CRITICAL REVIEW

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Advancing bioethanol: exploring feedstock diversity, production pathways, and environmental implications

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The depletion of finite fossil fuel reserves, coupled with the ever-growing global demand for energy, has raised significant concerns about the long-term sustainability of fossil fuel consumption. As these conventional energy sources become increasingly scarce, the need for viable alternatives has become more urgent. This pressing challenge has driven researchers, policymakers, and industries to explore and develop sustainable energy solutions that can reduce dependence on fossil fuels. Bioethanol, a sustainable substitute for gasoline, is produced globally, supporting economic growth in both developed and developing nations. It is derived from a wide range of feedstocks, including industrial waste and by-products like steel mill gases and glycerol from biodiesel production. Different bioethanol generations vary in technological readiness and environmental impacts, assessed using Life Cycle Assessment (LCA). This review explores the environmental consequences of different bioethanol production pathways, with a focus on advances in biotechnological methods. It also highlights the potential of lignin-rich biomass, which has been challenging to process but offers significant promise. The review underscores the importance of transparency in biorefinery LCA to fully understand the various environmental impacts. Additionally, it examines the role of genetic engineering, enzyme technologies, and government policies in promoting sustainable bioethanol production. Integrating bioethanol production with green chemistry and circular economy principles can strengthen its position in the bioeconomy, delivering long-term benefits to both the biofuel sector and society at large.

Sustainability spotlight

The transition to sustainable biofuels is critical to reducing dependence on finite fossil resources and mitigating climate change. This review highlights advancements in bioethanol production by exploring diverse feedstocks, including lignin-rich biomass and other industrial by-products, contributing to circular bioeconomy principles. The integration of genetic engineering, enzyme technologies, and green chemistry enhances process efficiency while minimizing environmental trade-offs. Transparent life cycle assessments (LCAs) are emphasized to ensure an accurate evaluation of sustainability metrics, including biogenic carbon storage. This work aligns with UN SDGs 7 (Affordable and Clean Energy), 12 (Responsible Consumption and Production), and 13 (Climate Action) by promoting renewable energy, resource efficiency, and reduced carbon emissions, fostering a more sustainable biofuel industry.

1. Introduction

Bioethanol, recognized as a renewable and sustainable biofuel, has garnered considerable attention as a viable alternative to fossil fuels in global efforts to mitigate greenhouse gas (GHG) emissions and combat climate change. As a biofuel, bioethanol is primarily produced through the fermentation of sugars derived from various feedstocks, ranging from food crops to agricultural residues and dedicated energy crops, with a technology readiness level (TRL) of 8–9.[1] The ongoing research into alternative feedstocks such as algae, genetically modified (GM) crops, industrial gas effluents, glycerol, and direct bioethanol-producing microbes, with TRLs ranging from 2 to 8, is evidence of the dynamic nature of bioethanol production.[1] The versatility of bioethanol production, coupled with its potential environmental benefits, positions it as a critical component of current and future energy needs. However, the production and utilization of bioethanol require careful assessment to ensure they deliver genuine environmental and economic advantages.

The selection of feedstocks is a crucial factor influencing the overall sustainability of bioethanol production. Feedstocks can be categorized into first-generation (*e.g.*, sugarcane, corn), second-generation (*e.g.*, lignocellulosic biomass (LCB), chitin, glycerol), third-generation (*e.g.*, algae and seaweed), and fourth-generation (GM feedstocks),[2–4] as summarized in the supplementary information (Table S1). Each type of feedstock presents unique advantages and challenges. For instance, while first-

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generation feedstocks are well-established and yield high bioethanol outputs, they often compete with food resources and may contribute to food insecurity.[5,6] Second-generation feedstocks offer greater sustainability potential since they use waste materials and non-food crops. However, their economic viability is hampered by the high costs of the processing technologies, which are still under development to reduce pretreatment expenses.[7–9] Dedicated energy crops under second-generation, explicitly grown for biofuel (biodiesel or bioethanol) production, have higher adaptability and biomass yield per hectare.[10–14] Examples include energy cane (sugarcane bred for higher fiber content), jatropha and camelina (oil-rich seeds for biodiesel, with remaining biomass for bioethanol), and grasses like napier grass, switchgrass, giant reed, and miscanthus, known for their high biomass yield and adaptability to various climates.[15] Third-generation feedstocks (summarized in Table S2 of SI) present

a promising frontier due to their high yield potential and minimal land use, yet their commercial viability is still being explored.[16] GM feedstocks are developed to maximize bioethanol yields using advanced biotechnological techniques that reduce lignin content while maintaining or improving other key agronomic traits to boost carbohydrate content.[15,17] This innovation aims to enhance bioethanol production efficiency and economic viability, though it presents potential environmental risks if not managed according to proper protocols.[18,19] Other unconventional feedstocks, such as industrial gas effluents and glycerol from biodiesel production facilities, offer innovative opportunities for bioethanol production, contributing to a circular economy by reducing waste.[20,21]

Although there are many pathways suitable for different feedstocks for the production of bioethanol, making the proper selection is challenging due to various factors like regional availability, ease of processing, economic factors, yield, and TRL. Bioethanol yield from various feedstocks is typically evaluated using two different metrics. The first expresses yield as liters of bioethanol produced per ton of biomass feedstock (L ton^{-1}, as shown in Table 1), reflecting conversion efficiency and process performance. The second measures bioethanol output (generally in liters) per hectare of cultivated land (kL ha^{-1}, as shown in Fig. 1), which integrates conversion efficiency, process performance, and the crop's agronomic productivity. Further, assessing the overall impact of a production pathway on the environment, from raw material extraction to processing, distribution, use, and disposal of the product, is vital for selecting a bioethanol process. LCA is a detailed methodology used to assess the overall environmental impacts throughout a product's life.[22] In bioethanol production, LCA is instrumental in evaluating and comparing the environmental advantages and trade-offs of various feedstocks and production processes.[23] It involves analyzing energy usage, GHG emissions, land-use changes (LUCs), water consumption, and other environmental effects.[24,25] Using LCA, stakeholders can make informed choices regarding the most sustainable and efficient feedstocks and production methods.

While several studies have explored various feedstocks for bioethanol production and their environmental impacts through LCA, no study has comprehensively addressed both feedstocks and their life cycle analysis in a unified manner. For instance, Uppalapati et al.[37] focused solely on the LCA of sugarcane molasses, while Osman et al.[38] broadened their scope to bioenergy as a whole, leaving bioethanol-specific discussions incomplete. Moreover, newer and more sustainable feedstocks were not explored in these studies, creating a gap in the literature. Zhan et al.[39] analyzed the environmental impact of cassava-based bioethanol production, but their focus was limited to first-generation bioethanol and a specific geographic context. Bernstad Saraiva[40] reviewed LCA studies in biorefineries, focusing mainly on feedstock provision and system boundary issues without delving into specific bioethanol feedstocks. Jain et al.[1] examined various feedstocks across multiple bioethanol generations, highlighting their technological readiness and economic aspects, but neglecting environmental

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Table 1 A summary of the current status of the production of different generations of bioethanol. L: liter

Generation	Intermediate processing	Yield (L ton^{-1})	Cost (US$ per gal)	TRL	Ref.
First	Milling, enzymatic hydrolysis, fermentation, distillation	70–590	~0.9	9	1 and 26–28
Second[a]	Route-1: pretreatment, enzymatic hydrolysis, fermentation, distillation Route-2: gasification, preconditioning, fermentation, distillation	40–350	~1.5	8–9	1, 7, 28 and 29
Third	Cultivation, harvesting, lipid extraction, fermentation, distillation	70–660	—	4–6	1, 30 and 31
Fourth	Novel pretreatment, engineered microorganisms/substrates, fermentation	Data not fully established[b]	—	2–3	1

[a] Excluding unconventional feedstocks like glycerol. [b] Highly variable and depends on the specific genetic modifications and synthetic biology strategies employed. However, theoretical yields suggest improvements over previous generations.

impact. Liu *et al.*[41] presented an LCA review on waste-feedstock biorefineries with a broader focus on various biofuels, diverting attention from bioethanol production.

This study contributes significantly to the existing literature by addressing gaps in current reviews and providing a comprehensive overview of bioethanol synthesis. It critically evaluates various feedstocks for bioethanol production, considering their suitability, associated yields, processing technologies, and TRL. A distinctive feature of this review is the tabulated comparison of the key intermediate steps, namely hydrolysis and fermentation, which play a crucial role in bioethanol production. The review also highlights the latest advancements, efficiencies, and challenges in the bioethanol production landscape. It goes further by examining the economic and policy factors that are often overlooked in other reviews. In particular, it underscores the importance of policies that promote sustainable practices and provide consistent incentives to ensure the long-term viability of bioethanol as a renewable energy source. A significant emphasis of the study is placed on the environmental impacts of bioethanol production, assessed through LCA. This approach enhances understanding of the bioethanol production process and aids in making informed decisions for researchers, engineers, and policymakers working to advance bioethanol technologies.

The review is organized as follows: Section 1 introduces the topic, outlining the scope and objectives of the review on sustainable bioethanol production. Section 2 examines current bioethanol production technologies from various feedstocks, highlighting advancements and challenges. Section 3 provides a detailed analysis of LCA studies on bioethanol production, assessing environmental impacts and identifying key factors influencing sustainability. Section 4 explores the economics and policies related to bioethanol production, including cost analysis and supportive policy frameworks. Section 5 integrates the findings, offering insights and strategies to improve the sustainability of bioethanol production. Section 6 concludes the review, emphasizing the outcome of the review. Through a comprehensive analysis of bioethanol production from various feedstocks and their environmental impacts, this review seeks to advance the dialogue on sustainable bioenergy and guide the development of more sustainable bioethanol production practices.
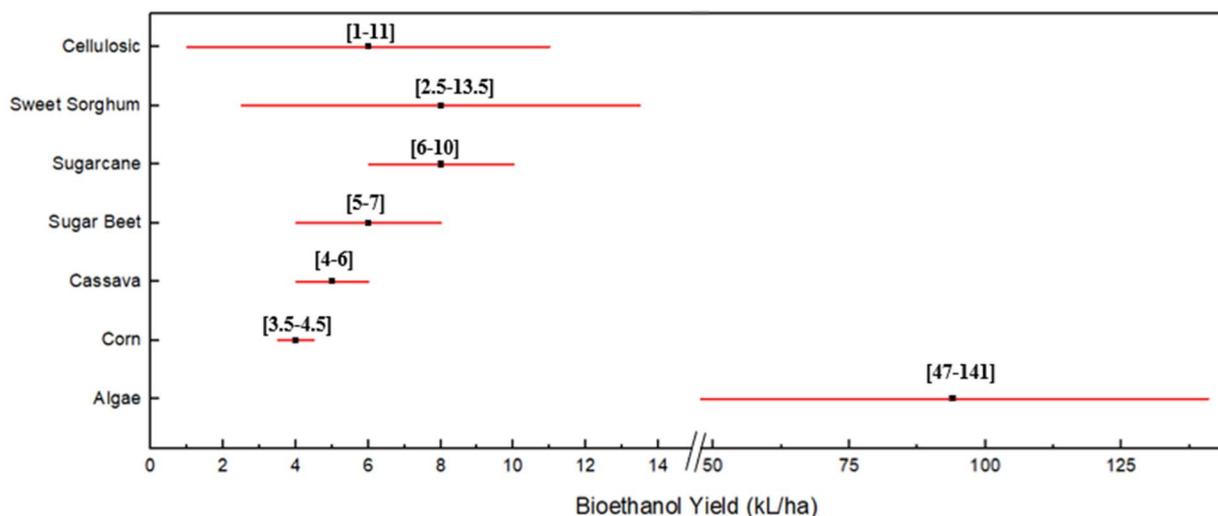

Fig. 1 Ethanol yield per hectare (this figure was drawn based on data provided in ref. 32–36).

2. Advancements in bioethanol production pathways

There are numerous feedstocks available for bioethanol production, as discussed in the supplementary information (Table S1). Various processing pathways can convert these carbon-rich feedstocks into bioethanol, as illustrated in Fig. 2. Typically, these feedstocks go through multiple processing stages, which depend on the biomass composition, including the weight percentages of carbohydrates, lignin, and other components such as ash, proteins, and oil, as well as the degree of polymerization of the carbon source. These include mono-saccharides (such as glucose, mannose, fructose, rhamnose, galactose, and others), disaccharides (like sucrose, lactose, maltose, and cellobiose), oligosaccharides like N-acetyl-D-glucosamine, and polysaccharides (such as starch, cellulose, and hemicellulose). In recent years, various waste streams from industries,[42] including effluent gases[43] and atmospheric carbon,[44] have been explored for bioethanol production potential. This section examines the significant advancements in the pathways for converting different feedstocks into bioethanol.

2.1 Advancements in biochemical conversion pathways

Bioethanol production through biochemical pathways involves several key steps applicable to all four generations of biomass feedstocks. The key conversion steps in bioethanol synthesis are hydrolysis and fermentation, which include using biological enzymes and microbes under mild processing conditions to convert the substrate into desired products.[45] The primary cost involved with hydrolysis and fermentation is due to the microbes and enzymes used,[46] as the operating conditions are close to ambient. First-generation feedstocks, which include sugar-based feedstocks, contain readily available monomeric and dimeric sugars that can be fermented to bioethanol using either fungal or bacterial fermenting microbes.[47] However, starch-based crops require additional processing steps to extract the starch through wet or dry milling.[48] The extracted starch is then hydrolyzed using the enzyme amylase, followed by fermentation to produce bioethanol.[49,50] The byproduct streams from wet milling, dry milling, and oil crop processing (such as hulls, DDG, or oilcake) are rich in lignocellulosic content. They are, therefore, used as second-generation feed-stocks.[14,51,52] However, their nutritional quality makes them more suitable as livestock feed.[53] Oil from the germ part of starchy crops and oil crops is transesterified into biodiesel,[54] and the byproduct stream (glycerol) has also been shown to have the potential for conversion to bioethanol,[55] thus being categorized as a second-generation feedstock.

Second-generation feedstocks require a crucial pretreatment step not needed for first-generation feedstocks.[7,56] These are generally categorized into four main types, with emerging innovations such as hybrid pretreatment and nanotechnology interventions (summarized in Fig. 3). The discussion about the
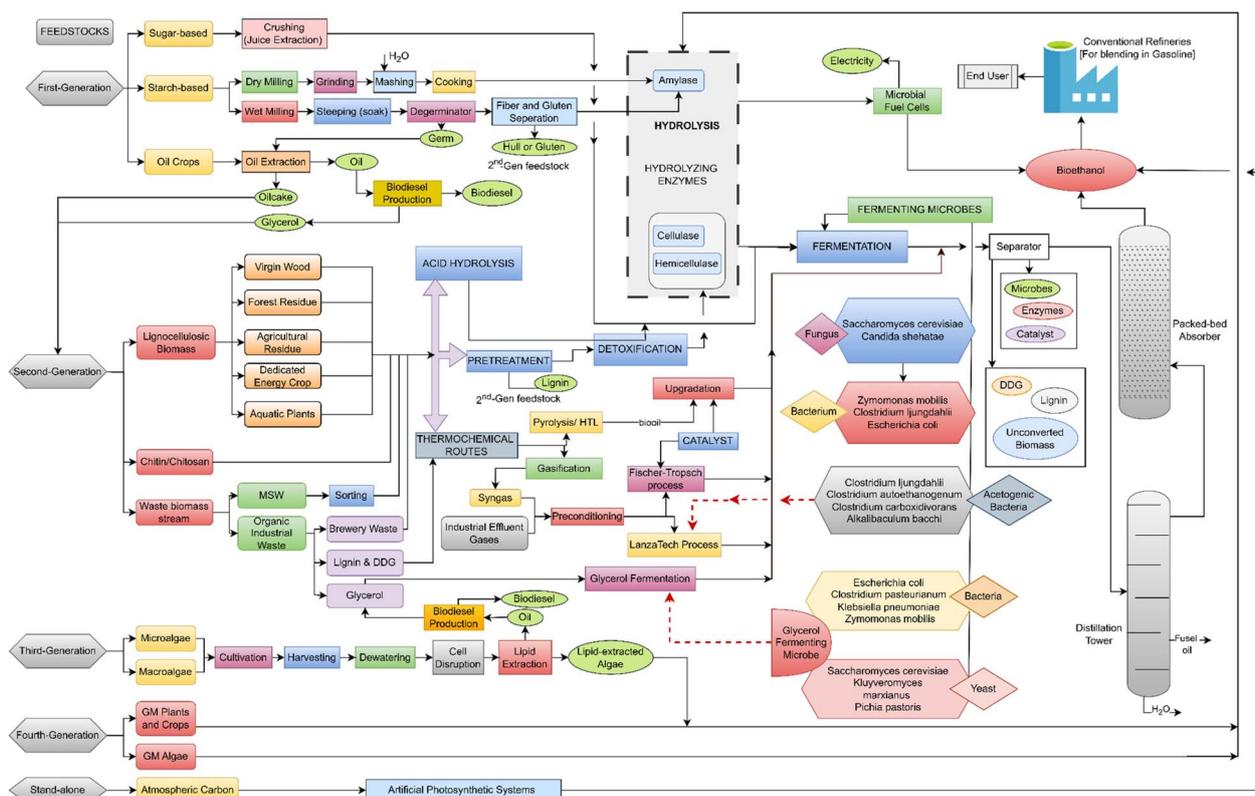

Fig. 2 Comprehensive overview of bioethanol production pathways from various raw materials. DDG: dry distillers' grains; GM: genetically modified.
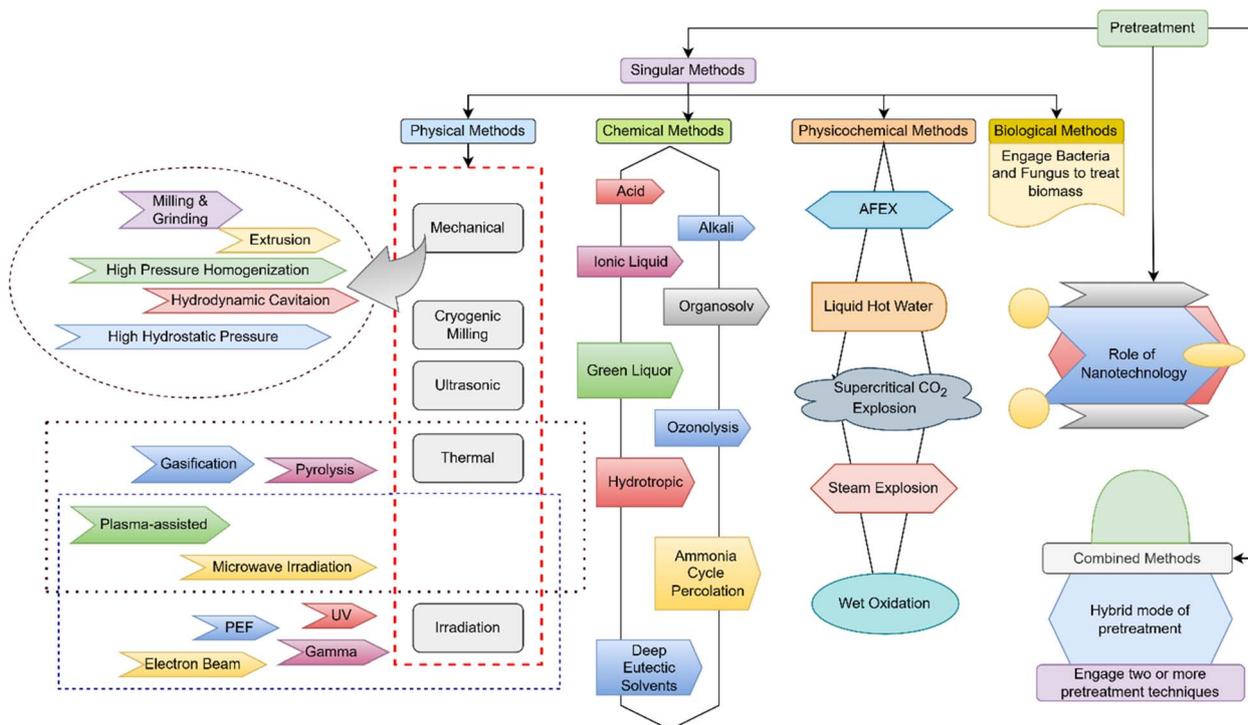
Fig. 3 Various pretreatment techniques for second-generation feedstocks. Reproduced from our previous work in *Sustain. Chem. Clim. Action*, 2024, **5**, 100053 (ref. 57) under a CC-BY license agreement.

pretreatment methods is beyond the scope of this review, but has been extensively covered in other studies.[57,58] Feedstocks such as LCB, chitin, chitosan, dry distillers' grains (DDG), oil-cake, and brewery waste undergo pretreatment to fractionate lignin and concentrate the cellulosic and hemicellulosic substrates.[59,60] This is followed by detoxification to remove toxicants such as hydroxymethylfurfural, acetic acid, and furfurals, which inhibit enzyme and microbial activity during hydrolysis and fermentation.[61,62] However, municipal solid waste (MSW) is initially sorted to gather only organic-rich material to ensure higher productivity and feed consistency.[63] The detoxified feed, from LCB or MSW, is hydrolyzed using cellulase and hemicellulase enzymes to convert cellulose and hemicellulose into fermentable sugars, followed by fermentation.[64,65] In some cases, acid hydrolysis is preferred over enzymatic hydrolysis for second-generation feedstocks,[66] followed by detoxification and fermentation, as depicted in Fig. 2.

Third-generation algal biomass feedstocks are grown in various open pond systems or more advanced photobioreactors, each with advantages and disadvantages.[67,68] After cultivation, the algae are harvested and dewatered, a process associated with high drying costs. Next, the cell walls are broken down to extract lipid content, leaving behind carbohydrate-rich feed-stocks that are hydrolyzed and fermented.[69] However, the lipid fraction is used in biodiesel production, and the glycerol side stream is used for bioethanol production, similar to the case with oil crops. Genetically engineered plants, crops, and algae are designed to have high carbohydrate content and are classified as fourth-generation feedstocks. These can be hydrolyzed

and fermented, producing higher bioethanol yields than previous generations.[18,70,71]

The various hydrolysis and fermentation pathways for bioethanol production, each with its own advantages and disadvantages, are shown in Table 2. Direct Fermentation (DF) is the most straightforward process with minimal processing steps, and it is best suited for first-generation sugar-based feedstocks.[72,73] Separate Hydrolysis and Fermentation (SHF) needs to be optimized for both hydrolysis and fermentation stages separately, and it takes a longer processing time.[109] Contrary to SHF, we have Simultaneous Saccharification and Fermentation (SSF), which reduces process time, is carried out in a single reactor, and potentially offers higher yields. Yet, it requires compatible enzymes and microbes, making it difficult to optimize both stages simultaneously.[75,80–82] Semi Simultaneous Saccharification and Fermentation (SSSF) is carried out in a single reactor for a set time for both hydrolysis and fermentation. However, SSSF gives a better yield than SSF, but the process control is more complex.[110] Solid-state Fermentation (SF) has low water requirements and is suitable for certain biomass types, but offers lower productivity.[85–87] Non-isothermal Simultaneous Saccharification and Fermentation (NSSF) optimizes temperature for each step, potentially increasing efficiency, but requires complex temperature control and higher energy.[111,112] Simultaneous Saccharification and Co-fermentation (SSCF) pathway is efficient for mixed sugar substrates, yielding higher bioethanol outputs.[88,90–93] Simultaneous Saccharification, Filtration, and Fermentation (SSFF) integrates filtration to reduce inhibitors, improving efficiency,

Table 2 Hydrolysis and fermentation (HnF) pathways utilized for different substrates from varied biomass sources for bioethanol production

Fermentation route	Feedstock	Overview	Pros	Cons	Ref.
DF	MDS & glycerol	Fermenting microbes directly convert the substrate to bioethanol	No complex hydrolysis step is needed	Substrate may inhibit microbes	72–74
SHF	PCs	PCs are hydrolyzed to MSs, followed by fermentation	HnF: operated individually at optimum conditions	Time-consuming and expensive; cellulase inhibition by sugars	75–79
SSF	PCs	Simultaneous HnF prevent sugar build-up and prevent cellulase inhibition	HnF carried out in a single reactor; better bioethanol yield	Operating reaction temperature may not be optimal for cellulase or yeast	75 and 80–82
SSSF	PCs	Similar to SSF with additional pre-saccharification using enzymes	Lower enzyme loading compared to SSF; pre-saccharification results in higher bioethanol yield	Higher operational complexity; longer process time	83 and 84
SF	PCs	Microbes thrive on solid, low-moisture substrates	No water required that reduces bacterial contamination and effluent generation; reduced space and energy requirements; consistent product formation	Smaller substrates hinder microbial growth due to limited aeration and heat dissipation	56 and 85–87
NSSF	PCs	Simultaneous hydrolysis (50 °C) and fermentation (30 °C) in separate reactors at respective optimals	Higher productivity, unlike isothermal SSF; requires fewer enzymes (30–40%) than SSF	Higher fixed capital cost compared to SSF	66, 81, 88 and 89
SSCF	PCs	Co-fermentation of xylose and glucose. Slow glucose release during hydrolysis maintains a high xylose/glucose ratio, compelling fermenting microbes to prioritize xylose consumption	Cost-effective as xylose is also converted to give high bioethanol yield; lowers inhibitory effect of xylose on fermenting microbes	Microorganisms will consume ample glucose in the broth, preventing co-fermentation	88 and 90–93
SSFF	PCs	Enzyme recycling post-filteration back to hydrolysis, while fermenting microbes are retained using flocculation and settling	Fermenting microbes and enzymes are reused; operates at optimum conditions; prevent enzymes inhibition, unlike in SSF	More complex than SSF due to an additional filtration step	89 and 94
CBP	PCs	Microbes directly convert biomass to bioethanol in a single reactor by releasing specific enzymes	Single microbe performs enzyme synthesis, along with HnF; enzyme inhibition by sugar is avoided; reduces capex and opex; no costly enzymes needed	Under research phase; lower process efficiency; GM of microbes could enhance productivity	92 and 95–98
VHG	MDS	Conventional process: normal to high sugar concentration. Here, >240 g L^{-1} sugar concentration is maintained (very high gravity)	High bioethanol yield (15 vol%) than conventional yield (<10 vol%); lower production cost, energy consumption, and effluents	Multiple stresses in yeast due to metal ions; yeast inhibition by product; lower pH below optimum; temperature rise	99–103
SGF	Syngas[a]	Wood–Ljungdahl pathway, employing acetogenic microbes under anaerobic conditions	Industrial exhausts rich in CO and H_2 can serve as feedstock; lower temperature and pressure requirement; tolerate sulfur in feed with no specific CO to H_2 ratio	Gas-liquid mass transfer resistance; lower productivity; microbes inhibition	104–108

[a] Typical composition: 30–60% CO, 25–30% H_2, 5–15% CO_2, and 0–5% CH_4; DF: direct fermentation; SHF: separate hydrolysis and fermentations; SSF: simultaneous saccharification and fermentation; SSSF: semi simultaneous saccharification and fermentation; SF: solid-state fermentation; NSSF: non-isothermal simultaneous saccharification and fermentation; SSCF: simultaneous saccharification and co-fermentation; SSFF: simultaneous saccharification, filtration, and fermentation; CBP: consolidated bioprocessing; VHG: very high gravity fermentation; SGF: syngas fermentation; MDS: monomeric and dimeric sugars; PCs: polymeric carbohydrates (e.g., starch, hemicellulose, and cellulose); MSs: monomeric sugars.

but involves higher operational complexity and potential membrane fouling.[89,94] Consolidated Bioprocessing (CBP) is a novel approach to bioethanol production. It is the best example of process integration and optimization, combining enzyme production, biomass hydrolysis, and fermentation in a single step.[97] This integration reduces the need for added enzymes and pretreatment steps, significantly reducing costs and process complexity. However, it requires highly efficient and robust microbes that can streamline all three processes: enzyme production, saccharification, and fermentation.[92,95–98] Very High Gravity Fermentation (VHG) results in higher bioethanol concentrations, reducing distillation costs, but imposes higher osmotic stress on cells, demanding robust microbes.[99–103] Lastly, it is essential to study bioreactor configurations in bioethanol production as it allows the optimization of yield, productivity, resource efficiency, process stability, scale-up feasibility, economic viability, and product quality, all of which are critical for the success and sustainability of bioethanol production processes, summarised in the supplementary information (Table S3).

The bioethanol produced from microbial fermentation is a lean solution containing less than 10 vol% bioethanol in water, as high ethanol concentrations cause product inhibition to fermenting microbes during fermentation.[113] However, novel strains of fermenting microbes have been developed to tolerate higher ethanol concentrations.[114] Divate *et al.*[115] performed metabolic engineering on the yeast *Saccharomyces cerevisiae* and produced a strain that demonstrated tolerance to ethanol concentrations as high as 14 vol%, whereas the growth of the wild strain was inhibited at 6 vol%. Finally, the lean bioethanol solution is concentrated and purified through distillation and dehydration to achieve fuel-grade standards.[116] Table 3 provides the recent advancements in bioethanol production and their yield through various techniques and innovations.

2.2 Advancements in thermochemical conversion pathways

The thermochemical conversion of biomass to bioethanol is a two-step process involving high temperatures.[127] Initially, biomass is converted into gas or bio-oil, which is then transformed into bioethanol *via* fermentation[128] or catalytic synthesis.[129,130] Gasification, pyrolysis, and hydrothermal liquefaction are employed to gasify or liquefy the biomass.[127] In gasification, biomass is converted to syngas (a mixture of CO, H_2, and CO_2) by gasifying the raw material at high temperatures (500–1200 °C) with controlled oxygen and/or steam.[131] The raw syngas and industrial effluent gases from steel mills or refineries contain impurities and undergo deoxygenation, tar removal, particulate and sulfur compound removal, and compression.[3] In the next stage, direct gas fermentation (similar to the LanzaTech process[132]) uses acetogenic bacteria like *Clostridium ljungdahlii*,[105] *Clostridium autoethanogenum*,[133] *Clostridium carboxidivorans*,[134] *Butyribacterium methylotrophicum*,[135] and *Alkalibaculum bacchi*,[136] leveraging their unique metabolic pathways to convert syngas into bioethanol efficiently.[104–108] It is compared with other fermentation technologies (Table 2) that vary in process complexity, efficiency,

and feedstock suitability. *Clostridium ljungdahlii* produced 198.76 liters of bioethanol per ton of syngas in a two-stage continuous fermentation process.[105] Alternatively, catalytic conversion involves passing cleaned syngas over a catalyst bed, typically composed of metals like copper, zinc, or their alloys supported on materials such as alumina or silica, including processes like Fischer–Tropsch synthesis[137] or catalytic methanol synthesis followed by methanol-to-ethanol conversion.[138] These versatile processes can utilize a variety of feedstocks, including LCB, MSW, and industrial byproducts such as DDG, lignin, or hulls. LanzaTech employs a proprietary strain of *Clostridium autoethanogenum*, genetically engineered to optimize the conversion of industrial waste gases (containing CO & CO_2) into bioethanol and valuable chemicals like 2,3-butanediol.[139] Ongoing research focuses on optimizing microbial and catalytic performance, improving yields, and enhancing the industrial viability of these processes to make bioethanol production more efficient and sustainable.

Another thermochemical conversion pathway is pyrolysis, which thermally degrades biomass without oxygen at temperatures between 400 and 600 °C, producing bio-oil, syngas, and char.[140] The bio-oil is then processed through catalytic upgrading or fermentation to produce bioethanol.[141] Similarly, hydrothermal liquefaction (HTL) converts wet biomass into bio-crude oil using high pressure (200–350 bar) and moderate temperatures (250–350 °C) in the presence of water.[142] The bio-crude is refined and catalytically upgraded to produce bioethanol, much like pyrolysis. Algal biomass, sewage sludge, and wet agricultural residues with high moisture content are particularly suitable for HTL, as they eliminate the need for costly drying, like in the biochemical conversion pathway.[143]

2.3 Advancements in non-conventional pathways

2.3.1 Microbial and chemical lignin degradation. Lignin presents a formidable challenge due to its diverse structure and resistance to degradation.[144] Nonetheless, recent advancements in biotechnological and chemical processes have significantly enhanced the efficiency of converting lignin into bioethanol. Certain microorganisms, including white-rot fungi like *Phanerochaete chrysosporium*[145] and *Trametes versicolor*,[146] bacteria such as *Pseudomonas putida*, and select species of Clostridium,[147] as well as various other genetically engineered microbes, have demonstrated the capability to degrade and/or metabolize lignin-derived compounds (either by thermal, chemical, or biological routes), such as aromatic substances, directly into bioethanol and other biofuels.[148,149]

Salvachúa *et al.*[147] recently showed that lignin with high molecular weight can be broken down into smaller oligomers and monomers using microbes' extracellular ligninolytic enzymes, such as laccases and peroxidases, through biological conversion pathways. Certain microbes can metabolize these lower molecular weight aromatic compounds (derived from lignin) and convert them into triacylglycerides,[150,151] or polyhydroxyalkanoates,[148] depending on the specific microbe. Triacylglycerides can be further processed into bioethanol, as discussed in other studies.[152,153] Several microbes, including

Table 3 Comparative analysis of bioethanol production from diverse biomass feedstocks: yields, methodologies, and innovations. SHF: separate hydrolysis and fermentation; SSF: simultaneous saccharification and fermentation

Raw material	Yield (L ton^{-1})	Methodology	Advancement/innovation	Ref.
Sorghum juice and sugarcane molasses	560[a]	Direct fermentation using yeast	Integrated biorefinery using a mix of juice and molasses	117
Water hyacinth	—	SSF without pretreatment	Bacterium or fungus-assisted fermentation without pretreatment	118
Soybean waste	550	Cell adhesion on soybean meal-coated (3D printed templates)	Recyclable 3D-printed systems; stable yields after 30 cycles of reuse	119
Oak	160	Steam explosion followed by SHF	Explored trade-offs between yields and pretreatment costs	120
Poplar	140			
Spruce	60			
Rice straw	300	Chemical pretreatment (H_3PO_4 and H_2O_2) followed by SHF	Mesophilic Aspergillus fungi showed high cellulase activity	121
Wheat straw	630	Acid hydrolysis, membrane-based acid recovery, fed-batch fermentation	Glucose yield (>90%) using fractional acid hydrolysis technology	122
Chitin	770[b]	Solid-state fermentation with *Pleurotus ostreatus*	Chitin to bioethanol and mushroom production	123
Glycerol	320	Direct microbial fermentation	Microbes from an anaerobic digester (wastewater treatment) convert glycerol to bioethanol	124
Kappaphycus alvarezii (red algae)	340	Fungal pretreatment followed by SHF	Fungal pretreatment increased sugar yields 2.3-fold and bioethanol yield by 38.23% at a lower cost	125
Genetically modified rice straw (Cesa7 mutant)	770[c]	Green liquor pretreatment followed by SHF	GM rice straw cellulose nanofibrils enhanced bioethanol yield and reduced production cost	126

[a] Bioethanol yield in L ton^{-1} of total reducing sugars in hydrolysate. [b] Theoretical yield. [c] Bioethanol yield in L ton^{-1} of cellulose obtained from rice straw.

Pseudomonas putida, *Rhodococcus opacus*, *Pseudomonas fluorescens*, and *Acinetobacter baylyi*, have shown potential for the valorization of lignin-derived aromatic monomers.[147,148,150,151,154] Zhao *et al.*[155] demonstrated that adding commercial laccase to degrade Kraft lignin into monomers, which were then consumed by *Rhodococcus opacus*, produced 145 mg of triacylglycerides per liter of solution. These ligninolytic microbes break down complex lignin substrates and catabolize the resulting monomers into target compounds such as bioethanol.

There are also chemical methods for processing monomeric lignin-derived compounds. Aromatic compounds can be transformed into cyclohexanol through hydrodeoxygenation, which involves reacting these compounds with hydrogen gas under controlled conditions, typically at temperatures between 100 and 250 °C and pressures of 10–50 atm, using catalysts.[156–159] Typical catalysts include metals like nickel (Ni), palladium (Pd), platinum (Pt), and ruthenium (Ru), which facilitate the hydrogenation of aromatic rings.[159] Once cyclic alcohols like cyclohexanol are produced, they are mixed with low-grade hydrous bioethanol for use in spark-ignition gasoline engines by combining gasoline, low-grade bioethanol, and cyclohexanol.[160–162] This oxygenated additive enhances performance and reduces emissions.[160] At the same time, biological methods tend to produce lower bioethanol yields from lignin due to less direct conversion. Chemical methods, while more efficient, often involve high energy demands and added complexity since they do not directly yield bioethanol.

2.3.2 Microbial glycerol fermentation. Glycerol ($C_3H_8O_3$), or glycerin, is a byproduct of biodiesel production resulting from the transesterification of triglycerides found in vegetable oils or animal fats.[163] Depending on its origin and purity level, glycerol may need pretreatment to remove impurities that could negatively impact fermentation, making it suitable as a feedstock for microbial processes.[164] Common microorganisms used are GM bacteria (such as *Escherichia coli*, *Clostridium pasteurianum*, *Klebsiella pneumoniae*, and *Zymomonas mobilis*)[74,165–167] and yeasts (like *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*, and *Pichia pastoris*)[168–170] that possess the metabolic pathways necessary for efficient glycerol conversion into bioethanol. Genetic engineering has improved these microbes for better glycerol utilization and higher bioethanol production rates.[168] The choice of microbes depends on factors like substrate concentration, pH, temperature, and environmental conditions, all of which affect fermentation efficiency.[153] The process typically starts with glycerol being converted into pyruvate through glycolysis, followed by bioethanol production *via* specific fermentation pathways of the microorganisms.[171] Depending on the method and microbe used, byproducts like acetate, lactate, or succinate may also be generated, which can be processed or repurposed for other industrial uses to improve overall efficiency and sustainability.[172] Liu *et al.*[173] reported the highest bioethanol yield of 0.27 g g^{-1} of glycerol by engaging the *Pachysolen tannophilus* fermenting microbe. Khattab *et al.*[174] reported a bioethanol yield of 0.47 g g^{-1} of glucose and glycerol

mixture by engaging engineered yeast *Saccharomyces cerevisiae*. Research continues to optimize these yields to make bioethanol production viable at the industrial level.

2.3.3 Microbial fuel cells (MFCs). Microbial fuel cells are bioelectrochemical systems that leverage microorganisms' catabolism to convert the chemical energy found in organic compounds into electrical energy.[175] In bioethanol production, MFCs offer an innovative approach by combining the microbial conversion of organic materials with electricity generation, all while minimizing GHG emissions.[176,177] These cells generate low to moderate electrical power, which is affected by microbial activity, substrate concentration, electrode design, and operating conditions.[177–179] The bioethanol yield from MFCs is influenced by how effectively microbes metabolizes substrates and the availability of appropriate feedstocks.[180–182] MFCs can utilize various organic substrates, including sugars like glucose, organic acids, and even ethanol.[183] For bioethanol production, LCB serves as an effective feedstock, which can be enzymatically broken down to release sugars that microorganisms in the MFC can then ferment into bioethanol. MFCs use microbial metabolism to oxidize organic compounds, releasing electrons that are transferred to an anode and generating an electric current.[184] At the cathode, these electrons combine with protons and an electron acceptor, such as oxygen, completing the electrochemical circuit and forming water or other reduced products.[185,186]

2.3.4 Artificial photosynthetic systems (APS). This cutting-edge method for bioethanol production is categorized as fourth-generation bioethanol production.[187] Unlike traditional algae cultivation and gas fermentation processes that rely on carbon-rich gas sources, this approach utilizes atmospheric CO_2 and H_2O.[188] These systems mimic natural plants, which convert sunlight into carbohydrates and lipids, but instead, they directly generate bioethanol. They consist of a carefully designed assembly of components housed in a reaction chamber known as photoelectrochemical cells (PECs) aimed at maximizing bioethanol yields.[189] Semiconductor materials like titanium dioxide serve as photocatalysts to capture sunlight and create electron–hole pairs.[190] These are enhanced by organic dyes or metal complexes that act as photosensitizers, efficiently absorbing light.[191–193] The solar energy harnessed by these components is then transferred to catalytic sites to drive reduction reactions. In PECs, the anode facilitates the oxidation of water to oxygen, while the cathode enables the reduction of CO_2 to bioethanol or other intermediates.[194] Specific enzymes and engineered microbes, which function as biocatalysts, are immobilized on the electrode surface to improve the selectivity and efficiency of CO_2 reduction to bioethanol.[195,196] This multi-step process involves creating intermediates like formate and carbon monoxide, which can be further converted chemically or biologically into bioethanol, often employing engineered microbes or specialized enzymes.[196]

3. Comparative life cycle assessment of various bioethanol generations

Several intermediate processing steps are required for bioethanol production, as discussed earlier. A comprehensive

LCA is essential to evaluate and optimize the sustainability of bioethanol production, guiding future research, policy development, and industrial practices toward more sustainable bioenergy solutions. This assessment must encompass all stages of production, from cultivating feedstocks to their final use and disposal. This section reviews the life cycle impact of first and second-generation bioethanol production. The limited literature on third-generation biorefineries focuses on LCA for micro and macroalgae growth and non-energy applications.[197,198] When energy generation is considered using algal feedstock, it often focuses on biodiesel due to the higher lipid content.[199–201] Third and fourth-generation bioethanol production, at lower TRL, lacks substantial data on environmental impact. This section provides a holistic understanding of LCA, encapsulating different feedstocks and system boundaries related to those biorefineries.

Various feedstocks are compared using different environmental impact parameters across the entire value chain, product, or service, as shown in Tables 4 and 5. All values in the tables are based on the production of one kg of bioethanol (functional unit chosen). Many system boundaries exist, and we have limited ourselves to a few, expressly: cradle-to-gate (all emissions from feedstock production to the end product), cradle-to-grave (emissions from feedstock production, refining, and disposal after the product's end of life), and well-to-tank (emissions from raw material acquisition, biorefining, and distribution to storage fuel tanks). Users are free to choose the system boundaries during the LCA study. However, the cradle-to-grave approach considers most emissions, from raw material generation to disposal.[202] The newly emerging cradle-to-cradle approach is increasingly appreciated due to its emphasis on sustainable development.[203,204] It aims to create products and systems with positive environmental and societal impacts throughout their life cycles.[203] Unlike traditional linear cradle-to-grave systems, where products end up as waste, cradle-to-cradle systems strive for continuous cycles of use and reuse.[205,206] Products are designed to return safely to natural systems (biological nutrients) or be perpetually recycled as technical nutrients in industrial processes.[207]

When assessing the impact of a process on carbon emissions, factors like LUCs must be considered, as they can significantly affect results.[216] Dłuzewski *et al.*[217] note that LUCs can disrupt carbon stocks in soil and vegetation, increasing GHG emissions. LUC is further subdivided into two: direct LUC and indirect LUC.[208] Direct LUC occurs when land is shifted for feedstock production (*e.g.*, converting forests or grasslands), leading to higher CO_2 emissions than in the typical case of moving toward renewable energy sources from conventional petroleum.[218] Indirect LUC happens when bioethanol production causes food shortages, prompting deforestation to expand croplands and further increase emissions.[219] LCAs with narrow boundaries may overlook these global impacts. For second-generation bioethanol, it is crucial to allocate the environmental effects from cultivation, including fertilizer and pesticide use, before conducting an LCA.[220]

We have compared the three major categories of the environmental impact of biorefineries: ecosystem quality, human

Table 4 Comprehensive life cycle impact assessment of bioethanol production from first-generation feedstocks (functional unit = 1 kg of bioethanol). C-GT: cradle to gate; C-GRV: cradle to grave; W-T: Well to tank; SE: system expansion; En: energy; EC: economic; BC: biochemical conversion

(A) Environmental impact category		Corn	Corn	Cassava	Cassava	Sweet potato	Sweet sorghum	Sugar beet	Wheat	Sugarcane	Sugarcane
(A1) Ecosystem quality											
GWP (kg CO_2-eq.)	Short term	91.48	—	—	0.69–1.30	2.53	—	—	1.26–2.04	0.53	—
	Long term	88.95	0.21	0.25	—	—	0.21	1.29	—	—	0.64
Acidification potential (kg SO_2-eq.)	Freshwater	1.13×10^{-6}	4.60×10^{-3}	4.70×10^{-3}	—	8.30×10^{-3}	2.05×10^{-2}	3.26×10^{-3}	—	2.41×10^{-2}	1.07×10^{-2}
	Terrestrial	9.49×10^{-4}	—	—	—	—	—	—	—	—	—
Eutrophication potential	Freshwater (kg PO_4-eq.)	2.6×10^{-3}	1.50×10^{-3}	1.70×10^{-3}	—	1.01×10^{-2}	4.30×10^{-3}	1.38×10^{-4}	—	5.57×10^{-5}	2.61×10^{-3}
	Marine (kg N-eq.)	1.31×10^{-2}	—	—	—	—	—	—	—	3.54×10^{-3}	—
Photochemical oxidant formation (kg ethylene-eq.)		7.34×10^{-1b}	7.60×10^{-3}	8.90×10^{-3}	—	6.70×10^{-3}	8.20×10^{-3}	1.46×10^{-4}	—	5.44×10^{-3b}	1.10×10^{-3}
Ozone layer depletion (kg CFC-11-eq.)		1.08×10^{-5}	—	—	—	—	—	2.40×10^{-7}	—	3.80×10^{-8}	4.26×10^{-8}
Ionizing radiation (kg U^{235} eq.)		8.69×10^{-6e}	—	—	—	—	—	—	—	4.59×10^{-7e}	3.14×10^{-7}
Land (m^2 year arable)	Transformation, biodiversity	2.40×10^{-2}	—	—	—	—	—	—	—	—	—
	Occupation, biodiversity	3.72	—	—	—	—	—	—	—	—	1.62
Water scarcity (m^3 world eq.)		11.94	—	—	—	—	—	—	—	—	—
(A2) Human health											
Freshwater ecotoxicity (kg 1,4-DB eq.)		—	—	—	—	—	—	2.09×10^{-2}	—	—	5.63×10^{-2}
Human toxicity potential (kg 1,4 DB eq.)	Cancer	2.04×10^{-5a}	1.07×10^{-2}	6.00×10^{-4}	—	4.60×10^{-3}	1.42×10^{-2}	2.85×10^{-1}	—	—	1.77×10^{-1}
	Non-cancer	1.34×10^{-5a}	—	—	—	—	—	—	—	—	—
Particulate matter formation (kg PM 2.5 eq.)		5.49×10^{-2}	—	—	—	—	—	—	—	1.39×10^{-3}	—
(A3) Resources											
Abiotic depletion (kg Sb eq.)	Fossil and nuclear energy use	1222.45^c	—	—	—	—	—	9.32×10^{-3}	—	9.24×10^{-6}	2.06×10^{-3}
	Mineral resources use	6.93^d	—	—	—	—	—	—	—	—	—
(B) System boundaries		C-GT	C-GRV	C-GRV	C-GRV	C-GRV	C-GRV	C-GT	W-T	C-GT	C-GRV
(C) Allocation methods		SE	SE	SE	SE	SE	SE	—	SE	En	EC
(D) Production pathway		BC	BC	BC	BC	BC	BC	BC	BC	BC	BC
(E) Ref.		208	209	209	210	209	209	211	212	213	214

[a] Reported in CTUh (Comparative Toxic Unit for humans). [b] Reported in kg NMVOC-eq. per kg bioethanol (NMVOC: Non-Methane Volatile Organic Content equivalent). [c] Reported in MJ deprived. [d] Reported in kg deprived. [e] 1 kg U^{235} = 80 011 kBq U^{235}.

Table 5 Comprehensive life cycle impact assessment of bioethanol production from second-generation feedstocks (functional unit = 1 kg of bioethanol). C-GT: cradle to gate; C-GRV: cradle to grave; SE: system expansion; En: energy; BC: biochemical conversion; TC: thermochemical conversion; eq.: equivalent; MSW: municipal solid waste

(A) Environmental impact category		Wheat straw	Switchgrass	Corn stover	Forest residue	MSW	Poplar			Bagasse
Blend		E100	E100	E100	E100	E100	E10	E85	E100	E100
(A1) Ecosystem quality										
GWP (kg CO_2-eq.)	Short term	0.87	0.84	1.14	0.23	0.14	0.246	0.125	0.096	0.24
	Long term	0.85	0.81	1.10	0.22	0.09	—	—	—	—
Acidification potential (kg SO_2-eq.)	Freshwater	7.98×10^{-9}	3.36×10^{-8}	3.97×10^{-8}	1.68×10^{-8}	1.27×10^{-8}	1.13×10^{-3}	5.3×10^{-3}	6.58×10^{-3}	7.09×10^{-3}
	Terrestrial	8.06×10^{-6}	3.64×10^{-5}	4.70×10^{-5}	1.42×10^{-5}	1.80×10^{-5}	—	—	—	—
Eutrophication potential (kg PO_4-eq.)	Freshwater	2.99×10^{-5}	1.08×10^{-5}	1.76×10^{-5}	8.86×10^{-6}	-3.75×10^{-5}	1.63×10^{-4}	1.09×10^{-3}	1.38×10^{-3}	2.03×10^{-5}
	Marine (kg N-eq.)	7.95×10^{-5}	3.44×10^{-4}	4.95×10^{-4}	1.14×10^{-4}	2.57×10^{-4}	—	—	—	1.65×10^{-3}
Photochemical oxidant formation (kg NMVOC-eq.)		2.31×10^{-3}	8.14×10^{-3}	9.24×10^{-3}	9.67×10^{-3}	2.33×10^{-3}	0.166^e	0.252^e	0.273^e	4.43×10^{-3}
Ozone layer depletion (kg CFC-11-eq.)		6.88×10^{-8}	5.73×10^{-8}	9.56×10^{-8}	5.47×10^{-8}	8.76×10^{-8}	3.07×10^{-8}	2.22×10^{-8}	2.00×10^{-8}	7.97×10^{-9}
Ionizing radiation (kg U^{235} eq.)		3.68×10^{-5}	3.24×10^{-5}	4.79×10^{-5}	2.17×10^{-5}	3.18×10^{-5}	—	—	—	2.21×10^{-7}
Land (m^2 year arable)	Transformation, biodiversity	1.50×10^{-4}	-5.99×10^{-3}	-2.06×10^{-3}	7.45×10^{-5}	-5.40×10^{-3}	—	—	—	—
	Occupation, biodiversity	0.11	0.06	0.04	0.01	−0.63				
Water scarcity (m^3 world eq.)		8.46	0.65	0.69	0.26	3.06	—	—	—	—
(A2) Human health										
Freshwater ecotoxicity (kg 1,4-DB eq.)		$15\,062.92^b$	$15\,069.82^b$	$15\,620.41^b$	6288.05^b	6204.06^b	2.43×10^{-3}	3.27×10^{-3}	3.55×10^{-3}	—
Human toxicity potential (kg 1,4 DB eq.)	Cancer	1.16×10^{-7a}	6.52×10^{-8a}	7.16×10^{-8a}	2.68×10^{-8a}	3.93×10^{-8a}	1.40×10^{-2}	1.90×10^{-2}	2.10×10^{-2}	—
	Non-cancer	2.83×10^{-7a}	1.59×10^{-7a}	1.66×10^{-7a}	2.09×10^{-7a}	6.79×10^{-8a}	—	—	—	—
Particulate matter formation (kg PM 2.5 eq.)		3.01×10^{-4}	5.53×10^{-3}	5.71×10^{-3}	2.06×10^{-3}	1.81×10^{-3}	—	—	—	2.41×10^{-4}
(A3) Resources										
Abiotic depletion (kg Sb eq.)	Fossil and nuclear energy use	3.74^c	7.84^c	11.53^c	3.87^c	12.73^c	1.58×10^{-3}	7.21×10^{-4}	4.55×10^{-4}	1.64×10^{-5}
	Mineral resources use	1.73×10^{-2d}	2.10×10^{-2d}	2.40×10^{-2d}	7.28×10^{-3d}	1.32×10^{-2d}				
(B) System boundaries		C-GT	C-GT	C-GT	C-GT	C-GT	C-GRV	C-GRV	C-GRV	C-GRV
(C) Allocation methods		SE	SE	SE	SE	SE	SE	SE	SE	En
(D) Production pathway		BC	BC	BC	TC	BC	BC	BC	BC	BC
(E) Ref.		208	208	208	208	208	215	215	215	213

^{a} Reported in CTUh (Comparative Toxic Unit for humans). ^{b} Reported in CTUe (Comparative Toxic Unit for ecosystem). ^{c} Reported in MJ deprived. ^{d} Reported in kg deprived. ^{e} Reported in g ethylene eq. per kg bioethanol.

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Table 6 Comparative analysis of different feedstocks.[256–266] GM: genetically modified; MSW: municipal solid waste

Severity	Low to negative	Low	Low to moderate	Moderate	High
Colour scheme	(dark green)	(light green)	(yellow)	(orange)	(red)

Feedstock	Bioethanol yield (kL ha⁻¹)	GHG emissions	Energy consumption	Water usage	Land use	Ref.
First-generation						
Sugarcane	5.9–9.9	Moderate	High	Moderate	High	227 and 256
Corn	3.8–4.2	High	High	Moderate	High	227, 257 and 258
Second-generation						
Syngas	Variable[a]	Low to negative	Moderate	High	Moderate	3 and 227
Lignocellulosic biomass	1–11	Low to negative	Moderate	High	Low	32, 33, 227 and 259–261
MSW[c]	—	Low to negative	High	Moderate	Low	227 and 259
Third-generation						
Algae[f]	47–141	Low to negative	High	High	Low	33, 227 and 262
Fourth-generation						
GM feedstock	Variable[d]	Low	Moderate	Low to moderate	Low	263
Non-traditional						
Effluent gases	Variable[b]	Low	Low	Low	Low	264
Glycerol[e]	Variable[b]	Low to negative	Low	Low	Low	265
Synthetic biology approaches	Variable[b]	Low	Low	Low	Low	266

[a] Depends on feedstock and gasification efficiency. [b] Depends on microbes/process involved based on their conversion efficiency. [c] Energy consumption is high due to additional sorting step. [d] Due to variable carbohydrate content. [e] Byproduct of oil crop to biodiesel production. [f] GHG emissions can vary significantly as shown in Fig. 4, but mainly it lies on the lower to negative side.

health, and resources. The occupational land use is significantly higher for first-generation feedstocks used in bioethanol production compared to second-generation feedstocks. Corn, first-generation feedstocks require 3.72 m² year of arable land per kilogram of bioethanol, which is more than twice that of sugarcane (Table 4). This is consistent with the fact that bioethanol yield from corn is almost half that of sugarcane (Table 6), making sugarcane more efficient in terms of arable land use for bioethanol production. However, the global warming potential (GWP) is higher for sugarcane-based bioethanol than corn-based bioethanol (Table 4). GWP, measured in CO_2 equivalents, reflects the potential of gases to cause global warming over a specified period, typically 100 years.[221] As shown in Tables 4 and 5, GWP is generally higher for first-generation feedstocks than second-generation feedstocks. Nevertheless, certain food-based feedstocks have exceptionally high bioethanol yields and thus exhibit a lower GWP, such as 0.21 kg CO_2-equivalent per kilogram of bioethanol from corn,[209] which

is significantly lower than corn stover's 1.4 kg CO_2-equivalent.[208] While the short-term effects of switching from conventional gasoline to renewable bioethanol are immediately noticeable within a decade, long-term GWP considers impacts over 100 years or more. Over the long term, renewable resources exhibit lower GWP and can contribute to environmental mitigation by reducing carbon emissions.[222]

The acidification potential from synthesizing one kilogram of bioethanol is higher with first-generation feedstocks, primarily due to the use of fertilizers and pesticides during crop cultivation.[38] This potential is measured in SO_2 equivalents, reflecting the cumulative effect of all acids released as if they were specific amounts of SO_2, detailed in Tables 4 and 5. Similarly, the eutrophication potential, which denotes nutrient overloading in water bodies causing excessive growth of aquatic plants and algae, ranges from 10^{-5} to 10^{-1} kg PO_4-eq. (phosphate equivalent) for freshwater and kg N-eq. (nitrogen equivalent) for marine water.[213] Alongside these, environmental

impact indicators like photochemical oxidant formation potential (measured in kg NMVOC-eq. or kg ethylene-eq.), ionizing radiation potential (measured in kg U^{235} eq.), and ozone layer depletion potential (measured in kg CFC-11-eq.) also vary between first and second-generation bioethanol (Tables 4 and 5). These impacts are more pronounced in first-generation feedstocks due to the larger agricultural inputs required, contributing significantly to acidification and eutrophication. Nitrogen-based fertilizers, in particular, can lead to nutrient loading in water bodies.[223] First-generation bioethanol production involves energy-intensive methods, often relying on fossil fuels for heat and power, leading to higher emissions of pollutants such as SO_2, NO_x, and VOCs, contributing to ground-level ozone formation.[224] Additionally, the expansion of agriculture for first-generation feedstock can lead to LUCs, including deforestation, impacting air quality, biodiversity, and carbon emissions.[219] Conversely, second-generation bioethanol technologies, such as enzymatic hydrolysis and fermentation of LCB, are more efficient and have lower energy requirements.[57] They often use marginal lands or agricultural residues, reducing competition with food crops and minimizing environmental impacts.[225] The transition from first to second-generation bioethanol production aims to mitigate these impacts while ensuring sustainable biofuel production. Moreover, cultivating high water-intensive crops like sugarcane and corn for first-generation bioethanol can exacerbate water scarcity, affecting ecosystem quality.[226]

Human health is also a significant consideration in bioethanol production from various feedstocks. Each feedstock involves processing units that use chemicals or produce by-products impacting the ecosystem, leading to freshwater eco-toxicity and human toxicity, ranging from 10^{-3} kg to 10^{-1} kg, and 10^{-8} kg to 10^{-1} kg 1,4-dichlorobenzene equivalents, respectively (Tables 4 and 5). Additionally, particulate matter (PM-2.5) emissions, ranging from 10^{-4} to 10^{-1} kg PM-2.5-equivalent per kg of bioethanol produced, pose risks, especially during the production of first-generation feedstocks, engaging transportation, ploughing, and harvesting.[213] Moreover, the production process affects abiotic resources (non-living natural resources), including minerals and energy resources like nuclear and fossil fuels, typically expressed in kilograms of antimony (Sb) equivalent per kilogram of bioethanol produced.[208] This category considers the extraction and depletion of various minerals and metals used in production processes, required energy generation, and bioethanol-related infrastructure. Abiotic depletion potential (ADP) is generally higher in first-generation bioethanol due to the intensive use of non-renewable resources such as fossil fuels, fertilizers, and minerals during feedstock cultivation, processing, and infrastructure development (Tables 4 and 5). In contrast, second-generation bioethanol, focusing on non-food biomass and advanced production technologies, tends to have a lower ADP by minimizing the depletion of abiotic resources and promoting sustainable resource management practices.

Furthermore, there are concerns about the actual environmental benefits of dedicated feedstocks. For example, an LCA study found that China's corn bioethanol and soybean biodiesel GWP was 40% and 20% higher than fossil petrol and diesel, respectively. This difference is mainly due to heavy fertilizer use, high energy consumption during processing, and China's reliance on coal-based energy.[227] However, using renewable energy for processing and adopting sustainable farming practices, like no-till farming and crop rotation, can help reduce emissions.[228] Additionally, higher-yielding crops can reduce the land needed for the same bioethanol production.[229] Non-traditional feedstocks, such as glycerol, industrial waste gases, and GM crops, possess significant potential for reducing GHG emissions.[230,231] Thus, the improved efficiency of the production processes and the co-product utilization can offset emissions and improve overall sustainability.

A comprehensive LCA study by Muñoz et al.[232] compared bioethanol production from various feedstocks, including sugarcane, maize, sugar beet, and wheat, across different regions. The cradle-to-gate approach had much lower GHG emissions of 0.7 to 1.5 kg CO_2-eq. per kg of bioethanol compared to both cradle-to-grave of 1.3 to 2 kg CO_2-eq. and fossil-based ethanol of 1.3–3.7 kg CO_2-eq. Moreover, maize stover in the USA (GWP: 1.25) and sugar beet in France (GWP: 1.27) were found to have the lowest impact from a GHG emission perspective, although when other impact categories were considered, trade-offs were encountered. Jeswani et al.[227] carried out an in-depth analysis of the environmental impact of producing different types of liquid biofuels, including bioethanol. Their findings indicated that, when LUC is excluded, first-generation biofuels generally emit fewer GHGs than conventional fossil fuels.[227] However, for the majority of feedstocks, the achieved emission reductions did not meet the 60% GHG savings threshold required by the European Union's Renewable Energy Directive (RED).[227,233] In contrast, second- and third-generation biofuels demonstrated a stronger capability to cut emissions, provided that no LUC takes place. The authors also emphasized that reductions in GHG emissions are frequently offset by increase in the other environmental impacts such as acidification, eutrophication, depletion of water resources, and loss of biodiversity.[227] For first-generation bioethanol, the GWP without accounting for LUC was found to range between 0.08 and 4.4 kg CO_2 eq. per kg of bioethanol produced (Fig. 4(a)). Among all the feedstocks analyzed, only sugarcane-based bioethanol achieved the 60% GHG reduction target set by the RED compared to fossil fuels (4.3 kg CO_2 eq.). This superior performance arises from sugarcane's relatively high crop yield, lower dependency on chemical fertilizers and pesticides, and the additional carbon credits gained from co-generated electricity within integrated biorefineries.[227] Nevertheless, when LUC impacts were considered, none of the first-generation pathways achieved the RED target.[227] In contrast, the second-generation pathways revealed a wider spectrum of GWP values (−3.1 to 4.7 kg CO_2 eq.), depending heavily on both the feedstock used and the processing technology applied (Fig. 4(a)). These advanced systems often utilise lignin byproducts as a source of electricity or as value-added products, which helps offset GHG emissions, sometimes even resulting in carbon-negative balances (Fig. 4(b)). For third-generation biofuels, the variability was even more pronounced, with reported
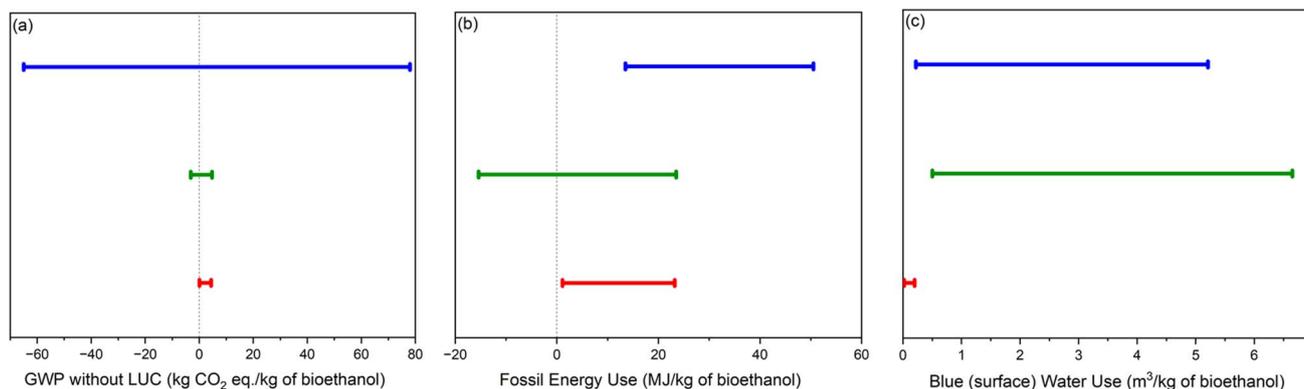
Fig. 4 Comparative life cycle impact ranges for (a) first-generation (red), (b) second-generation (green), and (c) third-generation (blue). Functional unit: 1 kg of bioethanol (this figure was drawn based on data provided in ref. 227). GWP: global warming potential; LUC: land-use change.

GWP values ranging from −65 to 78 kg CO_2 eq. (Fig. 4(a)). This broad variation stems largely from inconsistent methodological boundaries, differences in feedstock cultivation assumptions, nutrient inputs, and treatment of co-products across studies.[227]

Although second- and third-generation bioethanol pathways generally exhibit lower GWP than fossil fuels, their production is substantially more water-intensive, requiring approximately 55 to 246 times more water than fossil fuel production Fig. 4(c), which typically consumes about 3.5–8.5 liters per kilogram of gasoline.[234,235] Water use in bioethanol production varies widely with regional climatic conditions and irrigation practices, ranging from as low as 20 to 200 liters per kilogram of first-generation bioethanol in rain-fed systems Fig. 4(c). In contrast, corn-based bioethanol produced under intensive irrigation conditions, such as in Portugal, can require exceptionally high water inputs of around 2320 liters per kilogram of bioethanol.[235] Bioethanol production, regardless of generation (first to fourth), also poses significant threats to biodiversity. Large-scale cultivation of biofuel crops can cause habitat loss, soil and water pollution through nutrient runoff, and, in some cases, promote invasive species.[236–238] Intensive agricultural practices and heavy agrochemical use for first-generation feedstocks represent direct hazards to local flora and fauna.[236,239,240] The conversion of forests or other ecosystems into croplands for biofuel production further amplifies biodiversity loss by destroying wildlife habitats.[227,241] Compared to first-generation fuels, second-generation biofuels generally exhibit fewer negative ecological effects and may, under specific conditions, even enhance biodiversity.[1,227] Lignocellulosic crops, which typically require minimal fertilizers and pesticides and grow over longer cycles, can promote more sustainable land use, especially when cultivated on degraded or marginal land.[242–244] Utilizing agricultural residues or forestry byproducts as biofuel feedstocks generally causes less disturbance to ecosystems; however, excessive removal of such residues may deplete organic matter, disturb wildlife habitats, and increase herbicide use by encouraging weed proliferation.[245–247] The ecological consequences of third-generation (algal) biofuels are still uncertain. Algal cultivation could pose serious threats to coastal

biodiversity by introducing invasive algal species into sensitive ecosystems such as mangroves, coral reefs, seagrass beds, and mudflats.[227] Accurately quantifying biodiversity loss remains difficult due to the absence of standardized methods for assessing biofuel-related ecological changes. Overall, although biofuels can substantially reduce GHG emissions relative to fossil-based fuels, these benefits are often counterbalanced by other environmental drawbacks. For instance, first-generation bioethanol has been shown to possess up to three times greater acidification potential and between three and twenty times higher eutrophication potential than fossil fuels.[227]

Neto *et al.*[248] highlighted several industrial initiatives that convert effluent gases into bioethanol *via* fermentation, with LanzaTech emerging as a key leader. Large-scale facilities producing approximately 46 000 and 125 000 tons of bioethanol annually have been established in China and Europe, respectively, utilizing steel mill off-gases as carbon sources.[248] LCA studies indicate that the environmental performance of syngas fermentation is highly dependent on both the feedstock origin and the extent of process integration.[249,250] Comparative studies suggest that when syngas is derived from waste gases or biomass residues, and when energy recovery systems are incorporated, the overall impacts can be comparable or even superior to conventional biomass-to-biofuel pathways.[251,252] For MFCs and APS, LCAs consistently identify the electricity mix and the choice of reactor materials (such as electrodes and membranes) as the most influential factors. Under scenarios using renewable electricity and optimized system design, these technologies can achieve significantly reduced or even net-negative GHG emissions for certain products.[253,254] In contrast, reliance on grid electricity and current pilot-scale materials often results in higher impacts than traditional production methods.[255] Moreover, pilot-scale assessments emphasize that unrecovered off-gases and inefficient resource utilization can substantially increase environmental burdens unless effective capture or reuse strategies are implemented.[254]

After thoroughly examining the environmental impact parameters in Tables 4 and 5, Table 6 presents an in-depth comparative analysis of diverse feedstocks used in bioethanol

production. This analysis considers the yield and environmental effects to assess their sustainability. As shown in Table 6, the bioethanol yield is high in the case of first-generation feedstocks. Still, it has significant environmental impacts due to intensive agricultural practices and high water and energy consumption, leading to high GHG emissions. Second-generation feedstock utilizes waste materials and non-food crops, lowering environmental impacts compared to first-generation feedstocks. Third-generation also has higher yield potential with minimal land use requirements. The algal feedstocks possess tremendous potential for future scalability with advancements in cultivation and processing technologies. Fourth-generation feedstocks utilize advanced biotechnological approaches to improve efficiency and sustainability. The yield and environmental impacts vary significantly depending on specific genetic modifications and synthetic biology strategies. These are in early-stage development, having TRL 2, with the potential for significant future advancements.[1] Lastly, the fermentation of non-traditional industrial effluents offers solutions to cut down GHG emissions by sequestrating carbon right at its source, producing bioethanol. It lowers environmental impacts by utilizing GHGs as a feedstock. Integrating it with the iron and steel industries can help in significant ecological mitigation.

4. Economic and policy considerations

The global push towards sustainable energy solutions has brought bioethanol to the forefront of renewable fuel alternatives, influenced by economical, technological, policy framework, and environmental factors.[267,268] Feedstock availability and cost are crucial determinants of its economic feasibility. First-generation feedstocks, such as corn and sugarcane, dominate bioethanol production due to their high fermentable sugar content.[269] Corn, with costs typically ranging from 130 to 350 US$ per ton,[270] accounts for approximately 95% of bioethanol production in the United States.[271] However, their use raises concerns about food competition and environmental impacts.[272] Second-generation feedstocks derived from LCB offer a more sustainable option, although production costs remain high, i.e., ~1.5 US$ per liter of bioethanol.[28] Technological advancements have significantly enhanced the efficiency and cost-effectiveness of bioethanol production. Innovations in pretreatment, enzymatic hydrolysis, and consolidated bioprocessing have improved conversion efficiency and reduced costs (up to 25%).[57,268,273] Genetic engineering of microbial strains has further boosted bioethanol yields and tolerance, making bioethanol production more commercially viable.[274]

The economic viability of bioethanol is tied to market dynamics such as fluctuating oil prices, government subsidies, and renewable fuel demand.[275] Policy instruments like the U.S. Renewable Fuel Standard have increased demand,[272,276] contributing to a growing global market, projected to expand from US$ 83.4 billion in 2023 to US$ 114.7 billion by 2028.[277] However, oil price volatility and inconsistent policies (such as changes in the U.S. biofuel policy) remain a challenge.[278,279] Thus, policy frameworks play a pivotal role in the bioethanol industry. Initiatives like the EU's Renewable Energy Directive (RED II) set ambitious renewable energy targets (aiming for a 14% share by 2030 (ref. 280)), while tax incentives and subsidies encourage investment. However, inconsistencies in policies, particularly in emerging economies like India and China, pose challenges. For example, the consumption of fuel ethanol in these countries remains considerably lower than that of gasoline. To illustrate, in 2022, the market penetration of fuel ethanol in India was approximately 5.1 billion liters, with a forecast to reach around 6.2 billion liters in 2023. In contrast, the gasoline market penetration was projected to increase to 53 billion liters in the same year, nearly ten times higher than fuel ethanol.[281] This disparity underscores the need for targeted policies to expand bioethanol consumption in these regions.

Supportive public policies have been key to boosting bioethanol's cost competitiveness. The Proálcool program, launched in Brazil in 1975 to promote energy self-reliance, combined blending mandates, concessional financing, and infrastructure investments that encouraged large-scale bioethanol production.[282,283] Productivity increased dramatically (from ~2.4 to ~5 kL ha^{-1}) through improved crop varieties, efficient field management, and the reuse of stillage as fertilizer, leading to an average cost reduction of 3.5% per year between 1976 and 1994.[282] Goldemberg[284,285] estimated that combined savings from both agricultural and industrial improvements could further reduce bioethanol costs by 23%.[282] However, the withdrawal of public investment and subsidies in 1984 caused bioethanol-powered car sales to fall sharply from 94.4% to 51% of total vehicle sales.[282] In response, the government introduced new incentives to revive production, and by 1991, annual bioethanol vehicle output had doubled from 0.7 million to 1.4 million cars, reaffirming Brazil's global leadership in bioethanol development.[282] Chen et al.[286] found that technological improvements reduced overall bioethanol costs from sugarcane by 67% with a reduction of >70% in processing cost, stressing that factors like market competitiveness, economies of scale, and learning-by-doing are significantly shaped by policy measures.

In the U.S., the Renewable Fuel Standard (RFS) and accompanying tax credits spurred capacity expansion and process improvements, boosting bioethanol production and consumption nearly tenfold between 2002 and 2019.[287] Annual blending mandates and tradable Renewable Identification Numbers (RINs) reduced market uncertainty, attracting private investment, while learning-by-doing improved efficiency despite corn price fluctuations.[288–290] McPhail et al.[291] found that eliminating U.S. federal tax credits and tariffs would reduce bioethanol production by 18.6% and lower corn prices by 14.5%, underscoring that policy support rather than market forces alone has been a key driver of price and production dynamics of bioethanol. However, when gasoline prices exceed $3 per gallon, bioethanol production remains profitable without policy support, allowing output to rise from 6.5 to 14 billion gallons and corn prices to stabilize near $4 per bushel.[291] Timilsina and Shrestha[292] assessed the impacts of biofuel expansion on global

Table 7 Bioethanol policies: year, provisions, impact[a]

Nations	Policy	Year	Provisions/features	Impact	Remarks	Ref.
USA	Renewable Fuel Standards (RFS)	2007	Mandated annual renewable fuel volumes and blending; tradable RINs; tax credits	Increased bioethanol production and private investors	Updated annually	272, 276, 287–290 and 302
Brazil	Proálcool	1975	Energy self-reliance; blending mandates; concessional financing; infrastructure investments	Rapid industry build-out; cost-cutting due to improved farming; ethanol-based vehicles	Reduced oil import; long legacy as frontier in bioethanol	282 and 283
Brazil	RenovaBio	2017	Market for carbon-intensity certificates; efficiency certification for producers	Attracted investment; incentivised low-carbon bioethanol	Improved sustainability metrics	303 and 304
EU	Renewable Energy Directive	2009	Binding renewable energy targets; tax incentives; subsidies	Increased investment in low-carbon fuels; higher research funding for advanced and waste-derived bioethanol	Proactive updates on targets and ILUC rules	280 and 294–298
China	Bioethanol policy	2001	Pilot E10 rollouts; fuel-grade bioethanol standards; production and quality regulation	Scale-up of pilot plants; regional blending trials	Still in place	305
India	National Policy on Biofuels (NPB)	2018	Bioethanol from surplus/damaged grains; blending targets; stopped import and export of biofuels	Rapid blending growth; spurred 2G biorefinery growth in integration with petroleum refineries	Government procurement and support	1 and 306

[a] RIN: renewable identification numbers; ILUC: indirect land use change.

food prices, summarizing studies that projected increases of 23–72% for maize, 8–30% for wheat, 18–76% for oilseeds, and 11.5–66% for sugar under planned biofuel expansion scenarios by 2020. They further noted that the 2007–2008 food crisis was partly driven by the rapid growth of first-generation biofuel production.[292] In 2008, Rosegrant *et al.*[293] concluded that restricting biofuel production from food-based feedstocks could lower maize prices by 6% by 2010 and 14% by 2015, with smaller reductions for other crops, while a global moratorium could further decrease prices of maize, cassava, sugar, and wheat by 20%, 14%, 11%, and 8%, respectively. In the EU, RED II enhanced cost competitiveness through sustainability certification and technology differentiation, granting compliance credits and preferential market access to low-carbon fuels.[294–297] This boosted investor confidence and funding for advanced and waste-derived bioethanol.[298] Emerging economies like India and China adopt hybrid models with blending targets (E10–E20), concessional financing, subsidies, and infrastructure grants, and raising rural incomes *via* agricultural residues.[299–301] These examples show how early-stage policy interventions can offset high capital costs and accelerate industry development. Table 7 provides a consolidated overview of the various policies along with their corresponding summaries.

So in a nutshell, policy frameworks have made bioethanol economically viable through three synergistic mechanisms: (i) demand creation, (ii) cost mitigation, and (iii) market differentiation. Demand was driven by blending mandates and volume targets, ensuring stable markets, lowering investor risk, and encouraging capacity growth. Cost reduction involved

subsidies, concessional loans, and R&D support to foster technological learning and economies of scale. Market differentiation rewarded renewable markets *via* sustainability certifications like RED II and RFS.[307,308] Together, these measures have closed the cost gap with fossil fuels while delivering social and environmental benefits, including rural employment and lower import dependence.[60] Thus, the sustainability of bioethanol production is shaped by a complex interplay of factors, including feedstock availability, technological advances, economic viability, policy support, and environmental considerations. Overcoming the challenges in feedstock costs and efficiency through innovation, such as utilizing waste streams and advanced microbial technologies, holds promise for improving the competitiveness of bioethanol. Stable, transparent, and socially inclusive policy frameworks, supported by certification schemes ensuring environmental and economic integrity, will be essential to secure bioethanol's long-term viability as a cornerstone of the global renewable energy transition.

5. Potential improvements and future directions

A comprehensive summary of the four generations of bioethanol production is provided in Table 8, offering a holistic analysis that includes decision support, TRL, policies, and overall environmental impacts in terms of carbon emissions and GHG potential. As we progress from the first to the fourth generation, there is a noticeable increase in sustainability,

Table 8 Summary of bioethanol production from four generations of feedstock

Generation	Carbon emissions[a]	GHG reduction potential[b]	Holistic perspective	Technology readiness level (TRL)	Decision support	Policy and regulation	Ref.
First	0.08 to 4.4	Moderate to high[c]	Food-versus-fuel conflict, minimal environmental benefits	9	Established infrastructure, widely adopted	U.S. Renewable fuel standard supported blending in gasoline	225, 227, 272, 276, 309 and 314
Second	−3.1 to 4.7	High	Non-food-based feedstock offers higher sustainability	8–9	Costly pretreatment improving with R&D	EU renewable energy directive supported 2G bioethanol using incentives	1, 227, 311, 315 and 316
Third	−65 to 78	High	High yield per hectare; grow on non-arable land; reduces land-use impacts	4–5	Scale-up challenge, higher sustainability	Emerging technologies, limited policies, research funding and incentives required	1, 227 and 317
Fourth	—	Very high	Carbon-neutral/negative	2	Early-stage technology; can revolutionize sustainability	Currently speculative; require new regulatory frameworks	1

[a] kg CO_2-eq. per kg of bioethanol produced. [b] Compared to gasoline (94 g CO_2 eq. per MJ). [c] Data not well-established; sometimes even higher than gasoline.

reduced land use, and a shift toward carbon neutrality.[225,309] First-generation bioethanol sometimes has higher carbon emissions compared to gasoline.[310] Each generation presents evolving technologies and challenges, with later generations showing more promise but requiring continued research and development. Policies supporting bioethanol production have evolved, laying the foundation for more sustainable and environmental friendly alternatives. Notable policies, like the U.S. Renewable Fuel Standard (RFS),[272,276] the EU's Renewable Energy Directive (RED II),[311] Brazil's RenovaBio,[303] and others in China and India,[300,306] aim to reduce carbon emissions through the promotion of advanced biofuels. However, inconsistent regulations and policy uncertainties can hinder market stability, affecting long-term investments and planning.[312,313]

Recent innovations in bioethanol production have radically transformed the bioenergy landscape. For example, Artificial Photosynthesis systems, which harness sunlight directly,[187] significantly reduce external energy input and can be integrated with other renewable energy sources like solar or wind to build a more resilient energy infrastructure. Microbial Fuel Cells, generating both bioethanol and electricity, offer a novel approach to sustainable energy production.[175] Furthermore, advances in materials science, biotechnology, and process engineering are essential for overcoming current limitations. Biomass gasification and fermentation processes provide versatility, as syngas can be used as feedstock for multiple biofuels and chemicals,[318] offering greater efficiency and feedstock flexibility than conventional bioethanol production. Challenges remain, including the operational complexity of maintaining precise temperature, pressure, and gas composition conditions, as well as capital investment in specialized equipment.[319] Catalytic synthesis is also a promising avenue for

reducing carbon footprints in bioethanol production, although it faces hurdles such as catalyst performance and process integration.[320]

Consolidated bioprocessing, a key breakthrough, integrates enzyme production, biomass hydrolysis, and fermentation into a single step, eliminating the need for additional enzymes and pretreatment processes.[321] This innovation reduces both costs and process complexity. Additionally, continuous fermentation systems, which maintain optimal conditions for microbial growth, can enhance productivity and overall process efficiency.[92,322,323] Nanotechnology improves enzyme stability and reusability, particularly in biomass pretreatment and enzyme immobilization, making bioethanol production more cost-effective and sustainable.[57] Beyond its role as a biofuel, bioethanol is becoming an increasingly versatile chemical with applications in various downstream industries (discussed in SI). In synthetic biology, engineered microorganisms convert bioethanol into high-value chemicals and pharmaceuticals, reducing dependence on petrochemical routes.[324] This expands bioethanol's role as a sustainable carbon source for biomanufacturing.

Food-based, first-generation bioethanol has been produced for several decades, with Brazil and the United States leading global output through the use of sugarcane and corn as primary feedstocks, respectively.[325] This technology is fully mature and operates at a TRL of 9, with numerous commercial-scale plants established worldwide.[1] In contrast, large agricultural nations such as India and China are currently accelerating efforts to advance second-generation bioethanol technologies toward full commercialization (TRL 9), leveraging their abundant supplies of agricultural residues and other lignocellulosic feedstocks. In India, companies such as Praj Industries Limited[326] and Nuberg

Green Energy[327] have already developed and demonstrated technologies capable of converting these residues into bioethanol and are operating at TRL 9. Similarly, in Brazil, firms like GranBio and Raízen are pursuing second-generation bioethanol production and have achieved TRLs in the range of 8 to 9, reflecting near-commercial maturity.[328] Meanwhile, in the United States, companies including Mascoma Corporation[329] and Qteros Inc.[330] remain at comparatively earlier stages of technological deployment, operating at TRLs between 6 and 7, as their projects are still at the pilot or early demonstration level. Jain and Kumar[1] reported the TRL of 7–8 for bioethanol production via gas fermentation, with LanzaTech emerging as the leading industrial developer in this field. Algal-based third-generation bioethanol is still limited to the laboratory or pilot scale, corresponding to TRLs between 4 and 6.[1] Algenol Biofuels (USA), which demonstrated a semi-commercial photobioreactor system for bioethanol production, achieving a TRL of around 6 before scaling back operations due to economic constraints.[331] The European initiative under the EU Horizon framework, such as the BIOFAT, has reached similar pilot-scale advancements.[332] Lastly, fourth-generation bioethanol technologies remain predominantly at the research and laboratory scale, reflecting low TRL typically between 2 and 3.[1]

The central discussion ultimately revolves around identifying which type of biomass is most suitable for sustainable bioethanol production. There is, however, no universal answer, as the selection must be region-specific and guided by the availability of local resources, climatic conditions, and socioeconomic context. Such decisions should be supported by detailed life cycle and techno-economic analyses to ensure environmental sustainability and economic feasibility. Food-based first-generation feedstocks are generally less suitable due to their high agricultural input requirements and the ongoing food-versus-fuel debate.[1,219] Hence, alternative non-food biomass should be prioritised. Second-generation lignocellulosic residues hold strong potential for countries such as Brazil, China, and India, where vast amounts of agricultural residues are produced annually.[333,334] These lignocellulosic feedstocks can be efficiently utilized in decentralized biorefineries, which would minimize transportation costs and ensure a steady biomass supply by taking advantage of the abundant crop residues generated from multiple seasonal harvests throughout the year.[60,335] Also, it will help generate employment in the rural and remote areas.[60] In contrast, third-generation algal feedstocks may be impractical in regions with limited sunlight, as their cultivation in photobioreactors can demand significant energy inputs that compromise sustainability, particularly if derived from non-renewable sources. Even if renewable energy is used to produce algal biomass, subsequently converting that biomass back into bioethanol for energy use may not be carbon-intensive, but it represents a redundant and inefficient pathway. Fourth-generation and other advanced bioethanol technologies demonstrate promise at the laboratory scale but often face scalability and biosafety challenges, especially those involving GM organisms, necessitating rigorous protocols prior to deployment.[336–338] Despite this, bioethanol production also raises social and ethical concerns, particularly the "food vs. fuel" debate,[339] as large-scale production from food crops can exacerbate food insecurity and drive up prices.[340] Additionally, land-use changes can displace communities and harm biodiversity.[341] Balancing energy needs with food security and social equity is essential. Environmentally, bioethanol offers potential GHG reductions compared to fossil fuels, with cellulosic bioethanol providing greater benefits (~86% GHG reduction) than first-generation corn (~52% GHG reduction).[342] However, land-use changes can offset these gains,[341] emphasizing the need for sustainable land management and feedstock selection to maximize environmental advantages.

Continued research and development efforts are required to ensure complete resource utilization and to make the process economical both in terms of "atoms" and "capital." Jain et al.[343,344] proposed the complete valorization of LCB, and suggested converting holocellulose into bioethanol or bio-oil, inorganic ash into high-purity silica for applications such as catalysis or adsorption, and lignin into bio-oil for potential material uses, including biopolymers. Comprehensive techno-economic and life cycle assessments can further guide the identification of best practices for specific regions while considering system boundaries at a global scale. Prior feedstock characterization is essential for selecting the most suitable biomass for bioethanol production. For instance, second-generation feedstocks with high lignin content or third-generation feedstocks with high lipid content may not be suitable for standalone bioethanol production. However, these materials can be integrated with other processing units to achieve complete feedstock utilization and higher atom efficiency. Statistical tools like response surface methodology can optimize pretreatment conditions, minimize inhibitors, and improve process scalability. Ultimately, the successful scale-up of carbon-negative and integrative bioethanol technologies could offer the most sustainable route for future biofuel production.

A promising direction for bioethanol production involves integrating carbon capture and utilization (CCU) technologies, where CO_2 produced during fermentation is captured and repurposed for other industrial uses, creating a closed carbon loop.[345] However, unsustainable agricultural practices, deforestation, and land use changes can offset the environmental benefits of bioethanol production.[346] In the transportation sector, vehicle electrification is on the rise, but bioethanol still offers a viable alternative for powering electric vehicles. Technologies like solid oxide fuel cells that convert ethanol into electricity have demonstrated superior energy efficiency and sustainability compared to battery-powered electric vehicles (BEVs).[347] For instance, with its higher energy density, sugarcane bioethanol results in lower GHG emissions than BEVs, which in Brazil emit 65 grams of CO_2 per kilometer, compared to the 58 grams emitted by ethanol-powered flex-fuel vehicles.[348] In contrast, hybrid electric vehicles using bioethanol, already available in the Brazilian market, emit just 29 grams of CO_2 per kilometer.[348] Direct Ethanol Fuel Cells, still in the research phase, also hold promise for portable and stationary power applications, offering high energy density and low emissions.[347,349]

Fluctuations in fossil fuel prices affect the economic competitiveness of bioethanol, making it less attractive when oil prices drop. Therefore, supportive policies, subsidies, and incentives are necessary to drive the growth of the bioethanol industry. Genetic engineering in fourth-generation feedstocks, along with innovations in enzyme technology and process integration, holds the potential for significant advancements in production efficiency and sustainability. Additionally, future approaches, such as optimizing local biomass mixtures for improved lignin extraction and hydrolysis, can address challenges in feedstock collection and transportation.[60] Decentralized biorefineries and Industry 4.0 technologies can streamline the bioethanol value chain, improving operational viability.[335,350] Advanced research, such as molecular modeling and machine learning to optimize pretreatment processes, will drive further improvements in bioethanol production.[57] Life cycle and techno-economic assessments are essential to balance costs, energy use, and environmental impacts, guiding the development of more sustainable and economical biofuel solutions. As the technologies evolve, they promise the future of renewable energy and the bioeconomy.

6. Conclusion

The Circular Bio-society 2050 vision (Bio-based Industries Consortium, 2018) highlights a promising future where sustainable energy, food, and products are sourced through circular bioeconomy principles. This review underscores bioethanol production potential from diverse feedstocks, particularly from lignin-rich biomass, which has traditionally been a challenging material. However, recent breakthroughs in biotechnology and chemical processes have opened new pathways for efficiently converting lignin into bioethanol. The review also calls for more transparency in biorefinery life cycle assessments, emphasizing the need to evaluate the entire value chain to understand the full impact and benefits, including biogenic carbon storage. While fluctuations in fossil fuel prices can affect the economic feasibility of bioethanol, government support, subsidies, and incentives are essential to fostering growth in the bioethanol sector. Integrating bioethanol production into biorefineries and aligning it with green chemistry and the circular economy can further strengthen its role in the bioeconomy. Moreover, conducting detailed LCAs of first- and second-generation bioethanol enables the identification of environmental trade-offs and hotspots, providing critical insights to guide policy and technology development. Incorporating emerging metrics such as resource efficiency and socio-environmental benefits into LCAs can enhance decision-making and support the transition to sustainable biofuels. The continued advancement in genetic engineering, enzyme technology, and process integration is expected to significantly improve bioethanol production's efficiency and sustainability. As these innovations progress, they hold the potential to shape a more sustainable and renewable energy future, offering long-term benefits for both the biofuel industry and the broader bioeconomy.

Author contributions

Sanyam Jain: writing – original draft, visualization, validation, software, methodology, investigation, formal analysis, data curation, conceptualization. Shushil Kumar: writing – review & editing, visualization, validation, supervision, software, resources, project administration, methodology, investigation, funding acquisition, formal analysis, conceptualization.

Conflicts of interest

The authors have no relevant financial or non-financial interests to disclose.

Abbreviations

LCA	Life cycle assessment
DDG	Dry distillers' grains
LCB	Lignocellulosic biomass
MSW	Municipal solid waste
MFCs	Microbial fuel cells
APS	Artificial photosynthetic systems
PECs	Photoelectrochemical cells
GHG	Greenhouse gas
TRL	Technology readiness level
GM	Genetically modified
GWP	Global warming potential
LUC	Land-use change

Data availability

No primary research results, software, or code have been included, and no new data were generated or analyzed as part of this review.

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