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Review

Syngas Derived from Lignocellulosic Biomass Gasification as an Alternative Resource for Innovative Bioprocesses

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Abstract: A hybrid system based on lignocellulosic biomass gasification and syngas fermentation represents a second-generation biorefinery approach that is currently in the development phase. Lignocellulosic biomass can be gasified to produce syngas, which is a gas mixture consisting mainly of H_2, CO, and CO_2. The major challenge of biomass gasification is the syngas's final quality. Consequently, the development of effective syngas clean-up technologies has gained increased interest in recent years. Furthermore, the bioconversion of syngas components has been intensively studied using acetogenic bacteria and their Wood–Ljungdahl pathway to produce, among others, acetate, ethanol, butyrate, butanol, caproate, hexanol, 2,3-butanediol, and lactate. Nowadays, syngas fermentation appears to be a promising alternative for producing commodity chemicals in comparison to fossil-based processes. Research studies on syngas fermentation have been focused on process design and optimization, investigating the medium composition, operating parameters, and bioreactor design. Moreover, metabolic engineering efforts have been made to develop genetically modified strains with improved production. In 2018, for the first time, a syngas fermentation pilot plant from biomass gasification was built by LanzaTech Inc. in cooperation with Aemetis, Inc. Future research will focus on coupling syngas fermentation with additional bioprocesses and/or on identifying new non-acetogenic microorganisms to produce high-value chemicals beyond acetate and ethanol.

Keywords: gasification; syngas fermentation; lignocellulosic biomass; biorefinery; Wood–Ljungdahl pathway

1. Introduction

Nowadays, the current economic system is based on fossil resources, which include crude oil, coal, and natural gas. Crude oil is the most globally used resource. In order to produce energy, heat, and fuels (solid, liquid, gaseous), the global demand for oil is about 84 million barrels/day, which is estimated to reach approximately 116 million barrels/day in 2030 [1]. Moreover, commodity chemicals are mainly produced via oil refining [1]. However, fossil resources use is no longer economically and environmentally sustainable. From 1970 to 2017, the annual global extraction of materials (i.e., fossil resource, metals, non-metal minerals, and biomass) tripled. The latter continues

to grow due to the population's exponential growth, the increasing consumption of energy per capita, the economic and technological development, and the establishment of a new modern way of life [2]. Current scenarios show the increased fossil resources demand and the relative market price, and consequently, a negative impact on the environment due to the high amount of greenhouses gases (GHGs) emitted, i.e., carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), and water steam (H_2O), which accumulate into the atmosphere, promoting global warming with environmental and economic consequences via melting glaciers, rising sea levels, ocean acidification, desertification, acid rains, and extreme local climate events [3].

The industry, energy, building, and mobility sectors account for the majority of GHG emissions in the European Union (EU). In December 2019, the European Commission launched the New Green Deal, which is a roadmap for making the European Union economy modern, resource-efficient, and competitive [4]. The Green Deal is an integral part of the European Commission's strategy to implement the United Nations 2030 Agenda and the Sustainable Development Goals (SDGs) [5]. According to the European Green Deal, the EU will be climate neutral by 2050 and economic growth will be decoupled from fossil resources use. It is a new growth strategy that aims to guide the transition of all sectors into a circular economy, the phasing out of fossil fuels through the introduction of alternative renewable resources for energy and chemical production, and reducing the environmental footprint of human activities [6]. While energy and heat can be produced using different types of resources, such as solar, wind, hydropower, and geothermal energy, biomass is the only resource that can be used to produce chemicals and materials in addition to energy. Biomass is the only carbon-rich resource on Earth, in addition to fossil resources [7]. European Directive 2009/23/EC has defined biomass as "the biodegradable fraction of products, waste and residues from biological origin from agriculture (including plant and animal substances), forestry and related industries including fisheries and aquaculture, as well as the biodegradable fraction of industrial and municipal waste" [8]. International Energy Agency (IEA) Bioenergy Task 42 has defined biorefining as "the sustainable processing of biomass into a spectrum of marketable products (food, feed, materials, chemicals) and energy (fuels, power, heat)." Different biomass conversion processes are integrated with each other in this system to achieve a wide spectrum of products without waste generation [1]. Biorefining is one of the main drivers for the establishment of a bio-based economy. In the first global bioeconomy summit in Berlin in November 2015, the Food and Agriculture Organization (FAO) of the United Nations defined a bioeconomy as the "knowledge-based production and utilization of biological resources, biological processes and principles to sustainably provide goods and services across all economic sectors" [9]. Biorefining has the following goals: (i) increase industry competitiveness and prosperity, (ii) decouple the economy from fossil resources, (iii) reduce GHG emissions, and (iv) improve local and rural development. Through efficient strategies, biorefining should be designed to achieve economic, social, and environmental sustainability [10]. Based on IEA Bioenergy Task 42, biorefineries can be classified according to four features: feedstocks, conversion processes, products, and platforms [11]. Therefore, biorefining can be considered as a system in which products are obtained from feedstocks, through platforms and conversion processes. Interconnections between the four elements co-create integrated systems of two or more biorefineries (Figure 1).

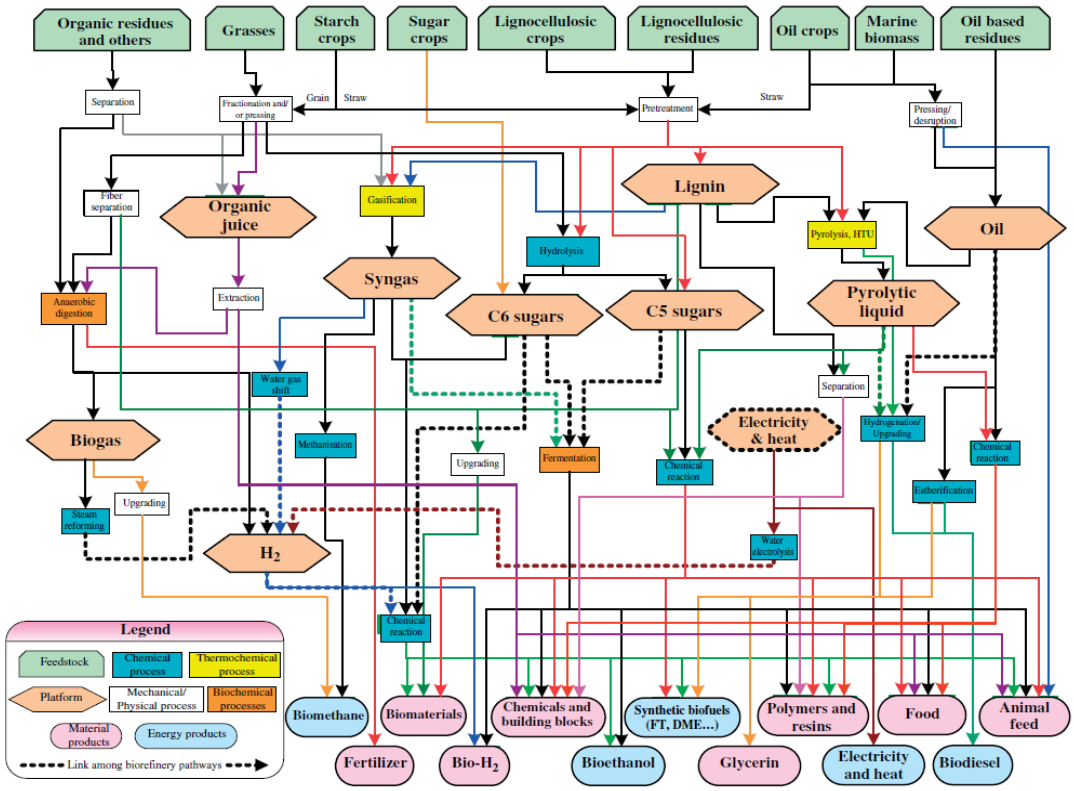

Figure 1. Overview of the biorefinery classification system. The network is organized into three levels: the upper level shows the specific feedstock which feeds each biorefinery, the intermediate level shows the platforms, and the lower level shows the products. Conversion processes can be applied to different feedstocks or platforms and they are linked through arrows ([11]; permission obtained from Wiley Online Library). HTU, Hydrothermal uprgrading process, FT, Fischer Tropsch biofuels, DME, Dimethyl ether.

Moreover, based on technology implementation, biorefineries can be classified as first-, second-, and third-generation biorefineries.

First-generation biorefineries, which are based on edible plant biomass as feedstock, have shown the possibility of large-scale production, distribution, and use of biofuels produced from biomass [12]. The agri-food competition of first-generation biorefineries raises social and ethical issues, which motivate the development of second-generation biorefineries, a more flexible platform based on non-edible lignocellulosic biomass with a wider spectrum of products [10,12,13]. Third-generation biorefineries, whose feedstock is macro- and microalgae, are advantageous due to there being no need for fertile soil and the ability of algae to grow in the presence of a high amount of carbon dioxide in wastewater from industrial processes or saltwater [14]. Nevertheless, technology scale-up and product commercialization are the major challenges that compromise their techno-economic feasibility [15].

Therefore, a second-generation biorefinery is the most promising system for a bio-based economy. Indeed, lignocellulosic biomass is an ideal feedstock because it is widespread, economical, and easy to use. In addition, the use of this biomass is more efficient in a second-generation biorefinery due to the simultaneous production of biofuels, chemicals, electricity, and heat [16].

Lignocellulosic biomass can be obtained from either dedicated crops, such as woody and perennial herbaceous crops, or agricultural and industrial residues [1]. Lignocellulosic feedstocks have crucial advantages over other biomass feedstocks. They are the non-edible portion of the plant, and therefore, they do not interfere with food supplies. Moreover, high amounts of this biomass can be produced quickly and cheaply. Lignocellulosic biomass is mainly composed of three polymers: cellulose (35–50%), hemicellulose (20–35%), and lignin (10–25%) [17]. In addition to these components, the biomass also contains proteins, minerals, extractable compounds, and ash at lower concentrations [18]. Based on

the type of lignocellulosic biomass, the three polymers are organized into non-uniform complex 3D structures that are characterized by their robustness, recalcitrance, and resistance to degradation.

Lignocellulosic gasification is a valid thermochemical approach for the conversion of organic solid matter into a gaseous mixture that is constituted of H_2, CO, CO_2, and CH_4, named synthetic gas or syngas. Although about 55% of syngas is still produced from coal, biomass utilization, especially lignocellulose, is constantly growing [19]. Indeed, gasification could be potentially applied to all different kinds of lignocellulosic biomass, unlike other conversion technologies [20,21]. Moreover, in the last few decades, a wide range of applications of syngas have been intensively studied. Syngas can be directly used as a combustible substance in power plants for heat and power production (steam cycle, co-combustion, combustion in gas turbines or internal combustion engines, high-temperature fuel cells), which represents the most common use of biomass-derived syngas. However, syngas also represents a platform that can be employed in a broad range of chemical and microbial processes, leading to gaseous and liquid fuels, as well as to chemicals [22]. Chemical process research has mainly focused on transportation fuel production from syngas, such as Fischer–Tropsch liquid fuels, hydrogen, methanol, dimethyl ether (DME), mixed alcohols, and synthetic natural gas (SNG) [23]. Instead, the biochemical conversion route consists of syngas fermentation in which obligate anaerobic microorganisms convert syngas into organic acids, alcohols, and other chemicals (Figure 2). The most commonly used microorganisms are acetogens, which use the Wood–Ljungdahl metabolic pathway. Syngas fermentation is defined as an indirect fermentation process because biomass is not fed directly into the fermenter, but it is previously converted into syngas through gasification [24]. Biological catalysts, especially acetogenic microorganisms, that are used in syngas fermentation enable high reaction selectivity and high conversion efficiency, with increased product formation [20]. The aim of this review was to investigate the hybrid system based on biomass gasification, with a specific focus on alternative feedstocks and on syngas fermentation, providing detailed information on acetogenic microorganisms and their metabolism, process optimization, and bioreactors design. Recent advances in the field of the metabolic engineering of acetogens for the production of wide-ranging valuable compounds are addressed. Currently, only two companies, LanzaTech Inc. and Aemetis, Inc. in cooperation, have scaled up this technology, thereby demonstrating the techno-economic feasibility of this new concept of a biorefinery at a large scale. A brief description of their industrial plants is shown. Finally, this review describes the challenges and highlights the research gaps for future work in the field of syngas biorefining.

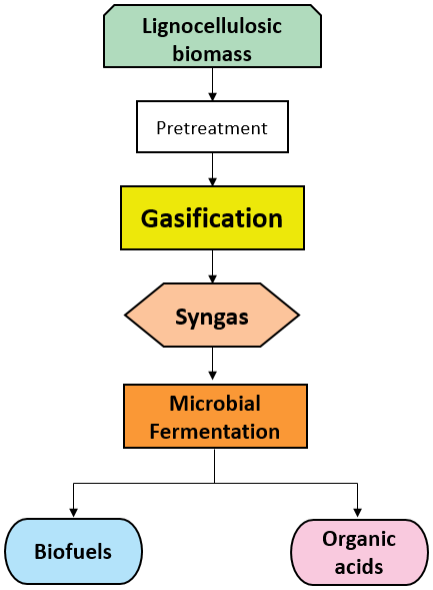

Figure 2. Syngas biorefinery concept.

2. Biomass Gasification

The gasification of carbonaceous feedstocks to syngas takes place inside a reactor, defined as a gasifier, at high temperatures (800–1500 °C). The feedstock is subjected to partial oxidation due to a lower concentration of oxygen than the stoichiometric requirement. Oxygen is supplied by a gasifying agent or carrier, such as air, pure oxygen, water steam, or their mixture. Although carbon dioxide can also be used as a gasifying agent, its use is less frequent. Moreover, the use of supercritical water is an innovative technology, without the need for pretreatment, which achieves a high H_2 yield and reduces tar and char production [25–27]. Compared with conventional methods, gasification is a more efficient process than combustion, which is the most common thermochemical route [28], and it can convert the entire carbon content in the biomass feedstock into gaseous compounds, unlike the biological or chemical hydrolysis that is adopted in biochemical processes [29]. According to the IEA Bioenergy Task 33, there are 114 working biomass gasification projects worldwide, 15 plants idle or on hold, and 13 are under construction or in planning (Figure 3) [30].

Figure 3. Geographical distribution of biomass gasification projects [30].

One main limitation of gasification technology is represented by the formation of tar. Tars are classified into primary, secondary, and tertiary tars. Primary tars consist of both oxygenated compounds (alcohols, carboxylic acids, ketones, aldehydes, etc.) and substituted phenols (cresol, xylenol, etc.). Secondary tars are alkylated aromatics, such as toluene, ethylbenzene, xylenes, styrene, and hetero-aromatics, such as pyridine, furan, dioxin, and thiophene. Finally, tertiary tars consist of aromatics and polycyclic aromatic hydrocarbons (PAH), such as benzene, naphthalene, phenanthrene, pyrene, and benzopyrene. While primary tars are produced directly from the pyrolysis of cellulose, hemicellulose, and lignin, secondary and tertiary tars are the result of several complex reactions that have not been fully clarified yet [22]. At the end of the entire process, two main product mixtures are present: a solid mixture and a gaseous mixture. The solid mixture contains the unreacted organic fraction and inert materials, such as tars and ashes. The gaseous mixture contains syngas and a small amount of impurities, such as light hydrocarbons (ethane, ethylene, acetylene), hydrogen sulfide (H_2S), sulfur dioxide (SO_2), hydrogen chloride (HCl), nitrogen oxides (NO_x), nitrogen (N_2), and ammonia (NH_3) [31]. The syngas's final composition and characteristics are related to the type of biomass, gasifying agent, gasifier type, and reactor's operational conditions, such as temperature, pressure, equivalence ratio (ER), residence time, and catalyst used [26,32–36]. For these reasons, in the last few decades, gasification has been intensively studied to investigate the effects of these factors, and thus, to identify the optimal conditions for the process. Regarding feedstock type, wood is the most commonly used feedstock in the gasification process. A representative component profile for syngas produced from several woody biomass types is shown in Table 1. In addition to woody biomass, other kinds of biomass have also been studied as gasification feedstocks. Agro-industrial

residues and perennial herbaceous crops (Table 2) represent promising feedstocks that can be used in a thermochemical conversion process to obtain both energy and chemicals. The use of agricultural and industrial wastes, as well as herbaceous crops, instead of woody feedstocks, extends the seasonal availability of biomass. A syngas's composition is highly dependent on the used feedstocks, as well as the gasification technology applied (Tables 1 and 2). It is worth pointing out that nitrogen (N_2) can represent a main syngas component when air is used as a gasifying agent, in addition to H_2, CO, CO_2, and CH_4. Therefore, air gasification results in N_2-diluted syngas with low H_2 and CO concentrations. Instead, when gasification is carried out with steam or oxygen, the syngas shows higher H_2 and CO concentrations. The latter condition is the most suitable for syngas fermentation due to the fact that microorganisms use H_2 and CO as primary substrates, in addition to CO_2. Although data on the negative effect of CH_4 have not been reported, this component is not used by microorganisms during syngas fermentation. Therefore, the CH_4 concentration in the syngas should be as low as possible. All the aforementioned impurities produced during the process can reduce the fermentability of syngas due to their negative effects on microorganisms. Therefore, one of the major challenges in biomass gasification is producing syngas with a low or absent impurities content. Biomass gasification needs further investigation studies to achieve an ideal syngas composition, thereby making the syngas fermentation process as efficient as possible [37].

Table 1. Syngas compositions that are obtained from the gasification of several woody biomass and their process characteristics.

Feedstock	Syngas Composition (% *v*/*v*)				Gasifier Type	Gasification Conditions	Reference
	H_2	CO	CO_2	CH_4			
Mesquite wood	1.6–3.0	13.0–21.0	11.0–25.0	1.0–1.5	Fixed bed gasifier	GA: air; T: 782 °C; ER: 2.70	[38]
Juniper wood	2.5–3.5	21.0–25.0	9.0–12.0	1.5–1.8	Fixed bed gasifier	GA: air; T: 713 °C; ER: 2.70	[38]
Pine wood	30.5	52.8	14.7	2.0	Downdraft fixed bed gasifier	GA: steam; T: 900 °C; ER: N.A.	[39]
Oak wood	18.0	21.0	12.0	2.0	Downdraft fixed bed gasifier	GA: air; T: N.A.; ER: N.A.	[40]
Poplar wood	45.5	23.1	20.8	8.6	Rotary kiln reactor	GA: steam; T: 1500 °C; ER: N.A.	[41]
Eucalyptus wood	10.7	20.2	9.1	8.6	Downdraft fixed bed gasifier	GA: air; T: 865 °C; ER: 0.31	[42]
Coffee wood	12.4	14.0	10.4	6.5	Downdraft fixed bed gasifier	GA: air; T: 813 °C; ER: 0.32	[42]
Rubber wood	6.0–8.0	10.0–14.0	16.0–18.0	N.A.	Bubbling fluidized bed gasifier	GA: air; T: 750–900 °C; ER: 0.38	[43]
Oil palm wood	60.0–70.0	10.0–30.0	20.0–50.0	5.0–10.0	N.A.	GA: steam; T: 800 °C; ER: N.A.	[44]
Spruce wood	10.7	25.9	9.7	3.8	Fixed bed reactor	GA: air; T: 800 °C; ER: N.A.	[45]
Wood residue	42.5	23.0	18.1	11.5	Fluidized bed gasifier	GA: air; T: 823 °C; ER: 0.17	[46]
Vermont wood [a]	28.6	23.5	24.0	15.5	Fluidized bed gasifier	GA: steam; T: 600–710 °C; ER: N.A.	[47]
Wood residue [b]	26.2–28.0	50.0–60.3	12.7–23.3	0.9–1.8	Entrained flow gasifier	GA: oxygen; T: 1200–1500 °C; ER: 0.44	[48]
SRF wood [c]	15.7–16.5	15.9–17.2	14.3–15.1	2.6–2.7	Downdraft fixed bed reactor	GA: air; T: 650–800 °C; ER: 0.25–0.26	[49]
Wood waste [d]	9.4–14.8	15.1–19.4	11.0–15.8	3.2–4.3	Bubbling fluidized bed gasifier	GA: air/air and steam mixture; T: 850 °C; ER: 0.20–0.29	[50]

GA: gasifying agent, T: temperature, ER: equivalence ratio, N.A.: data not available. [a] Vermont wood is a mixture of 25% red oak, 15% white pine, 15% maple, 15% ash, and 10% poplar, with the balance being cherry, birch, and cedar. [b] Wood residue is a mixture of 45% hardwood (birch) and 55% softwood (pine). [c] Solid recovered fuels (SRF) wood is composed of waste furniture and waste pallets from a waste collection site. [d] Wood waste that cannot be utilized to produce fuel for domestic heating because they come from potentially contaminated waste; it is made of sawdust from the wood packaging industry or it is obtained as a recycled product from furniture and from door and window frames.

Table 2. Syngas compositions obtained from the gasification of several agro-industrial residues and herbaceous crops and their process characteristics.

Feedstock	Syngas Composition (% *v*/*v*)				Gasifier Type	Gasification Conditions	Ref.
	H_2	CO	CO_2	CH_4			
Corn straw	48.5	33.9	12.2	5.3	N.A.	GA: N.A.; T: 750–900 °C; ER: N.A.	[51]
Wheat straw	25.4	27.5	22.0	16.3	Fluidized bed gasifier	GA: steam; T: 600–710 °C; ER: N.A.	[47]
Rice husk	5.0–8.0	16.0–21.0	15.0–16.0	46.0	Fluidized bed gasifier	GA: air; T: 700–800 °C; ER: 0.18–0.27	[52]
Coffee husk	6.6	13.8	12.1	14.8	Downdraft fixed bed gasifier	GA: air; T: 669 °C; ER: 0.12	[42]
Coconut coir	7.0–21.4	18.6–20.3	19.1–21.3	6.1–9.0	Entrained flow reactor	GA: air; T: 726–941 °C; ER: 0.21–0.30	[53]
Groundnut shells	13.8	13.0	13.5	5.7	Bubbling fluidized bed gasifier	GA: air; T: 714.4 °C; ER: 0.31	[54]
Almond shells	34.2–39.6	17.8–23.2	10.7–16.8	N.A.	Bubbling fluidized bed reactor	GA: N.A.; T: 820 °C; ER: N.A.	[55]
Hazelnut shells	11.1–14.7	8.6–20.7	9.5–16.3	1.4–2.5	Downdraft fixed bed gasifier	GA: air; T: 1000–1050 °C; ER: N.A.	[56]
Hay	8.8	19.7	14.4	3.0	Fixed bed reactor	GA: air; T: 800 °C; ER: N.A.	[45]
Corn stover	26.9	24.7	23.7	15.3	Fluidized bed gasifier	GA: steam; T: 600–710 °C; ER: N.A.	[47]
Olive kernels	5.4–9.3	6.9–8.6	19.0–21.7	1.8–3.0	Circulating fluidized bed gasifier	GA: air; T: 800 °C; ER: 0.4–0.7	[57]
Vine pruning	17.1–18.4	21.3–21.7	11.3–13.0	2.1–2.6	Downdraft fixed bed reactor	GA: air; T: N.A.; ER: 0.26	[58]
Corncobs	17.3	22.6	12.0	1.98	Downdraft fixed bed reactor	GA: air; T: N.A.; ER: 0.28	[59]
Citrus peels	60.0–65.0	15.0–25.0	15.0–23.0	<5.0	Fixed bed gasifier	GA: steam; T: 750 °C; ER: N.A.	[60]
Posidonia oceanica	11.8–24.9	4.1–12.7	14.1–20.0	2.0–3.0	Fluidized bed gasifier	GA: air; T: 750 °C; ER: 0.3	[61]
Empty fruit brunch	12.9–13.5	17.0–17.4	13.7–14.5	1.5–1.9	Downdraft fixed bed gasifier	GA: air; T: 650–825 °C; ER: N.A.	[62]
Sugarcane bagasse	7.4–8.0	8.0–12.9	15.9–18.7	1.4–2.5	Cyclone gasifier	GA: air; T: 600–950 °C; ER: 0.18–0.25	[63]
Sewage sludge	5.1–8.1	19.5–31.6	13.3–16.5	0.9–1.5	Fixed-bed gasifier	GA: air; T: 650–1100 °C; ER: 0.12–0.27	[64]
Miscanthus X giganteus	8.6	16.4	14.0	4.4	Bubbling fluidized bed reactor	GA: air; T: 800 °C; ER: 0.21	[65]
Switchgrass (*Panicum vigatum*)	23.5	33.2	19.4	17.0	Fluidized bed gasifier	GA: steam; T: 600–710 °C; ER: N.A.	[47]
Thistle (*Cynara cardunculus* L.)	36.6	8.5	50.4	4.5	Circulating fluidized bed gasifier	GA: steam and oxygen; T: 750 °C; ER: 0.3	[66]
Wheatgrass (*Elytrigia elongata*)	10.8	12.3	16.5	5.3	Bubbling fluidized bed gasifier	GA: oxygen-enriched air; T: 800 °C; ER: N.A.	[67]

GA: gasifying agent. T: temperature. ER: equivalence ratio. N.A.: not available data.

3. Syngas Fermentation

3.1. Metabolism Insight of Syngas Fermentation

Different types of microorganisms, bacteria, or archaea, whether aerobic or anaerobic, are known to be able to use CO or CO_2 as a carbon and/or energy source. These microorganisms are defined either as autotrophs when they use only C1 compounds as a carbon source and H_2 or light as an energy source, or as unicarbonotrophs when they use C1 compounds as a carbon and energy source. Among these microorganisms, acetogenic bacteria are the most used in syngas fermentation [20,68].

3.1.1. Acetogens and the Wood–Ljungdahl Pathway

Acetogens are obligate anaerobic microorganisms that are able to use CO and/or CO_2 with H_2 to produce organic acids, alcohols, and other industrially relevant chemicals. To date, more than 100 acetogenic species belonging to 22 genera are known, which have been isolated from different habitats. *Acetobacterium* and *Clostridium* are the most representative of these genera. Despite the wide diversity between these microorganisms, the Wood–Ljungdahl metabolic pathway is commonly used [69]. This pathway is a non-photosynthetic metabolic route that assimilates CO_2 into biomass and cell components. It is an irreversible, non-cyclic, strictly anaerobic pathway that consists of two branches: a methyl branch and a carbonyl branch (Figure 4) [20]. Acetogens' metabolism is characterized by two phases. The first phase, known as acidogenesis, is associated with growth. During this phase, microorganisms produce organic acids, mainly acetic acid, in addition to biomass. Acetate production is coupled with the substrate-level phosphorylation (SLP) mechanism, which generates one molecule of ATP per mole of acetate formed. The second phase, which takes place at the stationary growth phase, is named solventogenesis. When microorganisms enter this phase, alcohols are produced from both acetyl-CoA and from organic acids through their reduction. The main product of solventogenesis is ethanol. Therefore, ethanol, unlike acetic acid, is a non-growth-associated product. Solventogenesis is induced by several factors, such as a high concentration of protonated organic acids, non-optimal temperatures, a low pH, a limited concentration of sulfate and phosphate salts, and a high ATP/ADP ratio and/or NAD(P)H levels [70]. During autotrophic growth, reducing equivalents ($2H^+ + 2e^-$) can be provided from both CO and H_2 [71]. Before the beginning of the metabolic pathway, 1 mole of CO and 1 mole of H_2O are converted into 2 moles of reducing equivalents by monofunctional CO-dehydrogenase (CODH) through the biological water–gas shift reaction (Figure 4). Moreover, through the oxidation process catalyzed by hydrogenase (H_2ase), 1 mole of H_2 is converted into 2 moles of reducing equivalents (Figure 4) [72]. It is important to mention that H_2ase is CO-sensitive; therefore, H_2 consumption occurs only when CO is completely consumed or its concentration in the medium is low enough [73]. Acetate and ethanol production is possible from any combination of CO and H_2. Specifically, 4 moles of CO and/or H_2 are used to produce acetate, while 6 moles of CO and/or H_2 are used to produce ethanol [71,74,75]. Therefore, carbon fixation is independent of the origin of the reducing equivalents, although a higher H_2/CO molar ratio produces an improved CO conversion efficiency into acetic acid and ethanol [69]. Butyric acid, butanol, caproic acid, hexanol, 2,3-butanediol, and lactate can be produced in addition to acetic acid and ethanol (Figure 5).

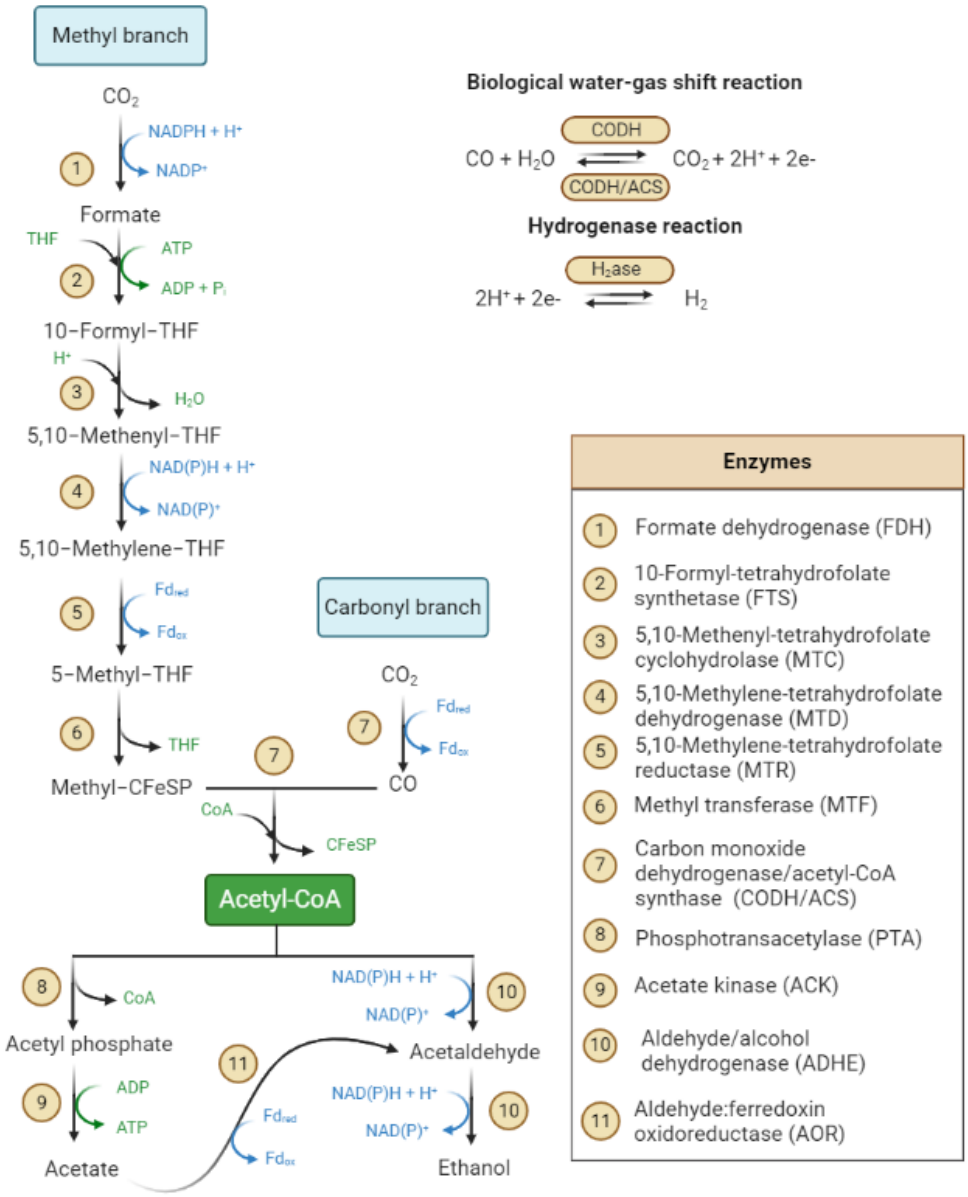

Figure 4. The Wood–Ljungdahl pathway: a schematic representation. THF, tetrahydrofolate; Fd_{red}, reduced ferredoxin; Fd_{ox}, oxidized ferredoxin; CoA, coenzyme A; CFeSP, corrinoid iron-sulphur-containing protein. Created in BioRender.com.

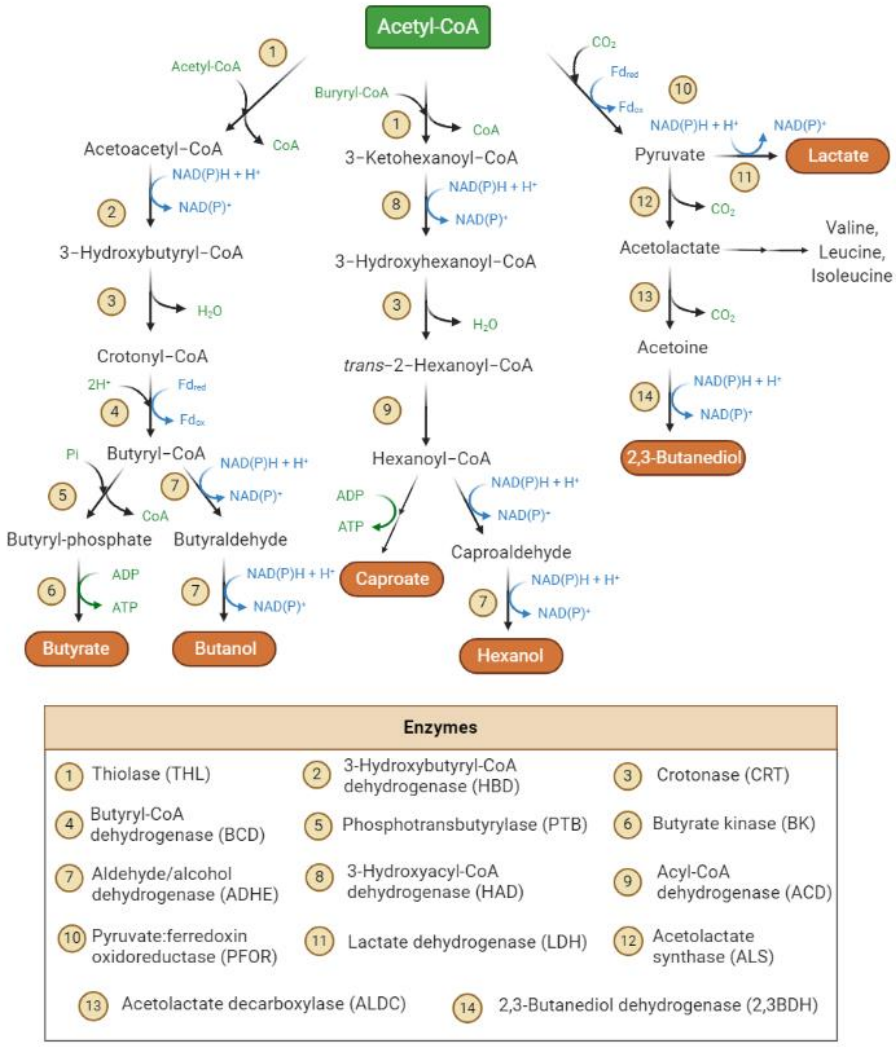

Figure 5. Acetyl-CoA metabolism for the production of butyrate, butanol, caproate, hexanol, 2,3-butandiol, and lactate. Fd_{red}, reduced ferredoxin; Fd_{ox}, oxidized ferredoxin; CoA, coenzyme A. Created in BioRender.com.

3.1.2. Energy Conservation Model in Acetogens

The ATP production of acetogenic microorganisms is based on the establishment of a chemiosmotic mechanism. Although the SLP mechanism, in which an exergonic chemical reaction is coupled with the phosphorylation of ADP, is used during the Wood–Ljungdahl pathway, no net ATP production is obtained [76]. The chemiosmotic mechanism, instead, consists of the establishment of an electrochemical ion gradient across the cytosolic membrane, which is used by a membrane-bound ATP synthase (ATPase) to produce ATP. This mechanism is coupled with cytosolic reactions of the Wood–Ljungdahl pathway, in which reduced ferredoxin (Fd^{2-}) is accumulated. Ferredoxin, which acts as an electron acceptor during the metabolic pathway, is then oxidized by membrane-bound protein complexes, creating a H^+ or Na^+ gradient [77]. Acetogens can be classified into two groups based on the bioenergetics mechanism: the ferredoxin:NAD^+ oxidoreductase (Rnf complex)-dependent group and the electron-bifurcating hydrogenase (Ech)-dependent group. The Rnf complex, and then ATPase, can use either protons (H^+) or sodium ions (Na^+), while Ech uses only H^+. The membrane-bound Rnf complex is proposed to couple the electron transfer from reduced ferredoxin to NAD^+ with the translocation of H^+ or Na^+ across the cytoplasmic membrane. Instead, the Ech is proposed to couple the electron transfer from reduced ferredoxin to NAD^+ with the translocation of H^+ across the cytoplasmic membrane, with the simultaneous H_2 formation [77].

3.2. Syngas Fermenting Microorganisms

3.2.1. Pure Culture Syngas Fermentation

The most commonly used microorganisms in pure culture syngas fermentation are Acetobacterium woodii [78], Clostridium aceticum [79], Clostridium autoethanogenum [80], Clostridium carboxidivorans [81], Clostridium ljungdahlii [82], and Clostridium ragsdalei [83], which were isolated from different habitats, such as marine sediments, soil, animal feces, agricultural settling lagoons, and chicken yard waste. All these microorganisms have a rod-shaped morphology with single, pairs, or chains organization, and are mostly motile due to flagella. The Gram-reaction is positive, except for C. aceticum and C. ljungdahlii. Moreover, these microorganisms are classified as risk group 1 organisms, and thus, they are suitable for biotechnological applications, with optimal temperatures between 30 °C and 37 °C and an optimal pH range between 5.8 and 6.8, except for C. aceticum, whose optimal growth pH is 8.3. Acetogenic microorganisms are obligate anaerobes that are able to use different syngas components (i.e., CO, CO_2, H_2) and organic compounds (i.e., sugars, amino acids, alcohols, carboxylic acids, and other substrates) as carbon and energy sources. The main end products are acetic acid and ethanol, but some strains are able to also produce butyric acid, butanol, caproic acid, hexanol, 2,3-butanediol, and lactate (Table 3).

3.2.2. Other Strains

Some other strains have shown the ability to use syngas as a carbon and energy source, including *Morella thermoacetica* [77,83], *Eubacterium limosum* [84], *Butyribacterium methylotrophicum* [85] and *Clostridium drakei* [81,86]. A brief description of these microorganisms is reported below but they will not be further addressed in this review. *M. thermoacetica* is a thermophilic microorganism that is isolated from horse feces. It was used as a model acetogen to elucidate the Wood–Ljungdahl pathway [77,83]. *E. limosum* and *B. methylotrophicum,* which are closely related to each other, are classified as hazardous and therefore allocated to risk group 2. These strains are autotrophic acetogens that can use H_2, CO_2, and CO, and produce acetate, butyrate, ethanol, butanol, and lactate [85,87]. *C. drakei* was isolated from acidic coal-mine pond sediments and it is an acetogen microorganism, an obligate anaerobe, and is able to grow both autotrophically with H_2 plus CO_2 and CO and heterotrophically with many organic substrates. Acetic acid, ethanol, butyrate, and butanol are the end-products of its metabolism. This strain is closely related to *C. carboxidivorans* [81,86].

3.2.3. Mixed-Cultures Syngas Fermentation

Mixed cultures are more suitable for continuous syngas fermentation processes since it expands the products spectrum due to the synergic action and increases the economic sustainability of the process due to less expensive culture nutritional requirements. In addition, microbial diversity allows for a high culture adaptation and high starvation resistance [68,88,89]. Mixed cultures can be obtained from sewage sludge used in wastewater treatment or in anaerobic digestion [89,90], from manure and/or animal feces [91], and from sediments or industrial wastes [69], as shown in several studies. In order to develop a mixed culture that is capable of efficiently using syngas, an enrichment approach based on operating parameters optimization is required. Mixed cultures produce not only acetate and ethanol [92] but also higher alcohols, such as butanol and hexanol [89].

Table 3. Overview of acetogenic microorganisms.

Strain	Gram	pH_{opt}	T_{opt}	Autotrophic Growth	Heterotrophic Growth	Products	Reference
A. woodii	+	6.8	30 °C	CO, CO_2, H_2	fructose, glucose, lactate, glycerate, formic acid, and o-methylated organic compounds	Acetate, EtOH	[78,93]
C. aceticum	–	8.3	30 °C	CO, CO_2, H_2	Fructose, ribose, glutamate, fumarate, malate, serine, pyruvate, formic acid, ethylene glycol, and ethanol	Acetate, EtOH	[79,94]
C. autoethanogenum	+	5.8–6.0	37 °C	CO, CO_2, H_2	Fructose, xylose, arabinose, rhamnose, glutamate, and pyruvate	Acetate, EtOH, 2,3-butanediol, lactate	[75,80]
C. carboxidivorans	+	6.2	38 °C	CO, CO_2, H_2	Ribose, xylose, fructose, glucose, galactose, arabinose, mannose, rhamnose, sucrose, cellobiose, trehalose, melezitose, pectin, starch, cellulose, inositol, mannitol, glycerol, ethanol, propanol, 2-propanol, butanol, citrate, serine, alanine, histidine, glutamate, aspartate, asparagine, casamino acids, betaine, choline, and syringate	Acetate, EtOH, butyrate, butanol, caproate, hexanol	[81,95,96]
C. ljungdahlii	–	6.0	37 °C	CO, CO_2, H_2	Fructose, glucose, xylose, arabinose, triose, erythrose, fumarate, formic acid, ethanol, and pyruvate	Acetate, EtOH, 2,3-butanediol, lactate	[82,97]
C. ragsdalei	+	6.3	37 °C	CO, CO_2, H_2	Pyruvate, threose, xylose, mannose, fructose, glucose, sucrose, ethanol, 1-propanol, casamino acids, glutamate, serine, choline, and alanine	Acetate, EtOH, butanol, 2,3-butanediol, and lactate	[75,83]

pH_{opt}, optimal pH; T_{opt}, optimal temperature.

Although mixed-culture fermentation represents a valid alternative in the large-scale production of alcohols in comparison to monocultures, in the enriched culture fermentation, human pathogens could be present, such as *Clostridium difficile* and *Clostridium sordellii*. For this reason, adequate enrichment protocols need to be developed to select and use microorganisms with extreme caution [71]. Instead of enriched mixed cultures, synthetic and defined co-cultures can be developed to overcome human health-related issues. Liu et al. demonstrated synergistic action in a mixed culture formed using *Alkalibaculum bacchi CP15* (56%), a CO-oxidizing, ethanol-producing acetogen [98], and *Clostridium propionicum* (34%), a microorganism that is able to convert alanine, serine, lactate, and other related compounds into propionate and acetate via the acrylate-CoA pathway [99], with the remaining 10% consisting of four other *Clostridium* strains for the production of acetate, ethanol, propanol, and butanol. Microorganism synergic action can achieve a 60% increase in alcohol production compared with monoculture syngas fermentation with only *A. bacchi* [100].

Diender et al. [101] developed a syngas fermentation process with a synthetic co-culture composed of *C. autoethanogenum* and *C. kluyveri* (1:1) to produce medium-chain fatty acids (MCFAs) and their corresponding alcohols. While *C. autoethanogenum* was able to produce acetic acid and ethanol from the CO present in the syngas, *C. kluyveri* was able to produce MCFAs, such as butyric acid and caproic acid, from ethanol via a reversed β-oxidation pathway. The MCFAs produced were further reduced by the enzymatic activities of *C. autoethanogenum,* thereby producing the corresponding higher alcohols, butanol, and hexanol. Richter et al. [102] carried out a further study with a defined co-culture of two strains, namely, *C. ljungdahlii* and *C. kluyveri*, to produce butanol and hexanol, although at a low specificity, with a low amount of octanol.

3.3. Strategies for Improving the Syngas Fermentation Process

The utilization of syngas in microbial fermentation is affected by several factors. In the last few years, intense research has been carried out in order to optimize the conditions of syngas fermentation to improve productivity and yield, as well as to reduce production costs. Research has primarily focused on the medium composition and operating conditions. Several studies have been carried out to investigate the effects of nutritional requirements, such as the organic source, vitamins, trace metals, and reducing agent, as well as temperature, pH, and gaseous substrate partial pressure. A common medium used by syngas fermenting microorganisms contains an organic carbon and nitrogen source, vitamins, mineral salts, and trace metals [72], and it has an appropriate oxidation–reduction potential (ORP) to avoid growth inhibition due to a high redox potential [103]. Media optimization studies have been carried out to ensure economic sustainability in a strain-specific manner [104]. As an organic carbon and nitrogen source, several studies reported the use of yeast extract (YE), which contains a wide range of organic substances, such as amino acids, carbohydrates, and nucleotides, as well as micronutrients, such as vitamins. The YE concentration was investigated on *C. autoethanogenum*, where high concentrations led to enhanced microbial growth with a positive effect on acetic acid production [105,106] and decreased production of more reduced products, such as ethanol. Ethanol production was tested with *C. ljungdahlii,* where traces of YE enhanced its production [107]. The effect of a mixture of proteose peptone, beef extract, YE, and NH_4Cl solution was investigated regarding the maintenance of ethanol and acetate production in non-growing cultures of *C. autoethanogenum* and *C. ljungdahlii*, which showed that an organic nitrogen source is necessary for microbial activity [108]. Recent studies showed no positive effects of vitamin supplementation on cell growth or on product formation, especially ethanol [106,109]. These results could have a huge impact on medium growth formulation because vitamins elimination could decrease the medium cost, and thus, the overall costs. An investigation on the effect of trace metals on ethanol production by *C. ragsdalei* showed that copper (Cu^{2+}) removal and higher nickel (Ni^{2+}), zinc (Zn^{2+}), tungstate (WO_4^{2-}), and selenate (SeO_4^{2-}) concentrations than those in the reference medium increased ethanol production [110]. The effect of molybdenum (Mo^{4+}) was also studied in syngas fermentation with *C. ragsdalei* [77] and *C. carboxidivorans* [111], with a positive and negative effect on ethanol production,

respectively. *C. autoethanogenum* was used to investigate the presence of tungsten, which increased the ethanol/acetate ratio, as well as the 2,3-butanediol/acetate ratio, and selenium, which partly counteracted the favourable effect of tungsten [109]. A zinc addition increased the expression of genes of alcohol biosynthesis and improved the carbon fixation and CO assimilation and conversion during syngas fermentation of *C. carboxidivorans* [112]. Finally, the iron (Fe) ions concentration in syngas fermentation medium must be higher than other metals due to their function as cofactors of several key enzymes of the acetyl-CoA pathway [111]. Since acetogens are not able to grow in a high-redox-potential environment [113], reducing agents, such as cysteine-HCl with or without sodium sulfide [113] and sodium sulfide ($Na_2S{\cdot}9H_2O$) [29], are required to induce a metabolic shift to a solventogenic phase [114] and to enhance ethanol production [105]. Several research studies have identified some alternative medium components. Cottonseed extract (CSE) had a similar chemical composition to the standard medium used for syngas fermentation by *C. ragsdalei*, with more specificity for ethanol production in comparison to the standard medium culture [115]. Corn steep liquor (CSL) is a major byproduct of the corn wet-milling industry. Experimental results obtained from bottle and 7.5 L reactor fermentations using either YE or CSL as a medium supplement showed improved butanol and ethanol production with a higher concentration of CSL [116]. Further studies, however, showed that CSL could be a substitute only for YE, vitamins, and minerals, whereas trace metals, ammonium, and a reducing agent should be added [117]. Remarkably, a CSL medium promoted the production of C4 and C6 alcohols and acids, while a CSE medium supported ethanol formation with *C. ragsdalei* and *C. carboxidivorans* [118]. Finally, biochar was also studied as an alternative medium component, which is a carbon-rich material that is obtained from biomass thermochemical conversion and is able to release alkaline elements and trace metals into liquid media. In the work of Sun et al. [71], syngas fermentation with four types of biochar produced from switchgrass (SGBC), forage sorghum (FSBC), red cedar (RCBC), and poultry litter (PLBC) gasification was investigated in comparison to YE. RCBC and PLBC showed an increase in ethanol production by 16.3% and 58.9%, respectively, compared to the YE medium. Moreover, PLBC enhanced the CO and H_2 consumption by 40% and 69% more than YE, respectively. On the other hand, the operating conditions are of utter importance. Temperature can play an important role due to ethanol production coupled with non-optimal growth conditions. In fact, during the syngas fermentation with *C. carboxidivorans* at the sub-optimal temperature of 25 °C, greater alcohol production was observed [96]. Sub-optimal temperatures prevent "acid crash," a phenomenon in which the fast organic acid accumulation in the medium causes a small or null alcohol production in the solventogenic phase. Moreover, sub-optimal temperatures could prevent cell flocculation that can occur at 37 °C [119]. The syngas fermentation medium pH has a strong influence on acetogens' metabolism, especially on the selectivity and distribution of end products. The pH effects on the metabolism shift have been widely investigated in several experiments carried out in the range between 4.5 and 6.8 with *C. ljungdhalii* [108], *C. autoethanogenum* [105,109], and *C. carboxidivorans* [120], and in the range between 6.9 and 8.0 with *C. aceticum* [121]. In order to promote ethanol production rather than acetic acid production, two approaches can be applied: (i) switching the pH from a value of 5.75 to the lower value of 4.75 in the same bioreactor [122] or (ii) separating the two phases of metabolism using two-stage bioreactors with different operating conditions, with the first supporting growth and the second promoting ethanol production [123–125]. In the latter case, the pH values are 5.5 and 6.0 for the first stages, while in the second stages, the pH values are lower, i.e., 4.5, 5.0, and 4.4–4.8. The partial pressure of gaseous substrates has a notable effect on acetogens' metabolism, thereby influencing the cell growth and product distribution [114]. In particular, the CO partial pressure defines the process efficiency, and thus, the utilization of other syngas components. *C. carboxidivorans* was used to investigate the increase of a gaseous mixture's partial pressure that was composed of CO and CO_2 during syngas fermentation, which allowed for an increased cell concentration of up to 440%. Moreover, it was highlighted that at higher CO partial pressures, the ethanol concentration increased. In this case, microorganisms were able to use excess electrons supplied by CO to reduce acetate into ethanol [126]. *C. aceticum* was tested at a high CO partial pressure (204.68 kPa), resulting in a high

cell concentration and good CO tolerance in a batch system [127]. Mayer et al. [128] showed a strong CO inhibition on *C. aceticum*, with reduced acetate production. However, high CO partial pressure can have negative effects on enzymes involved in the metabolic pathway since they are sensitive to substrate exposure, such as hydrogenase, as shown by *C. carboxidivorans*, in which the hydrogenase activity was reduced by 97% at a CO partial pressure of 202.7 kPa [24]. On the other hand, the increase of the H_2 partial pressure up to 170 kPa produced an increase in the productivity of acetate by *A. woodii* (7.4 g/L/day) [129]. A syngas fermentation in a batch using *C. ljungdahlii* as the biocatalyst was carried out to investigate the syngas total pressure effect without growth inhibition at high values [130]. Moreover, increased ethanol production was observed due to the simultaneous use of CO, CO_2, and H_2.

3.4. Mass Transfer Limitations and Bioreactor Optimization

One of the limiting factors in syngas fermentation is the mass transfer [131]; therefore, the choice of bioreactor is a key point to ensure a high cell concentration and productivity [114]. The most commonly used bioreactors (Table 4) are the continuous stirred tank reactor (CSTR), bubble column reactor (BCR), trickle-bed reactor (TBR), membrane bioreactor (MBR), and monolithic biofilm reactor (MLBR) (Figure 6). Several studies were carried out using CSTRs as bioreactors for syngas fermentation using different biocatalysts. *C. ljungdahlii* was used for ethanol and acetate production [132–135] at the laboratory scale with improved performances compared to CO fermentation. *C. carboxidivorans* was studied in CSTRs showing a shifting metabolism to a solventogenic phase with the production of butanol, as well as ethanol [136,137]. The highest ethanol and acetate productions were observed using *C. autoethanogenum* as a biocatalyst [138]. *C. ragsdalei* was studied in a CSTR with 10 g/L poultry litter biochar (PLBC), showing the feasibility of using a PLBC medium to enhance ethanol production from syngas for potential use at a commercial scale [139]. *C. ragsdalei* syngas fermentation was validated in a 100 L pilot-scale CSTR [140], showing a six-fold improvement in ethanol concentration compared to a serum bottle. A bubble column reactor (BCR) ensured an increased volumetric mass-transfer coefficient using a gas sparging system, which makes BCR more suitable for large-scale syngas fermentation than CSTR [21,141,142]. *C. carboxidivorans* provided a higher ethanol selectivity with respect to CO fermentation [142,143]. In a trickle-bed reactor (TBR) configuration, syngas is allowed to move either co-currently or counter-currently to the liquid medium with free or immobilized cells on the bed [21,141]. In a gas-continuous mode, there is a low mass transfer resistance, and thus, it is possible to operate with a low gas flow, thereby achieving a high volumetric mass transfer coefficient and high conversion efficiency. In continuous-liquid mode, instead, it is necessary to implement a gas recirculation system to achieve high conversion efficiency [20]. TBC is more advantageous than CSTR and BCR due to its high conversion rate of syngas and high productivity [105]. *C. ljungdahlii* was studied for acetic acid and ethanol production, while *C. aceticum* was investigated only for acetate production [144–146]. Membrane bioreactor (MBR) configurations with different types of membranes were developed [114], where the hollow-fiber membrane bioreactor (HFMBR) was the most commonly used [21,147]. In particular, this configuration with *C. ragsdalei* [148] was patented for an ethanol production process [149]. *C. ljungdahlii* [150], *C. carboxidivorans* [151], and *A. woodii* [152] have been studied with an HFM configuration with improved ethanol and acetate productivity. Monolithic biofilm reactor (MBR) configuration showed improved mass transfer characteristics [21,153], where *C. carboxidivorans* syngas fermentation obtained a higher ethanol productivity and final titer than other reactors [154].

Table 4. Syngas fermentation performance carried out in various reactor configurations.

Reactor Configuration	Syngas Composition	Operative Conditions	Microorganism	Products	Products Concentration-Productivity	Reference
CSTR	19% CO, 77% H_2, 4% CH_4	T: 38 °C pH: 5.0 Agitation rate: 1000 rpm	*C. ljungdahlii*	Ethanol	10.00 g/L 6.70 g/L/day	[134]
CSTR (2 L)	55% CO, 20% H_2, 10% CO_2, 15% Ar	T: 37 °C pHi: 6.8 Agitation rate: 500 rpm GFR: 14 mL/min K_La: 135 h^{-1}	*C. ljungdahlii*	Ethanol Acetate	6.50 g/L 5.43 g/L	[132,133]
CSTR (3 L)	60% CO, 35% H_2, 5% CO_2	T: 37 °C pH: 4.0–4.8 Agitation rate: 300–500 rpm GFR: 5–15 mL/min LFR: 0.25–0.75 mL/min K_La: 34.02 h^{-1}	*C. ljungdahlii*	Ethanol Acetate	3.75 g/L 14.97 g/L	[135]
CSTR (7.5 L)	16.5% CO, 15.5% CO_2, 5% H_2, 56% N_2	T: 37 °C pHi: 5.9 to pHf: 5.3 Agitation rate: 400 rpm	*C. carboxidivorans*	Ethanol Acetate	N.A. N.A.	[136]
CSTR (7.5 L)	20% CO, 5% H_2, 15% CO_2, 60% N_2	T: 37 °C pHi: 5.7 Agitation rate: 150 rpm GFR: 10 standard L/min	*C. carboxidivorans*	Ethanol Butanol	1.48–2.82 g/L 35.00–65.00 mM/g of cells/day 0.33–0.53 g/L	[137]
CSTR	2% CO, 65% H_2, 23% CO_2, 10% Ar	T: 37 °C pH: 5.0 Agitation rate: 800 rpm GFR: 30 mL/min D: 0.5 day^{-1}	*C. autoethanogenum*	Ethanol Acetate	9.69 g/L 5.97 g/L	[138]
CSTR (3 L)	40% CO, 30% H_2, 30% CO_2	T: 37 °C pHi: 5.9–4.8 Agitation rate: 200–300 rpm GFR: 1.3–1.8 mmol/min	*C. ragsdalei*	Ethanol	11.00 g/L	[139]

Table 4. *Cont.*

Reactor Configuration	Syngas Composition	Operative Conditions	Microorganism	Products	Products Concentration-Productivity	Reference
CSTR (100 L)	5% H_2, 15% CO_2, 20% CO, 60% N_2	T: 37 °C pHi: 5.9 to pHf: 4.7 Agitation rate: 150 rpm GFR: 0.9 standard L/min	*C. ragsdalei*	Ethanol Acetate 2-Propanol 1-Butanol	25.26 g/L 4.82 g/L 8.86 g/L 0.47 g/L	[140]
BCR (4.5 L)	25% CO, 15% CO_2, 60% N_2	T: 37 °C pH: 5.8 GFR: 200 ccm LFR: 200–300 mL/min D: 0.026 h^{-1}	*C. carboxidivorans*	Ethanol Acetate Butanol	N.A. N.A. N.A.	[143]
BCR (4 L)	14.7% CO, 4.4% H_2, 16.5% CO_2, 56.8% N_2, 4.2% CH_2, 2.4% C_2H_4, 0.8% C_2H_6	T: 37 °C pHi: 5.8–5.9 GFR: 180 ccm LFR: 1.5 mL/min	*C. carboxidivorans*	Ethanol Acetate Butanol Butyrate	1.60 g/L N.A. N.A. N.A.	[142]
TBR (1 L)	38% CO, 28.5% H_2, 28.5% CO_2, 5% N_2	T: 37 °C pH: 5.8 GFR: 2.3 or 4.6 sccm LFR: 200, 500, 700 mL/min (semi-continuous mode)	*C. ragsdalei*	Ethanol Acetate	5.70 g/L and 37.00 mg/L/h 12.30 g/L	[144]
TBR (1 L)	38% CO, 28.5% H_2, 28.5% CO_2, 5% N_2	T: 37 °C pHi: 5.8 GFR: 2.8–18.9 sccm LFR: 200–500 mL/min D: 0.012 h^{-1} (continuous mode)	*C. ragsdalei*	Ethanol Acetate	13.20 g/L and 158.00 mg/L/h 3.30 g/L	[155]
HFMBR	40% CO, 30% H_2, 30% CO_2	T: 37 °C pHi: 5.9 to pHf: 4.5 Agitation rate: 100 rpm GFR: 60 std L/min LFR: 180 mL/min	*C. ragsdalei*	Ethanol	10.00 g/L	[149]
HFMBR	50% CO, 30% H_2, 20% CO_2	T: 35 °C pH: 5.0GFR: 250 mL/min K_La: 385.2 h^{-1}	*C. ljungdahlii*	Ethanol Acetate	6.00 g/L 3.00 g/L	[150]

Table 4. *Cont.*

Reactor Configuration	Syngas Composition	Operative Conditions	Microorganism	Products	Products Concentration-Productivity	Reference
HFMBR (8 L)	20% CO, 5% H_2, 15% CO_2, 60% N_2	T: 37 °C pHi: 6.0 to pHf: 4.5–5.5 Agitation rate: 200 rpm GFR: 50–300 mL/min LFR: 50–200 mL/min K_La: 1096.2 h^{-1}	*C. carboxidivorans*	Ethanol Acetate	23.93 g/L and 3.44 g/L/day 5.00 g/L	[151]
CSTR (1 L) with a submerged HFMs module	40% H_2, 17% CO_2, 43% N_2	T: 30 °C pH: 7.0 Agitation rate: 1200 rpm GFR: 30 L/h D: 0.35 h^{-1}	*A. woodii*	Acetate	17.60 g/L and 148.00 g/L/day	[152]
MLBR (8 L)	20% CO, 5% H_2, 15% CO_2, 60% N_2	T: 37 °C pHi: 6.0 to pHf: 4.5–5.5 GFR: 50–300 mL/min LFR: 200–500 mL/min D: 0.48 day^{-1} K_La: 50–550 h^{-1}	*C. carboxidivorans*	Ethanol Acetate	4.89 g/L and 2.35 g/L/day 3.05 g/L and 1.46 g/L/day	[154]

GFR, gas flow rate; LFR, liquid flow rate; D, dilution rate; K_La, volumetric mass transfer coefficient; pHi, initial pH; pHf, final pH.

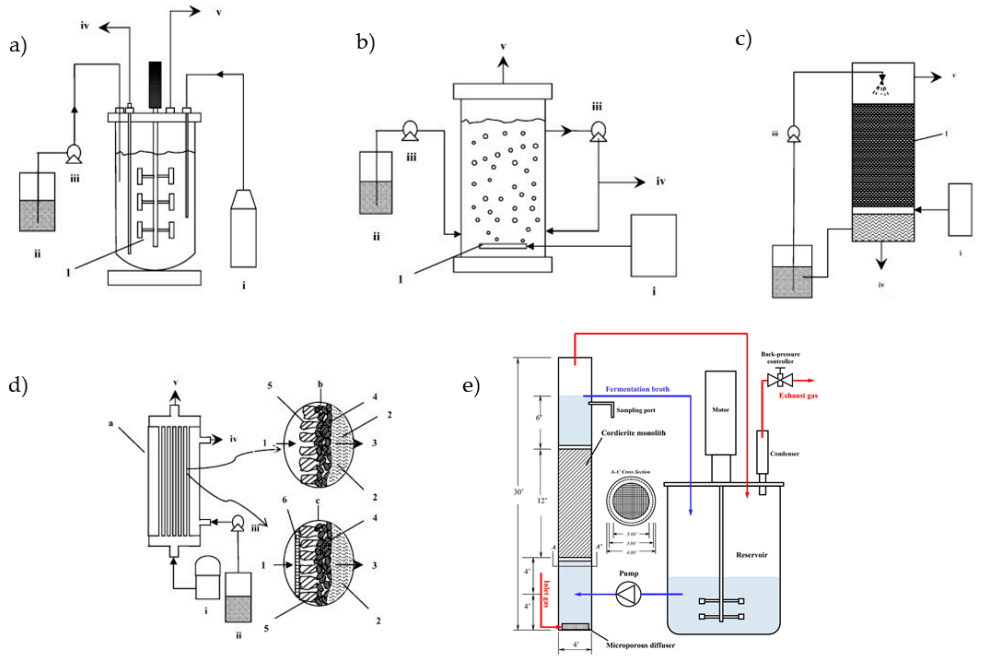

Figure 6. Schematic illustrations of common bioreactors for syngas fermentation: (**a**) continuous stirred tank reactor (CSTR), (**b**) bubble column reactor (BCR), (**c**) trickle-bed reactor (TBR), (**d**) hollow-fiber membrane bioreactor (HFMBR) ([114]; permission obtained by Wiley Online Library), and (**e**) MLBR ([154]; permission obtained by Elsevier).

4. Biomass Gasification and Syngas Fermentation at a Large Scale

The gasification of biomass followed by syngas fermentation to produce fuels and commodity chemicals is a developing technology. Nowadays, LanzaTech Inc. is the leading company regarding the syngas fermentation process. Founded in 2005, LanzaTech Inc. has developed a technology for the use of syngas and industrial waste gases to produce ethanol and 2,3-butanediol using a proprietary recombinant microorganism, i.e., *C. autoethanogenum.* LanzaTech Inc. holds a portfolio of over 100 patents that are related to genetic manipulation to enhance products of interest, reactor designs, process developments to enhance mass transfer and optimize operating conditions, process controls for stable operation and increased selectivity and productivity, syngas clean-up strategies, and product recovery technologies [156,157]. Nowadays, LanzaTech has four demonstration facilities using steel mill off-gases for the production of ethanol in New Zealand, China, and Taiwan. In particular, the two pre-commercial demonstration plants in China, built in partnership with BaoSteel and Shougang, the two major Chinese steel companies, have an ethanol production capacity of 300 metric tons/year each [157]. Moreover, the company is building a commercial plant with an ethanol production capacity of 62,000 metric tons/year in Belgium in partnership with the steel producer ArcelorMittal.

The patent specifications indicate the use of a loop reactor by LanzaTech Inc. for the syngas fermentation process. In a 2012 U.S. patent, a loop reactor was described for the steel mill off-gas (47% CO, 2% H_2, 21% CO_2, 30% N_2) fermentation using *C. autoethanogenum* DSMZ 19630 as the biocatalyst. This configuration consists of a 71 L vessel, a gas inlet, a gas outlet, and an external liquid pump for the circulation of the liquid phase. A vessel section is filled with Sulzer MellapakTM structured packing, which acts as a contact module between the gas and liquid phases. The process was carried out at a temperature of 37 °C and a pH of 5.3, with a centrifugal pump rate of 600–1250 m^3/h. The gas–liquid mass transfer was improved, achieving a volumetric mass transfer coefficient ($K_L a$) of 0.14 s^{-1} and a CO uptake of 5.66 mol/L/day. The final ethanol production was 25 g/L with a productivity of 23.9 g/L/h [158]. Moreover, the gas–liquid mass transfer was improved in forced circulating bubble column reactors by introducing a secondary circulating loop. This configuration consists of a vessel comprising two sections: a riser section (80% of the total reactor volume), wherein the liquid medium

and the syngas move concurrently upward, and a downcomer section, wherein the liquid medium and the syngas move downward. The vessel has a volume of either 390 L (height: 6 m) or 9800 L (height: 10 m). The liquid medium and the syngas are circulated with an external pump in a primary loop. An additional recirculation loop leads to improved gas conversion. The liquid flow rate in the downcomer section was 30 m^3/h, and in the second loop, it was 5.5 m^3/h. This configuration increased the gas retention in the reactor and avoided foaming issues during fermentation [159].

The process shows high feedstock flexibility, thereby allowing for the use of both industrial waste gases and syngas produced through the gasification of biomass wastes. In 2018, LanzaTech Inc. (Skokie, IL, USA) established a partnership with Aemetis, Inc. (Cupertino, CA, USA), which is an advanced renewable fuels and biochemicals company focused on the acquisition, development, and commercialization of innovative technologies that replace traditional petroleum-based products via the conversion of ethanol and biodiesel plants into advanced biorefineries. Aemetis, Inc. successfully developed an integrated demonstration unit for the hybrid process based on biomass gasification and syngas fermentation. The demonstration plant used waste orchard wood and almond and walnut shells as feedstock for the high-temperature plasma gasification system to produce syngas. Then, the cooled and cleaned syngas was supplied to a patented syngas fermentation bioreactor from LanzaTech Inc. to produce cellulosic ethanol. The broth was subsequently distilled to produce commercial-grade ethanol (Figure 7) [160]. Located at InEnTec's Technology Center in Richland, Washington, the plant was continuously operated for more than 120 days with a 94% uptime. This showed that Aemetis, Inc. can successfully produce high-value ethanol from waste orchard wood and other renewable feedstocks, developing a full-scale operating biorefinery [160]. Aemetis, Inc. planned to build a commercial plant in Riverbank, California, to produce 12 billion gallons per year of cellulosic ethanol from 1.6 million tons of waste orchard wood and other renewable feedstocks generated in the California Central Valley [160].

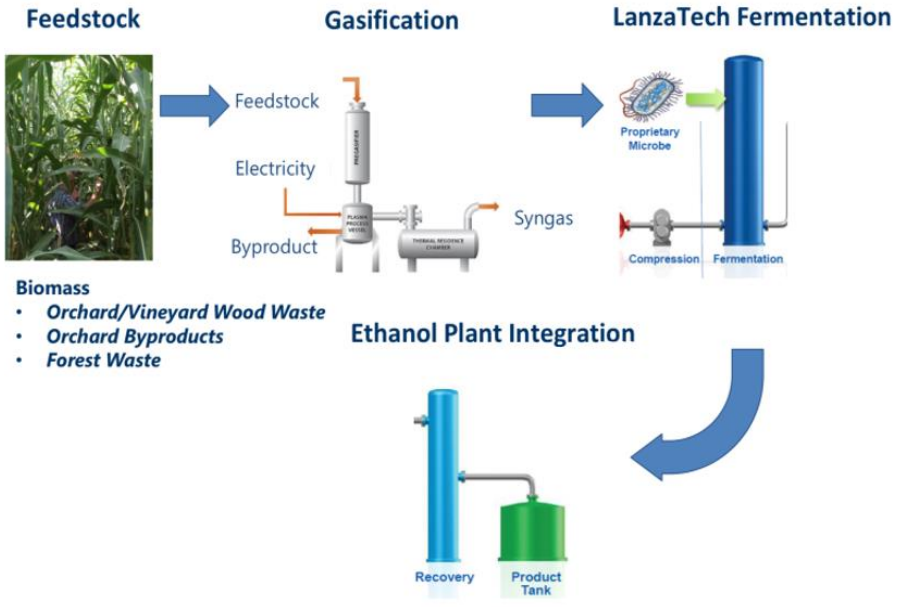

Figure 7. Schematic representation of the demonstrative plant developed by Aemetis, Inc. for ethanol production from syngas [160].

5. Future Perspectives on Strategies for a Sustainable Biomass-Derived Syngas Valorization

Biorefinery systems based on biomass gasification and syngas fermentation need further investigation, both to overcome challenges and increase its commercial interest. One of the major challenges affecting the integration of biomass gasification and syngas fermentation is the syngas final quality. Therefore, choosing the most suitable syngas clean-up strategy to remove impurities is a

key element in the development of this biorefinery system on a large scale. Moreover, so far, syngas fermentation has been studied for the production of a limited range of chemicals, mostly ethanol and acetate. In order to extend the target market of syngas-based biorefinery, further studies are required, both to identify new non-acetogenic syngas-fermenting microorganisms and to develop cascade approaches in which syngas fermentation is coupled with other bioprocesses.

5.1. Effects of Syngas Impurities and Syngas Clean-Up

Nowadays, gasification–fermentation research is commonly performed at the bench scale with clean syngas instead of biomass-derived syngas. The major difference between syngas produced from biomass gasification ("raw syngas") and purified, bottled, mixed commercial syngas ("clean syngas") is the presence of impurities and residues in addition to the main components (CO, H_2, and CO_2). Understanding of effects of syngas impurities on the fermentation process is critical for the design of an economically viable gasification–fermentation process, based on the development of syngas clean-up strategies [31].

Some impurities, such as light hydrocarbons, did not show any influence on the growth or the metabolism of *C. carboxidivorans* at concentrations below 5% [136,142]. Moreover, acetogens tolerate up to a 20% sulfur content, mainly in the form of hydrogen sulfide (H_2S) and carbonyl sulfide (COS) in syngas, which is useful during fermentation due to the reduction of the redox potential and removal of O_2 from the medium [37]. On the other hand, regarding the effects of tars, the properties and composition of the aromatic mixture are relevant rather than the quantity [161]. In particular, benzene, naphthalene, and polyaromatic hydrocarbons (PAHs) have an adverse effect on cell growth, as well as on hydrogenase activity due to their high solubility in the medium during interactions with microorganisms [31,37]. Tars can induce a steady or dormant state with a lack of H_2 uptake, followed by a metabolic shift from an acidogenesis to a solventogenesis phase, with higher ethanol production compared to acetate [136,142]. Similarly, nitrogenous species, such as nitric oxide (NO) and ammonia (NH_3), and thus, ammonium ions (NH_4^+), at concentrations of 0.4–0.15 g/L, 37% molar fraction, and 6.84 g/L, respectively, are enzyme inhibitors, especially ammonia for alcohol dehydrogenase and ammonium ions and nitric oxide for hydrogenase [162,163]. Interestingly, the presence of hydrogen cyanide (HCN) prolonged the lag phase of *C. ljungdahlii* [164].

There are different technologies for syngas clean-up (Table 5). The most suitable technology is dependent on affordability, environmental impact, and the final syngas quality required. Physical methods, such as cyclone, electrostatic precipitator (ESP), rotating particle separator (RPS), and filtration methods, are the simplest and the most mature technologies, but they are not suitable for tar removal due to the presence of very small particles [165]. Wet scrubbing technologies are effective in removing tars, as well as other impurities (i.e., NH_3, HCl). However, this technology needs additional steps, such as syngas cooling and wastewater treatment, which increase the overall energy input and costs [166]. Tars' thermal cracking is difficult to control due to the high process and equipment complexity and the process being highly dependent on many parameters [18,161]. The utilization of catalysts for hot gas cleaning is one of the most efficient technologies for impurities removal (i.e., tars, NH_3, NO_x, N_2O, H_2S) and the most viable option for the large-scale production of clean syngas [165]. Mineral-based catalysts are natural inexpensive minerals, including calcined rocks (calcined dolomite, magnesite, and calcite), olivine, clay materials, and iron ores. On the other hand, synthetic catalysts are char, alkali metal-based, activated alumina, and transition metal-based (Pt, Zr, Rh, Ru, Fe, and Ni) catalysts. Transition metals, as well as other synthetic catalysts, are relatively more expensive compared to the mineral catalysts, although they are more effective, especially for tar reduction [165,166]. However, synthetic catalysts undergo several deactivation mechanisms due to sulfur, chlorine, and alkali metals, which are present at high levels in biomass [167]. Further investigations are required to analyze the catalysts' performance, life cycle, costs and benefits, and techno-economic feasibility.

Table 5. Overview of syngas clean-up technologies.

Methods		Brief Description	Impurities Removed	Reference
Physical methods	Cyclone separators	A centrifugal force is applied to separate solids and aerosols from the gas.	Large particles (>5 μm diameter), tars	[161,166,168,169]
	Electrostatic precipitators (ESPs)	Dust particles and droplets of tar attach to gas ions produced in a corona discharge. Particles and droplets become charged and precipitate due to an applied electric field.	Particulate [a], tars	[161,166,168,169]
	Rotating particle separators (RPSs)	It is made of a rotating cylinder, where (i) tars are condensed and then the droplets are removed or (ii) a solvent is injected and then the saturated solvent is taken out.	Particulate [a], tars	[161]
	Filtration	Tars, dust, and particles are blocked on the filter surface. Different types of filters can be used, such as fabric filters, ceramic filters, activated carbon-based absorbers, sandbed filters, and catalytic filters.	Particulate [a] (tar elimination is not efficient)	[161,168,169]
	Wet scrubber (water, RME, OLGA scrubber [b])	It is composed of a gas cooler, fine tar mist separator, and occasionally, a solid particle separator. Impurities elimination occurs via condensation, precipitation, diffusion, solubility, and absorption.	Tars, NH_3, HCl	[166,169]
	Rectisol wash	This process takes place at a temperature below −40 °C and at high pressure. Methanol is used as a solvent due to its ability to allow for physical absorption of NH_3 and HCN, but also of H_2S and CO_2.	NH_3, HCN, H_2S, and CO_2	[168,169]
	Absorption	It is a physical phenomenon consisting of the penetration of a substance into a solid (polyethylene glycol; oxides of Fe, Mn, Zn, Cu, and Ca; molecular sieves) or liquid body (alkaline solution).	H_2S	[168,169]
	Membrane permeation	It is the separation of individual compounds on the basis of the difference in their rates of permeation through a thin membrane barrier.	H_2S	[170]
Thermal methods	Thermal cracking	It consists of a high-temperature (>1000 °C) treatment, during which heavy tar compounds are decomposed into lighter compounds.	Tars	[161,168,169]
Catalytic methods	Hot catalytic gas conditioning	Catalytic strategies provide the possibility to transform the impurities into useful gas compounds, such as CO and H_2. Catalysts can be natural minerals (calcined rocks, such as calcined dolomite, magnesite, and calcite; olivine; clay materials; iron ores) or metallic and metal oxide synthetic catalysts (alkali metal-based, activated alumina, and transition metal-based catalysts, such as Pt, Zr, Rh, Ru, Fe, and Ni).	Tars, NH_3, NO_x, N_2O, H_2S	[161,168,169]

[a] Particulates include char, ash, and alkalis present in the syngas. [b] A wet scrubber can use different liquids, such as water, rapeseed oil methyl ester (RME), or oil washing medium (OLGA).

5.2. Cascade Approaches

Coupling syngas fermentation with other bioprocesses can allow for the expansion of syngas fermentation products and improve product yields and economics. Coupled processes are defined as multistep bioprocesses that convert metabolites from a primary fermentation into valuable products in a secondary fermentation [103]. Acetic acid and ethanol, which are the main products of syngas fermentation, can be used as a substrate to produce C4–C6 carboxylic acids and their corresponding alcohols [171], dicarboxylic acids [172], lipids [51], and polyhydroxyalkanoates (PHAs) [173] (Table 6).

5.2.1. Syngas into Elongated Carboxylic Acids

Products from syngas fermentation can be converted by microorganisms other than acetogens into elongated carboxylic acids. Moreover, these carboxylic acids can be reduced into their corresponding alcohols using acetogens. Indeed, acetogens are able to reduce carboxylic acids first into the corresponding aldehydes and then into the corresponding alcohols via the aldehyde:ferredoxin oxidoreductase (AOR) and the aldehyde/alcohol dehydrogenase (ADHE) activity, respectively [104].

It has been demonstrated that some acetogens, such as *C. ljungdahlii* and *C. ragsdalei*, use syngas as a source of electrons and energy to reduce externally added acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, and caproic acid into their corresponding alcohols [174,175].

These alcohols are more energy-dense and easier to recover than ethanol; thus, they show great potentiality, both as drop-in fuels and as valuable commodity chemicals [104].

A proof-of-concept system in which syngas fermentation was coupled with a chain elongation process to avoid cost-intensive ethanol recovery step was shown [171]. Effluent from a syngas fermenting system with a pure culture of a *C. ljungdahlii* strain was used as a liquid feed medium for the chain elongation bioreactor. The syngas used in the first phase consisted of a gas mixture of 65% CO, 30% H_2, and 5% CO_2, and it was converted into 11.4 g/L of ethanol and 2.3 g/L of acetic acid. This effluent (2–4% *w*/*w* metabolite concentration) was used as a liquid medium in an anaerobic reactor with an open mixed culture. Ethanol was converted into caproic acid via chain elongation via the reversed β-oxidation pathway.

5.2.2. Syngas into Dicarboxylic Acids

Malic acid, together with fumaric and succinic acids, was selected by the United States Department of Energy to be one of the 12 most important platform chemicals produced from biomass. Malic acid can be used for the synthesis of polymers and for the food and pharmaceutical industries, as well as for many other bulk and fine chemicals. An integrated process in which acetic acid from syngas fermentation was converted into malic acid was developed [172]. Sequential production of malic acid from syngas took place in two different 2.5 L CSTRs. For the first step, *C. ljungdahlii* was used as a biocatalyst during syngas fermentation. The composition of syngas used inside the reactor was 32.5% H_2, 32.5% CO, 16% CO_2, and 19% N_2. This process took place under controlled operational conditions: a temperature of 37 °C, pH of 5.9, gas flow rate of 20 mL/min, and agitation speed of 800 rpm. During the 96 h process, *C. ljungdahlii* produced both acetate and ethanol, with final concentrations of 15.9 g/L and 2.0 g/L, respectively. Broth from the syngas fermentation was used for sequential fermentation conducted by *Aspergillus oryzae*. This fungal species was able to transform acetic acid into malic acid at a concentration of 1.83 g/L. The overall yield for the conversion of syngas in malic acid was 0.22 g/g (3.5 g malic acid per mol of syngas) [172].

5.2.3. Syngas into Lipids

Lipids are the main platform for the production of biodiesel, which is one of the most promising clean and renewable liquid fuels. Nowadays, lipids are produced from carbohydrate feedstocks via specialized crops or oleaginous microorganisms. An alternative gas-to-lipids approach that overcomes the limits of conventional processes was reported [51].

This approach was characterized by two stages. During the first stage, syngas was converted by the thermophilic acetogen *M. thermoacetica* into acetic acid. The acetic acid product was then fed as a substrate into a second bioreactor, where it was converted into lipids by the engineered oleaginous yeast *Yarrowia lipolytica.* The two stages were integrated into a single continuous-flow system (Figure 8), which consisted of an anaerobic BCR for the first stage and an aerobic CSTR for the second stage.

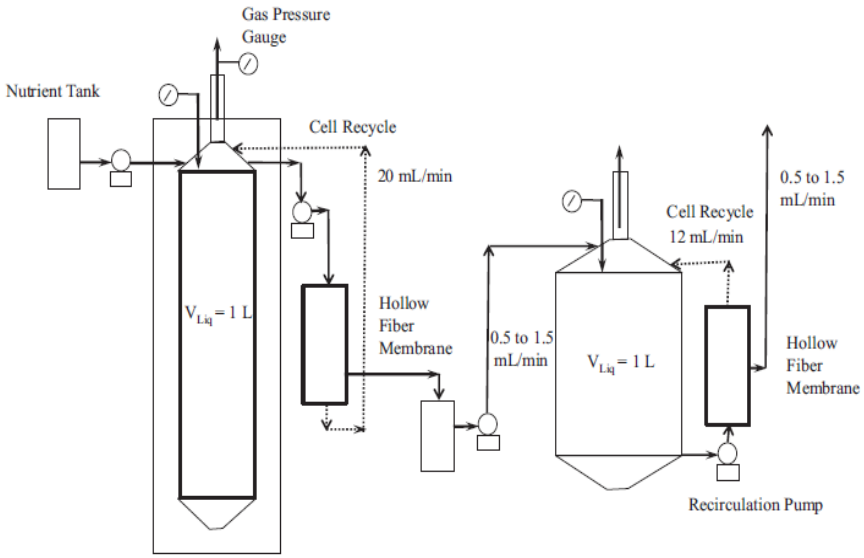

Figure 8. Schematic representation of a two-stage system for the production of lipids from syngas [51].

5.2.4. Syngas into Polyhydroxyalkanoates

PHAs are promising bioplastics due to having similar physico-chemical properties as conventional plastics. Several microorganisms are able to produce PHA, where the greatest yield is obtained using a pure culture of bacteria or archaea species. However, this bioprocess is not competitive with the fossil-based one due to high costs. A more convenient alternative is represented by the use of mixed microbial cultures, as well as cheaper substrates. Lagoa-Costa et al. [173] proposed an integrated two-stage bioconversion process for both bioethanol and PHA production. In the first stage, *C. autoethanogenum* carried out the anaerobic bioconversion of syngas, which consisted of a gas mixture of 30% CO, 10% CO_2, 20% H_2, and 40% N_2 in a 2 L CSTR. The final ethanol concentration was 3.79 g/L, while the acetic acid concentration dropped to 2.66 g/L. This effluent was used as a substrate to produce PHA in a fermentation process, which took place in a glass bioreactor filled with a mixed microbial culture (MMC). The second stage was operated in fed-batch mode. The maximum amount of PHA of 24% of the cell dry weight, in the form of polyhydroxybutyrate (PHB), was produced. Since acetic acid was only used as a substrate to produce PHA, alcohols such as ethanol could be recovered before the second stage. Moreover, a two-stage, whole-cell biocatalytic system for producing PHB from carbon monoxide via formate as an intermediate was developed [176]. In the first stage, *A. woodii* converted a different syngas mixture (20–65% CO, 2–14% H_2, 16–42% CO_2, 18–56% N_2) into a single final product, i.e., formate, achieving high selectivity due to the suppression of acetate production by removing Na^+. Formate was then supplied to a 1 L fermenter in which a genetically modified *Methylobacterium extorquens* strain converted it into PHB, with a concentration and cell contents of 0.097 g/L and 6.5%, respectively. The overall yield of PHB from CO was 2.24%.

Table 6. Cascade approaches overview.

• **Syngas into Elongated Carboxylic Acids**						
Stage 1: **Syngas fermentation**	**Microorganism**	**Substrate**	**Operative Conditions**	**Reactor**	**Products**	**Ref.**
	C. ljungdahlii	65% CO, 30% H_2, 5% CO_2	N.A.	N.A.	Ethanol (11.4 g/L) Acetate (2.3 g/L)	[171]
Stage 2: **Chain elongation process**	Mixed culture	Ethanol	T: 30 °C pHi: 6.5 to pHf: 5.5	Anaerobic filter reactor (700 mL)	Acetic acid (7.9 g/L) Butyric acid (19.4 g/L) Caproic acid (1.0 g/L)	
• **Syngas into Dicarboxylic Acids**						
Stage 1: **Syngas fermentation**	*C. ljungdahlii*	32.5% H_2, 32.5% CO, 16% CO_2, 19% N_2	T: 37 °C pH: 5.9 GFR: 0.02 L/min Agitation speed: 800 rpm	CSTR (2.5 L)	Ethanol (2.0 g/L) Acetate (15.9 g/L)	[172]
Stage 2: **Malic acid production**	*Aspergillus oryzae*	Acetic acid	T: 35 °C pH: 6.5 Aeration rate: 0.6 L/min Agitation speed: 300 rpm	CSTR (2.5 L)	Malic acid (1.8 g/L)	
• **Syngas into Lipids**						
Stage 1: **Syngas fermentation**	*M. thermoacetica*	CO/CO_2 (4/1) H_2/CO_2 (4/1)	T: 60 °C pH: 6.0 GFL: 1 L/min LFR: 0.5 mL/min	BCR (1 L)	Acetate (25.0 g/L)	[51]
Stage 2: **Lipids production**	*Y. lipolytica*	Acetic acid	T: 35 °C pH: 7.3 DO: 20%	CSTR (2 L)	Lipids (18.0 g/L)	
• **Syngas into Polyhydroxyalkanoates (PHAs)**						
Stage 1: **Syngas fermentation**	*C. autoethanogenum*	30% CO, 10% CO_2, 20% H_2, 40% N_2	T: 30 °C pH: 5.75 GFR: 0.01 L/min Agitation speed: 250 rpm	CSTR (2 L)	Acetate (2.7 g/L) Ethanol (3.8 g/L) 2,3-Butanediol (1.6 g/L)	[173]
Stage 2: **PHA production**	Mixed microbial culture (MMC)	Acetic acid	T: 30 °C	Glass bioreactor (1 L)	PHA (24% of cell dry weight)	
• **Syngas into PHAs**						
Stage 1: **Syngas fermentation**	*A. woodii*	20–65% CO, 2–14% H_2, 16–42% CO_2, 18–56% N_2	N.A.	CSTR	Formate (1.89–2.79 g/L)	[176]
Stage 2: **PHA production**	*M. extorquens*	Formate	N.A.	CSTR (1 L)	Polyhydroxybutyrate (PHB) (0.097 g/L)	

GFR: gas flow rate; LFR: liquid flow rate; DO: dissolved oxygen.

5.3. Non-acetogenic Microorganisms as a Valid Alternative to Acetogens

Non-acetogenic microorganisms would represent a great opportunity for biotechnological applications of syngas fermentation, thereby producing high-value chemicals beyond acetate and ethanol. Hydrogenogenic strains are able to use CO as a carbon source via the biological water–gas shift (WGS) reaction (Figure 4). Several strains have been identified as able to conduct the biological WGS reaction, both mesophilic and thermophilic species, such as *Rhodospirillum rubrum*, *Rhodopseudomonas palustris*, *Citrobacter* sp. *Y19*, *Rubrivivax gelatinosus*, *Carboxydothermus hydrogenoformans*, and *Bacillus simithii* [20,177]. In particular, *R. rubrum* is the most studied strain due to its ability to produce H_2 during syngas fermentation [178]. *R. rubrum* is a purple non-sulfur bacterium that grows heterotrophically or autotrophically, using light as a source of energy via the Calvin–Benson–Bassham cycle in aerobic or anaerobic conditions, as well as in darkness. When CO is used as the sole carbon and energy source, it induces the expression of CODH and the CO-sensitive hydrogenase [179]. These enzymes act in the WGS reaction. The CO_2 released from this reaction is partly assimilated for cell material production, while the remaining CO_2 and the produced H_2 are released in the medium. Thus, the growth medium is enriched with H_2 during syngas fermentation [68]. Moreover, PHA production, especially in the form of PHB and polyhydroxyvalerate (PHV), from syngas fermentation with *R. rubrum* was demonstrated using 3-ketothiolase, acetoacetyl-CoA reductase, and PHB synthase [68,180]. Several research studies have been carried out to investigate the effect of light intensity, organic carbon source (i.e., acetate), and nutrient-limited media cultivation on microbial cell growth, metabolism, and H_2 and/or PHA production. These works showed that both energy sources of light [181] and organic substrates [182,183] are required to provide electrons to produce H_2. Instead, nitrogen- [184] and/or phosphate-limiting [185] conditions are suitable for PHA accumulation in the cells.

Syngas fermentation with *R. rubrum* was also demonstrated in a 2 L CSTR for biological hydrogen production. The CSTR was operated with syngas (55% CO, 20% H_2, 15% Ar, 10% CO_2) under anaerobic conditions at various agitation speeds (150–500 rpm) and gas flow rates (5–14 mL/min), while the pH and temperature were set at constant values of 6.5 and 30 °C, respectively. Moreover, the fresh medium, which was fed at a 0.65 mL/min liquid flow rate, contained acetate as the organic carbon source. The H_2 productivity and yield were higher at the highest values of the agitation and gas flow rate [186,187].

The growth of *R. rubrum* was investigated using ground seed corn syngas and fermentation was initiated with an artificial syngas mixture (56.0% N_2, 17.2% CO, 16.3% CO_2, and 8.8% H_2) that was used only to reach the desired density. In the batch fermentations, 340 mg/g cell protein/day of PHA was produced and was composed of 86% β-hydroxybutyrate and 14% β-hydroxyvalerate [180].

Hydrogen-oxidizing bacteria, so-called Knallgas bacteria, are able to produce PHA during aerobic syngas fermentation. These microorganisms are able to fix CO_2 into cell material using H_2 as the electron donor and O_2 as the electron acceptor. Among them, the most known and studied is *Ralstonia eutropha*. The fermentation technology for this microorganism was investigated to solve two major problems: (i) the gas mixture required for cell growth has a ratio of H_2:CO_2:O_2 equal to 7:1:1, which is in the explosive range and (ii) H_2 and O_2 show a low solubility in a liquid medium.

An explosion-proof fermentation bench-plant for aerobic gas fermentation with *R. eutropha* was developed that contained an oxygen concentration in the gas phase below 6.0%. The results showed cell and PHB productivities of 2.28 g/L/h and 1.55 g/L/h, respectively [188]. An approach similar to two-stage syngas fermentation was also employed to grow bacterial biomass heterotrophically and in an inorganic medium (H_2, CO_2) with a safe O_2 concentration (below 6.9%), followed by PHB accumulation up to 82.1% (*w*/*w*). In addition to two-stage fermentation in batch mode, the continuous production of PHB by two-stage cultures was also investigated. In the first stage, fructose was continuously fed at a dilution rate of 0.1 h^{-1}; then, the culture was transferred into an airlift fermenter in chemostatic conditions, obtaining an autotrophic PHB accumulation. The PHB productivity (0.025 g/L/h) and final concentration (57.6% *w*/*w*) were lower than for the batch fermentation, probably due to the limited availability of O_2 in the medium [189,190]. Moreover, after the addition into the culture medium of 0.05%

(*w*/*w*) carboxymethylcellulose (CMC) as surface-active reagent, the cellular content and productivity of PHB increased to 81.4% (*w*/*w*) and 1.02 g/L/h, respectively [191]. As a CO-resistant strain, *R. eutropha* was investigated for its PHA production with a gas mixture containing 5–25% (*v*/*v*) CO, which resulted in 70–75% PHA accumulation [192]. Although CO did not show any negative effects on the key enzymes of PHAs synthesis, it was not used as a co-substrate. To enable the use of CO, *R. eutropha* was engineered with CODH from *Oligotropha carboxidovorans* using a syngas mixture (40% CO, 40% H_2, 10% CO_2, and 10% N_2) resulting in more than 20% PHB synthesis [193].

Finally, aerobic carboxydotrophs are microorganisms known for using the reducing power of CO and H_2 to fix carbon through the Calvin cycle, with O_2 as the electron final acceptor. *O. carboxidovorans* is the most investigated carboxydotroph. This microorganism grows in a CO, CO_2, and H_2 mixture, and on organic substrates [194,195]. Interestingly, the chemolithoautotrophic growth of *O. carboxidovorans* in a gas mixture containing 50% air and 50% syngas (3% CH_4, 18% CO_2, 41% CO, and 38% H_2) showed a modified viscosity of membrane lipids with longer chain fatty acids.

SYNPOL ("Biopolymers from syngas fermentation"), which was a European project that ended in 2016, aimed to improve acetogenic and non-acetogenic microorganisms' performance during syngas fermentation to produce biopolymers. Several efforts were made to analyze the capacity of various bacteria to ferment syngas and to create genetically modified strains. Studies were carried out mostly on *R. rubrum*, *R. eutropha*, and *O. carboxidivorans* [196].

6. Summary and Outlook

Biomass gasification integrated with syngas fermentation is a promising model of second-generation biorefining. In the first step, gasification can be fed with different kinds of lignocellulosic biomass, from woody biomass to agro-industrial residues and herbaceous crops, thereby showing tremendous feedstock flexibility. In the second step, syngas fermentation can be performed by several microorganisms for the production of fuels and chemicals. Acetogens are the most widely used due to the Wood–Ljungdahl metabolic pathway, which allows them to produce acetate and ethanol as the main end products, but also other products, such as butyrate, butanol, caproate, hexanol, 2,3-butanediol, and lactate. Moreover, a mixed culture can also be used in a syngas fermentation process to produce alcohols, from ethanol to propanol, butanol, and hexanol, but they could contain human pathogens, i.e., *C. difficile* and *C. sordellii*. Over the past few years, considerable research has been done in the area of the design and optimization of syngas fermentation processes in terms of microbial nutritional requirements (i.e., organic carbon and nitrogen source, vitamins, trace metals, and reducing agent) and operating conditions (i.e., temperature, pH, and gaseous substrate partial pressure). The ability to control the acetogens' metabolism through these parameters is important for improving the yields and productivity of the desired end product. The duration of the syngas fermentation processes reported in this review ranged from approximately 2 days (50 h) to 133 days (3192 h), with an average time of 35.5 days (852 h). The best fermentation performances were observed for *C. ragsdalei*, with a production of 25.26 g/L ethanol and an ethanol/acetate ratio of 5.24 in a 100 L CSTR during a 59-day process [140]. *C. carboxidivorans* showed production of 23.93 g/L of ethanol, an ethanol/acetate ratio of 6.96, and a productivity of 3.44 g/L/day in an 8 L HFMBR during a 19-day process1]. *A. woodii* showed the highest acetate production (17.6 g/L) without byproduct formation in a CSTR with a submerged HFM module during an 8-day process [152]. *C. ljungdahlii* produced 14.97 g/L of acetate and 3.75 g/L of ethanol in a 3 L CSTR during a 45-day process [135]. *C. ragsdalei* produced 12.30 g/L of acetate and 5.7 g/L of ethanol in a 1 L TBR in approximately 70 days [144]. Alternative reactor configurations should be considered for the syngas fermentation process, such as a bubble column reactor, trickle-bed reactor, membrane bioreactor, and monolithic biofilm reactor. In recent years, research activity has intensified in the field of biofilm reactors to achieve higher cell density than in suspended growth reactors. Among them, the hollow-fiber membrane bioreactor is the most promising configuration due to its high mass transfer rates and low energy consumption. The major challenge is the selection of a suitable membrane material. The latter should overcome the gas–liquid mass transfer

limitation and improve biofilm formation. Moreover, the membrane material should display long-term stability during the process, avoiding pore-wetting, biofouling, and other related problems. Therefore, further research studies are essential for developing an optimal reactor configuration that allows for high process performance, scalability, and implementation at an industrial scale. On the other hand, the development of genetically modified strains that are able to perform syngas fermentation represents a valid approach to improving process efficiency. Recent advances in the synthetic biology and metabolic engineering of acetogens are driven by two major objectives: to increase the yield and productivity of native products obtained from the Wood–Ljungdahl pathway, mainly acetate and ethanol, and to expand the products spectrum of acetogens, especially of *C. autoethanogenum* and *C. ljungdahlii*. In the latter case, the two strains were engineered to produce butanol, acetone, and isopropanol, along with other high-value compounds, such as 3-hydroxypropionate (3-HP), 2-butanol, methyl ethyl ketone (MEK), biodiesel, polyhydroxybutyrate (PHB), and terpenes. Therefore, genetic tools offer great opportunities to increase syngas fermentation valorization, although further efforts are required to provide a solid strain design strategy.

The potential of this second-generation biorefinery model is demonstrated by the large-scale projects of two companies in cooperation, LanzaTech Inc. and Aemetis, Inc. LanzaTech Inc. is mainly working in New Zealand, China, and Taiwan using steel mill off-gases for the production of ethanol through syngas fermentation with a proprietary microorganism. Aemetis, Inc., instead, developed a full-scale operating biorefinery for ethanol production using syngas derived from waste biomass gasification (i.e., waste orchard wood and almond and walnut shells).

In the future, additional approaches need to be developed to improve the potentiality and sustainability of this integrated biorefinery model, such as the development of efficient and affordable syngas clean-up strategies, the coupling of syngas fermentation with additional bioprocesses to produce high-value chemicals, and the investigation of promising non-acetogenic microorganisms to improve product formation and reduce the production cost.

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