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Potential utilization of dairy industries by-products and wastes through microbial processes: A critical review

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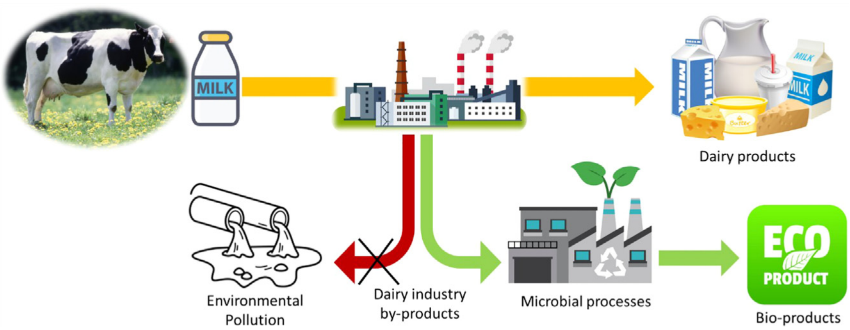
HIGHLIGHTS

- Excessive amounts of dairy industry wastes contribute to environmental pollution.
- Dairy wastes contain a high level of COD and nutrients (lactose, protein and fat).
- Effective biological products can be obtained by microbial processes.
- Anaerobic digestion efficiencies can be improved via addition of dairy wastes.
- Microbial processes can contribute to the global sustainable goals.

GRAPHICAL ABSTRACT



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ABSTRACT

The dairy industry generates excessive amounts of waste and by-products while it gives a wide range of dairy products. Alternative biotechnological uses of these wastes need to be determined to aerobic and anaerobic treatment systems due to their high chemical oxygen demand (COD) levels and rich nutrient (lactose, protein and fat) contents. This work presents a critical review on the fermentation-engineering aspects based on defining the effective use of dairy effluents in the production of various microbial products such as biofuel, enzyme, organic acid, polymer, biomass production, etc. In addition to microbial processes, techno-economic analyses to the integration of some microbial products into the biorefinery and feasibility of the related processes have been presented. Overall, the inclusion of dairy wastes into the designed microbial processes seems also promising for commercial approaches. Especially the digestion of dairy wastes with cow manure and/or different substrates will provide a positive net present value (NPV) and a payback period (PBP) less than 10 years to the plant in terms of biogas production.

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1. Introduction

The dairy industry has an important position among the food industries with the developed technology and widespread products such as cheese, yogurt, butter, cream, and ice cream (Demirel et al., 2005; Rivas et al., 2010). Milk and other processed dairy products can form the main components of food waste in significant amounts due to some problems such as incidents on farm (e.g. spillage), handling food for some analyses, transportation loss (e.g. damaged package), and obtaining poor quality products (Tostivint et al., 2017). In addition to these wastes, dairy industry by-products (cheese whey, spilled milk and curd chunks) are also potential pollutants due to presence of milk residues (Ahmad et al., 2019). Among them, the excessive amount of whey (approximately 9 L from 1 kg cheese production) released during cheese production to food waste creates an important environmental problem. Other wastes and by-products containing a high level of fat also make it difficult to treatment of these wastes. Furthermore, the wastes have high COD and BOD levels due to the milk proteins and lactose they contain. Considering their high organic contents, the treatment of dairy wastes should be considered a serious problem for the environment. The treatment of whey and other dairy products can be incomplete in the aerobic system or can be difficult in the anaerobic system due to their low bicarbonate alkalinity, high COD content, and a tendency to rapid acidifications (Awasthi et al., 2021).

Dairy industry wastes and by-products are potential raw materials to produce a variety of bio-based products meanwhile simultaneously reducing their carbon footprint. The integration of these wastes into the biorefinery will have contribution to the recovery/separation of bio-products, the growth of microorganisms, and the bioeconomy (Dahiya et al., 2018). However, for the development of waste-to-sustainable industrial processes, it is necessary to take into account (i) technical feasibility of industrial processes, (ii) potential of techno-economic analysis, and (iii) a life-cycle environmental assessment (Caldeira et al., 2020). For example, biogas and further electricity production will be limited due to high capital costs and long payback periods especially in countries in which there is a competition for natural gas (Liao et al., 2014). Therefore, the availability of the appropriate technological systems for integrated facility and its compatibility with the existing technologies should also be taken into consideration.

Expired milk, cheese whey and other dairy industry by-products, together with their rich contents, have been considered as inexpensive materials and evaluated as an alternative substrate in microbial production processes (such as bioenergy, enzymes, organic acids, biopolymers, biomass, etc.). By evaluating these wastes in microbial processes, four different “Global Sustainable Goals” can be contributed. The production of some value-added products (e.g., nisin, biomass, lactic acid) can be included in sustainable consumption and production goals. The production of other products such as bioethanol, biogas and biohydrogen can be suitable for affordable clean energy. Transferring these innovated technologies to industrial production could help solve both economic and environmental problems while providing new job opportunities within the goal of sustainable industrialization. Concomitantly with metabolite production, it contributes to the goal of preventing marine pollution by utilization of wastes used in microbial processes. In addition, the valorization of these wastes in bioprocesses will also contribute to the goal of zero-waste.

This work aimed to examine in detail the contents and varieties of the dairy industry by-products and wastes, their traditional valorization applications and their potential utilizations in biological systems. For this, it was comprehensively reviewed how dairy industry wastes and by-products can be evaluated in microbial production processes. In addition, the results of some techno-economic analyses of the value-added products made from dairy wastes were given.

2. Dairy industry by-products and wastes

The dairy industry is based on the production of various products (sterilized and pasteurized milk and processed dairy products) by processing raw milk (Rivas et al., 2010; Ahmad et al., 2019). Dairy industry side-streams released during processing can be divided into two groups: dairy industry by-products that can be considered edible (such as whey, ice cream, cheese, butter, milk residues), and dairy industry wastes which cannot be evaluated as food (expired, contaminated or spoiled dairy products). While both wastes and by-products can be used in such microbial processes like anaerobic digestion, it seems more feasible to use by-products for edible products such as lactic acid or biomass production.

The first wastewater generated during the process is released during the production and packaging of the products, especially during the cleaning of the equipment such as jar, tank, bottle, and the related equipment (Yonar et al., 2018). On the other hand, food-waste is also generated from milk and other dairy products (especially cream, yogurt, and butter) due to their expiration dates or improper production-transportation-storage processes (Tostivint et al., 2017).

Various by-products of processing dairy products (skim milk, spoiled milk, spilled milk, curd chunks, and whey) are also released during the productions (Ahmad et al., 2019). Dairy industry by-products are potential sources containing lactose, protein, and fat (Table 1), where these wastes can be categorized into two groups which are low-fat dairy substrates (e.g., milk 3%, fat) and fat-rich substrates (e.g., cream and crème fraiche, 40%–50%) (Mahboubi et al., 2017a, 2017b). Dairy by-products contain high COD values (1 kg of lactose, protein, and fat are equivalent to 1.13, 1, and 3 kg of COD, respectively) (Ahmad et al., 2019) due to their rich organic loads. These effluents, particularly high-fat-containing substrates, can cause serious organic loading problems in the local sewage drainage system (Perle et al., 1995).

According to the Food and Agriculture Organization (FAO), about a third (about 1.3 billion tons) of food produced for human consumption is lost or wasted (FAO, 2011). It has been reported that approximately 18% of milk production is wasted due to various reasons (Chang et al., 2018). In addition to this excessive amount of milk, large amounts of whey (9–10 L for 1 kg of cheese production) are also released during the cheese production process. Whey, which is greenish-yellow in color, varies in content depending on the type of milk (cow, goat, sheep, buffalo and other mammals) and the cheese production process (resulted in acid, sweet, and salty whey) (Blaschek et al., 2007). Although whey is reused for ricotta and cottage cheeses production, secondary cheese whey (SCW, Fig. 1) is generated during the process (Carvalho et al., 2013). Different types of by-products (whey powder, lactose, and whey protein, Fig. 1) can also be obtained by ultrafiltration of whey for various purposes, mainly to be used as food nutrients (Atra et al., 2005). Among these, whey powder provides higher efficiency than whey in microbial processes because it contains high concentrations of lactose and other nutrients (Dragone et al., 2011; Sar et al., 2017a, 2017b).

3. Traditional disposal approaches of dairy industry by-products and wastes

Dairy processing for various products such as cheese, yogurt, and butter etc. generate in waste streams with complex compositions. Each product processing and the milk depending on the source animal affect the composition of the dairy wastewater. Due to high organic compositions of dairy industry by-products, direct disposal of these products can cause environmental problems (Asunis et al., 2020).

Treating the streams, mechanical, chemical, physicochemical and biological treatments (aerobic and anaerobic) in-plant is a widespread method (Fig. 2) (Kolev Slavov, 2017). The mechanical treatment method provides the removal of suspended solids from dairy waste. This operation can be considered as a pre-treatment, but not as a treatment operation. Chemical treatment is a pre-treatment process to remove colloids and soluble contaminants from dairy processing waste, mostly by adding flocculants such as Fenton reagents ($FeSO_4$ and H_2O_2) (Vlyssides et al., 2012). Besides them, biological processes have been preferably applied to treatment system since physico-chemical method is also not effective for high soluble COD containing wastes and can require high-cost systems (Vidal et al., 2000).

Table 1
Characterization of dairy industry byproducts.

Dairy byproducts	pH	Total solid	Protein	Fat	Lactose	References
Milk	–	–	10–17 g/L	28–57 g/L	59–76 g/L	(Khan et al., 2013)
Untreated milk	–	114.8 g/kg	34.0 g/kg	–	47.3 g/kg	(Outinen et al., 2010)
High-heat-treated milk	–	113.8 g/kg	34.2 g/kg	–	45.0 g/kg	(Outinen et al., 2010)
Buffalo skim milk	–	10.20%	3.96%	–	5.22%	(Patel and Mistry, 1997)
Whey	6.57	6.31%	0.6%	0.2%	–	(Barile et al., 2009)
Cheese whey	–	68 g/L	2.0 g/L	–	39.60 g/L	(Obruca et al., 2011)
Cheese whey	3.30	59.76 g/L	1.48 g/L	–	35.28 g/L^{C}	(Treu et al., 2019)
Sweet whey	5.2 to 5.4	5.8 to 8.5%	0.6 to 1.0%	0.2 to 0.5%	–	(Blaschek et al., 2007)
Salty whey	5.2 to 5.4	8.6 to 12.4%	0.6 to 0.7%	0.6 to 0.8%	–	(Blaschek et al., 2007)
Whey (Edam)	–	52.3 g/kg	6.93 g/kg	1.8 g/kg	37.1 g/kg	(Outinen et al., 2010)
Caprine cheese whey	6.34	7.07%	0.63%		5.02%	(Sanmartín et al., 2012)
Whey protein concentrate	5.96	18.73%	6.37%	0.4%	–	(Barile et al., 2009)
Whey permeate	6.50	4.87%	0.17%	0.1%	–	(Barile et al., 2009)
Whole milk powder	–	–	24.9%	27%	40.5%	(Silva and O'Mahony, 2017)
Skim milk powder	–	–	33%	0.6%	56.6%	(Silva and O'Mahony, 2017)
Cheese whey powder	–	–	19%	2.6%	48%^{TS}	(Ozmihci and Kargi, 2007)
Cheese whey powder			12.5%	2.2%	67%^{TS}	(Kargi et al., 2012)
Cheese whey powder			14%	2.3%	75%^{TS}	(Sar et al., 2017a, 2017b; Sar et al., 2019)
Sweet whey powder	–	–	11%	1%	74%	(Felix da Silva et al., 2018)
Cream	–	–	2%	40%	3%^{C}	(Mahboubi et al., 2017a)
Crème fraiche	–	–	2%	34%	3%^{C}	(Mahboubi et al., 2017a)

C: Carbohydrates, TS: Total sugar.

Biological treatment is based on microorganisms grown on organic materials in an aerobic and/or anaerobic conditions. Sequencing batch reactor (SBR), membrane bioreactor (MBR) and biofilm reactor and rotating biological contactor (RBC) have been frequently used techniques in aerobic processes (Li and Zhang, 2002; Swain et al., 2018; Collivignarelli et al., 2019). Currently, wetland treatment is one of the applied methods as an aerobic method. After the treatment of dairy wastewater in aerobic ponds for 5 days incubation, it can be discharged into the water basin (Britz et al., 2004). However, this methodology has some disadvantages such as contamination of water resources and a bottleneck of large surface area requirement. Moreover, some components in dairy waste like fat and protein couldn't be completely degraded in aerobic systems (Omil et al., 2003). Alternatively, two thermophilic waste treatment methods which are two anaerobic and aerobic, or only anaerobic digestion using continuous-stirred tank reactors were reported (Arvanitoyannis and Giakoundis, 2006). Although combinations of aerobic and anaerobic systems are application methods for the treatment of dairy waste, these multistage processes can be cost-effective (Masters, 1993; Demirel et al., 2005). An anaerobic sludge blanket (UASB) reactor consisting of sludge blanket, influent-distribution system, gas-solid separator, and the effluent-withdrawal system is the typical application of choice for anaerobic treatment (Kushwaha et al., 2011). Anaerobic system has more advantageous than the aerobic system because it does not require aeration and large surface area. However, anaerobic processes can be limited by the low bicarbonate alkalinity and high COD content of the substrates, as well as the tendency to rapid acidification (Demirel et al., 2005; Awasthi et al., 2021).

As an alternative to these treatment methods, waste management of dairy industry by-products and wastes can also be carried out by determining their use in microbial processes.

4. Microbial processes

The food industry is one of the significant waste producers in the world. Which major part related to the dairy industry. The most striking of the dairy industry wastes is whey, which is discarded from milk and threatens environmental problems and human health (Chandra et al., 2018). Dairy

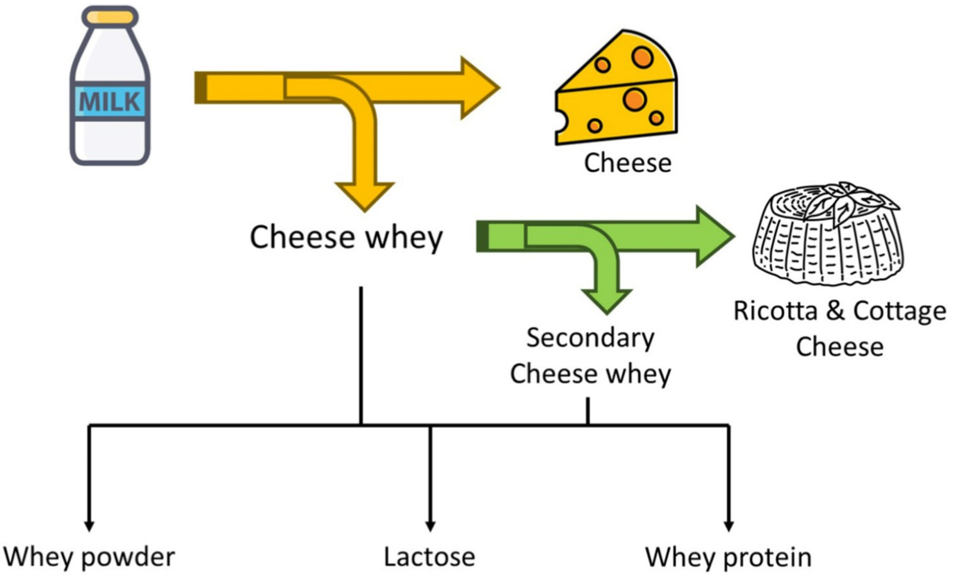

Fig. 1. Generation of whey, secondary whey and their by-products during the cheese production process.

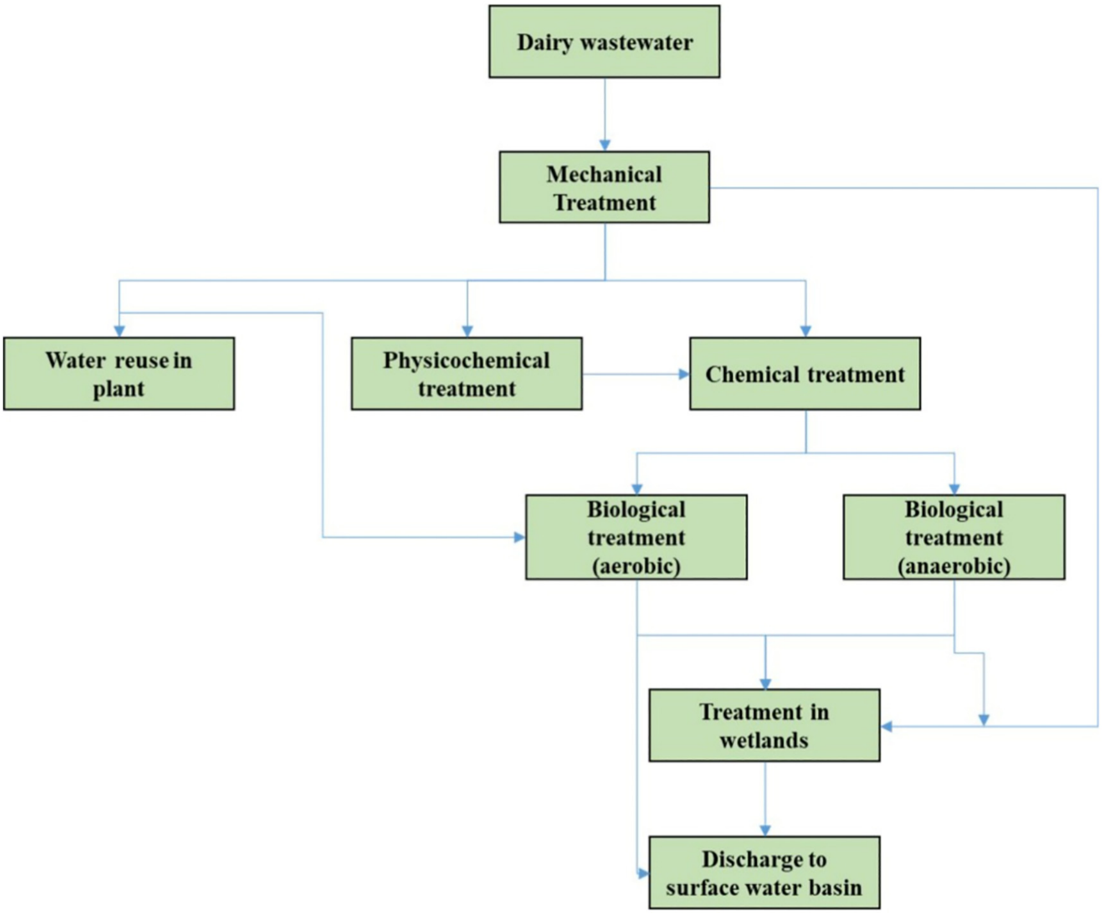

Fig. 2. Treatment methods of dairy effluents. Adapted from Kolev Slavov (2017).

waste and related by-products can be purified and used in other industries by development of some technologies such as reverse osmosis (RO), drying, hydrolysis, ion exchange, nanofiltration (NF), ultrafiltration (UF) and electrodialysis (Yang et al., 1992; Ostojić et al., 2005; Ryan and Walsh, 2016). However, biological methods such as microbial fermentation or anaerobic digestion can be preferred because these methods can be more sustainable than traditional ones and produce more valuable compounds (Chandra et al., 2018). Bioconversion of milk, whey and other dairy products into value-added products is an essential solution in the modern era. Despite their polluting nature, dairy industry by-products are an excellent source of lactose and some other nutrients that can support microbial growth for production of bio-products (Fig. 3). A list of bio-products produced from dairy waste by microorganisms was shown in Table 2 and the production of some value-added substances was described in the following sections.

4.1. Biofuels

4.1.1. Biomethane

Dairy industry by-products with high levels of chemical oxygen demand (COD) can be used as a possible feedstock for biogas production via anaerobic digestion (AD) (Kozłowski et al., 2019). AD is mainly consist of three stages which are (1) hydrolysis of the complex materials into soluble materials, (2) volatile fatty acids (VFAs) and hydrogen production and (3) biogas production from VFAs and hydrogen (Wainaina et al., 2019). *Acetivibrio*, *Bacillus*, *Peptococcus*, and *Vibrio* are responsible for the hydrolysis, *Butyribacterium*, *Propionibacterium* and *Clostridium* are VFAs producers, and some of methanogenic bacteria and archaea are also biogas producers in AD system (Wainaina et al., 2019).

From a circular economy perspective, produced biogas can be consumed as a thermal energy for steam production in the cheese manufacturing process. Otherwise, it can be used as a source of electrical power for the wastewater treatment unit (Comino et al., 2009). Moreover, solid and liquid fractions of the anaerobic digesters contain valuable nutrients that can be served as soil fertilizers (Kavacik and Topaloglu, 2010). Consequently, the anaerobic digestion approach not only can control the wastewater pollution by the dairy industry but also can save energy (Asunis et al., 2020; Hublin et al., 2012).

Many types of bioreactors have been used for the anaerobic digestions of dairy effluents including stirred anaerobic sequencing batch reactor (ASBR), upflow anaerobic sludge blanket (UASB), plug flow (PF), fluidized-bed reactor (FBR), rotating anaerobic contact, and upflow fixed-film loop reactors (Gelegenis et al., 2007; Kavacik and Topaloglu, 2010; Alayu and Yirgu, 2018). Dairy industry by-products usually contain a high organic load and could be a suitable substrate for methane production in anaerobic processes. However, anaerobic treatment of whey is a challenge due to its high COD, low pH value and lack of alkalinity (Kavacik and Topaloglu, 2010). On the other hand, other dairy wastes having low COD values (milk and yogurt) can be non-suitable regarding their anaerobic digestions in conventional continuous stirred tank reactor (CSTR)-type biogas plant (Karadag et al., 2015). The obtained results from various reactors have shown the UASB reactor is an appropriate system with the highest COD removal efficiency.

Besides, the carbohydrates in the dairy effluents stimulate the growth of acid-forming bacteria but inhibit methanogens that cause instability of the reactors. In order to overcome this problem, a co-digestion of dairy effluents with other wastes such as manure, goat straw bedding, brewery spent grain, cattle dung, vinasse, poultry, or livestock wastes has been proposed by many researchers during last decades (Lovato et al., 2019; Szaja and Montusiewicz, 2019; Fernández-Rodríguez et al., 2021). These combinations can retain the carbon/nitrogen (C/N) ratio and microbial synergism in a proper state that supports methanogens' growth and increases biogas

Fig. 3. Potential bio-products produced by using by-products of dairy industry through microbial processes.

production indirectly (Gelegenis et al., 2007; Comino et al., 2009; Comino et al., 2012). Moreover, it is also significant to test the concentrations of metal ions such as copper, zinc, and nickel with mixed substrates as they may limit methanogenesis (Hublin et al., 2012; Panesar and Kennedy, 2012). The acetogenesis and methanogenesis phases can be separated to prevent instability and enhance biomethane yield. This strategy is known as a two-stage anaerobic digestion that needs a lower hydraulic retention time (HRT) compared to traditional anaerobic digestion (less than 5 days) (Asunis et al., 2020).

Electricity and heat generations by anaerobic digestion of various types of dairy by-products (milk, yogurt, whey, dairy sludge and fat sludge) would be beneficial to integrate a biogas system into a dairy plant (Kozłowski et al., 2019).

4.1.2. Biohydrogen

In addition to biomethane, biohydrogen is a feasible alternative to combustion of fossil fuel. It is a clean energy resource as its combustion generates only water and does not emit any greenhouse gases into the atmosphere (Das, 2009). However, the routine hydrogen production process generates carbon dioxide which is the main greenhouse gas (Yang et al., 2007; Show et al., 2012). Regarding this problem, anaerobic fermentation could be a suitable substitute for hydrogen production from wastes with high organic content.

Dairy wastewater, whey, whey powder and milk powder have been used for the hydrogen production (Kargi et al., 2012; Karadag et al., 2014; Chandra et al., 2018). Theoretically, 8 mol of hydrogen can be produced from a unit of lactose consumption, while in current technology, only 3 mol of hydrogen can be achievable (Sherif et al., 2014; Ryan and Walsh, 2016). This may be due to the difficulty of hydrolysis of protein-rich dairy wastes by hydrogen-producing microorganisms (Okamoto et al., 2000). To improve the hydrogen production yield from dairy wastes, different fermentation conditions have been applied. One of them is hydrogen production in a continuous stirred tank reactor (CSTR), a molar yield of 2.8 $molH_2$/mol lactose hydrogen can be obtained when the organic loading rate (OLR) was 138.6 g lactose/L/d (Davila-Vazquez et al., 2009). Other methods for hydrogen production are dark fermentation under anaerobic conditions that is probably a feasible method for hydrogen production at the industrial level if especially it is integrated into other methods (Rao and Basak, 2021; Asunis et al., 2019). This process includes three stages: 1) hydrolysis of lactose into its monomers, 2) homofermentation of glucose and galactose to lactate by lactic acid bacteria (LAB) and 3) anaerobic fermentation of lactate to hydrogen and volatile fatty acids (VFAs) (Asunis et al., 2020). By using this method, the optimum yield of biohydrogen was 4.13 H_2 mol/mol lactose by a microbial consortium (Romão et al., 2014). However, it should be considered that the dark fermentation is intrinsically a complicated process that depends on many factors such as feedstock composition, pretreatment, inoculum size, reactor type, and operating conditions i.e. temperature, pH, fermentation time, OLR, HRT, etc. (Asunis et al., 2019; Dessì et al., 2020). Additionally, the co-fermentation of crude glycerol and cheese whey in an expanded granular sludge bed reactor (EGSBR) was investigated and the results showed maximum hydrogen yield (0/120 mmol H_2/g COD) when the cheese whey/crude glycerol ratio was 5:1. In this reactor configuration, propionic acid was also produced by increasing crude glycerol concentration that resulted in the repression of H_2 production (Lopes et al., 2017).

Microbial electrohydrogenesis cells (MECs) are other strategies for biohydrogen production from cheese whey under controlled conditions. In this system, as the low pH values may affect the function of exoelectrogens and the pH control is required. Moreover, for valorization of cheese whey is

Table 2
A list of some bio-products produced from whey by microbial fermentation.

Bio-product types	Bio-products	Microorganism domains	Examples of microbial strains	References
Biofuels	Biomethane	Yeasts	*Kluyveromyces lactis* *Kluyveromyces marxianus*	(Guimarães et al., 2010)
	Bioethanol	Yeasts	*Kluyveromyces marxianus* *Kluyveromyces lactis*	(You et al., 2017; Sampaio et al., 2020; Yamahata et al., 2020; Tesfaw et al., 2021)
		Bacteria	*E.coli*	(Akbas et al., 2014; Sar et al., 2017a, 2017b: Sar et al., 2019)
	Biohydrogen	Archaea	*Methanobacterium* sp.	(Rosa et al., 2014)
	Biodiesel	Algae	*Chlorella protothecoides*	(Espinosa-Gonzalez et al., 2014)
		Bacteria	*Streptococcus thermophiles* *Lactobacillus delbrueckii* subsp. *bulgaricus*	(Vasiljevic and Jelen, 2001)
Enzymes	*β*-galactosidase	Filamentous fungi	*Aureobasidium pullulans*	(Kaur et al., 2015)
		Yeasts	*Kluyveromyces marxianus* *Candida pseudotropicalis*	(Kaur et al., 2015)
	α-amylase	Bacteria	*Serratia marcescens*	(Romero et al., 2001)
Solvents	Biobutanol	Bacteria	*Clostridium acetobutylicum*	(Foda et al., 2010)
Vitamins	Vitamin B12	Bacteria	*Propionibacterium shermanii*	(Bullerman and Berry, 1966)
Surfactants	Biosurfactant	Bacteria	*Lactococcus lactis* *Streptococcus thermophilus*	(Rodrigues et al., 2006)
Polyols	Glycerol	Yeasts	*Kluyveromyces marxianus*	(Rapin et al., 1994)
Organic acids	Propionic acid	Bacteria	*Propionibacterium* strains	(Atasoy and Cetecioglu, 2021; Atasoy et al., 2020a)
	Acetic acid	Yeasts	*Kluyveromyces fragilis*	(Mostafa, 2001)
	Gluconic acid	Filamentous fungi	*Aspergillus niger*	(Mukhopadhyay et al., 2005)
	Lactic acid	Bacteria	*Lactobacillus casei* *Lactococcus lactis*	(Panesar et al., 2010; Prasad et al., 2014)
	Citric acid	Filamentous fungi	*Aspergillus niger*	(El-Holi and Al-Delaimy, 2003)
	Succinic acid	Bacteria	*Anaerobiospirillum succiniciproducens* *Actinobacillus succinogenes*	(Lee et al., 2000; Louasté and Eloutassi, 2020)
	Pyruvic acid	Bacteria	*Klebsiella oxytoca*	(Cao et al., 2020)
Biopolymers	Poly-3-hydroxybutyric acid (PHB)	Bacteria	*Pseudomonas hydrogenovora*	(Koller et al., 2008)
	polyhydroxyalkanoates (PHAs)	Bacteria	*Thermus thermophilus* *Ralstonia eutropha* *Alcaligenes latus* *Aeromonas hydrophila* *Pseudomonas putida*	(Sudesh et al., 2000; Pantazaki et al., 2009)
	Poly(β-L-malic acid) (PMA)	Filamentous fungi	*Aureobasidium pullulans*	(Xia et al., 2021)
Hormones	Gibberellic acid	Filamentous fungi	*Fusarium moniliforme* *Aspergillus niger*	(Kahlon and Malhotra, 1986; Cihangir and Aksöza, 1997)
Ribonucleotides	5′-guanosine monophosphate 5′-inosine monophosphate	Yeasts	*Kluyveromyces marxianus*	(Húngaro et al., 2013)
Biomass	Single-cell protein (SCP)	Yeasts	*Kluyveromyces marxianus* *Kluyveromyces fragilis*	(Schultz et al., 2006)
	Fungal biomass	Filamentous fungi	*Aspergillus oryzae*	(Mahboubi et al., 2017a, 2017b; Thunuguntla et al., 2018)
Aroma compounds	Acetoin Diacetyl	Bacteria	*Lactobacillus casei*	(Zotta et al., 2020)
Bioactive compounds	Lactobionic acid	Bacteria	*Pseudomonas taetrolens*	(Goderska et al., 2014)
	Nisin Z	Bacteria	*Lactococcus lactis*	(Amiali et al., 1998)
	Plantaricin	Bacteria	*Lactobacillus plantarum*	(Zotta et al., 2020; Sharma et al., 2021)
	Pediocin	Bacteria	*Pediococcus acidilactici*	(Pérez Guerra et al., 2005)
	Enterocin AS-48	Bacteria	*Enterococcus faecalis*	(Ananou et al., 2008)
		Yeasts	*Kluyveromyces lactis*	
		Filamentous fungi	*Aspergillus oryzae*	
	Glactooligosaccharides (GOSs)	Bacteria	*Bacillus circulans* *Lactobacillus reuteri* *Bifidobacterium bifidum* *Bifidobacterium infantis* *Lactobacillus acidophilus* *Lactobacillus pentosus* *Bifidobacterium longum*	(Nath et al., 2016)
Exopolysaccharides (EPS)	Dextran Xanthan Cellulose	Bacteria	*Leuconostoc mesenteroides* *Xanthomonas campestris* *Komagataeibater xylinus*	(Zotta et al., 2020)
Fusel alcohols	2-phenylethanol	Yeasts	*Kluyveromyces marxianus* *Debaryomyces hansenii* *Galactomyces geotrichum*	(Szudera-Kończal et al., 2020; Valdez Castillo et al., 2021)

better to use anaerobic digestion effluent as a suitable choice for MEC process (Rivera et al., 2017).

4.1.3. Bioethanol

At present, the use of low-cost feedstocks for bioethanol production is encouraged, and lactose-containing whey/expired milk has an advantage over lignocellulosic feedstocks since it does not need special pretreatment (Akbas et al., 2014; Pescuma et al., 2015; Ryan and Walsh, 2016; Okamoto et al., 2019). In the production of ethanol from lactose-containing dairy waste, microorganisms that can naturally consume lactose can be used or a chemical hydrolysis can be performed using the enzyme *β*-galactosidase (Guimarães et al., 2010). However, diauxic growth of microorganisms in the presence of both glucose and galactose after hydrolysis reduces the ethanol production efficiency (Panesar and Kennedy, 2012). The most common yeasts are *Kluyveromyces marxianus* and *K. lactis* which can ferment lactose to ethanol directly (You et al., 2017; Sampaio et al., 2020; Yamahata et al., 2020; Tesfaw et al., 2021). *Kluyveromyces* growth could be limited by salt and moderate sugar concentrations, and tolerance to produced ethanol is lower than *S. cerevisiae* (Mawson, 1994; Akbas et al., 2014). The lactose consumption and ethanol productivity yields were achieved through cloning of the ethanol producing and lactose consumption genes to some microorganisms such as *Saccharomyces cerevisiae*, *E. coli*, and *Corynebacterium glutamicum* (Dien et al., 2000; Zou et al., 2013; Shen et al., 2019). Some fungal species (*Neolentinus lepideus*, *Aspergillus oryzae* and *Neurospora intermedia*) can naturally consume lactose and produce ethanol using the dairy industry wastes (Thunuguntla et al., 2018; Okamoto et al., 2019). Many factors like strain type, aeration rate, substrate concentration, cell immobilization, temperature, initial pH and the type of fermentation systems, etc. affect the bioethanol production from the whey, whey powder and expired milk (Pescuma et al., 2015; Sar et al., 2017a, 2017b; Sar et al., 2019; Asunis et al., 2020).

4.1.4. Biodiesel

One of the alternatives for fossil fuels is methyl ethyl esters extracted from plants, microalgae or oily fungal biomass which are known as biodiesel (Kumar et al., 2020a, 2020b; Mathew et al., 2021). Growing microalgae (*Chlorella*, *Anabaena*, *Scenedesmus*, *Chlamydomonas* and *Acutodesmus* etc.) and fungal (*Mortierella isabellina*, *Thamnidium elegans*, *Mucor* sp., and *Fusarium*) species in dairy wastes can encourage the treatment of wastewater, supports the production of oily biomass and contributes bioenergy generation (Chokshi et al., 2016; Chan et al., 2018; Brar et al., 2019; Roy et al., 2021). Among them, *Chlorella protothecoides* is known as the best lipid producing strain from dairy by-product stream using batch (42% lipids, dry weight basis) and fed-batch (20.5 lipids, dry weight basis) fermentation (Espinosa-Gonzalez et al., 2014). *Apiotrichum curvatum*, *Scenedesmus* sp. and *Cryptococcus laurentii* strains are also other oleaginous microorganisms that can be able to produce lipids from whey (Pescuma et al., 2015; Borges et al., 2016). Oily biomass containing oleic and palmitic acids can be evaluated for biodiesel production with two steps including extraction of oil from biomass and obtaining fatty acid methyl esters (FAME) via acid-catalyzed transesterification and esterification steps (Vicente et al., 2009; Chan et al., 2018).

4.2. Organic acid(s) production

Organic acids are valuable microbial products with acidic properties. The most common organic acids are acetic, lactic, propionic, succinic, citric, oxalic, valeric, and capric acids (Pandey et al., 2016; Louasté and Eloutassi, 2020). These acids known as carboxylic acids are considered as important raw materials in various industries, and their global demand have been gradually increased. Consequently, finding renewable, inexpensive, and accessible resources like dairy wastes for high yield production of organic acids attracts researchers' attention.

4.2.1. Acetic acid

The traditional acetic acid (vinegar) production includes fermentation of the carbohydrates to ethanol and then oxidation of ethanol to acetic acid by acetic acid bacteria (AAB). However, the aerobic step of this process is not affordable due to its high energy input. Hence, in past decades, it was proposed to perform anaerobic homo-fermentative production of acetic acid from whey by *Clostridia*. However, the theoretical yield of acetate concentration in this process was approximately 3% while, in practice, acetate yield was less than 1% (Yang et al., 1992; Tang et al., 1988). Recently, Veeravalli and Mathews (2018) used an acid-tolerant lactic acid bacterium, *Lactobacillus buchneri*, to produce acetic acid from whey powder in an immobilized system. The results showed that 57% of the lactose in the whey powder was converted into acetate and propylene glycol. It seems *L. buchneri* has industrial potential for acetic acid production that can be improved through metabolic or evolutionary engineering (Veeravalli and Mathews, 2018).

4.2.2. Lactic acid

Lactic acid is a natural preservative acid and flavoring agent used in food, pharmaceutical, cosmetic, chemical and textile industries (Trakarnpaiboon et al., 2017). It is also a precursor for synthesis of polylactic acid (PLA) in chemical industry (Liu et al., 2018). Traditionally, lactic acid is produced by mainly LAB, some fungal strains (*Rhizopus microspores*) and engineered bacterial strains (*Bacillus* sp. and *E. coli*) (Trakarnpaiboon et al., 2017; Harirchi et al., 2020). Dairy industry wastes such as skim milk and whey can be considered as suitable medium for LAB cultivation. Many researcher have shown that L(+)isomer of lactic acid were produced instead of D(−) isomer of lactic acid when bacterial strains (*Lactobacillus casei*, *L. paracasei* subsp. *tolerans*, *L. rhamnosus*, *L. lactis*, and *Streptococcus thermophiles*) were cultivated in whey media (Pescuma et al., 2015; Ghasemi et al., 2017). The L(+) lactic acid has a better feature that makes it suitable to be used in the food and pharmaceutical industries (Zhang et al., 2007). Among the LAB, *Lactobacillus helveticus* can produce more lactic acid compared to other LAB, but the final product is a racemic mixture of (DL) lactic acid (Panesar et al., 2007; Shiphrah et al., 2013). However, microbial production of lactic acid is not cost-effective, thus; the most investigations are conducted to find high yielding strains, low-priced raw materials, and novel technological production methods (Cui et al., 2012).

Lactic acid bacteria are naturally grown in various types of milk (cow, goat, sheep, camel) and used for production of probiotics (Ao et al., 2012; de Almeida Júnior et al., 2015; Fguiri et al., 2016). In addition, the presence of lactic acid bacteria has been detected in dairy products such as yogurt, ice-cream, cheeses, sweet creams and sweet kajmaks (a kind of cream) (Terzic-Vidojevic et al., 2014; Góral et al., 2018). For this purpose, cheese whey and expired milk as dairy products have been used to produce lactic acid. However, since the composition of cheese whey is not enough for lactic acid production, it should be enriched with minerals (potassium phosphate, ammonium sulfate, magnesium chloride, or manganese sulfate) and nitrogen (yeast extract, peptones, molasses, corn steep) to support bacterial growth and the yields of lactic acid production (Gupta and Gandhi, 1995; Panesar and Kennedy, 2012). Moreover, many cultivation parameters (temperature, pH, oxygen requirement, agitation) should be also investigated to improve the lactic acid production (Zayed and Winter, 1995; Panesar et al., 2007). For example, *L. helveticus* showed maximum lactic acid production at 42 °C while *L. casei* displayed the highest rate of lactic acid production at 37 °C (Roy et al., 1986; Büyükkileci and Harsa, 2004). Since lactic acid bacteria undergo a long lag period to start fermentation of whey, a larger fermenter capacity is required that increases operational costs. On the contrary, high productivity occurs even in low-volume fermenters when continuous production systems were applied (Aeschlimann and von Stockar, 1991; Panesar et al., 2007; Soriano-Perez et al., 2012). In continuous batch systems, immobilized cells can be successfully applied for lactic acid production using by dairy wastes (Kourkoutas et al., 2005; Panesar and Kennedy, 2012). Comprehensively, lactic acid production from dairy effluents by microbial fermentation has been increased recently. However, downstream processing may increase the costs and this problem could be solved by using integrated processes (Phanthumchinda et al., 2018; Ahmad et al., 2020; Li et al., 2021).

4.2.3. Citric acid

Citric acid is a common organic acid found naturally in citrus fruits that is used in pharmaceutical, beverage, and food industries (Najafpour, 2015). Moreover, a well-known yeast species, *Yarrowia lipolytica*, has an exclusive ability in its production and secretion into the medium (Kamzolova et al., 2005). Among filamentous fungi, *Aspergillus niger* is frequently used for citric acid production (Panesar and Kennedy, 2012). Due to wide-ranging applications of citric acid, novel and low-priced process technologies are required for higher production yield. Generally, for a high yield production of citric acid, raw materials with high sugar content are needed. Many inexpensive wastes such as starch hydrolysates, date seeds, molasses, grape must, and apple pomace have high carbohydrate content employed for citric acid production. Yalcin et al. (2009) studied citric acid production in whey supplemented with fructose using the yeast strains. Maximum acid concentrations were 32.65 g/L and 49.23 g/L produced by *Y. lipolytica* NBRC 1658 and *Y. lipolytica 57*, respectively.

The improvements in the citric acid industry will both overcome the waste disposal problems and reduce the dependency of industry over other producers. Thus, ecological and economic benefits will be provided to the citric acid industry (Sawant et al., 2021). Citric acid, added as an emulsifier, acidifier, or antioxidant during the processing of ice creams and cheese products, can be regenerated from mixture of whey and different substrates (Soccol et al., 2006; Yalcin et al., 2009). Thus, citric acid produced by dairy industry can be reused to produce dairy products, and this process may result in decreasing total costs in the cheese production unit.

4.2.4. Propionic acid

Another important organic acid is propionic acid which is used to produce cellulosic plastics, perfumes, herbicides, and other chemicals (Ahmadi et al., 2017). Moreover, it is a significant fungicide and food preservative that is also produced in petrochemical refineries. However, some efforts have been made to produce propionic acid from whey containing lactose under anaerobic conditions and continuous fermentation by *Propionibacterium acidipropionici* ATCC 4875 (Gupta and Srivastava, 2001). Moreover, this acid is known as one of the VFA that can be produced during acidogenic fermentation in the anaerobic digestion process. Propionic acid with other VFAs (acetic and butyric) was produced by mixed culture in the presence of *Propionibacterium acidipropionici* from dairy industry wastewater (Atasoy et al., 2020a; Atasoy and Cetecioglu, 2021). Anaerobic sequencing batch reactor (ASBR) was successfully applied to produce VFA and bioenergy from cheese whey. Moreover, Atasoy et al. (2020b) reported that the propionic acid produced through *Desulfovibrionaceae* and *Synergistaceae* from cheese processing wastewater was found to be main organic acid (80%) when the VFA production was performed in ASBR. However, the bottleneck of this method is the purification and recovery of produced propionate (Lagoa-Costa et al., 2020).

4.2.5. Succinic acid

Succinic acid is the final product of anaerobic metabolism in the Krebs cycle. This acid is frequently used in various industries (detergent, cosmetic, herbicides, fungicides, etc.) that result in high demand for its production (Zeikus et al., 1999). Chemical production of succinic acid is not only an expensive process, but also contributes to greenhouse gases emission. It is the main reason why the green production of this acid via whey valorization considered recently. The yield of succinic acid (0.58 g h^{-1} L^{-1}) was found to be high when the initial pH of the medium was 6.80 in 50 g/L cheese whey medium with 5% inoculation (Wan et al., 2008). Louasté and Eloutassi (2020) studied microbial production of succinic acid from whey under anaerobic conditions. In batch fermentation, the succinic acid yield and productivity of *Actinobacillus succinogenes cultivation on whey* were 62.1% and 0.81 g/L/h, respectively. The use of immobilization system with batch, repeated-batch and continuous batches can also increase the succinate productivity (0.89–1.09 g h^{-1} L^{-1}) (Uysal and Hamamcı, 2021). In addition, formic and acetic acids were also by-products that can be detected during the fermentation of whey at 48 h (Wan et al., 2008).

4.3. Enzymes production

It is not exaggerated to say the heart of biotechnological processes is enzymes. They have been employed during human history directly and indirectly. Industrial-scale production of enzymes was started in the 1960s and rapidly grew. Most industrial enzymes are produced by microorganisms that may be genetically modified (Singhania et al., 2010). Despite extensive research about the enzyme production process, it has remained an expensive process in many cases. The selection of substrates can influence enzyme production and affect production costs significantly. One of the low-priced resources for enzyme production is dairy industry by-product (Ryan and Walsh, 2016). Moreover, enzyme production from these substrates may be a sustainable way that solves the pollution problem of the dairy industry. Some yeasts, molds, and bacteria can grow on wastes containing lactose and produce enzymes such as β-galactosidase (Alves et al., 2019), α-amylase (Jabeen et al., 2019), manganese peroxidase (Feijoo et al., 1999), protease (Romero et al., 2001), penicillin acylase (De León-Rodríguez et al., 2006), penicillin amidase (Tahir et al., 2009), polygalacturonase (Panesar and Kennedy, 2012), cutinase (Watanabe et al., 2014), inulinase (Singh et al., 2019), lipase (Knob et al., 2020), nattokinase (Sahoo et al., 2020), or α-galactosidase (Álvarez-Cao et al., 2020).

4.3.1. Beta-galactosidase

Traditionally *Kluyveromyces* and *Aspergillus* species exhibit excessive potential for β-galactosidase (lactase) production (Oliveira et al., 2011). While some microbial strains (*Streptococcus*, *E. coli*, *Lactobacillus*, *Leuconostoc*) are able to produce this enzyme, the enzyme obtained from *Bacillus* sp. has been commercialized (Panesar et al., 2006; Oliveira et al., 2011). Alternatively, a novel strain of *Paracoccus marcusii* strain was identified to produce β-galactosidase from lactose (Kalathinathan and Muthukaliannan, 2021).

The potential use of whey among dairy wastes has been intensively investigated for enzyme production (Viana et al., 2018; Alves et al., 2019; Bosso et al., 2019). The initial pH of the whey and incubation temperature can influence the β-galactosidase production rate (Alves et al., 2019). Additionally, a drawback exists in lactase production that related to its intracellular nature and rises downstream processing costs. For this, many types of research were conducted to find simple, operative, and affordable purification methods for lactase (Bansal et al., 2008).

4.3.2. Alpha-amylase

The α-amylase is another commercial enzyme that may be produced from dairy waste. Production of α-amylase can be performed by using agro-dairy wastes (wheat bran, cheese whey, soybean cake, hazel nut oil cake) through a semi-solid-state fermentation (Selen and Saban Tanyildizi, 2017). Moreover, a thermo stable amylase can be also produced from agro-dairy wastes, which is a mixture of sugarcane bagasse and whey, by *Anoxybacillus beppuensis* (Jabeen et al., 2019). Alternatively, dairy wastes mixed with fruit industry by-products, such as orange peels, can also be used for α-amylase production (Uygut and Tanyildizi, 2018).

4.3.3. Proteases

Proteases are hydrolytic enzymes broadly used in various fields including, molecular biology, food and pharmaceutical industries, textile, leather processing, and detergents. Their production from dairy waste is a promising way in biotechnology and resource recovery. It was investigated that the various strains of *Serratia marcescens* produced extracellular metalloprotease when the bacterium was grown on the whey (Romero et al., 2001; Panesar and Kennedy, 2012). Similarly, some fungal species, such as *Aspergillus* and *Mucor*, can be grown in whey and produce the protease enzyme (El-Shora and Metwally, 2008).

4.4. Bioactive compounds

The importance of bioactive compounds on human health has been increased recently. Therefore, many efforts have been made to find novel and cost-effective ways for their production on a large scale (Kaur et al., 2020; Sebastián-Nicolás et al., 2020). Healthy products similar to bioactive peptides in milk that provide nutraceutical and functional foods can be obtained using dairy products such as whey by microbial fermentation (Moslehishad et al., 2013; Yadav et al., 2015; Lucarini, 2017).

Galacto-oligosaccharides (GOSs) are the conversion of lactose (defined lactose solutions, milk, cheese whey and acid whey from yogurt) using the endoenzyme β-galactosidase (Deng et al., 2020; Duan et al., 2021; Zerva et al., 2021). The GOSs are non-digestible oligomers of up to eight saccharide units that can be served as prebiotic for colon health by promoting the growth of *bifidobacteria* (Mano et al., 2019; Wiciński et al., 2020).

However, the loss of the high rate of commercial enzymes is the main drawback of this valorization method that may be overcome by using the immobilized enzymes. Similarly, microbial production of GOSs has still many challenges associated with productivity, yield and final product quality (Chandra et al., 2018; Suwal et al., 2019; Rico-Rodriguez et al., 2021). Despite these challenges, microalgae are promising microorganisms for GOSs production from whey. In a study, *Tetradesmus obliquus* was grown in a medium containing sweet whey permeate. The obtained results showed a substantial quantity of *β*-galactosidase after 7 days of cultivation. Moreover, lactose hydrolysis was occurred under mixotrophic and heterotrophic conditions in this species (Suwal et al., 2019).

Among yeasts and bacterial strains, *Kluyveromyces marxianus* showed the ability for high production of *β*-lactoglobulin from whey. Additionally, *S. cerevisiae* and *L. helveticus* have converted whey into a potent antihypertensive peptide. Didelot et al. (2006) indicated a co-culture of *Candida parapsilosis* and *Lactobacillus paracasei* could produce a bioactive peptide with angiotensin converting enzyme (ACE) inhibitory function that consisted of Tryptophan-Leucine-Alanine-Histamine-Lysine (Trp-Leu-Ala-His-Lys) and resistant to trypsin and pepsin in-vitro (Didelot et al., 2006). Moreover, *Pseudomonas taetrolens* strain has capability of lactose oxidation to a bioactive compound, lactobionic acid (LBA). This derivative of gluconic acid is a polyhydroxy acid and exhibits acidulant, humectant, antioxidant, anti-ageing, and chelating properties which are applicable in the food, cosmetic, and pharmaceutical industries. The chemical methods used for LBA production are so expensive and, hazardous by-products may produce in side reactions. Hence, green production of LBA from low-cost raw materials is in the spotlight of research. Cheese whey and bovine scotta are raw materials that can be considered proper substrates for LBA production via microbial fermentation. The optimal conditions for LBA production by *P. taetrolens* was determined to be a 48-h oxidation process at 30 °C from cheese whey (Goderska et al., 2014; De Giorgi et al., 2018). Previously, the concentration of produced LBA by *Pseudomonas* sp. LS13-1 was 175 g/L when the cells were grown on spray-dried whey. Moreover, the production of LBA is reported in other microbial species such as *Zymomonas mobilis*, *Acetobacter orientalis*, *Microdochium nivale*, *Myriococcum thermophilum*, *Sclerotium rolfsii*, and *Pseudomonas graveolens* (Pescuma et al., 2015).

Fascinatingly, edible fungus, *Pleurotus* spp. can produce bioactive compounds. These compounds display properties that may improve heart health and immune system, decrease cancer risk, or balance blood sugar. In a recent study, the mycelial growth of *Pleurotus djamor* PLO13 was performed in the liquid medium supplemented with whey and selenium. In this medium, mycelial biomass, including antioxidant activity and contents of *β*-glucans and, ergosterol was increased that indicated *P. djamor* could be employed as a nutritional supplement (Velez et al., 2019).

Additionally, antimicrobial peptides such as bacteriocins can be produced by using whey. These compounds may be effective on Gram-negative or Gram-positive bacteria or both of them. Nisin and pediocin are two common examples of bacteriocins produced in the media containing whey. These bacteriocins are promising antibiotics for the treatment of drug-resistant infections (Ryan and Walsh, 2016). Panesar and Kennedy (2012) suggested that the diluted whey could be a suitable substrate for bacteriocin production by *Lactococcus lactis* subsp. *lactis* CECT 539 and *Pediococcus acidilactici* NRRL B-5627 strain.

4.5. Single-cell protein

Since dairy wastes are suitable substrates for bioprocessing, several studies focused on the microbial fermentation for single-cell protein (SCP) production that can be used as the human foods or animal feeds (Ryan and Walsh, 2016). Most studies used *K. fragilis*, which is lactose-utilizing yeast and known as generally recognized as safe (GRAS) (Ghaly and Kamal, 2004; Ghaly et al., 2005; Schultz et al., 2006). Industrial production of SCP from deproteinized whey was initiated in France in the 1950s and three yeasts, *K. marxianus* var. *marxianus*, *K. marxianus* var. *lactis*, and *Candida pintolopepsii* were used (Panesar and Kennedy, 2012; Pescuma et al., 2015). Among yeast strains used for SCP production, *Debaryomyces robertsiae* (Synonym: *Wingea robertsii*) showed a maximum yield of 89% with higher protein content (Sandhu and Waraich, 1983). Moreover, in batch fermentation system, baker's yeast (*S. cerevisiae*) can be produced from whey that its lactose content hydrolyzed by immobilized lactase and supplemented with minerals (Castillo, 1990).

Filamentous fungi are also attractive microorganisms for protein-rich biomass production. *Aspergillus*, *Rhizopus*, *Neurospora*, *Monascus*, *Fusarium*, designated as GRAS, are the most common fungal genera mainly used for fungal biomass production from different types substrates (Souza Filho et al., 2019; Sar et al., 2020a, 2020b; Sar et al., 2021). As an alternative to the substrates used for fungal biomass production, the potential use of various dairy wastes (such as cheese whey, milk, yogurt, cream) was investigated through cultivation of *Aspergillus oryzae* and *Neurospora intermedia* strains. Fungal biomass production from expired milk was found to be 11 g/L by *A. oryzae* and 7 g/L by *N. intermedia* (Thunuguntla et al., 2018). However, the bioconversion of substrates containing high fat content (e.g., cream) could be difficult process. For this, two-step fermentation which is consisted of *A. oryzae* (a fat-degrader) in the first reactor and then *N. intermedia* (a lactose consumer) in the following reactor, has been recommended. It has been suggested that the biomass produced by these fungi contains 30–40% protein and that can be used as animal feed (Mahboubi et al., 2017a, 2017b). Similarly, production of oil-rich biomass from dairy wastes like whey by cultivation of various fungal species can also be possible (Kakkad et al., 2015; Chan et al., 2018; Ibarruri and Hernández, 2019; Chan et al., 2020). These oily fungal biomass containing 24–32% oil can also be used in biodiesel production or nutraceutical applications (Chan et al., 2018; Chan et al., 2020).

4.6. Biopolymers

Dairy waste is an appropriate substrate for biopolymer productions (Pandian et al., 2010; Dutt Tripathi et al., 2021). Biopolymers have a wide range of applications. They can have emulsifying, biocatalyst activities, gel formation and antitumor activities, etc. and they are considered appropriate alternatives to oil-based polymers (Zikmanis et al., 2020). Some of the biopolymers such as PLA and polyhydroxyalkanoates (PHAs) exhibit plastic and mechanical properties that are comparable with oil-based plastics (Gironi and Piemonte, 2011). In addition, they can also be employed for clinical care applications, food packaging, insulation, or disposable tableware and garments (Khanafari and Sepahei, 2007; Ryan and Walsh, 2016). There were three promising strategies suggested for the bioconversion of dairy processing wastes to PHA: 1) Direct bioconversion of lactose to PHA that is limited to few microorganisms such as *Hydrogenophaga pseudoflava*, *Thermus thermophilus*, *Pseudomonas hydrogenovora*, *Bacillus megaterium* or engineered strains bearing lactose-degrading genes (e.g. engineered *Cupravidus necator*) (Asunis et al., 2020; Israni et al., 2020), 2) Enzymatic or chemical bioconversion of whey to break lactose into glucose and galactose, which are usable by a wide range of microorganisms, and 3) Two-stage bioconversion includes fermentation of lactose into lactic acid and then conversion of lactic acid to PHA by PHA-producing strains (Mollea et al., 2013; Ryan and Walsh, 2016). Practically, using mixed microbial cultures (MMC) are a suitable strategy for the high yield production of PHAs by enhancing of complex substrates (Zikmanis et al., 2020).

Similarly, dairy wastes can be bioprocessed to produce exopolysaccharides (EPS). These extracellular polymeric substances are used commonly in food products to improve food texture (Iliev et al., 2001). Some bacterial EPS that can be produced from whey and its derivatives including alginate, dextran, levan, xanthan, pullulan, cellulose, curdlan, hyaluronic acid, or gellan (Zikmanis et al., 2020). The bacterial strains of *Pseudomonas*, *Azotobacter chrococcum*, *A. vinelandii*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *L. rhamnosus*, *Xanthomonas cucurbitae*, *X. campestris*, *Rhizobium radiobacter* S10, *Zunongwangia profunda*, and *Sphingomonas paucimobilis* have substantial benefits for EPS production from dairy wastes (Panesar and Kennedy, 2012; Pescuma et al., 2015; Wang et al., 2020). Attractively, Grigorova et al. (1994) reported that a lactose-negative *Rhodotorula acheniorum* strain grown with *L. casei* 91 strain produced a high level of EPS indicating an effective synergism between both strains. However, the production of the microbial EPS can be an expensive process and this drawback can be overcome by using low-priced raw materials and optimization of the production process.

4.7. Pigments

In general, pigments are colored materials found in the plant, animal, or microbial cells. One of the best-known pigments is carotenoids that are used as antioxidants, anticancer agents, coloring and flavoring additives in the food, feed, pharmaceutical, and cosmetic industries. Carotenoids are derivatives of unsaturated isoprene (Bakhtiyari et al., 2020; Liu et al., 2021) and the biosynthesis of this group of pigments is discovered in molds, algae, lichens, yeasts, and some bacterial genera (Bhosale and Bernstein, 2005; Varzakakou et al., 2010a). *Rhodosporidium*, *Rhodotorula*, and *Phaffia* are common examples of yeasts for carotenoid production. The first two genera produce *β*-carotene, torularhodin, and torulene, while astaxanthin is produced by *Phaffia* (Perrier et al., 1995). Additionally, *Sporobolomyces*, *Xanthophyllomyces*, *Sporidiobolus*, and *Cryptococcus* are also known as carotenoid-producing yeasts (Nasrabadi and Razavi, 2011). They can ferment mono- and disaccharides to produce these pigments; however, there are a few reports about the utilization of lactose as a carbon source for the biosynthesis of carotenoids in yeasts. Co-cultivation of lactose-positive yeasts or bacteria with carotenoid producers resulted in carotenoid production in whey ultrafiltrate (Panesar and Kennedy, 2012; Frengova et al., 2004). The molds have an applicable capacity for pigment production. For instance, *Blakeslea trispora* is a filamentous fungal species from the phylum *Zygomycota* that showed the highest yield for carotenoid production from deproteinized whey in the shake flask, airlift bioreactor, and bubble column bioreactor (Varzakakou et al., 2010a, 2010b; Roukas et al., 2015). In a recent study, a freshwater microalgal strain, *Desmodesmus* sp. L2B Bold, was grown on cheese whey and produced 0.5 μg/mL carotenoids under environmental conditions (Bonett et al., 2020). Furthermore, the bacterial genus *Cellulosimicrobium* showed the ability of carotenoid production from whey. This strain produced 17.5 mg/L carotenoids when the concentration of whey in the medium was adjusted at 60% w/v (Bakhtiyari et al., 2020). Melanin is a black-brown pigment with various functions included metal ion chelation, photo-protection, thermoregulation, etc. that makes it a suitable choice in the cosmetic, pharmaceutical and food industries (Eskandari and Etemadifar, 2021). Eskandari and Etemadifar (2021) optimized growth conditions of the bacterial strain, *Dietzia schimae* NM3 by response surface methodology (RSM) to increase melanin production in the whey-containing medium.

Comprehensively, microbial production of the pigments can be feasible industrially if the process costs can be minimized as far as possible. One of the factors that may aid in this issue is the use of low-priced raw materials like dairy whey. Moreover, optimization of environmental factors and growth conditions that affect carotenoid production could accommodate this platform more feasible.

4.8. Fuel cells

Remarkably, dairy wastewater, cheese wastes/wastewaters, cheese whey and whey powder have the potential to act as an electron donor and stimulate the dissolution of the toxic solvent trichloroethene (TCE) (Sekar et al., 2019; Veeramani et al., 2020). By adding whey powder (10% w/w) to the designed microcosms, the solubility of TCE increased by a factor of three and six in laboratory and field experiments, respectively. It indicates the extensive applications of dairy wastes can be performed for various purposes (Macbeth et al., 2006). In this regard, one of the other applications of dairy wastes is related to electricity generation via microbial fuel cells (MFCs). In MFC, organic wastes are anaerobically oxidized by microorganisms in the anode and, produced electrons are conducted to the external circuit (Tremouli et al., 2013; Ghasemi et al., 2017). In a study conducted by Antonopoulou et al. (2010), a two-chamber mediator-less MCF was run using a diluted cheese whey that generated a voltage of 0.23 V, a current density of 80 mA/m^2, and a power density of 18.4 mW/m^2 (Antonopoulou et al., 2010). Furthermore, Ghasemi et al. (2017) applied two different methods to treat cheese whey and concentrated whey. In an immobilized cell reactor (ICR), fermentation of whey was carried out by immobilized *L. bulgaricus* cells and the maximum concentration of lactic acid (10.7 g/L) obtained at a dilution rate of 0.125/h when the initial concentration of lactose was 50 g/L. The COD removal was 95% and 86% for cheese whey-fed and concentrated whey-fed, respectively by applying MFC. Moreover, a power density of 288.12 mW/m^2 achieved for concentrated whey-fed MFC (Ghasemi et al., 2017). Similarly, Wenzel et al. (2017) used the reactor effluent which was 1000 times more than raw cheese whey, and the power density were reached to 439 mW/m^2.

In addition to MFC, enzymatic fuel cells (EFC) can be used for power generation from whey. This system is based on redox-active enzymes and is thought of as an innovative technology for green power generation. In a study, EFC with immobilized cellobiose dehydrogenase was employed for the power generation from cheese whey. This enzyme is known as a unique anodic enzyme that produces bioelectrical energy from lactose, cellodextrins, or cellobiose and can transfer electrons prominently. Under optimal conditions, a maximum power density of 1.839 μW/cm^2 was obtained when cheese whey was demonstrated in the EFC (Choi et al., 2020). It seems modern biological treatments are more efficient than traditional ones for the recovery and conversion of wastes into products and energy resources.

4.9. Biosurfactants

Biosurfactants are biological alternatives to surfactants and display more selectivity and specific activity under harsh conditions. Generally, surfactants are synthesized chemically and employed in the detergent, oil, food, pharmaceutical and cosmetic industries. Most known biosurfactants such as emulsan, surfactin, or rhamnolipids are produced by bacteria especially, *Bacillus*, *Acinetobacter*, and *Pseudomonas* (Decesaro et al., 2020). Recently, biosurfactant production from whey was investigated by Decesaro et al. (2020). In this study, *Bacillus methylotrophicus* and *Bacillus pumilus* were grown on permeate from ultrafiltration of whey to produce biosurfactant and some important factors were tested by using a fractional factorial design.

4.10. Miscellaneous

Microbial fermentation is considered a green technology for dairy waste valorization. Recently, some efforts have been made to produce other chemicals through this platform. Various microbial strains from the bacterial genera *Lactococcus*, *Lactobacillus*, and *Enterococcus* can utilize lactose at carbon source. During lactose fermentation, several products such as alcohol, ketones, aldehydes, organic acids, and aroma compounds may produce in the medium. Partial bio-oxidation of alcohol results in ketones and aldehydes; that their stability in the fermentation medium depends on environmental factors such as pH values. For instance, bacterial fermentation of whey at pH 5.8 results in the production of diacetyl and acetoin. However, when the pH value decreases to 4.5, acetoin is transformed into diacetyl. Despite many studies performed on whey valorization methods and their final products, some technical challenges are remained to be solved. The most important challenges are microbial growth inhibition, catabolism repression, low production yield that affect economic aspects of whey biorefineries (Kasmi, 2018; Valdez Castillo et al., 2020; Zotta et al., 2020; Awasthi et al., 2021).

5. Microbial mechanisms for converting dairy wastes into bio-products

The main components found in the dairy industry wastes and by-products are lactose, fat, and protein. Initially, these complex organic compounds need to be converted into monomeric compounds to produce bioproducts. Although dairy wastes are considered suitable raw materials for microbial products, lactose is the main component that cannot be naturally consumed by some microorganisms, e.g. *S. cerevisiae*, due to lack of lactose hydrolysing enzymes (Pescuma et al., 2015). *S. cerevisiae*, known as GRAS, is mainly used microorganism for ethanol and single-cell protein production (Jones et al., 2020). Alternatively, engineered *S. cerevisiae* strains carrying lactose hydrolase and lactose transporter genes (Zou et al., 2021), or other microorganisms such as *E. coli*, *Bacillus*, *Lactobacillus*, *Kluyveromyces*, *Aspergillus*, *Fusarium*, *Neurospora*, *Mucor*, or *Penicillium*, which are natural lactose consumers, can be used to produce different metabolites (Table 2; Fig. 4) (Singh et al., 1992; Silvério et al., 2018; Liu et al., 2019). Fat, another valuable substrate found in the dairy wastes, plays an important role in both microbial metabolite production and COD levels, as approximately 1 kg of fat is equivalent to 3 kg of COD (Ahmad et al., 2019). It is more attractive to use microorganisms capable of synthesizing lipase in microbial processes of dairy wastes containing fat. *Bacillus*, *Staphylococcus*, *Enterococcus*, *Aspergillus*, *Neurospora*, *Rhizopus*, *Xanthomonas*, *Rhodotorula*, *Candida*, *S. cerevisiae*, and *Y. lipolytica* are reported as microbial lipase producers (reviewed in Bharathi and Rajalakshmi (2019)). Another step in the hydrolysis of the main components of dairy wastes is the conversion of proteins to amino acids by microorganisms capable of synthesizing the protease enzyme (Jiang et al., 2017). Additionally, other nitrogen sources such as nucleic acids, urea, and some ion forms (NO_2^-, NO_3^-, NH_4^+), organic and inorganic phosphorous, and some metals (Na, Cl, K, Ca, Mg, Fe, Cu, Ni, Mn) found in the dairy wastes can help to the growth of microorganisms and productions of their value-added products (Demirel et al., 2005; Kushwaha et al., 2011; Lappa et al., 2019). Then, the products of the hydrolysed lactose, fats, and proteins (Fig. 4) and other nutrients are used in different metabolic pathways depending on the type of microorganism (bacteria, yeast, fungus, microalgae), the type of targeted product (ethanol, pigment, enzyme, organic acid, etc.) and the culture condition (aerobic or anaerobic).

VFAs (acetic, propionic, isobutyric, butyric, isovaleric, valeric and caproic acid), other carboxylic acids (succinic and lactic acids) and hydrogen can be generated by blocking methane production in anaerobic digestion (Fig. 4). However, in the absence of this inhibition, biogas can be produced by methanogens, which consume hydrogen and VFAs (Wainaina et al., 2019). VFAs produced in AD can be considered as a precursor to the formation of PHA (Vu et al., 2020). On the other hand, pure cultures can produce PHA using by pure VFAs (Vu et al., 2021) or dairy wastes (Pagliano et al., 2017; Koller et al., 2010).

Monomeric compounds can be transformed into essential nutrients that supplies energy for microbial metabolism (Chen and Wang, 2017). Some microbial primary metabolites (i.e., ethanol, biomass, acetic acid, citric acid etc.) released during the growth of microorganisms can be produced by cultivating microorganisms on the dairy waste or by-products (Behera et al., 2019). Additionally, microorganisms produce a wide variety of secondary metabolites (i.e., pigments, antibiotics, vitamins, toxins, alkaloids, fatty acids, etc.) during active cell growth (Devi et al., 2020). These valuable metabolites can be produced similar to primary metabolite production (Fig. 4). However, in some processes, stress conditions such as the addition or removal of metal ions, carbon sources and nitrogen sources might be required to improve the secondary metabolite production (Rao et al., 2017).

To obtain products beneficial for health, fermented bio-products or functional food additives (such as galacto-oligosaccharides, lactobionic acid and bacteriocins) can be obtained by fermentation of dairy by-products by microorganisms such as whey (Fig. 4).

6. Contributions of dairy by-products and their value-added products to the bioeconomy

Dairy industry results in several waste streams such as cheese whey, milk whey, cream, etc. Among the studies on valorization of dairy waste, some studies also focus on integration of the valorization process into the existing dairy plants. Although not many, there are few studies in the literature about techno-economic analysis of integrated processes (Table 3). Anaerobic digestion and biogas production for dairy waste valorization are

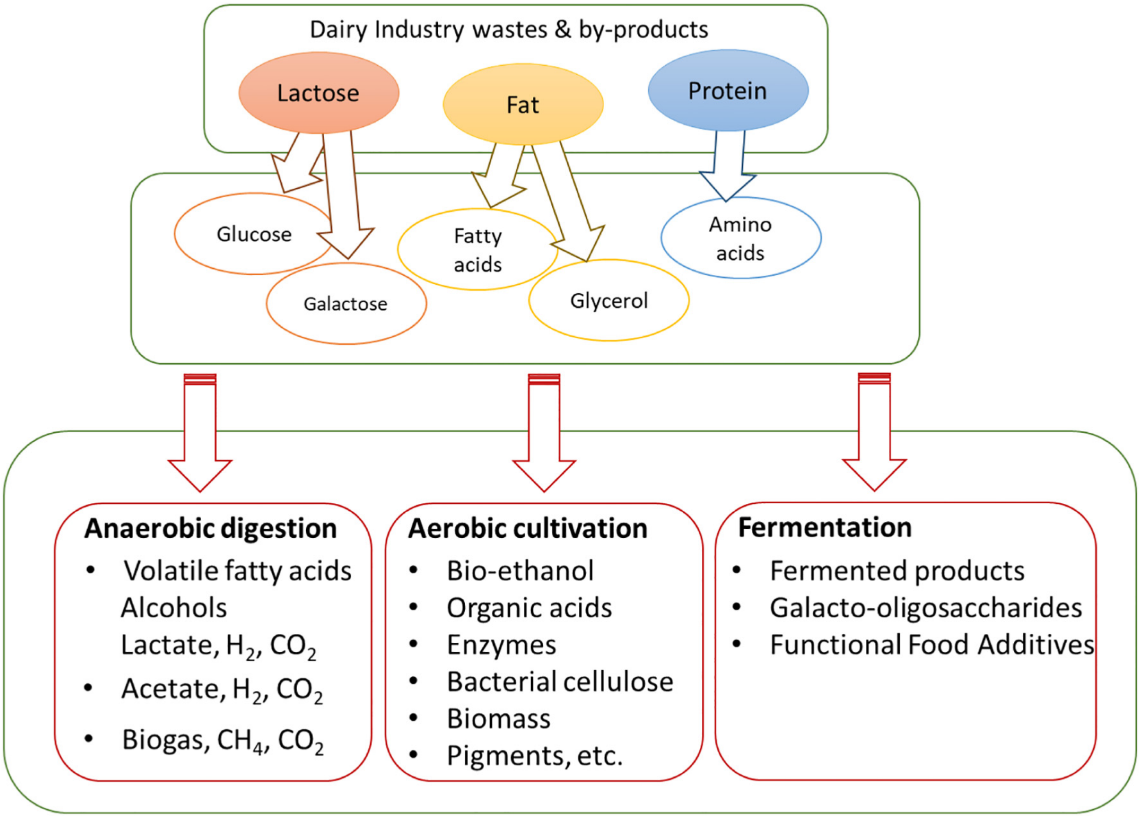

Fig. 4. Bioconversion of dairy industry wastes and by-products into microbial products.

widely studied from the techno-economic perspective of integrated processes.

Mainardis et al. (2019) studied biomethane production by ultrasound pretreatment from cheese whey by focusing on improving methane yield, and energy analysis of anaerobic digestion process integrated dairy plant. It was reported that the ultrasound pretreatment enhanced the methane yield by 16% when the energy applied was 251.4–693.7 Wh/kg VS. The energy need of dairy plant could be supplied mostly through anaerobic digestion of dairy waste. Biogas production from dairy waste was also studied as co-digestion of cheese whey and other waste streams such as agricultural waste, livestock manure (Imeni et al., 2019; Papirio et al., 2020). Papirio et al. (2020) stated that anaerobic digestion process of hemp hurds was improved by codigestion of hemp hurds with cheese whey. Cheese whey valorization can be implemented while improving hemp production process economically. Similarly, anaerobic digestion process and the economy of the farm were improved by codigestion of animal manure and cheese whey at by 70:30 ratio. A positive net present value (NPV) and less than 10 years of payback period (PBP) were obtained, while small dairy farms with more than 115 livestock were found to be feasible (Imeni et al., 2019). Co-digestion of milk whey and potato stem was found to be a promising valorization method in terms of energy production through biogas (Martínez-Ruano et al., 2019).

Production of nisin from cheese whey is another valorization strategy. Nisin is a compound, consist of peptides, produced by bacteria as a part of their defense mechanism against other bacteria. This group of compounds was named as bacteriocins. Nisin is a commonly studied antibacterial compound, harmless to human. Therefore, it is a candidate of natural preservative for food, although it is very expensive (Shin et al., 2016; Arias et al., 2021). Arias et al. (2021) studied techno-economic analysis of nisin production from cheese whey via *Lactococcus lactis*. Production of nisin from a waste material such as cheese whey was resulted in economically feasible. Net present value (NPV) with 14 million Euro and seven years of payback period (PBP) was reported to be an alternative way following the nisin production from sugar beet pulp with 68.5 million Euro NPV and 9 years of PBP.

Studies about biodiesel production by yeast and microalgae production for further biofuel application from dairy effluents were also showed economic viability of the alternative processes (Summers et al., 2015; Kumar et al., 2020a, 2020b). Summers et al. (2015) stated that diesel production from de-lactosed whey permeate can be profitable when the biofuel is sold above 4.78 USD/gal. Another method for dairy effluent treatment is through microalgae production which results in microalgae biomass and clean water while providing 1.9 years PBP and 118% IRR with 20 years of process life time (Kumar et al., 2020a, 2020b).

Considering the several microbial processes mentioned in this review, techno-economic analysis of other methodologies to the best of our knowledge is not available in the literature. Therefore, the studies on techno-economic analysis that has potential to be applied in industry should be further investigated.

7. Conclusion and future perspectives

Dairy industry wastes and by-products are defined as the most important environmental pollutants with their lactose, protein and fat contents. A suitable treatment strategy has not been determined for the treatment of these wastes with high COD values. The potential use of dairy wastes as a carbon source in microbial production processes (such as biofuels, organic acids, enzymes, polymers, and biomass) has been frequently evaluated and its usability as the substrate has been determined. Additionally, dairy wastes can be mixed with other substrates (such as cow manure or

Table 3
Some example studies on techno-economic assessment of dairy waste valorization.

Bioproduct	Waste stream	Conditions/capacity	Analysis tool	Findings/economics	References
Biogas	Cheese whey	• Low-cost digesters	• N/A[a]	• Providing Electricity and heat need of the plant • Surplus energy • Low transport and management costs	(Mainardis et al., 2019)
Biogas	Co-digestion of whey and livestock manure	• Medium cattle farms (250 cattle heads)	• Analytica free 101	• Best economic result: Co-digestion of manure with cheese whey (70:30) • Profitable for small farms (115 cow heads) • NPV > 0 • PBP < 10 years	(Imeni et al., 2019)
Biogas	Mixture of milk whey and potato stem	• Cost of whey: 0.01 USD/L • Cost of potato stem: 0.04 USD/kg • Fertilizer selling price: 30 USD/t	• Aspen plus/aspen plus economic analyzer	• NPV: 33.93–41.05 Million USD	(Martínez-Ruano et al., 2019)
Biogas	Mixture of whey and industrial hemp residues	• Co-digestion of whey and hemp residuals (70:30)	• N/A[a]	• Enhanced biomethane production by 10.7% • Total biomethane yield potential up to 296 MNm^3/year in Italy • Overall profit: up to 6124 € ha^{-1}(per hectare of hemp production area)	(Papirio et al., 2020)
Diesel	Delactosed whey permeate	• Large-scale dairy processing facility • Processing capacity: 3.8 million L of dairy effluent, delac/day	• N/A[a]	• Minimum fuel selling price: $4.78/gal renewable diesel	(Summers et al., 2015)
Nisin & lactic acid	Cheese whey	• Nisin production:100 kg/batch • Operating time: 330 days/year	• SuperPro designer	• Nisin production: 25.5 ton/year • Lactic acid Production: 281.2 ton/year • NPV: 14.386 million Euro • Return on Investment: 6.67% • PBP: 14.8 years.	(Arias et al., 2021)
Biomass (microalgae)	Dairy effluent	• Capacity >1 million L (MLD)/day • Lifetime: 20 years • Microalgae biomass selling price: $482/ton	• N/A[a]	• Microalgal production: 504 ton/year • IRR: 118% • PBP:1.9 years	(Kumar et al., 2020a)
Fermented drinks	Cheese whey + carrot juice	• Integrated cheese/whey-carrot beverage process • Production capacity: 332640 m^3 whey-carrot beverage/year	• SuperPro designer	• NPV: 10,464.04 million USD • IRR: 384.61% • PBP: 0.15 years	(Arsić et al., 2018)

[a] N/A: not available.

potato stem) and integrated into anaerobic digestion processes, and some studies have shown that this co-digestion could be profitable for facilities depending on techno-economic analysis. However, it is necessary to do some further research is needed for the recovery of products from dairy wastes since the techno-economic analyses are mostly for biogas production: it is necessary to (1) investigate of the use of expired or discarded dairy products such as milk, yogurt and especially fatty dairy products (e.g., cream, butter) in microbial processes, (2) expand the potential use of dairy industry wastes for both biometabolite production and waste treatment, (3) to determine how dairy waste can be used more efficiently in biorefinery, (4) develop a bioprocess design to improve the existing dairy/food industry by identifying new processes to be integrated into the dairy industry, and (5) carry out the techno-economic analyses of production of some important microbial products such as bioethanol and biohydrogen.

CRediT authorship contribution statement

Taner Sar: Conceptualization, Writing - original draft, Visualization. **Sharareh Harirchi**: Writing - original draft. **Mohadasseh Ramezani**: Writing - original draft. **Gülru Bulkan**: Writing - original draft. **Meltem Yesilcimen Akbas**: Writing - review & editing, Supervision. **Ashok Pandey**: Writing - review & editing. **Mohammad J. Taherzadeh**: Conceptualization, Writing - review & editing, Supervision, Project administration.

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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