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Progress in lignocellulosic biomass valorization for biofuels and value-added chemical production in the EU: A focus on biochemical conversion processes

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ABSTRACT

In light of the growing environmental challenges associated with rapid industrialization, climate change, and escalating global energy demand, the transition to sustainable and renewable energy sources has become increasingly urgent. The European Union (EU) Renewable Energy Directive targets 42.5% renewable energy by 2030, compared with about 25.2% in 2024, highlighting the urgent need to expand sustainable energy technologies. The valorization of lignocellulosic biomass for the production of bioenergy and value-added chemicals is particularly critical for advancing sustainable waste management practices and enabling a low-carbon circular bioeconomy. In the EU, biomass supply is dominated by agriculture (70%) and forestry (27%). Lignocellulosic biomass availability increased from approximately 492 million tons of dry matter in 2015 to 513 million tons in 2020 and is projected to reach nearly 551 million tons by 2030. This study provides a comprehensive overview of advanced biochemical conversion pathways for lignocellulosic biomass, with a specific focus on anaerobic digestion, dark fermentation, and syngas fermentation. These processes are examined in terms of their underlying mechanisms, operational conditions and product yields alongside the unique challenges and opportunities associated with each technology. The emphasis on biochemical routes is motivated by the fact that our previous work extensively covered thermochemical conversion processes, and this review aims to provide a complementary perspective. To set the foundation for future research in biochemical and integrated lignocellulosic biomass valorization, this study also synthesizes prior research efforts, identifies key knowledge gaps, and highlights emerging prospects for sustainable bioprocess development.

1. Introduction

With industrialization and rapid population growth, the global energy demand has increased quickly over the past few years and is the most serious challenge of the modern era. The world population is forecasted to be 9 billion people by 2050, and the energy demand will increase by 50% [1]. Around 80% of the global energy supply and 66% of electricity generation depend on traditional resources such as fossil

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fuels, while these fuels release a lot of greenhouse gas (GHG) to the atmosphere.

According to data released in 2023, the amount of atmospheric CO_2 was 423.9 ppm, which is 144 ppm higher than that reported during the preindustrial period [2]. According to the Intergovernmental Panel on Climate Change Sixth Assessment Report, high and very high greenhouse gas emission scenarios (SSP3-7.0 and SSP5-8.5) project sustained increases in CO_2 emissions, potentially doubling relative to current levels by mid-century or by 2100. In contrast, intermediate scenarios (SSP2-4.5) are projected to stabilize emissions, whereas low-emission pathways (SSP1-1.9 and SSP1-2.6) are expected to result in net-zero or net-negative CO_2 emissions after 2050[3]. At the 2015 Paris Climate Conference, the UN countries agreed to limit the climate warming to below 2 °C compared to the preindustrial levels [4]. The average temperature for 2020 is reported to be 1.2 ± 0.1 °C higher than the baseline of 1850-1900 [5].

Considering this global problem associated with our overdependence on consuming fossil fuels, the replacement of these traditional fuels with renewable and sustainable sources could alleviate this problem [2]. In March 2023, the European Union (EU) Renewable Energy Directive (RED) stated that at least 42.5% of the EU's energy needs to be provided by renewable sources by 2030 [6]; in 2024, approximately 25.2% of the consumed energy in EU countries was provided by renewable energy [7], and 17.3% is needed to reach the 2030 targeted value. Energy Roadmap 2050 predicted that about 30% of the whole energy consumption in the EU can be produced by renewable energy by 2030 [8].

Biofuels, with the same properties as petroleum-derived fuels, are used as important sustainable sources for different applications such as transportation, energy generation, industrial boilers, etc. [9]. A life cycle assessment (LCA) of this fuel implies that during the whole process of biofuels production, to the last use of burning biofuels in an engine, the amount of CO_2 released is lower than that of traditional sources [10]. In Europe, biofuel production has increased substantially since the early 2000s, rising from about 13 thousand barrels of oil equivalent per day in 2000 to approximately 294 thousand barrels of oil equivalent per day in 2024. Germany produced the highest amount of biofuels among European countries (66 thousand barrels of oil equivalent per day), followed by the Netherlands (39) and France and Spain (29) in 2024 [11]. Biofuels production in some European countries is shown in Fig. 1.

The successful development of biofuels production is highly dependent on the type of feedstock. Biofuels can be divided into different generations according to the biomass sources. Food-based biofuels production, called first-generation biofuels, have been associated with environmental and economic risks, such as increased use of land, food crisis, enhanced food prices, damage to ecology and biodiversity, and the amount of GHG emissions [12]. According to the European Commission RED in 2012, the number of food-based biofuels approved to be reduced to 5% of the targeted 10% renewable energy [13]. The growing conflict of first-generation biofuels production with food supply has stimulated attention toward the second-generation biofuels, which are based on lignocellulosic biomass and produced from non-edible biomass.

The main feature of lignocellulosic biomass is that they are abundant around the world with relatively low cost, tackles the direct competition with edible food, and does not need to be bred on arable land[14.] Despite the growing number of review papers on lignocellulosic biomass valorization, most existing reviews focus on a single conversion route [14–16], specific reactor configurations [15], pretreatment methods [17], or global biomass-to-energy pathways [18], rather than providing an integrated assessment of anaerobic digestion, dark fermentation, and syngas fermentation within the EU context [19]. Recent reviews have, for example, discussed decentralised lignocellulosic biomass conversion pathways at a broad systems level and the importance of techno-economic and environmental assessment for sustainable bio-based processing. Still, they do not specifically examine how EU biomass availability, utilization patterns, policy drivers, and circular bioeconomy objectives shape the development of biochemical conversion technologies.

In this review, the EU context refers to studies, datasets, and reports selected based on one or more of the following criteria: (i) feedstocks originating from EU countries or representative of major European lignocellulosic biomass streams, including agricultural residues, forestry residues, energy crops, and organic biowastes; (ii) experimental, pilot, industrial, statistical, or policy evidence relevant to deployment in the EU; and (iii) discussion of process opportunities and constraints linked to EU decarbonisation targets, renewable energy policies, and circular bioeconomy strategies. Building on our earlier review of thermochemical conversion pathways [14], this article provides a complementary biochemical perspective by integrating the three key biological routes into a single review. These technologies include AD, dark fermentation and syngas fermentation. The review also highlights the prospects and potential of these technologies for the future EU biorefineries and low-carbon resource recovery systems.

This integrated EU-focused perspective constitutes the main novelty of the present review. More importantly, the EU-specific, cross-pathway synthesis of lignocellulosic biomass valorization through AD, dark fermentation, and syngas fermentation, with additional emphasis on biofilm-based enhancement and implications for circular biorefinery development, presents new insights and novel contributions presented in this review.

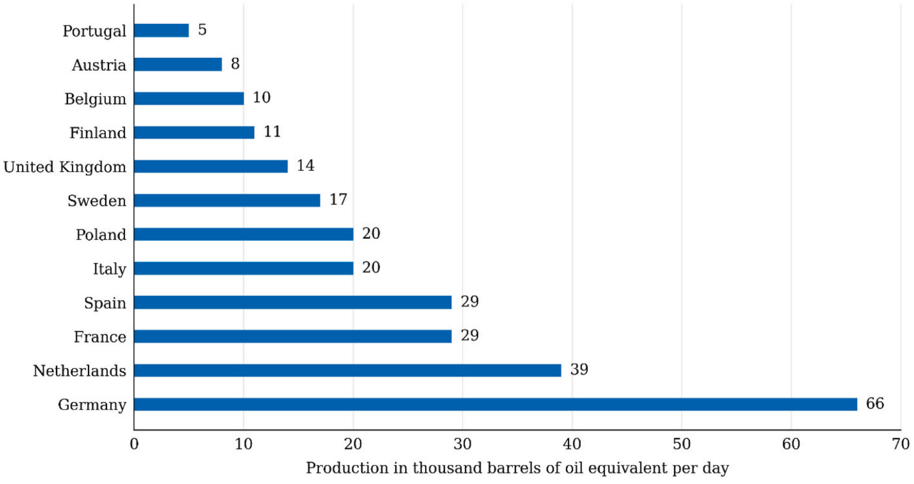

Fig. 1. Biofuel production in selected European countries 2024 [11].

2. Biomass available in Europe

In many developing countries, the main source of energy is provided by burning biomass. However, this traditional way is not sustainable, and inefficient combustion of biomass can be associated with severe environmental implications. Due to the emergence of new technologies, biomass can change to different forms (solid, liquid and gaseous) and be utilized to produce energy more effectively. At the EU level, biomass supply is dominated by the agricultural and forestry sectors. Approximately 70% of the total biomass supply originates from agriculture, including food crops, agricultural residues, and grazed biomass, while about 27% is derived from forestry-related sources [20]. Biomass produced in the EU for food purposes amounts to roughly 500 million tons of dry matter per year, of which approximately 100 million tons correspond to plant-based food [20]. Forestry represents a major source of biomass in the EU, with approximately 551 million m^3 of wood harvested from forests in 2017, in addition to about 180 million m^3 of secondary wood by-products generated during wood processing [20]. These large biomass flows from agricultural and forestry systems represent an important source of lignocellulosic residues that can be utilized for bioenergy and bioproduct production. Lignocellulosic biomass, a renewable resource with high energy density (6-6.5 kWh/m^3), is produced in large quantities, about 181.5 billion t/year globally [14], and comes mainly from agricultural and forestry residues and energy crops [21].

Maize, wheat, rice, and sugarcane are among the most widely cultivated agricultural crops worldwide and occupy the largest share of global cropland. These crops contribute significantly to the generation of lignocellulosic residues in the agricultural sector, while other agricultural wastes represent only a relatively small fraction of total agrowaste production [22]. The residues produced from these crops are substantial; for example, corn cultivation generates approximately 4 tons per acre of corn stover, whereas wheat production typically produces about 1–3 tons per acre of wheat straw annually [19,22]. The global availability of rice straw is 731 million tons/year, and sugarcane bagasse is 180 million tons/year [22]. The global production of plant biomass is reported to be around 200 billion tons per year, although only about 8-20 billion tons of this biomass is potentially accessible for biofuels production [23].

To assess temporal trends in lignocellulosic biomass availability in Europe, EU-level biomass potential estimates from the literature were compiled for multiple time points [24]. Fig. 2 presents the evolution of total lignocellulosic biomass availability in the EU from 2015 to 2030, expressed in million tons of dry matter. Only the lignocellulosic fraction, including agricultural and forestry residues and dedicated lignocellulosic crops, was considered in this analysis.

The results show a gradual increase in lignocellulosic biomass availability over time, rising from approximately 492 Mton dry matter in 2015 to about 513 Mton dry matter in 2020, with further increases projected to reach approximately 537 and 551 Mton dry matter by 2025 and 2030, respectively [24]. This trend reflects an increasing pool of lignocellulosic feedstocks available in Europe and highlights their growing relevance for bioenergy and bioproduct applications. These estimates represent aggregated EU-level sustainably removable lignocellulosic biomass availability derived from published scenario-based assessments and are intended to illustrate temporal trends rather than actual utilization [24].

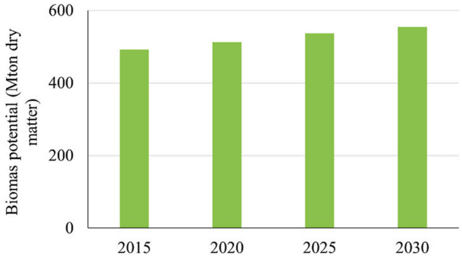

Fig. 2. Lignocellulosic biomass availability in the EU from 2015 to 2030, expressed in million tons of dry matter [24].

In Europe, lignocellulosic biomass availability varies with geography, with forestry sources including aspen, alder, birch, willow, and poplar the most predominant [25]. Willow dominates in Denmark, the Netherlands, the UK, and Ireland, while poplar is common in warmer areas like France, Italy, and Spain [26]. Among energy crops, miscanthus is considered a suitable source for biofuel production in Europe due to its wide climate adaptability [27]. Regardless, the EU croplands dedicated to lignocellulosic crops remain limited [28].

Wheat straw is the most available lignocellulosic biomass produced from plant residue [29]. Agricultural crop residues, including stalks and leaves of cereals and vegetable crops, have an estimated realistic potential of 74.89 million tons/year in the EU, while the technical-sustainable potential for residues excluding grassland is estimated at about 104 million tons per year, which exceeds the contribution from forestry residues [30]. Considering environmental constraints, the estimated amount of straw and stover potential in EU-27 was 45-215 million tons/year (2017), 115-185 million tons/year (2020), and 110-165 million tons/year (2030) [31].

Key biofuel crops in the EU include miscanthus and short rotation coppice in the UK/Europe [32], sorghum and short rotation forestry in Italy [33], and maize in Germany [34]. In Europe, switchgrass, reed canary grass, miscanthus, and giant reed are considered the most promising perennial grasses for biofuel production [35]. The biomass yield of lignocellulosic feedstocks used for biofuel production varies widely, largely due to differences in environmental conditions and species-specific growth potential [36]. For instance, reported yields for switchgrass, reed canary grass, miscanthus, and giant reed across Europe range from 9 to 25, 3–14, 10–30, and 7–61 Mg/ha, respectively [37]. The yield level of some agricultural crop residues in EU-27 is represented in Appendix A**,** appsec1Table A.1 and A.2 [30].

Actual crop yield refers to the amount of the primary agricultural product harvested from a crop. This value reflects its productivity under given agronomic and environmental conditions. Residue yield represents the plant material remaining after harvest of the main product, such as straw, stalks, or leaves, and is directly linked to the yield of the primary crop. The significant difference between these biomass yields is due to their adaptability to specific conditions of regional climate. It is reported that reed canary grass is more adaptable to northern European climate conditions. On the contrary, the adaptability of miscanthus and switchgrass is higher in northern and central Europe, and giant reed seems to be more adapted in the Mediterranean zone [36].

3. Biofuel production technologies: a focus on biological conversion processes iological conversion processes

Lignocellulosic biomass can be converted to biofuels using two ways: Biological processes including fermentation and anaerobic digestion (AD) and thermochemical processes such as pyrolysis (See Fig. 3). The thermochemical processes require more energy input including energy for biomass drying [39].

In the biological conversion technologies (often called biochemical conversion processes), microbial organisms (mainly anaerobic bacteria) carry out the multi-phase reactions for the production of biofuels such as methane, hydrogen, diesel, ethanol, etc., along with value-added chemicals like acetic acid, propionic acid, etc. The inherently flexible nature of these organisms in terms of adaptability to the inhibitory compounds formed during the biochemical reactions and low energy requirement presents a compelling advantage of biological processes compared to the thermochemical processes.

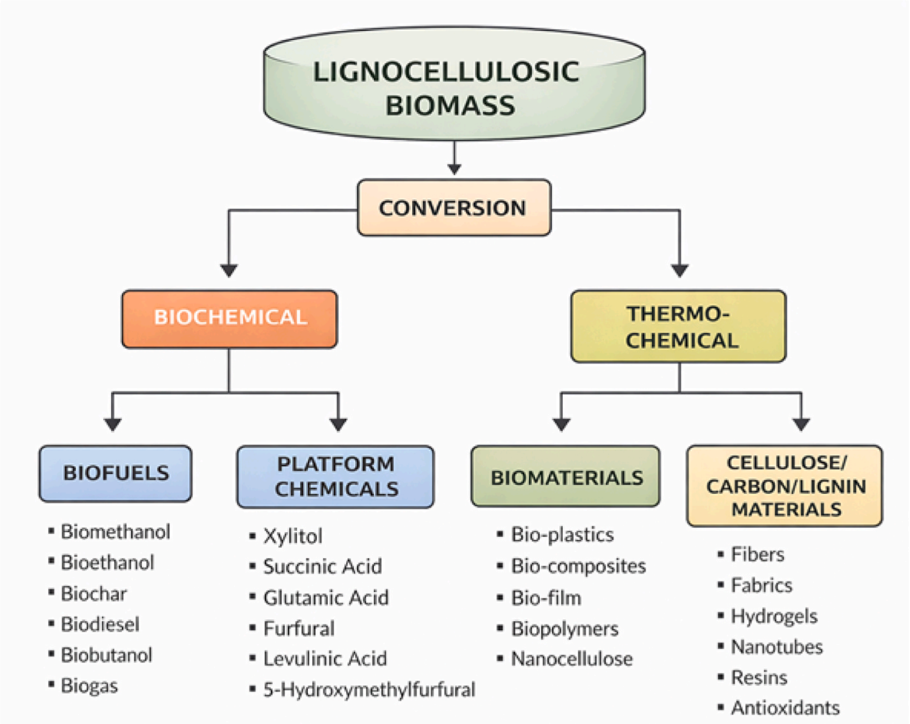

Fig. 3. Biochemical and thermochemical conversion of lignocellulosic substrate into biofuels and value-added bioproducts (Sharma et al. [38]).

3.1. *Anaerobic digestion (AD)*

Anaerobic digestion (AD) is a biological process which transforms the organic and inorganic matter of wastes into biogas using different groups of microorganisms in the absence of oxygen (Fig. 4). The main content of the produced biogas is methane and carbon dioxide (around 50-70% CH_4 and 30-50% CO_2). Biogas may also contain little amount of other gases such as hydrogen, hydrogen sulphide, ammonia and nitrogen depending on the type of utilized feedstock and the operating conditions. AD process includes four steps including hydrolysis, acidogenesis, acetogenesis and methanogenesis. It is important to note that different microbial communities are responsible for each stage [41].

The first step of the AD process is hydrolysis, where undissolved organic compounds, including lipids, carbohydrates, polysaccharides, and proteins that cannot be taken up and digested by microorganisms directly, are converted to water-soluble compounds by the hydrolytic anaerobic microorganisms. The hydrolysis of carbohydrates is faster than proteins and fats, as the oxygen dissolved in the liquid is utilized by the facultative anaerobic bacteria, and this leads to a reduction in the redox potential, which is required for obligatorily anaerobic bacteria

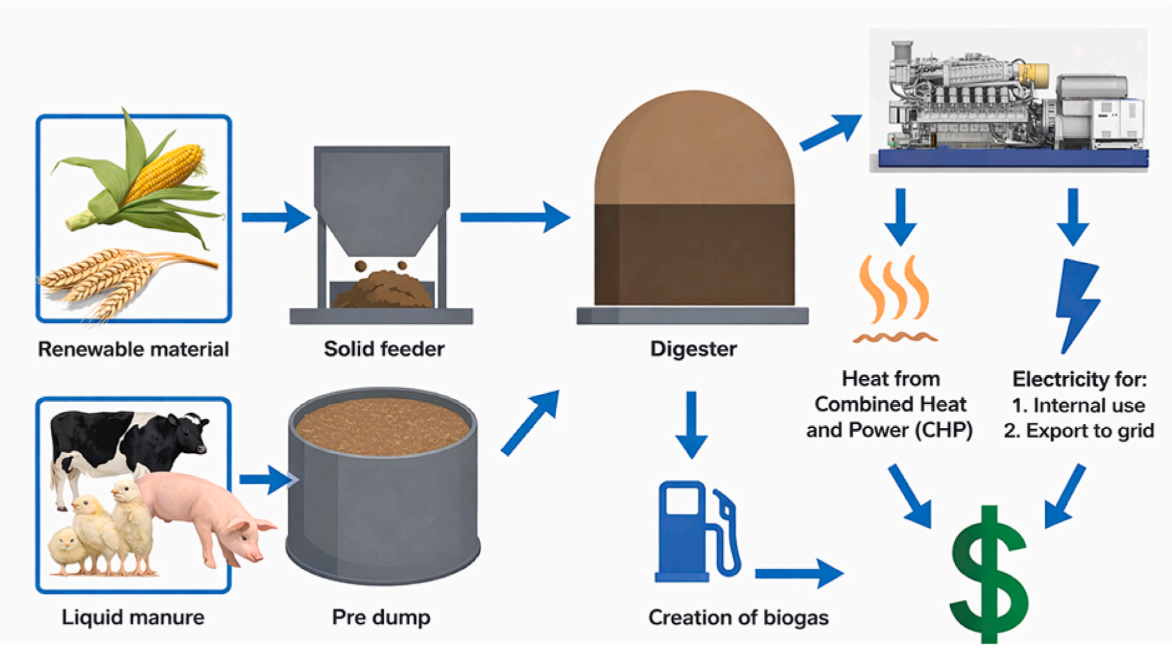

Fig. 4. Anaerobic Digestion Process (André et al. [40]).

[42]. In the second step, acidogenic microorganisms take up hydrolytic products such as monomers, sugar, amino acids and long-chain fatty acids and degrade them into volatile fatty acids, carbon dioxide, and hydrogen. Then, the terminal products formed by acidogenic bacteria are turned into acetate, carbon dioxide, and hydrogen by acetogenic microorganisms. In this stage, the dominant group of acidogenic bacteria, namely obligate hydrogen-producing acetogens, metabolize fatty acids to acetate, CO_2, and H_2, while a small number of homoacetogens produce acetate from CO_2 and H_2. During the final stage of the AD process, methane (biogas) is produced by two groups of methanogenic bacteria. Hydrogenotrophic methanogens utilize carbon dioxide (as an electron acceptor) and hydrogen (as an electron donor) to form methane, and Acetoclastic methanogens consume acetate to produce biogas [41,42].

Due to the complex function of different microbial communities, imbalanced interactions between microbial groups could change the reaction rate and result in accumulating inhibitory matters [39]. Among different groups of microorganisms, methanogenesis has the lowest growth rate. Therefore, the growth of methanogens is considered the rate-limiting step during the AD process [39]. However, the rigid structure of lignocellulose wastes makes hydrolysis the rate-limiting stage due to the recalcitrant nature of lignocellulosic-containing feedstocks [39].

Biogas generation via AD is an environmentally friendly way that was utilized as an alternative source to around 36 Mtoe of fossil fuels in 2018 [43]. Biogas can be used for a broad range of applications, including producing thermal energy, steam, and electricity [44]. In the EU, the main application of biogas is primarily for generating electricity and/or heat. According to the data released by the European Biogas Association (EBA), the EU witnessed a 67% growth (+7699 units) in biogas plants between 2009 and 2016 (from 6227 to 17,662 units), and this was due to the increasing use of agricultural biomass in biogas plants [45]. The produced biogas in Germany comprised 64% of the total generated biogas in the EU in 2015, and it is considered a leading contender in biogas production from the fermentation process of agricultural crops and wastes [46].

The produced biogas from lignocellulose biomass can be upgraded to biomethane and utilized as a fuel for different purposes as an alternative source to natural gas [44]. In Europe, biomethane production has increased from 2.5 × 109 m^3 to more than 18 × 109 m^3 between 2000 and 2015 [47]. The average biomethane potential (BMP) of different lignocellulosic biomass is shown in Fig. 5. All BMP values presented in the figure are normalized to volatile solids (VS) and expressed as mL/g VS to allow direct comparison among substrates. According to this figure, ensiled by-products, grasses and leaves, and oil seeds, fruit shells and wastes produce the highest amount of biomethane, ranging from 250 to 300 mL/g VS. Agricultural by-products and waste (e.g., different types of straws, stover, bagasse) and hardwoods have the average BMP within the range of 150- 200 mL/g VS^{40}. The higher BMP values observed for ensiled by-products, grasses and leaves, and oil seeds and fruit wastes are mainly attributed to their higher content of readily degradable organic compounds and lower structural recalcitrance. In contrast, agricultural residues such as straw, stover, and bagasse, as well as woody biomass, contain more complex lignocellulosic structures and relatively higher lignin fractions, which limit microbial access to cellulose and hemicellulose and reduce methane yields during AD [41]. However, the amount of produced biogas and the content of biomethane can be improved by applying a suitable pre-treatment method based on the characteristic variations of lignocellulose substrate [48]. Fig. 5 demonstrates a significant increase in the average of BMP for some substrates after utilizing pre-treatment. For example, pre-treatment can increase the average BMP for grasses and leaves (with a higher percentage of cellulose and hemicellulose) to more than 400 mL/g VS. It can be noted from the figure that the maximum BMP enhancement after pre-treatment is related to pulp and papering residues changing from 130 to over 200 mL/g VS. The increase in BMP for these substrates is mainly associated with enhanced enzymatic hydrolysis, greater water solubility, lignin solubilization, and reduced crystallinity of cellulose [41]. On the other hand, flowers and seeds, as well as soft woods, have the lowest potentiality of biomethane production before and after pre-treatment [41]. This behavior is primarily associated with their higher lignin content and the presence of recalcitrant structural compounds that limit microbial hydrolysis and biodegradation during AD [49]. Interestingly, not all woody biomass behaves similarly during AD. Hardwoods and softwoods differ significantly in lignin structure, which influences their biodegradability [50]. Softwood lignin consists predominantly of guaiacyl units that form highly condensed structures, whereas hardwood lignin contains both guaiacyl and syringyl units that are typically less condensed and therefore more susceptible to microbial degradation, leading to higher biomethane potential before and after

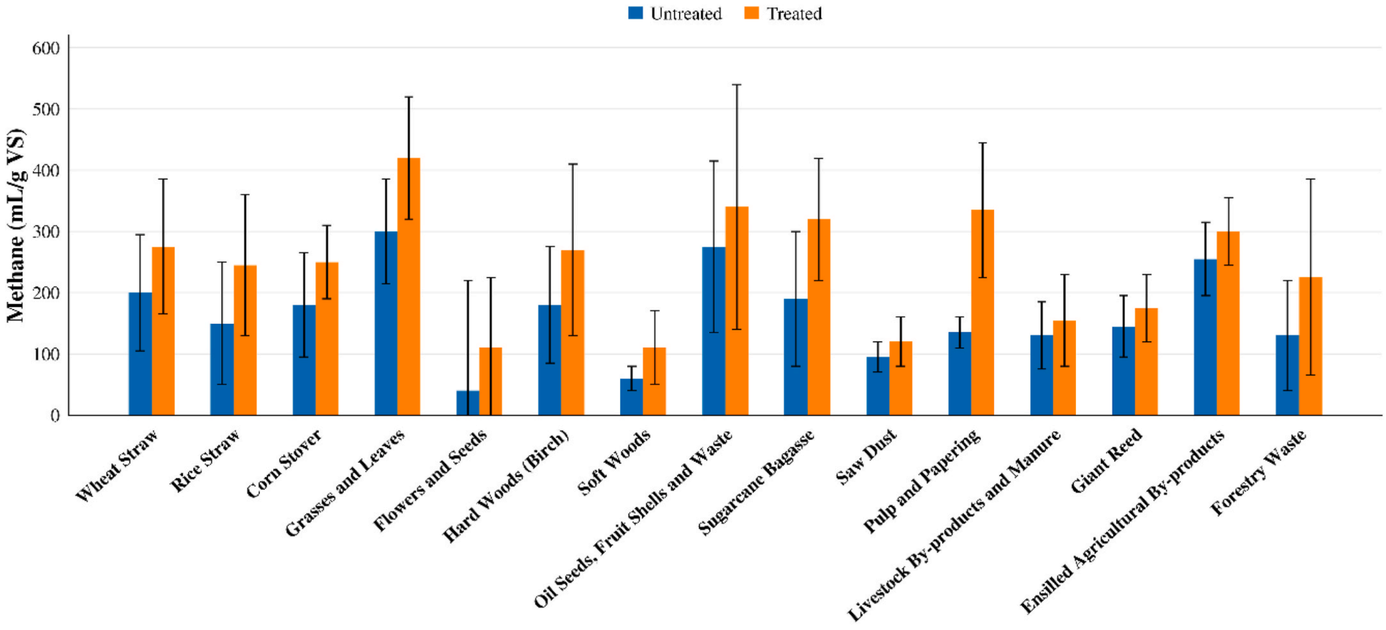

Fig. 5. Biomethane potential of various lignocellulosic substrates [41].

pre-treatment [50].

Table 1 summarizes various pre-treatment strategies reported to enhance the biodegradability of lignocellulosic biomass and improve methane production during AD. The main objective of these pre-treatment techniques is to reduce cellulose crystallinity, disrupt the lignin barrier, and increase the accessible surface area for microbial and enzymatic activity [41]. By modifying the structural complexity of lignocellulosic materials, pre-treatment can significantly enhance substrate hydrolysis, which is often the rate-limiting step in AD [68].

As shown in Table 1, pre-treatment approaches can generally be classified into mechanical, thermal, chemical, and biological methods. Among these, thermal pre-treatment has frequently demonstrated substantial improvements in methane production and has been widely applied to different lignocellulosic substrates. For instance, hydrothermal pre-treatment of safflower straw has been reported to increase methane production by 406.9 mL/g VS during AD [56], highlighting the effectiveness of thermal pre-treatment in improving substrate digestibility.

Table 1
Different pre-treatment methods for lignocellulosic materials.

Substrate	pretreatment	Pretreatment condition	Methane improvement (mL CH_4/g VS)	References
Physical Pre-treatment				
Sugar beet pulp	Grinding	2.5 mm particle size	100.7	Kowalska-Wentel and Ziemiński [51]
Wheat straw	Cavitation	-	17.9 mL/g TS	Patil et al. [52]
Lay silage	Grubben deflaker and a Krima disperser	<2 mm	104	Lindmark et al. [53]
Paper waste	Hollander beater	-	44	Rodriguez et al. [54]
Thermal Pre-treatment				
Rice straw	Microwave	190 °C/4 min	325.2	Kainthola et al. [55]
Safflower straw	Hydrothermal	180 °C/60 min	406.9	Hashemi et al. [56]
Rice straw	Steam explosion	280 °C/15 bar/ 10 min	486	Aski et al. [57]
Saw dust	Autoclave	120 °C/15 min	315	Bala and Mondal [58]
Chemical pre-treatment				
Cotton waste	Acidic	Citric acid, 0.5 mmol/gVS	54	Pellera and Gidarakos [59]
Wheat straw	Alkaline	6%KOH, 3days, room temperature	75	Jaffar et al. [60]
Birch wood	Redox reactions/ Fenton reactions	0.001M $FeCl_3$, 0.01M H_2O_2, 2h	17	Lamb et al. [61]
Cocoa shell	Ionic liquid	NMMO,120 C,3h	27	Mancini et al. [62]
Biological pre-treatment				
Wheat straw	Ensiling	Lab-scale ensiling in plastic bag	96	Gallegos et al. [63]
Corn stover	Fungi	Pleuroluseyngii, 30 days	59	Wyman et al. [64]
Corn straw	Micro-aeration	5mLO_2/gVS	40	Xu et al. [65]
Wheat straw	Microbial consortium	Originated from decomposed wood, 50 C, 7days	136	Tantayotai et al. [66]
Enzymatic	Birch	H_2O_2, 85% Celluclast and 15% NcLPMO9C (Cell+9C) blend	−71	Costa et al. [67]

Despite the significant benefits of pre-treatment in improving methane yields and digestion rates, several limitations remain. Chemical and enzymatic pre-treatment methods, particularly those involving alkaline reagents such as NaOH or specialized enzymes, are often associated with relatively high operational costs and require additional steps for chemical recovery and downstream processing [41]. Consequently, some of these pre-treatment methods have not yet been widely implemented at an industrial scale for AD.

In contrast, biological pre-treatment methods represent a relatively low-cost and environmentally friendly alternative for enhancing methane production. However, their application is often limited by slow treatment rates. One potential strategy to overcome this limitation is to integrate the pre-treatment process with biomass storage, allowing partial degradation of lignocellulosic structures prior to AD [41]. The advantages and disadvantages of each pretreatment method is shown in Table 2.

3.2. Dark fermentation

Dark fermentation is one of the significant and promising methods for bio-H_2 production over the past two decades, due to its higher production rates and its effectiveness for the utilization of various organic wastes (Fig. 6). An overview of studies that utilized dark fermentation for biofuel production with lignocellulosic biomass precursor is shown in Table 3. It is important to note that lignocellulosic biomass comprises of lignin, cellulose, and hemicellulose [77]. However, using lignocellulosic feedstocks for bio-H_2 production in large-scale industries is quite complicated due to the cost management of pre-treatment arising from the recalcitrant lignin present in the biomass. An overview of biomass pretreatment methods is outside the scope of the current review. However, several reviews have been documented that explain these processes [78,79]. Regardless, different pretreatment methods as well as their advantages and limitations has been documented in Table 2.

In the process of dark fermentation, organic substrates are converted into products like volatile fatty acids (VFA), ethanol, and bio-H_2 by the obligate anaerobic and facultative anaerobic bacteria, through the carbon chain reactions [80]. A wide range of anaerobic bacteria, like *Enterobacter, Klebsiella, and Clostridium*, have been used in the production of bio-H_2 in dark fermentation. Among the different methods employed for bio-H_2 production, dark fermentation is promising due to its relatively higher rate of production of bio-H_2.

The principal mechanism of H_2 production could be achieved through the removal of excess electrons by hydrogenase enzyme activity, where protons act as electron acceptors in an anaerobic environment, which eliminates the electrons created by oxidation of organic substrates [81,82]. Hoang et al. [83] observed that in the dark fermentative H_2 production process, carbohydrates were directly fermented into H_2, CO_2, and organic acids. Notably, the dark fermentation process results in the release of short-chain organic acids such as formic acid, acetic acid, lactic acid, butyric acid, and alcohols, which leads to the better production of bio-H_2. Hydrogen produced from dark fermentation can be applied for various industrial purposes, particularly fuel cells that produce electricity.

Fermentation is a metabolic process that occurs under anaerobic conditions to regenerate the cell's energy currency, adenosine triphosphate (ATP). Moreover, under anaerobic conditions, the tricarboxylic acid (TCA) cycle is blocked [84]. Bio-H_2 production from dark fermentation is mainly associated with the action of the hydrogenase enzyme. Three important biochemical reactions are mostly involved in dark fermentation. The first reaction, which mainly occurs in *E. coli* and *Enterobacteriaceae*, is catalyzed by the two major enzymes, pyruvate formate lyase (PFL) and formate hydrogen lyase (FHL); the second reaction involves pyruvate: ferredoxin oxidoreductase (PFOR) and Fd-dependent [Fe-Fe] hydrogenase (HydA); and the third reaction

Table 2
Advantages and limitations of different pretreatment methods.

Pretreatment Strategy	Classification	Examples/Methods	Advantages	Limitations
Grinding/Milling	Mechanical (Physical)	Ball milling, hammer milling, shredding, chopping	Increases surface area and porosity; improves microbial accessibility; improves mass transfer	High energy consumption; operational costs; may not sufficiently break lignin structures
Ultrasonication	Mechanical/Physical	Ultrasonic cavitation treatment	Breaks cell walls; increases solubilization of organic matter; improves hydrolysis rate	Energy intensive; scale-up challenges
High Pressure Homogenization	Mechanical/Physical	High-pressure disruption of biomass cells	Enhances substrate solubilization; improves biodegradability	High capital cost; high energy demand
Steam Explosion	Thermal	High-pressure steam followed by rapid decompression	Effective lignocellulose disruption; increases cellulose accessibility; improves methane yield	Equipment cost; formation of inhibitory compounds
Thermal Hydrolysis	Thermal	Heating at 100–200 °C before digestion	Breaks down complex polymers; improves sludge digestibility; increases biogas yield	High energy requirement; potential formation of inhibitors
Hydrothermal Pretreatment	Thermal	Hot water treatment under pressure	No chemicals required; improves hemicellulose solubilization	Requires specialized equipment; energy intensive
Alkaline Pretreatment	Chemical	NaOH, $Ca(OH)_2$, ammonia	Effective lignin removal; improves cellulose digestibility	Chemical cost; need for neutralization; potential environmental issues
Acid Pretreatment	Chemical	H_2SO_4, HCl	Breaks hemicellulose and lignocellulose bonds; enhances hydrolysis	Corrosion; formation of inhibitors (furfurals); chemical recovery required
Oxidative Pretreatment	Chemical	Ozone, hydrogen peroxide	Delignification; improved biodegradability	Chemical costs; safety concerns
Organosolv Pretreatment	Chemical/Thermochemical	Organic solvents (ethanol, methanol)	Efficient lignin removal; high-quality lignin recovery	Solvent recovery needed; high operational cost
Enzymatic Pretreatment	Biological	Cellulase, hemicellulase enzymes	Environmentally friendly; high specificity; minimal inhibitor formation	High enzyme cost; slower reaction rates
Microbial Pretreatment	Biological	Fungi (white-rot fungi), bacteria	Selective lignin degradation; low energy input; environmentally sustainable	Long processing time; sensitive to environmental conditions
Microaerobic Pretreatment	Biological	Controlled oxygen exposure before AD	Enhances microbial degradation; reduces toxic metabolite formation	Requires careful oxygen control
Combined Pretreatment	Hybrid (e.g., Thermochemical, Physicochemical)	Steam + alkaline; ultrasound + alkaline; mechanical + biological	Higher degradation efficiency; synergistic effects; improved methane yields	Higher complexity; higher cost and process control requirements

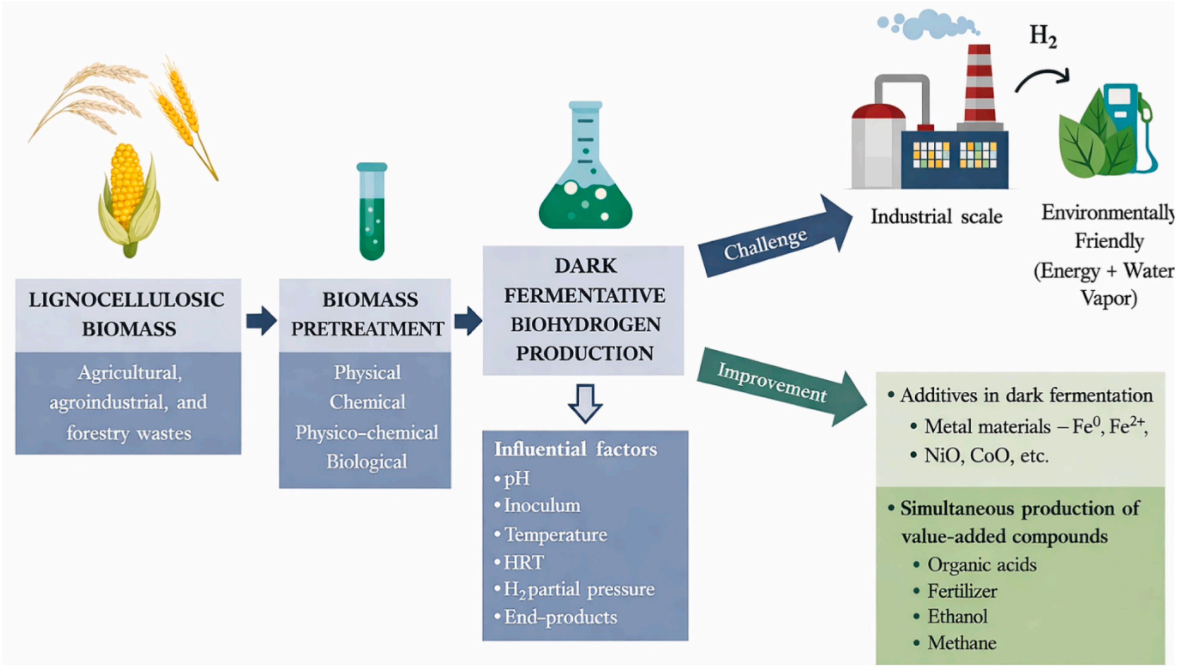

Fig. 6. Dark Fermentation, challenge & value-added compound (Soares et al. [69]).

involves NADH to increase bio-H_2 production.

In the first type of reaction, pyruvate formed via the Embden-Meyerhof pathway (EMP) breaks into acetyl-CoA and formate by PFL, which catalyses the reaction as represented in equation (1).

$$\text{Pyruvate} + \text{CoA} \rightarrow \text{Acetyl-CoA} + \text{formate} \quad (1)$$

$$HCOOH \rightarrow CO_2 + H_2 \quad (2)$$

Formate is then cleaved to form CO_2 by the FHL enzyme, as represented in equation (2). In these reactions, high production of formate activates the FHL transcription by formate-bound FHLA, and the resulting incomplete degradation of formate can cause less hydrogen production. Simultaneously, lactate dehydrogenase induced in the acidic conditions can convert the pyruvate into lactate, which results in

Table 3
Studies of dark fermentation for lignocellulosic biomass.

Substrate	Temperature (°C)	Reactor configuration, operation vol.	Inoculum pre-treatment	Substrate pre-treatment	[a]COD feedstock (mg l-1)	HRT (d)	[b]OLR	[c] ηH2	[d]εCOD (%)	Ref.
Rice straw	35	20L fed batch	Heat treatment	-	-	-	-	40mLH_2/g VS removed	-	Sattar et al. [70]
Rice straw	35	250 ml Batch	Heat treatment	Acid -Thermal pre-treatment	-	-	-	771 ml/L	-	Sen et al. [71]
Rice husk	35-37	100 ml Batch	Heat treatment	Acid -Thermal pre-treatment	-			3.3LH_2/L^{-}d	-	Gonzales et al. [72]
Acid algal hydrolysate	35-3 7	ASBR	Immobilized beads	Acid hydrolysis	16000	N/A	N/A	4.8 LH_2/L^{-}d	N/A	Kumar et al. [73]
Wheat straw	70	UASBR	Heat treated	Hydrothermal pretreatment	2900	1	N/A	821.4 mLH_2/L^{-}d	100	Kongjan et al. [74]
Oat straw hydrolysate	28	Trickling filter	Heat treated	Acid hydrolysis	35000	1	2.96	1.95 LH_2/ L^{-}d	N/A	Arriaga et al. [75]
Acid hydrolysate	35	FBR	Immobilized beads	Acid hydrolysis	16000	6	N/A	20 LH_2/L^{-}d	96	Anburajan et al. [76]

[a] Influent chemical oxygen demand.
[b] Organic loading rate in kg COD/m^3day.
[c] Hydrogen yield coefficient in (%; m^3 H_2/kg COD $_{\text{removal}}$).
[d] COD removal efficiency.

less production of hydrogen.

The second type of reaction mostly occurred in the *Clostridium species*. Pyruvate undergoes oxide reduction reactions, through oxidation of pyruvate to acetyl-CoA, which requires a reduction of ferredoxin (Fd) by PFOR, and then reduced Fd is oxidized by [FeFe] hydrogenase, which catalyses the formation of H_2 as represented in equation (3) and 4 [85]’ [86]. During this process, depending upon the various conditions, the reduced products of butanol, ethanol, and acetone can be produced [87, 88].

$$\text{Pyruvate} + \text{CoA} + 2\ \text{Fd (ox)} \rightarrow \text{Acetyl-CoA} + 2\ \text{Fd (red)} + CO_2 \tag{3}$$

$$2\ \text{H+} + \text{Fd (red)} \rightarrow H_2 + \text{Fd (ox)} \tag{4}$$

The third type of reaction, which probably occurs in the thermophilic bacteria and several *Clostridium species*, was mainly done by two enzymes NADH: ferredoxin reductase (NFOR) and HydA, and the oxidized Fd is reduced by NADH in the carbon metabolism from the reaction, which is described in the biochemical equations of 5-7 [87,89].

$$\text{Glucose} + 2\ \text{NAD+} \rightarrow \text{EMP}\ 2\ \text{Pyruvate} + 2\text{NADH} \tag{5}$$

$$2\ \text{NADH} + 4\text{Fd (ox)} \rightarrow \text{NFOR}\ 2\ \text{NAD+} + 4\ \text{Fd (red)} \tag{6}$$

$$4\ \text{Fd (red)} + 4\ \text{H+} \rightarrow \text{HydA}\ 4\ \text{Fd(ox)} + 2H_2 \tag{7}$$

Theoretical yield of a maximum of 4 mol of H_2 per mole of glucose is formed when pyruvate is oxidized to acetate as the sole metabolic end product from Eq (8). However, only 2 mol of H_2 per mole of glucose is produced when pyruvate is oxidized to butyrate, also known as the hydrogen production pathway shown in Eq. (9)[82]. Hence, the higher A/B (acetate/butyrate) ratio of organisms following a mixed acid pathway is essential for greater H_2 production, as represented in the following equations [90,91].

$$C_6\,H_{12}\,O_6 + 2H_2O \rightarrow 2\ CH_3COOH + 2\ CO_2 + 4H_2 \tag{8}$$

$$C_6\,H_{12}\,O_6 \rightarrow CH_3CH_2CH_2COOH + 2\ CO_2 + 2H_2 \tag{9}$$

In the mixed culture dark fermentation, anaerobic consortia from open environmental sources contain H_2-consuming microorganisms, e.g. methanogens, propionate-, lactate-, valerate-, and caprate-. During the H_2 production, ethanol-producing microorganisms consume the H_2 as an electron donor thereby leading to a decrease in production yield.

The H_2-consuming reaction occurs in two ways: as reduction equivalents (NADH2; potential H_2) and as molecular hydrogen or other acids fermentation [92]. Also, some facultative-anaerobic bacteria, such as enteric bacteria, can perform anaerobic respiration rather than fermentation with nitrate, fumarate as terminal electron acceptors. To control the hydrogen-consuming reaction, many strategies are applied from the various reports. Propionic acid inhibition, known as one of the hydrogen-consuming pathways, is mainly caused by two reasons: one is the formation of hydrogen partial pressure, and the other is the domination of propionic acid-producing bacteria in the community. Heat treatment is one of the strategies to effectively inhibit the propionic acid formation and control the domination of propionic acid-forming bacterial species, as reported by Sivagurunathan et al. [93], and it improved the population of hydrogen-producing bacteria, while it inhibited the growth of hydrogen-consuming bacteria. On the other hand, sparging N_2 gas in the dark fermentation reduces the partial pressure and maintains the proper anaerobic condition [94].

Inhibition of hydrogen production occurs when the accumulation of various bacteria in the anaerobic consortium results in unbalanced chemical or kinetic conditions, leading to the alteration in the electron flux between the various bacterial communities, such as acidogens and acetogens involved in reactions of equations (9)–(16). Significant accumulation of VFA could inhibit the metabolic activity due to the increase in ionic strength and inhibition by undissociated acids. Considering that acetate/butyrate are the major VFA produced in the dark fermentation from glucose. Lactic acid bacteria (production of bacteriocins), sulfate-reducing bacteria (SRB), and homoacetogenesis have been recently observed by the researchers in AD and particularly affecting hydrogen production [95]. Acidogenesis is a process where acetate is produced by heterotrophic microorganisms through the oxidation of organic acids; meanwhile, in homoacetogenesis, the acetate is produced by autotrophic bacteria through CO_2 reduction along with H_2. This homoacetogenesis process competes with other microorganisms in H_2 dark fermentation. For example, homoacetogens grow faster than butyrate- and propionate-degrading acetogens because their affinity to H_2 is higher when compared to the affinity of acetogens to propionate and butyrate [96]. The dark fermentation for hydrogen production also involves the transformation of organic compounds into various organic and inorganic compounds that are commercially valuable products, such as acetic acid, butyric acid, propionic acid, caproic acid, lactic acid, ethanol, and formic acid. Applying the suitable LCB in the biobased economy could increase the global efficiency of biomass-based production of valuable products, and it might be attributed to the huge impact or an alternative to the petroleum-based industries.

Table 3 shows the various lignocellulosic biomass that were used as a substrate in biohydrogen production. Kongjan et al. [74] performed the biohydrogen production from oat straw hydrolysate in the UASBR (upflow anaerobic sludge blanket reactor), along with thermophilic conditions. The thermophilic condition enhances hydrolysis activity and the biohydrogen production system in the microbiome. On the other hand, Kumar et al. [73] biohydrogen production by using pre-treated AAH (acid algal hydrolysate) in ASBR (anaerobic sequencing batch reactor) with immobilized bead system and Anburajan et al. [76] using the same feed stock for hydrogen production in the continuous production with the immobilized beads and the specific reactor configuration FBR (fixed bed reactor). This study proved the immobilized system and the reactor configuration, trapped the active biomass and increased hydrogen production and showed the inhibitory tolerance.

3.3. Syngas fermentation

In syngas fermentation, industrial exhaust gases and gasified lignocellulosic biomass (rich in CO, CO_2 and H_2) are fermented into energy

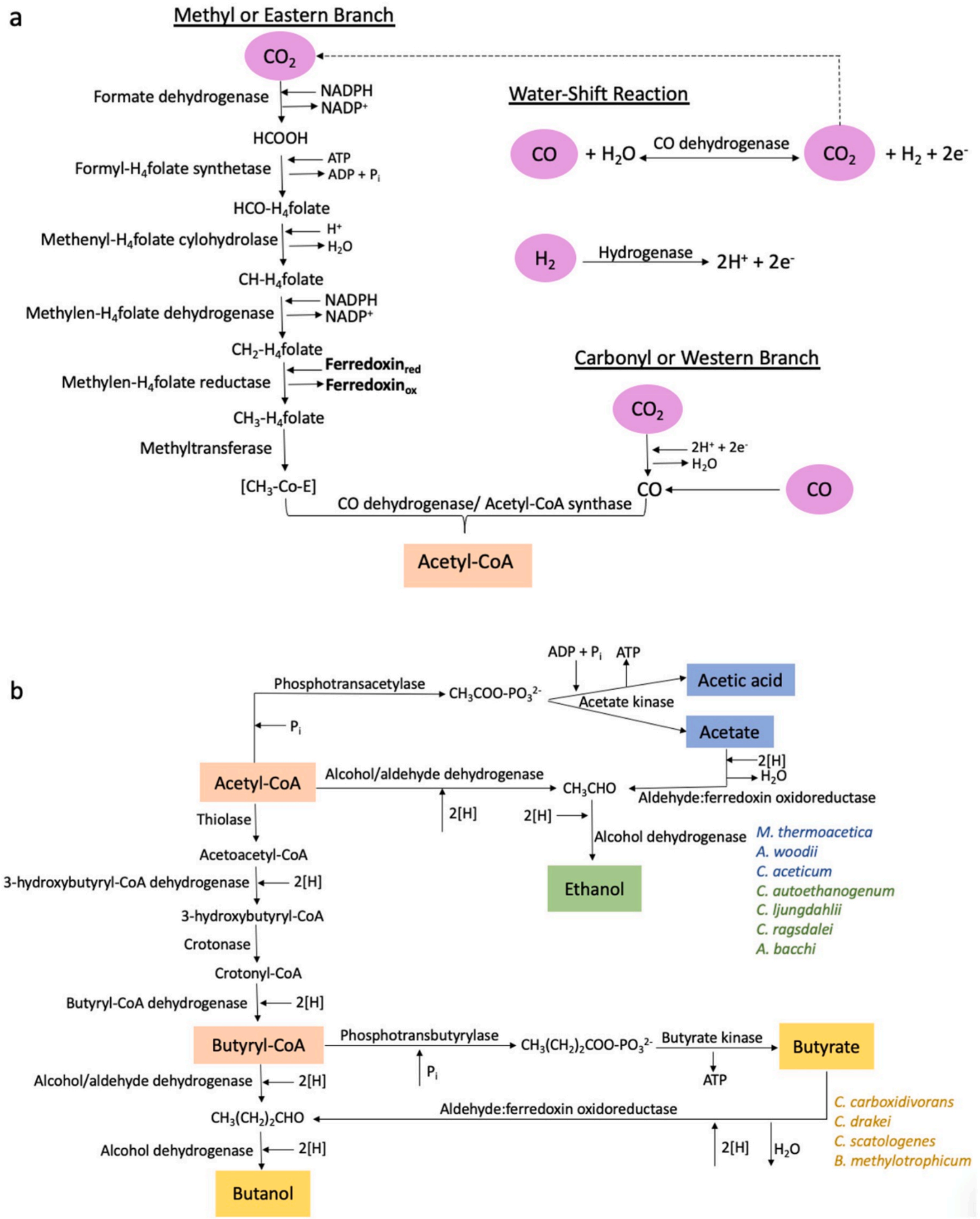

Fig. 7. (a) Acetogenesis and (b) Solventogenesis phases of Wood-Ljungdahl metabolic pathway adopted from Ref. [97]. The product of interest is colour-coded based on the type of bacteria involved.

carriers like ethanol, butanol, hexanol, as well as value-added chemicals such as acetic acid, formic acid, and biopolymers by acetogenic bacteria, namely *Clostridium ljungdahlii, Clostridium autoethanogenum, Acetobacterium woodii* and *Clostridium carboxidivorans*, through Wood-Ljungdahl (also known as the reductive acetyl-CoA pathway) in the mesophilic temperature range. Furthermore, the gaseous H_2 fuel can also be synthesized through water gas shift (WGS) reactions where CO oxidation to CO_2 is coupled with proton reduction as a result of CO dehydrogenase enzyme activity [97,98]. Despite being a relatively new technology, it is well studied and commonly accepted owing to its inherent advantageous including i. high enzymatic selectivity for enhanced product yield [99], ii: flexibility on feedstock composition in terms of H_2:CO ratio [100], iii: achieving multiple end products when a mixed culture is used [101], iv ability to adopt presence/formation of inhibitory compounds [102], v: lower operation costs due to the mild process conditions with regards to other syngas utilizing technologies [103], vi: elimination of complicated pre-treatment steps [104]. On the other hand, poor mass transfer rate at the gas-liquid interface due to limited CO and H_2 solubility and low ethanol conversion yield of biocatalysts are the major process challenges [105,106]. Some mass transfer limitations include (i) diffusion of gaseous substrates into the gas-liquid interface, (ii) their transfer into liquid media, (iii) the gaseous substrate diffusion into the stagnant liquid surrounding of the microbes and (iv) the transport of the diffused gases into the cells. Traditionally, volumetric mass transfer rate (kLa) is increased corresponding to the increased agitation speed in continuous stirred tank reactor, however, the recent progress in biofilm reactor design addressed this challenge in a more energy efficient way [97] has recently reported the operational principles of biofilm reactors employed for syngas fermentation and compared the impact of the different biofilm technologies on the mass transfer rates.

The metabolic pathway can be divided into two main stages known as acetogenesis and solventogenesis [107]. The metabolic reactions take place in both stages of syngas fermentation and are given in Fig. 7. In the acetogenesis step, CO and CO_2 undergo a series of reductive reactions catalyzed by hydrofolate-dependent enzymes to form a methyl group in Eastern Branch. Subsequently, methyl-CoA and carbonyl groups, originating from Western Branch, are combined by CO dehydrogenase/Acetyl-CoA synthase enzyme complex to form Acetyl-CoA. Acetyl-CoA is the main intermediate product of the process as it is further reduced to produce ethanol, butanol and value-added chemicals in the Solventogenesis phase [108,109]. Acetogenesis reactions are often redox imbalanced, as an electron donor (ferredoxin) with an extremely low reduction potential (<-500) is required for the activity of CO dehydrogenase/Acetyl-CoA synthase enzyme complex [110–112]. Therefore, extracellular electron transfer mediators like NADPH, NADH, with various redox potentials, determine the Acetyl-CoA production yield and correspondingly the syngas fermentation efficiency [113,114]. Advances of bio-electro fermentation processes in terms of electron transfer mechanisms and syngas conversion yield have recently been reported by Barbosa et al. [115].

The common biochemical reactions that occur in syngas fermentation processes are given in Table 4. 66% of the carbon in the form of CO present in syngas can theoretically be converted into ethanol based on Eqs (1) and (2). Moreover, 50% of the carbon in CO form can be utilized for acetic acid production based on Eq. (3).

The H_2 content of the syngas is the major factor affecting the overall fermentation yield, as it provides the required electrons and H^+ ions for the hydrogenase enzymatic activity for ethanol conversion. In case of hydrogenase enzyme inhibition due presence of nitrous oxides, ethylene, ethane or acetylene when biomass-derived syngas is used as a substrate [116,117], the electron production from H_2 is blocked; therefore, the CO utilization is diverted to electron production from product formation [118]. Furthermore, ethanol and organic acid accumulation are associated with inhibitions as they impair the microbial cell integrity due to hyperpolarisation of the lipid layer of the cell membrane. Selective ethanol recovery can stimulate bacterial activity and increase the reaction rates at the same time [119].

Table 4
Common reactions in syngas fermentation with Gibbs free energy under standard conditions adopted from [97].

Product	Biochemical Reaction	Eq.	ΔG° (kJ/mol)
Ethanol	$6CO + 3H_2O \rightarrow C_2H_5OH + 4\,CO_2$	(1)	−220.6
	$2\,CO_2 + 6H_2 \rightarrow C_2H_5OH + 3H_2O$	(2)	−96.0
Acetic acid	$4CO + 2H_2O \rightarrow CH_3COOH + 2\,CO_2$	(3)	−154.9[a]
	$2\,CO_2 + 4H_2 \rightarrow CH_3COO^- + H^+ + 2H_2O$	(4)	−95.0
Acetate	$4H_2 + 2\,CO_2 \rightarrow C_2H_3O_2^- + H^+ + 2H_2O$	(5)	−74.4
	$4H_2 + 2HCO_3^- + H^+ \rightarrow C_2H_3O_2^- + 4H_2O$	(6)	−114.5
	$4CO + 2H_2O \rightarrow C_2H_3O_2^- + H^+ + 3\,CO_2$	(7)	−172.2
Butanol	$12H_2 + 4\,CO_2 \rightarrow C_4H_9OH + 7H_2O$	(8)	−486
	$12CO + 5H_2O \rightarrow C_4H_9OH + 8\,CO_2$	(9)	−245
Butyrate	$C_2H_3O_2\text{-} + C_2H_5OH \rightarrow C_4H_2O_2^- + H_2O$	(10)	−38.5
	$C_2H_3O_2\text{-} + 6H_2 + 2\,CO_2 \rightarrow C_4H_7O_2^- + H_2O$	(11)	−143.0
Water shift reaction	$CO + H_2O \rightarrow CO_2 + H_2$	(12)	−20.1

[a] ΔH value.

The concentration and composition of impurities in syngas can vary significantly depending on the biomass feedstock, gasification technology, and operating conditions, with reported levels ranging from trace concentrations (ppm) to up to 15% [120,121]. Raw syngas produced during biomass gasification may contain contaminants such as particulates, tar compounds, sulfur species, ammonia, and light hydrocarbons that can inhibit microbial activity during fermentation [122]. Therefore, gas-cleaning steps are typically required prior to biological conversion. Cyclones and filters are commonly used for particulate removal, while tar compounds can be reduced through catalytic cracking or scrubbing processes [123]. Sulfur compounds are typically removed using sorbents such as zinc oxide or through amine treatment, whereas water scrubbers can be applied to remove ammonia and other trace impurities [123]. Despite these gas-cleaning strategies, trace impurities may still enter the fermentation system and influence microbial activity. Once impurities present in syngas are transferred into the fermentation medium, they may directly affect microbial activity through mechanisms such as cell toxicity, enzyme inhibition, or shifts in metabolic pathways [123]. In addition, these impurities can indirectly influence the fermentation process by altering environmental conditions within the bioreactor, including pH, osmolarity, and redox potential [123]. Many compounds commonly found in syngas are known enzyme inhibitors and may disrupt key metabolic pathways in acetogenic microorganisms, thereby affecting overall fermentation efficiency and product formation [123].

Despite these technical challenges associated with syngas quality and gas-cleaning requirements, significant progress has been made in recent years toward the practical implementation of syngas fermentation technologies. Several EU-funded initiatives are exploring syngas fermentation technologies. For example, the BioSFerA (2020–2024) project aims to convert biogenic residues and wastes into sustainable aviation and maritime fuels through an integrated process combining biomass gasification and syngas fermentation. In this approach, syngas is first biologically converted to acetate and subsequently transformed into microbial oils (triacylglycerides), which are hydrotreated to produce drop-in biofuels. The project is currently advancing the technology from laboratory scale (technology readiness levels 3) toward pilot-scale validation (technology readiness levels 5) [124]. Another notable EU initiative is the STEELANOL project, which aims to demonstrate large-scale industrial syngas fermentation by converting steel mill waste gases into advanced bioethanol at the ArcelorMittal plant in Ghent, Belgium. The process, developed in collaboration with LanzaTech, uses specialized microorganisms to ferment syngas or syngas-like industrial gases (CO, CO_2, and H_2) into ethanol that can be blended with gasoline for transportation fuels. The project represents one of the first industrial-scale applications of gas fermentation technology in Europe [125]. Beyond these initiatives, several companies and startups at different technology readiness levels are also exploring syngas fermentation for the production of a wide range of products, including animal feed proteins, biofuels, biochemicals, and biomaterials [126]. These projects highlight the growing interest within the EU in developing syngas fermentation technologies that can convert industrial waste gases and biomass-derived syngas into valuable fuels and chemicals, contributing to circular carbon utilization and reducing greenhouse gas emissions.

4. Roles of biofilm in biomass valorization

Various biological methods of lignocellulosic biomass valorization were discussed in the earlier sections, and this section briefly explains the important role of biofilm formation in the conversion of lignocellulosic biomass to biofuels through biological processes. Around 85% of the organic material can be degradable by various microbiomes or microbial communities. Generally, biofilms are considered microbial communities that selectively attach to the supporting materials. Biofilm formation contributes to various functions of microbial communities, such as developing the survival or the adaptation of microbial cells in challenging environmental conditions (pH, oxygen, etc.) and tolerance to toxic inhibitors. The formation of biofilm is involved in the multiphase process, the first step in the attachment of planktonic cells, irreversible adhesion, followed by microcolony formation, and the final phase of maturation and dispersal. During the process, the cells are stabilized by the multicellular arrangements in the biofilm matrix and maintain the production of exoenzymes and cell debris [127]. Extracellular polymeric substances (EPS), consisting of proteins, polysaccharides, and extracellular DNA (eDNA), are produced by a variety of microbial cells and play a significant role in biofilm formation, aggregation of microbial cells, and providing viscoelasticity to the biofilm.

Beyond structural formation, biofilms provide several mechanistic advantages that significantly improve lignocellulosic biomass conversion efficiency. One of the primary benefits is high microbial cell density retention, which prevents biomass washout and enables reactors to operate at higher organic loading rates and shorter hydraulic retention times [128,129]. The EPS matrix also acts as a protective barrier, improving microbial tolerance to inhibitory compounds such as volatile fatty acids, ammonia, and other fermentation intermediates [130–132]. In addition, the spatial organization of microbial cells within the biofilm enhances syntrophic interactions between different microbial groups, facilitating efficient metabolite exchange and electron transfer [133,134]. This spatial proximity is particularly important in AD and dark fermentation systems, where coordinated interactions between hydrolytic bacteria, fermentative microorganisms, and methanogens, including direct interspecies electron transfer (DIET), determine the overall conversion efficiency [135].

The rumen bacterial system was found to be the first complex microbiome, which presents in the ruminant animal that converts the complex LCB into simple monomers (e.g. short-chain fatty acids). The rumen bacterial biofilm ecosystems contained the dominant bacterial species *Fibrobacter Clostridia* [136,137]. In general, anaerobic biofilm contains the cellulolytic bacterial species of *Clostridia*, such as *C. phytofermentanus, C. thermocellum, and C. cellulolyticum* [138–140]and the non-Clostridia species such as *Fibrobacter succinogenes and Ruminococcus albus* [141,142]. In the biological conversion and degradation of LCB, biofilms play a crucial role; in particular, anaerobic bacteria form a thin layer on the surface of cellulose and accelerate the solubilization. Cellulolytic biofilms allow for a high concentration of enzymes at the boundary layer between the solid substrate and the liquid phase, and the fast capture of hydrolysis products directly at the hydrolysis site. In other words, biofilm allows for the high concentration of cellulases at the boundary layer and more complete capture of hydrolysis products directly at the energetically favourable site [143].

In the anaerobic digester, the role of biofilms has been less investigated compared to the rumen microbiome. Several reports verified the existence of biofilms containing *Fibrobacter or Clostridia* on solid substrates in AD experiments, but their specific role in LCB conversion was not investigated [136,137,144].

Several studies have quantitatively demonstrated the performance advantages of biofilm-based systems compared with suspended microbial cultures. Biofilm reactors typically achieve significantly higher biomass retention and metabolic activity. For example, biofilm-based hydrogen production systems have reported hydrogen yields of approximately 1.17 mol H_2/mol substrate and productivities of 2.4 mmol $H_2\,L^{-1}\,h^{-1}$, which are substantially higher than those obtained in conventional suspended culture reactors [145,146]. Similarly, fixed-bed biofilm reactors have achieved hydrogen production rates of up to 60 L $H_2\,L^{-1}\,d^{-1}$, highlighting the ability of immobilized microbial systems to maintain high metabolic activity and long-term operational stability [147,148]. In AD systems, biofilm or granular sludge reactors such as UASB and trickling filters also enable stable operation under high organic loading conditions by retaining dense microbial communities [128,129].

Within the European bioenergy sector, biofilm-based reactors have been increasingly applied for lignocellulosic biomass conversion due to their operational stability and high biomass retention. For example, UASB reactors, widely implemented in several European wastewater treatment facilities, rely on granular biofilms to retain high-density microbial communities and achieve efficient methane production from complex organic substrates. Similarly, fixed-bed and trickling filter biofilm reactors have been investigated in European research programs for hydrogen production and AD of lignocellulosic hydrolysates. These reactor configurations promote microbial immobilization on support materials, enabling stable long-term operation and higher biofuel productivity compared with conventional suspended-growth systems. Such biofilm-based technologies are increasingly recognized as promising strategies for improving process efficiency in the emerging EU circular bioeconomy, where efficient conversion of agricultural residues and forestry biomass into renewable fuels and chemicals is a major priority.

The bioconversion process of the lignocellulose process requires a huge expense for the pretreatment and detoxification of lignocellulose. To overcome this issue, consolidated bioprocessing (CBP) offers a promising strategy, which combines hydrolytic enzymes production, substrate saccharification, and microbial fermentation in a one-step process, which is done by the mixed bacterial community in biofilm. CBP was mostly applied in the thermophilic condition to achieve direct biochemical production from lignocellulosic biomass. Several strategies have been exploited, including the “native strategy” and “recombinant strategy”, e.g. *Clostridium thermocellum*, one of the cellulolytic anaerobic bacteria, which directly convert the lignocellulosic biomass into the value-added products with the maximised growth rate of 0.1-0.16 h-1 in crystalline cellulose, on the other hand, metabolic engineered *C. thermocellum* produced 14 g/L ethanol from the pure microcrystalline cellulose. Moreover, applying this strategy can convert the hard biomass into simple monomers by the cellulolytic enzymes as previously reported [149,150]. In the current development stages of CBP, much attention has been given to designing a robust recombinant microbial species, with the help of genetic engineering tools, in the biofilm system to either express hydrolytic enzymes in a fermenting organism or transfer the biofuel-synthesizing pathway into a hydrolytic organism.

4.1. Roles of biofilm in AD and dark fermentation

In the past several decades of research into biofilm or granule-based technologies, granules have attracted microbial ecologists and environmental engineers due to their high stability and capability to improve microbial function. As discussed, biofilm is important in the LCB conversion into various products such as fuels and chemicals, and furthermore, biofilm plays a crucial role in converting the carbon source into the product. Since LCB is a solid, microorganisms play a role in the form of a biofilm on the surface of LCB, whereas microbial granules, which are formed by the network of biofilms or aggregates of microbial cells, play an important role in the process of converting dissolved carbon sources into biogas or biofuels.

In the AD process, microbial biofilm or granules improve the microbial process productivity and maintain stability. In a biofilm-based methanogenic reactor, the biofilm provided the maintenance of active biomass by controlling their washout, which leads to the stabilization of the reactor at a higher loading rate [151]. The various microbial communities, along with the methanogenic bacterial species, formed as filamentous and improved the microbial quality, e.g., M. harundinacea dominates biofilm and granule communities in all reactors, showing the ability to grow at a sodium concentration up to 20 g Na+/L, and its filamentous form is important for biofilm and granule formation [152]. The various studies proved that the high-density biofilms in the continuous methanogenic reactor improved biogas production and utilized the higher organic loading rate.

In the dark fermentative biohydrogen production, also biofilm system also played a crucial role. Biofilm increased the cell viability in attached bacteria growth, and particularly Bacillus attached to granular activated carbon could maintain the hydrogen production capability for over 40 days [153]. The hydrogen production yield and productivity reached 1.17 mol/mol and 2.4 mmol H_2/L/h, respectively, in the biofilm reactor compared with the suspended growth reactor [154].

5. Comparative assessment of biochemical conversion pathways

The three main biochemical pathways for converting lignocellulosic biomass, including AD, dark fermentation, and syngas fermentation, all differ substantially in terms of substrate utilization, process efficiency, and technological maturity. These differences arise from variations in microbial metabolism and different reactor configurations used. As a result, the performance of each pathway varies depending on operational conditions, pretreatment strategies, and the composition of the biomass.

AD is the most mature and widely implemented technology for the conversion of organic waste into bioenergy. Its main advantage lies in its process stability and its ability to treat a wide range of wet organic residues while producing methane-rich biogas. However, when lignocellulosic biomass is used as the primary substrate, the complex structure of cellulose, hemicellulose, and lignin significantly limits microbial hydrolysis, which is typically the rate-limiting step in AD [155]. Consequently, pretreatment processes are often required to enhance biodegradability. Variations in methane yields reported in the literature are largely associated with differences in feedstock composition, pretreatment methods, reactor configurations, and microbial community structures.

Dark fermentation provides an alternative biological route for hydrogen production and is generally characterized by faster reaction rates than AD. Nevertheless, its overall conversion efficiency is often limited by the incomplete degradation of substrates and the formation of organic acids and other metabolic by-products [156]. These by-products can inhibit hydrogen-producing microorganisms and reduce overall hydrogen yields. Reported results in the literature vary widely due to differences in microbial consortia, fermentation conditions, and substrate characteristics, which influence the metabolic pathways of hydrogen-producing bacteria.

Syngas fermentation represents a distinct approach, as it utilizes gaseous substrates derived from biomass gasification rather than solid lignocellulosic feedstocks. This pathway allows the conversion of otherwise recalcitrant lignocellulosic materials into fuels and chemicals such as ethanol and acetate. However, its performance is strongly influenced by gas–liquid mass transfer limitations and the low solubility of key syngas components such as CO and H_2 in aqueous media [157]. Consequently, reactor design and gas transfer efficiency play critical roles in determining productivity. While syngas fermentation offers significant flexibility in feedstock utilization and operates under relatively mild conditions, its large-scale implementation remains limited compared with AD.

Among the three technologies, AD currently represents the most industrially established technology for converting biomass into renewable energy due to its operational robustness and technological maturity. In contrast, dark fermentation and syngas fermentation offer promising alternatives for hydrogen and liquid biofuel production, respectively, but require further optimization to improve conversion efficiency, reactor design, and process economics. The differences in reported performance among these pathways highlight the importance of feedstock characteristics, microbial activity, and reactor engineering in determining the overall efficiency of the lignocellulosic biomass conversion system.

6. Challenges and future prospects

Biomass conversion to value-added products in the European Union has been a sustainable drive over several decades. Existing and start-up academic, research and industrial institutions such as BIOS BIOENERGIESYSTEME GmbH (Austria), Ashwell Biomass Ltd. (United Kingdom), Vekamaf Services B.V. (Netherlands), Bioendev AB (Sweden) and Aalborg Energie Technik a/s (AET) (Denmark), etc., have adopted several thermochemical and biochemical technologies to convert their enormous amount of geographically distributed biomass to valuable products. With the consciousness of the drawbacks associated with biomass upgrading, these and many other institutions across the globe are currently seeking alternatives to address challenges of biomass pretreatment or preprocessing, issues relating to costs, technological designs/heat transfers, market and demands and eventually government policies. The primary issues of initial biomass pretreatment account for a greater part of the cost, as the biomass required for upgrade undergo screening, size reduction via crushing/densification, drying and other treatment with chemicals such as acid, alkaline [158,159]. For biochemical conversion pathways such as AD and dark fermentation, the recalcitrant structure of lignocellulosic biomass limits microbial accessibility and reduces conversion efficiency. Therefore, the development of cost-effective and environmentally sustainable pretreatment strategies remains a key research priority. During processing, the reactor plays an essential role in the transformation of biomass. For biochemical processes, reactor configuration strongly influences microbial activity, substrate conversion efficiency, and gas–liquid mass transfer. In particular, improving reactor designs for high-solids AD systems, optimizing fermentation conditions for hydrogen production in dark fermentation, and enhancing gas–liquid mass transfer in syngas fermentation reactors remain critical technological challenges. Future research should therefore focus on the development of advanced bioreactor designs, improved microbial consortia, and optimized operating conditions to enhance the performance and scalability of biochemical lignocellulosic biomass conversion systems. [148]Another challenge associated with biomass upgrade is the quality of products generated. Bio-oil obtained from biomass upgrades is mostly not ‘drop-in’, hence it cannot be used directly in fuel engines. Therefore, the biofuel obtained is further upgraded via catalytic processes (hydrodeoxygenation and decarboxylation) to obtain petroleum-like fuels [160]. Biochar has a promising potential, since it can be applied in areas such as biofertilizer, biopesticide, and catalyst. Recently, biochar has been co-processed with other feeds in AD, due to its properties, to augment the optimum yield of methane [161]. This suggests that integrating biochar with AD systems could enhance microbial activity, improve process stability, and increase methane production, representing a promising direction for future research. It is essential to note that the use of aquaculture solid waste (e.g., shellfish shells and sea feed) as a potential co-processed feedstock can be employed in the biorefinery system. Substrates required for co-processing in digesters need to be diversified. For example, cow manure, kitchen wastes and waste-activated sludge are excellent co-substrates, which result in increased microbial population (and activity). The feedstock range utilized for biomass conversion appears to be limited to just forestry, municipal solid and food wastes, etc. Other range of biomass/wastes needs to be explored, especially aquatic biomass. Future research should therefore focus on expanding the range of co-substrates for biochemical conversion processes, including aquatic biomass and other underutilized organic wastes, to improve substrate availability and process efficiency. For example, 90 million tons of manure and slurry in the UK remain hugely untapped. This is of great concern because these wastes can contribute to the production of ammonia, methane and nitrogen oxide for energy generation in the UK [162]. In addition, the existing range of biomass readily available for upgrade needs to be co-processed with other low-amount biomass available, to augment the total biomass amount required for upgrade. Catalysis is also an important aspect of thermochemical conversion that cannot be ignored, as many catalysts such as zeolites, MgO, CaO, K_2CO_3, etc [163], have been widely applied in the upgrade of microalgae biomass, lignocellulosic biomass and municipal wastes and have been exceptional [164]. In parallel with catalytic developments, further research is needed to improve biocatalysts and microbial consortia for biochemical pathways such as AD, dark fermentation, and syngas fermentation in order to enhance substrate utilization and product yields. New catalysts (both homogeneous and heterogeneous catalysts) with bi-functional properties, high activity and selectivity need to be designed, synthesized and employed for biomass upgrade. The goal is to ensure a circular economy, where the wastes generated are utilized in the processing loop. Essential areas of concern where scientists and engineers have little or no influence are government policies, which in turn affect market supplies and demands. The government need to support the EU policy reforms to promote a transition from traditional energy to sufficiently advanced bioenergy from waste and residues, but such a smooth transition can only be made possible when the right policies are made. For example, farmers of energy crops are a group of people who are mostly influenced by government, international and local policies, because if they are limited by a policy that discourages them from exporting their produce, or importing the items required to grow these crops, they will stop growing the energy crops. The government and legislatures need to review land acquisition policies that can favour interested parties and institutions to easily acquire these lands and grow energy crops. In order to understand the economic and environmental impacts involved in biomass conversion, researchers are encouraged to utilize and employ life-cycle analysis and techno-economic analysis tools, which will help elucidate the economic feasibility and carbon neutrality of their processes. Future studies should also integrate techno-economic and life-cycle assessments with reactor design optimization and microbial process development to identify scalable solutions for biochemical biomass conversion. Finally, to ensure a circular economy, researchers must rely on utilizing their process wastes or by-products as fuels for self-energizing the processes, or as raw materials to augment the total biomass efficiency. Such integrated biorefinery strategies can significantly improve the sustainability and economic viability of AD, dark fermentation, and syngas fermentation systems.

7. Conclusions

Biological conversion technologies have emerged as highly promising approaches for the valorization of lignocellulosic biomass (LCB) into biofuels and value-added chemicals within the European Union. AD stands out as an efficient and environmentally friendly pathway, converting diverse feedstocks including forest residues, agricultural by-products, and organic wastes into biogas and biomethane through its four-stage process of hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Optimized pretreatment, careful microbial community management, and feedstock diversification are critical to overcoming limitations such as the recalcitrant nature of lignocellulose and the slow growth of methanogens, thereby maximizing yields and process stability. Dark fermentation offers a complementary avenue for sustainable biohydrogen production, leveraging rapid substrate utilization and hydrogenase-mediated pathways to generate hydrogen and valuable co-products. Advances in thermophilic processing, reactor design, and microbial enrichment strategies have demonstrated the potential to address current challenges, including lignocellulose recalcitrance and hydrogen losses to competing pathways. Biofilm-mediated systems further enhance LCB conversion by promoting high cell density, enzyme localization, and tolerance to inhibitory conditions. By enabling consolidated bioprocessing and improving mass transfer in syngas and fermentation reactors, biofilms contribute to more robust, efficient, and specific production of biofuels and chemicals. Despite these advances, several overarching challenges remain, including high pretreatment costs, reactor design limitations, feedstock variability, and catalyst development. Addressing these barriers, alongside expanding underutilized biomass streams and integrating co-processing strategies, is essential to meet biomass demand efficiently. Coupled with supportive policy frameworks, market incentives, and rigorous life-cycle and techno-economic analyses, these technological innovations can drive the EU toward a low-carbon, circular bioeconomy. Ultimately, coordinated efforts in biological conversion technologies, process optimization, and sustainable resource management will position the EU as a global leader in renewable bioenergy and value-added chemical production.

CRediT authorship contribution statement

Yasna Mortezaei: Formal analysis, Investigation. **Parthiban Anburajan:** Data curation, Formal analysis, Investigation, Methodology. **Great Umenweke:** Data curation, Formal analysis. **Umaru Musa:** Data curation, Formal analysis. **Fatih Güleç:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation. **Orla Williams:** Conceptualization, Data curation, Formal analysis. **Wesam Hassan:** Data curation, Formal analysis. **Hyun Suk-Oh:** Conceptualization, Data curation. **Edward H. Lester:** Conceptualization, Data curation, Formal analysis. **Chukwuma C. Ogbaga:** Data curation, Formal analysis, Investigation. **Burcu Gunes:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology. **Jude Okolie:** Conceptualization, Data curation, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A

Table A
1The yield level of some agricultural crop residues in EU-27[30].
Table A1

Crop	Yield range (t/ha)				
	low	medium	high	lowest	highest
Wheat	Bulgaria, Cyprus, Czech Republic, Estonia, Greece, Portugal, Romania, Spain,	Austria, Finland, Hungary, Italy, Latvia, Lithuania, Luxembourg, Malta, Poland, Slovakia, Slovenia, Sweden	Belgium, Denmark, Germany, Ireland, Netherlands, United Kingdom, France	Portugal (1.6)	Ireland (8.8)
Barley	Cyprus, Estonia, Greece, Latvia, Lithuania, Portugal, Romania, Spain	Austria, Bulgaria, Czech Republic, Denmark, Finland, Hungary, Italy, Luxembourg, Poland, Slovakia, Slovenia, Sweden, United Kingdom, Malta	Belgium, France, Germany, Ireland, Netherlands	Cyprus (1.6)	Belgium (8.0)
Rye	Bulgaria, Lithuania, Romania, Spain, Hungary, Portugal, Greece	Austria, Finland, Czech Republic, Italy, Latvia, Poland, Slovakia, Slovenia, Estonia, Netherlands, Ireland	Denmark, France, Germany, Luxembourg, United Kingdom, Belgium, Sweden	Portugal (0.9)	United Kingdom (9.6)
Oat	Bulgaria, Lithuania, Romania, Cyprus, Estonia, Latvia, Spain, Greece, Portugal, Slovakia	Austria, Czech Republic, Hungary, Italy, Luxembourg, Poland, Slovenia, Finland, Sweden	Denmark, Belgium, Germany, Netherlands, Ireland, United Kingdom, France	Cyprus (1.0)	Ireland (7.4)
Maize	Bulgaria, Lithuania, Romania, Poland	Czech Republic, Denmark, Hungary, Italy, Luxembourg, Slovakia, Slovenia, Portugal	Austria, Belgium, France, Germany, Netherlands, Spain, Greece	Romania (3.6)	Netherlands (11.8)
Rapeseed	Bulgaria, Estonia, Finland, Italy, Lithuania, Romania, Spain	Austria, Belgium, Czech Republic, Greece, Hungary, Latvia, Poland, Slovenia, Slovakia, Sweden	Denmark, France, Germany, Ireland, Luxembourg, Netherlands, United Kingdom	Estonia (1.4)	Belgium (4.0)
Sunflower	Portugal, Romania, Slovenia, Spain	Bulgaria, Germany, Greece, Poland, United Kingdom	Austria, Czech Republic, France, Hungary, Italy, Slovakia	Portugal (0.56)	Austria (2.6)
Sugar beet	Bulgaria, Finland, Latvia, Lithuania, Romania, Slovenia	Czech Republic, Denmark, Germany, Hungary, Ireland, Poland, Portugal, Slovakia, Sweden, United Kingdom, Italy	Austria, Belgium, France, Greece, Netherlands, Spain	Bulgaria (18.7)	France (85.3)
Wine	Bulgaria, Cyprus, Malta, UK	Austria, Czech Republic, France, Hungary, Portugal, Romania, Slovakia, Slovenia, Spain	Germany, Italy, Greece, Luxembourg	Bulgaria (3.3)	Luxembourg (14.5)

Table A.2
The yield level of some agricultural crop residues in EU-27[30].

Crop	Yield level	Country	Crop yield (t/ha)	Residue yield (t/ha)
Wheat	Lowest	Portugal	1.64	0.79
	Medium	Poland	4.03	2.90
	Highest	Ireland	8.78	8.43
Barley	Lowest	Cyprus	1.6	0.77
	Medium	Denmark	5.27	3.32
	Highest	Belgium	7.96	6.21
Rye	Lowest	Portugal	0.94	0.51
	Medium	Luxembourg	6.25	4.69
	Highest	UK	9.6	9.17
Oat	Lowest	Cyprus	0.96	0.52
	Medium	Denmark	4.7	3.25
	Highest	Ireland	7.4	6.17
Maize	Lowest	Romania	3.6	3.24
	Medium	Luxembourg	7.86	8.26
	Highest	Netherland	11.75	14.10
Rapeseed	Lowest	Estonia	1.4	
	Medium	Poland	2.72	3.37
	Highest	Belgium	4	5.79
Sunflower	Lowest	Portugal	0.56	0.90
	Medium	Poland	1.7	3.27
	Highest	Austria	2.6	6.02
Sugar beet	Lowest	Bulgaria	18.7	1.49
	Medium	Lithuania	37.4	6.13
	Highest	France	85.3	12.79
Wine	Lowest	Bulgaria	3.3	9.78
	Medium	Greece	8.7	31.28
	Highest	Luxembourg	14.5	60.83

Data availability

No data was used for the research described in the article.

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