

Bioremediation of dairy wastewater using bacteria: a panoramic review

Utilização de bactérias para biorremediação de resíduos lácteos: uma revisão panorâmica

Uso de bacterias para la biorremediación de desechos lácteos: una vista panorámica

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Abstract

The Bioremediation consists of applying microorganisms to reduce, eliminate or transform organic or inorganic polluting compounds into inert substances. Solid and liquid wastes are generated daily in industrial activities and, among the food processing industries, the dairy sector presents a large number of liquid effluents due to its extensive use of water in various activities and sectors. For an efficient treatment of effluents, it is essential to understand the microbiota of the wastewater, its biochemical characteristics and metabolic activity, as well as the origin of the polluting compounds. The growing demand for products derived from livestock and agricultural activity drives the search for environmental technologies capable of providing sustainable development. In this scenario, biotechnological studies and bioremediation applications are increasingly gaining ground both for environmental control and for application in value-added products. The purpose of this review is to present and discuss the most recent and used technologies for bioremediation of effluents from dairy industries using bacteria.

Keywords: Bioremediation; Bacteria; Dairy wastewater; Treatment; Effluents; Sustainable.

Resumo

A biorremediação consiste na aplicação de microrganismos para reduzir, eliminar ou transformar compostos poluentes orgânicos ou inorgânicos em substâncias inertes. Resíduos sólidos e líquidos são gerados diariamente nas atividades industriais e, dentre as indústrias de processamento de alimentos, o setor de laticínios apresenta um grande número de efluentes líquidos devido ao seu amplo uso de água em diversas atividades e setores. Para um tratamento eficiente de efluentes, é essencial conhecer a microbiota do efluente, suas características bioquímicas e atividade metabólica, bem como a origem dos compostos poluentes. A crescente demanda por produtos derivados da atividade pecuária e agrícola impulsiona a busca por tecnologias ambientais capazes de proporcionar o desenvolvimento sustentável. Nesse cenário, estudos biotecnológicos e aplicações de biorremediação ganham cada vez mais espaço tanto para controle ambiental quanto para aplicação em produtos de valor agregado. O objetivo desta revisão é apresentar e discutir as tecnologias mais recentes e utilizadas para biorremediação de efluentes de indústrias de laticínios utilizando bactérias.

Palavras-chave: Biorremediação; Bactérias; Efluentes lácteos; Tratamento; Efluentes; Sustentabilidade.

Resumen

La biorremediación consiste en la aplicación de microorganismos para reducir, eliminar o transformar compuestos contaminantes orgánicos o inorgánicos en sustancias inertes. Los residuos sólidos y líquidos se generan diariamente en las actividades industriales y, dentro de las industrias de procesamiento de alimentos, el sector lácteo cuenta con una gran cantidad de efluentes líquidos debido a su amplio uso del agua en diversas actividades y sectores. Para un tratamiento eficaz de los efluentes es fundamental conocer la microbiota del efluente, sus características bioquímicas y actividad metabólica, así como el origen de los compuestos contaminantes. La creciente demanda de productos derivados de la actividad ganadera y agrícola impulsa la búsqueda de tecnologías ambientales capaces de proporcionar un desarrollo sostenible. En este escenario, los estudios biotecnológicos y las aplicaciones de biorremediación ganan cada vez más espacio tanto para el control ambiental como para su aplicación en productos de valor agregado. El objetivo de esta revisión es presentar y discutir las tecnologías más recientes y utilizadas para la biorremediación de efluentes de industrias lácteas utilizando bacterias.

Palabras clave: Biorremediación; Bacterias; Efluentes lácteos; Tratamiento; Efluentes; Sostenibilidad.

1. Introduction

The industrialization growth in developed and developing countries has resulted in the emersion of many types of diseases and pollutants that contaminate the environment. One way to solve this problem is through public awareness associated with a multidisciplinary approach by scientific experts to national and international organizations claiming they must address the emergence of this threat and propose sustainable solutions (Manisalidis et al; 2020). The reality today, regarding natural resources, is seeking to redress the numerous aggressions caused by the population due to the imposition of environmental legislation that requires efficient treatments to its effluents (Ferronato & Torretta 2020). For this, new technologies have been searched for, as ordinary treatment systems often used by industries do not fit in their financial plan, and are, for the most part, economically unviable. In this context, biodegradation using microorganisms has stood out as an efficient and promising technology to treat effluents, with the possibility of being effective in both: reducing the environmental impact and making the process economically viable (Goud et al; 2020; Rocuzzo et al; 2020).

Considered as a renewable natural resource, water for industrial and domestic use can only recover its qualities if properly treated, making the process of residual management the dominant step in the whole cycle of water application (Chripim et al; 2020). According to Slavov, 2017, most of the wastewater in the dairy industry comes from industrial manufacturing processes. In numbers, from the total water used in the entire process in this industry, including sanitary activities, contaminated water represents 50% to 80% of the total water consumed, while the remaining 20-50% represents potentially clean water (Tsachev, 1982). Studies estimated that the amount of wastewater is approximately 2.5 times higher than the total of milk processed in units of volume (Ashkuzzaman et al; 2019). The water discarded by the industry does not have the quality and necessary parameters for human consumption, but it can be reapplied for other purposes, for example, the industry applies in washing reactors and mobile tanks, cooling towers, boilers, washing machines, and yards, among others (Ahmad et al; 2019).

According to CONAMA Resolution No. 357/05 (Brazil) effluents from any polluting source may only be released, directly or indirectly, into bodies of water after proper treatment, and the effluent must not cause or have the potential to cause anything that results in toxic effects, or other effects that harm the environment (Conama Resolution No. 357/05, 2011). Effluents from dairy industries have a high polluting potential, as the amount of organic matter and its derivatives are very high and there is an excess of nutrients such as lactose, potassium, fats and proteins, chemicals in general from cleaning equipment, and these effluents present a very high chemical oxygen demand (COD) (Goli et al; 2019).

For an efficient treatment, it is essential to know the microbiota of the wastewater and its biochemical characteristics, as well as the origin of the polluting compounds and ideal metabolic activity (Janczukowicz et al., 2008). On top of that, microorganisms need to withstand the concentrations of the residual pollutants and also be able to degrade them (Faria et al., 2017). Bioremediation, as an emergent technology, can use live, dead, or even parts of the microorganisms (Gaylarde et al., 2005), and these microorganisms can be autochthonous (native) or allochthonous (from another location) microorganisms (Francisco & De Queiroz, 2018).

As it presents a loss of organic and inorganic products, the biological treatment is very adequate to treat dairy wastewater. With the ability to decompose organic matter and removes nutrients, bioremediation has been widely indicated for the treatment of dairy effluents. This technology makes use of microorganisms or their enzymes to convert the polluted environment into its original conditions. For presenting an easy maintenance option, having a low cost of implantation compared to conventional treatments, and being able to remove eutrophic nutrients and organic matter, bioremediation is a safe and economically viable technology (Al-Wasify et al; 2017).

Bioremediation works by applying in the effluents microorganisms with degradation and metabolic use of pollutants capacities (as in the case of the dairy industry, proteins, fatty substances, and lactose) and it also has the advantage of treating the contamination in the same place, so that large amounts of soil, sediment or water do not need to be dug up or pumped out of the soil forums for treatment (Gaur et al; 2018).

2. Methodology

In this context, to address the state of the art in the application of bacteria in bioremediation of dairy wastewater, we searched for different types of publications, including books, scientific reports, patent requests, amongst others. The reports were chosen with the objective to give a panoramic view of the application of bacteria in this important biotechnological sector. Later on, we constructed a table (Table 2) addressing some of technologies mentioned in the text.

3. Water Quality Parameters and Composition of Dairy Wastewater

For the United Nations, the water quality criteria must be based on scientific information that presents "numerical data or recommended narrative statements" to support the use of water. It also points out that water can be recycled from different sources, such as effluents, that is, recovered and treated after use and destined for other purposes (Un-Water, 2015). The World Health Organization (WHO) cites, in its water quality guide, a variety of parameters; such as pH control, turbidity, dissolved oxygen, for monitoring the quality of the water used (WHO, 2017). These guidelines are used as a baseline for the regularity of the various forms of water use in Brazil (Un-Water, 2015).

According to the National Council of Environment – CONAMA (Brazil), "effluent is the term used to characterize liquid discharges from different activities or processes". Solid and liquid wastes are generated daily during industrial activities, and among the food processing industries, the dairy sector has a large production of liquid effluents due to its extensive use of

water in various activities and sectors. It is estimated that water consumption in Brazil in dairy products can reach up to 10 liters for each liter of processed milk (Akansha et. Al, 2020; Santos, Queiroz & Neto, 2018; Conama, 2011; Cetesb, 2008).

Among the points of generation of dairy wastewater, it is possible to mention the residues from the washing of lines, process tanks, machines, and floors, milk residues from spills or losses in the process, serum and clots, and solutions containing surfactants involved in cleaning. As mentioned above, the main pollutants of these residues are proteins, fats, lactic acid, and sugars, as well as cleaning and sanitizing products (Borges et al., 2019; Joshiba et al., 2019; Soares et al., 2019).

About 90% of the milk used becomes whey (liquid remaining from the milk's coagulation), further increasing the generation of liquid effluents. Cheese production also generates whey, resulting from the coagulation of milk, which has high electrical conductivity due to the cheese salting process. Buttermilk, a product that originated from the processing of pasteurized milk cream during the butter-making process, has a composition similar to skimmed milk with a high phospholipid content (Bosso et. Al, 2019; Santos, Queiroz & Neto, 2018; Cetesb, 2008). The volume of effluent generated in the dairy industry varies according to the type of product processed, manufacturing technology employed, production capacity, and application of good manufacturing practices, as shown in Table 1.

Table 1. Average volume of effluents generated in different production lines.

Product type	Volume (L) of liquid effluent/liter of processed milk
Milk, creams, and yogurts	3
Butter and cheese	4
Concentrates of milk or whey and dehydrated dairy products	5

Source: Adapted from CETESB (2008).

The inadequate disposal of wastewater in the environment represents a great environmental concern. The high load of organic matter present in the effluents can cause a high environmental imbalance, such as alteration of the concentration of dissolved oxygen in the water (COD) in the receiving water bodies, resulting in environmental damage and even possible lethal risk for local aquatic fauna and flora (Akansha et. Al, 2020; Moreira et al., 2020). Besides, according to the FAO (Food and Agriculture Organization), the wastewater treated to acceptable parameters to be reused in other sectors, such as agriculture, for example, is seen as a source of reuse of the large volume of water applied in the industrial scope, thus avoiding the waste of natural resources and aggregating economic and social benefits (FAO, 2010).

In general, the wastewater generated by dairy products is characterized by presenting high concentrations of organic matter, high content of biochemical and chemical oxygen demand (BOD and COD), high concentrations of oils, greases, sedimentable materials, suspended solids, and even have surfactant substances. However, its composition, as well as its concentrations and volume, may vary according to the size of the industry, seasonality, types of products generated, manufacturing process, and methods of controlling industrial waste (El-Sesy & Mustafa, 2020; Ahmad et al., 2019; Zablocka et al., 2019; Dias et al., 2018).

4. Bioremediation of Dairy Wastewater

As mentioned above, the dairy industry generates effluents with a high load of organic matter that, if released into the soil, will cause damage such as salinization, sodicization, changes in pH, among other damages, and if released directly into water, bodies can cause high levels of contamination. For this, it is necessary to treat the effluents before their release into the environment (Figueiredo et al., 2015). The effluents generated by the dairy industries contain milk and milk products, sugar,

pieces of fruit, essences, condiments, various chemical products used in hygiene procedures, sand, and lubricants that are diluted in the water used for equipment cleaning. In many dairy industries, whey is discharged directly into the effluent, being approximately one hundred times more polluting than domestic sewage (Silva, 2011).

Wastewater can only be discharged directly into the receiving water bodies after due treatment and according to all the rules imposed by regulatory agencies. In addition to the pre-established parameters, the regulatory agencies may also add other conditions and quality parameters, in compliance with possible seasonal or local specificities (Conama, 2011; Copam / Cerh, 2008).

The most used processes for the treatment of dairy wastewater are: aerobic treatment (activated sludge, batch reactors, treatment in lakes, membranes), anaerobic treatment (sludge blanket reactors, batch reactors, and filters), built wetlands, coagulation/electrocoagulation/flocculation, precipitation, bioremediation (Arvanitoyannis & Tserkezou, 2008; Justina et al., 2017), the latter being the object of the present study.

Bioremediation is an emerging and innovative technology due to its economic viability, improved competence, and compatibility with the environment, it is a process in which living organisms, usually plants (phytoremediation) or microorganisms (fungi, bacteria, and their enzymes), are used to remove or reduce pollutants in the environment. Some technologies use physical and/or chemical processes to decontaminate polluted environments, but the biological bioremediation process is an ecologically and effective approach in the treatment of environments contaminated with organic molecules that are difficult to degrade (Gaylarde et al., 2005; Singh, 2020).

Bioremediation can be classified as "*in-situ*" when the transformation or destruction of contaminants occurs at the site, generating water and carbon dioxide as products, for example, in a process that has no toxicity and can be incorporated into the environment without damage to organisms. On the other hand, "*ex-situ*" bioremediation consists of removing the contaminating material for treatment in another location, outside of the original place. It can be applied for the treatment of contaminated soils, groundwater, and wastewater. Removal is necessary when there is a possibility of contamination of people and/or the environment which is being bioremediated (Lacerda et al., 2019; Silva; Santos & Gomes, 2014).

Microorganisms used for bioremediation promote the biodegradation of toxic pollutants to obtain energy, carbon, and nutrients, their efficiency as biodegradable depends, in many cases, on the structure of the molecules and the ability to produce enzymes capable of carrying out the degradation process, these enzymes are very specific for the most of substrates (Costa et al., 2010; Pereira & Freitas, 2012; Silva; Santos & Gomes, 2014).

Bioremediation has occupied a prominent position as a technology in the treatment of contaminated environments, including its use in domestic and industrial Sewage Treatment Plants (Oliveira Netto et al., 2015). Techniques such as *in-situ* bioremediation, bio-enhancement, and biostimulation, have been used, applying native or selected microorganisms, accelerating the biodegradation process of organic matter (Lacerda et al., 2019). Among the bioremediators, it is possible to highlight biosurfactants, bacteria, and fungi as the most commonly used agents.

Biosurfactants are produced by animals, plants, bacteria, filamentous fungi, and yeasts, where the application of microorganisms is more promising due to the short growth period. Surfactants are agents that reduce the surface tension, with the ability for detergency, emulsification, phase dispersion, and are mostly applied in environments polluted with lipids. Its demand has increased as it has low toxicity, is biodegradable in nature, and promotes the recovery of ecosystems contaminated by hydrocarbons from leaks (Gasparin et al., 2012; Mallmann et al., 2010).

Microorganisms such as bacteria, filamentous fungi, yeasts, or a consortium of different microorganisms are specialized in degrading or tolerating various compounds present in the contaminated effluent (Paula et al., 2013). Fungi are microorganisms capable of degrading chemical substances of complex structure, to produce simpler and being easily assimilable molecules, being a viable alternative for bioremediation (Silva & Rondon, 2013).

Bacteria are the most used microorganism in bioremediation processes, due to their biochemical diversity and ability to destroy or transform dangerous contaminants into compounds that are less harmful to humans and the environment (Andrade et al 2010). Microbial consortia are constituted by a complex population of species that, in synergism, are potentially applied in the biodegradation of pollutants, and can be used as inoculants in biological treatments, presenting the advantage of reducing the time of waste degradation (Costa et al., 2007). Metabolic pathways of degradation dependent on a set of factors determined by the functional chemical structure of the compound, by the ability of the microorganism to perform a degradation, as well as the environmental conditions for the microorganism growth (Leonel et al., 2010)

Lipolytic enzymes constitute the most important group of biocatalysts for biochemical applications due to their use in a wide range of substrates, stability to temperature, pH, and organic solvents, and their asymmetric synthesis. The effluents from the dairy industries have a high-fat content, which can cause several operational problems, in this context, a consortium with microorganisms and enzymes have been used for the degradation of these compounds, presenting an interesting alternative to avoid operational problems (Cammarota et al., 2002).

5. Bacterial Bioremediation

Many bacterial species can efficiently degrade the residual charges of dairy wastewater by adsorption, accumulation, and other intra and extracellular enzymatic mechanisms (Rocuzzo et al., 2020; Kasmi et al., 2020; Mazzucotelli et al., 2013). The use of microorganisms such as bacteria has additional advantages of being renewable, providing treated effluents of good quality, converting the dairy processing residues into useful products of high value as a by-product or even as a raw material for other industries, and also, the process can generate biomass, which is commercially valuable (Mazzucotelli et al., 2013; Chandra et al., 2018; Ahmad et al., 2019).

Microorganisms used in the bioremediation process can be isolated from different sources or even be native to a contaminated area such as dairy wastewater (Porwal et al., 2015). Many studies have investigated species of bacteria present in dairy effluents to obtain efficient wastewater treatment agents (Alaa et al., 2019; Keffala et al., 2017; Gulhane & Shome, 2019).

Bacterial species *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Lactobacillus delbrueckii*, *Staphylococcus aureus*, and *Enterococcus hirae*, isolated from dairy effluents, were effective in the process of treating dairy wastewater in a study published by Al-Wasify et al., 2017. A laboratory-scale model was designed in two stages, one of aeration, with the five species of bacteria prepared with an equal percentage of inoculum, and the stage of filtration, in which rice straw and layers of activated carbon were used as a means of filtration. The results of this study by Al-Wasify et al., 2017 showed a considerable improvement in the color of dairy wastewater, pH values close to neutrality, efficiency in reducing BOD by 78.7%, and improvement of the physical-chemical quality with 99.3% of removal of suspended solids, attributed to the degradation of organic materials by bacterial consortium.

The biodegradation of dairy wastewater is an often-effective technology, especially in the case of native bacterial strains such as protease-producing bacteria. Among the various bacteria, *Bacillus sp.* has shown to be a relevant genus producing proteases (Madhu, 2016). Garcha et al., 2016, isolated and identified from samples of wastewater and dairy sludge, species of *Bacillus sp.*, demonstrating high bioremediation efficiency to reduce BOD, COD, and suspended solids. The strain *Bacillus thuringiensis*, isolated by Garcha et al., 2016, showed an efficiency of 89.8% in the reduction of BOD, and 88.6% in the reduction of suspended solids. Chandran et al., 2014, reported a high level of protease activity from bacterial strains isolated from dairy wastewater, with *Bacillus sp.* being the predominant bacteria found. Bacterial proteases effectively lysates milk proteins such as casein and other carbohydrates (Chandran et al., 2014). In another study by El-Sesy and Mustafa (2020), the BOD and COD values in the dairy wastewater were reduced by 82% and 85% respectively, demonstrating a high capacity

for biodegradation by the bacteria *Bacillus subtilis*, endorsing it as a promising microorganism for degrading high concentrations of organic compounds found in wastewater from dairy effluents, such as lactose, casein, and fats.

An interesting strategy is the application of lipase-producing bacterial strains to degrade oil and fat, reducing lipid residues. *Pseudomonas aeruginosa*, isolated from dairy wastewater showed high rates of lipid degradation with high lipase activity, generating 17% free fatty acids and reducing COD rates to 250mg O₂ / L after 12 hours of incubation (Sandaruwani et al., 2018). A consortium with selected lipase-producing bacteria, comprising of *Bacillus subtilis*, *Bacillus. licheniformis*, *B. amyloliquefaciens*, *Serratia marsescens*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* for application in the treatment of effluents rich in lipid content such as milk effluents, was described by Prasad et al., 2011. The formulated mixed culture proved to be effective in treating wastewater, BOD values were reduced from 3200 mg / L to less than 40 mg / L, lipid concentrations were reduced from 25,000 mg / L to approximately 80 mg / L after 12 days of incubation. In the results described by Prasad et al., 2011, *S. marsescens*, when tested alone, showed good lipid degradation from 25,000 to 280 (mg / L) in dairy wastewater. Another study by Balaji et al., 2020, investigated the production and use of an extracellular lipase from *Bacillus* sp. Haltolerant VITL8 in dairy wastewater treatment. The enzyme contributed as an adjuvant by reducing the levels of BOD and COD by approximately 40%, being correlated with the efficiency in the hydrolysis of lipids present in the effluents, reducing 45% of the concentration of the initial lipid within a short period of 8 hours.

Bioaugmentation strategies, such as the addition of external microorganisms with a high ability to degrade a specific substrate represent a promising alternative for the treatment of effluents (Keffala et al., 2017). Dairy wastewater is an enriched medium for microbial growth, and native bacterial strains, when combined with external microorganisms, improve treatment performance with high rates of degradation (Gulhane & Shome, 2019; Teixeira et al., 2019).

The use of mixed cultures can have a cumulative effect in increasing biomass generation, growth efficiency, and enzyme production (Roccuzzo et al., 2020). Numerous researches emphasize the importance of various bacterial species in the treatment of industrial wastewater. Different bacteria were isolated from by-products and agro-industrial waste by Mazzucotelli et al., 2013. Four strains were selected as potential members of the microbial consortium: *Lactococcus garvieae*, *Bacillus thuringiensis*, *Escherichia coli*, and *Stenotrophomonas* sp. The strains selected in the study by Mazzucotelli et al., 2013, were reported to have a multienzyme capacity, highlighting amylolytic, proteolytic, caseinolytic, lipolytic, cellulolytic, and hydrolytic abilities. The consortium significantly improved the degradation of wastewater from dairy products, with high BOD degradation rates of 80.67% and 83.44% at 24 and 48 h, respectively, demonstrating an important technological potential in the degradation of dairy effluents (Mazzucotelli et al., 2013)

Technologies such as the invention BR1020160218934A2 also show a bioremediation composition, which comprises two species of the genus *Serratia* sp. and spores of two bacteria of the genus *Bacillus* sp. with high potential for degradation of industrial effluents that contain fats or lipids, such as the dairy industry. The bioremediation composition in liquid form, comprises of 3.5x10⁶ to 3.5x10¹⁰ CFU / mL of *Serratia* sp. isolated RL1; 3.5x10⁶ to 3.5x10¹⁰ CFU / mL of *Serratia* sp. isolated MC2; 3.5x10⁶ to 3.5x10¹⁰ CFU / mL of *Bacillus* sp. CG3 and 3.5x10⁶ to 3.5x10¹⁰ CFU / mL of *Bacillus* sp. CGIB isolate, as well as a dispersant, a conservative agent, a thickener, and a stabilizer with a pH between 5.0 to 6.0 in water.

Immobilization techniques are also used and considered an effective strategy to obtain better degradation results. The advantage of cell immobilization is that the enzymes remain active and stable for a longer time due to their natural environment, besides, their use facilitates the separation of biomass from the liquid medium and decrease the susceptibility to contamination by unwanted organisms (Roccuzzo et al., 2020; Srivasrava et al., 2016). Srivasrava et al., 2016, isolated bacterial strains from dairy wastewater and tested their bioremediation efficiency in immobilization state. Two efficient strains were identified as *Bacillus* sp. and *Citrobacter freundii* and its biodegradation potential was evaluated as a consortium of cells immobilized in sodium alginate. The immobilized bacterial consortium showed a reduction in the physical-chemical

parameters from 19 mg / L to 7.3 mg / L of oil and grease (O&G), and efficiency in the removal of organics products with a BOD reduction greater than 90% after three incubation days.

In another technology, the invention WO2012162530A2 presented a wastewater treatment system including a secondary treatment system that comprises an aeration basin with polymer substrates and/or ceramic substrates in fixed positions, in which consortia of specific bacterial strains contribute to the formation of biofilms. The consortium of bacterial strains is composed of bacteria of the genus *Caulobacter sp.*, *Enterobacter sp.*, *Pseudomonas sp.*, *Gordonia sp.*, *Bacillus sp.*, *Agrobacterium sp.* and *Zoogloea sp.* The bacteria used to form the biofilm were selected to maximize the reduction of wastewater contaminants, including application in dairy effluents.

Amado et al., 2016, developed a low-cost culture medium using whey as a nitrogen source for the production of hyaluronic acid by *Streptococcus zooepidemicus*. The results showed high hyaluronic acid yields of 3.2 g / L, which is comparable to synthetic media using culture media containing whey (4.0 g / L) or whey hydrolyzate (3.2 g / L), confirming the suitability of this alternative source of nitrogen for bioproduction. Besides that, the study showed a reduction in production costs up to 70%, resulting in a promising strategy for the valorization of dairy residue such as whey.

A study reported by Halder et al., 2020, and Biswas et al., 2019 described a system of management of dairy wastewater in a self-sustaining zero-discharge process, using a consortium with six bacterial isolates including proteobacteria (*Aeromonas sp.*, *Acinetobacter sp.* and *Thauera amino aromatica sp.*), and *Bacillus sp.* The consortium converted dairy wastewater into a biofertilizer, using a steel bioreactor and polypropylene rings to form a biofilm, guaranteeing a custom-made immobilized system that was able to convert dairy wastewater into a by-product with ammonium. The total effluent was converted into biofertilizer, with a reduction of 41.83% in nitrate, 45.83% in phosphate, and 82.6% in BOD levels after 16 hours of incubation. The process was staged in a 72 L bioreactor with an ammonia production rate of 3×10^{-8} moles⁻¹ 100 mL⁻¹. The dairy effluent was converted into biofertilizer for use as a substitute for chemical fertilizer and fresh water for irrigation. Dairy wastewater contains a high nutrient composition (especially nitrogen, phosphorus, and potassium), some species of bacteria selectively convert nitrate, hydroxylamine, nitrile, nitrite, and glutamate into ammonia (Wang & Serventi, 2019).

Behera et al., 2019, studied the production of bacterial lipids using dairy wastewater for biodiesel applications. Oleaginous bacteria with high lipid accumulation were isolated from dairy effluents. A bacterial strain described in the study as DS-7 efficiently used dairy wastewater to accumulate lipids during exponential growth. The bacilliform oleaginous bacteria can accumulate 90% of lipids with lipid productivity of 1.2 g / L·d using lactose as the only carbon source and resulting in a ~ 50% reduction in BOD. The lipids produced were characterized by their potential as low-cost alternative sources of biodiesel, demonstrating the appreciation of dairy wastewater as a renewable raw material for the production of biodiesel.

The use of microorganisms, especially bacterial species, for the treatment of wastewater plays an important role in reducing pollution to the environment, also, to increase the added value of wastewaters such as dairy products, contributing to the diversification of by-products and sustainable environmental applications (Chandra et al., 2018; Ahmad et al., 2019). In Table 2, we highlight all the technologies mentioned in this article, reporting the bacterial species used and the result achieved in each approach.

Table 2. Technologies applied for bioremediation of dairy wastewater using bacterial cultures.

Technology	Applied microorganism	Results achieved
Native bacteria identified and isolated for the dairy wastewater treatment process. (Al-Wasify et al., 2017).	<i>Pseudomonas aeruginosa</i> , <i>Bacillus subtilis</i> , <i>Lactobacillus delbrueckii</i> , <i>Staphylococcus aureus</i> , and <i>Enterococcus hirae</i>	<ul style="list-style-type: none"> - Efficiency in the reduction of 78.7% in BOD levels, improvement of the physical-chemical quality of 99.3% in the removal of suspended solids, and pH values close to neutrality. - Results attributed to the degradation of organic materials by bacteria,
Protease-producing native bacterial strains, isolated and identified for bioremediation of milk effluents. (Garcha et al., 2016).	<i>Species of Bacillus sp. (Bacillus thuringiensis)</i>	<ul style="list-style-type: none"> - 89.8% efficiency in the reduction of BOD, and 88.6% in the reduction of suspended solids.
The protease-producing native bacterial strain is used to degrade high concentrations of organic compounds found in wastewater from dairy effluents. (El-Sesy and Mustafa, 2020)	<i>Bacillus subtilis</i>	<ul style="list-style-type: none"> - 82% reduction in BOD and 85% COD. - Potential to degrade high concentrations of organic compounds found in wastewater from dairy effluents, such as lactose, casein, and fats.
Bacterial strain producing lipases applied to degrade oil, fat, and lipid residues from milk effluents. (Sandaruwani et al., 2018).	<i>Pseudomonas aeruginosa</i>	<ul style="list-style-type: none"> - High rates of lipid degradation, with higher lipase activity, generating 17% of free fatty acids - COD rate reduction to 250mgO₂ / ltr after 12 hours of incubation.
Bacterial strains producing lipases, for use in the treatment of wastewater or industrial effluents rich in lipid content such as milk effluents. (Prasad et al., 2011).	<i>Bacterial consortium: Bacillus subtilis, Bacillus licheniformis, Bacillus amyloliquefaciens, Serratia marsescens, Pseudomonas aeruginosa and Staphylococcus aureus.</i>	<ul style="list-style-type: none"> -Reduction of BOD values from 3200 mg / L to less than 40 mg / L. - Lipid degradation has been reduced from 25,000 mg / L to approximately 80 mg / L after 12 days of incubation. - <i>Serratia marsescens</i>, when tested alone, showed good lipid degradation from 25,000 to 280 (mg / L) in milk effluents.
Production and use of extracellular lipase from a halotolerant bacteria in the wastewater treatment of the dairy processing industry. (Balaji et al., 2020)	<i>Bacillus sp. VITL8</i>	<ul style="list-style-type: none"> - Reduction of BOD and COD levels by approximately 40%, being correlated with the efficiency in the hydrolysis of lipids present in the effluents. - 45% reduction in initial fat within a short period of 8 hours.
Bio-augmentation strategy using mixed cultures, for the treatment of dairy effluents. (Mazzucotelli et al., 2013).	<i>Lactococcus garvieae, Bacillus thuringiensis, Escherichia coli, and Stenotrophomonas sp.</i>	<ul style="list-style-type: none"> - High BOD degradation rates of 80.67% and 83.44% at 24 and 48 h. - Multienzyme capacity, highlighting amyolytic,

Use of bio-augmentation technology using mixed cultures for the development of a bioremediation composition. BR1020160218934A2.	<i>Serratia sp. RLI.</i> , <i>Serratia sp. MC2.</i> , <i>Bacillus sp. CG3.</i> , <i>Bacillus sp. CGIB.</i>	proteolytic, caseinolytic, lipolytic, cellulolytic, and hydrolytic capacity.
Use of the technique of cellular immobilization in sodium alginate of bacterial strains for bioremediation of milk effluents. (Srivastava et al., 2016)	<i>Bacillus sp. and Citrobacter freundii.</i>	- Technological invention that comprises a bioremediation composition with two bacteria of the genus <i>Serratia sp.</i> and spores of two bacteria of the genus <i>Bacillus sp.</i> - High potential for degradation of industrial effluents that contain fats or lipids, such as the dairy industry
Wastewater treatment system (dairy effluents), using a consortium of bacterial strains immobilized on polymer substrates and/or ceramic substrates to form a biofilm. WO2012162530A2	<i>Caulobacter sp.</i> , <i>Enterobacter sp.</i> ; <i>Pseudomonas sp.</i> ; <i>Gordonia sp.</i> ; <i>Bacillus sp.</i> ; <i>Agrobacterium sp.</i> ; <i>Zoogloea sp.</i>	- Reduction from 19 mg / L to 7.3 mg / L of oil and grease (O&G). - Efficiency in removing organics with a BOD reduction greater than 90% after three days of incubation.
Alternative bioremediation strategy for dairy wastewater by a bacterial strain using whey as a culture medium for the production of hyaluronic acid. (Amado et al., 2016)	<i>Streptococcus zooepidemicus.</i>	- Technological invention that comprises a wastewater treatment system, including dairy effluents. - Secondary system with aeration basin with polymer substrates and/or ceramic substrates in fixed positions in which the bacterial pool formed biofilms to maximize the reduction of contaminants.
Dairy effluent management using a consortium of bacterial strains for converting dairy wastewater into a biofertilizer for use as a chemical fertilizer substitute. (Halder et al., 2020)	<i>Bacterial consortium: proteobacteria (Aeromonas sp., Acinetobacter sp. and Thauera amino aromatica sp.) and Bacillus sp. (Biswas et al., 2019)</i>	- Production with high yields of hyaluronic acid (3.2 g / L), confirming the adequacy of residues from the dairy industry as a nitrogen alternative for bioproduction. - Viability of the medium formulated by the by-product, and reduction of production costs by up to 70%.
Production of bacterial lipids using dairy wastewater for biodiesel applications. (Behera et al., 2019)	<i>Bactérias oleaginosas DS-7</i>	- Reduction of 41.83% of nitrate, 45.83% of phosphate, and 82.6% in BOD levels after 16 hours of incubation. - Ammonia production rate of 3×10^{-8} moles ⁻¹ 100 mL ⁻¹ . - Dairy effluent was converted into biofertilizer, making the management process self-sustainable.
		- Bacillum-shaped oleaginous bacteria can accumulate 90% of lipids with lipid productivity of 1.2 g / 1-d. - ~ 50% reduction in BOD levels. - The lipids produced were characterized by their potential as low-cost alternative sources of biodiesel.

Source: Authors.

6. Advances and Challenges in the Bioremediation Process in the Treatment of Dairy Wastewater

Dairy industries are major producers of wastewater. These dairy effluents have various compositions, depending on the dairy product in which they originated, but they share the characteristic of having high levels of chemical and biochemical oxygen demand (COD and BOD) in addition to the presence of lipids, suspended solids, high concentrations of nitrogen and different pH ranges. These characteristics make these wastes highly problematic for untreated disposal in native watercourses, but they also make treatment extremely complex and financially costly (Britz et. Al., 2006).

Currently, the treatment of dairy effluents generally occurs from a pre-treatment stage, in which larger solids, plastics, milk clots, etc. are removed. In some cases, this pre-treatment may also involve a pH treatment (Britz, 2008). After pH treatment, a treatment is carried out to remove oils and suspended particles through physical-chemical processes of decantation, filtration, coagulation, flocculation, and flotation (Otaibi et al; 2019). Secondary treatment is carried out in cases where the effluents have a very high concentration of organic matter. For this phase of treatment, the most used types of treatment are aerobic biodegradation processes (Dinakar, 2019). Despite these current techniques, many of them are excessively costly, ineffective, and generate secondary pollutants that are problematic for the environment, highlighting the importance of new technologies for bioremediation processes (Tchamango, et al 2020).

In recent years, new strategies for treatment have been thought of. The extract of the yeast *Yarrowia lipolytica* for digestion of the fatty residues present in the wastewater proved to be extremely promising in the reduction of lipids found in the tailings as well as in the COD and BOD levels, according to Dunoyer, 2019. Extracellular lipases from *Bacillus sp* are also promising in this area, since the treatment can be done using the microorganism itself, without the need for enzyme extraction and purification (Balaji, 2020).

7. Conclusion and Future Perspectives

The present review brought together a part of the state of the art regarding the bioremediation of dairy wastewater, which covers wide areas and techniques used for this purpose. Although many practices are already described regarding the use of microorganisms in the bioremediation process, there are still scientific and technological advances that need to be achieved to improve the understanding of the mechanisms and controlled reproductions of the reactions that occur during the bioremediation processes.

Geological characteristics of polluted sites, including the type of waste, depth of pollutants, products to be manufactured, cleaning chemicals, and location of the establishment, are key factors in deciding on the most appropriate treatment and the most efficient method for effectively treating industrial dairy wastewater.

There has been rapid and great progress in the development of effective, economical, and environmentally friendly bioremediation processes. In the future, bioremediation using microorganisms is expected to be widely used to significantly reduce contamination in polluting residues that cause negative and destructive impacts on the environment.

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