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Scaling bioethanol for the future: the commercialization potential of extremophiles and non-conventional microorganisms

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Unlike conventional bioethanol production, which raises environmental concerns such as a high carbon footprint from resource-intensive crops, deforestation, and food security issues, non-conventional bioethanol production offers a more sustainable alternative. However, non-traditional feedstock availability and its pretreatment are the main challenges, importantly feedstock availability is either underreported or poorly forecasted, while pretreatment is costly, reaching up to 40% of the overall process or it might generate inhibitors that hamper ethanol production in commercial scale, as well as environmental impact. The literature further lacks the recent update for conventional and non-conventional microbial ability to ferment these feedstocks or their tolerance for inhibitors compared with the conventional yeast. Therefore, this review discusses Europe's non-conventional feedstock availability in national levels and pretreatment, highlighting pretreatment's cost industrially, scalability, and its impact on microbial fermentation and the environment. Moreover, recent European policies that might impact the commercialization of non-conventional bioethanol are discussed, emphasizing the revised RED III policy, certification scheme, and how to eliminate fraudulent biofuel imports to boost advanced ethanol production. Finally, this review discusses the pilot-scale case studies that investigated the non-conventional methods besides the recent update on non-conventional microbes' ability, inhibitors, and the techniques such as the immobilization to improve ethanol yield.

KEYWORDS

bioethanol production, extremophiles, non-conventional microorganisms, sustainable energy, cell immobilization

1 Introduction

The type of feedstock and fermenting microorganisms are the primary factors that determine whether bioethanol production is conventional or non-conventional; the procedure used is the secondary factor. Bioethanol derived from non-food crops, such as food waste, agricultural residues, and gaseous by-products, utilizing either conventional or non-conventional fermenting microorganisms, or both, is known as non-conventional bioethanol. Similarly, non-conventional bioethanol is also produced when non-conventional microorganisms are employed, regardless of the type of feedstock (International Energy Agency, 2022; Ndubuisi et al., 2023; Sun et al., 2024).

Non-conventional feedstocks, such as lignocellulosic materials, food waste, and agricultural residues, offer significant advantages over conventional crops by utilizing renewable, low-cost, and widely generated. However, utilizing these feedstocks at the commercial scale remains challenging due to many technical and economic constraints, particularly feedstock availability, the biochemical complexity of the feedstock, the cost compared with the traditional ways, the microbial potential to utilize these feedstock, and the recent policies that are related to commercialization of advanced biofuels (Novia et al., 2025).

First of all, feedstock availability including in the developed countries is generally not reported or forecasted comprehensively, and these feedstocks often require advanced pretreatment techniques to break down lignin and hemicellulose to enhance sugar yields, potentially increasing the overall production cost. Furthermore, the non-conventional pretreatment processes such as deep eutectic solvents, organic solvents, and ionic liquids often introduce inhibitory by-products that can impede fermentation efficiency. On the other hand, other processes, particularly the conventional such as acid or alkaline pretreatment, could have a direct environmental impact, while the cost on industrial level of these processes are varied and some of them are not feasible for the industrial scale (Shukla et al., 2023).

To overcome the inhibitory factor that was generated during or after the pretreatment or by other sources, environmental impact, and reduce the overall production cost, many strategies are suggested. Non-conventional microorganisms are increasingly engineered or selected for their ability to withstand these inhibitors while maintaining robust metabolic activity, paving the way for higher bioethanol yields under industrially relevant conditions. The use of extremophiles in bioethanol production adds a unique dimension to the process by exploiting their natural adaptability to extreme environments, such as high temperatures, salinity, or acidic conditions. These characteristics reduce the need for stringent sterile conditions, which can significantly lower operational costs in industrial applications. For example, thermophilic bacteria and thermotolerant yeasts, like *Kluyveromyces marxianus*, can ferment diverse sugars, including pentoses and hexoses, at elevated temperatures, improving process integration and efficiency. Beyond feedstock and microbial selection, process innovations such as pretreatment process (Shukla et al., 2023), employing mixed or sequential fermentation (Estrada-Martínez et al., 2019), cell immobilization (Sertkaya et al., 2021), and consolidated bioprocessing (Singh et al., 2022) could be a keypoint to commercialize the non-conventional bioethanol by overcoming the mentioned challenges.

This review fills the existing research gap regarding the feedstock availability in Europe as indicated earlier and classifies the pretreatment processes and the potential of each process in industrial scale, environmental impact, and inhibitory generation along with other inhibitory factors that hinder non-conventional ethanol production. The most updated European policies are discussed as well since it could play a significant role in non-conventional ethanol adoption. Furthermore, this review highlights the latest technical advancements, challenges, and potential of utilizing extremophiles and non-conventional microorganisms/methods in bioethanol production compared with the traditional pathways, focusing on innovative approaches like cell immobilization. Finally, detailed case-studies are provided regarding the scaled (pilot-scale) non-conventional ethanol production and their feasibility is reviewed.

This paper offers a bridge with existing knowledge gaps and provides actionable insights for researchers, industry stakeholders, and policymakers. By outlining the opportunities and barriers in non-conventional bioethanol production, this work contributes to the broader bioenergy literature, fostering innovation and collaboration in the field. Furthermore, the strategies discussed here may accelerate the development of sustainable bioethanol production processes, offering viable solutions to global energy challenges and advancing the transition toward a circular bioeconomy.

2 Opportunities extremophiles/non-conventional microbes bring to bioprocesses

Conventional methods for bioethanol production have limitations, leading to the adoption of non-conventional organisms. Generally, yeasts and bacteria are preferred for bioethanol generation due to their broad substrate range and optimal fermentation conditions. *Saccharomyces cerevisiae* and *Saccharomyces uvarum* dominate industrial ethanol production due to their ability to ferment glucose, maltose, and fructose. However, as ethanol accumulates, product formation is inhibited, and these species cannot utilize xylose sugars, a major component of hemicellulose and lignocellulosic biomass (Ibrahim, 2023).

Commonly used bacterial strains include *Zymomonas mobilis* and *Escherichia coli* indicated higher production yields relative to yeast species. However, most bacterial strains cannot ferment pure ethanol, necessitating additional purification processes (Bayrakci and Koçar, 2013). While conventional yeasts and bacteria offer some benefits, their limitations, such as substrate specificity and process inefficiencies, underscore the need for innovative approaches.

From an environmental perspective, extremophiles and non-conventional microorganisms recover waste products, such as sugarcane bagasse, pine needles, and sugar beet pulp, through the valorization of agricultural and forest wastes. These processes support bioethanol production and mitigate environmental hazards, such as forest fires, while reducing waste accumulation (Sharma and Chauhan, 2024).

Thermophilic organisms hold significance for bioethanol production due to their unique capabilities, including their enzymatic system and the advanced adaptation to harsh conditions. The enzymatic system in the thermophiles involve hemicellulases and/or cellulases, where they do not exist naturally in *S. cerevisiae* (Chang and Yao, 2011; den Haan et al., 2021). Although genetic engineering has made it possible to produce such enzymes in *S. cerevisiae* (Li et al., 2022), trial-and-error approach is still necessary because the successful production of such enzymes is still unpredictable according to den Haan et al. (2021). Other advantages of using thermophilic organism's enzymes are that they can tolerate severe industrial conditions, such as high temperatures, excessive pH, the presence of organic solvents, lengthy processing times, and a prolonged half-life at a particular elevated temperature. Despite numerous attempts, the expense of the enzymes frequently limits their use today. However, it is anticipated that the cost will drop as the market for the enzymes grows and bigger volumes of production result. Furthermore, it is anticipated that the need for microbial catalysts will rise in tandem with the industry's paradigm shift away from fossil fuels and toward the use of renewable resources. Additionally, the requirement for thermostable selective biocatalysts will undoubtedly continue to grow in the future since genetic engineering is growing for the thermophiles (Zuliani et al., 2021).

Using a whole cell is another approach that could be applied. Thermophiles such as *Thermoanaerobacter* sp., can utilize a wide range of sugar types such as pentoses, hexoses, disaccharides, and polysaccharide depending on the strain, makes them well-suited for agricultural residue valorization and ethanol production (Patelski et al., 2024). This genus has been investigated extensively due to the fact that its species display the highest ethanol yields exhibited by a thermophile, can function at elevated temperatures up to 85°C, which reduces contamination risks in non-sterile conditions while allowing for a cost-effective process. However, the wild strains are not very attractive for commercial ethanol production compared with *S. cerevisiae* because 62%–90% of theoretical maximum can be produced, while 90%–93% in *S. cerevisiae* (Ahmad et al., 2024; Kazemi Shariat Panahi et al., 2022; Ruchala et al., 2020; Zuliani et al., 2021) due to metabolic pathways, leading to mixed-products fermentation such as acetate, lactate, and hydrogen instead of only ethanol (Chang and Yao, 2011). Genetic engineering tools are available for a wide range of thermophiles and ethanol yield was reported to reach up to 92%–94% in *Thermoanaerobacterium* sp. and *Thermoanaerobacter mathranii*, respectively. Yet, more studies and validation is required and the literature lacks the relevant research in large scales (Kazemi Shariat Panahi et al., 2022).

Unlike thermophiles, wild *Kluyveromyces marxianus,* a thermotolerant species, exhibits superior fermentation performance at higher temperatures, reaching up to 52°C, compared to *S. cerevisiae* (Park et al., 2015). *K. marxianus* grows more quickly at elevated temperatures at growth rate of 0.80 h^{-1} compared with 0.37 h^{-1} in *S. cerevisiae* (Mo et al., 2019) and other studies have also revealed that *K. marxianus* exhibits superior behavior in producing ethanol under inhibitory existence (furans) in contrast to a commercial strain of *S. cerevisiae* (Amaya-Delgado et al., 2018). However, because of its weak ethanol tolerance, which is only 6% (v/v) (Ha-Tran et al., 2020), *K. marxianus* is presently unsuitable for commercial usage despite the fact that it was scaled to a pilot level since the commercial *S. cerevisiae* can tolerate up to 18% (v/v) (Sahana et al., 2024). Still, Bilal et al., (2022) indicated that *K. marxianus* can be restructured to have a better tolerance to ethanol than *S. cerevisiae*, making it a more resilient host that produces ethanol.

Similarly, *Pichia stipitis* or known as *Scheffersomyces stipitis*- a mesophilic species, has the maximum native xylose fermentation capacity among known microorganisms and scaled into a pilot level; yet, glucose non-competitively limits xylose transport. Furthermore, *S. stipitis* is less resistant to ethanol than *S. cerevisiae* and the requirement to preserve microaerophilic conditions make it difficult to apply on a commercial scale (Ishizaki and Hasumi, 2013). Therefore, it is suggested to apply *S. stipitis* sequentially or co-cultivation with other microbes since 88% of ethanol efficiency was produced via sequential fermentation with *Z. mobilis* and valorize (>95%) of the added sugars (Singh et al., 2014a), while ethanol titer can be improved by 1.56%–4.59% and 46.12%–102.14% of *Z. mobilis* and *P. stipitis*, respectively, compared with monocultures (Sun et al., 2021).

Co-fermentation method is widely used for optimal results from lignocellulosic biomass toward circular economy and waste valorization as well as boost ethanol yield. Co-culture or mixed cultures are reported to be suitable for industrial applications (Goers et al., 2014). This method separates enzymatic breakdown and microbial conversion steps, minimizing inhibitory effects and enabling high sugar process yields. For instance, microbial hydrolysis of sugar beet pulp with *Trichoderma viride*, which is more affordable than commercial enzymes followed by co-fermentation using *S. cerevisiae* and *P. stipitis* achieved 5.38 kg of ethanol per 100 kg of substrate, highlighting its efficiency (Patelski et al., 2024). *S. stipitis* co-cultivation with *S. cerevisiae*, improved ethanol yield to reach 87.54% compared with only *S. cerevisiae* 84.20% when co-cultivated using *Prosopis juliflora* (Naseeruddin et al., 2021). Importantly, *S. stipitis* did not show a significant competition with *S. cerevisiae* since it became a predominant strain after the glucose consumption (Wu et al., 2023). Likewise, the co-culture of wild and engineered *Thermoanaerobacter* strains with other strains such as *Caldicellulosiruptor* sp. and *Clostridium thermocellum* improved the ethanol concentrations compared with monocultures by 2–8.2-fold and 194%–440%, respectively, and showed a good potential for consolidated bioprocessing (Svetlitchnyi et al., 2013; He et al., 2011). Furthermore, Hawaz et al. (2024) reported that *S. cerevisiae* and *Pachysolen tannophilus* achieved a maximum of 77% ethanol yield under optimum conditions of a 46°C reaction temperature. While Mondal et al. (2024) reported that sugarcane molasses fermentation by *S. cerevisiae* and *Wickerhamomyces anomalus* increased ethanol yields by 29% and 53%, respectively, compared to single-species yields, demonstrating the benefits of microbial synergy.

Integrated methods or biorefinery approach another promising strategy. As stated earlier, *Thermoanaerobacter* sp. is able to produce multiple chemicals at the same time, making it suitable for biorefinery and reducing the overall production cost (Wu et al., 2021). *C. thermocellum* can produce bioethanol and biohydrogen

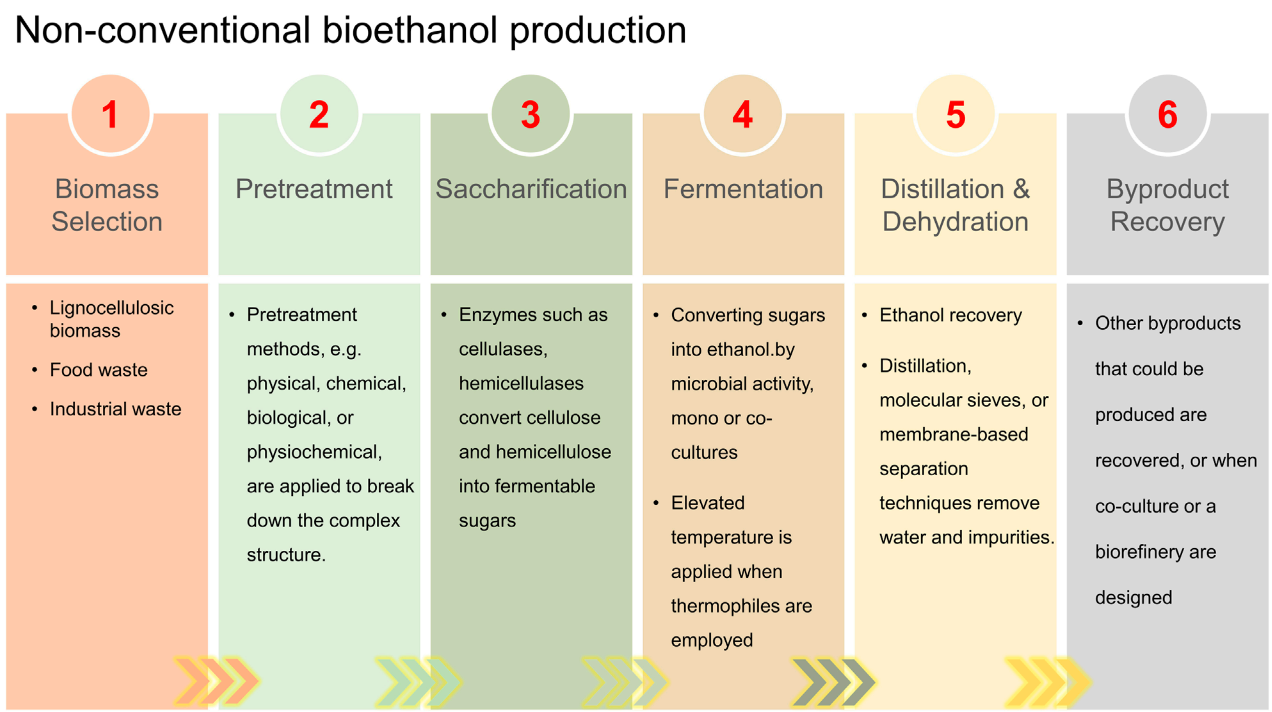

FIGURE 1
The process of non-conventional bioethanol production.

from sugarcane bagasse in non-sterile conditions simultaneously. This process lowers costs while delivering substantial yields (Ahmad et al., 2024). Crucially, *C. thermocellum* can be applied for hydrogen production industrially (Gallo et al., 2024). *K. marxianus* seems to be attractive for a biorefinery due to the possibility of producing heterologous proteins, enzymes, fatty acids, and lactic acid. Furthermore, genetic engineering tools are available to manipulate this strain (Reina-Posso and Gonzales-Zubiate, 2025). *K. marxianus* and *Bacillus coagulans* co-cultivation could improve lactic acid and ethanol by 90% using pomegranate peels, reaching 92% and 98% of the theoretical maximum ethanol and lactic acid, respectively (Demiray et al., 2024). Yet, competition from known microbiological platforms such as *S. cerevisiae* and *E. coli* is one of the main challenges. The switch to production systems based on various strains such as *K. marxianus* is a logistical and financial challenge because the current industrial infrastructure is primarily optimized for these microorganisms. Metabolism engineering techniques that increase substrate conversion efficiency and product optimization must be used in conjunction with efforts to incorporate lignocellulosic hydrolysates and agro-industrial wastes in order to employ *K. marxianus* on biorefinery applications (Reina-Posso and Gonzales-Zubiate, 2025). Importantly, the literature lacks these studies and the major research is being concentrated on a single product optimization, particularly ethanol, along with slow advancement in genetic engineering (Baptista and Domingues, 2022). The non-conventional ethanol production process is summarized in Figure 1 below.

3 Key scaling parameters, challenges and innovations in commercialization of bioethanol focusing on our main topic

3.1 Technical barriers for upscaling

3.1.1 Feedstock availability

One of the main challenges of ethanol production is the feedstock availability and variability. Therefore, it is necessary to categorize the available feedstock, particularly bio-waste such as agricultural residue, forestry, and food waste, to recognize waste generation and to predict the availability of this waste in the future.

Agricultural residue was expected to have a theoretical potential of 291–367 million tons of dry matter (Mt DM) per year in the EU and 253–483 Mt in Europe prior to 2021 (Scarlat et al., 2019), and the actual amount of agricultural residue was estimated to be 439.76 million tons by 2021 in Europe (European Commission, 2021). Over 212 Mt DM is thought to have the technical capacity to be utilized in Europe (Scarlat et al., 2019). However, the technological, financial, and future risks that are anticipated by components of climate change hinder residue utilization for biofuel production. By 2030, only 83.3 Mt of agricultural residues from wheat, soybeans, sunflower, rye, olives, barley, rice, oats, triticale, rapeseed, and corn could be processed into various biofuels including bioethanol (O'Malley and Baldino, 2024).

TABLE 1 Potential feedstock for non-conventional bioethanol production across Europe.

Potential feedstock	Producing country	Crop Production amount (Mt)	References
Corn stover	Ukraine	31	Statista (2024a)
	France	14.3	Statista (2022)
	Romania	8.5	Romania Insider (2024)
Wheat straw	France	25.7	Argus (2024a)
	Ukraine	22.5	USDA (2023)
	Germany	22.1	Lyddon (2022)
	UK	14	Department of Environment (2024)
	Poland	12.6	USDA (2024)
Barley straw	France	11.3	World Population Review (2024)
	Germany	11.2	
	UK	7.4	
	Spain	7	
Oat straw	Poland	1.6	Kobuszynska (2021)
	Finland	1.1	Business Finland (2023)
Olive oil waste	Spain	5.9	Alkhalidi et al. (2023)
	Italy	2.1	
	Greece	1.2	
Soybean waste	Italy	1	Businesscoot (2022)
Forestry residues	Finland, Austria Sweden	3.5–16	Di Gruttola and Borello (2021)
Vegetable waste	Europe	80.7	Statista (2024b)
Fruit waste	EU	Around 35.9	Eurostat (2024b)
Seaweed	Europe	0.2	European Parliament (2023)

France, Germany, and Romania are the major key players in agricultural residue production in the EU as they produced 59.78, 39.07, and 30.89 million tons in 2020, compared to 19.44 million tons in the UK (Carraro et al., 2021). Nonetheless, the production rate of certain agricultural residues in European countries is variable depending on the total amount of crops produced in each country. The summary of the produced crops is given in Table 1.

Cereals make up 50% of the EU's economic production and 74% of its residual output (European Commission, 2021). Between now and 2035, the amount of agricultural and forest land in the EU is expected to stay constant. Despite climate change and limitations on the accessibility and affordability of certain agricultural inputs (such as plant protection products), cereal and oilseed yields are expected to stay steady due to short-term beneficial developments like precision farming, increased crop rotation, and better soil health. However, the EU will produce less sugar beet as a result of the gradual drop in sugar consumption (European Commission, 2023). Furthermore, the Representative Concentration Pathway (RCP) 8.5 scenario predicts that by 2050 the EU's corn production will decline by 1%–22%, while southern Europe's wheat yields may drop by as much as 49%, underscoring the Mediterranean regions' extreme vulnerability due to reduced water supplies, continuously rising temperatures, and an increase in the frequency of heat waves and droughts (Hristov et al., 2020). Noteworthy, the complexity of agricultural systems and the variety of influencing factors naturally constrain the precision of estimates of agricultural residue, despite the fact that these estimates are essential for assessing the availability of resources (such as bioenergy) (European Commission, 2021). In contrast to the agricultural residues through 2050, the biomass resource for forestry residues is expected to remain stable at 11.2 Mt. However, alternative uses for these residues are still lacking, particularly for the byproducts of roundwood production (O'Malley and Baldino, 2024). Nevertheless, recent studies indicated that these residues have a high potential to be utilized as feedstock for bioethanol production in the form of beech wood chips (see case studies section below).

The potential for food waste in Europe is being lost, much like forestry waste. Although it was acknowledged that, from an economic and environmental perspective, food waste might be utilized to create biofuels such as bioethanol (Fagundes et al., 2024a; 2024b). Furthermore, the most readily available, reasonably priced, and plentiful feedstock for bioethanol production is food waste. The increasing rate of food waste generation and the depletion of energy supplies are real concerns, even though application technology is still in its infancy. One effective strategy is the bioconversion of waste at different stages of the food value chain (Bibra et al., 2023).

Over two-thirds of the 118–138 million tons of bio-waste produced yearly in the EU originate from municipal sources, with the remaining portion coming from the food and beverage sector (Ecostar, 2024). Depending on the Member State, bio-waste can range from 18% to 60% of municipal solid waste (Stylianou et al., 2020), and only 40% of this waste is effectively recycled in the EU (European Compost Network, 2022). In 2022, around 75% of food waste in Europe was either incinerated or deposited in landfills, and only 26% (around 5 million tons) of food waste was captured (Coombe, 2024). Certain European countries, namely, Romania, Cyprus, and Malta are struggling with waste recycling (European Environment Agency, 2023). Surprisingly, Romania lacks a working recycling infrastructure, clear legislation, and—above all—traceability and control systems. Organic waste is frequently intermingled, and there is little chance of composting or creating biofuel utilizing this waste (Ecostar, 2024). Nevertheless, beginning on 1 January 2024, bio-waste collection will be mandatory for EU Member States in accordance with the Waste Framework Directive (WFD) (Favoino and Giavini, 2024). Food waste would rank fifth in the EU for greenhouse gas emissions if they were a member state (Eufic, 2024). The total amount of food waste recorded at the EU level in 2022 was just over 59 million tons of fresh mass. 32 million tons of fresh mass, or 54% of the total (accounting for 72 kg per inhabitant), were made up of household food waste (Eurostat, 2024a; 2024b). Sweden, Croatia, and Slovenia had the lowest waste output per person, whereas Cyprus had the largest quantity of food waste *per capita*, at over 400 kg. Belgium, Denmark, Greece, and Portugal are likewise at the top of the scale (Fleck, 2024). In terms of mass, fruit accounted for 27% of all food waste in the EU, followed by vegetables (20%) and grains (13%) (Eufic, 2024). Notably, more than half of the production of fruits and vegetables comes from Spain, Italy, France, Poland, the Netherlands, and Germany. According to recent data, Spain produced 13.87 million tons of fresh fruit and vegetables in 2022, making it the EU's highest producer. With a 2022 output of 12.35 million tons, Italy comes in second, followed by France (5.9 million tons), Poland (5.3 million tons), the Netherlands (4.8 million tons), and Germany (3.7 million tons) (Europe Data, 2024). In Spain, fresh fruits and vegetables account for 80% of food waste (Foodrus, 2020), over 260 million kilograms of wasted fruits and vegetables were discarded only between spring and summer in 2019 (Fresh Plaza, 2024). The possibility for instant conversion of fruit waste into bioethanol without any sort of pretreatment makes fruit wastes quite intriguing feedstocks. Still, the process requires a lot of effort to optimize ethanol production and to compare it with the conventional pathways (Basaglia et al., 2021).

The seaweed (algae) industry has been gaining significant attention recently in obtaining bioethanol because the agar-based algae industry generates 60%–75% of solid waste biomass which is easier to hydrolyze than some other plants (Muryanto et al., 2024). Moreover, the algae industry is expected to expand in the coming years, potentially resulting in a substantial increase in biomass availability (Al-Hammadi and Güngörmüşler, 2024). In Europe, Norway is the leading country in algae production (Cai, 2021), and is remarkably expanding its algae industry. In 2018, it cultivated 169 tons, and by 2050, it will have the capacity to produce more than 20 million metric tons of macro and microalgae annually (Bazil and Krogstie, 2020). Therefore, this feedstock should be considered and investigated to produce bioethanol and to analyze various types of algal biomass since the studies are limited regarding bioethanol production.

Syngas is an excellent raw material for generating bioethanol due to its adaptability and accessibility (Gungormusler et al., 2022) which consists of a mixture of CO, H_2, N_2, CH_4, and CO_2 (Sertkaya et al., 2021), and this type of feedstock is already being utilized by LanzaTech in Belgium for bioethanol production commercially (LanzaTech, 2023). However, there is a lack of publicly available statistics, making it difficult to pinpoint the precise yearly production volume of syngas in the world including the EU. On the other hand, relevant marketplaces and studies can provide insights. For instance, Europe shows a high capacity for producing syngas since it generated 22 billion cubic tons in 2023 (European Biogas Association, 2024), and renewable gas generation is projected to increase in the coming years which could be implemented in non-conventional ethanol production (Al-Hammadi and Güngörmüşler, 2025).

3.1.2 Policies and regulatory

The Renewable Energy Directive was amended by the European Union (EU) in 2023 and is known as "RED III." As a result, the overall goal for the use of renewable energy in all sectors of the European Union was raised to at least 42.5% by 2030. Although the advanced biofuel has only a share of 4.5%, it is increased by 1.2 times compared with RED II in 2018 (3.5%), encouraging the utilization of the biowaste (The European Parliament, 2023). Notably, waste and residue utilization are double-counted toward the renewable energy goal, which significantly encourages the utilization of the biowaste as well. As for 2024, France, Finland, and the Netherlands are the most countries that produce advanced biofuel in the EU, accounting for 16.6, 4, and 2.9% cal of the total advanced biofuel production for each country, respectively. Importantly, the advanced bioethanol production in France represents 1.2% cal and is expected to be 3.8% cal by 2028. In the meantime, EU countries are lowering the cap (with an upper limit of 7%) of ethanol production from food and feed resources. Additionally, France and the Netherlands have already banned or started to ban some conventional feedstock for biofuel production such as soybean oil which encourages the non-conventional feedstock for ethanol production (Lieberz and Rudolf, 2024). Likewise, Germany's environment ministry is intending to submit a draft law to prohibit the usage of crop and feed-based biofuels "as soon as possible" (Argus, 2023).

Although RED and the Common Agricultural Policy (CAP) aim to encourage the use of bioenergy, neither the RED Reform (RED III) nor the National Strategic Plans in the CAP contain a precise set of "binding" regulations to facilitate this shift towards the use of agricultural waste. Future studies should look more closely at how these frameworks might be used in concert to address the problem of indirect land-use change (ILUC) and enhance the utilization of agricultural waste streams in the direction of a more circular energy economy (Alessandrini et al., 2023).

EU biofuels policy is unstable, primarily due to sustainability issues and the fact that the majority of member states had failed to meet the 2020 targets. Over time, the types of biofuels that are prioritized have changed. Unpredictability in policy may make the sector less appealing to private investors and raise risks. Long-term investments may be at risk due to ambiguities surrounding the classification of advanced biofuels. Moreover, these policies lack a definite policy direction after 2030. There is currently no specific aim for road transport, but there is a 2030 target for the use of renewable energy in all transport sectors combined (RES-T). While the growth of crop-based and mature biofuels in road transportation is being restricted, a significant portion of this increase may be absorbed by the aviation and maritime industries. This does not allow for the increased use of biofuels in transportation by road. Furthermore, the European Commission has not implemented a comprehensive biomass policy according to the recent data in 2023, despite its stated need for resource efficiency and fair competition. The main tools to limit biomass overexploitation for biofuels are target caps and sustainability criteria. Despite the Commission's studies, there has been no EU biomass strategy since the 2005 biomass action plan and no assessment of biomass availability and its potential in relation to renewable targets. Member states have left biomass availability assessment to their national energy and climate plans, and a study by the Commission found that only a small majority of member states refer to their domestic biomass production potential (European Court of Auditors, 2023). Crucially, certain advanced ethanol plants/companies in Europe such as St1 in Finland were enforced to terminate their service due to many reasons including feedstock availability (St1 Nordic Oy, 2023).

For advanced biofuel production in the EU, credits and certification such as ISCC, RSB, and greenhouse gas (GHG) savings are essential for guaranteeing sustainability, adherence to legal requirements, and market access, further to guarantee that imported biofuels in the EU do not originate from deforested or high-carbon stock areas. Lower certification prices or higher credits along with policy improvements are anticipated to increase demand for waste-based ethanol with greater GHG savings in 2025. Ethanol consumption in 2024 was impacted by the drop in GHG ticket costs, especially in Germany, which reduced the price gap between high and low GHG savings ethanol. Physical blending and premiums for high-GHG savings ethanol are expected to rise, and certain countries such as Germany have increased the GHG quota in 2025 and the use of carried-forward GHG certificates is proposed to be suspended, aiming to lessen dependency on previous credits, promote more mixing of low-carbon fuels such waste-based ethanol, and boost immediate compliance pressure. On the other hand, other countries such as the Netherlands have a minor decrease in the carry-forward allowed for tickets (Argus, 2024a; 2024b).

Recently, the certification scheme was criticized and flagged to be "inadequate" to combat the fraudulent (Moskowitz et al., 2023), and European biofuel producers have strongly criticized the delay in putting in place a mechanism to safeguard the EU market from fraudulent biofuel imports, which could lead to a significant climate damage and deforestation in non-EU producers in addition to the biofuel market since the fraudulent biofuel is cheaper which lead to unfair competition among the prices (Advanced Biofuel USA, 2023), and this unfair competition has already contributed to the shut down of advanced ethanol plants/companies such as Clariant in Romania (Clariant, 2023), and currently, France and Germany are calling on the EU to improve the policies to prevent the importation of fraudulent biofuel (AgWeb, 2024). The European Commission announced that it is creating a database to track the supply chains of feedstock for the renewable fuels used in the EU, as for January 2024, the EU announced that the database has become open for registration, and it will fully operate in 2026. The complete implementation is anticipated to hasten commercialization by fostering an environment for advanced ethanol production that is more transparent and conducive to investment (GoodFuels, 2024).

3.1.3 Pretreatment methodologies

The high cost of bioethanol production stems from biomass resistance and expensive pretreatment, which consumes the most energy and accounts for up to 40% of total costs (Zhang et al., 2024; Singh et al., 2014b; Bender et al., 2022; Awoyale and Lokhat, 2021). Pretreatment methods, classified as conventional or non-conventional (Saad and Gonçalves, 2024), present challenges—conventional methods are unsustainable due to harsh conditions and low productivity, while non-conventional methods have application limitations.

Physical pretreatment increases the surface area of the biomass and enhances hydrolysis yields. In many cases, physical pretreatment is necessary before or after other pretreatment processes (Kassim et al., 2022). Among the green-physical processes, milling is the most used technique according to Arce and Kratky (2022) and Bender et al. (2022), and this technology does not generate inhibitors. However, milling was generally considered non-feasible economically due to high energy consumption (Beluhan et al., 2023). Alternatively, extrusion has become one of the most attractive technologies because it can combine thermal, mechanical, and chemical pretreatments (Shukla et al., 2023) with various feedstock (Duque et al., 2017), and it has a low cost (Zheng and Rehmann, 2014). There is currently little information available about the expansion of extrusion for lignocellulosic biomass pretreatment, despite the fact that it is currently used on pilot scales (Vandenbossche et al., 2016), and can be easily modified for commercial use (Kuster Moro et al., 2017). Recently, plasma, microwave, and ultrasonic-assisted pretreatments have gained noticeable attention. However, high energy consumption and equipment cost are the main obstacles that hinder their implementation on the industrial level besides the high demand for advanced engineering and process optimization (Abolore et al., 2024). Simonetti et al. (2022) indicated that microwave pretreatment could be a feasible technique if electricity was provided via renewable sources.

The most used technique on a commercial scale is chemical pretreatment, particularly acid and alkali due to their high efficiency and low cost on an industrial basis (Verma and Shastri, 2020; Wang et al., 2022; Fagundes et al., 2024a). However, they are associated with inhibitor formation, corrosion, or slow reaction time (if they are diluted), respectively (Kumar and Sharma, 2017;

Johannes and Xuan, 2024). More importantly, none of these methods is eco-friendly. The strong bases and acids utilized in these procedures are corrosive and toxic, and after pretreatment, neutralizing the acids or bases produces chemical waste that could contaminate soil and water (Jönsson and Martín, 2016; Wang et al., 2019; Hongbo et al., 2020). The most efficient and environmentally friendly chemical techniques are organic solvents (organosolv) and deep eutectic solvents (DESs). In the biomass, both solvents can dissolve lignin and hemicellulose leaving cellulose intact (Abolore et al., 2024). However, the organic solvents have an inhibition impact on the enzymatic hydrolysis and their removal is necessary (Maurya et al., 2015). Unfortunately, the low recovery rate of organic solvents makes this process exceedingly expensive, making it unsuitable for large-scale and commercialization (Mielenz, 2020), and currently, there are only four operational pilot plants that operate with organic solvents according to Tofani et al., (2023). Unlike organic solvents, DESs were reported to be more advantageous in terms of cost because they are easy to recycle (Mielenz, 2020). Still, DESs are relevantly a new technology and are still more likely to be used at the laboratory scale. In order to be sustainable, DES-based biorefineries must be technically scalable at the industrial level (Satlewal et al., 2018). Additionally, even though DESs were widely claimed to have low toxicity and are biodegradable, they are not always environmentally benign and their residue might inhibit enzymatic saccharification (Jose et al., 2024; Yao et al., 2024). Similar to DESs, ionic liquids (ILs) were acknowledged to be one of the most "green" and efficient solvents for lignocellulosic biomass since they dissolve lignin at room temperature (Xu et al., 2017; Zhao et al., 2022). However, it was demonstrated that some ILs are toxic to the microorganisms depending on the solvent's type and concentration, and since ethanol has a low energy density of combusting, the procedures must be performed consecutively in the same reaction pot to make ILs application commercially viable (Kuroda, 2024). Barcelos et al. (2021) used cholinium lysinate in a single-pot pretreatment, demonstrating its effectiveness, biocompatibility, and efficiency in a pilot-scale system. However, improvements in the total yield and solid and enzymatic loading are needed (Barcelos et al., 2021). Another strategy to overcome the ILs toxicity is using non-conventional microorganisms with higher ILs tolerance such as *Kazachstania telluris* and *Wickerhamomyces anomalus*. Yet, the studies have not focused on this strategy (Kuroda, 2024).

Since its development in 1925, steam explosion has emerged as one of the most popular techniques for pretreating biomass and food residue; in fact, it has been effectively used as the primary pretreatment technique in commercial projects in the USA and China to produce bioethanol from lignocellulosic biomass (Chung and Washburn, 2016; Yang et al., 2023) and already scaled to industrial level (Oliveira et al., 2013; Chen, 2015) because it is effective, environmentally friendly, typically chemical-free, and industrially scalable (Guigou et al., 2023). This technology has a low cost and minimal energy requirements (Dziekońska-Kubczak et al., 2018), and is less expensive than biological, physical, and non-conventional chemicals pretreatments (Chen, 2015; Baral and Shah, 2017). Although this technology is the most successful and promising to be applied industrially, it still requires improvements to overcome the main challenges such as incomplete removal or disruption of lignin and inhibition generation (Behera et al., 2014). The latter can be minimized via steam explosion modification through the replacement of atmospheric air with CO_2, in a pretreatment method known as supercritical CO_2 explosion (Ravindran and Jaiswal, 2016). This modification allows for a better pretreatment of high lignin content (Alam et al., 2024). CO_2 itself has a low cost and works in mild conditions unlike the steam explosion, however, it requires high pressure and high capital cost for carbon capture and storage, making it a moderate costing technology (Gu et al., 2013).

Another technique to overcome the limitation of the steam explosion is the ammonia expansion/explosion (AFEX), also referred to as the ammonia-catalyzed steam explosion. This technique is nearly identical to steam explosion technology excluding the harsh operational conditions that are applied in the steam explosion and the applied liquid anhydrous ammonia instead of atmospheric air to serve as a catalyst (Bundhoo et al., 2015; Meraj et al., 2023; Yang et al., 2023). Even though the AFEX offers industrial advantages such as negligible inhibitor impact, and water washing elimination, and is already scaled on a pilot basis (Shukla et al., 2023), it is costly due to the expense of ammonia and its recovery, and ammonia necessitates extra safety precautions and equipment. As a result, its higher efficiency might not be enough to balance these costs (Menon and Rao, 2012).

The liquid hot water method is comparable to a steam explosion, except the water is kept liquid by applying pressure (Keskin et al., 2019). LHW merely employs water as a reagent and requires less amount of energy compared with the steam explosion (on small scales) for its heating and cooling processes since lower pressure is required and more advantageous over the steam explosion in terms of inhibitors formation which is mild (Serna-Loaiza et al., 2022). Nevertheless, this process is not feasible on the industrial scale compared with the steam explosion because it requires a massive amount of water (Pachapur et al., 2020) with the possibility of generating wastewater which adds additional cost (Mujtaba et al., 2023).

Biological pretreatment is environmentally friendly, uses less energy, does not require chemicals, and does not generate inhibitors in most cases (Wu et al., 2022). It consists of microbial and enzymatic methods, with white, brown, and soft rot fungi being the most commonly investigated microbes (Maurya et al., 2015; Singh et al., 2022). However, microbial pretreatment requires a long time due to a slow conversion rate, eventually lowering the overall productivity (Mishra et al., 2018; Zhang et al., 2023). Moreover, this process requires massive bioreactors with sterile conditions and continuous monitoring that add additional cost which reaches 4-15 times greater than conventional methods besides the sugar consumption by the microbes which might lower the sugar availability for the fermentation, limiting the scaling up to an industrial scale (Ummalyma et al., 2019; Vasco-Correa and Shah, 2019), and Vasco-Correa and Shah, (2019) indicated that fungal pretreatment in a biorefinery scale might be not feasible economically in contrast to enzymatic pretreatment which is preferred for scaling up. However, enzymatic pretreatment has a low efficiency (Porninta et al., 2023), and enzyme costs can account for up to 48% of the final product's total cost (Ramos et al., 2024). According to recent studies, on-site enzyme synthesis may drastically lower enzyme prices, and reducing enzyme loading is another strategy for bringing down the price. Yet, these studies are still awaiting industrial data validation (Liu et al., 2016).

Combining physical, chemical, physiochemical, and biological techniques is a new approach to overcoming the mentioned challenges (Ummalyma et al., 2019). The combined techniques could improve sugar yield, effectively handle different kinds of biomass, increase versatility and scalability, and reduce the inhibitors (Shukla et al., 2023), Moreover, numerous research suggested that the combination of pretreatment techniques could lower expenses and energy usage (Jiradechakorn et al., 2023). However, further research is necessary because the combined methods have higher operational costs and require optimization according to various feedstock and combined methods, which makes scalability more difficult and complex, requiring additional equipment that might raise the initial cost investment (Dimos et al., 2019).

3.1.4 Inhibitors

As already indicated in Table 2, certain chemicals that are widely applied for bioethanol production may inhibit the production, additionally, inhibitor formation such as furan derivatives, carboxylic acids (Al-Hammadi and Güngörmüşler, 2025), phenolic compounds (Wang et al., 2017), or glycolaldehyde is challenging during biomass pretreatment since they could disrupt glycolytic pathway and ethanol fermentation (Jayakody et al., 2011). These inhibitors could be avoided via an appropriate biomass pretreatment as previously discussed. Chemical residue, feedstock variability, and ethanol itself are other major inhibitors as well (see Figure 2). The feedstock that contains heavy metals was recommended to be processed before bioethanol production if it contains heavy metals. Noteworthy, many agricultural regions are prone if not already contaminated with heavy metals due to industrial waste, fertilizer, pesticides, and herbicides leaching into water and soil (Zohri et al., 2022). The amount of sugar loading for the fermentation process is very crucial since osmotic pressure-induced stressors on yeast cells reduce the efficiency of ethanol synthesis (Thatiyamanee et al., 2024). Similarly, high ethanol concentrations that are yielded through fermentation can inhibit the process because it reduces water activity nearby yeast cells, thereby removing hydrate layers from the medium (Nguyen et al., 2015). Furthermore, it affects the enzymes that are involved in the glycolysis process and reduces the ability of the plasma membrane to function as a semipermeable barrier, leading to cofactors and coenzymes leakage through the membrane (Osman and Ingram, 1985). In the case of utilizing syngas for ethanol production, syngas impurities such as furans, hydrogen sulfide (H_2S), hydrocarbon and tar, particulate matter, metals, catalyst residues, and nitrogen oxide (NO_x) should be removed or decreased (Al-Hammadi and Güngörmüşler, 2025), and CO and CO_2 concentrations should be controlled so that fermenting microbes can tolerate them, otherwise, they can impact microbial growth (Gungormusler and Keskin Gundogdu, 2020).

3.2 Innovations in commercialization via immobilization

Cell immobilization confines viable microbes in a matrix, preserving their activity while enhancing protection, localization, and reusability, which improves the sustainability (Lapponi et al., 2022; Hassan et al., 2019). Various immobilization strategies such as cross-linking, aggregation and biofilm-mediated immobilization, covalent bonding, encapsulation or entrapment, and adsorption are well-defined in the literature (Sagir and Alipour, 2021; Mohidem et al., 2023) besides the potential of the developed immobilized reactors Willaert, (2011) and Wouters et al. (2021). These technologies have shown significant promise in enhancing bioethanol production, offering several advantages over traditional free-cell fermentation methods. They are being applied to immobilize at both research and commercial scales, with varying degrees of implementation (Karagoz et al., 2019; Erkan-Ünsal et al., 2023).

One of the most notable advantages of the cell immobilization procedure is the increased tolerance of cells to various lignocellulosic inhibitors. Chacón-Navarrete et al. (2021) and Rakin et al. (2009) indicate that cells immobilized in various supports, such as calcium alginate, are capable of maintaining their viability even in the presence of phenols, furans, and high ethanol concentration. Immobilized cells show greater physiological stability associated with the production of protective compounds such as trehalose or glycogen. These compounds favor an increase in cell viability with respect to fermentations with free cells by limiting the toxicity of ethanol and other lignocellulosic inhibitors (Chacón-Navarrete et al., 2021).

Furthermore, immobilized cells in alginate have demonstrated efficient fermentative activity for at least two fermentation cycles before the degradation of the support material (Rakin et al., 2009). Nevertheless, in this study, alginate support demonstrated some limitations in its mechanical stability under high cell density and CO_2 release conditions. On the other hand, polyvinyl-alcohol (PVA) showed major mechanical resistance, although it exhibited less fermentation efficiency. This, along with other studies showing the potential for reusing immobilized cells and their suitability for use, highlights numerous industrial benefits. These include reduced downtime between cycles, and enhanced suitability for large-scale bioethanol production plants. Additionally, these systems offer economic advantages, such as notable reductions in operational costs. Immobilization systems also improve molecular transport between immobilized cells and the medium due to the adjustable porosity of materials like alginate and biochar systems. This allows substrates to penetrate toward the cells while metabolic products are subsequently released into the medium (Chacón-Navarrete et al., 2021). Another significant advantage of cell immobilization is the optimization of space, as the supports can accommodate a higher number of cells in a reduced volume. This increases cell density, volumetric productivity, and consequently fermentation efficiency (Chacón-Navarrete et al., 2021; Rakin et al., 2009).

The exploration of cell immobilization technologies for bioethanol production is opening new avenues, particularly with the use of non-conventional microorganisms. Although *S. cerevisiae*—in both its engineered and wild forms—remains the dominant organism for bioethanol production across all generations, these emerging technologies are still primarily in the research and development phase (Jansen et al., 2017; Soleimani et al., 2017; Moremi et al., 2020). Encouragingly, initial strides toward commercial implementation are now being observed. Table 2 provides an overview of various non-conventional microorganisms,

TABLE 2 Pretreatment technologies for bioethanol production from agricultural residue and food waste. The cost is determined based on the industrial scale.

Method category	Pretreatment method	Process type	Scale	Cost	Inhibitor formation	Green
Physical						
	Milling	Conventional	Pilot	High	No	Yes
	Extrusion	Non-conventional	Pilot	Low	No	Yes
	Microwave	Non-conventional	Pilot	High	Mild	Yes
	Ultrasound	Non-conventional	Pilot (limited)	High	Mild	Yes
	Plasma	Non-conventional	Laboratory	High	No	Yes
Chemical						
	Acid	Conventional	Commercial	Low	Yes	No
	Alkali	Conventional	Commercial	Low	No	No
	Deep eutectic solvents (DESs)	Non-conventional	Laboratory	Low	Yes	Conditionally
	Organic solvents	Non-conventional	Pilot (limited)	High	Yes	Yes
	Ionic liquids (ILs)	Non-conventional	Pilot (limited)	High	Yes	Conditionally
Physio-chemical						
	Steam Explosion	Conventional	Commercial and limited industrially	Low	Yes	Yes
	CO2 explosion	Non-conventional	Laboratory	Moderate	No	Yes
	Liquid Hot Water	Non-conventional	Pilot	Moderate	Mild	Yes
	Ammonia-based	Non-conventional	Pilot	High	Negligible	No
Biological						
	Enzymatic	Non-conventional	Pilot	High	No	Yes
	Microbial	Non-conventional	Laboratory	High	Mild	Yes

including extremophiles, that have been immobilized within cell carriers to optimize bioethanol production.

Table 3 highlights various non-conventional microorganisms employed for bioethanol production using diverse feedstocks, emphasizing extremophiles and experiments approaching the performance of the conventional microorganism *S. cerevisiae*. This species achieves productivities of 3–4 g/Lh with ~75% fermentation yield for first-generation bioethanol and 1–2 g/Lh with ~65% yield for second-generation production (Macrelli et al., 2014; Narisetty et al., 2022; Devi et al., 2023; Hans et al., 2023). Extremophilic microorganisms such as *Z. mobilis* under repeated batch fermentation with mesoporous silica and glucose achieved a productivity of 0.39 g/Lh and a fermentation efficiency of 56.70% (Niu et al., 2013). Although these values fall below those of *S. cerevisiae*, they highlight the potential of extremophiles when fermentation conditions are further optimized.

Kamelian et al. (2022) demonstrated a sequential fermentation strategy combining *Z. mobilis* (strain ATCC 10,988) with *S. stipitis* (ATCC 58,376), achieving a productivity of 0.29 g/Lh and a fermentation yield of 78.43%. This study illustrates the advantages of using extremophilic and non-conventional microorganisms in tandem to enhance bioethanol production efficiency, especially when targeting specific substrates or conditions. While the productivities remain lower than the benchmarks of *S. cerevisiae*, such approaches show promise in advancing non-conventional systems for sustainable bioethanol production. In addition, Stepanov and Efremenko (2017) achieved a significant milestone by attaining a productivity of 0.64 g/Lh using *Pachysolen tannophilus* Y-475, a yeast known for its capacity to ferment pentose sugars. This was accomplished through the use of an innovative bioreactor system with immobilized cells, nearly doubling the productivity reported in many other non-conventional setups.

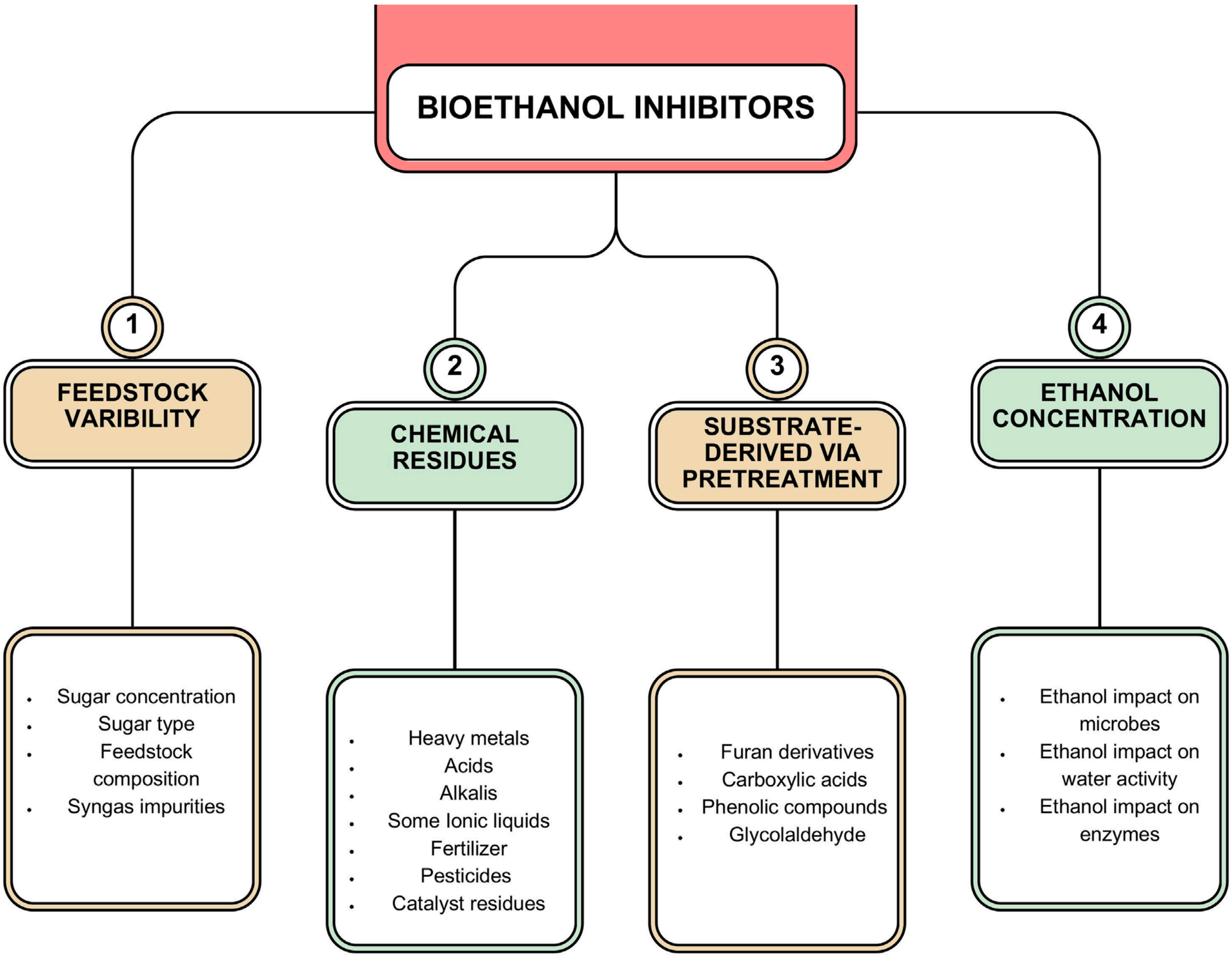

FIGURE 2
Bioethanol inhibitors that are resulted before, during, or after fermentation process.

Other extremophiles such as *Candida shehatae*, known for its ability to metabolize and ferment pentose sugars and to survive in environments with high concentrations of inhibitors typically present in hydrolysates, achieved notable substrate utilization efficiencies. In a batch fermentation process using rice straw autohydrolysate, it delivered a fermentation efficiency of 92.16%, but its productivity was relatively low at 0.20 g/Lh (Abbi et al., 1996). Another experiment featuring a co-culture of *S. cerevisiae* and *S. stipitis* using wheat straw hydrolysate achieved 0.1 g/Lh productivity and a 68.10% fermentation efficiency (Karagöz and Özkan, 2014). Although the productivities fall short of conventional values, the efficiency levels approach or exceed *S. cerevisiae* in certain cases, showcasing potential in specialized conditions.

Notably, some systems utilizing immobilized cells enhanced productivity under industrially challenging feedstocks. For example, 81.11% fermentation efficiency was reached with pretreated wheat straw, though productivity remained at 0.06 g/Lh (Brethauer and Studer, 2014). These results highlight the innovative cell immobilization techniques applied to improve performance, even though further optimization is required to meet the standards of conventional production systems.

4 Pilot case studies on extremophiles/non-conventional microbes/methods on ethanol production

Globally, non-conventional bioethanol production faces several challenges limiting their readiness for scale-up as previously indicated in this article. This limitation involves the developed countries as well. The European market shows Surprisingly, only a few companies in European countries have scaled the production to a pilot or commercial scale (see Table 4). Nevertheless, recent studies in the last decade conducted abroad investigation regarding non-conventional bioethanol production using various feedstocks and microorganisms. The feedstocks included lignocellulosic biomass (Limayem and Ricke, 2012), industrial waste (Alfonsín et al., 2019), and urban and municipal waste (Meng et al., 2021), whereas microbial strains included primarily yeast (Nandal et al., 2020) and secondly bacteria (Tang et al., 2021).

For non-conventional feedstocks and microbes processes, Lin et al. (2012) demonstrated that employing *S. stipitis* for xylose fermentation from rice straw has a potential for commercial ethanol

TABLE 3 Immobilized whole cells in cell carriers for enhanced bioethanol production with non-conventional methods, feedstocks and/or microorganisms.

Non-conventional microorganism	Cell carrier	Feedstock and concentration in g/L	Fermentation conditions	Bioethanol titer (productivity in g/Lh and fermentation efficiency in %)	References
Z. mobilis ATCC 10988, NCIM 2428	Pectin beads	Glucose (150)	pH 7, 30°C, 16 h, continuous fermentation	NP, 90.2	Kesava et al. (1995)
Candida shehatae NCL-3501	Alginate beads	Rice straw autohydrolysate (20)	pH 5.5, 30°C, 150 rpm, 120 h, batch fermentation	0.20, 92.16	Abbi et al. (1996)
Z. mobilis	Mesoporous silica	Glucose (100)	pH 7.0, 30°C, 24 h, repeated batch fermentation	0.39, 56.70	Niu et al. (2013)
S. cerevisiae VTT C-79095 and *S. stipitis* VTT C-07806T	Multispecies biofilm membrane	Washed pretreated wheat straw supplemented with xylose (17.5 cellulose and 22 xylose)	pH 5.0, 28°C, 144 h, batch consolidated bioprocessing	0.06, 81.11	Brethauer and Studer (2014)
S. cerevisiae ATCC 26602 and *S. stipitis* DSM 3651	Alginate beads	Wheat straw hydrolysate (30)	pH 4.8, 30°C, 0.75 h, continuous fermentation	0.1, 68.10	Karagöz and Özkan (2014)
P. tannophilus MTCC 1077	Alginate beads	Peels of pineapple *Ananas cosmosus* (51.7)	pH 5.0, 50°C for 24 h (saccharification) and 32°C for 96 h (fermentation), batch simultaneous saccharification and fermentation	0.15, 39.82	Bhatia and Johri (2015)
S. stipitis NCIM 3498				0.15, 41.34	
Pachysolen tannophilus Y –475	Poly (vinyl alcohol) cryogel	Crude glycerol (25)	pH 5.6, 28°C, 15–24 h, repeated batch fermentation	0.64, NP	Stepanov and Efremenko (2017)
K. marxianus	Biochar	Valencia orange peel hydrolysates (90)	pH 4.8, 42°C, 100 rpm, 10 h, batch fermentation	NP, 7.3	Kyriakou et al. (2019)
Pichia kudriavzevii KVMP10				NP, 2.5	
S. cerevisiae YPH499 and *Pachysolen tannophilus* ATCC 32691	Alginate beads	Pretreated cotton stalk lignocellulosic biomass (20)	pH 5.0, 30°C, 150 rpm, 96 h, batch simultaneous saccharification co-fermentation	0.10, 90.29	Malik et al. (2020)
Spathaspora passalidarum UFMG-CM-469	Lentikats® discs	Oat and soybean hulls enzymatic hydrolysate (51.57)	pH 5.5, 28°C, 120 h, batch fluidized-bed fermentation	0.29, 78.43	Dall Cortivo et al. (2021)
Spathaspora passalidarum UFMG-CM-469	Lentikats® discs	Oat and soybean hulls enzymatic hydrolysate (42.012)	pH 5.5, 28°C, 216–312 h, continuous fermentation	0.26, 66.67	
S. stipitis (ATCC 58784)	Artificial biofilm support materials	Detoxified and glucose- or xylose-enriched rice husk hydrolysate (NP)	pH 6.2, 30°C, 150 rpm, 48 or 120 h, batch (repeated) fermentation	0.02, 46.08	Bader et al. (2022)

(Continued on the following page)

TABLE 3 (*Continued*) Immobilized whole cells in cell carriers for enhanced bioethanol production with non-conventional methods, feedstocks and/or microorganisms.

Non-conventional microorganism	Cell carrier	Feedstock and concentration in g/L	Fermentation conditions	Bioethanol titer (productivity in g/Lh and fermentation efficiency in %)	References
Z. mobilis ATCC 10,988 and *S. stipitis* ATCC 58,376	Alginate beads	Synthetic glucose xylose medium (50)	pH 6.7, 30°C, 150 rpm, 70 h, sequential fermentation	0.29, 78.43	Kamelian et al. (2022)
S. cerevisiae KCTC 7906 and *S. stipitis* KCTC 7228	Alginate beads	Bamboo (50)	pH 5.0, 30°C, 200 rpm, 48 h (*S. cerevisiae*), and 120 h, 0.25 vvm (*S. stipitis*), separate hydrolysis and fermentation and sequential fermentation	0.11, 70.59	Song et al. (2022)

NP: not provided; vvm: volumes of air per volume of liquid per minute [Wu and Maravelias (2018)].

production. Importantly, the ethanol yield was affected directly via biomass pretreatment directly. Unlike rice straw hydrolysates which were conditioned via ammonia that yielded 0.39 g/g of ethanol, the highest ethanol yield and productivity were 0.44 g/g and 0.22 g/Lh, respectively, when rice straw hydrolysates were obtained via the overliming-detoxification process. The authors stated that there is a strong relationship between the initial cell density and the concentration furfural on one hand, and the pretreatment process on the other hand. Hence, all these considerations may further improve ethanol yield depending on the applied pretreatment process and conditioning (Lin et al., 2012).

In a different strategy to utilize the rice straw, hydrolyzed rice straw was added to bamboo, plywood, and bagasse xylose fermentation by *P. stipites*. Similar to the previous study, overliming and ammonia were selected as the detoxification procedure to remove inhibitory compounds present in hemicellulosic hydrolysates and for neutralizing, respectively. Factually, this strategy increased *S. stipitis* cell mass, leading to higher ethanol yield by 20%–51% compared to the method when hydrolyzed rice straw was not added into the xylose, and the overall ethanol yield and productivity were 0.45 g/g and 0.25 g/Lh when ammonia pretreatment was conducted, and 0.43 g/g and 0.27 g/Lh, respectively. The yield and productivity were slightly better than rice straw-based xylose when it was solely utilized for the fermentation, indicating the high potential of rice straw to be the main source of xylose (Lin et al., 2016).

The potential of utilizing beech wood chips as a source of xylose and *Spathaspora passalidarum* capacity for bioethanol production was investigated by de Vrije et al. (2024). The authors employed organosolv fractionation method based on acetone for the pretreatment process. The medium also contained organic acids, furans, and phenolics. The ethanol yield of *S. passalidarum* was 0.38 g/g, which was less than that of *S. stipitis* (de Vrije et al., 2024). In contrast to *P. stipites*, which had ethanol productivity of 0.22 and 0.25 g/Lh (Lin et al., 2012; 2016), *S. passalidarum* had superior ethanol productivity of 0.78 g/Lh. However, YP + salt medium was added along with the extracted sugars for the fermentation process. Interestingly, the up-scaled reactor exhibited a higher ethanol yield compared with the flask scale which was 0.34 g/g which encourages conducting further pilot-scale analysis regarding xylose fermentation via *S. passalidarum*. In the same study, glucose was extracted from beech wood chips along with xylose. The extract was added to the YP + salt medium and was fermented by *S. cerevisiae.* Ethanol yield and productivity were 0.48 g/g and 3.9 g/Lh, respectively (de Vrije et al., 2024), showing the high capacity of wood chips to be applied with the ordinary medium since the yield is near the theoretical value of 0.51 $g_{ethanol}/g_{sugars}$ (Krishnan et al., 1999). Sugarcane bagasse utilization by *Kluveromyces marxianus* showed less potential for ethanol production. Lin et al., (2013) conducted alkaline pretreatment followed by a fermentation process using a rotary drum reactor which is rarely reported. The authors achieved the highest ethanol concentration and productivity of 24.6 g/L and 0.342 g/Lh, respectively. Importantly, the results showed an effective scaling up since the obtained outcomes were similar to the laboratory scale. However, significant improvements are required to achieve better productivity.

Industrial waste, namely, avocado seeds, oat hulls, empty fruit bunches from palm oil, sugarcane bagasse, the potato processing

TABLE 4 Non-conventional bioethanol producing companies across Europe.

Company	Country	Company type	Feedstock	Production capacity (ton/year)	References
Versalis	Italy	Chemical	Lignocellulosic	25,000	Eni (2022)
Celtic Renewables	Scotland	Acetone-Butanol-Ethanol (ABE)	Whisky byproducts	1000 including chemicals	Celtic Renewables (2021)
Vertex Bioenergy	Spain, France	Bioethanol, feed, electricity, wine alcohohl	Cereals, sugar beet, agricultural residues	Around 615,420	Acciona (2021), Vertex Bioenergy (2022)
NordFuel Oy	Finland	Biorefinery	Sawdust, forest residue	70,000	NordFuel (2022), Bioenergy Insights (2024)
LanzaTech, ArcelorMittal	Belgium	Steel production, bioethanol	syngas	63,120	LanzaTech (2023)
AustroCel Hallein GmbH	Austria	Pulp industry, bioethanol	Wood-based residual	30,000	Austrocel (2024)

industry, and the seaweed industry was investigated using *S. cerevisiae* (see Table 5 below). Among these non-conventional feedstocks, potato waste and avocado seeds exhibited the highest ethanol concentration and the most promising feedstocks for ethanol production. Various potato wastes were investigated separately to figure out the highest ethanol-producing feedstock, although the pretreatment methods were not identical. Potato peels were pretreated with alkaline while the potato tubers and slices were pretreated hydrothermally since these pretreatment methods were favored for each group. All groups were fermented using conventional yeast. The maximum ethanol concentration obtained from potato tubers and slices was 64 g/L, followed by potato peels which was 9 g/L, and these results were similar to those of a laboratory scale. Further, the authors utilized potato starch waste and chips directly via simultaneous saccharification and fermentation without any additional pretreatment process. Both of them exhibited high ethanol concentrations of 50 g/L and 57.5 g/L for the starch and chips, respectively. However, the ethanol productivity of the starch (0.69 g/Lh) was low compared with the chips (2.13 g/Lh) (Felekis et al., 2023). Similarly, avocado seeds-derived starch showed a very competitive ethanol concentration and productivity of 50.94 g/L and 2.11 g/Lh to potato starch and chips, respectively, after dilute acid pretreatment and conventional yeast fermentation. Further, the authors stated that the byproducts that could inhibit the fermentation process were very low (Caballero-Sanchez et al., 2023).

Lastly, food waste was investigated using *S. cerevisiae* and mixed strains as well. In the single-strain case study, food waste was pretreated physically and biologically using milling and enzymatic, respectively. Following that, *S. cerevisiae* was employed for the fermentation in laboratory, pilot, and semi-pilot scales to compare the outcomes. Interestingly, the pilot scale resulted in the highest ethanol yield, concentration, and productivity of 0.48 g/g, 96.46 g/L, and 1.79 g/Lh, respectively, with the lowest fermentation time. The authors stated that food waste utilization can be economic. However, important factors such as nitrogen source and substrate loading must be considered since high loading of food-based sugar might inhibit or impact the fermentation process, and the absence of nitrogen in the food-based waste might require nitrogen supplementation and sugar reducing technique, respectively, prior to the fermentation process (Yan et al., 2013). In the case of mixed cultures, *S. cerevisiae, Schwanniomyces occidentalis,* and *p. stipites* were applied for food waste, particularly, solid mixtures of fruits and vegetables residues. A mild thermal pretreatment was followed initially and then fermented by the mixed cultures. However, the ethanol yield of 0.19 g/g was lower than the laboratory scale of 0.22 g/g, and lower than in the previous studies of food waste utilization. Notably, a reasonable pilot scale yield of more than 0.40 g/g indicates that the method may be scalable to a commercial scale, particularly if other parameters such as cost-effectiveness and productivity are positive (Macrelli et al., 2012).

5 Roadmap for commercialization of bioethanol with extremophiles/non-conventional microbes

One of the commercialization roadmap for bioethanol production using extremophiles and non-conventional microorganisms focuses on leveraging cell immobilization technologies to enhance productivity and fermentation efficiency under industrially challenging conditions. Extremophilic microorganisms, such as *Z. mobilis*, *S. stipitis*, and *P. tannophilus*, demonstrate unique capabilities in tolerating harsh environments, efficiently fermenting diverse substrates, and metabolizing pentose sugars. Innovative approaches like sequential fermentation and co-culture systems have achieved fermentation efficiencies comparable to or exceeding conventional systems, though productivities remain below the benchmarks of *S. cerevisiae* (Karagöz and Özkan, 2014; Kamelian et al., 2022; Song et al., 2022). Techniques such as immobilized cell have shown promise in optimizing non-conventional setups, with notable

TABLE 5 Pilot scale ethanol production with non-conventional microbes/feedstocks.

Method Category	Microbial strain	Feedstock type	Pilot Reactor size (L)	Ethanol concentration (g/L)	Ethanol yield (g/g)	Ethanol productivity (g/Lh)	References
Non-conventional feedstock, separate hydrolysis and fermentation	*S. cerevisiae*	Oil palm empty fruit bunches	350	40.6–36.9	Around 0.26	Around 0.84	Jeon et al. (2014)
Non-conventional microbes and feedstock	*S. stipitis*	Rice straw	100	n.d	0.44	0.22	Lin et al. (2012)
Non-conventional feedstock	*S. cerevisiae*	Starch of avocado seeds	40	50.94	0.45	2.11	Caballero-Sanchez et al. (2023)
Non-conventional mixed microbes and feedstock	Mixed cultures of *S. cerevisiae* *Schwanniomyces occidentalis* and *S. stipitis*	Solid standard mixture (fruits and vegetables Residues)	70	n.d	0.19	n.d.	Estrada-Martínez et al. (2019)
Non-conventional feedstock, Simultaneous saccharification and fermentation with delayed inoculation	*S. cerevisiae* Y-1693	Oat hulls	63	5.4	0.09	0.07	Skiba et al. (2017)
Non-conventional microbes and feedstock	*S. stipitis*	Addition of hydrolyzed rice straw in xylose fermentation	100	n.d	0.45	0.25	Lin et al. (2016)
Non-conventional feedstock, simultaneous saccharification and fermentation	*S. cerevisiae*	Potato peels	200	9	n.d.	0.18	Felekis et al. (2023)
		S. cerevisiae Potato tubers and slices	200	64	n.d.	1.33	
		S. cerevisiae Potato starch waste	200	50	n.d.	0.69	
		S. cerevisiae Upscaled potato chips	200	57.5	n.d.	2.13	
Non-conventional microbes and feedstock, simultaneous saccharification and fermentation	*K. marxianus*	sugarcane bagasse	100	24.6	n.d.	0.51	Lin et al. (2013)

(Continued on the following page)

TABLE 5 *(Continued)* Pilot scale ethanol production with non-conventional microbes/feedstocks.

Method Category	Microbial strain	Feedstock type	Pilot Reactor size (L)	Ethanol concentration (g/L)	Ethanol yield (g/g)	Ethanol productivity (g/Lh)	References
Non-conventional feedstock	*S. cerevisiae*	Seaweed waste	350	6.31, 4.77	n.d.	0.08, 0.06	Muryanto et al. (2024)
Non-conventional feedstock	Recombinant *S. cerevisiae* MA-R4	Milled eucalyptus	70	53.5	n.d.	0.74	Fujii et al. (2014)
Non-conventional feedstock, separate hydrolysis and fermentation	*S. cerevisiae* H058	Food waste	50, 1000	93.79, 96.46	0.47, 0.48	1.56, 1.79	Yan et al. (2013)
Non-conventional feedstock	*S. cerevisiae*	YP + salts medium with beech wood extract	20	103.3	0.48	3.9	de Vrije et al. (2024)
Non-conventional microbes and feedstock	*Spathaspora passalidarum*	YP + salts medium with beech wood extract	20	20.8	0.38	0.78	de Vrije et al. (2024)

advances like a productivity of 0.64 g/Lh achieved with immobilized *P. tannophilus* and efficiencies exceeding 90% in specific setups (Kesava et al., 1995; Abbi et al., 1996; Brethauer and Studer, 2014; Stepanov and Efremenko, 2017; Malik et al., 2020). To accelerate the commercialization of bioethanol production with extremophiles and non-conventional microbes, a potential roadmap must incorporate multi-disciplinary collaboration among academia, industry, and policymakers. Developing robust pilot-scale demonstrations to validate laboratory findings is crucial for ensuring industrial scalability. Additionally, establishing regulatory frameworks and incentivizing investments in advanced biotechnologies can help overcome financial barriers. Moving forward, the roadmap emphasizes further optimization of fermentation conditions, integration of extremophiles tailored for specific feedstocks, and industrial-scale adaptation of immobilization technologies to bridge the gap between non-conventional and conventional bioethanol production benchmarks. These efforts must be supported by comprehensive life cycle assessments to evaluate the environmental and economic benefits of non-conventional bioethanol production systems. By aligning technological innovation with policy support, the pathway to widespread adoption of these advanced methods can be effectively realized.

6 Conclusion

The recent concerns regarding food security and environmental impact are urging for using non-food feedstock for biofuel production and supported by policy update. However, using non-conventional feedstock is challenging since the well-recognized microbial strains are unable to ferment these types of sugars. As a result, non-conventional thermophiles are suggested as a sustainable alternative since their enzymes have a potential to valorize these types of sugars such as pentose in elevated temperature, potentially to be cost-effective where sterilization is eliminated. Importantly, certain engineered strains such as *Thermoanaerobacter mathranii* could have ethanol yield of 94%. However, the literature lacks scaling up and validation studies. Similarly, other non-conventional strains, particularly, *P. stipitis*, and *K. marxianus* showed the best xylose fermentation and fast growth compared with *S. cerevisiae*, respectively, along with ethanol production. However, they are less resistant to ethanol, making them less attractive. Therefore, new techniques such as genetic engineering which its tools are available for many strains such as *K. marxianus*, co-culture, immobilization, and pretreatment selection could overcome or improve the tolerance along with ethanol yield improvement. Notably, co-culture could improve ethanol yield by 440% with some strains. Yet, more research should be conducted on large scales. Additionally, the literature lacks very important data that are related to a single strain, co-culture, or immobilized cells in biorefinery. For instance, *K. marxianus* is well-known for its potential to produce multiple valuable products and its co-culture with *Bacillus coagulans* could improve lactic acid and ethanol by 90%.

Feedstock availability and its pretreatment which accounts for up to 40% of ethanol production are other challenges. Unfortunately, feedstock availability is not reported properly including in the developed countries, while the novel eco-friendly pretreatment techniques are not scaled and/or formulate inhibitors, making the commercialization of the advanced ethanol very challenging and less viable. This paper provides a comprehensive review of the current advancements, challenges, and future directions for leveraging extremophiles and non-conventional microorganisms in bioethanol production. It serves as a valuable resource for researchers, industry stakeholders, and policymakers to drive innovation and collaboration, ultimately accelerating the transition to sustainable energy solutions.

Author contributions

MA-H: Data curation, Investigation, Visualization, Writing – original draft. GA: Investigation, Writing – original draft. FM-G: Investigation, Writing – original draft. JM-G: Investigation, Writing – original draft. TK: Investigation, Writing – original draft. MG: Conceptualization, Supervision, Validation, Writing – review and editing.

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