through drying for easier transport and prolonged shelf-life. The moisture removal of whey permeate enables the production of lactose concentrate. In its natural state, whey permeate is a liquid with white to yellowish colour. The moisture removal of whey permeate allows the production of lactose concentrate. Furthermore, this drying process also results in the crystallization of lactose (Ibach and Kind, 2007). The occurrence of lactose crystallization in dried whey products can act as a limiting factor in certain applications, such as when used as pig feed (Woyengo et al., 2015), while simultaneously offering beneficial properties in other contexts, such as its utilization as a licking block for cattle (Lynch and McDonough, 1979). Aside from drying, directly incorporating whey permeate into other products has also been done to obtain better valued and/or alternative products. This approach is commonly found in food, beverages, and animal feed.

The utilization of whey permeate for food and beverage products is among the initial efforts to valorize this byproduct. However, even though whey permeate is generated in a food grade environment, its poor nutritional value limits its utilization for food and beverage products. Apart from its nutritional value, organoleptic properties also play an important role in the utilization of whey permeate as food and beverage products. Organoleptic properties provide relevant information on how the product can be valorized as food and beverage, especially with consumers acceptability. A study by Babenyshev et al. (2016) has investigated the organoleptic properties of raw whey permeate from cow milk. Their study reported that whey permeate has the appearance of homogenous liquid, clear, with greenish to yellow colour, sour taste with strong whey-ish aftertaste, has fermented-milk odour and clean flavour. The use of whey permeate as food product primarily leverages lactose as a reducing sugar, facilitating Maillard browning when combined with proteins, thereby enhancing the flavour of the final product. Milner et al. (2020) explored the use of whey permeate as a substitute for sucrose in sponge cakes, achieving a 21.5 % reduction in total sugar without significantly altering the cakes' sensory characteristics compared to control, while also increased the cakes' overall moisture content. For production of foods that involve yeast fermentation (e.g., bakery products), lactose remains unfermented by the yeast. This allows lactose to take part in Maillard browning, which enhances both colour and flavour of the finished product.

Moreover, several studies have also explored the use of whey permeate as salt replacer/reducer. As previously discussed, certain types of whey permeate could have high mineral composition, making it an ideal material for sodium-reduced foods production. Jiang (2011) conducted a study in which whey permeate powder was used as a partial replacement for salt in a smoked sausage at 0 %, 25 %, 50 %, 75 %, and

Table 2
Terminologies used to identify whey permeate in published works.

Name	Substrate	Process pre-treatments	Filtration and separation method	References
Acid whey permeate	Greek yogurt	Pasteurization of skim milk at 90 °C for 5 min, followed with direct vat set culture inoculation to produce Greek yogurt.	Ultrafiltration (Koch HF, 1018–1.0-43-PM50)	Bentahar et al. (2019)
Cheese whey permeate	Cheddar cheese whey	n/a	Ultrafiltration (HF-15-43-PM 50, Romicon HF 2 SSS)	Boyaval and Goulet (1988)
	Gorgonzola cheese whey	Inoculation of whole cow milk with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* along with spores of *Penicillium roqueforti.*	Cross-flow filtration (Ultran®-miniflex, Whatman, Switzerland)	Barile et al. (2009)
	Cheddar cheese whey powder	Rehydration of spray-dried cheese at in a 1.5 % (w/v) yeast extract solution.	Ultrafiltration (Romicron HF2SSS equipped with 2 p.m.–50 hollow-fiber cartridges)	Roy et al. (1987)
Deproteinized milk serum	Cheddar cheese	n/a	Ultrafiltration (not specified)	Hobman (1984)
	Lactic acid casein	n/a	Ultrafiltration (not specified)	Hobman (1984)
Deproteinized whey	Whey (not specified)	n/a	Cross-flow filtration and sterile filtration	Daniel et al. (1999)
	Sweet whey and sour whey	n/a	Cross-flow filtration and sterile filtration	Schultz et al. (2006)
	Cheese whey	Process separation (not specified) to obtain crude whey	Thermal filtration of the crude whey at 90 °C for 15 min	El-Gindy (2003)
Salted whey permeate	n/a	n/a	Ultrafiltration (not specified)	Murad and Foda (1992)
Sweet whey permeate	n/a	n/a	Ultrafiltration (not specified)	Murad and Foda (1992)
Ultrafiltered whey	Sweet cheese whey	Sterilization at 105 °C for 20 min, followed with *Lactobacillus helveticus* inoculation at 42 °C	Ultrafiltration at 15 kDa (P = 3 bar; v = 3/ms; temperature at 40 °C.	González et al. (2007)
Whey lactose	Whey	n/a	Microfiltration by using 0.22 μm membrane (Pellicon system, Millipore) followed with 0.45 μm membrane filtration and 10 kDA ultrafiltration (Pellicon system)	Mariotti et al. (2008)
Whey ultrafiltrate	Cheddar cheese whey	n/a	Ultrafiltration (Abcor HFA 180 membranes)	Cox and MacBean (1977)
	Cheddar cheese whey powder	Dissolution of cheddar cheese whey powder in distilled water with 6.5 % w/v concentration.	Ultrafiltration (Romicron HF2SSS; with PM-50 hollow-fiber cartridges and molecular weight cut-off of 50,000)	Roy et al. (1986)
	Cheese whey	n/a	Ultrafiltration (Millipore, Pellicon cassette system)	Chiarini et al. (1992)
	Sweet whey	Phospholipoportein (PLP) elimination through calcium precipitation and thermal treatment.	Ultrafiltration (not specified)	Kulozik and Wilde (1999)
	White brined cheese whey	n/a	Ultrafiltration (GR61PP membranes)	Frengova et al. (2004)

n/a not available.

100 % substitution levels. Their consumer survey involving 100 participants revealed no significant differences in overall liking, flavour, saltiness, texture, firmness, or juiciness between sausages with salt replacement ratio of 25 % whey permeate powder and 75 % salt as compared to those without salt replacement (100 % salt). However, it is important to consider the type of permeate used for a specific application, as various permeates exhibit distinct flavour profiles. For example, acid whey permeate tend to have a more sour taste than sweet whey permeate due to higher levels of acids and therefore lower pH values (Smith et al., 2016).

Besides its application in food, there is also an attractive option to utilize whey permeate as a functional beverage for sport drinks due to its mineral and lactose content. Sports drinks are formulated with minerals and carbohydrate aiming to both restore fluid balance and energy to support exercise performance (Sawka et al., 2007). Given its mineral and carbohydrate content, whey permeate serves as a suitable ingredient for sports drink formulations. Several studies have conducted sensory evaluations on various electrolyte and sports drink formulations incorporating permeates. These beverages typically consist of water, whey permeate, and flavouring or fruit juice extracts, such as orange (Olson, 2003), pineapple or passion fruit (Ferreira et al., 2021), and jabuticaba berry and red fruit (Ferreira et al., 2020). Beucler et al. (2005) examined the substitution of water with whey permeate at different levels (0 %, 25 %, 50 %, 75 %, and 100 %) in a fruit-flavoured, noncarbonated sports drink designed for hydration. Sensory analysis revealed that beverages with lower whey permeate concentrations (25 % and 50 %) had good consumer acceptability, comparable to commercial products. However, higher whey permeate levels resulted in undesirable "brothy" and "dairy-sour" flavours, posing a significant challenge for manufacturers in developing permeate-based beverages.

Another use of whey permeate in beverage industry is by inoculating the byproduct with microorganisms in order to improve the nutritional and/or economical values through fermentation. Aamer and El-Kholy (2017) developed functional beverages rich in phenolic compounds by combining whey permeate with kumquat fruit paste and fermenting the mixture using yogurt cultures (*Streptococcus thermophilus* and *Lactobacillus delbrüeckii* subsp. *bulgaricus*). Similarly, M'hir et al. (2021) explored the use of kefir grains, which naturally contain various lactic acid bacteria (LAB) and yeasts, in carob-based health beverages formulated with different ratios of oat flour and whey permeate. Their findings indicate that the optimal formulation for LAB and yeast growth, as well as consumer acceptability, consisted of 11.51 % whey permeate and 4.77 % oat flour. Aside from functional beverages, whey permeate has also been used to produce low-alcoholic beverages. The concept of fermenting whey permeate into alcoholic beverages was initially started from the whey-based spirit production, which has been commercially developed and commercialized in 1978 (Hughes et al., 2018). The lack of protein content in whey permeate minimizes precipitation during distillation process, thus overcoming challenges associated with precipitation during alcohol distillation. The industrial production stream of whey permeate into alcoholic beverages typically follows a process similar to microbial ethanol production by following the Carbery process (Fig. 2).

Whey permeate has also been observed for its potential to be used in

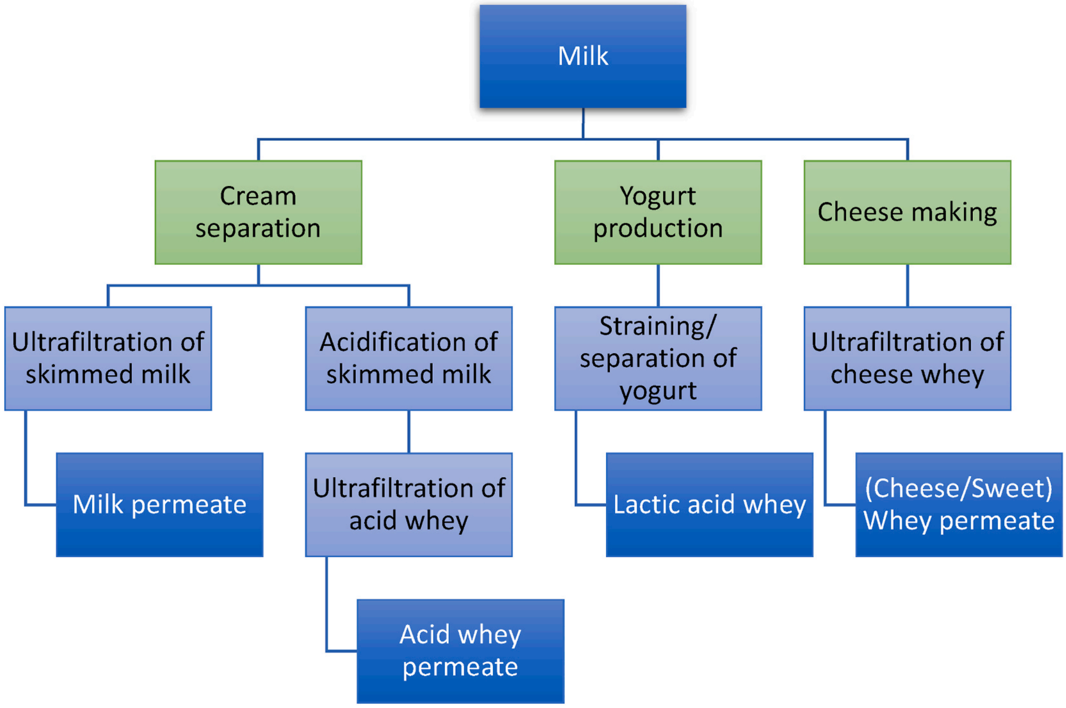

Fig. 1. Simplified process flow chart for whey permeate production. Adopted and modified from (O'Donoghue and Murphy, 2023).

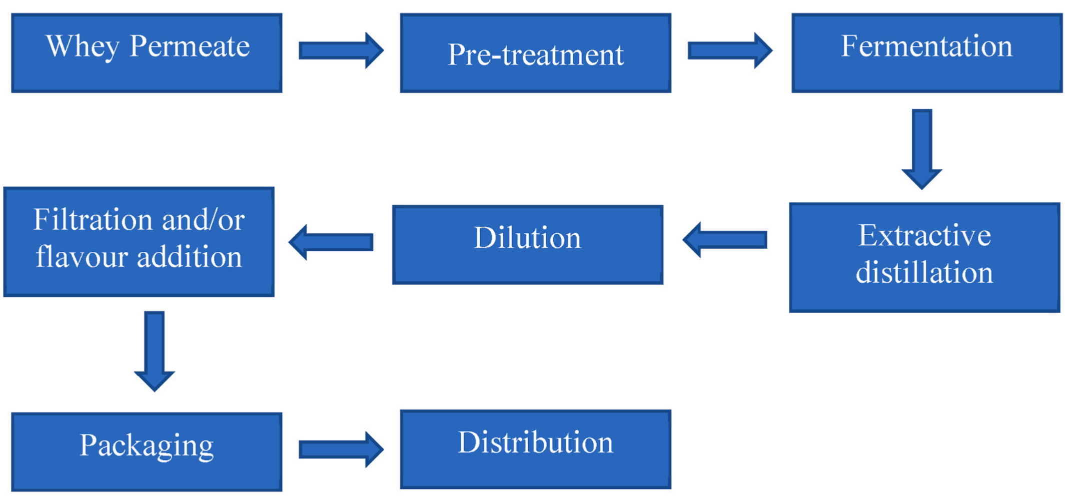

Fig. 2. Processing stream of whey permeate into alcoholic beverages via the Carbery process. Recreated from Hughes et al. (2018).

animal feed. Similar to human consumption, the utilization of whey permeate for animal feed is limited by its poor nutritional value (Diblíková et al., 2013). This leads to the utilization of whey permeate as feed supplement instead of major source of energy in animal feed. Initial work on whey permeate utilization for animal feed has been reported by Lynch and McDonough (1979). Their research showed that feeding Holstein steer calves with whey permeate and liquid protein supplement produced lower body weight gain compared to control, despite the similar dry matter intake conversion to body weight ratio between control and experimental calves. Furthermore, their research also processed whey permeate into cattle lick blocks by varying total solids, temperature, pH, and agitation. These blocks contained 0.6 % nitrogen, 70–72 % lactose, and 12 % ash, with some treatments involved increasing nitrogen level to 1.7 % by the addition of ammonia (NH_3) or urea. When it was fed to four young calves each or 60 days, it was shown that growth rates and feed efficiencies for calves fed ammonium supplemented whey permeate blocks were similar to those of calves fed with control. Research on feeding whey permeate to dairy cattle was also done by De Seram et al. (2019), where dry whey permeate was used to substitute barley grain. However, their finding also demonstrated unsatisfying results. It was demonstrated that whey permeate substitution did not affect dry matter intake, milk yield, and milk composition of the cow. Similarly, such problems and complexities of feeding whey permeate for ruminants have also been reported for liquid whey (Schingoethe, 1976), whereas the best feeding regime often does not show significant positive effects towards feeding efficiencies, while also having the risk of excessive urination, teeth erosion, as well as bloating when liquid whey was not given properly.

3.2. Indirect application of whey permeate

As mentioned earlier, whey permeate is an abundant source of lactose, offering significant potential for developing value-added products that can serve as ingredients in food industries. Aside from direct whey permeate utilization, the byproduct can be indirectly valorized to generate novel products. However, the sole carbon content in the form of lactose limits the bioconversion of whey permeate. To overcome this, whey permeate is typically fermented with lactic acid bacteria. During this process, lactose undergone several pathways, such as tagatose, glycolytic, Leloir, and phosphoketolase pathway (Fig. 3). The smaller carbon compounds (glucose and galactose) from lactose degradation can then be further utilized by other microorganisms, whether through multi-steps fermentation and/or co-fermentation, leading to the generation of final products with added value. In this scenario, the final products are generated in two ways, the first is by converting lactose into other useful carbon compounds (organic acids, biomethane, polymers, and etc.), and the second is by using whey permeate as a growth medium for fungi and microalgae to produce targeted products, such as bacteriocins, prebiotic carbohydrates, and microalgae. A summary of indirect utilization into novel products (organic acids, prebiotic carbohydrates, bacteriocins, and microalgae) is presented in Table 3.

Indirect applications of whey permeate typically allow manufacturers to produce higher-value products or ingredients compared to direct applications. In most cases, lactose is converted into more valuable organic acids. However, the biotransformation of lactose from whey permeate still creates significant amounts of side products (O'Donoghue and Murphy, 2023). In addition, although whey permeate can be regarded as a cheap starting material, most of the indirect applications require complex processing, such as lactose purification as well as supplementation of certain additives. These extra processing steps would increase costs and pose economic challenges when whey permeate processing is the main focus of the system. Another indirect application that could both manage the need for waste processing and eliminate the need for complex valorization process of whey permeate is through anaerobic digestion (AD). However, different to whey and other dairy wastes, sustainable operation of whey permeate AD for methane production is rarely observed (Bella and Rao, 2023). In the next section, theories on AD technology and operational set ups are presented. In addition, current applications of whey permeate as AD feedstock are reviewed.

4. Anaerobic digestion

Anaerobic digestion (AD) is a well-established waste-to-energy technology that facilitates the biological transformation of organic matter into energy sources (Angelidaki et al., 2018). Furthermore, AD utilizes a simpler and more cost-effective technology that demands less energy and space compared to aerobic treatment systems. Dairy industry effluents with high organic content can serve as a valuable feedstock for biogas production through anaerobic digestion. Biogas is primarily composed of methane (CH_4) and carbon dioxide (CO_2), and can be utilized as a combustion gas to power generators for heat and electricity production. Additionally, it can serve as an alternative to natural gas for cooking, be upgraded into biomethane for fuel, or be employed in chemical synthesis (Vasudevan et al., 2020). The AD process compromises four key stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. These sequential steps outline the overall pathway involved in the breakdown of organic matter under anaerobic conditions (Fig. 4).

In general, microorganisms cannot directly utilize complex organic matter, as it must first be degraded into simpler, soluble compounds (Gujer and Zehnder, 1983). A group of bacteria known as hydrolytic bacteria facilitates the breakdown of carbohydrates, proteins, and lipids into simpler compounds such as sugars, amino acids, and long-chain fatty acids. This process occurs through the secretion of extracellular enzymes (Li et al., 2011). During the acidogenesis stage, these products undergo fermentation, leading to the formation of various short-chain volatile fatty acids (VFAs) such as formic, propionic, butyric, lactic, and succinic acids, along with ketones (e.g., methanol, ethanol, glycerol, and acetone) and alcohols. The VFA concentration is highly influenced by various factors, including pH, hydraulic retention time, organic loading rate, temperature, and nutrient availability (Ajayi-Banji and Rahman, 2022; Cysneiros et al., 2012; Jain et al., 2015; Mata-Alvarez et al., 2014; Zhang et al., 2014). Acidogenesis typically progresses at a faster rate than methanogenesis (Zhou et al., 2018). As a result, there is a possibility of VFA accumulation, leading to a decrease in pH and an increased concentration of non-dissociated volatile fatty acids. This condition can inhibit the methanogenesis process (Fezzani and Cheikh, 2010; Wang et al., 1999; Xu et al., 2014). The acids and other compounds produced during the acidogenesis are still unfavourable by methanogens. However, during acetogenesis, most of the long-chain VFAs from acidogenesis are further metabolized into acetic acid (or its salts), that is thermodynamically more favoured by methanogens to produce CH_4, along with CO_2, H_2 and H_2O. Furthermore, it should also be noted that a rise in H_2 concentration can inhibit the activity of acetogenic bacteria. To prevent this, hydrogen partial pressure must be kept low, which is regulated by the presence of hydrogen-scavenging bacteria (Harper and Pohland, 1986). During the methanogenesis stage, all intermediate products generated in the previous stages are transformed into CH_4, CO_2, and H_2O. Approximately two-thirds of the CH_4 is produced through acetate conversion and alcohol fermentation, while the remaining one-third results from CO_2 reduction.

5. Key factors influencing anaerobic digestion process

The effectiveness of AD process depends largely on several operational factors, including operating pH, organic loading rate (OLR), hydraulic retention time (HRT), temperature, and nutrient availability. These factors are essential for the design and operation of a full-scale anaerobic digester. Industrial wastewaters with high organic loads are particularly suitable for anaerobic treatment due to their significant pollutant load, potential for energy recovery, and lower sludge production. However, in practical applications, AD can face process instabilities due to the slow microbial growth rate, suboptimal digester conditions (such as pH, temperature, OLR, and HRT), and the presence of inhibitory compounds like ammonia. Problems such as the accumulation of VFA and ammonia, pH reduction, and alkalinity depletion arise due to insufficient understanding and control of the chemical reactions involved in anaerobic digestion (Lv et al., 2018; Poirier et al., 2017; Yuan and Zhu, 2016). Many of these challenges have been extensively documented in previous studies. Additional factors influencing AD include feedstock moisture content, flow patterns (such as stirred and unstirred fluid flows), and reactor configurations. However, addressing all these aspects in this review may compromise its clarity. Therefore, only selected factors are discussed below.

5.1. Temperature

Temperature plays a crucial role in determining the rate of biogas production, CH_4 content in biogas, and the overall heat requirements of the system. Anaerobic digestion can be carried out within three distinct temperature ranges: psychrophilic (15–25 °C), mesophilic (35–40 °C), and thermophilic (50–60 °C) conditions. This is due to that different microbial genera in the AD process could thrive and multiply at varying temperature ranges (Nie et al., 2021). Nevertheless, it is crucial to maintain a stable temperature within the selected range. In thermophilic digestion, even a ±2 °C fluctuation can lead to a nearly 30 % reduction in CH_4 production. In contrast, mesophilic microbes are more resilient, tolerating variations of ±3 °C without significantly impacting CH_4 yield (Zupančič and Roš, 2003). A previous study has highlighted that increasing the temperature from 20 to 40 °C and beyond 55 °C enhances the rate of gas production (Desai et al., 1994). However, in terms of energy efficiency, mesophilic conditions are recommended for acidogenic reactors. Adopting thermophilic conditions can be beneficial for

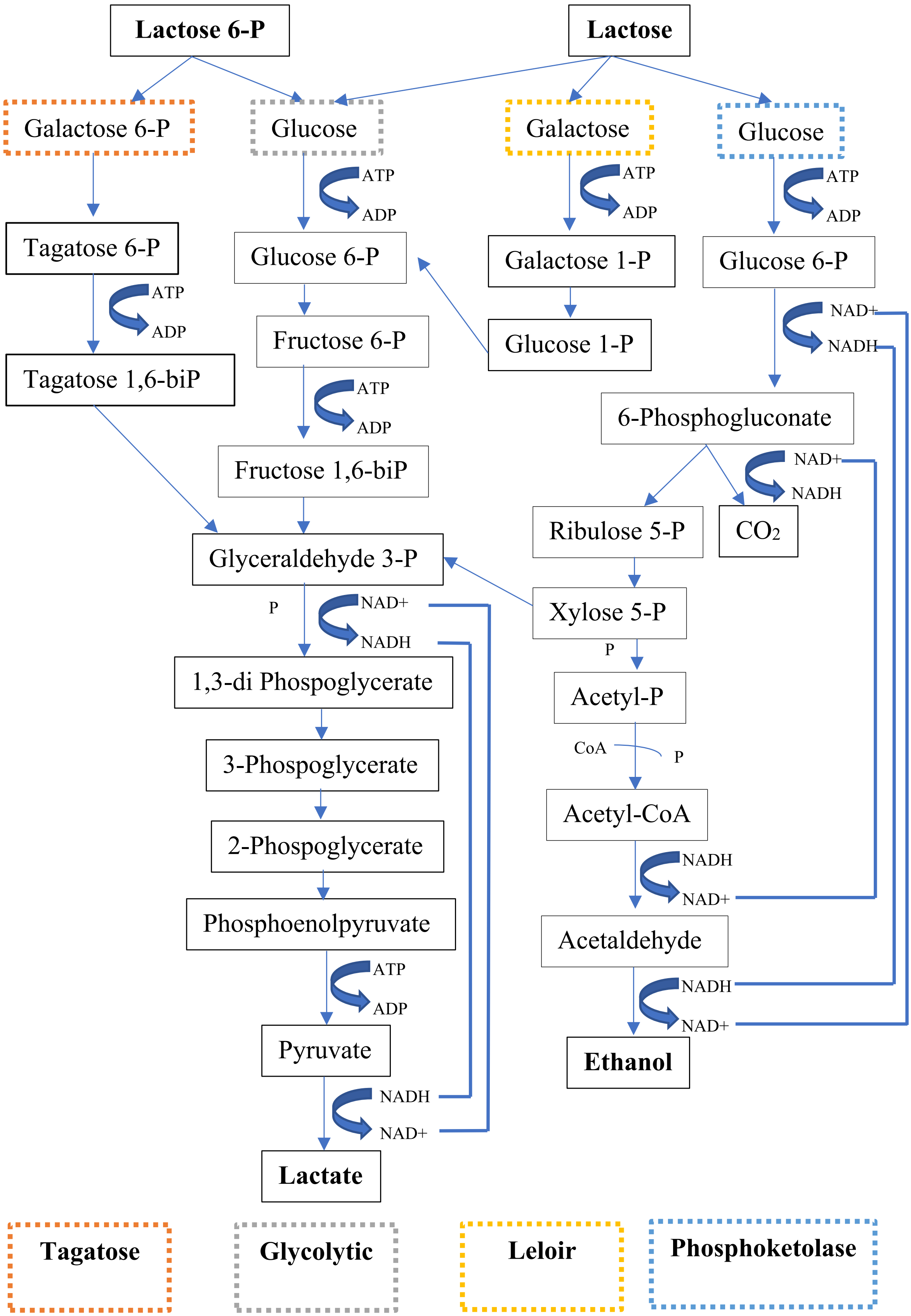

Fig. 3. Lactose metabolism pathway by lactic acid bacteria. Reconstructed from Bintsis (2018).

Table 3
Indirect applications of whey permeate for organic acids, galacto-oligosaccharides, prebiotic carbohydrates, bacteriocins, and microalgae production.

Target compound	Processing conditions	Results	Reference
Organic acids	5 % lactose, static state, 1.5 % $CaCO_3$ addition, 37 °C for 36 h	*Lactobacillus rhamnosus* B-445 and *Enterococcus faecium* strains produced the highest lactic acid yields (27 and 23.9 g/L, respectively)	Dosuky Atiat et al. (2019)
	5 % initial sugar concentration, with nutrient supplementation, 37 °C for 36 h	*Enterococcus hirae*-68 produced the highest lactic acid yield at 14.8 ± 0.09 g/L	Dosuky et al. (2022)
	Small scale (200 mL) batch fermentations in flask shaker, 30 °C for 14 h, no supplementation	*Lactobacillus casei* MT682513 produced the highest lactic acid yield (44.87 mg/mL) of the five isolates tested	Sayed et al. (2020)
	Pre-treatment with β-galactosidase to hydrolyse lactose (30 min at 50 °C). Fermentation was carried out for 48 h at either 35 or 37 °C	Results demonstrated that *P. acidilactici* had a higher lactic acid yield than *L. bulgaricus* after 24 h of fermentation	Klupsaite et al. (2019)
	100 mL batch fermentations, 20 g/L initial lactose concentration, shaking (120 rpm) on a rotary shaker at 44 °C	Lactic acid yields of 27.5 g/L were achieved using supplementation with 5 g/L peptone, 8 g/L egg extract and 0.2 g/L YE after 52 h	Lech (2020)
	Continuous and batch, two phase operation in lab scale bioreactors using silica granules as the support media for biofilm growth (for lactic acid production *Lactobacillus helveticus* was used). Lactose concentration of 9.0 g/L	For a two-phase batch system with a volume of $1.7m^3$, conversion rates of lactose to lactic acid of >90 % were possible in approximately 3.5 h	Narayanan and Narayan (2021)
	250 mL fermentations, static conditions, pH 7.0, 37 °C for approximately 25 h. The addition of a nitrogen source (whey protein hydrolysate) at a range of concentrations, as well as varying the whey permeate concentration, was examined	Whey permeate with an initial carbon concentration of 22.56 g/L produced a lactic acid of 19.14 g/L	Sharma et al. (2021)
	600 L pilot-scale batch reactor fitted with a rotary jet head system, initial lactose content of 50 g/L, pH maintained at 6.4, 38 °C for ~ 10 h	Achieved 98 % conversion of lactose to lactobionic acid	Hua et al. (2007)
	400 kg batch reactor. 49 °C for 5 h. Addition of air into substrate. Varying levels of enzyme addition under pH-static conditions (5.0–6.0) or with no pH control	Maintaining the pH (between 5 and 6 using NaOH) increased the degree of lactose conversion to >90 %, compared to 41 % with no pH control	Budtz et al. (2007)
	Initial lactose concentration of 40 g/L. Fermentation with *P. taetrolens* at 30 °C for 32 h at 250 rpm shaking. Subsequent fermentation with *L. casei*: 100 rpm shaking at 37 °C for 48 h.	Final solution contained probiotic *L. casei* strain, lactobionic acid as a prebiotic (9.52 g/L after 48 h) and a small amount of lactic acid	García et al. (2017)
	Supplemented with nitrogen sources (diammonium phosphate and Fermaid K). Fermentation conditions: anaerobic (static) and aerobic (shaking at 185 rpm) conditions, 30 °C for 34 days	Highest acetic acid yield (9.18 ± 3.38 g/L) was achieved using the yeast *Dekkera anomala* under aerobic conditions	Marcus et al. (2021)
	Enzyme inhibitors calcium-EGTA and o-iodosobenzoate were examined. Fermentation conditions; 300 mL solution, 30 °C, shaking at 200 rpm, anaerobic conditions	o-iodosobenzoate addition (0.3 mM) resulted in a 2.4-fold increase in the propionic acid production rate and 70 % reduction in the acetic acid production rate	Morales et al. (2006)
	Crude glycerol (50 g/L), CaCl2 (0.15 g/L) and yeast extract (15 g/L) addition. Initial lactose concentration of 32.5 g/L, 34 °C at pH 7.0 for 168 h	Achieved a succinic acid yield of 54 g/L	Podleśny et al. (2019)
	Batch fermentation, 34 °C, pH 7, 168 h, anaerobic conditions, initial lactose concentration of ~100 g/L and yeast extract supplementation	Successfully produced 57.7 g/L of succinic acid (represents a yield of 62 %)	Szczerba et al. (2020)
	Variety of initial lactose concentrations and supplementation with yeast extract (5 g/L) examined. Fermentation conditions: 3 L bioreactor, 46 h, 37 °C, pH maintained at 6.7	Highest succinic acid yield of 0.57 g/g achieved with an initial sugar concentration of 43 g/L using *A. succinogenes*	Terboven et al. (2021)
Prebiotic carbohydrates	Examination of two initial lactose concentrations (50 and 200 g/L) and temperatures (37 °C and 50 °C)	Achieved GOS yield of 50 % (of total sugar) after 5 h at 50 °C	Geiger et al. (2016)
	Fermentations were carried out at two different pH values (4.5 and 7.0) and subjected to shaking (200 rpm) at 35 °C for 12 h.	The highest GOS yield (25 g GOS/100 g lactose) was achieved using Lactozyme™ from *Kluyveromyces lactis*	Mano et al. (2019)
	Whey permeate was concentrated to 30 % lactose using nanofiltration and evaporation. Enzymatic reaction completed at 55 °C for 2 h	Maximum GOS yield of 74 % (g GOS/g lactose) achieved after 0.5 h	Orrego and Klotz-Ceberio (2022)
	Initial lactose content of 205 g/L (pure lactose solution) or 194 g/L (whey permeate). Reactions carried out at 25 L scale at 17 °C for 6 h	Lactose conversion rate was slightly higher in whey permeate but had a slightly lower final GOS yield (25 % of total sugar) compared to pure lactose solutions (27–28 %)	Splechtna et al. (2007)
	Batch reactions were carried out at 37 °C and pH 6, with initial lactose concentrations of 200 g/L	Total GOS production was 7 % higher in the pure lactose solution compared to the whey permeate	Cho et al. (2003)
	β-galactosidase from *Aspergillus oryzae*. A variety of flow rates (0.5–6 mL/h) were investigated at 60 °C and pH 5.2	Achieved a maximum GOS yield of 39.3 % (56.4 % lactose conversion) in whey permeate after the second cycle reaction using immobilized beads in the packed-bed bioreactor	Eskandarloo and Abbaspourrad (2018)
	A variety of batch and continuous experiments were carried out at a range of initial lactose contents (14, 20 and 23 %), temperatures (35 or 45 °C) and enzyme concentrations	Maximum oligosaccharide yield of 31 % was achieved using the pilot scale UF-hollow fiber membrane reactor and whey permeate with 20 % lactose concentration	Foda and Lopez-Leiva (2000)
	Reactions carried out at a variety of residence times and initial lactose concentrations (6, 10 and 20 %), 40 °C at pH 4.5	Achieved lactose conversion rates (to GOS) between 25 and 45 %, depending on initial lactose concentration	Leiva and Guzman (1995)
	Batch process: varying lactose concentrations (5–30 %) and temperatures, pH 6.6 for 5 h. Recycled membrane reactor: flow rate of 2×10^{-8} m^3/s, transmembrane pressure of 0.15 MPa	GOS production was approximately 54 % higher in the membrane reactor compared to the batch reactor	Das et al. (2011)
	Isomerisation (using eggshell at 98 °C and pH 6.8) followed by enzymatic transgalactosylation (using β-galactosidase from *B. circulans*)	Maximum GOS yield (40 % GOS and 11 % lactulose) was achieved using isomerised whey permeate with an initial carbohydrate concentration of 300 g/kg at pH 6.5 and 50 °C after 5 h	Corzo-Martínez et al. (2013)

(*continued on next page*)

Table 3 (*continued*)

Target compound	Processing conditions	Results	Reference
	Enzymatic transgalactosylation (pH 6.5, 50 °C, 4 h) followed by isomerisation using sodium aluminate as catalyst (40 °C for 24 h)	Nearly 50 % of lactose was converted into prebiotic carbohydrates after 6 h of isomerisation	Padilla et al. (2015)
	Catalytic conversion using 0.5 g/L ruthenium on carbon (Ru/C) in a continuous stirred-tank reactor at 60 °C, pressure of 60 bar, stirring rate of 600 rpm for 210 min	Maximum lactose conversion into prebiotic carbohydrates after 210 min of 34 and 37 % for acid and sweet WP respectively	Enteshari and Martínez-Monteagudo (2020)
	Samples (6 % whey permeate solution or 5 % lactose solution) were subjected to high alkaline conditions in the reactor for 63 min	39.78 % of lactulose from whey permeate after 35 min of at 330 mA	Djouab and Aïder (2019)
Bacteriocins	Whey permeate concentration of 6 % (w/v), Supplementation with yeast extract or Tween 80, batch fermentation (50 mL) at 30 °C	Maximum nisin Z content (4100 IU/mL) in non-aerated cultures was achieved after 8 h with pH between 5.5 and 6.5	Amiali et al. (1998)
	Cells immobilized in k-carrageenan/locust bean gum gel beads. Whey permeate concentration 6 % (w/v), supplementation with KCl, yeast extract and Tween 80. Fermentations at pH 6 and 30 °C	Maximum nisin Z production (8200 IU/mL) achieved after 1 h cycles	Bertrand et al. (2001)
	Whey permeate concentration of 6 % (w/v), supplementation with KCl, yeast extract and Tween 80. Immobilization in k-carrageenan/locust bean gum gel beads. Fermentations at 30 °C and pH maintained at 6	Highest nisin Z production (2560 IU/mL) from an aerated, continuous free cell fermentation with a dilution rate of 0.15 h	Desjardins et al. (2001)
	Whey permeate was supplemented with yeast extract or casein hydrolysate at different levels. Variety of fermentation conditions (temperature, pH etc.) examined	Maximum nisin (5.1×10^4 AU/mL) was obtained using continuous fermentation (pH 5.5, 31 °C) of whey permeate supplemented with casein hydrolysate	Liu et al. (2005)
	Supplementation with yeast extract at varying concentrations and Tween 80 (0.1 %) addition. Fermentation at pH 6.5 and 37 °C for 24 h	Highest pediocin C20 yield (150×10^3 AU/mL) was achieved in whey permeate supplemented with 2 % yeast extract	Halami and Chandrashekar (2005)
	6 % (w/v) whey permeate, supplemented with yeast extract, Tween 80 and glucose. Incubation with *P. acidilactici* for 8 h, followed by addition of *L. lactis.* Temperature of 37 °C and pH maintained at 5.5	Highest nisin (730 AU/mL) and pediocin (1360 AU/mL) achieved in mixed strain fermentation after 18 or 16 h incubation, respectively	Goulhen et al. (1999)
	Supplementation with yeast extract, Tween 80, $MgSO_4$ and $MnSO_4$ at varying concentrations. Fermentations (50 mL vol) at 30 °C for 24 h	Highest bacteriocin production in WP (2048 AU/mL) after 9 h occurred in a sample supplemented with 2 % yeast extract and 0.1 % Tween 80	Daba et al. (1993)
	Variety of supplements (meat extract, yeast extract, arginine, Tween 80, vitamins and minerals) were investigated. Fermentation at 26 °C with varying percentages of inoculum addition	Optimum formulation (based on cost effectiveness) for bacteriocin production (yield of 0.270×10^6 Al/L) was whey permeate supplementation with meat extract (4 g/L) and yeast extract (8 g/L)	Musatti et al. (2020)
	Whey permeate (3 %) with approximately 24 mg/mL lactose. Addition of yeast extract (0.5 %). Variety of organic buffer salts tested. Fermentations at 37 °C and pH 6.5 for 12 h	The highest thermophilin yield (27,000 TAU/mL) was achieved after 10 h in whey permeate with yeast extract and addition of 1 % (w/v) 2-[*N* morpholino]-ethane sulfonate	Somkuti and Gilbreth (2007)
	Variety of supplements (yeast extract and mineral salts) at various concentrations examined. Fermentation at pH 6.5, 30 °C for 16 h	Highest plantaricin production (7000 AU/mL) was achieved in whey permeate containing yeast extract and mineral salts under controlled pH (6.5) conditions after 16 h	Enan and Amri (2006)
Microalgae	Whey permeate concentrations from 0 to 40 % were examined in a shake flask (pH 2, 12 d, 45 °C, orbital agitation) and scaled up to a 2.5 L bioreactor (8 d, 45 °C, pH 2)	Optimum whey permeate concentration was 20 %. Highest phenolic yields were catechin (3.56 mg/g) and ellagic acid (0.87 mg/g), which were obtained in the shake flask and bioreactor, respectively	Zimermann et al. (2020)
	Substitution of Bold's basal medium with acid whey permeate at varying levels (20–80 %). Shake flasks: 250 mL, pH 6.8–7.0, 21 °C for 13 d. Bioreactor: 21 °C, pH 7.2, stirring at 150 rpm, 16 d	20 % (v/v) substitution of acid whey permeate was found to be optimal. Optimal enzyme productivities were achieved at day 7 for both shake-flask (14.60 UL/d) and photobioreactor (16.26 UL/d)	Bentahar et al. (2019)
	Partial replacement of Bold's basal medium with sweet whey permeate at 0, 60 and 100 % (v/v). 300 mL volume, 21 °C, agitation at 120 rpm, 1 % CO_2 injection, 16 d	Optimum culture conditions for biomass and enzyme production was 60 % sweet whey permeate substitution for 8 days	Bentahar and Deschênes (2022)

increasing organic loading rates (OLR) and reducing construction costs, but since methanogens are highly sensitive, advanced technology is required to maintain stable operation at higher temperatures.

The need for relatively high temperatures to ensure optimal anaerobic digester performance poses a significant challenge. Diluted liquid wastes often do not produce enough CH_4 to sustain the required heating, making the process less efficient. This limitation has driven research into the feasibility of operating anaerobic digesters at lower temperatures (Collins et al., 2006). A study on expanded granular sludge bed reactor to treat diluted dairy wastewater was done to assess its feasibility for operation at 10 °C, microbial composition and bioreactor dynamics under these low-temperature conditions (Bialek et al., 2013). It has been shown that it is difficult to maintain biomass retention at such low temperatures. To address this, a higher height-to-diameter ratio has been implemented. Their study achieved an 85 % chemical oxygen demand (COD) removal rate, demonstrating that AD at low temperatures is feasible in temperate climate zones, reducing heating energy demands and enhancing overall energy balance. A study on treating dairy wastewater at lower temperatures using upflow anaerobic sludge blanket (UASB) and expanded granular sludge bed reactors revealed that at 15 °C, the diversity of the available microbial consortium decreased. Furthermore, this study also revealed that the experimental reactors maintained good performance with UASB reactor out-performing the expanded granular sludge bed reactor (McAteer et al., 2020). Nevertheless, the majority of studies have shown that biogas production tends to be decreased when AD is performed outside mesophilic conditions (Jiang et al., 2020; Nie et al., 2021; Zamanzadeh et al., 2016). Therefore, AD operators are advised to identify the optimal temperature at which CH_4 production can be maintained without unnecessary heating consumption, ensuring an efficient anaerobic digestion process.

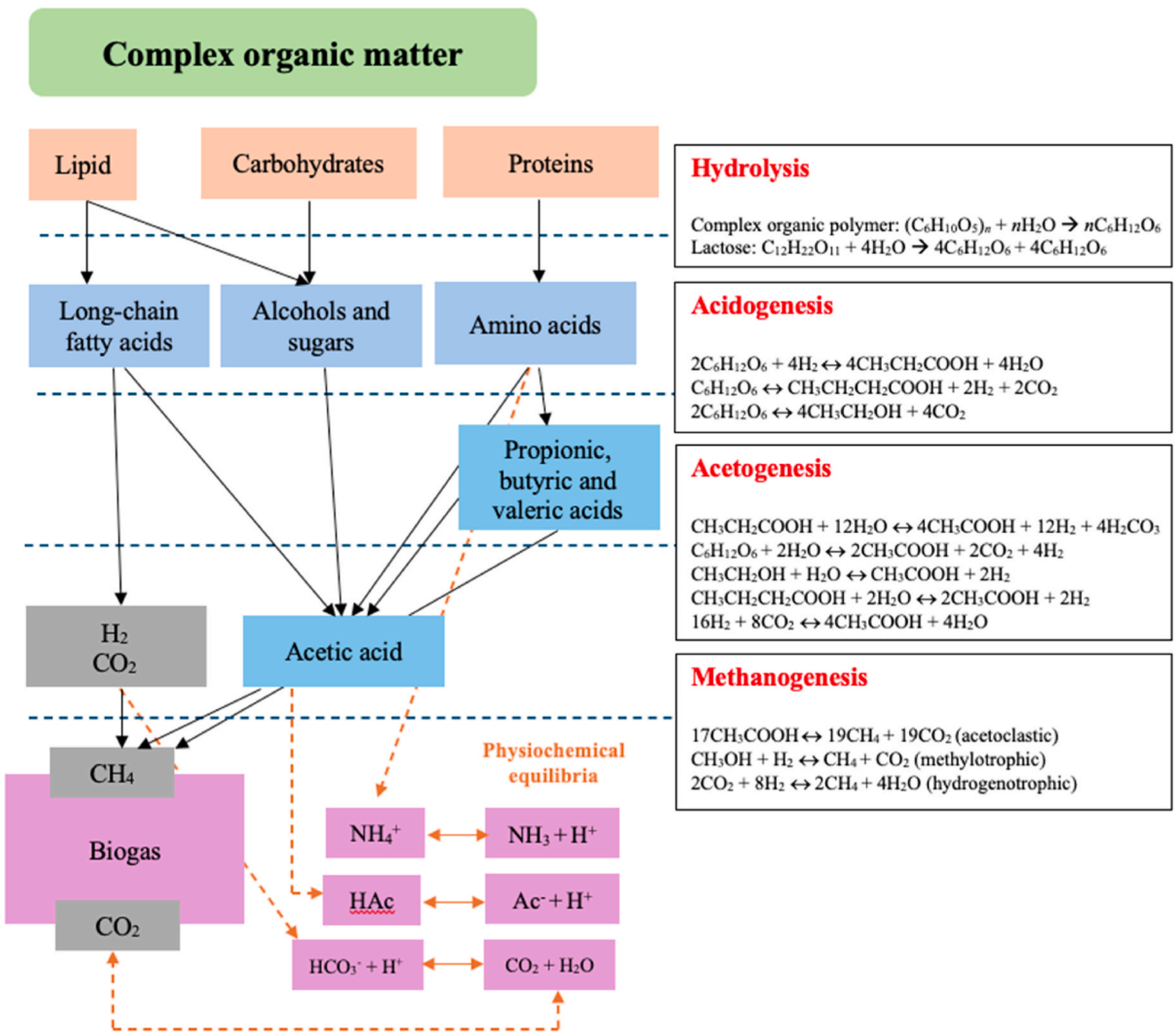

Fig. 4. Anaerobic digestion process.

5.2. Hydraulic retention time

Hydraulic retention time (HRT) or hydraulic residence time refers to the duration for which the soluble substrate remains within the reactor. Acidogenic and methanogenic microorganisms exhibit different growth rates. Consequently, in single-stage reactors, it is crucial to regulate the growth period of both microbial groups, as acidogenic bacteria thrive under shorter HRT and lower pH conditions, while methanogenic microbes can be inhibited under these conditions (Demirer and Chen, 2004). Conventional anaerobic digesters require a long HRT ranging from 20 to 200 days, resulting with the need for a large area for feedstock storage. This issue can be mitigated by employing high-rate digesters, which require a shorter retention time. Some researchers have demonstrated steady-state conditions in anaerobic bioreactors by employing multiple HRTs. These conditions are determined by assessing the standard deviations in CH_4 production and organic matter removal efficiency. Various studies have observed AD performance under different HRT periods, such as 5 HRTs (Göblös et al., 2008), 7–17 HRTs (Cota-Navarro et al., 2011), or 2 HRTs (Kundu et al., 2013). In a study by Cota-Navarro et al. (2011), where whey residues were used as substrate, authors found that gradually reducing HRTat regular intervals while maintaining a constant substrate concentration supports microbial community development. However, their study also revealed that a sudden decrease in HRT could lead to biomass washout.

A study on a continuous-flow completely mixed reactor for treating dairy wastewater has shown that both organic loading rate (OLR) and VFA production increased as HRT gradually decreased until 12 and 24 h without causing methanogen washout (Demirel and Yenigun, 2004). In addition, it has been demonstrated that a stable operation was achieved through a continuous recirculation system and by maintaining a relatively high pH. Fang and Yu (2000) also observed that acidification rates increased significantly when HRT was reduced to 12 h, while further reductions led to only minimal changes in acidification rates. Overall, reducing HRT can contribute to lower capital costs and a smaller digester volume as long as stable AD system can be maintained. Therefore, it is essential to assess whether the primary limitation of implementing shorter HRTs is the regeneration time of the relevant microorganisms. In more recent studies, researchers have suggested that digestate recirculation could be an option to achieve shorter HRT. It has been reported that digestate recirculation could maintain the digester alkalinity (Ratanatamskul and Saleart, 2016; Zuo et al., 2015), alleviate ammonia inhibition (Nie et al., 2015), as well as enhanced proliferation of methanogens (Shao et al., 2022). Satisfactory results of applying digestate recirculation at shorter HRT in AD have also been reported by other studies (Chen et al., 2021; Qin et al., 2024; Salehiyoun et al., 2025). However, it should be noted that the positive result from these studies also depends on the technical operation of the digestate recirculation, such as recirculation rate and solid/liquid fraction of the recirculated digestate. In general, all studies above collectively indicate that HRT plays a crucial role in influencing degradation efficiency, CH_4 production, and the distribution of microbial communities.

5.3. Organic loading rate

Organic loading rate (OLR) represents the quantity of volatile solids (VS) introduced into the reactor and it is expressed in terms of COD or VS per reactor volume per unit of time (Nkuna et al., 2022). The desired OLR in an AD system determines the appropriate start-up strategy. Systems with low target OLR are more stable and can typically be initiated using any inoculum source, followed by a gradual increase in substrate feeding. However, this conventional approach may not be effective for systems that target high OLR, as the risk of acidification and toxicity can hinder the growth of inoculum and substrate degradation. To ensure a smooth and efficient startup in high OLR systems, the most widely used method involves employing a mixed inoculum source or pre-adapted/acclimatized microbial cultures followed by gradual increase until target OLR is reached (Neves et al., 2010; Singh et al., 2010). A two-stage continuous stirred tank reactor-upflow anaerobic sludge bed (CSTR-UASB) system was investigated at OLRs ranging from 6.7 to 23.4 $kg_{COD}.L^{-1}.\ d^{-1}$, with HRT of 9.5 h, to evaluate the corresponding COD removal efficiency (Diamantis et al., 2014; Gavala et al., 1999). In this study, the recirculation of acidifying biomass was performed, achieving an 87 % COD removal rate, which was comparatively lower than results reported in other studies (Antonopoulou et al., 2008; Gavala et al., 1999). Both studies demonstrated that waste from cheese whey undergoes higher fermentation rate at low OLRs, regardless of the use of single- or two-stage digester, due to the highly biodegradable nature of whey.

5.4. Mixing

Effective mixing plays a critical role in ensuring homogeneous blending of the substrate, facilitating the uniform distribution of microorganisms, preventing scum formation, minimizing grit deposition, maintaining chemical consistency, and eliminating temperature gradients within the digester (Kaparaju et al., 2008). Proper mixing is essential for promoting adequate contact between microbial populations and nutrients, which is vital for optimal digestion performance (Parkin and Owen, 1986). However, excessive mixing can disrupt slow-growing microorganisms and induce shear stress (Karim et al., 2005), potentially destabilizing the digester by altering key operational parameters such as pH and moisture content. The duration, intensity, and method of mixing are also significant factors to be considered. Common mixing techniques include mechanical agitation, manual stirring, recirculation of digester contents, and biogas injection at the base of the reactor (Karim et al., 2005). However, the effect of mixing to the overall AD performance still needs to be further observed, especially with regards of energy consumption of AD systems.

5.5. Carbon to nitrogen ratio

Carbon to nitrogen (C/N) ratio is an important parameter of the AD process as it directly affects microbial activity within the digester. Inappropriate C/N ratios may lead to the accumulation of excessive volatile fatty acids (VFAs) and/or elevated ammonia levels within the digester, both of which can act as significant inhibitors in the AD process (Azkarahman et al., 2020; Jain et al., 2015). In general, a C/N ratio of 25–30 is recommended for the effective AD operation (Hassan et al., 2017; Hills, 1979; Jain et al., 2015; Xu et al., 2018), as this range ensures optimal conditions for the degradation of the majority of carbon present in the substrate pH and alkalinity. Since such ratios are not always naturally present, it is advisable to co-digest with other compatible substrates. Typically, feedstocks with a C/N ratio below 40 are recommended for mixing with dairy waste to maintain reactor stability and ensure a balanced nutrient profile. An optimal C/N ratio can be achieved by combining feed materials with high and low C/N ratios, such as blending municipal waste with animal manure.

In a study involving the co-digestion of cheese whey waste, poultry waste, and cattle manure in a ratio of 3:2:1, CH_4 yield of 62 % was achieved (Desai et al., 1994). The study highlighted that cheese whey, owing to its high carbohydrate content, enhances the growth of acid-forming bacteria. Conversely, poultry waste contributes to increased nitrogen levels, which mitigates the inhibitory effects of acid-forming microbes on methanogenic activity. The bioavailability of carbon and nitrogen in a substrate can be significantly influenced by operational parameters such as temperature and pH, as well as by factors like VFA accumulation and ammonia concentration. For instance, in a study utilizing cow manure as the substrate, a reduction in temperature from 60 to 37 °C in a digester with elevated ammonia levels resulted in an increase in biogas production (Angelidaki and Ahring, 1994). The same study revealed that higher C/N ratios reduce the risk of ammonia inhibition. This indicates that the concentration of total ammonia nitrogen (TAN) and VFA directly influence nitrogen availability, while may also be affected by the operating temperature. Their study also revealed that ammonia levels between 0.05 and 0.2 g of NH_4-N/L can cause significant inhibition of the digestion process. Moreover, it has been reported that when carbon is supplemented during anaerobic digestion, the majority is utilized for biogas production, assuming the C/N ratio remains within the optimal range of 40 and 53 (Gil et al., 2019). Therefore, it is important to determine nutrients composition of the feedstocks at the initial stages of the process to ensure optimal AD performance.

5.6. pH

The pH value, or hydrogen ion concentration, of a solution determines its acidity or alkalinity. Microorganisms are generally sensitive to extremely acidic or alkaline conditions. In AD, three key microbial groups (acidogenic, acetogenic, and methanogenic) exhibit distinct pH preferences for optimal growth. Acidogenic bacteria, which grow relatively faster, thrive at a pH range of 5.2–6.5. Acetogenic bacteria prefer a slightly higher pH range of 6.6–7.6, while methanogenic archaea, which grow relatively slower, favour a pH range of 7.5–8.5 (Meegoda et al., 2018). Therefore, the pH of the AD feedstock significantly influences digester performance. For instance, lactose-rich wastes have low pH and encourage the proliferation of acidogenic bacteria under anaerobic conditions (Kisaalita et al., 1987). This results in the excessive production and accumulation of VFA, causing a sharp decline in pH. A notable example is the digestion of cheese whey, which due to its low initial pH and high biodegradability, generates substantial VFA through lactose degradation. This VFA can accumulate in the system, further lowering the pH. When coupled with low bicarbonate alkalinity (e.g., 50 meq/L), this pH drop can inhibit methanogenic activity, ultimately leading to digester failure (Charalambous et al., 2020).

The addition of buffering agents could be used to overcome the problem with rapid acidification. Dairy wastewaters typically exhibit alkalinity levels below 1000 mg $CaCO_3$/L in most instances (Demirel and Yenigun, 2004; Demirel and Yenigün, 2006), which is insufficient to support stable AD. In cases where natural alkalinity is inadequate, external alkaline agents such as lime ($CaCO_3$), sodium hydroxide (NaOH), or sodium carbonate (Na_2CO_3) must be supplemented to stabilize the system. Researchers have investigated the addition of buffering agents such as NaOH, $NaHCO_3$, and Na_2CO_3, during the start-up phase to achieve the desired pH levels (Bezerra et al., 2007). The study suggests that once an appropriate amount of the buffering agent is introduced, the AD system gradually stabilizes at higher pH values as the AD progresses. Besides the addition of buffering agents, another commonly applied solution to overcome the problem of over-acidification is the use of multi-stage AD reactor set-ups, such as those introduced by (Kisaalita et al., 1987), Dareioti and Kornaros (2014), and Gensollen et al. (2022). In these set-ups, the reactor at the initial-stage is conditioned to optimize hydrolysis and acidification process of the feedstock. The produced organic acids from the first-stage are then pumped or transferred to another reactor that is optimized for methanogenesis process. In this scenario, acidifying bacteria and methanogen archaea activity can be separately optimized suiting their respective optimum pH range.

5.7. Trace elements

Methanogenesis is responsible for biogas production and is carried out by a specialized group of anaerobic microorganisms known as methanogens, which belong to the archaea domain. In addition to essential macronutrients such as C, H, N, P, and S, microbial activity is also highly dependent on trace elements for survival and metabolic functions. Therefore, maintaining adequate levels of trace elements in AD system is vital to ensure optimal and stable performance. This aspect has been emphasized by numerous studies conducted to underscore the significance of trace elements in anaerobic digestion. For instance, the supplementation of iron (Fe), nickel (Ni), cobalt (Co), molybdenum (Mo), and other trace elements in digestion systems has been shown to enhance anaerobic digestion performance across various substrates (Pobeheim et al., 2010; Schmidt et al., 2014; Zhang et al., 2015). Among various trace elements, Fe is the most extensively studied for its supplementation effects on AD, primarily due to its high demand as indicated by fundamental research. Notably, Park and Novak (2013) investigated the direct addition of Fe(III) at 1.25 % (by weight) into a sewage sludge digestion system to mitigate odour-causing byproducts. Their findings revealed a reduction of over 65 % in the production of hydrogen sulfide (H_2S) – a problematic and corrosive compound. This reduction was attributed to the precipitation of FeS, which consumed bisulfide (HS^-), a metabolite generated by sulfate-reducing bacteria. One of many challenges that hinder the widespread adoption of full-scale biogas plants is the high capital cost, which extends the return on investment period. Therefore, reducing H_2S levels in biogas can significantly lower the costs associated with its removal, making biogas utilization more economically viable. However, Gustavsson et al. (2013) highlighted that the precipitation of FeS can influence the solubility of other essential nutrient metals, such as Ni and Co.

A balanced interplay between hydrolysis, acidogenesis, acetogenesis, and methanogenesis is essential for the successful operation of an AD system. Specifically, VFA must be degraded at a sufficient rate to ensure a stable environment for microbial activity and overall digester stability. Trace elements supplementation has been shown to enhance VFA degradation, thereby helping to control VFA levels (Climenhaga and Banks, 2008; Ortner et al., 2014; Wall et al., 2014). Such improvements often lead to more efficient organic matter digestion. For example, in a co-digestion system combining food waste and swine wastewater, the addition of Fe, Ni, Mo, and Co resulted in negligible VFA concentrations, reduced COD, and increased solid degradation (Zhang et al., 2011). Yirong et al. (2015) demonstrated that a combination of trace elements facilitated the start-up of a thermophilic digester by reducing initial VFA levels and preventing VFA accumulation during the digestion process. Moreover, trace element supplementation has also proven effective in restarting failed or unfed digesters (Bayr et al., 2012; Wei et al., 2014), indicating its importance in maintaining sustainable AD operation.

6. Overview of anaerobic digestion studies on whey permeate

As highlighted in earlier sections, the utilization of whey permeate has been constrained by its low nutritional values and challenges associated with managing the secondary waste generated during processing. An option to tackle these problems is through the utilization of whey permeate as AD feedstock. Through AD, lactose as the major organic compound in whey permeate can be converted into renewable energy in the form of CH_4 as well as valuable organic acids in the form of VFA. Consequently, the AD process effectively reduces the organic load of whey permeate, thereby significantly lowering its environmental impact and polluting potential. The AD of lactose proceeds through the following stages.

1. Hydrolysis

$$4C_{12}H_{22}O_{11}\ (lactose) + 4H_2O \rightarrow 4C_6H_{12}O_6\ (glucose) + 4C_6H_{12}O_6\ (galactose) \tag{Eq. 1}$$

2. Acidogenesis

$$2C_6H_{12}O_6 \rightarrow 4C_2H_5OH\ (ethanol) + 4CO_2 \tag{Eq. 2}$$

$$2C_6H_{12}O_6 + 4H_2 \rightarrow 4C_2H_5COOH\ (propionic\ acid) + 4H_2O \tag{Eq. 3}$$

3. Acetogenesis

$$C_2H_5OH + H_2O \rightarrow CH_3COOH\ (acetic\ acid) + 2H_2 \tag{Eq. 4}$$

$$4C_6H_{12}O_6 + 8H_2O \rightarrow 8CH_3COOH + 8CO_2 + 16H_2 \tag{Eq. 5}$$

$$4C_2H_5COOH + 12H_2O \rightarrow 4CH_3COOH + 12H_2 + 4H_2CO_3 \tag{Eq. 6}$$

$$16H_2 + 8CO_2 \rightarrow 4CH_3COOH + 4H_2O \tag{Eq. 7}$$

4. Methanogenesis

$$CH_3COOH + 8CH_3COOH + 8CH_3COOH + 2CH_3COOH \rightarrow 19CH_4 + 19CO_2 \tag{Eq. 8}$$

$$2CO_2 + 8H_2 \rightarrow 2CH_4 + 4H_2O \tag{Eq. 9}$$

Lactose is a milk disaccharide consisting of glucose and galactose (Fox, 2009). During hydrolysis stage, lactose is broken down by the lactase enzyme produced by the hydrolytic bacteria (Eq. (1)). In the subsequent acidogenesis phase, it further degrades into ethanol (Eq. (2)) or CO_2 and propionic acid (Eq. (3)). Ethanol then reacts with H_2O to produce acetic acid (Eq. (4)). Additionally, glucose which has been partially digested by hydrolytic bacteria, generate CO_2, H_2, and acetic acid as byproducts (Eq. (5)). Similarly, propionic acid undergoes hydrolysis to form acetic acid (Eq. (6)). Some of the CO_2 and H_2 produced also contribute to acetic acid formation (Eq. (7)). In the methanogenesis stage, acetic acid from previous phases dissociates to produce CH_4 and CO_2 (Eq. (8)) through acetoclastic methanogens activity. Another pathway include H_2 utilization as electron donor by hydrogenotrophic methanogens to reduce CO_2 and producing CH_4 and H_2O as the final product (Eq. (9)).

Aside from understanding the AD process of lactose, the theoretical CH_4 yield from lactose AD can be calculated through the Buswell equation (Eq. (10)) based on the elemental compositions of general compound as follows:

$$C_aH_bO_c + \left(\frac{a - \frac{b}{4} - \frac{c}{2}}{2}\right) H_2O \rightarrow \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{2}\right) CH_4 + \left(\frac{a}{2} - \frac{c}{2}\right) \tag{Eq. 10}$$

By using this equation, it can be calculated that 1 mol of lactose ($C_{12}H_{22}O_{11}$) equal to 3.25 mol of CH_4. Furthermore, at standard temperature and pressure (STP) condition (1 mol CH_4 = 22.4 L), 1 kg of lactose could theoretically yield ±212.6 L of CH_4.

The AD of whey permeate is less observed as compared to other dairy products such as cheese whey or other dairy wastewaters (Bella and Rao, 2023). In Table 4, a more detailed experimental designs, yields and objective of the study from published whey permeate AD works is presented. Current research offers limited insights into the optimal operational conditions for AD of whey permeate and its efficiency in CH_4 production, particularly in terms of practicality and reproducibility. Earlier studies of whey permeate AD, such as by Boyaval and Corre (1987) and Colomban et al. (1993), were focused on VFA production by using *Propionibacterium* genera. Furthermore, other studies observed the

Table 4
Current findings on the AD of whey permeate.

Research objectives	Reactor type	Inoculum	Operational condition	AD process yields	References
Propionic acid production	Continuous stirred tank reactor (CSTR) coupled with ultrafiltration cell recycle	*Propionibacterium acidipropionici* (ATCC 4695)	Working volume of 3 L; temperature at 30 °C and pH maintained at 7	Propionic acid = 25 g.L^{-1}; biogas or CH_4 production is not assessed	Boyaval and Corre (1987)
	Batch and continuous membrane bioreactor	*Propionibacterium acidipropionici* (ATCC 4695)	Working volume of 2, 3, 50, 700, 5200 L; temperature at 20–35 °C; pH maintained at 6.5, and pressure at 0.2 bar	Propionic acid = 30–40 g.L^{-1}; biogas or CH_4 production is not assessed; complete removal of lactose content is achieved	Colomban et al. (1993)
Hydrogen gas production	Semi-continuous packed bed bioreactor	Biosolid pellets from wastewater treatment plant	Initial feeding with synthetic water prior to using whey permeate as AD feedstock; working volume of 4 L; OLR of 18.8–6.3 g_{COD}.L^{-1}d^{-1}; HRT of 2–1 d; temperature at 35 °C; pH maintained at 4.5–5.5;	H_2 = 18.1–28.6 L_{H2}.kg_{COD}^{-1}; biogas = ~8.8 L d^{-1}; acetic acid = ~2800 mg_{COD}.L^{-1}; propionic acid = ~1800 mg_{COD}.L^{-1}; butyric acid = 5750 mg_{COD}.L^{-1};	Fernández et al. (2014)
Study on methanogenetic kinetics of whey permeate AD	Packed bed immobilized cells bioreactor	Sewage sludge	Working volume of 0.5 L; temperature at 30–37 °C; and pH maintained at 7	Propionic acid = ~0.28 g g^{-1}; acetic acid = 0.18 g g^{-1}; CH_4 = 300 mL g^{-1}	Yang and Guo (1990)
	Packed bed immobilized cells bioreactor	Sewage sludge	Working volume of 0.5 L; temperature at 30 °C; and pH maintained at 7–7.5	Biogas = ±0.6 L d^{-1}	Yang and Guo (1991)
	Continuous upflow anaerobic sludge blanket (UASB) reactors	Not specified	OLR of 0.26–0.87 kg_{COD}.kg_{VSS}^{-1}d^{-1}; HRT of 5.0–0.4 d; reactor volume at 7.2-L; temperature at 35 °C; pH maintained at ≥6.6	VFA = 36.7–1673 $mg_{acetate}$.L^{-1}; CH_4 = ~2.67 L.L^{-1}.d^{-1}; substrate removal = 64.2–99 %	Hwang et al. (1992)
Study on methanogen/ microbial community on AD system	Batch mixed tank reactor	AD effluent from full-scale plant treating municipal wastewater	Working volume of 6 L; substrate concentration at 5 g_{SCOD}.L^{-1}; temperature at 35 °C; pH maintained at ≥7	Biogas production = ±1.7 L.L^{-1} (of which 73–86 % CH_4); complete removal of substrate COD after 42 d	Lee et al. (2010)
	CSTR	AD effluent from full-scale plant digesting cow manure and food waste at mesophilic temperature (37 °C; pH 7.6)	Co-digestion of cow manure with whey permeate (48 % from manure then changed to 64.2 % from manure, with operational changes depending on reactor condition); working volume of 6 L; OLR of 0.5–2.9 g_{VS}.L^{-1}.d^{-1}; HRT of 25 d; temperature at 37 °C; initial pH at 7.5; and stirring at 180 rpm	CH_4 = up to ±225 mL.g_{VS}^{-1}.d^{-1}; acetic acid = ~67.9 mM; propionic acid = ~123.5 mM	Hagen et al. (2014)
Observation and/or optimization for AD performance	Upflow fixed-bed reactor coupled with or without clay beds for microbial immobilization	Sewage sludge	OLR of 24–36 kg.L^{-1}.d^{-1}; HRT of 3.5–2.1 d; temperature at 37 °C; pH maintained at 6.5–6.7 and 4.9–5.3; recirculation of suspended flora at 6 times.h^{-1}	Acetate = 6–65 mmol.L^{-1}; propionate = 0–10 mmol.L^{-1}; n-butyrate = 0–35 mmol.L^{-1}; biogas production = 2.7–7.3 L.L^{-1}.d^{-1}; COD removal = 90–95 %	Zellner et al. (1987)
	UASB and multiplate anaerobic reactor	Granular sludge from UASB used to treat cheese whey wastewater	Working volume of 19.2 L; OLR of 20 kg_{COD}.L^{-1}.d^{-1}; HRT of 1 d; COD/N adjusted to 100/1; pH maintained at 5; addition of $Ca(OH)_2$ as research treatments	Total VFAs (sum of acetate, propionate and butyrate) = 5.18–7.78 kg.L^{-1}; CH_4 = ~6.7 L.L^{-1}.d^{-1} COD; removal = 92–97 %	El-Mamouni et al. (1995)
	Two-stage CSTR	Granular sludge from full-scale UASB reactor treating fruit juice wastewater	Working volume of 5 L; OLR of 20, 25,30, and 35 kg_{COD}/L^{-1}.d^{-1}; HRT of 1 d; temperature at 35 °C; pH maintained at 5.7–5.8 for first reactor and 7.4 for second reactor	CH_4 = 0.12 L_{CH4}.kg_{COD}^{-1}; COD removal = ~98 %; H_2 = 4.19 mol_{H2}.$kg_{CODremoved}^{-1}$	Kisielewska et al. (2014)
	Single- and two-stage CSTR	AD effluent from full-scale plant operating at thermophilic condition for livestock manure and food waste treatment	Working volume of 3 L; OLR of 3.6–2.4 g_{COD}.L^{-1}.d-1; HRT of 15 d; temperature at 55 °C; pH maintained at ≥6.5; continuous stirring at 150 rpm	CH_4 = ~333 mL_{CH4}.g_{COD}^{-1}; acetate = ~10 g.L^{-1}; propionate = <1 g.L$^{-1;}$ butyrate = ~4.6 g.L^{-1}	Fontana et al. (2018a)
	Single- and two-stage CSTR	AD effluent from full-scale plant operating at thermophilic conditions for livestock manure and food waste treatment	Working volume of 3 L; OLR of 2.4 g_{COD}.L^{-1}.d-1; HRT of 15 d; temperature at 55 °C; pH maintained at ≥6.5; continuous stirring at 150 rpm; gas recirculation at 0.7 mL.L^{-1}; H_2 gas injection at 1.7 mL min^{-1}	CH_4 = ~270 mL_{CH4}.g_{COD}^{-1}; acetate = ~7.5 g.L^{-1}; total VFA = ~10.8 g.L^{-1}	Fontana et al. (2018b)
	Partitioned UASB	Granular sludge from UASB reactor used in treating effluent from distillery industry	Co-digestion of whey permeate and cow slurry at the ratio of 1:1, 2:1, 3:1, and 4:1; working volume of 4 L; OLR of 6.25 g_{COD}.L^{-1}.d-1; HRT of 10 d; temperature at 37 °C; pH maintained at 6.5–7.8;	Biogas = ~3.5 L d^{-1} (of which ~51 % CH_4); total VFA = ~0.37 mg.L^{-1}; sugar removal = ~99 %	Fagbohungbe et al. (2019)
	Single and two-stage batch stirred tank reactor	AD digestate from large scale agricultural plant	Co-digestion of whey permeate (26 % w/w) with slaughterhouse sludge (31 % w/w), green beans (20 %	CH_4 production not assessed; H_2 = ~ ±380 mL_{H2}.L^{-1}; acetic acid = ~ ±8 g.L^{-1}; propionic acid =	Gensollen et al. (2022)

(continued on next page)

Table 4 (*continued*)

Research objectives	Reactor type	Inoculum	Operational condition	AD process yields	References
			ww), water (14 % w/w) and cow manure (9 % w/w); working volume of 5 L; continuously stirred; temperature at 40 °C; pH regulated at 4.5, 5, 5.5, 6.5 and 7	~7.3 g.L^{-1}; butyric acid = ~ ±5.2 g.L^{-1}	
	Batch and semi-CSTR	AD effluent from full scale plant fed with energy crops	Batch:Working volume of 70 mL; temperature at 20, 30, and 37 °C; inoculum to substrate ratio at 0.5, 1, and 2 (w/w); initial pH at 7, 7.5, and 8. Semi-CSTR:Reactor volume of 5 L; OLR of 2.5 $g_{VS}L^{-1}d^{-1}$; HRT of 30 d; continuously stirred at 30 rpm; temperature at 37 °C; pH maintained at 7	Batch: CH_4 = ~653.64 ± 12.16 $NL_{CH4}kg_{VS}^{-1}$; VS degradation = ~85.85 ± 2.46 Semi-CSTR: CH_4 = ~227.84 ± 83.49 NL_{CH4}.kg_{VS}^{-1}.d^{-1} during first 3 HRT and ~40.37 ± 6.28 NL_{CH4}.kg_{VS}^{-1}.d^{-1} during inhibited-steady state; total VFA = ~ ±75 g.L^{-1}; organic carbon degradation = ~35 %	Azkarahman et al. (2025a)
	Batch reactor	AD effluent from full scale plant fed with energy crops	Working volume of 70 mL; temperature at 20, 30, and 37 °C; inoculum to substrate ratio at 0.5, 1, and 2 (w/w); initial pH at 7, 7.5, and 8	CH_4 = ~466.29 ± 13.71 NL_{CH4}.kg_{VS}^{-1}input; total VFA = 2.85 ± 0.35 g.L^{-1}; VS degradation = ~81.14 %; organic carbon degradation = ~77.92 ± 1.44 %	Azkarahman et al. (2025b)

AD process and kinetics by using whey permeate as the substrate to understand its overall biochemical process. In a study conducted by Yang and Guo (1990), the kinetics of methanogenesis from whey permeate were investigated using a batch packed bed bioreactor with immobilized cells. Their research elucidated the kinetic pathways of lactose degradation into VFAs and methane CH_4.

Similarly, a kinetic study of whey permeate AD by using packed bed immobilized cell bioreactor revealed acetate and propionate as the majorly produced VFAs, which later VFA has also been shown to inhibit the AD process at the concentration of more than 10 g/L (Yang and Guo, 1991). Another kinetic study was done by Hwang et al. (1992), where kinetic parameters (maximum substrate utilization rate, constant, and half saturation coefficient) of whey permeate AD were determined under continuous UASB reactors. Aside from AD process and its kinetics, other studies observed microbial aspects during the AD of whey permeate to understand the possible biochemical process behind the process. A study by Lee et al. (2010) revealed that the methanogens community shifted during batch AD of whey permeate. In their experiment, methanogen community showed dynamic changes, particularly between acetoclastic *Methanosarcinaeae* and hydrogenotrophic *Methanomicrobiales*. Furthermore, a study by Hagen et al. (2014) observed microbial community profiles of whey permeate AD under two identical operations of CSTR systems. Both studies highlighted the challenge in controlling microbial communities of whey permeate AD, even with identical setup which was performed by the later study.

An early observation by Zellner et al. (1987) focused on the performance of whey permeate AD with different reactor types (suspended and immobilized complex) and defined consortia. Their study revealed the accumulation of acetate and propionate within the digester, which led to a decline in the rate of biogas production. Another study by El-Mamouni et al. (1995) observed multiple anaerobic reactors for whey permeate AD and revealed COD removal up to 97 % with CH_4 production rate of 6.7 m^3 m^{-3}. d^{-1} at an OLR as high as 20 kg_{COD}.L^{-1}. d^{-1}. In a different operational set up, Kisielewska et al. (2014) used two-stage anaerobic fermentations of whey permeate and showed maximum CH_4 yield of 0.12 L_{CH4}. kg_{COD}^{-1} with COD removal up to 98 %. A study by Fontana et al. (2018a) demonstrated that a two-stage reactor for whey permeate AD outperformed a single-stage reactor due to the inclusion of a pre-acidification step. This step facilitated a more effective distribution of microbial species across environments suited to their metabolic functions. Additionally, the study highlighted that the accumulation of acetate in the single-stage reactor was primarily attributed to the low abundance of acetoclastic methanogens and the partial inhibition of hydrogenotrophic methanogens, which hindered the activity of syntrophic acetate-oxidizing bacteria. In a different study, Fontana et al. (2018b) further investigated the AD performance of the two different reactor operations treating whey permeate by H_2 injection for biogas upgrading. Their findings demonstrated that H_2 injection enhanced CH_4 content by 7 % in single-stage configuration, attributed to the upregulation of the hydrogenotrophic pathway by *Methanothermobacter wolfeii* UC0008. However, their study also revealed no positive effect of H_2 injection towards CH_4 production in two-stage reactor. Alternatively, Fernández et al. (2014) investigated the AD of whey permeate for H_2 production using a packed bed reactor with polyurethane foam as the support material. Their findings indicated the presence of non-H_2-producing microorganisms that likely compete for substrates, thereby negatively impacting the dominance of H_2-producing bacteria. Additionally, their study emphasized the need for further evaluation of operational conditions to improve process productivity. A more recent study on AD of whey permeate was done by Fagbohungbe et al. (2019). Their study employed a co-digestion process combining whey permeate and cattle slurry in a partitioned upflow anaerobic digestion tank. The results demonstrated that the highest CH_4 production was achieved at a feeding ratio of 2:1 (whey permeate to cattle slurry, w/w), with the cattle slurry contributing sufficient buffering capacity to stabilize the process. A study by Gensollen et al. (2022) applied co-digestion of whey permeate with green beans, cow manure and slaughterhouse sludge in a two-stage batch AD and revealed that the pH regulation (4.5–7) affected the metabolic pathways as well as microbial community involved. Other studies have explored the process optimization of whey permeate AD under batch reactors and its performance under continuous systems (Azkarahman et al., 2025a, 2025b). Their studies highlighted that kinetic parameters from batch reactors cannot be directly applied to the continuous system. Furthermore, inhibited-steady state of whey permeate AD under continuous system was demonstrated. This indicates further optimization strategies are still required to maintain optimum CH_4 production. Alternatively, their studies also indicate that shifting the main focus of whey permeate AD from CH_4 generation into VFA production without a bioaugmentation step could be an interesting option.

Anaerobic digestion holds significant potential for treating effluents with high organic load, such as whey permeate. While fundamental factors influencing AD have been discussed earlier in this article, several critical aspects must be considered to ensure the successful implementation of these techniques. Current studies using AD for whey permeate valorization have employed several methods, such as employing different AD reactor configurations (El-Mamouni et al., 1995; Fontana et al., 2018a, 2018b; Kisielewska et al., 2014), co-digestion with other substrates to balance the feedstock nutrients as well as buffering agent (Fagbohungbe et al., 2019; Gensollen et al., 2022), bioaugmentation with determined AD microbes (Boyaval and Corre, 1987; Colomban et al., 1993), as well as innovative techniques to also recover H_2 aside from CH_4 (Fernández et al., 2014; Fontana et al., 2018a, 2018b). While these methods showed promising results for whey permeate utilization, there is a gap in the reproducibility and practicality of the methods. For example, the availability of feedstock for co-digestion is frequently subject to seasonal or regional limitations, creating challenges for maintaining consistent performance in large-scale anaerobic digestion (AD) systems (Xie et al., 2016). Additionally, a study by Hagen et al. (2014) revealed variations in microbial populations even in identical AD systems, highlighting the difficulties in achieving reproducible bioaugmentation outcomes. Moreover, reactor configurations and H_2 injection might add process complexity in the whey permeate AD.

The application of whey permeate AD has been investigated under diverse operational conditions and reactor configurations. Among published studies, it is found that the CH_4 production is mostly over the theoretical value of CH_4 production from lactose degradation. This is due to several reasons, such as degradation of other organic matters aside from lactose from whey permeate, additional effect from co-digestion, and probable overestimation of the CH_4 measurement. The highest CH_4 yield from whey permeate AD is found to be under batch systems. In this reactor set-up, whey permeate AD is operated in a closed system without continuous feeding. This explains the high CH_4 yield due to complete degradation of the substrate but limited for its practicality when AD scale-up is the objective. It is then suggested that research using batch AD is done for initial assessment of the substrate potential for AD feedstock (including assessment of the pre-treatment effect towards substrate degradability) and/or microbial evaluation such as that performed by Lee et al. (2010) and Azkarahman et al. (2025b). Furthermore, comparative analyses between reactor types reveal distinct advantages and limitations. Continuous stirred tank reactor (CSTR), the most commonly reported systems, provide simplicity, operational robustness and applicability for scaling up the process. However, CSTR is susceptible to microbial washout under high OLR and short HRT due to soluble/liquid characteristics of whey permeate, thus the substrate cannot provide an adequate matrix for the microbial community to attach and be retained within the reactor (Azkarahman et al., 2025a). Published works using different reactor designs, such as UASB or sequencing batch reactors, might be an option to tackle this problem, although their complexity may limit widespread adoption in small-to medium-scale dairy operations.

Beyond the reactor type, comparisons across different waste streams, including cheese whey (Flores-Mendoza et al., 2020), waste milk (Adghim et al., 2020), and mixed dairy effluents (Chou and Su, 2019), have demonstrated that whey permeate could generate higher AD yields. Moreover, we also found that higher yield of whey permeate AD when compared to energy crops typically used as AD feedstocks, such as corn stover (Lu et al., 2022) and wheat straw (Kaldis et al., 2020), which is probably due to the absence of lignocellulosic materials as it is the case with the crops that limit AD biodegradability. However, while whey permeate is highly degradable, it lacks buffering capacity and essential nutrients, making it more sensitive to process perturbations compared to more heterogeneous wastes. Furthermore, there is also a key barrier to drawing robust conclusions across studies arising from the lack of standardization in data reporting. AD yields are often expressed in different units (e.g., mL CH_4 g^{-1} COD, m^3 CH_4 kg^{-1} VS, or L CH_4 L^{-1} substrate for CH_4 production and $g.L^{-1}$, $g.g^{-1}$, or mM for VFA), thus limiting cross-comparability. To address this, we propose systemic calculation of mass balances that account for substrate input and process outputs, including CH_4 and VFA generation, and organic matter degradation. Such approaches would not only enhance comparability but also provide mechanistic insights into organic matter conversion efficiencies. In addition, while AD has demonstrated its feasibility for waste treatments, the techno-economic assessment of the whey permeate AD remains underexplored. Such study will be critical to evaluate the scalability and commercial viability of whey permeate AD.

7. Future outlook

Advancing the valorization of whey permeate through AD requires targeted research efforts to overcome persisting technical and methodological challenges. First, the inconsistent use of terminology across publications—particularly regarding the definitions of whey permeate, deproteinized whey, and lactose-rich effluents—could hinder reproducibility and comparability of results. Establishing clear and standardised terminology will be essential to ensure that findings can be accurately interpreted and meaningfully compared across studies. Second, future research should consider comprehensive mass balance assessments of AD processes applied to whey permeate. Many existing studies only report partial yields from the AD system (e.g., VFAs, CH_4, H_2, or organic load removal), depending of its respective aim of the study. Developing systematic mass balance frameworks will not only improve comparability between studies but also provide deeper insights into nutrient recovery potential, digestate quality, and overall process efficiency. In order to give a more comprehensive picture from the AD system, we then suggest to include at least CH_4, total VFA production, and substrate input or removal (COD or VS) to the mass balance calculation. Third, there is a critical need for techno-economic assessments tailored to whey permeate AD systems. While AD is widely recognised as a cost-effective technology for waste valorization, the scalability and commercial feasibility for dairy by-product streams remain underexplored. Among published studies on whey permeate AD, it has been demonstrated that each different experimental design (reactor types, operational conditions, co-digestion, etc.) significantly affect AD outputs. Integrating techno-economic with life cycle assessment will be vital for identifying cost bottlenecks, guiding process optimization, and evaluating the environmental trade-offs of full-scale deployment. Finally, advancing whey permeate AD requires a multidisciplinary approach that unites microbiological insights, process engineering, and economic evaluation. Addressing these aspects will accelerate translation from laboratory-scale findings to industrial applications, ultimately supporting sustainable dairy waste management and renewable energy generation.

8. Conclusions

This review has highlighted the characteristics, production, and applications of whey permeate, ranging from direct uses aimed at enhancing its nutritional or physical properties to indirect applications for developing novel products. It is evident that whey permeate holds significant potential for utilization, and its disposal through wastewater systems indicates inefficient use of the byproduct with potential environmental consequences. Among various utilization strategies discussed, anaerobic digestion (AD) emerges as a promising approach to fully harness the value of whey permeate, offering a sustainable solution that aligns with the principles of a circular economy. The key characteristics of whey permeate, including its high organic load, biodegradability and lactose content make it particularly suitable for anaerobic treatment. A thorough understanding of the biochemical transformations occurring during the AD of whey permeate is essential for controlling the accumulation of intermediate products, especially with the acidic nature of whey permeate, which can inhibit the AD process. Furthermore, careful optimization of the key operational parameters affecting AD performance is essential for ensuring stable and efficient performance within AD reactors. Current studies on using whey permeate as AD feedstock have revealed promising results, both in CH_4 production for renewable energy production, as well as COD removal to minimize the environmental impact. However, significant gaps remain in the existing literature on the AD of whey permeate. Many studies present challenges in reproducibility due to variations in the terminology used to define whey permeate, co-substrate characteristics in co-digestion systems, difficulties in controlling microbial communities for defined consortia, and the inherent complexities of multi-stage AD reactor configurations. Further research is needed to optimize the overall performance of whey permeate AD, overcoming limitations between each reactor type, with a particular focus on enhancing the practicality and reproducibility of the operational strategies employed. This will pave the way for achieving sustainable AD of whey permeate.

CRediT authorship contribution statement

Aldyon Restu Azkarahman: Writing – original draft, Methodology, Investigation, Conceptualization. **Denise Cysneiros:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. **Afroditi Chatzifragkou:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. **Kimon Andreas G. Karatzas:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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