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Whey to Ethanol: Unlocking the Potential of Cheese Whey for Sustainable Bioethanol Production

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Abstract

Nowadays pharmaceutical water contamination is a worldwide problem. Resulting from an absence of appropriate legislation, policies, and efficient treatment technologies, especially those developed to remove pharmaceuticals from wastewater, has led to the further contamination of soil and natural water bodies. The adverse impact this has on wildlife/human species reinforces the need to develop proper monitoring and detection techniques for pharmaceutical water contamination. This article focuses on the sources, fate, monitoring, and negative effects of pharmaceuticals globally and compares it with data from Kazakhstan and bordering Commonwealth of Independent States (CIS) countries. In general, Kazakhstan/CIS countries are demonstrating similar trends with other developed comparison countries in terms of types and concentrations of pharmaceuticals found. The major difference between Kazakhstan/CIS and developed countries is the absence of matured monitoring and legislation systems. On the other hand, removal of pharmaceuticals at wastewater treatment plants, which precede most sources of soil and natural water way pollution, is intensified by the development of the pharmaceutical industry and approval of novel drug types. This work shows that proper disposal, monitoring and legislation, efficient wastewater treatment, and development of accurate drug determination methods have significant potential in controlling pollution of the environment, including the water–soil nexus.

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1. Introduction

The environmental impacts of fossil fuel consumption, including air pollution and greenhouse gas emissions leading to climate change, have raised serious concerns.[1] Burning fossil fuels for energy is the largest contributor to human-generated greenhouse gas emissions, particularly carbon dioxide (CO_2). The combustion of fossil fuels releases not only CO_2 but also harmful air pollutants, including sulfur dioxide (SO_2), nitrogen oxides (NO_x), and particulate matter. These emissions are the primary driver of global climate change and the associated impacts, including increasing temperatures, rising sea levels, extreme weather events, and ecosystem disruption.[2-4] Additionally, these pollutants adversely affect the environment and human health, leading to respiratory diseases.[5,6]

In an increasingly saturated global society, where the transport sector accounts for more than 40% of the total fossil fuel consumption, fossil fuel reserves are estimated to be used up in the next 40-50 years. Over the past several decades, the depletion of fossil fuel reserves, rising global demand for energy, and concerns about environmental sustainability have driven increased attention to developing alternative energy sources and technologies. This has led to a greater focus on cleaner and more sustainable energy sources. Consequently, technologies to improve the energy efficiency of industrial processes, buildings, and transportation have become a priority. Moreover, energy conservation and energy-efficient technologies play a crucial role in reducing the overall energy

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demand.[7] Finally, developing alternative renewable fuel sources has become a global priority due to concerns about energy security, environmental sustainability, and the depletion of finite fossil fuel reserves. Over the years, significant progress has been made in the development of various renewable fuel sources.

Advances in bioenergy include the development of advanced biofuels such as cellulosic ethanol and renewable diesel. Biomass feedstocks are being optimized, and biorefinery technologies are being deployed to convert various organic materials into biofuels and bioproducts. Many countries and regions, aiming to meet ambitious renewable energy and carbon reduction goals, are turning to bioethanol as a domestically produced, low-carbon fuel, thereby enhancing energy security and capitalizing on advancements in biotechnology and agricultural practices to make production more efficient and sustainable. Lastly, this has increased its competitiveness as an alternative fuel, with global ethanol production amounted to more than 100 billion liters.[7,8] Global output generally rose over time, but the COVID-19 epidemic caused a worldwide decline in production in 2020. Production has increased, but has not yet returned to pre-pandemic levels. In 2021, the United States generated more than 15 billion gallons of ethanol, making it the world's greatest producer. 82% of the world's ethanol is produced by the United States and Brazil together. Brazil predominantly employs sugarcane, whereas the bulk of ethanol produced in the United States comes from corn.[9-14]

Traditional raw materials used to produce alcohol, specifically ethanol, include a variety of plant materials that contain sufficient fermentable sugars or other carbohydrates that can be converted into alcohol through the fermentation process. The most widely used starch-containing materials are grains (rye, wheat, corn, barley, oats, millet) and potatoes, sugar-containing materials, such as molasses (waste from sugar and starch production), defective sugar beets, as well as wood and waste from agricultural plants.[15-18] In addition to the traditionally used starch-containing raw materials, which are used to produce bioethanol through the enzymatic conversion of starch into glucose using amylolytic enzymes, followed by the conversion of glucose into the target product ethanol, there is another promising source for the biotechnological production of fuel ethanol - whey. This method is currently one of the promising methods for completely processing enzymatic hydrolysates from dairy production.[19,20]

The dairy sector generates diverse waste streams, and their management profoundly influences the environment, with dairy processing plant wastewater containing organic matter, chemicals, and nutrients, posing a risk of water pollution and disruption to aquatic ecosystems if not properly treated before discharge into natural water bodies. Disposing whey as waste can be challenging and costly due to increasingly strict environmental regulations. For instance, whey is a byproduct of cheese and yogurt production and contains high levels of organic matter, which, if not properly managed, can lead to environmental issues, particularly when it enters water bodies. Dairy industry wastewater is claimed to be 10 times more polluted than household wastewater. This implies that it contains higher concentrations of pollutants, including organic matter, nutrients, suspended solids, pathogens, and potentially harmful substances.[21-23] Hence, adequate treatment can remove or reduce the levels of organic matter, nutrients, and chemical contaminants in wastewater before it is discharged into natural water bodies. Compliance with environmental regulations and best practices in wastewater management is critical for minimizing the environmental impact of dairy processing operations and safeguarding aquatic ecosystems.

Milk whey, a byproduct of the dairy industry, can indeed be used in bioethanol production. The key idea is that lactose can be fermented by certain microorganisms, typically bacteria and yeast, to produce ethanol and carbon dioxide through alcoholic fermentation. It is important to note that the fermentation of lactose in whey requires specific microorganisms capable of metabolizing lactose, and conditions such as temperature, pH, and oxygen levels need to be controlled to effectively facilitate the fermentation process effectively.[24] There has been much research on ethanol production from whey, and the central aspect of these investigations was to study the formation of ethanol from crude whey using yeast strains, leading to maximum lactose utilization and ethanol. Although a great deal of research has been accomplished with the cultures of yeast strains, such as K. marxianus, Kluyveromyces lactis, Kluyveromyces fragilis, Candida pseudotropicalis, Candida intermedia, and Torula cremoris, on deproteinized whey, the use of crude whey as a culture medium has not been explored comprehensively.[25,26] There is an opportunity for further research and exploration in utilizing crude whey as a culture medium for yeast strains. Such research could help understand how yeast strains perform and adapt in a more complex and less processed growth environment, which may have implications for various bioprocessing applications, including bioethanol production.

In recent years, there has been a growing interest in using immobilized microbial cells for biocatalysis in various biotechnological industries. Immobilized cells, trapped within support materials, offer advantages such as increased stability, longer lifespans, and higher productivity, making them ideal for continuous or repeated batch processes. Furthermore, their ease of separation from the final product and the potential for reuse reduce production costs and enhance the control of bioprocess conditions.[27]

The development and research on immobilized microbial cells as biocatalysts have indeed been actively conducted over the last two decades. This area of biotechnology has seen significant advancements, and researchers have explored various methods and applications for immobilizing microbial cells. Research and development in the field of immobilized microbial cells have shown promising potential for cost-effective ethanol production based on whey and other substrates.[28] The low cost of whey as a substrate, combined

with the increased productivity of bioreactors based on immobilized microbial cells, presents a compelling case for the cost-effectiveness of this approach compared with traditional bioreactors using free cells. As reported by various authors, the significant increase in productivity underscores the economic advantages of utilizing immobilized microbial cells for various bioprocesses, including ethanol production from whey. The examples provided suggest that a bioreactor with immobilized yeast cells offers several benefits for the continuous fermentation of whey to produce bioethanol. Researchers have explored various methods and applications for immobilized microbial cells during this time. This research has expanded the potential applications of immobilized microbial cells in multiple bioprocesses. Immobilized microbial cells show promise for cost-effective ethanol production, mainly using whey and other substrates.[29,30]

This review aims to describe the current knowledge of bioethanol production from whey, fermentation technologies, and the advantages of using whey as a fermentable material. In addition, this review considers various other factors that influence ethanol yield from whey.

2. Historical background of bioethanol production

Throughout history, one of the earliest biotechnologies used by humans was the fermentation of sugar to produce ethanol. Humans have been using ethanol as an intoxicating component of alcoholic beverages since prehistory. Consequently, its ingestion has long been known to have intoxicating effects. Dry residue on 9,000-year-old pottery discovered in China implies that alcoholic beverages were consumed by Neolithic people.[31,32]

Alcohol use increased dramatically because of many factors. Early in the 1800s, to substitute whale oil in lamps, ethanol made from corn feedstock was mainly used.[33] The American inventor Samuel Morey is credited with one of the early experiments involving ethanol in an internal combustion engine. In 1826, Morey built a prototype of a "gasoline" engine that used ethanol as a fuel source. This experiment marked one of the earliest known uses of ethanol in an engine. However, the widespread use of ethanol in automobiles came much later. In the late 19th and early 20th centuries, various alternative fuels, including ethanol, were experimented with as internal combustion engine technology evolved. In the 1880s, grain ethanol was used as a fuel source for some early automobiles. Henry Ford introduced Model T in 1908, which played a pivotal role in making automobiles more accessible to the public.[34] Brazil has a significant history of using bioethanol, primarily derived from sugarcane, as an automobile fuel. While the specific date for introducing ethanol as a fuel in Brazil might vary slightly, it is generally accepted that Brazil began experimenting with ethanol as a fuel source in the 1920s.[35] The installation of the first ethanol-gasoline blend fueling station in Nebraska by the Earl Coryell Company in 1933 is an interesting historical development in the use of ethanol as a fuel.[36] Indeed, ethanol has a long and storied history as a fuel and chemical compound (Fig. 1).

The discovery of cheap oil fields in 1940 certainly had a significant impact on the global energy landscape. The availability of abundant and low-cost petroleum resources from these oil fields, along with the development of efficient refining and distribution systems, contributed to a period of relative abundance and stability in the supply of gasoline and other petroleum-based fuels.[36] The production and use of ethanol as a fuel experienced fluctuations over the years, and the 1970s marked a significant turning point for the ethanol market, primarily due to the global oil crisis and rising petroleum oil prices.[31] In the 1970s, the United States experienced a substantial increase in ethanol production, primarily from corn. The government introduced various policies and incentives to promote ethanol production and use as a biofuel. By the late 1970s, the U.S. ethanol industry had grown, and the country was producing nearly 90 million gallons of ethanol per year. This marked the beginning of the modern ethanol industry in the United States. In response to rising oil prices and the need for energy security, the Brazilian government launched the "National Alcohol Program" (Programa Nacional do Álcool or Proálcool) in 1975. Proálcool was a strategic plan aimed at promoting the production and use of ethanol as a fuel. Brazil was particularly well-suited for ethanol production due to its abundant sugarcane crops, which could be used as a feedstock for ethanol production. As a result, Brazil rapidly expanded its ethanol industry during this period.[36] The aim of Brazil's "National Alcohol Program" (Proálcool), launched in 1975, was the large-scale production of ethanol from sugarcane as an alternative to gasoline.

Ethanol-gasoline blends were used in several countries, including European nations, in the early to mid-20th century. There were several reasons for adopting these blends, including safety and environmental benefits.[37] Later, the Energy Tax Act of 1978 significantly promoted the production and consumption of bioethanol-gasoline blends in the United States. This legislation introduced tax incentives to encourage the use of biofuels, including ethanol, as part of the country's response to energy security concerns and rising oil prices. In 1984, Brazil introduced the use of hydrated bioethanol (bioethanol with a high-water content, typically around 96% ethanol and 4% water) as a car fuel.[38]

Ethanol production remains a significant industry worldwide, with various countries continuing to use it as a biofuel to reduce greenhouse gas emissions, promote energy security, and support agricultural sectors since 2000. Significant progress has been made in developing enzyme and microbial technologies to break down lignocellulosic biomass into fermentable sugars. This period appears to be the emergence of several biotechnology companies focused on second-generation ethanol production. For instance, the first commercial-scale cellulosic ethanol plant, Poet-DSM's

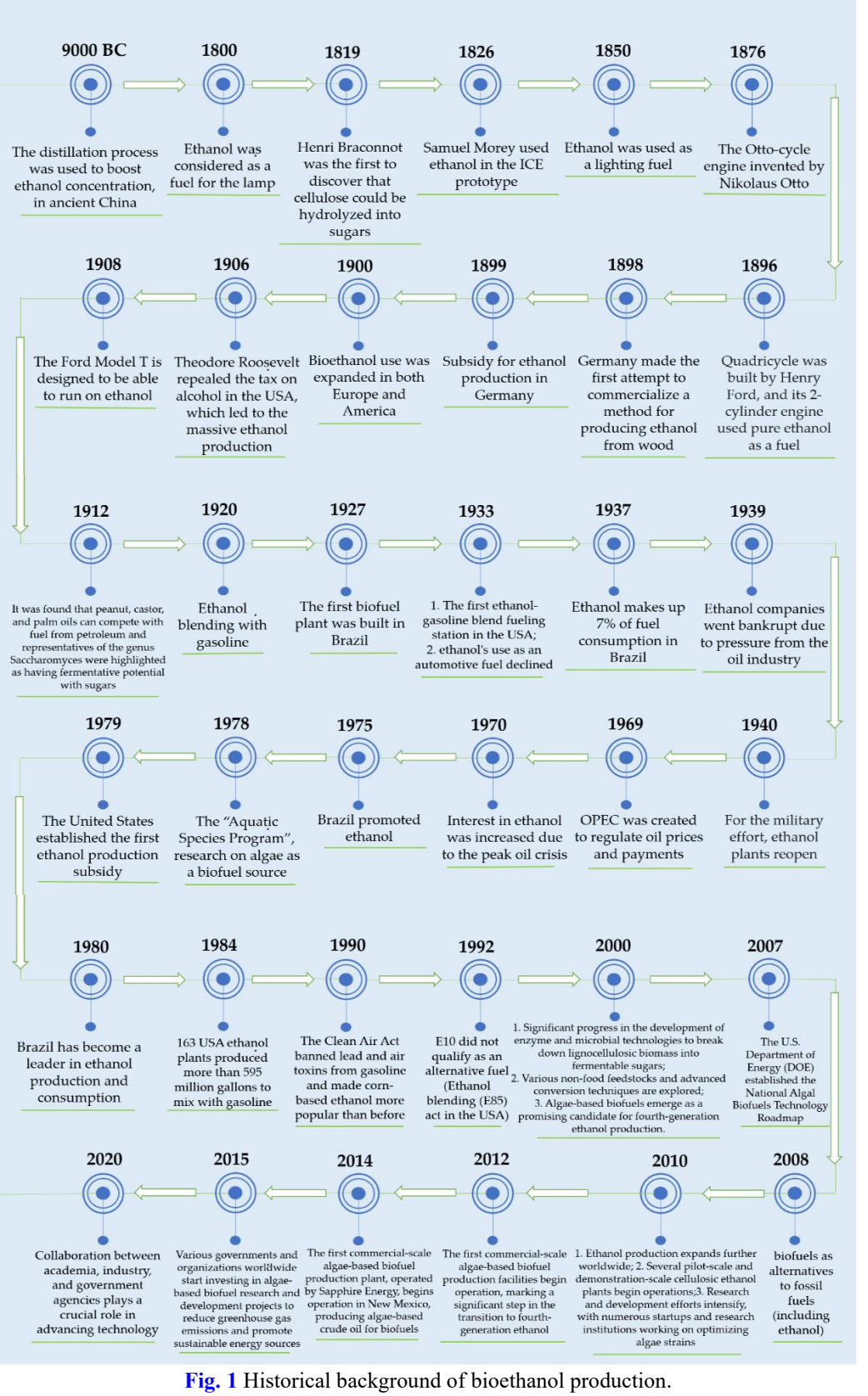

Fig. 1 Historical background of bioethanol production.

Project Liberty in Iowa, USA, began producing ethanol from corn stover. This marks a significant milestone in the commercialization of second-generation ethanol.[39]

Between 2015 and 2019, continued research and development efforts improved the efficiency and viability of third-generation ethanol production, making it more competitive with first- and second-generation ethanol. Since 2020, third-generation ethanol technologies and production methods have continued to advance, with increased emphasis on sustainability, carbon capture, and utilization. However, the

transition to fourth-generation has occurred since the first commercial-scale algae-based biofuel production facilities began operation, marking a significant step in the transition to fourth-generation ethanol in 2012.[40]

Liquid biofuels, such as biodiesel or bioethanol, were the subject of intense research throughout the later decades of the 20th century as potential replacements for fossil fuels. Research and development efforts are underway to explore alternative feedstocks for ethanol production beyond traditional corn and sugarcane. These efforts focus on lignocellulosic biomass, such as crop residues (e.g., corn stover) and woody materials. After investigating the chemical composition of milk whey, researchers and engineers have found ways to convert lactose-rich whey into bioethanol, which is a renewable and sustainable energy source. Previously, milk whey was a byproduct of the dairy industry and has traditionally been considered a waste product. Bioethanol production from milk whey is a relatively recent development in the field of biofuels.[38]

Research in the 1970s and 1980s focused on the microbial fermentation of whey to produce bioethanol. Scientists experimented with various microorganisms, including yeast strains, to convert lactose in whey into ethanol. During this period, immobilization techniques for microbial cells were also being developed and refined. These techniques played a crucial role in improving the efficiency of ethanol production from whey. From the 1990s onward, interest was increased in developing cost-effective and sustainable bioethanol production methods using whey as a substrate. Research efforts have expanded to optimize fermentation conditions, select suitable yeast strains, and design efficient bioreactors. In recent years, some companies and research institutions have successfully commercialized bioethanol production from whey on a larger scale. These efforts involve the use of immobilized yeast cells as well as the optimization of downstream processing and product recovery. Today, bioethanol production from whey remains an active area of research and development, with a growing focus on improving efficiency, reducing costs, and ensuring environmental sustainability. It represents a valuable example of converting waste streams into valuable products per the principle of the circular economy.[40]

3. Whey processing alternatives

Whey is a byproduct of cheese production and is rich in protein, lactose, minerals, and vitamins. Due to its large organic load, whey is frequently regarded as a waste. It can be processed and utilized in various ways, depending on the desired end product and the specific components. Initially, milk is fermented by naturally occurring undefined bacteria that have been introduced into the milk through milk processing, cow handling, and the natural microbes that acidify the milk for cheese manufacture. However, the industrially designed starter cultures are added to increase the required cheese characteristics and aroma. The whey that is drained off has various types as a result. Additionally, Asunis *et al.* found that the amount of water, detergents, and sanitizing agents used to clean the milk or cheese whey container impacted the cheese whey's chemical composition.[41] The composition of whey can vary significantly based on several factors, including milk composition, cheese type, cheese-making process, cheese milk treatment, and post-curd processing.[42] Moreover, the composition of cheese whey depends on factors involving milk quality, animal feed, and animal breed.

Cheese whey generally retains 20% of milk proteins and 55% of lactose, vitamins, and minerals from milk.[41,43] Whey has a lactose level that ranges between 4.5% and 6.0%.[44–46] Owing to the conversion of lactose to lactic acid during the fermentation process in acidic whey, its lactose level is lower than that of sweet whey. The only carbon source for ethanol generation, lactose in whey, is the rate-limiting step in the process. Lactose also inhibits the growth of other microbes. Cold preservation of whey can extend its shelf life. However, it is essential to consider the growth of lactic acid bacteria and other microbes, which can impact the composition of the whey, particularly the lactose content.[47,48] Processing whey offers multiple options to reduce waste and create value-added products. Processing whey offers various alternatives to reduce waste and create value-added products. Whey protein isolate production involves the removal of most non-protein components (e.g., fat and lactose) from whey, producing a high-protein powder. It is commonly used in sports nutrition and food products. Depending on the desired end product and market demand, there are many whey processing alternatives (Fig. 2).

These alternative methods of whey processing showcase the potential for transforming what was once considered waste into valuable resources. They not only reduce environmental impact but also create opportunities for innovative and sustainable applications across various industries, from food to energy and agriculture. As technology advances, we can expect even more creative uses for whey, further promoting eco-friendly and responsible practices. Whey is rich in high-quality proteins, lactose, and other valuable substances, such as vitamins and fats. One of the most common methods of whey processing is to isolate these proteins. For instance, whey protein isolates are widely used in the food industry, especially in sports nutrition products and protein supplements. This method not only reduces waste but also generates a valuable product. Moreover, lactose, the primary sugar in whey, can be enzymatically hydrolyzed to produce lactose-free whey. This benefits individuals with lactose intolerance and expands the potential applications of whey in various food products.

4. Ethanol production from lactose by microorganisms

Few yeasts ferment lactose even though many yeasts can digest lactose aerobically.[49] Kluyveromyces species have been widely employed to produce bioethanol from cheese

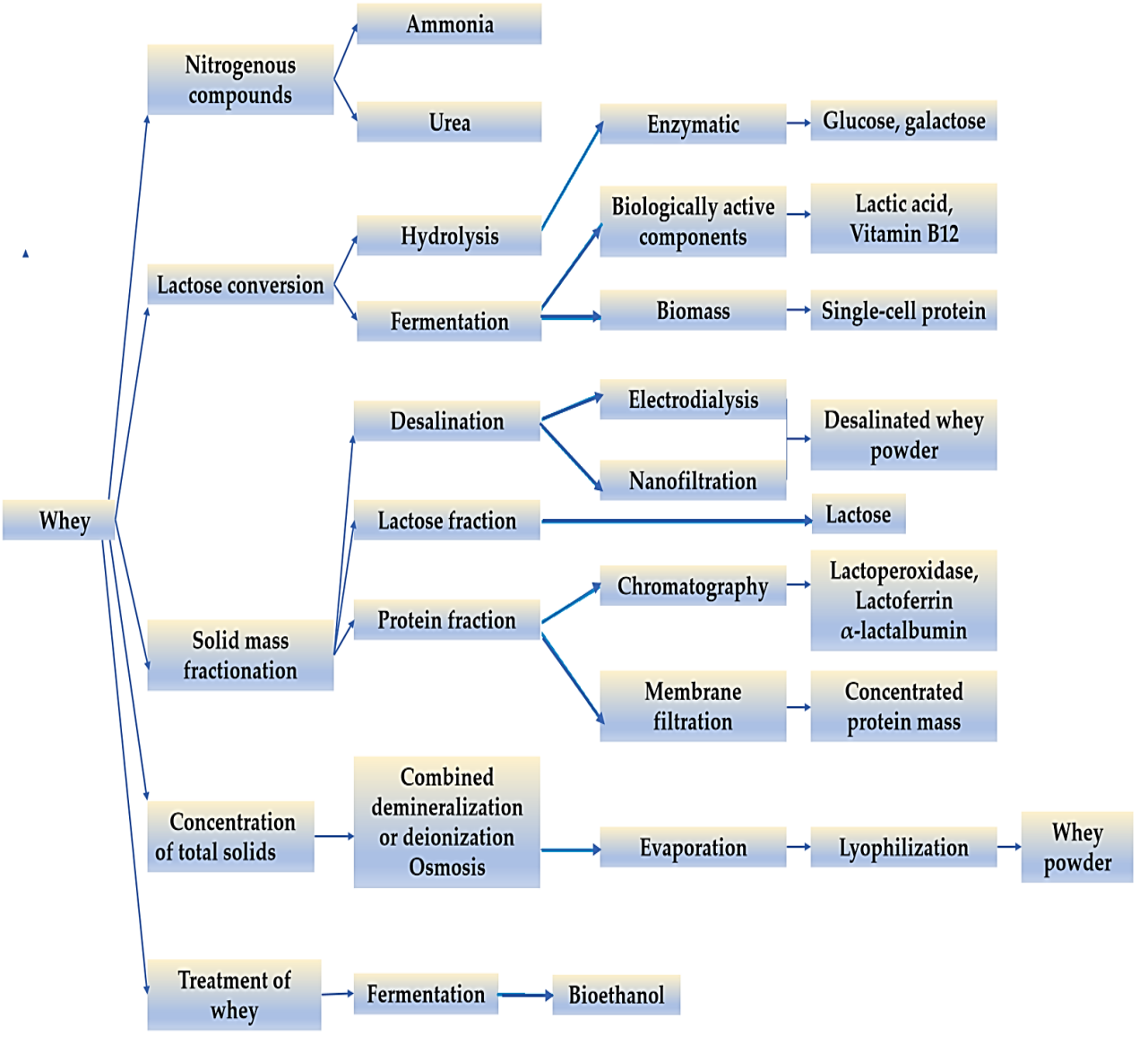

Fig. 2 Alternative ways of whey processing.

whey, including K. lactis, K. marxianus, and K. fragilis.[44,49-56] Along with Kluyveromyces, various Candida species strains, including C. pseudotropicalis, C. krusei, C. inconspicua, C. xylopsoci, and C. kefyr, are additionally recognized for their capacity to metabolize lactose into ethanol.[42,56-59] Pichia kudriavzevii was isolated being isolated from kefir, it was found that the yeast can ferment lactose. Despite this, yeast grows slowly on media containing lactose, making it less applicable in bioethanol production.[60]

Since they are effective and tolerant of whey with a lower pH, *K. marxianus* and *K. lactis* are most frequently used for the generation of ethanol.[61] Despite their close evolutionary relationships, *K. marxianus* and *K. lactis* produce more ethanol than the latter and engage in better fermentative metabolism.[62] This is especially true at higher temperatures. The thermotolerant yeast *K. marxianus* exhibits high growth yield and -galactosidase activity. Even at high temperatures, the ability to produce ethanol from whey is a strong contender. It was a suitable bacterium, with a maximum alcohol production efficiency of 96.5%, for lactose fermentation to produce ethanol. *K. marxianus* is effective at producing ethanol because it consumes all of the lactose in whey within 16 h. In another investigation, "coalho" cheese whey was used as the substrate, and it was discovered that the yeast *K. lactis* NRRL Y-8279 was more effective than *K. marxianus* ATCC 36907 for the co-production of -galactosidase and ethanol.[63] In addition to *Kluyveromyces species*, some *Candida* species, such as *C. kefir*, *C. inconspicua*, and *C. xylopsoci,* are promising in lactose hydrolysis and ethanol production, although *Candida* species have a lower capacity to convert sugars into ethanol and can use more lactose than *Kluyveromyces* species.[64] In addition, the fastidious *Candida* species has numerous dietary requirements, which reduces the competitiveness of its ethanol production. Additionally, most *Candida* species are susceptible to ethanol concentrations, inhibited by low ethanol concentrations with a low conversion

yield (40%).[65-67]

Some *Kluyveromyces* species exhibit lower ethanol tolerance and are inhibited by moderate sugar and salt contents of whey compared with *S. cerevisiae*.[68] The cost-effective production method for ethanol from lactose-containing whey involves using *S. cerevisiae*.[69] Protoplast fusions of *S. cerevisiae* and *Kluyveromyces* spp. and exogenous expression of the lactose hydrolase gene in S. cerevisiae (genes from *E. coli*, *A. niger*, and *Kluyveromyces* spp.) are methods to confer lactose utilization to *S. cerevisiae* in addition to pre-hydrolysis via chemical or biological methods.[70,71] Additionally, *Lactococcus lactis* has been genetically altered to create ethanol from the lactose found in whey; however, this method has several disadvantages due to the exact nature of the organism's nutritional needs.[72]

Yeasts, genetically modified bacteria, and yeast are not the only organisms that can naturally use lactose and produce ethanol using cheese whey as a carbon source. However, some filamentous fungal species, including *Aspergillus oryzae*, *Neurospora intermedia*, and *Neolentinus lepideus*, have been found to be beneficial in ethanol production.[73] In addition to glucose, galactose, and lactose, *N. lepideus* could also make ethanol from the five-carbon sugar xylose.[74] Additionally, *Aspergillus niger* may make ethanol from sweet whey because it can do so at a pH of 5.9 and a temperature of 30°C when lactose-dominant lactoserum is used as the starting material.[75] In addition to making ethanol from whey, the fungus can also make ethanol from lignocellulose that has been degraded chemically or biologically. The capacity of *N. lepideus* to ferment lactose in the presence of high calcium concentrations, a mineral frequently present in whey, gives this organism an advantage when producing ethanol.[76] They also claimed that *N. lepideus* is capable of ethanol fermentation from milk-rich lipids.

The growth circumstances of the target yeast have a significant impact on the production of ethanol. The primary determinants of bioethanol generation from whey are temperature, pH, incubation period, salinity, solid load, and carbon source concentration (Fig. 3). After ranking the growth parameters, it was concluded that temperature, followed by pH, inoculum size, and lactose concentration, was the most crucial factor determining the synthesis of ethanol from cheese whey using *K. marxianus* UFV-3.[77]

Temperature: The advantages of high-temperature fermentation for ethanol production include reduced cooling costs, decreased danger of contamination, continuous transition from fermentation to distillation, and simultaneous saccharification and fermentation. The capacity of *K. marxianus* ETP87 to create ethanol from whey at 45 °C is encouraging to use the yeast for ethanol production from warm whey before it is dominated by lactic acid bacteria.[78] However, greater temperature amplifies the inhibitory effects of additional elements such as salt and ethanol. At 38 °C and 42 °C, *Candida* species (*C. inconspicua* and *C. xylopsoci*) successfully generated ethanol from lactoserum. For the synthesis of ethanol from cheese whey, *C. kefyr* requires a temperature of 30 °C and pH.[79] Compared with *Candida* species, *Kluyveromyces* species are more thermotolerant and can survive high temperatures.

pH: In yeast cells, the process of producing energy, enzyme activity, other protein structures, cell membrane permeability, and metabolite transport are all significantly impacted by the divergence of external pH from its ideal condition.[80] To compensate for pH deviation, yeast cells have developed a

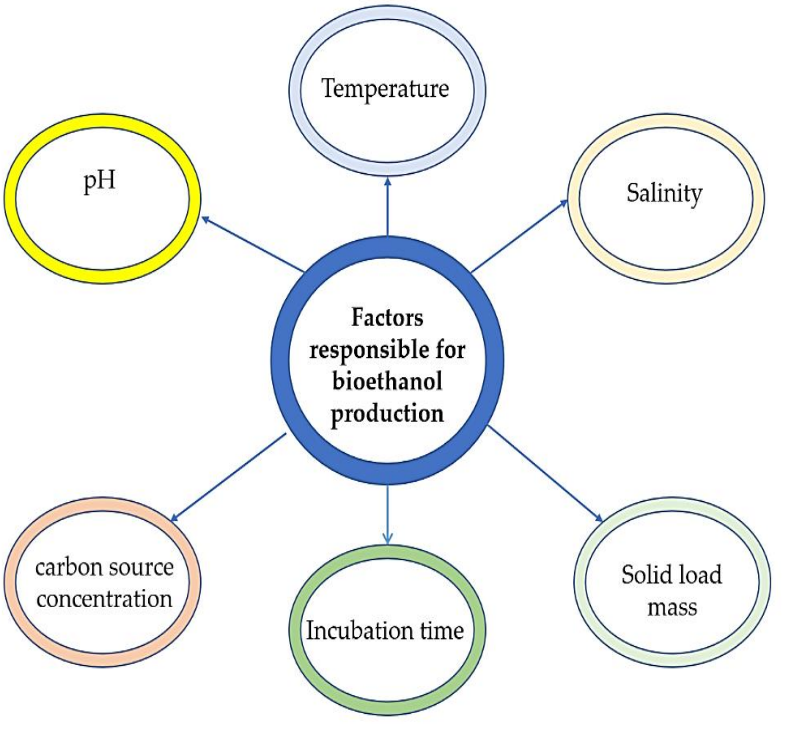

Fig. 3 Factors affecting bioethanol production.

variety of mechanisms, including pH compartmentalization, proton-translocating ATPases that control cytosolic pH, cation transporters that control cytosolic pH, and regulation of organellar pH.[81] Ethanol-producing yeasts from cheese whey must tolerate a pH range of 3.5 – 6.5. It was found that pH 5 – 6 is the best for producing ethanol.

Salinity: With different salts, the yeasts showed variable sensitivity. $CaCl_2$ and NaCl concentrations between 10 and 40 g/L were observed to have a negative correlation with the rate of ethanol generation. When ethanol was produced from cheese whey with lower salt concentrations (less than 2 g/L) compared with cheese whey without external salts, there was no discernible difference, indicating that the cheese whey contained enough NaCl and $CaCl_2$ for the growth of microorganisms. At higher lactose concentrations or under other less-than-ideal growth circumstances, the effects of salts on the synthesis of ethanol were more prominent.[82]

Incubation time: The ethanol fermentation process is negatively impacted by solid particles found in whey. According to Koushki *et al.*, lactose absorption and ethanol production rates increased linearly with increasing whey solid concentrations.[83] Waiting for more incubation time reduces the ethanol titers because *Kluyveromyces* spp. use ethanol as a carbon source when there is a lack of a carbon source, which can take up to 12 or 16 h if the whey is not concentrated or an external carbon source is added. According to a different study, lactose usage started within 24 hours and ended after 72 hours.[84]

The presence of oxygen may affect ability of microorganisms to ferment whey lactose. One of the factors influencing sugar consumption preferences is oxygen. For instance, when *K. lactis* was grown on a medium comprising carob sugars mixed with whey, lactose was first digested under high aeration, followed by glucose and fructose. Similarly, when the oxygen concentration increases, a high lactose medium creates sufficient ethanol; however, under anaerobic conditions, no ethanol is formed from high lactose.[85]

According to some investigations, $(NH_4)_2SO_4$ and soy wheat have been used as nitrogen sources to create ethanol from dilute acid (1.5%) hydrolyzed carrot pomace using *Pichia stipites*.[86] They discovered that cheese whey-supplemented carrot pomace hydrolysate had a greater ethanol content than soy wheat. Although $(NH_4)_2SO_4$ is more expensive than its organic competitors, such as cheese whey and soy wheat, it produced more ethanol than cheese whey when used as a nitrogen source. In the work by Ferreira *et al.*, thermotolerant *K. marxianus* CCT 7735 fermented a mixture of ricotta cheese and sugarcane bagasse to create bioethanol.[87]

5. The crucial role of catabolite repression

Catabolite repression can be strategically used in bioethanol production to enhance the efficiency of the fermentation process and improve ethanol yield. Multiple studies have been conducted to boost ethanol production using certain yeast strains. These studies involved supplementing cheese whey with external carbon sources as a strategy to enhance ethanol productivity.[88] Nevertheless, the presence of glucose can negatively impact ethanol yield in a fermentation process by repressing the utilization of other sugars, resulting in a diauxic growth pattern.[53] Advances in genetic engineering have made it possible to alleviate challenges related to the co-fermentation of glucose and other sugars in microorganisms (Table 1). For instance, according to some research, K. marxianus has been modified to overexpress a specific xylose transporter (GAL2-N376F). This genetic modification enhances the yeast's ability to ferment xylose, a sugar derived from lignocellulosic biomass. As a result, this genetically engineered yeast strain can produce 50.10 g/L from the co-fermentation of glucose and xylose.[89] Additionally, the mutant strain K. marxianus SBK has demonstrated its capability to produce ethanol under specific conditions. It was proven to produce 23.82 g/L of ethanol at 40°C from a mixture containing 40 g/L of glucose and 28 g/L of xylose.[90] This indicates the strain's potential for ethanol production from lignocellulosic biomass.[91,92] Likewise, the successful genetic modification of K. marxianus strain KCTC 17555ΔURA3 by introducing a mutant sugar transporter, ScGAL2-N376F, derived from Saccharomyces cerevisiae had a positive consequence. Thus, introducing the mutant sugar transporter resulted in a remarkable improvement in xylose utilization. Specifically, the mutant strain demonstrated a 195% increase in xylose utilization compared with the parental strains.[91,93] The ScGAL2-N376F mutant strain exhibited an enhanced sugar consumption rate. Moreover, the mutant strain significantly increased ethanol production rates by 52%. The report by Hua et al. underscores the importance of genetic modifications to enhance xylose utilization in microorganisms, particularly when a mixture of xylose and glucose is used as the carbon source.[94] By improving the utilization of xylose and other non-glucose sugars, research like this contributes to the sustainability of biofuels. It enables the utilization of a wider range of feedstocks and reduces waste in bioethanol production. This and other related studies exemplify the field of metabolic engineering, where genetic modifications are used to optimize the metabolic pathways of microorganisms for specific tasks, such as improved sugar utilization in biofuel production. Moreover, the methods mentioned are indeed significant for bioethanol production, especially when lignocellulosic feedstocks are involved.[95-97]

Removing glucose repression is another essential strategy for improving the fermentation of non-glucose sugars in microorganisms, particularly in mixed-sugar fermentation processes. Catabolite repression is a regulatory mechanism that prioritizes glucose utilization over other sugars when both are present. Mig1 transcription factor is central to glucose repression in yeast and other microorganisms. It regulates the expression of genes associated with the transport and utilization of alternative carbon sources, such as galactose, sucrose, maltose, and lactose. When glucose is present, particularly at higher concentrations, Mig1 acts to repress the

Table 1. The genetic modifications in yeasts for bioethanol production.

The yeast species	Target gene (s)	Genetic manipulation methods	Goal(s)	The volume of produced ethanol	Positive results
K. lactis	*KlNDI1*	The removal of a specific gene called *KlNDII*	*shift* from nonfermentative to fermentative	45 g/L	Enhanced bioethanol synthesis from whey lactose.[98]
	(*TtTHC1*) Squalene-tetrahymanol cyclase	Target gene heterologous expression	Oxygen-independent sterol production		At a greater temperature, the mutant thrived anaerobically.[99]
	TATA-binding protein (TBP) Spt15	Combinatorial mutagenesis collection	Raising ethanol threshold	57 g/L	One amino acid modification (Lys to Glu at position 31) in TBP caused the expression of hundreds of genes to change, which enhanced ethanol tolerance and output.[100]
K. marxianus	A wide range of genes	The specific concentration of ethanol (6% v/v) for an extended period (100 days) to encourage adaptive changes or evolution	Enhancing ethanol tolerance	120 g/L	It helped to analyze the upregulation of multiple cellular pathways in response to ethanol exposure. These pathways may include anti-osmotic, anti-oxidative, and anti-thermic process development.[101]
K. marxianus SBK1	Glycolytic enzyme	To create a thermotolerant mutant capable of simultaneous co-fermentation of glucose and xylose by relieving catabolite suppression (2-DG)	Alleviating catabolite repression (simultaneous utilization of glucose and xylose)	23.82 g/L	Except for glucokinase-1, glycolytic enzymes were significantly downregulated.[90]
K. marxianus ScGal2_N376F	Galactose permease (*SCGAL2-N376F*) transporter	Introducing extra copies of a specific gene into an organism or increasing the expression level of that gene	Enhancing xylose uptake and glucose/xylose co-utiliztion		The 195% improvement in xylose consumption.[102]
	Galactose permease (*ScGAL2-N376F*) transporter	Overexpressing the target gene	Efficient utilization of galactose in the presence of glucose	36.16 g/L	Enhancing the ethanol production rate by 52% without a significant change in ethanol concentration but with a shorter production time.[102]
K. marxianus DMKU 3–1042	Glucose transport	Multiple genetic mutations with Ultraviolet radiation	Efficient utilization of cellobiose in the presence of glucose		Glucose uptake was considerably reduced due to frame-shift mutations in 26-28 genes, including three genes encoding glucose transporters, yet the mutant strain's ethanol content was lower, and ethanol output was higher when mixed with the parent strains.[105]

expression of genes such as *GAL83, LAC4, SUC2, LAC12, and MAL62*.[103] A specific genetic manipulation in the yeast species K. marxianus, where the gene KmMIG1 has been disrupted, alleviated glucose repression on certain non-glucose sugars like galactose, raffinose, and sucrose.[94,104] Another study conducted by Murata et al. involving K. marxianus, UV mutagenesis, specifically at the LAC12-CEL2 cluster was used, and their findings suggest that this mutagenesis has an effect on the repression of glucose on cellobiose sugar transport.[105]

Disrupting or modifying the Mig1 gene using different techniques can reduce glucose repression on the transport and metabolism of other sugars in yeast and similar microorganisms. Mig1 is a transcriptional repressor that plays a central role in glucose repression. When glucose is present in the environment, Mig1 helps repress the expression of genes involved in the utilizing alternative sugars. For instance, the study by Nurcholis et al. is consistent with the concept of disrupting the Mig1 gene to alleviate glucose repression by utilizing other sugars in K. marxianus.[103] However, disrupting the Mig1 gene can have broader effects on central metabolic pathways, including the glycolytic pathway, in yeast and similar microorganisms. The glycolytic pathway is a central metabolic pathway that plays a crucial role in the breakdown of glucose and the production of energy (in the form of ATP) and precursor molecules for various cellular processes. Overall, overexpressing genes associated with specific non-glucose sugar transporters and simultaneously removing glucose repression by disrupting regulator-associated genes can effectively enhance the transportation, utilization, and fermentation of non-glucose sugars in microorganisms like yeast.[106,107] Moreover, genetic engineering of lactose-fermenting yeasts to enhance ethanol production from lactose is an important aspect of metabolic engineering and biotechnology aimed at improving the efficiency and sustainability of bioethanol production processes.[108]

6. Immobilization of yeasts

Yeast immobilization refers to physically confining intact yeast cells to a specific region or support matrix while preserving their biological activity. This immobilization can be achieved through various techniques and materials, including gels, membranes, beads, and other solid supports. Using immobilization methodologies in alcoholic fermentation (AF) offers several advantages over conventional free yeast cell methods, and various immobilization systems have been proposed for different applications. The utilization of natural supports (such as fruit pieces), organic supports (such as alginate), inorganic supports (such as porous ceramics), membrane systems, and multi-functional agents are among the yeast immobilization techniques that have received the most research.[109] Immobilization allows for the concentration of yeast cells in a relatively small space, resulting in high cell densities. This can lead to increased metabolic activity and higher fermentation rates, ultimately improving productivity. Moreover, Immobilized yeast systems often lead to improved product yields. The controlled environment and enhanced metabolic activity can result in higher conversion rates of substrates into desired products, such as ethanol. In addition, immobilization can act as a barrier against unwanted microbial contamination. The physical confinement of yeast cells helps protect them from competing microorganisms that might interfere with fermentation. Finally, immobilized systems offer better control over fermentation conditions. Parameters, such as temperature, pH, and nutrient levels, can be consistently maintained, leading to increased reproducibility of the fermentation process and product quality. These advantages make immobilized yeast systems highly valuable in various biotechnological and industrial applications, including bioethanol production, winemaking, brewing, and other valuable chemicals and products.[110,111]

6.1 Immobilization with classical carriers

Immobilization on a support surface refers to the attachment of yeast cells to a carrier through covalent bonds between the cell and the support or through adsorption (ionic bonds or electrostatic forces). The choice between covalent bonding and adsorption depends on the specific requirements of the immobilization process and the desired outcomes. Covalent immobilization offers high stability; however, it may be more complex and involve modification of the support surface with suitable functional groups. On the other hand, adsorption is relatively simple and may not require extensive surface modification; however, it may be less stable and may require optimization to achieve the desired level of attachment and stability.[112] Some examples of notably-known support surfaces include cellulosic materials, such as diethylamino-ethyl-cellulose (DEAE-cellulose), honorable sawdust, and wood, as well as materials like hydromica, montmorillonite, palygorskite, porous glass, and porous porcelain. DEAE-cellulose is a commonly used organic support material in biotechnology. Due to its anion-exchange properties, it is often employed for the adsorption-based immobilization of biomolecules, including yeast cells. These support surfaces provide a physical matrix for yeast cell attachment and growth. The choice of support material depends on factors such as the specific application, the desired characteristics of the immobilization system (e.g., stability, surface properties, porosity), and compatibility with the yeast strain being used. The strength of the bonding and the stability of the biofilm formed by the yeast cells can vary and may be challenging to predict. Additionally, detachment and relocation of yeast cells within the immobilization matrix can occur, particularly when the cells are actively growing.[113]

When making ethanol from whey, immobilizing chemicals such as carboxymethyl cellulose, olive pits, sodium alginate, and calcium alginate are typically employed. Cell immobilization promotes ethanol yield, productivity, and substrate use. It further decreases production costs, maintains

yeast activity stability, and increases ethanol yield. It also lowers production costs, keeps yeast activity stable, and makes it easier to separate yeast biomass from the bulk during morning processing.[114] However, limited nutrient transport, chemical and physical instability of the gel, high cell densities in the gel beads, metabolites owing to the gel matrix, non-renewability of the beads, and increasing immobilization expenses are some disadvantages of using immobilized yeasts for the generation of ethanol.[115]

Continuous fermentation offers several advantages over batch operations, especially regarding to ethanol production.[116-118] The most popular bioreactor types for producing ethanol through continuous fermentation are fluidized bed, packed bed ICR, and packed column.

6.2 Nanoparticle- and metal-organic framework (MOF) based immobilization

Nanoparticle and metal-organic framework (MOF)-based immobilization are two distinct approaches used in various fields of science and technology to anchor or immobilize specific molecules, compounds, or particles for various applications. Immobilization protects ethanol-producing yeasts and their enzymes from the inhibitory effects of intermediate metabolites such as alcohols, organic acids, and external inhibitors. It allows cells and enzymes to withstand harsher environmental conditions than free cells and enzymes. Notably, the immobilization of β-galactosidase from *Kluyveromyces* species with nanoparticles represents a significant advancement in enzyme technology. This approach enhances the reusability of the enzyme across multiple cycles while retaining a higher percentage of its original activity. By employing nanoparticles as immobilization supports, researchers have effectively addressed one of the critical challenges in enzyme applications: enzyme stability and longevity. The study conducted by Beniwal *et al.* represents a significant advancement in bioethanol production from whey through the immobilization of β-galactosidase on silicon dioxide nanoparticles and the co-immobilization of cultures of *Kluyveromyces marxianus* and *Saccharomyces cerevisiae*. This research demonstrates a successful single-stage batch process with a high bioethanol yield of 63.9 g/L, highlighting the potential for efficient and sustainable ethanol production from whey.[85] Additionally, another study conducted by Liu *et al.* is a notable contribution to the field of enzyme immobilization, focusing on β-galactosidase from *Kluyveromyces fragilis* and using magnetic poly (glycidyl methacrylate–ethylene glycol dimethacrylate–hydroxyethyl methacrylate) nanobeads with reactive epoxy groups as immobilization support. The magnetic nanobeads-immobilized biocatalyst demonstrated excellent operational stability. Retaining 81.5% of its original activity after 10 reaction cycles is a significant achievement. This stability is crucial for practical applications because it reduces the need for frequent enzyme replacement and enhances the economic viability of biocatalytic processes.[119] The encapsulation of β-D-galactosidase from *Kluyveromyces lactis* using silicon dioxide nanoparticles represents another significant advancement in enzyme immobilization technology. This approach has demonstrated several noteworthy outcomes, including the retention of enzyme activity and reduced hydrolysis time.[120] Retaining half of the initial activity of β-galactosidase after being reused 20 times when immobilized by a polysiloxane–polyvinyl alcohol magnetic (mPOS–PVA) composite is a remarkable achievement in enzyme immobilization technology.[121]

Research on immobilizing yeast cells using nanoparticles to enhance ethanol production from whey is a relatively limited but promising area. The immobilization of whole microbial cells, such as *Saccharomyces cerevisiae* with nanoparticles, offers several advantages for biotechnological applications, including bioethanol production. This highlights a significant achievement in this field, where immobilized *S. cerevisiae* cells using magnetic nanoparticles resulted in higher bioethanol production with an impressive productivity of 264 g/L/h.[122] Likewise, the immobilization of *Saccharomyces cerevisiae* with dark brown chitosan-coated manganese ferrite nanoparticles has shown notable benefits for ethanol production, including reduced fermentation time, higher ethanol titer, and increased productivity.[123] The investigation conducted by Firoozi *et al.* focusing on the immobilization of Saccharomyces cerevisiae with L-lysine-coated magnetite nanoparticles (MNPs) represents a significant contribution to biotechnological research, particularly in the context of bioethanol production. This study aimed to evaluate the performance of immobilized yeast cells in ethanol production using molasses as a carbon source. In addition to facilitating magnetic separation, amino acids such as L-lysine have been used to improve the biocompatibility, high surface activity, and lower toxicity of iron oxide nanoparticles.[124,125]

MOFs are a class of porous materials composed of metal ions or clusters coordinated with organic ligands. MOFs are characterized by their well-defined and regular crystalline structures, which give them a high degree of porosity and a huge internal surface area. This unique structure allows MOFs to hold and adsorb a wide range of compounds, making them valuable materials in various applications, including gas storage, separation, catalysis, drug delivery, and sensing. MOFs have garnered significant attention as encapsulating agents in various fields of science and technology due to their remarkable properties and versatility.[126,127]

The immobilization of various substances, particularly catalysts and biomolecules, into MOFs has gained significant attention due to its potential applications in catalysis, environmental remediation, and drug delivery.[125,126] MOFs are a class of highly porous materials constructed from metal ions or clusters coordinated with organic ligands. The porous nature and tunable properties of MOFs make them ideal host matrices for immobilization, offering protection to the immobilized molecules while enabling controlled release.

This essay will delve into the concept of immobilization in MOFs, providing insights into the common MOFs used, criteria for material selection, immobilization mechanisms, and potential applications.[126]

Common types of MOFs for immobilization. Several MOFs have demonstrated their effectiveness in immobilizing a wide range of substances. Some common examples include UiO-66, MIL-101, HKUST-1, MOF-74, etc. UiO-66, composed of zirconium nodes and organic linkers, is well-known for its exceptional chemical and thermal stability.[128] It has been used to immobilize catalytic active sites and various guest molecules, while MIL-101 features large pores and high thermal stability, making it suitable for immobilizing enzymes, nanoparticles, and drug molecules.[129] Its tunable pore size is advantageous in accommodating a variety of guest species. Comprising copper paddlewheel nodes and carboxylate linkers, HKUST-1 is known for its catalytic activity. It is often utilized to immobilize catalytic centers, making it a valuable material for heterogeneous catalysis.[130]

Criteria for choosing MOFs for immobilization. The selection of MOFs for immobilization depends on several key factors:

1. The size of the MOF's pores must be compatible with the dimensions of the substance to be immobilized. An appropriate match ensures that the guest molecules are accommodated within the MOF structure.[127,131]
2. MOFs vary in their stability and chemical resistance. Choosing an MOF with the necessary stability is crucial to maintaining the integrity of the immobilized molecules.[131]
3. Some MOFs possess specific functional groups on their ligands that can interact with guest molecules, enabling tailored immobilization.[124,131]
4. The nature of interactions between the MOF and the guest molecules, such as host-guest interactions and electrostatic forces, should be considered for successful immobilization.[131]

Immobilization Mechanisms. The immobilization of substances within MOFs can occur through various mechanisms, depending on the nature of the guest molecules and MOF structure.[127] Some common mechanisms include:

1. Adsorption: Physical trapping of guest molecules within the MOF pores. It relies on weak interactions, such as van der Waals forces and hydrogen bonding, and is reversible.
2. Coordination: In MOFs with metal nodes, coordination bonds can form between the metal centers and specific functional groups on the guest molecules. This method is often employed to immobilize catalytic species.
3. Encapsulation: Guest molecules can be completely encapsulated within the MOF structure, protecting them from the external environment. Encapsulation is particularly relevant for drug delivery applications.

Applications of immobilization in MOFs. The immobilization of substances into MOFs has a wide range of applications:

1. Catalysis: MOFs can serve as hosts for catalytic centers, providing a platform for heterogeneous catalysis. This has applications in green chemistry, industrial processes, and fuel production.[127]
2. Drug delivery: MOFs with controlled release properties are used in drug delivery systems to ensure targeted and sustained drug release for therapeutic purposes.[125,127]
3. Gas storage and separation: MOFs have been explored for gas storage and separation, particularly in hydrogen storage for fuel cell applications and carbon dioxide capture from industrial emissions.[124,127]
4. Environmental remediation: Immobilization in MOFs can facilitate the removal of heavy metals and organic pollutants from contaminated water sources.[127]

In conclusion, the immobilization of substances within MOFs presents a promising avenue for various applications. Common MOFs, such as UiO-66, MIL-101, HKUST-1, and MOF-74, provide versatile platforms for immobilization. The criteria for selecting an appropriate MOF include pore size, stability, functional groups, and guest interaction. The immobilization mechanisms can vary, including adsorption, coordination, and encapsulation. With applications spanning catalysis, drug delivery, gas storage, and environmental remediation, the field of MOFs immobilization continues to evolve, offering innovative solutions to various challenges in chemistry, materials science, and environmental sustainability.

7. Economically feasible benefits of whey in ethanol production

Small- and medium-sized dairy industries often have limited financial resources to invest in advanced technologies for processing and converting cheese whey into value-added products. They may explore cost-effective and scalable solutions to address this challenge, such as simpler fermentation and separation processes. Converting cheese whey into powder and condensed whey is a common practice, but it primarily reduces the volume and preserves the whey's nutritional components. However, these products may have limited market value compared with other value-added products, such as ethanol and organic acids.[132] The economic viability of producing ethanol from cheese whey can be influenced by several factors, including the cost of equipment, the scale of production, and the market demand for the final product. Although there may be limited studies on cheese whey-based ethanol compared with cellulosic, molasses, and starch-based ethanol, assessing its economic feasibility is essential for informed decision-making.

The low lactose content in milk whey, which typically produces only 2%-3% (v/v) ethanol, poses economic challenges for direct fermentation to ethanol. Milk whey, as a starting material, contains various components, including milk proteins, fats, minerals, and water, in addition to lactose.[24] Recent research into the economic feasibility of transforming byproducts from cheese production into bioethanol has shown that this approach can yield monthly financial gains of as much as US$ 3816.96. This is achieved through ethanol sales at a rate of US$3.02 per liter, which is hypothetically above the market price of US$2.21 per liter, while production costs decrease to US$0.81 per liter after a certain point in the

process. As a result, this not only offsets manufacturing expenses but also reduces variable costs.[133]

The primary challenge in cost-effectively producing ethanol from cheese whey lies in the whey's low lactose content. Nevertheless, when the lactose concentration is elevated to 200 g/L, it becomes possible to generate ethanol economically, even if the whey initially contains 40–50 g/L of lactose.[134] Alternatively, for economic feasibility, it is essential to utilize biogas from effluent treatment for alcohol recovery, including dehydration and distillation processes. The plant must achieve a daily ethanol production rate of 60,000 L (equivalent to 5 million gallons per year). To achieve this, the fermentable sugars in the feedstock should be at a concentration of 15% (w/v), which would allow to produce ethanol with an alcohol content ranging from 9% to 10% (v/v). Additionally, the cost of recovery processes must be reduced to below $0.2 per liter to ensure economic viability.[135] These factors contribute to increased manufacturing costs, primarily due to the need for external lactose and whey supplement with low-cost sugar sources like molasses and other industrial byproducts. However, when combining various sugars, catabolite repression can occur. This repression can potentially be mitigated through genetic engineering, especially in sugar transport systems. Furthermore, achieving a high tolerance to ethanol and osmotic pressure is essential to effectively increase the sugar content of cheese whey effectively.[24]

In summary, enhancing multiple aspects of the ethanol production process can contribute to the economic feasibility of producing ethanol from whey. These improvements include increasing the availability of fermentable sugars, optimizing yeast growth conditions, developing efficient yeast strains through traditional and metabolic engineering approaches, achieving higher ethanol concentrations, leveraging distillery waste as an additional income source to reduce production costs, and considering increased investment costs. Together, these strategies can enable cost-effective ethanol production from whey.

8. Conclusions

The main and significant waste produced during the preparation of dairy products is cheese whey. Since it possesses a high oxygen demands, releasing it into the environment unregulated causes environmental damage. The best option is to valorize cheese whey for different biological products, but this paper concentrated on the current trends of bioethanol production from whey via traditional fermentation technologies and recently developed ethanol optimization methods, such as immobilizing the microbes with MOFs and nanoparticle-coated supporting material. The study on combining biochemical pathways from different organisms to produce ethanol from biodegradable wastes, like whey, is crucial for reducing the environmental effects of food production as well as increasing the efficiency and sustainability of other industrial sectors (like the production of commercial polymers and fuel). Despite the significant advancements discussed here, whey fermentation's promise for producing renewable energy and other uses is mostly unrealized. Therefore, as developing countries adopt these cutting-edge technologies, research aimed at increasing production efficiency and energy consumption through the fermentation of agricultural byproducts like cheese whey will provide significant economic benefits while reducing energy dependence and mitigating the effects of pollution. One of the primary challenges in ethanol production from cheese whey is its low lactose content, which results in a lower ethanol titer. To economically manufacture ethanol from cheese whey, it becomes necessary to introduce inexpensive external carbon sources into the whey. However, this often results in catabolite suppression, necessitating the use of genetic engineering techniques to overcome this issue. While genetic engineering can enhance ethanol yield, it also raises the expense of distilling at the industrial level. Nevertheless, cost-effective ethanol manufacturing from cheese whey is attainable by optimizing various production processes and carefully managing investment costs. This involves a holistic approach that encompasses not only fermentation technology but also downstream processes, separation techniques, and waste management.

In conclusion, bioethanol production from cheese whey holds tremendous potential for sustainable and renewable energy generation and reduces the environmental impact of dairy waste disposal. As research continues to advance and technology adoption spreads, overcoming challenges such as low lactose content and cost-efficiency will be critical to unlock the benefits of this promising bioprocess fully.

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Conflict of Interest

There is no conflict of interest.

Supporting Information

Not applicable.

Abbreviations

AF – alcoholic fermentation
CO_2 – carbon dioxide
DEAE-cellulose – diethylamino-ethyl-cellulose
mPOS–PVA – polysiloxane–polyvinyl alcohol magnetic
MNPs – magnetite nanoparticles
MOF – Metal-Organic Frameworks
NO_x – nitrogen oxides
SO_2 – sulfur dioxide

References

[1] C. Pratt, K. Tate, Mitigating methane: emerging technologies to combat climate change's second leading contributor, *Environmental Science & Technology*, 2018, **52**, 6084-6097, doi: 10.1021/acs.est.7b04711.

[2] K. Skrable, G. Chabot, C. French, World atmospheric CO_2, its 14C specific activity, non-fossil component, anthropogenic fossil component, and emissions (1750-2018), *Health Physics*, 2022, **122**, 291-305, doi: 10.1097/hp.0000000000001485.

[3] A. El-Sayed, M. Kamel, Climatic changes and their role in emergence and re-emergence of diseases, *Environmental Science and Pollution Research*, 2020, **27**, 22336-22352, doi: 10.1007/s11356-020-08896-w.

[4] A. Zhang, A. L. Carroll, S. Atsumi, Carbon recycling by cyanobacteria: improving CO_2 fixation through chemical production, *FEMS Microbiology Letters*, 2017, **364**, fnx165, doi: 10.1093/femsle/fnx165.

[5] X. Xu, S. Nie, H. Ding, F. F. Hou, Environmental pollution and kidney diseases, *Nature Reviews Nephrology*, 2018, **14**, 313-324, doi: 10.1038/nrneph.2018.11.

[6] J. Ameeramja, E. Perumal, Pulmonary fluorosis: a review, *Environmental Science and Pollution Research*, 2017, **24**, 22119-22132, doi: 10.1007/s11356-017-9951-z.

[7] A. Bušić, N. Marđetko, S. Kundas, G. Morzak, H. Belskaya, M. Ivančić Šantek, D. Komes, S. Novak, B. Šantek, Bioethanol production from renewable raw materials and its separation and purification: a review, *Food Technology and Biotechnology*, 2018, **56**, 289-311, doi: 10.17113/ftb.56.03.18.5546.

[8] T. Falano, H. K. Jeswani, A. Azapagic, Assessing the environmental sustainability of ethanol from integrated biorefineries, *Biotechnology Journal*, 2014, **9**, 753-765, doi: 10.1002/biot.201300246.

[9] Goldy, D. Bhowmick, Lignocellulosic biorefinery as a model for sustainable development of biofuels and value added products, *Bioresource Technology*, 2018, **247**, 1144-1154, doi: 10.1016/j.biortech.2017.09.163.

[10] R. K. Prasad, S. Chatterjee, P. B. Mazumder, S. K. Gupta, S. Sharma, M. G. Vairale, S. Datta, S. K. Dwivedi, D. K. Gupta, Bioethanol production from waste lignocelluloses: a review on microbial degradation potential, *Chemosphere*, 2019, **231**, 588-606, doi: 10.1016/j.chemosphere.2019.05.142.

[11] S. Shah, V. Venkatramanan, R. Prasad, Sustainable Green Technologies for Environmental Management. Singapore: Springer Singapore, 2019, doi: https://doi.org/10.1007/978-981-13-2772-8.

[12] V. Venkatramanan, S. Shah, R. Prasad, Global Climate Change and Environmental Policy: Agriculture Perspectives. Singapore: Springer Singapore, 2020, doi: https://doi.org/10.1007/978-981-13-9570-3.

[13] V. Venkatramanan, S. Shah, R. Prasad, Exploring Synergies and Trade-offs between Climate Change and the Sustainable Development Goals. Singapore: Springer Singapore, 2021, doi: 10.1007/978-981-15-7301-9.

[14] D. Zolotareva, A. Zazybin, Y. Belyankova, A. Dauletbakov, S. Tursynbek, K. Rafikova, A. Ten, V. Yu, S. Bayazit, A. Basharimova, M. Aydemir, Increasing sugar content in source for biofuel production using agrochemical and genetic approaches at the stages of BioMass preharvesting and harvesting, *Molecules*, 2022, **27**, 5210, doi: 10.3390/molecules27165210.

[15] S. G. Karp, J. D. C. Medina, L. A. J. Letti, A. L. Woiciechowski, J. C. de Carvalho, C. C. Schmitt, R. de Oliveira Penha, G. S. Kumlehn, C. R. Soccol, Bioeconomy and biofuels: the case of sugarcane ethanol in Brazil, *Biofuels, Bioproducts and Biorefining*, 2021, **15**, 899-912, doi: 10.1002/bbb.2195.

[16] A. Tosi, Furtado, Of actors, functions, and fuels: exploring a second generation ethanol transition from a technological innovation systems perspective in Brazil, *Energy Research & Social Science*, 2020, **70**, 101706, doi: 10.1016/j.erss.2020.101706.

[17] J. K. Saini, R. Saini, L. Tewari, Lignocellulosic agriculture wastes as biomass feedstocks for second-generation bioethanol production: concepts and recent developments, *3 Biotech*, 2015, **5**, 337-353, doi: 10.1007/s13205-014-0246-5.

[18] A. Y. Krylova, E. A. Kozyukov, A. L. Lapidus, Ethanol and diesel fuel from plant raw materials: a review, *Solid Fuel Chemistry*, 2008, **42**, 358-364, doi: 10.3103/s0361521908060062.

[19] N. Phukoetphim, A. Salakkam, P. Laopaiboon, L. Laopaiboon, Kinetic models for batch ethanol production from sweet sorghum juice under normal and high gravity fermentations: Logistic and modified Gompertz models, *Journal of Biotechnology*, 2017, **243**, 69-75, doi: 10.1016/j.jbiotec.2016.12.012.

[20] A. Pandey, R. Höfer, M. Taherzadeh, K. M. Nampoothiri, C. Larroche, Preface. Industrial Biorefineries & White Biotechnology. Amsterdam: Elsevier, 1st Edition - May 8, 2015, doi: 10.1016/b978-0-444-63453-5.05001-1.

[21] C. Onwulata, P. Huth, Whey Processing, Functionality and Health Benefits. New York: John Wiley & Sons, 2009.

[22] M. Peplow, Cellulosic ethanol fights for life, *Nature*, 2014, **507**, 152-153, doi: 10.1038/507152a.

[23] R. Van Noorden, EU debates U-turn on biofuels policy, *Nature*, 2013, **499**, 13-14, doi: 10.1038/499013a.

[24] J. Zou, X. Chang, Past, present, and future perspectives on whey as a promising feedstock for bioethanol production by yeast, *Journal of Fungi*, 2022, **8**, 395, doi: 10.3390/jof8040395.

[25] Z. Salman, O. Mohammad, Ethanol production from crude whey by *Kluyveromyces marxianus*, *Biochemical Engineering Journal*, 2006, **27**, 295-298, doi: 10.1016/j.bej.2005.05.009.

[26] J. S. S. Yadav, J. Bezawada, C. M. Ajila, S. Yan, R. D. Tyagi, R. Y. Surampalli, Mixed culture of *Kluyveromyces marxianus* and Candida krusei for single-cell protein production and organic load removal from whey, *Bioresource Technology*, 2014, **164**, 119-127, doi: 10.1016/j.biortech.2014.04.069.

[27] D. Borovikova, R. Scherbaka, A. Patmalnieks, A. Rapoport, Effects of yeast immobilization on bioethanol production, *Biotechnology and Applied Biochemistry*, 2014, **61**, 33-39, doi: 10.1002/bab.1158.

[28] T. Sar, B. C. Stark, M. Yesilcimen Akbas, Effective ethanol production from whey powder through immobilized *E. Coli* expressing *Vitreoscilla* hemoglobin, *Bioengineered*, 2017, **8**, 171-

181, doi: 10.1080/21655979.2016.1218581.
[29] Z. Fang, J. Lyu, J. Li, C. Li, Y. Zhang, Y. Guo, Y. Wang, Y. Zhang, K. Chen, Application of bioreactor technology for cell culture-based viral vaccine production: present status and future prospects, *Frontiers in Bioengineering and Biotechnology*, 2022, **10**, 921755, doi: 10.3389/fbioe.2022.921755.
[30] J. I. Betts, F. Baganz, Miniature bioreactors: current practices and future opportunities, *Microbial Cell Factories*, 2006, **5**, 21, doi: 10.1186/1475-2859-5-21.
[31] A. Demirbas, Biorefineries: for biomass upgrading facilities. Green Energy and Technology, London: Springer, 2009.
[32] V. M. Pathak, Navneet, Handbook of Research on Microbial Tools for Environmental Waste Management, Noted as an IGI Global Core Reference Title in Environment & Agriculture for 2019, doi: 10.4018/978-1-5225-3540-9.
[33] M. B. Sticklen, Feedstock crop genetic engineering for alcohol fuels, *Crop Science*, 2007, **47**, 2238-2248, doi: 10.2135/cropsci2007.04.0212.
[34] B. D. Solomon, J. R. Barnes, K. E. Halvorsen, Grain and cellulosic ethanol: history, economics, and energy policy, *Biomass and Bioenergy*, 2007, **31**, 416-425, doi: 10.1016/j.biombioe.2007.01.023.
[35] M. Balat, H. Balat, Recent trends in global production and utilization of bio-ethanol fuel, *Applied Energy*, 2009, **86**, 2273-2282, doi: 10.1016/j.apenergy.2009.03.015.
[36] E. Gnansounou, A. Dauriat, Ethanol fuel from biomass: a review, *Journal of Scientific & Industrial Research*, 2005, **64**, 809-821.
[37] F. Rosillo-Calle, A. Walter, Global market for bioethanol: historical trends and future prospects, *Energy for Sustainable Development*, 2006, **10**, 20-32, doi: 10.1016/S0973-0826(08)60504-9.
[38] H. C. Bagheri, E. Espi, M. Teresa Larre Cacho, Fuel ethanol at a key economic and technological junction in the USA, *SSRN Electronic Journal*, 2016, doi: 10.2139/ssrn.2789612.
[39] V. Sharma, A review on current technological advancement of lignocellulosic bioethanol production, *Journal of Applied Biotechnology & Bioengineering*, 2016, **1**, 61-66, doi: 10.15406/jabb.2016.01.00011.
[40] P. Cavelius, S. Engelhart-Straub, N. Mehlmer, J. Lercher, D. Awad, T. Brück, The potential of biofuels from first to fourth generation, *PLoS Biology*, 2023, **21**, e3002063, doi: 10.1371/journal.pbio.3002063.
[41] F. Asunis, G. De Gioannis, P. Dessì, M. Isipato, P. N. L. Lens, A. Muntoni, A. Polettini, R. Pomi, A. Rossi, D. Spiga, The dairy biorefinery: integrating treatment processes for cheese whey valorisation, *Journal of Environmental Management*, 2020, **276**, 111240, doi: 10.1016/j.jenvman.2020.111240.
[42] I. M. Banat, D. Singh, R. Marchant, The use of a thermotolerant fermentative *Kluyveromyces marxianus* IMB3 yeast strain for ethanol production, *Acta Biotechnologica*, 1996, **16**, 215-223, doi: 10.1002/abio.370160223.
[43] A. Poladyan, K. Trchounian, A. Paloyan, E. Minasyan, H. Aghekyan, M. Iskandaryan, L. Khoyetsyan, S. Aghayan, A. Tsaturyan, G. Antranikian, Valorization of whey-based side streams for microbial biomass, molecular hydrogen, and hydrogenase production, *Applied Microbiology and Biotechnology*, 2023, **107**, 4683-4696, doi: 10.1007/s00253-023-12609-x.
[44] D. Ariyanti, H. Hadiyanto, Ethanol production from whey by *Kluyveromyces marxianus* in batch fermentation system: kinetics parameters estimation, *Bulletin of Chemical Reaction Engineering & Catalysis*, 2013, **7**, 179-184, doi: 10.9767/bcrec.7.2.4044.179-184.
[45] M. Pescuma, G. F. Valdez, F. Mozzi, Whey-derived valuable products obtained by microbial fermentation, *Applied Microbiology and Biotechnology*, 2015, **99**, 6183-6196, doi: 10.1007/s00253-015-6766-z.
[46] A. Deen Christensen, Z. Kádár, P. Oleskowicz-Popiel, M. H. Thomsen, Production of bioethanol from organic whey using *Kluyveromyces marxianus*, *Journal of Industrial Microbiology & Biotechnology*, 2011, **38**, 283-289, doi: 10.1007/s10295-010-0771-0.
[47] A. Tesfaw, D. Kosebent, E. T. Oner, F. Assefa, Bioethanol production from grass pea and wild oat hydrolysates using S. cerevisiae ETP53, K. marxianus ETP87, and P. fermentans ETP22, *Discover Energy*, 2021, **1**, 1-12, doi: 10.1007/s43937-021-00005-3.
[48] A. Karim, M. Aider, Comprehensive utilisation of electro-activated whey-based media in cell growth, metabolite production and aroma compounds synthesis using a starter culture originated from kefir grains, *International Dairy Journal*, 2022, **126**, 105276, doi: 10.1016/j.idairyj.2021.105276.
[49] T. Tavares, F. X. Malcata, Whey and whey powders: fermentation of whey. Encyclopedia of Food and Health. Amsterdam: Elsevier, 2016: 486-492, doi: 10.1016/b978-0-12-384947-2.00749-2.
[50] M. Das, A. Raychaudhuri, S. K. Ghosh, Supply chain of bioethanol production from whey: a review, *Procedia Environmental Sciences*, 2016, **35**, 833-846, doi: 10.1016/j.proenv.2016.07.100.
[51] Xin, Zhou, Bio-utilization of cheese manufacturing wastes (cheese whey powder) for bioethanol and specific product (galactonic acid) production via a two-step bioprocess, *Bioresource Technology*, 2019, **272**, 70-76, doi: 10.1016/j.biortech.2018.10.001.
[52] A. Karim, N. Gerliani, M. Aïder, *Kluyveromyces marxianus*: an emerging yeast cell factory for applications in food and biotechnology, *International Journal of Food Microbiology*, 2020, **333**, 108818, doi: 10.1016/j.ijfoodmicro.2020.108818.
[53] A. Tesfaw, E. T. Oner, F. Assefa, Evaluating crude whey for bioethanol production using non-Saccharomyces yeast, *Kluyveromyces marxianus*, *SN Applied Sciences*, 2021, **3**, 1-8, doi: 10.1007/s42452-020-03996-1.
[54] C. Iraporda, D. E. Romanin, M. Rumbo, G. L. Garrote, A. G. Abraham, The role of lactate on the immunomodulatory properties of the nonbacterial fraction of kefir, *Food Research International*, 2014, **62**, 247-253, doi: 10.1016/j.foodres.2014.03.003.

[55] G. Dragone, S. I. Mussatto, J. B. Almeida e Silva, J. A. Teixeira, Optimal fermentation conditions for maximizing the ethanol production by Kluyveromyces fragilis from cheese whey powder, *Biomass and Bioenergy*, 2011, **35**, 1977-1982, doi: 10.1016/j.biombioe.2011.01.045.
[56] M. Bilal, L. Ji, Y. Xu, S. Xu, Y. Lin, H. M. N. Iqbal, H. Cheng, Bioprospecting *Kluyveromyces marxianus* as a robust host for industrial biotechnology, *Frontiers in Bioengineering and Biotechnology*, 2022, **10**, 851768, doi: 10.3389/fbioe.2022.851768.
[57] T. Ikegami, T. Morita, S. Nakayama, H. Negishi, D. Kitamoto, K. Sakaki, Y. Oumi, T. Sano, K. Haraya, H. Yanagishita, Processing of ethanol fermentation broths by *Candida krusei* to separate bioethanol by pervaporation using silicone rubber-coated silicalite membranes, *Journal of Chemical Technology & Biotechnology*, 2009, **84**, 1172-1177, doi: 10.1002/jctb.2151.
[58] D. Azzouni, A. Lahkimi, B. Louaste, M. Taleb, M. Chaouch, N. Eloutassi, Performance of aspergillus Niger and *Kluyveromyces marxianus* for optimized bioethanol production from dairy waste. Advances in Intelligent Systems and Computing. Cham: Springer International Publishing, 2019: 162-175, doi: 10.1007/978-3-030-11881-5_14.
[59] C. Farkas, J. M. Rezessy-Szabó, V. K. Gupta, E. Bujna, T. M. Pham, K. Pásztor-Huszár, L. Friedrich, R. Bhat, V. K. Thakur, Q. D. Nguyen, Batch and fed-batch ethanol fermentation of cheese-whey powder with mixed cultures of different yeasts, *Energies*, 2019, **12**, 4495, doi: 10.3390/en12234495.
[60] H. Hoshida, R. Akada, High-temperature bioethanol fermentation by conventional and nonconventional yeasts. Biotechnology of Yeasts and Filamentous Fungi. Cham: Springer International Publishing, 2017: 39-61, doi: 10.1007/978-3-319-58829-2_2.
[61] F. Roohina, M. Mohammadi, G. D. Najafpour, Immobilized *Kluyveromyces marxianus* cells in carboxymethyl cellulose for production of ethanol from cheese whey: experimental and kinetic studies, *Bioprocess and Biosystems Engineering*, 2016, **39**, 1341-1349, doi: 10.1007/s00449-016-1610-0.
[62] T. Zotta, L. Solieri, L. Iacumin, C. Picozzi, M. Gullo, Valorization of cheese whey using microbial fermentations, *Applied Microbiology and Biotechnology*, 2020, **104**, 2749-2764, doi: 10.1007/s00253-020-10408-2.
[63] C. Teixeira de Carvalho, S. Dantas de Oliveira Jr, W. B. de Brito Lima, F. G. Macêdo de Medeiros, A. Laura Oliveira de Sá Leitão, E. S. dos Santos, G. R. de Macedo, F. C. de Sousa Jr, Potential of "coalho" cheese whey as lactose source for β-galactosidase and ethanol co-production by *Kluyveromyces* spp. yeasts, *Preparative Biochemistry & Biotechnology*, 2020, **50**, 925-934, doi: 10.1080/10826068.2020.1771731.
[64] B. W, Silveira, Ethanol production from cheese whey permeate by *Kluyveromyces marxianus* UFV-3: a flux analysis of oxido-reductive metabolism as a function of lactose concentration and oxygen levels, *Enzyme and Microbial Technology*, 2005, **36**, 930-936, doi: 10.1016/j.enzmictec.2005.01.018.
[65] J. M. Rocha, A. Guerra, On the valorization of lactose and its derivatives from cheese whey as a dairy industry by-product: an overview, *European Food Research and Technology*, 2020, **246**, 2161-2174, doi: 10.1007/s00217-020-03580-2.
[66] M. M. Lane, N. Burke, R. Karreman, K. H. Wolfe, C. P. O'Byrne, J. P. Morrissey, Physiological and metabolic diversity in the yeast *Kluyveromyces marxianus*, *Antonie Van Leeuwenhoek*, 2011, **100**, 507-519, doi: 10.1007/s10482-011-9606-x.
[67] D. Mehri, N. Altinay Perendeci, Y. Goksungur, Utilization of whey for red pigment production by monascus purpureus in submerged fermentation, *Fermentation*, 2021, **7**, 75, doi: 10.3390/fermentation7020075.
[68] M. Y. Akbas, B. C. Stark, Recent trends in bioethanol production from food processing byproducts, *Journal of Industrial Microbiology and Biotechnology*, 2016, **43**, 1593-1609, doi: 10.1007/s10295-016-1821-z.
[69] X. Yuan, X. Chen, G. Shen, S. Chen, J. Yu, R. Zhai, Z. Xu, M. Jin, Densifying lignocellulosic biomass with sulfuric acid provides a durable feedstock with high digestibility and high fermentability for cellulosic ethanol production, *Renewable Energy*, 2022, **182**, 377-389, doi: 10.1016/j.renene.2021.10.015.
[70] A. Devi, A. Singh, S. Bajar, D. Pant, Z.U. Din, Ethanol from lignocellulosic biomass: an in-depth analysis of pre-treatment methods, fermentation approaches and detoxification processes, *Journal of Environmental Chemical Engineering*, 2021, **9**, 105798, doi: 10.1016/j.jece.2021.105798.
[71] T. Su, D. Zhao, M. Khodadadi, C. Len, Lignocellulosic biomass for bioethanol: recent advances, technology trends, and barriers to industrial development, *Current Opinion in Green and Sustainable Chemistry*, 2020, **24**, 56-60, doi: 10.1016/j.cogsc.2020.04.005.
[72] H. Park, D. Jeong, M. Shin, S. Kwak, E. J. Oh, J. K. Ko, S. R. Kim, Xylose utilization in Saccharomyces cerevisiae during conversion of hydrothermally pretreated lignocellulosic biomass to ethanol, *Applied Microbiology and Biotechnology*, 2020, **104**, 3245-3252, doi: 10.1007/s00253-020-10427-z.
[73] N. Priharto, F. Ronsse, G. Yildiz, H. J. Heeres, P. J. Deuss, W. Prins, Fast pyrolysis with fractional condensation of lignin-rich digested stillage from second-generation bioethanol production, *Journal of Analytical and Applied Pyrolysis*, 2020, **145**, 104756, doi: 10.1016/j.jaap.2019.104756.
[74] J. Zhang, J. Liu, L. Kou, X. Zhang, T. Tan, Bioethanol production from cellulose obtained from the catalytic hydro-deoxygenation (lignin-first refined to aviation fuel) of apple wood, *Fuel*, 2019, **250**, 245-253, doi: 10.1016/j.fuel.2019.03.020.
[75] S. Rezania, B. Oryani, J. Cho, A. Talaiekhozani, F. Sabbagh, B. Hashemi, P. F. Rupani, A. A. Mohammadi, Different pretreatment technologies of lignocellulosic biomass for bioethanol production: an overview, *Energy*, 2020, **199**, 117457, doi: 10.1016/j.energy.2020.117457.
[76] J. Chen, B. Zhang, L. Luo, F. Zhang, Y. Yi, Y. Shan, B. Liu, Y. Zhou, X. Wang, X. Lü, A review on recycling techniques for bioethanol production from lignocellulosic biomass, *Renewable*

and Sustainable Energy Reviews, 2021, **149**, 111370, doi: 10.1016/j.rser.2021.111370.
[77] D. Moysés, V. Reis, J. Almeida, L. Moraes, F. Torres, Xylose fermentation by saccharomyces cerevisiae: challenges and prospects, *International Journal of Molecular Sciences*, 2016, **17**, 207, doi: 10.3390/ijms17030207.
[78] J. T. Cunha, P. O. Soares, A. Romaní, J. M. Thevelein, L. Domingues, Xylose fermentation efficiency of industrial Saccharomyces cerevisiae yeast with separate or combined xylose reductase/xylitol dehydrogenase and xylose isomerase pathways, *Biotechnology for Biofuels*, 2019, **12**, 1-14, doi: 10.1186/s13068-019-1360-8.
[79] G. M. Martins, D. Alonso Bocchini-Martins, C. Bezzerra-Bussoli, F. C. Pagnocca, M. Boscolo, D. Alves Monteiro, R. da Silva, E. Gomes, The isolation of pentose-assimilating yeasts and their xylose fermentation potential, *Brazilian Journal of Microbiology*, 2018, **49**, 162-168, doi: 10.1016/j.bjm.2016.11.014.
[80] N. Phaiboonsilpa, T. Chysirichote, V. Champreda, N. Laosiripojana, Fermentation of xylose, Arabinose, glucose, their mixtures and sugarcane bagasse hydrolyzate by yeast Pichia stipitis for ethanol production, *Energy Reports*, 2020, **6**, 710-713, doi: 10.1016/j.egyr.2019.11.142.
[81] S. R. Macwan, B. K. Dabhi, S. C. Parmar, K. D. Aparnathi, Whey and its utilization, *International Journal of Current Microbiology and Applied Sciences*, 2016, **5**, 134-155, doi: 10.20546/ijcmas.2016.508.016.
[82] S. Sansonetti, S. Curcio, V. Calabrò, G. Iorio, Bio-ethanol production by fermentation of ricotta cheese whey as an effective alternative non-vegetable source, *Biomass and Bioenergy*, 2009, **33**, 1687-1692, doi: 10.1016/j.biombioe.2009.09.002.
[83] M. Koushki, M. Jafari, M. Azizi, Comparison of ethanol production from cheese whey permeate by two yeast strains, *Journal of Food Science and Technology*, 2012, **49**, 614-619, doi: 10.1007/s13197-011-0309-0.
[84] S. H. Mohd Azhar, R. Abdulla, S. A. Jambo, H. Marbawi, J. A. Gansau, A. A. Mohd Faik, K. F. Rodrigues, Yeasts in sustainable bioethanol production: a review, *Biochemistry and Biophysics Reports*, 2017, **10**, 52-61, doi: 10.1016/j.bbrep.2017.03.003.
[85] A. Beniwal, P. Saini, A. Kokkiligadda, S. Vij, Physiological growth and galactose utilization by dairy yeast *Kluyveromyces marxianus* in mixed sugars and whey during fermentation, *3 Biotech*, 2017, **7**, 1-13, doi: 10.1007/s13205-017-0985-1.
[86] E. Demiray, S. E. Karatay, S. Dönmez, G. Dönmez, The usage of carrot pomace for bioethanol production, *Journal of the Chilean Chemical Society*, 2016, **61**, 2996-2998, doi: 10.4067/S0717-97072016000200029.
[87] P. G. Ferreira, F. A. Silveira, R. C. V. Santos, H. Leonardo André Genier, R. H. Santos Diniz, J. I. Ribeiro, L. G. Fietto, F. M. L. Passos, W. B. Silveira, Optimizing ethanol production by thermotolerant *Kluyveromyces marxianus* CCT 7735 in a mixture of sugarcane bagasse and ricotta whey, *Food Science and Biotechnology*, 2015, **24**, 1421-1427, doi: 10.1007/s10068-015-0182-0.
[88] A. E. Mahmoud, S. A. Fathy, M. M. Rashad, M. K. Ezz, A. T. Mohammed, Purification and characterization of a novel tannase produced by *Kluyveromyces marxianus* using olive pomace as solid support, and its promising role in Gallic acid production, *International Journal of Biological Macromolecules*, 2018, **107**, 2342-2350, doi: 10.1016/j.ijbiomac.2017.10.117.
[89] J.-S. Kim, J.-B. Park, S.-W. Jang, S.-J. Ha, Enhanced xylitol production by mutant *Kluyveromyces marxianus* 36907-FMEL1 due to improved xylose reductase activity, *Applied Biochemistry and Biotechnology*, 2015, **176**, 1975-1984, doi: 10.1007/s12010-015-1694-z.
[90] S.-B. Kim, D.-H. Kwon, J.-B. Park, S.-J. Ha, Alleviation of catabolite repression in *Kluyveromyces marxianus*: the thermotolerant SBK_1 mutant simultaneously coferments glucose and xylose, *Biotechnology for Biofuels*, 2019, **12**, 1-9, doi: 10.1186/s13068-019-1431-x.
[91] N. W. Y. Ho, Z. Chen, A. P. Brainard, Genetically engineered *Saccharomyces* yeast capable of effective cofermentation of glucose and xylose, *Applied and Environmental Microbiology*, 1998, **64**, 1852-1859, doi: 10.1128/aem.64.5.1852-1859.1998.
[92] N. W. Y. Ho, Z. Chen, A. P. Brainard, M. Sedlak, Successful design and development of genetically engineered saccharomyces yeasts for effective cofermentation of glucose and xylose from cellulosic biomass to fuel ethanol. Recent Progress in Bioconversion of Lignocellulosics. Berlin, Heidelberg: Springer Berlin Heidelberg, 1999: 163-192, doi: 10.1007/3-540-49194-5_7.
[93] M. Kuyper, M. Toirkens, J. Diderich, A. Winkler, J. Vandijken, J. Pronk, Evolutionary engineering of mixed-sugar utilization by a xylose-fermenting strain, *FEMS Yeast Research*, 2005, **5**, 925-934, doi: 10.1016/j.femsyr.2005.04.004.
[94] Y. Hua, J. Wang, Y. Zhu, B. Zhang, X. Kong, W. Li, D. Wang, J. Hong, Release of glucose repression on xylose utilization in *Kluyveromyces marxianus* to enhance glucose-xylose co-utilization and xylitol production from corncob hydrolysate, *Microbial Cell Factories*, 2019, **18**, 1-18, doi: 10.1186/s12934-019-1068-2.
[95] X. Hou, Anaerobic xylose fermentation by spathaspora passalidarum, *Applied Microbiology and Biotechnology*, 2012, **94**, 205-214, doi: 10.1007/s00253-011-3694-4.
[96] Y.-K. Su, L. B. Willis, T. W. Jeffries, Effects of aeration on growth, ethanol and polyol accumulation by *Spathaspora passalidarum* NRRL Y-27907 and *Scheffersomyces stipitis* NRRL Y-7124, *Biotechnology and Bioengineering*, 2015, **112**, 457-469, doi: 10.1002/bit.25445.
[97] N. K. Harner, X. Wen, P. K. Bajwa, G. D. Austin, C.-Y. Ho, M. B. Habash, J. T. Trevors, H. Lee, Genetic improvement of native xylose-fermenting yeasts for ethanol production, *Journal of Industrial Microbiology & Biotechnology*, 2015, **42**, 1-20, doi: 10.1007/s10295-014-1535-z.
[98] M. I. González-Siso, A. Touriño, Á. Vizoso, Á. Pereira-Rodríguez, E. Rodríguez-Belmonte, M. Becerra, M. E. Cerdán, Improved bioethanol production in an engineered *Kluyveromyces lactis* strain shifted from respiratory to fermentative metabolism

by deletion of *NDI1*, *Microbial Biotechnology*, 2015, **8**, 319-330, doi: 10.1111/1751-7915.12160.
[99] W. J. C. Dekker, R. A. Ortiz-Merino, A. Kaljouw, J. Battjes, F. W. Wiering, C. Mooiman, P. de la Torre, J. T. Pronk, Engineering the thermotolerant industrial yeast *Kluyveromyces marxianus* for anaerobic growth, *Metabolic Engineering*, 2021, **67**, 347-364, doi: 10.1016/j.ymben.2021.07.006.
[100] P. Li, X. Fu, S. Li, L. Zhang, Engineering TATA-binding protein Spt15 to improve ethanol tolerance and production in *Kluyveromyces marxianus*, *Biotechnology for Biofuels*, 2018, **11**, 1-13, doi: 10.1186/s13068-018-1206-9.
[101] W. Mo, M. Wang, R. Zhan, Y. Yu, Y. He, H. Lu, *Kluyveromyces marxianus* developing ethanol tolerance during adaptive evolution with significant improvements of multiple pathways, *Biotechnology for Biofuels*, 2019, **12**, 1-15, doi: 10.1186/s13068-019-1393-z.
[102] D.-H. Kwon, S.-B. Kim, J.-B. Park, S.-J. Ha, Overexpression of mutant galactose permease (*ScGal2*_N376F) effective for utilization of glucose/xylose or glucose/galactose mixture by engineered *Kluyveromyces marxianus*, *Journal of Microbiology and Biotechnology*, 2020, **30**, 1944-1949, doi: 10.4014/jmb.2008.08035.
[103] M. Nurcholis, S. Nitiyon, Suprayogi, N. Rodrussamee, N. Lertwattanasakul, S. Limtong, T. Kosaka, M. Yamada, Functional analysis of Mig1 and Rag5 as expressional regulators in thermotolerant yeast *Kluyveromyces marxianus*, *Applied Microbiology and Biotechnology*, 2019, **103**, 395-410, doi: 10.1007/s00253-018-9462-y.
[104] Marlene, Baptista, *Kluyveromyces marxianus* as a microbial cell factory for lignocellulosic biomass valorisation, *Biotechnology Advances*, 2022, **60**, 108027, doi: 10.1016/j.biotechadv.2022.108027.
[105] M. Murata, S. Pattanakittivorakul, T. Manabe, S. Limtong, M. Yamada, Mutants with enhanced cellobiose-fermenting ability from thermotolerant *Kluyveromyces marxianus* DMKU 3-1042, which are beneficial for fermentation with cellulosic biomass, *Fuels*, 2022, **3**, 232-244, doi: 10.3390/fuels3020015.
[106] F. Zoppellari, L. Bardi, Production of bioethanol from effluents of the dairy industry by *Kluyveromyces marxianus*, *New Biotechnology*, 2013, **30**, 607-613, doi: 10.1016/j.nbt.2012.11.017.
[107] A. Deen Christensen, Z. Kádár, P. Oleskowicz-Popiel, M. H. Thomsen, Production of bioethanol from organic whey using *Kluyveromyces marxianus*, *Journal of Industrial Microbiology & Biotechnology*, 2011, **38**, 283-289, doi: 10.1007/s10295-010-0771-0.
[108] A. M. Ohstrom, A. Elizabeth Buck, X. Du, J. Wee, Evaluation of Kluyveromyces spp. for conversion of lactose in different types of whey from dairy processing waste into ethanol, *Frontiers in Microbiology*, 2023, **14**, 1208284, doi: 10.3389/fmicb.2023.1208284.
[109] F. Roohina, M. Mohammadi, G. D. Najafpour, Immobilized *Kluyveromyces marxianus* cells in carboxymethyl cellulose for production of ethanol from cheese whey: experimental and kinetic studies, *Bioprocess and Biosystems Engineering*, 2016, **39**, 1341-1349, doi: 10.1007/s00449-016-1610-0.
[110] M. I. González-Siso, A. Touriño, Á. Vizoso, Á. Pereira-Rodríguez, E. Rodríguez-Belmonte, M. Becerra, M. E. Cerdán, Improved bioethanol production in an engineered *Kluyveromyces lactis* strain shifted from respiratory to fermentative metabolism by deletion of *NDI1*, *Microbial Biotechnology*, 2015, **8**, 319-330, doi: 10.1111/1751-7915.12160.
[111] B. Rodrigues, M. E. Lima-Costa, A. Constantino, S. Raposo, C. Felizardo, D. Gonçalves, T. Fernandes, L. Dionísio, J. M. Peinado, Growth kinetics and physiological behavior of co-cultures of Saccharomyces cerevisiae and Kluyveromyces lactis, fermenting carob sugars extracted with whey, *Enzyme and Microbial Technology*, 2016, **92**, 41-48, doi: 10.1016/j.enzmictec.2016.06.012.
[112] G. L. Utama, T. B. A. Kurnani, S. Sunardi, M. F. Cahyandito, R. L. Balia, Joint cost allocation of cheese-making wastes bioconversions into ethanol and organic liquid fertilizer Bulgarian, *Journal of Agricultural Science*, 2017, **23**, 1016–1020, doi: 10.1371/journal.pone.0277134.
[113] D. M. Ha-Tran, R.-Y. Lai, T. T. M. Nguyen, E. Huang, S.-C. Lo, C.-C. Huang, Construction of engineered RuBisCO *Kluyveromyces marxianus* for a dual microbial bioethanol production system, *PLoS One*, 2021, **16**, e0247135, doi: 10.1371/journal.pone.0247135.
[114] A. Tesfaw, F. Assefa, Current trends in bioethanol production by *Saccharomyces cerevisiae*: substrate, inhibitor reduction, growth variables, coculture, and immobilization, *International Scholarly Research Notices*, 2014, **2014**, 1-11, doi: 10.1155/2014/532852.
[115] A. Deen Christensen, Z. Kádár, P. Oleskowicz-Popiel, M. H. Thomsen, Production of bioethanol from organic whey using *Kluyveromyces marxianus*, *Journal of Industrial Microbiology & Biotechnology*, 2011, **38**, 283-289, doi: 10.1007/s10295-010-0771-0.
[116] Y.-H R. Hung, M. Chae, D. Sauvageau, D. C. Bressler, Adapted feeding strategies in fed-batch fermentation improve sugar delivery and ethanol productivity, *Bioengineered*, 2023, **14**, 2250950, doi: 10.1080/21655979.2023.2250950.
[117] S. Gabardo, R. Rech, M. A. Z. Ayub, Performance of different immobilized-cell systems to efficiently produce ethanol from whey: fluidized batch, packed-bed and fluidized continuous bioreactors, *Journal of Chemical Technology & Biotechnology*, 2012, **87**, 1194-1201, doi: 10.1002/jctb.3749.
[118] S. Gabardo, G. F. Pereira, M. P. Klein, R. Rech, P. F. Hertz, M. A. Z. Ayub, Dynamics of yeast immobilized-cell fluidized-bed bioreactors systems in ethanol fermentation from lactose-hydrolyzed whey and whey permeate, *Bioprocess and Biosystems Engineering*, 2016, **39**, 141-150, doi: 10.1007/s00449-015-1498-0.
[119] H. Liu, J. Liu, B. Tan, F. Zhou, Y. Qin, R. Yang, Covalent immobilization of *Kluyveromyces fragilis* β-galactosidase on magnetic nanosized epoxy support for synthesis of galacto-oligosaccharide, *Bioprocess and Biosystems Engineering*, 2012, **35**, 1287-1295, doi: 10.1007/s00449-012-0716-2.

[120] M. Lal Verma, C. J. Barrow, J. F. Kennedy, M. Puri, Immobilization of β-d-galactosidase from *Kluyveromyces lactis* on functionalized silicon dioxide nanoparticles: characterization and lactose hydrolysis, *International Journal of Biological Macromolecules*, 2012, **50**, 432-437, doi: 10.1016/j.ijbiomac.2011.12.029.
[121] D. F. M. Neri, V. M. Balcão, M. G. Carneiro-da-Cunha, L. B. Carvalho Jr, J. A. Teixeira, Immobilization of β-galactosidase from *Kluyveromyces lactis* onto a polysiloxane-polyvinyl alcohol magnetic (mPOS-PVA) composite for lactose hydrolysis, *Catalysis Communications*, 2008, **9**, 2334-2339, doi: 10.1016/j.catcom.2008.05.022.
[122] P. T. Sekoai, C. N. M. Ouma, S. P. du Preez, P. Modisha, N. Engelbrecht, D. G. Bessarabov, A. Ghimire, Application of nanoparticles in biofuels: an overview, *Fuel*, 2019, **237**, 380-397, doi: 10.1016/j.fuel.2018.10.030.
[123] A. Núñez Caraballo, A. Iliná, R. Ramos González, C. N. Aguilar, G. Michelena Álvarez, A. C. Flores Gallegos, J. Sandoval-Cortés, M. A. Aguilar-Gonzalez, N. O. Soto-Cruz, J. Daniel García García, J. L. Martínez-Hernández, Sustainable ethanol production from sugarcane molasses by *Saccharomyces cerevisiae* immobilized on chitosan-coated Manganese ferrite, *Frontiers in Sustainable Food Systems*, 2021, **5**, 683170, doi: 10.3389/fsufs.2021.683170.
[124] A. Isaac, Sanusi, Impact of nanoparticle inclusion on bioethanol production process kinetic and inhibitor profile, *Biotechnology Reports*, 2021, **29**, e00585, doi: 10.1016/j.btre.2021.e00585.
[125] D. Verma, J. S. Paul, S. Tiwari, S. K. Jadhav, A review on role of nanomaterials in bioconversion of sustainable fuel bioethanol, *Waste and Biomass Valorization*, 2022, **13**, 4651-4667, doi: 10.1007/s12649-022-01843-5.
[126] T.-H. Wei, S.-H. Wu, Y.-D. Huang, W.-S. Lo, B. P. Williams, S.-Y. Chen, H.-C. Yang, Y.-S. Hsu, Z.-Y. Lin, X.-H. Chen, P.-E. Kuo, L.-Y. Chou, C.-K. Tsung, F.-K. Shieh, Rapid mechanochemical encapsulation of biocatalysts into robust metal-organic frameworks, *Nature Communications*, 2019, **10**, 5002, doi: 10.1038/s41467-019-12966-0.
[127] A. Silva, J. Alexandre, J. Souza, J. Neto, P. de Sousa Jr, M. Rocha, J. dos Santos, The chemistry and applications of metal-organic frameworks (MOFs) as industrial enzyme immobilization systems, *Molecules*, 2022, **27**, 4529, doi: 10.3390/molecules27144529.
[128] M. Pourmadadi, M. M. Eshaghi, S. Ostovar, A. Shamsabadipour, S. Safakhah, M. S. Mousavi, A. Rahdar, S. Pandey, UiO-66 metal-organic framework nanoparticles as gifted MOFs to the biomedical application: a comprehensive review, *Journal of Drug Delivery Science and Technology*, 2022, **76**, 103758, doi: 10.1016/j.jddst.2022.103758.
[129] M. Zou, M. Dong, T. Zhao, Advances in metal-organic frameworks MIL-101(Cr), *International Journal of Molecular Sciences*, 2022, **23**, 9396, doi: 10.3390/ijms23169396.
[130] Junyao, Pan, HKUST-1 derived carbon adsorbents for tetracycline removal with excellent adsorption performance, *Environmental Research*, 2022, **205**, 112425, doi: 10.1016/j.envres.2021.112425.
[131] K. Shortall, F. Otero, S. Bendl, T. Soulimane, E. Magner, Enzyme immobilization on metal organic frameworks: the effect of buffer on the stability of the support, *Langmuir*, 2022, **38**, 13382-13391, doi: 10.1021/acs.langmuir.2c01630.
[132] C. S. Osorio-González, N. Gómez-Falcon, S. K. Brar, A. A. Ramírez, Cheese whey as a potential feedstock for producing renewable biofuels: a review, *Energies*, 2022, **15**, 6828, doi: 10.3390/en15186828.
[133] G. Lara Utama, Reducing cheese-making by-product disposal through ethanol fermentation and the utilization of distillery waste for fertilizer, *International Journal of Geomate*, 2017, **13**, 103–107, doi: 10.21660/2017.37.2737.
[134] K. Valta, P. Damala, E. Angeli, G. Antonopoulou, D. Malamis, K. J. Haralambous, Current treatment technologies of cheese whey and wastewater by Greek cheese manufacturing units and potential valorisation opportunities, *Waste and Biomass Valorization*, 2017, **8**, 1649-1663, doi: 10.1007/s12649-017-9862-8.
[135] K. C. Ling, Whey to ethanol: A biofuel role for dairy cooperatives? Washington DC: University of Minnesota, Rural Business and Cooperative Programs, 2018.