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COMPREHENSIVE REVIEW

Towards sustainable Cleaning-in-Place (CIP) in dairy processing: Exploring enzyme-based approaches to cleaning in the Cheese industry

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Abstract

Cleaning-in-place (CIP) is the most commonly used cleaning and sanitation system for processing lines, equipment, and storage facilities such as milk silos in the global dairy processing industry. CIP employs thermal treatments and non-biodegradable chemicals (acids and alkalis), requiring appropriate neutralization before disposal, resulting in sustainability challenges. In addition, biofilms are a major source of contamination and spoilage in dairy industries, and it is believed that current chemical CIP protocols do not entirely destroy biofilms. Use of enzymes as effective agents for CIP and as a more sustainable alternative to chemicals and thermal treatments is gaining interest. Enzymes offer several advantages when used for CIP, such as reduced water usage (less rinsing), lower operating temperatures resulting in energy savings, shorter cleaning times, and lower costs for wastewater treatment. Additionally, they are typically derived from natural sources, are easy to neutralize, and do not produce hazardous waste products. However, even with such advantages, enzymes for CIP within the dairy processing industry remain focused mainly on membrane cleaning. Greater adoption of enzyme-based CIP for cheese industries is projected pending a greater knowledge relating to cost, control of the process (inactivation kinetics), reusability of enzyme solutions, and the potential for residual activity, including possible effects on the subsequent product batches. Such studies are essential for the cheese industry to move toward more energy-efficient and sustainable cleaning solutions.

KEYWORDS
biofilms, cleaning-in-place (CIP), enzymes, fouling, sustainability

1 | INTRODUCTION AND GENERAL CONSIDERATIONS FOR CIP

Formation of deposits or fouling of processing equipment within the dairy industry is a major difficulty, particularly within the inner surfaces of machinery and pipes, and it depends on the type of treatment involved in the particular process (temperature, pH, time, etc.) (Guerrero-Navarro et al., 2019). As an example, the fouling on heat exchanger surfaces during dairy processing can be classified into two categories depending on the heat intensity of the process during which it is formed: Type A, when the temperature range is 75–110°C and the fouling material comprises 4%–8% fat, 50%–70% proteins, and 30%–40% minerals; and type B with temperatures above 110°C, and it contains 4%–8% fat, 15%–20% protein, and 70%–80% minerals (Bansal & Chen, 2006). Generally, the issues caused by fouling are diverse and impact various aspects of dairy processing, such as food safety, operation efficiency, and product quality. Fouling can result in the formation of biofilms, decreased heat transfer coefficients for equipment, increased energy usage, modification of processing equipment surfaces, pressure drops, product loss, and increased environmental load of wastewater generated. Additionally, they can also affect the functional properties of the final products and increase the microbial load leading to decreased shelf life of products (de Jong, 1997; Guerrero-Navarro et al., 2019) and underlining the need for effective cleaning and sanitation processes.

Currently, the most used and effective industrial sanitation system is cleaning-in-place (CIP). It is defined as a circulatory washing system with sanitizing liquid running through enclosed machines and pipes without needing to dismantle or open those (Bremer et al., 2009; Memisi et al., 2015). CIP may also involve spraying and circulating cleaning liquids through the equipment surfaces under increased turbulence and flow velocity conditions. It is a five-step process that employs alkaline, acidic detergents, and disinfectant (if required) (Bremer et al., 2009). A schematic representation of a typical CIP process is shown in Figure 1, and the most common chemicals used for CIP are described in Table 1. CIP systems are widely used in the dairy industry and other food sectors, such as breweries, edible oils, or fat manufacture, where pipe-based systems are utilized (Gugała et al., 2015). Globally, the dairy industry is known for its high water consumption, and it has been estimated that 5000 L of water is needed to produce just 1 kg of cheese. The major contributor to this usage within dairy processing facilities is the extensive CIP required to maintain the facilities to meet the necessary food production criteria (Finnegan et al., 2018). A typical CIP setup comprises tanks with mixing units and heaters (for water, disinfecting liquids, and cleaning chemicals), pumps, a pipe system consisting of valves, and a control unit (Gugała et al., 2015).

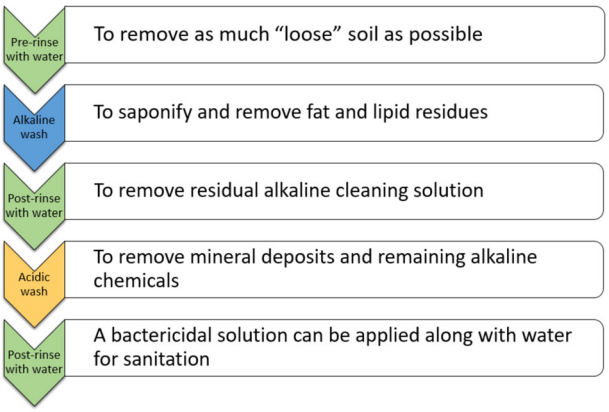

FIGURE 1 Flowchart of a typical five-step cleaning-in-place (CIP) process employed in dairy. The rinsing steps are performed using water at 20°C, alkaline washing step is typically performed at temperatures of 70–80°C, and acid washing step is carried out at 55–80°C. Adapted from Bremer et al. (2009).

After the CIP process, the levels of cleaning efficacy can be divided into physically clean, microbiologically clean (i.e., disinfected), and chemically clean. Physically clean refers to the aesthetic attribute or visual cleanliness of the surface, but although the surface can appear clean, chemical residues may remain. Microbiologically clean refers to the absence of microbiological contamination after the disinfection step in CIP, and chemically clean refers to surfaces with no traces of chemical residues left after CIP (Thomas & Sathian, 2014).

Of the cleaning agents discussed in Table 1, chlorine-based agents are under extensive scrutiny. Chlorine readily reacts with microorganisms (active chlorine) and is usually introduced in the cleaning process as hypochlorite or gaseous chlorine. It is a strong oxidizing agent and can be involved in rapid side reactions that can overcome the chlorine demand of the system after only minimum disinfection is achieved (McCarthy et al., 2018). Sodium hypochlorite-based sanitizers show activity against a wide range of microorganisms like bacteria, spores, and viruses (Thomas & Sathian, 2014). Hekmati and Bradley (1979) reported a loss of effectiveness of chlorine-based sanitizers on contact with various dairy products including whole milk, skim milk, and 30% cream. However, the rate of inactivation of chlorine varies depending on the milk component, with lactoglobulin having the most rapid effect, followed by lactalbumin, casein, lactose, insoluble lipoprotein, soluble lipoprotein, and fat globule membrane lipids. When all the milk components are considered together, the loss of sanitizing activity appears to be cumulative and thus can increase the demand for sanitizer amounts required. Similarly, using chlorine as a sanitizing

TABLE 1 Commonly applied chemicals for CIP across the dairy industry.

Type	Chemicals used	Working parameters	Function	Reference
Alkali detergents	Sodium hydroxide (NaOH), potassium hydroxide (KOH), sodium carbonate (Na_2CO_3), sodium silicates (Na_2SiO_3), etc.	0.15%–5% of alkali detergent is used at 70–80°C for 10–30 min, depending on the level of soiling.	Saponification of fats, which are converted to soaps and can be easily removed with water.	Bremer et al., 2006, 2009; Featherstone, 2015; Thomas & Sathian, 2014
Acidic detergents	Nitric acid (HNO_3), phosphoric acid (H_3PO_4), hydrochloric acid (HCl), sulfuric acid (H_2SO_4), etc.	0.5%–1% of acidic detergent is used at 55–80°C for 5–20 min, depending on the level of soiling.	Removal of residual alkaline detergents, removal of minerals, and bacteriostatic action.	Bremer et al., 2006, 2009; Featherstone, 2015; Thomas & Sathian, 2014
Sanitizers	Chlorine-based sanitizers (hypochlorite or organic chlorine releasing sanitizers), iodine-based sanitizers, peracetic acid ($C_2H_4O_3$), hydrogen peroxide (H_2O_2), etc.	The required concentration, temperature, and time of contact for sanitizers depend on the sanitizer employed and the plant's requirements.	Destroy vegetative cells of pathogenic organisms and significantly reduce the number of unwanted microorganisms.	Alasri et al., 1993; Featherstone, 2015; Thomas & Sathian, 2014

agent in the dairy industry can result in the undesired formation of chlorate. As reported by McCarthy et al. (2018), the primary source of chlorate in milk and other dairy products can be attributed to disinfection byproducts generated as a result of using chlorinated water and residues from chlorine-based detergents for cleaning. Major nutritional issues like inhibition of iodine uptake and methemoglobin formation can occur because of the presence of oxychlorine and other chlorate species. Infants and young children are the most susceptible to such risks in the general population. Ingestion of inorganic chlorine derivatives can also cause nephrotoxic and hematotoxic effects and inhibit thyroid functioning (EFSA, 2015). Thus, there is a move away from the use of chlorine-based sanitizers.

Dairy producers looking to incorporate alternatives to chlorine-based sanitizer chemicals have focused on peracetic acid and hydrogen peroxide. While they are slow-acting sanitizers, the related health and environmental benefits are greater as compared to traditional chlorine-based sanitizers (Gleeson et al., 2013). They are highly effective, are biodegradable, and produce water, oxygen, and acetic acid upon degradation. The primary advantage of peracetic acid over chlorine-based chemicals is the absence of chlorine as a byproduct, any associated noncorrosive vapors after mixing with water at high temperatures, and the lack of phosphates. However, certain factors need to be considered before employing peroxyacetic acid, such as the production of acetic acid after addition to water that can increase the biological oxygen demand (BOD) of wastewater generated and hence increase the wastewater treatment plant (WWTP) costs. It can be toxic to human and aquatic life at higher concentrations and cause severe damage (ECHA, 2023; Prasad et al., 2004).

It is vital to evaluate various factors such as the type and quantity of chemicals required, water usage, disposal methods, and overall costs to determine the most effective approach for achieving sufficient cleaning in the dairy processing industry. The chemicals used for the current CIP processes are predominantly nonbiodegradable and, thus, must be neutralized before discharge. Irrespective of the cleaning agent and process used, issues like disposal, negative environmental impact, and high costs are prevalent for all these processes (Grasshoff, 2005). It is also imperative to consider that using subinhibitory doses of disinfectant can cause antibiotic resistance development in the bacteria. The subinhibitory selection allows the selection of the most fit resistant mutants, which can persist and spread in the system. These can grow in the absence of antibiotics, leading to the creation of highly fit mutants that become stable in the population. Thus, caution and careful monitoring are required when using disinfectants to prevent the development of antibiotic resistance in bacteria (Sandegren, 2019).

Within the industrial processing scale, the primary environmental impacts on milk processing and dairy production are associated with electricity, thermal energy, water, and cleaning products, without considering the impacts of milk production. An attributional life cycle assessment (LCA) conducted by Alves et al. (2019) evaluated the environmental performance of producing 1 kg of organic mozzarella cheese, focusing on the cradle-to-gate stage, which revealed that the use of cleaning products accounts for 5.4%–18.9% of the environmental burdens in

all categories, with sodium hydroxide-based acid cleaning being the primary contributor. Thus, using enzymes as an alternative to chemical agents in CIP has certain advantages, such as reducing the potential pollution caused by chemical waste and avoiding the problems associated with the corrosive nature of chemical agents (Guerrero-Navarro et al., 2019).

Apart from being a major source of contamination, biofilms are also known to enhance corrosion rates, decrease heat transfer, and increase fluid friction in dairy equipment (Bremer et al., 2006). Biofilms adhering to equipment surfaces, even when dispersed, can reform on the same surface from the remains of the previously formed cells. Thus, cleaning of the equipment is required after every production cycle. CIP, although the major process employed to remove milk fouling and biofilms, is not always completely efficient against biofilms (Gonçalves et al., 2020). CIP regimes used regularly in the dairy industry have proven to show variable results against biofilms due to resistance to CIP-based chemical and physical treatments (Kumari & Sarkar, 2016). One of the strategies for efficient biofilm removal is enzyme-based preparations, in combination with biocides, which is an attractive approach to addressing biofilm problems in the dairy industry (Bridier et al., 2015).

This review aims to comprehensively assess CIP practices in the dairy processing industry, including challenges associated with the use of chemicals for CIP such as high energy and water consumption, environmental concerns, efficacy in biofilm removal, and their overall impact on sustainability. Furthermore, novel strategies such as enzyme-based cleaning agents as promising alternatives to replace or reduce the use of chemicals during CIP within the cheese industry are considered. By exploring these aspects, valuable insights can be gained into the current CIP practices and potential developments for enhanced sustainability and efficiency in the dairy processing sector.

2 | UNDERSTANDING THE IMPACT OF ENERGY AND WATER USAGE FOR CIP IN DAIRY PROCESSING

The quantity of water used and associated high temperatures during cleaning are responsible for the highest energy consumption during CIP (Gugała et al., 2015; Ramírez et al., 2006; Wojdalski et al., 2013). This may suggest that a reduction in temperatures should be considered, but studies by Davey et al. (2013), using 1% (w/v) NaOH at 50°C to remove milk protein deposit with a thickness of 0.15 mm, showed that if the temperature of CIP cleaning fluid falls from 65–80°C to 50°C, CIP will fail in 4.2% of all operations, and that equates to 15 fails per annum.

2.1 | Energy consumption during CIP

An intercountry analysis of energy consumption and efficiency for the dairy industry between Germany (1993–2000), the United Kingdom (1990–2000), the Netherlands (1989–2000), and France (1986–2000) revealed that CIP is responsible for a significant part of total plant operating costs, especially during CIP of evaporators and dryers where it can account for up to 10%—26% of the total energy used for processing (Ramirez et al., 2006). The CIP processes in the Canadian dairies consume energy in the range of 0.0001–0.0930 kWh/L of milk (Wojdalski et al., 2013). CIP ranks third in terms of energy usage (9% of total energy used) in an integrated cheese, powder, and whey plant after spray dryers and evaporators in typical Australian diary (Rad & Lewis, 2013). In the U.K. dairy industry, the thermal needs of dairy processing to produce hot water and steam for processes such as pasteurization, evaporation, drying, and CIP contribute to approximately 80% of energy use (Rad & Lewis, 2013). According to the values estimated by Ramirez et al. (2006), CIP is responsible for 9.5%, 19%, and 26% of energy use in Dutch fluid milk processing, cheese, and butter processing industries, respectively. Natural gas is a fuel widely employed for producing thermal energy (hot water and steam) in dairy processing, and electricity is also employed for processes like pumping, storage, separation, and cleaning (Xu et al., 2009). According to Chamberland et al. (2019), CIP consumes 352.79 m^3 of natural gas per 10,000 kg of soft cheese produced, which is much higher than the natural gas consumption for pasteurization steps, which ranges from 71.28 to 222.51 m^3 per 10,000 kg of cheese. Additionally, CIP consumes 776.97 kWh of electricity, which is similar to the highest consumer of electricity, pasteurization, at 794.55 kWh. Similarly, Aguirre-Villegas et al. (2012) identified pasteurization and cleaning as the most energy-intensive process in a life cycle impact assessment of cheese industry in Wisconsin, USA. The study estimated that producing 1 kg of cheese in the industry required 3.19 MJ of electricity and 3.35 MJ of thermal energy, contributing to a high potential for global warming of 0.459 kg CO_2-eq per kg of cheese produced. Therefore, reducing the energy consumption of CIP processes can have a significant impact on the overall energy efficiency of dairy processing industry. It should also be considered that as the chemicals used in CIP require high temperatures, they can also increase the corrosion of processing equipment and increase maintenance costs.

2.2 | Water consumption during CIP

Another crucial aspect of dairy processing that contributes significantly to environmental impacts is the high use of

water. The food industry is positioned third after the chemical and refinery industries in terms of water consumption and wastewater discharge, and for dairy processing industries, water is highly consumed for CIP practices in contrast to use as an ingredient (Rad & Lewis, 2013). Worldwide, dairy processing utilizes 0.2–11 L of water per liter of milk processed, and CIP contributes to 28% of this (Prasad et al., 2004). Yan and Holden (2019) reported water consumption in four different Irish dairy processing industries in 2014 and 2015 to be in the range of 1–60 L of water per kilogram of milk processed, of which 28% was used for CIP. The cleaning of evaporators and driers consumes the highest amount of water in the dairy industry (225.4–309.5 L/t of total milk processed) followed by CIP of feeding systems and silos (104.7–387.7 L/t of total milk processed). According to Rad and Lewis (2013), Dairy Australia reported the highest water utilization by CIP (28%), followed by pasteurization (25%) in dairies. It is important to note that the water used during pasteurization for steam generation can be reused in some cases for subsequent pasteurization, while the water used for CIP is typically discarded after use. In an LCA of 44 different artisanal French cheeses for their environmental impact, it was revealed that CIP was responsible for consumption of 10%–29.5% of total water utilized during cheese production (Cortesi et al., 2022). Hence, we can consider CIP to be a major consumer of water during processing.

Water plays an important role in dairy processing, as it serves multiple functions such as carrying energy for heating or cooling, acting as a cleaning material, and transporting essential nutrients and chemicals such as protein, fats, and sugars. Thus, due to high demand, wastewater management is essential from a cost perspective (Yan & Holden, 2019). However, it should also be noted that not all water is sourced externally. Water may be extracted from milk and other ingredients, such as condensates from evaporation, which can be recycled for dairy processing (Prasad et al., 2004). The inflow of water to WWTP is composed of condensate in the range of 23%–41% and effluents in the range of 59%–77% (Yan & Holden, 2019). It is imperative to maintain the water inflow within the limits of the capacity of any WWTP, including focusing on minimizing CIP-derived water, recovery of CIP solutions, and reduction in condensate losses or a combination of them all.

2.3 | Tackling the problem of high energy and water consumption during CIP

There is a major shift toward reducing water and energy consumption during dairy processing, including the introduction of alternative cleaning products, specific reduction in water and energy use during CIP, and potential for recovery of CIP chemicals by membrane filtration. Similarly, there is also substantial potential for the reuse of water recovered from milk in evaporation for use in CIP processing. Various pioneering dairy industries in New Zealand have determined that water from condensates alone can satisfy the water requirements of CIP in dairy processing (Yan & Holden, 2019). Membrane technology is required for the recovery of this water, and thus, its cost efficiency should be considered (Rad & Lewis, 2013). Other ways to reduce water utilization for CIP is to use steam boiler water and water recovered from reverse osmosis of permeates from membrane filtration of rennet whey (Meneses & Flores, 2016; Wojdalski et al., 2013). The latter study, in particular, shows that a combination of reverse osmosis and ultrafiltration can recover 47% of the water from whey, and an analysis of the microbiological and physiochemical quality of the recovered water showed that it had huge potential for use in CIP systems without compromising product quality and safety. It was also suggested that this could also protect the processing equipment from the negative effects of hard water.

Reduction in energy and water usage for CIP can also be achieved by the use of cleaning solutions that require less water for their activity and disposal, such as enzymes. On examining the use of enzymatic CIP in the textile industry, Graßhoff (2002) suggested its potential to substantially reduce the number of cleaning chemicals required and water and energy consumption in the dairy industry. Enzymes work under mild temperature and pH, thus utilizing less energy and reducing wastewater costs, and are also noncorrosive (Prasad et al., 2004).

3 | ENVIRONMENTAL CONSIDERATIONS FOR CIP

The traditional approach to cleaning dairy processing equipment is a five-step process using alkali and acidic chemicals. This use of chemical products involves additional costs for their neutralization and elimination before being released into the wastewater streams. The major environmental impacts of chemicals used in CIP of dairy processing plants are discussed below.

3.1 | Salt load

The current CIP process involves the circulation of 0.5%–1.5% NaOH solution or other formulated alkaline detergents at a temperature range of 70–80°C under high flow velocity and turbulence (Paul et al., 2014). In Germany, the salt load of the wastewater generated by CIP operations was reported to be approximately between 2000 and 6000

tonnes/year as the chemical agents have to be neutralized before being sent into the effluent streams. This salt is nonbiodegradable and thus can cause severe environmental problems (Graßhoff, 2002). Dairy plant wastewaters are typically characterized by a substantial organic load owing to the presence of diluted milk and milk products. Additionally, these wastewaters contain significant amounts of cleaning agents and sanitizers, and are rich in sodium content due to the use of caustic soda for cleaning purposes (Patra & Duary, 2020). The effects like high soil salinity and groundwater contamination are a major concern for land used for irrigation near dairy plants (Prasad et al., 2004).

3.2 | Nutrient load and potential for eutrophication

Nitric acid, phosphoric acid, nitrogen, and phosphorous from caustic detergents and surfactants utilized in CIP contribute to the increased BOD of wastewater (Finnegan et al., 2018; Wojdalski et al., 2013), with the potential to lead to eutrophication, algal blooms, and detrimental effects on aquatic life (Prasad et al., 2004). CIP materials constitute a significant component of the eutrophication potential of dairy processing waste, contributing to 80% of the total eutrophication potential. Thus, replacement or reduction of caustic chemicals during CIP could prove to be the best alternative in terms of controlling eutrophication potential (Eide et al., 2003). The environmentally harmful effect of CIP on dairy production has been found to be important. No study has been conducted on CIP contribution to cheese production. It may be necessary to conduct such studies given the large variety of cheese products and associated processing pathways (Finnegan et al., 2018).

3.3 | Health implications associated with CIP chemicals

One of the most commonly used chlorine-based sanitizers is sodium hypochlorite, which has significant benefits in disinfecting processing lines, but if it comes in contact with organic materials such as in milk, it has the potential to produce total organic chlorine, which is made up of both volatile organic chlorine (VOX) and nonvolatile organic chlorine. Trichloromethane (TCM), also known as chloroform, is the most significant VOX contaminant. Acetoin, diacetyl, and other methyl ketones found in milk and milk products can react with chlorine to form VOX in the form of TCM. If the TCM is not completely removed from the processing line after rinsing, it accumulates as TCM residues in further processing cycles and can cause contamination in high-fat dairy products like cheese, butter, and so forth and can also result in undesirable food taints. TCM has been added as a Group 2B carcinogen by the International Agency for Research on Cancer. Therefore, it is important to take measures to control the use of chlorine in food processing to avoid the formation of VOX, including TCM, and to monitor the presence of these contaminants in milk and milk products (Gleeson et al., 2013; Ryan et al., 2012).

The cheese industry, which falls under the broader umbrella of dairy processing, utilizes roughly a quarter of the world's raw milk production and is known to be an energy-intensive process (Xu et al., 2009) and particularly, in the European Union over 39% of the total milk produced is utilized for cheese making (Cortesi et al., 2022). The prevalent impacts of energy and water usage, as well as the use of chemicals in CIP processes, on the broader dairy industry also have implications for the cheese industry. Therefore, understanding and implementing sustainable CIP practices are crucial for the viability and success of cheese industry. Enzyme-based approaches to CIP are one potential avenue for reducing the environmental impact of cheese production while maintaining quality and safety standards.

4 | ENZYME-BASED CIP APPROACHES: A POTENTIAL SUSTAINABLE ALTERNATIVE TO CHEMICAL-BASED CIP

Enzyme-based CIP approaches have emerged as a promising alternative to traditional chemical-based CIP methods in dairy processing. These approaches utilize enzymes to break down organic deposits on processing surfaces, reducing the need for harsh chemicals and improving sustainability. This section will explore the potential of enzyme-based CIP approaches in the dairy processing industry, including their effectiveness, challenges, and future prospects.

CIP by a combination of enzymes and acid-based chemical disinfection proved to have the lowest environmental impact and acidification potential on an LCA of cleaning procedures used in dairy industries, including energy and water utilization by Wirtanen (2002). The different protocols of cleaning assessed were conventional CIP (alkaline/acid) with hot water disinfection, single-phase CIP with acidic chemical disinfection, and conventional CIP with cold nitric acid disinfection. However, certain limitations were noted with that LCA, including the use of data from different origins and noncharacterization of certain chemical emissions like phosphonates and tensides. Graßhoff (2002) advised that pending optimization of enzyme dose, process control, and cost, enzyme-based

cleaning agents can prove to be an effective replacement for chemical cleaning agents and their hazardous effects and can ultimately reduce the chemical loading of wastewater due to cleaning effluents. Additional advantages of using enzymes for CIP include decreased handling of corrosive and hazardous cleaning chemicals by workers and reduction in chemical and thermal stress on the processing equipment (Paul et al., 2014).

However, there is a need for a comparison of the efficacy of different cleaning strategies for the cheese industry with model systems offering an efficient means to achieve this. Using a lab-scale milk fouling model with an average fouling content of 52.8 mg/cm^2 per test coupon and using turbidity measurement to monitor cleaning efficacy, Guerrero-Navarro et al. (2019) evaluated the removal of protein and carbohydrates involved in fouling with proteases and amylases. The wettability of enzymes was improved by the addition of nonionic surfactants, and the traditional cleaning products achieved the removal of 72% of fouling, in comparison to 78% for the enzymes. There was also a reduction in cleaning time (33%), wastewater generated (33.3%), and energy consumption (enzymes worked at temperatures 28.5% lower than chemical cleaning products).

A study of the efficacy of various commercial enzymes alcalase, esperase, savinase, purafect, properase, and neutrase (all proteases), flavorzyme (mixture of exopeptidase and endopeptidase), and CIPzyme (mixture of protease and lipase), for 45 min during CIP of heat exchangers, showed that only treatments with esperase, properase, and savinase resulted in optically clean surfaces (Graßhoff, 2002). Process optima for these enzymes were established as 0.05% at pH 12 and 60°C (esperase), 0.05% at pH 10 and 50°C (properase), and 0.025% at pH 9.5 and 55°C (savinase) with only properase and savinase considered as potential cleaning agents for the dairy industry. Paul et al. (2014) evaluated the capacity of a keratinolytic protease isolated from *Bacillus tequilensis* strain hsTKB in model studies to remove milk fouling on stainless steel panels similar to the deposits observed in dairy plants. In that study, immobilization of the enzyme by fixing with 3% glutaraldehyde on alginate beads improved the cleaning activity exponentially and showed potential for its use as a CIP agent. The use of the enzyme was followed by a sodium carbonate rinse eliminating the use of corrosive caustic chemicals. Similarly, due to immobilization, it was possible to recover the enzyme after five continuous cycles, and it showed 65.55% of its initial activity. Neutralization of enzymes does not require acid, thus avoiding salt generation. Future, pilot-scale and industrial trials were advised to appraise this technology for scale-up fully.

Proteases have proven useful in CIP across various industries but, for higher levels of efficacy, Graßhoff (2002) suggested the need to be complemented with a mixture of other enzymes, the addition of surfactants, or implementation of an additional cleaning step, for example, precleaning step with the use of an acid treatment for 15 min with 0.5% nitric acid at 60°C, rinsing, and enzymatic treatment for 45 min. However, in the interest of long-term sustainability, the latter use of acid treatment is a less preferred option. However, if the parameters such as processing installations, production methodologies, type of food product, and the specific risks of residual enzyme activity on food products can be addressed, enzymatic cleaning has great potential to be used for CIP in combination with traditional sanitizing methods to enhance plant hygiene (Delhalle et al., 2020).

5 | EFFECTS OF BIOFILM FORMATION ON DAIRY PROCESSING LINES

Bacteria associated with dairy processing can adhere to equipment surfaces, thus enabling biofilm formation in process tanks, lines, and heat exchangers (Guerrero-Navarro et al., 2019). Biofilms can be defined as a community of various strains of microorganisms that are immobile, adhered to a solid support, and characterized by the protective covering of an extracellular polymeric matrix or EPS (Gonçalves et al., 2020). The cohesion of cells in a biofilm is enabled by the presence of gelatinous organic matrices made up of complex mixtures of self-created biopolymers and comprises microcolonies that have water channels in between an assortment of cells and an EPS layer. The EPS layer consists of homo- and heteropolysaccharides like mannose, fucose, glucose, fructose, galactose, mannuronic acid, or glucuronic acid-based complexes or pyruvate. The saccharides form polysaccharides like polymannans, cellulose, levans, dextrans, alginate, glycogen, and amylopectin (Bridier et al., 2015; Johansen et al., 1997). These biofilms release bacteria after maturation that can compromise the safety and quality of the final product, and they are one of the major causes of fouling in the dairy processing equipment surfaces. The bacteria present in biofilms can alter their microenvironments and thus can easily resist traditional antibiofilm chemicals (Parkar et al., 2004).

Biofilms contain milk spoilage-causing bacteria and potentially pathogenic organisms (Guerrero-Navarro et al., 2019; Lequette et al., 2010) and are a major source of contamination in the cheese industry. The major pathogenic organisms of concern that form biofilms in processing equipment for the dairy industry are *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, and *Pseudomonas* spp. (Gonçalves et al., 2020), and more specifically in the cheese

industry *L. monocytogenes*, *B. cereus*, *Streptococcus thermophilus*, and other Gram-negative bacteria (Marino et al., 2013). The bacteria are present on wet surfaces, drains, conveyor belts, walls, the regenerative section of pasteurizers, refrigerated tanks, ultrafiltration, and on reverse osmosis membranes of cheese plants. Pathogens present in a biofilm are 100–1000 times more resilient to cleaning chemicals than the subsequent planktonic cells (Boyce & Walsh, 2012; Bremer et al., 2009; Delhalle et al., 2020). The efficacy of disinfection of biofilms during CIP is dependent on interference by organic substances, pH, temperature, hardness of water, presence of inhibitors, concentration, and time of contact (Simões et al., 2010). In cleaning protocols currently employed in the dairy industry, the main biocides used for the elimination of biofilms are peroxygen, quaternary ammonia, halogens, and organic acids, but due to the presence of EPS, the cells in the biofilm show greater resistance to biocides than their planktonic counterparts. This resistance can be attributed to the protective nature of EPS matrix, which interferes with the penetration of biocides and limits their interaction with bacterial cells (Gonçalves et al., 2020). Typically, in biofilms, EPS constitutes 90% of the biomass and bacteria make up the rest 10% (Fleming et al., 2017). The reason for their defense mechanism against biocides is not yet fully understood. Still, it has been hypothesized that the structure and composition of the EPS are mainly responsible for their resistance. Fully matured biofilms contain EPS with multiple molecules like eDNA, proteins, and various polysaccharides, depending on the nature of their resident microorganisms (Gonçalves et al., 2020).

6 | ENZYMES AS BIOFILM REMOVAL AGENTS

A potential solution for the biofilm resistance crisis would be replacing or combining chemical-based cleaners with natural enzyme-based cleaners. The complexity of EPS composition in biofilms may vary among bacterial species. EPS is an essential target for sanitization procedures in dairy processing environments to tackle biofilm issues. Enzymes can degrade the matrix components of EPS in biofilms, which facilitates the inactivation and removal of detached cells during industrial cleaning and disinfection procedures (Bridier et al., 2015). Recent studies on enzyme-based preparations have demonstrated promising outcomes for the removal of microbial biofilms formed on various surfaces. Enzymes themselves sometimes might not be able to eliminate the biofilms but rather enable the entry of disinfectant into the biofilm matrix. As shown in Figure 2, enzymes work by targeting the matrix of biofilms and loosening it to trigger the release of planktonic cells; this helps the standard disinfection agents to reach their planktonic target cells (Bridier et al., 2015; Coughlan et al., 2016; Kumar et al., 2021). Some of the advantages of using enzymes to degrade biofilms are their high specificity and fast reaction rates under moderate conditions of temperature, pH, and concentration (Oulahal et al., 2007). Johansen et al. (1997) showed a combination of oxidoreductases with polysaccharide-hydrolyzing enzymes worked efficiently against model biofilms of pathogenic organisms such as *Staphylococcus epidermis*, *Pseudomonas aeruginosa*, *S. aureus*, and *Pseudomonas fluorescens* on polypropylene and steel surfaces, as confirmed using fluorescence microscopy and an indirect conductance test or carbon dioxide emission test. However, enzymes have been widely studied as an antibiofilm strategy in mono-species biofilms, but mixed biofilms are more common in real environments. A study by Puga et al. (2018) evaluated the effectiveness of nine commercial enzymatic preparations in treating seven types of dual-species biofilms containing *L. monocytogenes* and accompanying bacteria from food processing plants. The study found that *L. monocytogenes* strains were equally susceptible to enzymatic attack in both mono- and dual-species young biofilms, but the effect of the association was beneficial for some of its partners such as *E. coli* and *S. saprophyticus*. While the use of enzymes did not achieve good results in terms of viable attached cell log reductions in dual-species biofilms, confocal laser scanning microscopy (CLSM) images showed significant structural damage after enzymatic treatment with DNase I, pronase, and pectinase. The study concludes that enzymes could be a useful tool for weakening the structure of *L. monocytogenes*-carrying biofilms on food processing plant surfaces, in combination with disinfection treatment, and can be used to probe the biofilm's external or accessible structure.

The classes of enzymes with the most potential that can be used for biofilm elimination are cellulases, proteases, amylases, lipases, or DNases, each having their specific characteristics as further considered as follows.

6.1 | Protease activity against biofilms

Proteins are the most vital structural component, especially in the matrix of dairy biofilms. Thus, proteases are regarded as the most potential enzymes against diary biofilms. Proteases bind and hydrolyze protein molecules and convert them into smaller units that move out through the cell membrane and are metabolized (Phyllis Molobela et al., 2010). Orgaz et al. (2007) used a delayed response nonspecific protease and immobilized it in alginate beads combined with polysaccharide-degrading enzymes like pectin lyase, cellulase, or pectin esterase to disrupt biofilm

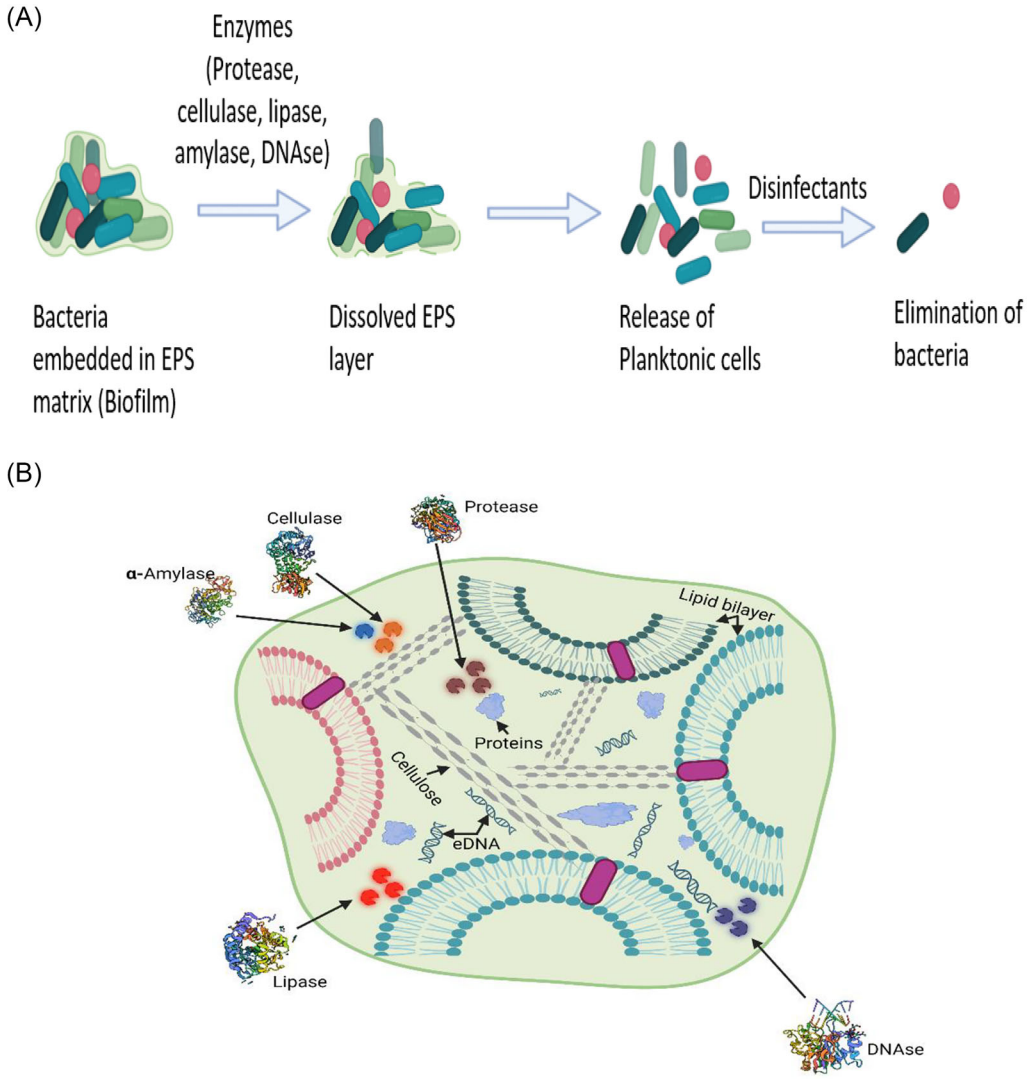

FIGURE 2 (a) Schematic representation of the action of enzymes on biofilms. Enzymes target the extracellular matrix of biofilms, leading to the release of planktonic cells. This can facilitate the action of standard disinfectants by exposing the planktonic cells to a greater concentration of the disinfectant, allowing for more effective elimination. (b) Mechanism of action of enzymes (protease, cellulase, amylase, lipase, and DNAse) on major target components in the biofilm matrix. Adapted from Kumar et al. (2021) and recreated with BioRender.com.

formed by *P. fluorescens*. Those authors suggest that the protease works more optimally independently, thus requiring a delay in its release to allow polysaccharide-degrading enzymes to work. Although the enzymes were partially able to degrade biofilms on their own, the combination of the protease and polysaccharide degrading enzymes resulted in a 4-$\log_{10}$ reduction of biofilm cells and 96% removal of biofilm mass. Also, when compared to amylases, commercial proteases (everlase and savinase) were able to reduce about 90% of the biofilm biomass of *P. fluorescens*, whereas amylases reduced the biofilm biomass by 40%–50% (Saggu et al., 2019). Parkar et al. (2004) used a commercial protease-containing enzyme system consisting of two components, an enzyme/surfactant (0.8%) and alkali/chelants (0.09%), at 60°C for 30 min, and showed that it was successful in eliminating biofilms on test coupons in lab-scale trials. In larger pilot-scale trials, the enzymatic solutions fully eliminated the biofilm cells and matrices, but there was a residual presence of ~20% fluorescent material on the striations of the test SS discs. Parkar et al. (2004) also evaluated the activity of different commercial enzymes like CellulaseL, Mutanolysin, Purastar™, and Purafect against thermophilic biofilm-forming bacteria, and although unable to completely inhibit the biofilms, they were able to decrease the viability by 5.8, 4.3, 6.6, and 3.6 $\log_{10}$ cells/cm^2, respectively, with the presence of residual polysaccharides. This may indicate either the presence of residual enzymes or fouling deposits on the surfaces.

Various proteases and polysaccharidases were evaluated for their ability to remove biofilms of 16 bacterial species

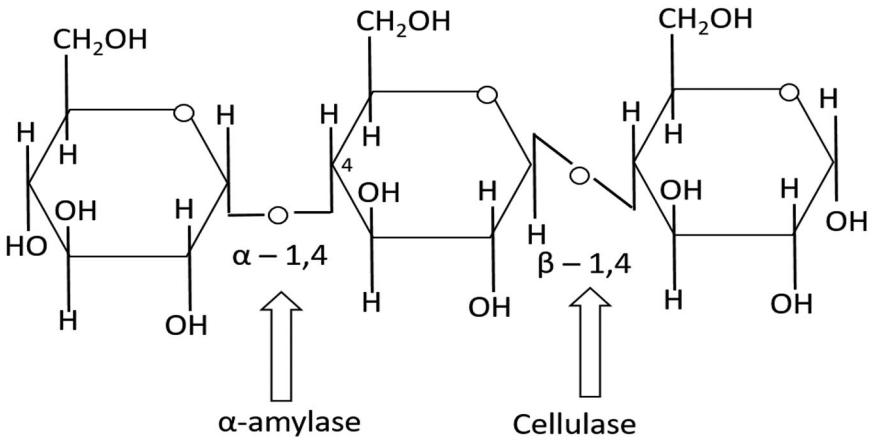

FIGURE 3 Action of enzymes on glycosidic linkages of exopolysaccharides in biofilm EPS. Adapted from Fleming et al. (2017).

using microtiter plate studies by Lequette et al. (2010). The enzymes evaluated were as follows: papains P1 (Enzybel) and P3 (Blue Star Chemicals); two serine proteases P2 and P4 (Subtilisin, Novozymes); and polysaccharide mixes of A (cellulase and hemicellulases [Novozymes]) and B (mixture of one third of mix A, one third of α-amylase S1, and one third of β-glucanase from Novozymes). NaOH and water were used as controls, stainless steel slides were prepared with 48-h-old biofilms, and CIP with enzymes was carried out at 45°C for 30 min under a laminar flow rate of 68 liters per hour (Reynolds number = 1500). The two serine proteases and α-amylase showed the highest potential for the removal of biofilms developed and had similar effects to the NaOH control, although NaOH was not able to remove EPS from biofilms as efficiently as enzymes. In another study, Pirlar et al. (2020) showed that trypsin was able to disrupt the biofilms formed by five out of six combinations of biofilm of *P. aeruginosa* and *S. aureus*.

6.2 | Cellulase activity against biofilms

Cellulases damage cellulose that is one of the major components of biofilms. The three groups of cellulases that can disrupt cellulose are exoglucanase, endoglucanase, and β-glucosidase. The major target for cellulases is the β-1,4-linkages in the cellulose structure as shown in Figure 3 (Jayasekara & Ratnayake, 2019; Menendez et al., 2015). Cellulase has significant efficacy against *Pseudomonas* sp. biofilms (Jayasekara & Ratnayake, 2019), while Loiselle and Anderson (2003) demonstrated that it can partially inhibit the biofilm biomass and colony formation of *P. aeruginosa*. Pirlar et al. (2020), on studying the synergistic and individual effects of enzymes on dual-species biofilms with various strains of *P. aeruginosa* and *S. aureus*, observed that β-glucosidase was effective in inhibition of the strains with high polysaccharide content. Although cellulase itself is unable to completely inhibit biofilm formation, a combination of cellulase with other enzymes could be effective in biofilm remediation (Loiselle & Anderson, 2003).

6.3 | Amylase activity against biofilms

The most researched group of enzymes in enzymatic cleaning procedures are amylases. Glucoside amylase and α-amylase represent 25% of the global market for enzymatic cleaning (Gonçalves et al., 2020). A study by Craigen et al. (2011) showed the ability of α-amylase for rapid detachment of *S. aureus* biofilm and release of planktonic bacteria. Amylases hydrolyze the α-1,4- glycosidic bonds in biofilm structures as seen in Figure 3 and result in disruption of biofilms. The effect of α-amylase and cellulase individually and in combination against biofilms of *S. aureus* and *P. aeruginosa* was studied by Fleming et al. (2017); treatment with enzymes resulted in a significant reduction of biofilm biomass due to breakage of glycosidic linkages in EPS matrix and dispersal and release of planktonic bacteria and thus increased the effectiveness of ensuing antibiotic treatments. Similarly, studies by Lahiri et al. (2021) reveal that a commercial and partially purified α-amylase from *Bacillus subtilis* can inhibit biofilm formed by *P. aeruginosa* and *S. aureus*. Spectrophotometric studies and microscopic observation confirmed that the biofilm inhibition of partially purified α-amylase was higher ($89.14 \pm 6.3\%$ and $99.8 \pm 6.3\%$) than the commercial α-amylase ($86.5 \pm 6.3\%$ and $94.5 \pm 6.3\%$) for reduction of *P. aeruginosa* and S. *aureus*.

6.4 | Lipase activity against biofilms

Lipids are also a major component of EPS structure in biofilms and contribute to the structure and stability of bacterial biofilms and form the lipid bilayer of

bacterial membranes; thus, lipases have a high potential for the destruction of biofilms (Di Martino, 2018; Vu et al., 2009). A lipase isolated from *Oceanobacillus* sp. PUMB02 and a marine sponge-derived lipase (Lpc53E1) showed 90%–95% disruption of biofilms of various pathogens such as *B. cereus*, *Listeria* sp., *E. coli*, *Serratia* sp., and *Vibrio parahaemolyticus* (Kiran et al., 2014). Similarly, Yassein et al. (2021) demonstrated that a lipase isolated from *Aspergillus niger* MW029470 achieved a 77.1%, 74.9%, 93.6%, and 95.3% inhibition of the biofilms against biofilms of *Proteus mirabilis*, *P. aeruginosa*, methicillin-resistant *S. aureus*, and *E. coli*, respectively.

6.5 | DNase activity against biofilm

Extracellular DNA (eDNA) is another major component of biofilms as it facilitates adhesion, gene transfer, and aggregation of bacteria and acts as a structural scaffolding for them. The use of DNases could prove to be an effective tool against biofilms (Jiang et al., 2020). Optimized treatment of *P. aeruginosa* biofilms with 10 μg/mL of DNase I with the inclusion of Mg^{2+} and 5 min of contact time resulted in a 90% reduction of the biofilm mass. However, when the same treatment was used against mixed-species biofilms consisting of *Klebsiella* spp., *Enterococcus faecalis*, *Salmonella typhimurium*, and *S. aureus*, the biofilm was only reduced by 36%−37% (Sharma & Pagedar Singh, 2018). *Listeria monocytogenes* have shown high sensitivity to DNase treatment in low ionic strength environmental conditions or under conditions with high osmotic pressure and low nutrients (generally reflecting industrial settings), suggesting the potential of DNase application. However, it should also be noted that the degradation of biofilms by DNase depends on the maturity of biofilms; young biofilms are primarily susceptible to DNase treatment, whereas as the biofilm gets older, the susceptibility to DNase decreases (Okshevsky et al., 2015).

6.6 | Case study: *B. cereus* and biofilm control

The presence of *B. cereus* in the processing environment is a major spoilage concern in the dairy industry due to the production of enterotoxins responsible for food poisoning (Kumari et al., 2014). They also produce extracellular enzymes, which decrease the organoleptic quality of milk products—especially, lipases produced can cause defects like bitty cream and unpleasant flavor, rancidity, butyric, buttery, unclean, and soapy texture in milk (Kumari et al., 2014). It has a high capacity to adhere to stainless steel surfaces and form a biofilm, leading to large economic losses due to spoilage (Kumari & Sarkar, 2016). *Bacillus* isolates showed high resistance to typical CIP process parameters in cases of biofilm formation (Ostrov et al., 2019). The efficacy of cleaning and disinfection of spores in a dairy environment can be determined by evaluating the number of germinating spores and the amount of milk soil left. Wirtanen (2002) used this method and, after the application of three CIP runs, showed that a standard alkaline/acid wash was the most effective, followed by ozonized water and enzyme-based CIP in determining the efficacy against two strains of *B. cereus* and under two different soiling conditions using an artificial heating procedure to create fouling on stainless steel surfaces and using a pilot-scale rig to create soiled surfaces. Although mono-component enzymatic cleaning was not as effective as other methods as enzymes are substrate specific, the use of a mixture of enzymes may be more effective in obtaining efficient cleaning. For example, oxidases are bactericidal but are not able to remove biofilms from polypropylene and steel surfaces; alternatively, a complex mixture of polysaccharide-hydrolyzing enzymes is not bactericidal but can remove biofilms from surfaces. Using a combination of these enzymes can potentially result in efficient removal and elimination of *B. cereus* spores and biofilms.

The use of enzymes as biofilm removal agents in dairy processing has shown promising results and can potentially improve the overall hygiene of processing lines and equipment. However, more research is needed to fully understand the effectiveness of different classes of enzymes and optimize their use in industrial settings. Biofilms are complex structures comprising various bacteria that poses a significant challenge in devising enzymatic control, which is due to the absence of specific targets or substrates for biocidal activity in biofilms. Therefore, it is essential to understand the properties of the biofilms and their resident bacteria and tailor the enzymatic CIP protocol accordingly. Despite these challenges, the use of enzymes in biofilm removal offers a sustainable and eco-friendly alternative to traditional chemical agents and could lead to significant improvements in the overall sustainability of the dairy processing industry.

7 | ENZYMATIC CIP OF SURFACES ACROSS VARIOUS INDUSTRIES

Commercial protease enzyme solutions are regularly employed in the cleaning of medical apparatus, laundry, and contact lenses (Kumari & Sarkar, 2016), and similarly, enzymatic cleaning is applied to membranes used in microfiltration, nanofiltration, ultrafiltration, and reverse osmosis of dairy-based streams as well as in egg and meat processing and ice cream manufacturing. The primary soil

in these industries contains non-denatured animal proteins which can be easily degraded by enzymes (Grasshoff, 2005).

Tsiaprazi-Stamou et al. (2019), utilizing a parallel-plate flow chamber, studied the effects of enzymes (protease, lipase, and amylase) individually and in combination on a mixed-microbial sample isolated via swabbing from a meat packaging line and growing its biofilm on stainless steel and polyethylene surfaces in vivo. The enzymes used in the study were obtained by using standard enzymatic formulations (Itram Hygiene S.L. Company, Barcelona, Spain). After CIP, the enzyme preparations effectively removed biofilms, and a combination of protease, lipase, and amylase was most effective with synergistic effects to remove total biofilm biomass and reduce bacterial viability by 82.9%, whereas the combination of amylase–lipase reduced bacterial viability by 73.5% and combination of amylase-protease reduced the viability by only 14.4% on stainless steel surfaces.

The use of an ultrasonic transducer for the standardization of biofilm removal and hygiene testing on curved food contact surfaces in food processing equipment showed that ultrasound (40 kHz for 10 s) in combination with amyloglucosidase (50 U/mL) and EDTA (0.025 mol/L) was most effective (100%) in the removal of a *S. aureus* biofilm from stainless steel surfaces, and the most successful combination for removal (75%) of *E. coli* biofilm was protease (18 U/mL), papain (3 U/mL), and EDTA (0.025 mol/L) (Oulahal et al., 2007). A study by Fenton et al. (2013) showed a peptidase enzyme ($CHAP_K$) derived from a bacteriophage to be effective against a biofilm of *S. aureus* commonly associated with bovine mastitis. A concentration of 31.25 μg/mL completely eradicated staphylococcal biofilms and prevented further biofilm formation by this strain, proving its efficacy in the removal of staphylococcal biofilms and as surface decontamination spray in food processing environments. Similarly, Delhalle et al. (2020) demonstrated the efficacy of enzymatic cleaning in enhancing the hygienic conditions of commercial ready-to-eat lasagna food processing plants. They also monitored the microbial community using 16s rDNA metagenomics and traditional microbiology methods, both in the processing plants and in the lasagna products throughout their shelf life.

8 | CONSIDERATIONS FOR ENZYMATIC CIP IN THE CHEESE INDUSTRY

Since there has been little research on the practicality of enzymatic CIP across the broader dairy industry, enzymes are currently predominantly used in the cleaning of membranes. Even though lab- and pilot-scale studies have revealed great potential in enzymes as CIP agents, there is a need for more information on the application of enzymes for CIP, particularly in the cheese industry. Proteases are the most widely studied enzymes for CIP as they are efficient in removing protein deposits formed in dairy plants and also help remove the EPS layer of biofilms; they work at mild conditions of 60°C, pH 7–9, and 20–40 min of working time. Before enzymatic CIP protocols can be established in the cheese industry, several advantages and disadvantages need to be considered, including cost, the potential for reuse, the impact of residual enzymatic activity on subsequent products, and inactivation/removal of enzymes after use. These may be considered as follows.

8.1 | Cost

Alkaline CIP solutions were costed at €0.047/L and €0.011–0.019/L and enzymatic CIP solutions at €0.045/L and €0.015/L by Guerrero-Navarro et al. (2019) and Boyce et al. (2010), respectively. In a cost analysis of CIP in South African dairies (Graz et al., 2003), it was suggested that enzymatic CIP would decrease chemical costs by 7%, energy and WWTP costs would be halved, and any penalties incurred due to improper effluent treatment would drop by 90%. More recently, Guerrero-Navarro et al. (2022) reported alkaline treatment costs in a dairy plant at €0.047/L, alkaline-acid treatment at €0.061/L, and enzymatic treatment at €0.09/L, but suggested that dilution of buffers used in enzymatic cleaning would reduce costs to €0.0495/L. Boyce et al. (2010) also indicated that the low-temperature use of enzymatic cleaning would reduce the energy costs associated with enzymatic CIP.

8.2 | Reuse of enzyme solutions

The fouling material removed during cleaning with enzyme solutions shows no effect on the cleaning efficiency of enzymatic cleaners, and they show the potential to be reused without the need for treatment (Boyce et al., 2010). Argüello et al. (2003) reported that a 30% activity loss occurred in enzyme solutions used for CIP of inorganic ultrafiltration membranes. Still, the flux capacity of membranes was high despite the reuse of enzymatic cleaning solutions in subsequent cleaning cycles. However, Grasshoff (2005) observed complete inefficiency of the reused enzyme solution to clean fouling from coupons replicating plate heat exchanger fouling, but when a fresh concentrate of enzymes was added to the used solution, the activity was regained for cleaning. Adding fresh enzyme concentrates to used enzymatic solutions can save the cost of use of buffers and surfactants for several cleaning cycles.

There is a broader scope for in-depth research on the reuse of enzyme solutions for cleaning—even if their practical reusability is limited, the environmental and cost benefits of the enzymatic CIP process should be evaluated.

8.3 | Effect of residual enzymatic activity on subsequent products

In the context of limited published research on the application of enzymatic cleaning agents in the cheese industry, a concern remains around the potential for residual enzyme activity to persist in cheese-making equipment after cleaning and to transfer to cheese curd with potential for negative impacts on ripened cheese quality. Enzymes deliberately added during cheese making are responsible for cheese matrix formation, aging, and maturation of the cheese. It has also been shown that some enzymes that persist in milk after heat treatment cause extensive proteolysis and lipolysis and can result in off flavors, rancidity, soapy taste, reduced yield, loss of structure, bitterness, and loss of consumer acceptability (El Soda & Pandian, 1991; Lemieux & Simard, 1991; Paludetti et al., 2020; Wilkinson et al., 1992).

Studies are required to determine whether residual enzyme levels may remain after CIP, at what level, and whether they may influence ripened cheese quality. Grasshoff (2005) did simulate a worst-case scenario of enzymatic cleaning solutions persisting in cheese plant equipment by adding different dilutions of 5% savinase (a majorly used enzyme for cleaning) into cheese milk. The dilutions were 0.1, 1, and 10 mg of enzyme in 100 mL of milk. It was observed that the coagulation time was reduced by 8 min in the 10-mg solution of savinase, while other dilutions had no visible effects. However, it is doubtful that enzyme contamination would occur at this magnitude with equipment rinse after every use with water, a decrease in the activity of diluted enzyme over the cleaning period, and the use of an acid cleaning step to decrease enzyme activity immediately to low or negligible levels. However, further studies should consider low residual levels, particularly of different enzymes and combinations, and the absence of acid wash and their potential impact over long ripening times.

Additionally, another factor that needs to be considered is the use of enzymes produced by genetically modified microorganisms (GMOs); according to the rules established under EU GMO Regulation (EC) No 1829/2003, food or feed made with a GMO (only used as an aid for processing) and not from a GMO (as a source) is exempted from the scope of GM Food and Feed regulation, which makes the use of GMO enzymes acceptable in CIP as long as it does not persist in the final product (Wesseler et al., 2022).

8.4 | Inactivation/removal of enzymes after use

To establish enzymes as industrial CIP agents, complete inactivation of enzymes after CIP is necessary. Boyce et al. (2010) employed various proteases (0.05 units/mL) and lipases (1% [w/v]) under varying conditions of temperatures (40–60°C) and optimum pH (according to manufacturer's specification) to analyze their efficacy in removal of fouling generated by heating raw milk at 84–89°C. It was suggested that that no additional enzyme inactivation step is needed after enzymatic CIP as residual enzyme activity can be removed easily by rinsing with water, which was confirmed by CSLM and loss of protease activity in the final rinse. Also, exposing the enzyme to 0.5% and 0.1% nitric acid at room temperature completely inactivated the enzyme after 1 min and the proteases lost all activity when treated at high temperatures of 80°C for 3 min or 90°C for 1 min. However, in an era of increased awareness of sustainability, there is a need to limit the use of water, acid rinses, and energy use in heating water.

Similarly, in the study by Boyce et al. (2010), the proteases lost all activity when exposed to hypochlorite at the concentration recommended for CIP of dairy plants. Similarly, with the current use of peracetic acid as an accepted alternative to chlorine-based chemicals, low enzyme stability at low pH suggests that peracetic acid would inactivate the enzymes. Graz et al. (2003) also reported a complete inactivation of protease enzyme after incubation at 75°C for 10 min or exposure to 0.1% peroxide-based industrial sanitizer for 10 min. However, again there is a move away from the use of hypochlorite and the need to limit the use of acids.

The use of UV light may offer an alternative approach. Lante et al. (2013) combined proteases with subsequent UV-inactivation for CIP in the dairy industry and showed that UV light inactivates enzymes in solution and thus allows for a controlled enzymatic CIP process. However, its energy requirements should be quantified.

8.5 | Health implications associated with enzymes

Enzymes of plant, bacterial, and fungal origin have been known to have the potential to cause asthma and occupational respiratory allergy, depending on levels of exposure and condition. Among these enzymes, proteases are the most commonly used in detergents worldwide, but sensitization is not limited to these enzymes. High-molecular-weight proteins, such as lipases, cellulases, and other enzymes in newer detergents, also have the potential to cause sensitization. Thus, controlling exposure to enzymes

is vital to efficiently manage the risk. It is also necessary to monitor the working environment and workforce to make sure that exposure control is adhered to, and impacts on employee health are minimized. Although the boundary between inadequate practice and best practice varies depending on ingredients, industrial setting, and specific detergent formulation applied, exposure to these enzymes presents potential risk to human health. This can be readily controlled in industrial settings if exposure is monitored tightly (Basketter et al., 2015; Flindt, 1995).

The detergent industry proposed guidelines for safety assessment of enzymes, exposure control, and medical surveillance of workers exposed to these enzymes. Manufacturing sites that failed to adhere to these guidelines have reported cases of diseases, whereas manufacturing sites adhering to these guidelines have had much fewer cases of asthma and allergy among workers (Sarlo, 2003).

9 | CONCLUSIONS

Caustic and acid-based chemicals typically applied during CIP are relatively cheap, but considering the CIP process as a whole, from procurement to use, and disposal costs and high energy and water usage requirements, questions arise as to their levels of sustainability. Similarly, with increased awareness of the role of biofilms leading to potential downstream quality issues in products such as powders and cheese, the limited permeability of biofilms to traditional CIP chemicals and continued questions as to their capacity to remove biofilms in the disinfection of processing lines and equipment are of concern to the dairy industry.

Enzymatic cleaning is of interest as an effective sanitizing agent for the cheese and wider dairy industries with benefits like enhanced sustainability, including improved waste management/biodegradability, energy and water use, and relative economic costs in comparison to traditional CIP. Other advantages include substrate-specific CIP processes, the potential for targeted breakdown of biofilms for entry of disinfectants, and so forth. Although routinely employed in various commercial cleaning applications such as CIP in textile plants, membrane plants, contact lenses, and so forth, enzymatic cleaning has not been widely applied to replace CIP chemicals in the cheese industry, mainly due to the need to develop further protocols for use, knowledge gaps relating to their dosage requirements, information on process control, and economic sustainability.

Future research is suggested to facilitate the partial or total replacement of chemical CIP practices and combine or replace them with enzymatic cleaning protocols involving minimal use of chemicals. Similarly, there is a need to investigate the capacity of enzymes to target the EPS layer of biofilms and isolate planktonic cells. The undesirable effects of any residual enzyme activity that may arise in the drive to increase the sustainability of CIP should be quantified and eliminated, and finally, there should also be a focus on combining different enzyme types, such as proteases, lipases, polysaccharidases, and so forth, to maximize their efficacy in eliminating fouling and biofilms.

Overall, enzymes offer a sustainable alternative to the resource-hungry cleaning methods currently used in the cheese industry. However, in order to promote their wider adoption, there is a need for the development of comprehensive knowledge and understanding of their effectiveness, safety, and impact on the quality of ripened cheeses produced in plants where enzyme-based CIP approaches are implemented.

AUTHOR CONTRIBUTIONS

Karan J. Pant: Conceptualization; writing—original draft; visualization; methodology. **Paul D. Cotter**: Writing—review and editing; validation. **Martin G. Wilkinson**: Writing—review and editing; validation. **Jeremiah J. Sheehan**: Writing—review and editing; supervision; methodology; validation.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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