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Towards syngas biorefineries: The potential of microbial consortia for syngas valorisation

Silvia García-Casado [a,b], Raúl Muñoz [a,b], Raquel Lebrero [a,b,*]

[a] Institute of Sustainable Processes, Dr. Mergelina, s/n, 47011 Valladolid, Spain
[b] Department of Chemical Engineering and Environmental Technology, School of Industrial Engineering, University of Valladolid, Dr. Mergelina, s/n, 47011 Valladolid, Spain

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ABSTRACT

Gasification has emerged as a promising platform to cope with recalcitrant organic waste within the framework of biomass-based biorefineries, producing syngas that can be fermented into valuable bioproducts. Despite its potential, syngas fermentation is based predominantly on pure cultures, which faces significant challenges, including the limited portfolio of generated compounds (primarily acetate and ethanol) and their low productivity. To address these bottlenecks, the potential of microbial consortia as effective platforms for syngas conversion has been evaluated. Syngas biomethanation using mixed cultures is a well-established process, with pilot-scale implementations yielding promising results. Alternatively, the production of carboxylic acids has emerged as an interesting option compared to pure cultures, as comparable acetate productivities can be achieved along with the possibility for chain elongation to butyrate or caproate. However, the feasibility of using mixed cultures to produce alcohols and other high-value compounds from syngas remains underexplored. Advancing the field will also require the development of innovative technologies to overcome inherent barriers and fully unlock the potential of syngas-based bioprocesses. Key challenges include the presence of impurities and variability in syngas composition, mass transfer limitations in bioreactors, and the need for efficient downstream effluent purification. In this context, mixed cultures emerge as a robust approach capable of buffering syngas fluctuations and tolerating certain impurities. At the same time, the development of novel gas phase bioreactors and innovative membrane-based systems for effluent purification is crucial for enhancing CO and H_2 mass transfer and improving products titers, respectively.

1. Biomass as a sustainable feedstock for syngas production

The global environmental situation, largely driven by the extensive reliance on fossil fuels, is accelerating the transition to sustainable production systems. The development of novel low-carbon technologies that rely on clean sources challenges conventional processes to adapt existing infrastructures and process units to accommodate new raw materials and process requirements, such as those associated with the production of synthesis gas (syngas). Syngas is a gas mixture composed of carbon monoxide (CO), hydrogen (H_2) and carbon dioxide (CO_2), along with some minor compounds, including methane (CH_4), hydrogen sulphide (H_2S) or tars. Traditionally, steam reforming, auto-thermal reforming and partial oxidation of hydrocarbons have been the most widely used processes for syngas production at large-scale (Aasberg-Petersen et al., 2003; Bakkerud, 2005; Dybkjær and Aasberg-Petersen, 2016). They primarily use coal, natural gas, and petroleum, which account for 97 % of the feedstock used for syngas generation (Centi and Perathoner, 2020). However, biomass gasification has emerged as a promising alternative to these traditional processes, enabling the production of syngas and supporting the transition of this sector toward a biorefinery concept.

Biomass is defined as the "biodegradable fraction of products, waste and residues of biological origin from agriculture (including vegetal and animal substances), forestry and related industries including fisheries and aquaculture, as well as the biodegradable fraction of industrial and municipal waste", according to the European Directive 2009/28/EC. Within the biorefinery concept, these resources are used as feedstock in several processes to obtain building blocks for the synthesis of chemicals and biofuels (Cherubini, 2010). Biomass offers numerous advantages as a promising raw material in sectors traditionally dominated by fossil

* Corresponding author.
E-mail address: raquel.lebrero@uva.es (R. Lebrero).

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fuels, mainly due to its diverse sources and carbon neutrality. Due to its high carbon, hydrogen, and volatile matter content, biomass is particularly advantageous for gasification processes, where any carbon-based material can be transformed into syngas through partial combustion and hydrogenation using an oxidant (gasifying agent) (Reaction (1)) (Mondal et al., 2011; Sikarwar and Zhao, 2017). Several parameters influence the final syngas composition and the conversion efficiency of the process, including the operational conditions, the reactor configuration, and the feedstock characteristics.

$$Biomass \xrightarrow{oxidant} CO_{(g)} + CO_{2\,(g)} + H_{2\,(g)} + CH_{4\,(g)} + H_2O_{(l)} + H_2S_{(g)} + C_{(s)} + Tar_{(l)} + Trace\ species \tag{1}$$

The gasification temperature and the gasifying agent play a major role in determining the final ratio and composition of syngas. Elevated temperatures (800–1000 °C) are crucial to increase the efficiency of the process by promoting the thermal cracking of the tars into gas products, and therefore enhancing syngas ratio (Gao et al., 2023). Conversely, the gasifying agent mainly determines the final composition and heat content of the syngas. A wide variety of oxidants can convert heavier hydrocarbons and solid fractions into H_2 and CO, with steam and air being the most popular oxidants, as shown in Table 1. While air has traditionally been the preferred gasifying agent due to its low-cost, its high N_2 content reduces the energy content of the resulting syngas. In contrast, the use of steam offers a distinct advantage by increasing the CO and H_2 content in the final syngas while maintaining similar operating costs (Islam, 2020; Samimi et al., 2020).

Reactor configuration also plays a critical role in determining the properties of syngas and can be classified into three main types: entrained flow, fixed-bed, and fluidized-bed gasifiers. Among these, the conventional fixed-bed gasifier is the most widely implemented, owing to its high carbon conversion efficiency, minimal ash generation, simple operation, and limited requirements for feedstock pretreatment (Sikarwar and Zhao, 2017; Tezer et al., 2022). Fluidized-bed gasifiers have gained attention as an efficient and flexible configuration for handling various feedstocks (Loha et al., 2018), which promotes their implementation for large-scale applications. Nevertheless, they also present some drawbacks, such as the high final ash content and the elevated capital investment in equipment (Gao et al., 2023; Mishra and Upadhyay, 2021). Finally, entrained flow reactors are commonly used for coal gasification, accounting for 70 % of the syngas produced worldwide (Sripada et al., 2017). However, the use of biomass in this type of gasifier has been mainly tested at a small scale, while studies at larger scale are limited and not very promising. In fact, entrained flow reactors require pulverized feedstock, so the difficulty of grinding the biomass compromises the process (Basu, 2013). Overall, industrial-scale biomass gasification is predominantly carried out using fixed-bed and fluidized-bed reactors (Tremel et al., 2013), as shown in Table 1.

Biomass characteristics should also be considered during the gasification process. Moisture, hydrogen and carbon content, and heating value (HV) of biomass are the main parameters that determine both the energy balance of the process and the final syngas yield (Gil et al., 2019; Molino et al., 2016). A high moisture content generally results in a decrease in syngas yield and heating values. While higher moisture can promote the production of H_2 through steam reactions, it often leads to a reduction in CO content, ultimately diminishing the overall energy content of the syngas (McKendry, 2002). Therefore, the maximum moisture content in raw biomass is commonly set at 30 %, being 10–15 % the optimal percentage (Bermudez and Fidalgo, 2016). These values are in accordance with the percentages compiled in Table 1 for different biomass types. Similarly, the HV and the carbon and hydrogen content of biomass are correlated parameters that significantly influence the potential of biomass as a gasifiable feedstock, along with the final composition of the syngas produced. González-Vázquez et al. (2018) compared the influence of these parameters for different biomass types. The results indicated that a higher carbon content of the biomass, which corresponds to a higher HV, favours an increase in both CO concentration and syngas yield. Conversely, a high hydrogen content in the

Table 1
Effect of biomass type and gasification conditions on the final syngas composition.

Biomass type		Feedstock characteristics			Gasification conditions		Syngas composition (%v/v)					Ref.
		Moisture (%wb)	Carbon content (%wt)	HV (MJ/kg)	Reactor type	GA and temperature	CO	H_2	CO_2	CH_4	N_2	
Agro-industrial and forestry residues	Wheat straw	8.0–10.0	43.2	-	Fluidized bed	Steam 824°C	24.7	19.5	49.5	4.7		Rasmussen and Aryal (2020)
	Herbaceous crop	-	43.5	18.3	Fixed bed	Steam 850°C	37.0	55.6	7.3	0.0		Vamvuka et al. (2023)
	Sunflower crop	-	41.0	19.7	Fixed bed	Steam 850°C	27.6	52.2	19.6	0.6		
	Almond shells	9.0–12.1	46.4–48.1	18.5–19.1	Fluidized bed	Air 600–750°C	17.2	15.9	16.8	3.4	46.7	McCaffrey et al. (2019)
						Steam 750–900°C	20.0	38.3	17.1	6.1	18.6	
	Digestate	13.2	37.6	14.8	Fluidized bed	Air 745°C	17.3	9.6	14.2	2.0	56.8	Baláš et al. (2022)
	Wood Chips	20.0	54.6	19.2	Fixed bed	Air 815–980 °C	19.8	9.1	11.9	1.3	57.3	Oveisi et al. (2018)
Municipal wastes	Sewage sludge	20.0	51.2	15.0	Fixed bed	Air 950°C	9.6	3.8	13.4	0.8	68.0	Seggiani et al. (2012)
	Municipal solid waste	10.2	51.8	20.3*	Fixed bed	Steam 900°C	22.7	54.2	20.6	1.3		Luo et al. (2012)
	Plastic wastes	0.5–1.5	66.6–76.5	34.9–42.7	Fluidized bed	Air 850°C	11.5	6.0	11.5	6.6	64.8	Arena and Di Gregorio (2014)

VM: Volatile Matter Content, GA: Gasifying Agent, %wb: wet basis, %dm: dry matter, HV: Higher Heating Value.
[*] Lower heating value.

biomass appears to enhance the H_2 concentration in the syngas generated.

Finally, it is interesting to compare the composition of the syngas resulting from fossil fuels and biomass gasification. Syngas derived from coal, natural gas and petroleum coke is mainly composed of CO and H_2 (25–65 % v/v), while low concentrations of CO_2, H_2O, and CH_4 are also present in the gas mixture, typically below 15 % v/v each (Ba et al., 2020; H. Xu et al., 1999; Zhang et al., 2024). In contrast, the syngas obtained from biomass exhibit a lower CO content, typically ranging from 20 to 30 % v/v, along with highly variable H_2 percentages (Table 1). Additionally, this syngas often contains CH_4 levels up to 10 %, whereas fossil fuel-derived syngas typically has trace amounts of this gas. Data compiled in Table 1 reveal significant variability in syngas composition, which must be carefully considered when using this gaseous mixture, as will be discussed in the following section.

2. Syngas bioconversion to added-value compounds

As an essential intermediate for bulk chemicals and energy production, the syngas market is expected to grow in the following years. More precisely, synthesis gas stands out as a raw material in the manufacture of methanol, ammonia and products derived from the Fischer-Tropsch (FT) process, among others (Centi and Perathoner, 2020; González-Vázquez et al., 2018). Prior to its use, syngas must meet several specifications to avoid operational problems, such as catalyst poisoning or equipment corrosion and clogging, and to increase the conversion yields (Abdoulmoumine et al., 2015). Both syngas derived from fossil fuels and biomass share similar conditioning requirements, including adjusting the H_2:CO ratio via water gas-shift reactions, and the elimination of CO_2 and impurities (Swain et al., 2011). However, the inherent variability and composition of syngas derived from biomass gasification limit its use in conventional transformation processes. Thus, the high CO_2 content and low H_2 levels negatively affect the H_2:CO ratio of the gas mixture, which must be adjusted to 2:1 for applications such as methanol production and the FT process (Dry, 2002; Yin et al., 2005).

In this context, gas fermentation has emerged as an attractive alternative to thermo-chemical conversion, where several anaerobic bacteria utilize CO, CO_2 and H_2 as carbon and/or energy sources. Compared to conventional processes, gas fermentation presents a greater tolerance to syngas impurities, reducing the quality requirements for raw syngas and, consequently, the cost of the conditioning step. Additionally, a strict H_2:CO ratio is not essential for gas fermentation. However, this ratio plays a crucial role in determining the bioproducts distribution, as it affects the conversion of CO and CO_2 into acids or reduced products (Teixeira et al., 2018). Several other advantages can be highlighted, including mild operational conditions that lead to lower energy costs, high conversion efficiency and product selectivity, or the wide variability of interesting compounds that can be synthetized (Liew et al., 2016). Therefore, coupling biomass gasification to gas fermentation processes represents a novel biorefinery concept, where several bio-based products can be generated (Fig. 1).

However, some limitations must be overcome to optimize and ensure the feasibility of syngas bioconversion. On the one hand, gas fermentation processes commonly employ pure cultures as microbial catalyst. This ensures obtaining a specific compound at a high selectivity and yield, since their optimal growth conditions can be determined, facilitating process control (Parera Olm and Sousa, 2022). Nevertheless, high productivities at large-scale have been only reported for acetate and ethanol synthesis (Takors et al., 2018). To achieve high concentrations and productivities for other compounds, the use of mixed cultures is becoming an interesting alternative, as it is possible to maximize the production of a target compound by optimizing the environmental conditions (pH, temperature, etc.) (Baleeiro et al., 2019). On the other hand, the low solubility of H_2 and CO poses a significant challenge in gas fermentation, leading to substrate limitation that restrict microbial growth and ultimately decrease system productivity (Gavala et al., 2021). To address this, several strategies have been explored to increase the gas-liquid mass transfer of CO and H_2, typically focused on reactor configurations designed to maximise volumetric mass transfer efficiency.

In this scenario, this review focuses on the potential of mixed cultures as a biocatalyst in gas fermentation processes. A compilation of those gas fermentation studies that employ syngas mixtures similar to that obtained from biomass gasification has been carried out, in order to compare the potential of microbial consortia versus monocultures in terms of productivity. In addition, the critical technological challenges associated with integrating this bioprocess and biomass gasification have been addressed. Different strategies to support the process viability at a large scale are proposed.

3. The potential of mixed cultures for syngas bioconversion

Traditionally, the mixed culture biotechnology (MCB) has focused mainly on wastewater treatment, bioremediation and fermented food generation (Bader et al., 2010). Its adoption in industries such as pharmaceutical or biochemical has been hampered by the complexity of controlling product distribution, so that the use of pure cultures is widely extended in these processes (Hoelzle et al., 2014). However, MCB

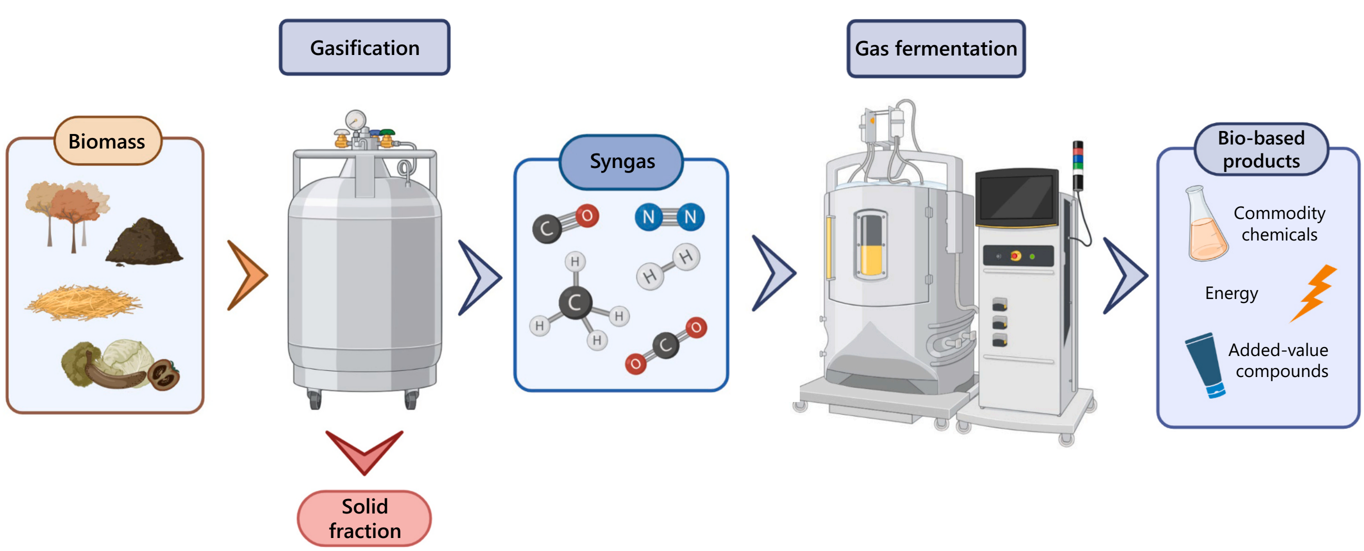

Fig. 1. Scheme of a syngas biorefinery, where biomass gasification is coupled to a gas fermentation system to obtain bio-based products (created in Biorender®).

offers several advantages that support its broader application across bioprocesses, i.e. no sterile conditions requirement, higher robustness, or the capacity to adapt to environmental and substrate variations (Kleerebezem and van Loosdrecht, 2007).

Four main types of microorganisms can use syngas as an energy and carbon source: methanogens, acetogens, hydrogenogens and sulphate reducers (Jeoung et al., 2014). This wide variety results in the production of different compounds of interest, including CH_4, short- and medium-chain acids, and simple and complex alcohols. In addition, several works have reported the synthesis of added-value products, such as lipids, pigments or biopolymers. The following sections summarize the metabolic pathways responsible for their synthesis, the pure cultures commonly used for their production, and a comparison with mixed cultures in terms of productivity.

3.1. Methane

Anaerobic digestion is the classical biological process for CH_4 generation, being the primary component of biogas (50–80 % v/v) along with CO_2 (Chen et al., 2015). This widely implemented technology is based on the anaerobic microbial conversion of an organic substrate through four well-defined stages: hydrolysis, acidogenesis, acetogenesis, and methanation (Weiland, 2010). In methanation, acetate and CO_2:H_2 generated in previous steps are converted into CH_4 through the Reactions (2) and (3), respectively (Angelidaki et al., 2011).

$$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2 \quad (2)$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (3)$$

$$4CO + 2H_2O \rightarrow CH_4 + 3CO_2 \quad (4)$$

Both pathways are involved in syngas biomethanation (Fig. 2). Indeed, acetogenic bacteria can synthesize the acetate required in Reaction (2) from CO, CO_2 and H_2, while the H_2 and CO_2 in Reaction (3) can either be directly derived from syngas or generated by converting CO into CO_2 and H_2 via the water gas-shift reactor (Paniagua et al., 2022). An additional pathway enables the direct conversion of CO into CH_4, known as carboxydotrophic methanation (Reaction (4)). However, CH_4 production through this pathway is negligible compared to the previously mentioned processes, as carboxydotrophic methanogens have significantly longer doubling times than acetogens and hydrogenotrophic bacteria, thus their activity is not reported in most studies (Grimalt-Alemany et al., 2018a).

In this context, the use of co-cultures emerges as an interesting alternative, where known species are used for obtaining first the intermediate compound (acetate or H_2:CO_2) and then, CH_4. For example, a tri-culture of *Rhodospirillum rubrum*, *Methanosarcina barkeri* and *Methanobacterium formicicum* was studied by Kimmel et al. (1991) for syngas biomethanation in small and large scale trickle-bed reactors (TBR). The small TBR (1.1 L), packed with Intalox saddles, reached a volumetric CH_4 production of 1.2–1.8 L_{CH4} L^{-1} d^{-1} and a low CO removal of 40–60 %. Conversely, the larger TBR (26 L) showed an unstable CH_4 production and, despite a higher CO removal was observed (up to 80 %), the volumetric CH_4 production was considerably lower (0.3 L_{CH4} L^{-1} d^{-1}) under similar inlet gas composition. Better results were obtained by Diender et al. (2018) using a co-culture composed of *Carboxydothermus hydrogenoformans* and *Methanothermobacter thermoautotrophicus* in a continuous 1.5-L stirred-tank reactor (STR) fed with a ≈30:70 CO:H_2

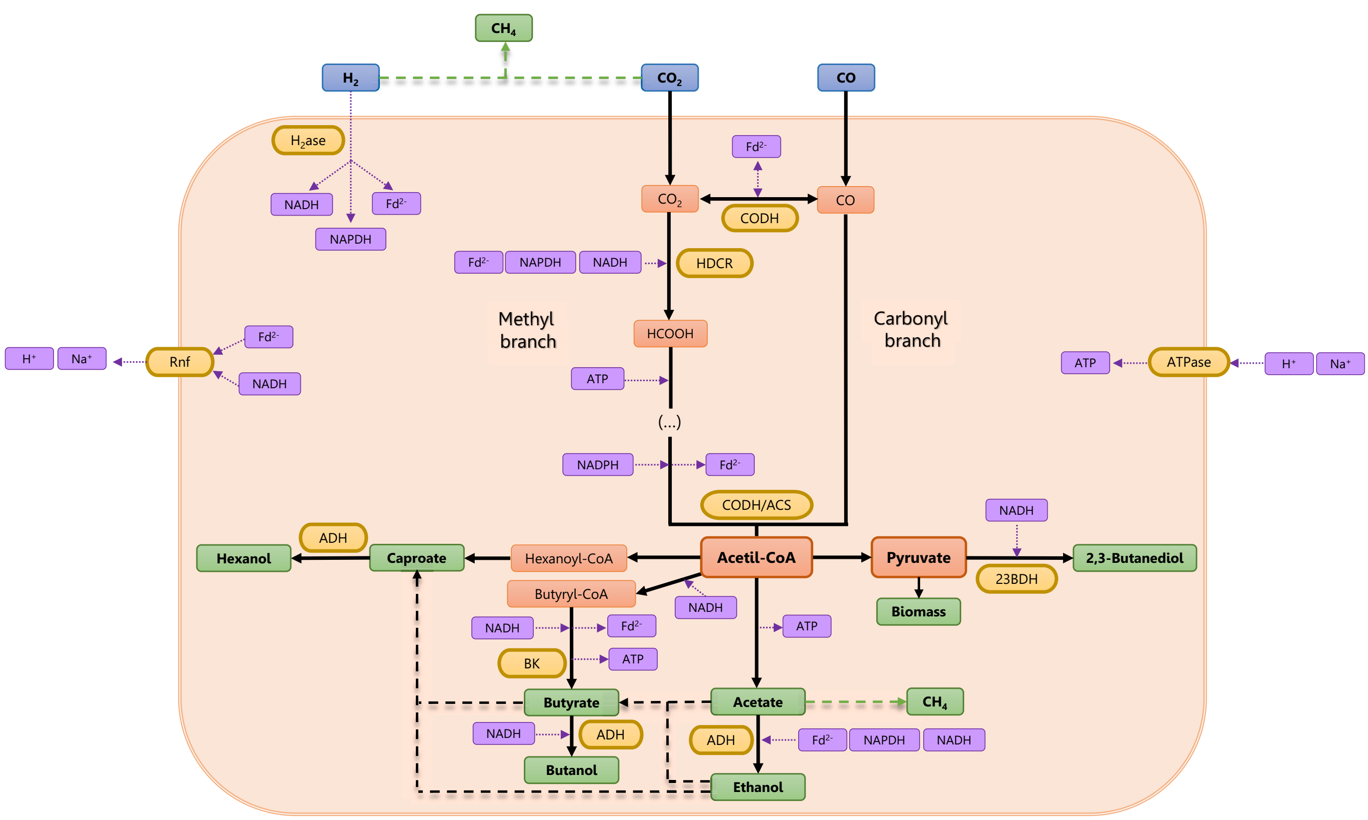

Fig. 2. Schematic representation of the key metabolic pathways and main products derived from syngas fermentation or biomethanation. Blue boxes indicate the substrates, yellow boxes the main enzymes involved, red boxes the intermediates and green boxes the final products. Continuous arrows indicate the direct pathways, while dashed arrows indicate chain elongation or methanation pathways. The main energy-conserving mechanisms are also represented in the diagram, where purple boxes indicate the primary electron and energy carriers. The following abbreviations have been used: 23BDH: 2,3-butyrate dehydrogenase, ADH: alcohol dehydrogenase, BK: butyrate kinase, CODH: CO dehydrogenase, CODH/ACS: CO dehydrogenase/acetyl-CoA synthase, HDCR: hydrogen-dependent carbon dioxide reductase, H_2ase: hydrogenase (adapted from Katsyv and Müller, 2020; Kennes-Veiga et al., 2023; Molitor et al., 2017; Sun et al., 2019).

mixture at inlet flow rates of 7 and 6 mL min^{-1}. Under the first condition, a volumetric CH_4 production rate of 3.5 L_{CH4} L^{-1} d^{-1} was achieved, with up to 90 % removal of CO and H_2. The authors attributed this instability to the sensitivity of *M. thermoautotrophicus* to light. However, there are discrepancies in the literature regarding the extent of the negative impact of light on CH_4 production by *M. thermoautotrophicus* (Olson et al., 1991; Tada and Sawayama, 2004). In the second stage, when the H_2 flow was reduced and the reactor was shielded from light, the system stabilized, leading to a slight increase in CH_4 production (4.0 L_{CH4} L^{-1} d^{-1}) and higher CO and H_2 removals (up to 93 %). The authors also highlighted the impact of stirring speed, as higher velocities enhanced the CO and H_2 solubilization, thus CH_4 production. However, it was observed that values exceeding 400 rpm resulted in the inhibition of methanogenic activity, possibly due to shear stress or a high CO accumulation in the medium. In fact, the sensitivity of pure cultures to shear stress is often considered during the operation with bioreactors (Converti et al., 1993), as will be discussed in Section 4.1.

Although the application of co-cultures proved to be an interesting way of syngas biomethanation, better results have been obtained by using mixed cultures in this field (Table 2). For example, Li et al. (2022) obtained similar results to those reported by Diender et al. (2018) for the same bioreactor configuration. A 4.5-L STR was inoculated with anaerobic sludge and operated at different gas flowrates (10–35 mL min^{-1}) and agitation speeds (300–800 rpm). Best results were obtained at 35 mL min^{-1} and 800 rpm, as the conversion efficiency was favoured due to mass transfer enhancement. This allowed a near complete CO and H_2 removal and a volumetric productivity of 3.0 L_{CH4} L^{-1} d^{-1}, with the gas outlet containing 45.4 % v/v CH_4. The authors attributed the increased CO consumption to H_2 generation at higher agitation rates, which favoured the occurrence of hydrogenotrophic methanogenesis. Indeed, taxonomic analysis demonstrated that *Methanothermobacter* was the predominant methanogenic genus, commonly associated with the transformation of CO_2 and H_2 into CH_4. Slightly lower values were reported by Asimakopoulos et al. (2020a) in a 180-mL TBR with polypropylene/polyethylene packing material, inoculated with a previously enriched syngas-converting mixed culture. The study evaluated the effect of liquid recirculation and inlet gas flow rates on CH_4 production, identifying optimal conditions at 1600 L L_{bed}^{-1} d^{-1} and 1.30 mL min^{-1}, respectively. Process operation under these parameters resulted in a CH_4 productivity of 2.0 mmol $_{CH4}$ L_{bed}^{-1} d^{-1} (1.2 L_{CH4} L^{-1} d^{-1}), with a CO and H_2 removal efficiencies exceeding 90 %. The highest productivities during the experiment (3.3 $mmol_{CH4}$ L_{bed}^{-1} d^{-1}; 2.0 L_{CH4} L^{-1} d^{-1}) were achieved at the expense of reduced conversion efficiency.

Despite these results were in accordance with typical CH_4 volumetric productivities reported for anaerobic digestion (0.1–2.0 L_{CH4} L^{-1} d^{-1}) (Yang et al., 2016), higher productivities have been obtained at larger reactor volumes. For example, Figueras et al. (2023) achieved a maximum CH_4 volumetric productivity of 23.2 L_{CH4} L^{-1} d^{-1} from syngas biomethanation in a STR pressurized at 4 bar. The 10-L reactor was fed with syngas at an inlet flowrate of 1.4 m^3 d^{-1}. Under these conditions, the mixed culture showed H_2 and CO removals of 89 and 82 %, respectively. Higher values up to 96 % were recorded when the reactor was operated at the lowest gas inlet flowrate (180 L d^{-1}), but CH_4 productivity was significantly reduced (4.0 L_{CH4} L^{-1} d^{-1}). Promising results were also obtained by Asimakopoulos et al. (2021) with a H_2-rich syngas fed at similar flowrates. In this study, a TBR packed with polypropylene/polyethylene materials was used to evaluate the influence of gas inflow rates under constant liquid recirculation (1.5 L min^{-1}) and liquid inlet flow rate (0.5 L d^{-1}). Results demonstrated that operating at the highest syngas inflows had a negative impact on CO removal rates but let to an increase in CH_4 productivity. Therefore, the maximum CH_4 volumetric productivity of 17.6 mmol $_{CH4}$ L_{bed}^{-1} h^{-1} (10.3 L_{CH4} L^{-1} d^{-1}) was reached at the highest syngas flowrate (360 L d^{-1}). However, the authors indicated that operating at lower syngas flowrates, which allowed for a complete CO and H_2 depletion, is preferable to minimize downstream processing. In a final experiment, the TBR was connected in-line with a real fluidized gasifier fed with wood pellets. Similar trends to those obtained with the synthetic syngas were observed, with the highest CH_4 productivity reaching 14.4 $mmol_{CH4}$ L_{bed}^{-1} h^{-1} (8.5 L_{CH4} L^{-1} d^{-1}) at a syngas inflow rate of 200 L d^{-1}. Although this study did not report a taxonomic analysis of the microbial culture, the authors emphasized the activity of hydrogenotrophic methanogens.

Table 2
Overview of bioreactor configurations and conditions for syngas biomethanation performed by mixed cultures.

Reactor type Working volume	Conditions	Configuration		Syngas composition (%v/v)	Products concentration	CH_4 productivity (L_{CH4} L^{-1} d^{-1})	Ref.
		Liquid phase	Gas phase				
STR 4.5 L	55°C pH 6.8-7.0	Batch	Cont. 10–35 mL min^{-1}	40:50:10 $CO:H_2:CO_2$	53.6 % CO_2 45.4 % CH_4	3.0	Li et al. (2022)
TBR 180 mL	37°C No pH control	Cont. 10 mL d^{-1}	Cont. 0.5–1.6 mL min^{-1}	20:45:25:10 $CO:H_2:CO_2:CH_4$	30.5 % CO_2 49.5 % CH_4	1.2	Asimakopoulos et al. (2020a)
STR 1.8 L	55°C No pH control	Cont. 60 mL d^{-1}	Cont. 5–20 mL min^{-1}	50:20:30 $CO:H_2:CO_2$	-	13.2	Jiang et al. (2023)
BCB 1.8 L	55°C No pH control	Cont. 60 mL d^{-1}	Cont. 5–20 mL min^{-1} Gas recirculation	50:20:30 $CO:H_2:CO_2$	-	**36.7**	
TBR 180 mL	37°C(1) - 60°C(2) No pH control	Cont. 22.5 mL d^{-1}	Cont. 1–5 mL min^{-1}	20:45:25:10 $CO:H_2:CO_2:CH_4$	29.1(1)–40.7(2)% CO_2 6.7(1)–22.5(2)% CH_4	2.1(1) - 5.0(2)	Asimakopoulos et al. (2020b)
STR 10 L	55°C No pH control	Cont. 0.5 L d^{-1}	Cont. 3.2–15.8 L d^{-1}	45:55 $CO:H_2$	25.7 % CO_2 43.1 % CH_4	0.57	Andreides et al. (2022)
TBR 35 L	37°C No pH control	Cont. 140–1000 mL d^{-1}	Cont. 175 L d^{-1}	30:56:14 $CO:H_2:CO_2$	-	0.9–1.15	Cheng et al. (2022)
STR 10 L	55°C pH 6.0 4 bar	Batch	Cont. 180 L d^{-1}	40:40:20 $CO:H_2:CO_2$	-	4.0	Figueras et al. (2021)
STR 10 L	55°C pH 6.0 4 bar	Batch	Cont. 180–1356 L d^{-1}	40:40:20 $CO:H_2:CO_2$	-	**23.2**	Figueras et al. (2023)
TBR 5 L	60°C pH 7.0 Up to 0.8 bar	Cont. 0.5 L d^{-1}	Cont. 20–360 L d^{-1}	20:45:25:10 $CO:H_2:CO_2:CH_4$	44.2 % CO_2 26.4 % CH_4	10.3	Asimakopoulos et al. (2021)

(1) Mesophilic conditions, (2) Thermophilic conditions, STR: Stirred Tank Reactor, TBR: Trickle Bed Reactor, BCB: Bubble Column Bioreactor.

Overall, biomethanation by mixed cultures is a well-established bioprocess for syngas valorisation. The above-summarized studies reveal that it is possible to reach CH_4 productivities similar or higher than those reported for conventional anaerobic digestion, mainly through hydrogenotrophic methanogenesis. In addition, syngas composition does not appear to be a process limitation, as demonstrated by the consistent results across different gas mixtures (Table 2). It is particularly noteworthy that lower productivities are not associated with higher CO concentrations, which is one of the main limitations commonly indicated for syngas bioconversion. Therefore, syngas biomethanation by open mixed cultures proved to be a robust process at small and medium scales. Further work must validate these promising results through pilot-scale implementation and the use of real syngas mixtures.

3.2. Volatile fatty acids

Syngas fermentation has demonstrated its potential to synthesize a wide range of carboxylic acids and alcohols. Acetogens are highly versatile microbes capable of metabolizing syngas components through the Wood-Ljungdahl pathway (WLP). For instance, CO serves as both carbon and energy source (Fig. 2). It can enter directly into the carbonyl branch of the WLP, bypassing the need for CO_2 reduction. CO also supports the methyl branch by supplying reducing equivalents required for the stepwise reduction of CO_2 to a methyl group. The two branches of the WLP converge in the synthesis of acetyl-CoA via the carbon monoxide dehydrogenase/acetyl-CoA synthase (CODH/ACS) complex. CODH also catalyses the oxidation of CO to CO_2 within the methyl branch (Neto et al., 2024). In fact, hydrogenases and dehydrogenases play key roles in the WLP. While CODH is essential for CO assimilation, several hydrogenases are involved in CO_2 reduction, most notably the hydrogen-dependent carbon dioxide reductase (HDCR) complex. HDCR catalyses the reduction of CO_2 to formate, coupled to H_2 oxidation (Schuchmann and Müller, 2013) and has been identified as a key inhibition point in the presence of CO (Kwon et al., 2022). It is known that CO toxicity is a common bottleneck in syngas bioconversion processes, as it significantly impairs the activity of hydrogenases. The iron atom in the active sites of hydrogenases exhibits a high affinity for CO, leading to inhibition of their catalytic function (Bürstel et al., 2016). To avoid this potential inhibition, CO_2 can be used as the carbon source in the WLP, as it enters directly into the methyl branch and is also reduced to CO via CODH in the carbonyl branch (Liew et al., 2016). In this case, H_2 supplies the energy required in the WLP, which also occurs when CO concentration decreases (Daniell et al., 2012).

Energy metabolism plays a crucial role in determining microbial preference for either CO or H_2:CO_2 as substrates, as explained by Bertsch and Müller (2015). WLP is essentially a reductive pathway for acetyl-CoA synthesis, where H_2 or CO can act as an e^- donor. When e^- are provided by H_2 via the HDCR enzyme, ferredoxin (Fd) reduction ($E^{0'} = -500$ mV) is coupled to H_2 oxidation ($E^{0'} = -414$ mV), which also requires a parallel e^- transfer from H_2 to NAD^+ ($E^{0'} = -320$ mV). In contrast, when CO acts as e^- donor via CODH, its oxidation ($E^{0'} = -520$ mV) can directly reduce Fd, making CO a more energetically favourable electron source. Ultimately, reduced ferredoxin (Fd^{2-}) is the key e^- donor in acetogens and its oxidation is mediated by the respiratory enzyme ferredoxin:NAD^+ oxidoreductase (Rnf). Specifically, the e^- transfer from Fd^{2-} to NAD^+ catalysed by Rnf is coupled to the traslocation of protons (H^+) or sodium ions (Na^+) across the membrane, thereby generating an electrochemical gradient that drives ATP synthesis (Fig. 2) (Katsyv and Müller, 2020; Molitor et al., 2017).

Overall, this section is focused on the production of acetate, butyrate and caproate, as they have been reported as natural carboxylic acids derived from gas fermentation (Gavala et al., 2021; Schiel-Bengelsdorf and Dürre, 2012).

Acetate is the main volatile fatty acid (VFA) derived from the WLP and its biosynthesis from syngas is well studied, either synthesised from CO (Reaction (5)) or from CO_2 and H_2 (Reaction (6)). Although several studies have demonstrated the ability of different species for acetate synthesis in batch experiments, there are few works where the continuous production of acetate has been evaluated with promising results. Novak et al. (2021) studied the synthesis of this VFA by *Acetobacterium woodii* in a continuous 200-mL STR. A gas flowrate of 50 mL min^{-1} was used to feed the bioreactor with two different syngas mixtures, with high (10.6:60.1:9.5 % v/v CO:H_2:CO_2) and low (18.6:29.7:16.7 % v/v CO:H_2:CO_2) H_2 content. The results revealed that a higher H_2 content enhanced acetate production, as steady acetate productivities of 12.6 and 16.6 mmol L^{-1} h^{-1} (17.9 and 23.5 $g_{acetate}$ L^{-1} d^{-1}) for low and high H_2 concentrations were observed, respectively. The acetate concentration in the liquid medium was 17.8 $g_{acetate}$ L^{-1} under high H_2 concentration conditions, while a lower concentration was observed when using the low H_2 content syngas (13.4 $g_{acetate}$ L^{-1}). Lower acetate productivities were reported by Hu et al. (2013) employing *Moorella thermoacetica* in a 1.3 L bubble column bioreactor (BCB). Despite a higher accumulation of up to 26.0 $g_{acetate}$ L^{-1}, the maximum acetate productivity achieved was 3.12 $g_{acetate}$ L^{-1} d^{-1}.

$$4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2 \tag{5}$$

$$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O \tag{6}$$

Apart from studies using pure cultures, to the best of our knowledge there are no investigations involving open mixed cultures aimed at enhancing acetate productivity in a continuous bioreactor. However, the presence of this carboxylic acid has been detected in most gas fermentation studies conducted with mesophilic and thermophilic microbial consortia, together with high productivities. For example, Quintela et al. (2024) employed two 400-mL TBRs packed with polypropylene/polyethylene materials for the evaluation of a microbial consortia at pH values ranging from 4.5 to 7.5 under mesophilic conditions (37°C). The inoculum was sourced from syngas-fermenting bioreactor effluent, with methanogenic activity suppressed via heat-shock pretreatment. Results indicated that neutral pH values favoured acetate production, with the highest pH evaluated (7.5) yielding a steady acetate productivity of 5.75 $g_{acetate}$ L^{-1} d^{-1} and a concentration of 17.2 $g_{acetate}$ L^{-1}, respectively. The analysis of the microbial community revealed that *Acetobacterium* and *Clostridium* were the major genera, and their abundance depended on the pH used. When a higher acetate accumulation was observed (pH 7.5), the genus *Acetobacterium* dominated. Superior results were obtained by Shen et al. (2018) in a hollow-fiber membrane biofilm reactor (HFMBR), who evaluated the spectra of products synthetized by an enriched culture fed with H_2:CO under thermophilic conditions (55°C). In this case, the reactor was inoculated with anaerobic sludge from a mesophilic anaerobic digester and the methanogenic activity was inhibited by the addition of bromoethanesulfonate (BES). In continuous mode, pH optimization demonstrated that the highest pH value (6.5) maximized acetate synthesis over other VFAs, consistent with the findings of Quintela et al. (2024). More specifically, an acetate productivity of 16.4 $g_{acetate}$ L^{-1} d^{-1} was observed, corresponding to an acetate concentration of 24.6 $g_{acetate}$ L^{-1}. In this case, acetate production from CO was associated with the presence of *Thermoanaerobacterium*.

As showed in Fig. 2, butyrate can also be produced naturally in gas fermentation (Reactions (7) and (8)) by several microbes that are capable of transforming acetyl-CoA into butyryl-CoA, and then into butyrate via butyrate kinase enzyme (BK) (Heiskanen et al., 2007; Jeong et al., 2015). However, to our knowledge, only the study from Pacheco et al. (2021) focused on the pure-strain synthesis of this carboxylic acid from syngas. These authors evaluated the production of butyrate by "*Butyribacterium methylotrophicum*" in a batch experiment using a 1.65-L STR fed with different syngas mixtures. The long-term experiment was carried out in two sequential cycles to enhance first acetate synthesis (pH 7.0) and then butyrate production (pH 6.0). The maximum butyrate concentration of 0.28 $g_{butyrate}$ L^{-1} was obtained at the end of the second stage, which was relatively low compared to the 3.5 $g_{acetate}$ L^{-1}

accumulated. This low yield, along with the inhibitory effect of butyrate accumulation in the liquid medium at low pH values (Agler et al., 2011), has contributed to a low interest in the use of pure cultures for butyrate bioproduction.

$$10CO + 4H_2O \rightarrow C_3H_7COOH + 6CO_2 \tag{7}$$

$$4CO_2 + 10H_2 \rightarrow C_3H_7COOH + 6H_2O \tag{8}$$

In contrast, butyrate represents a secondary product in anaerobic fermentations by open mixed cultures as a result of chain elongation processes. This pathway for carboxylate production occurs via reverse β-oxidation (RBO), where short-chain carboxylates are elongated employing an e^- donor (Baleeiro et al., 2019). In this case, acetate is elongated to butyrate using ethanol as e^- donor (Reaction (9)). The use of ethanol as e^- donor involves its initial oxidation to acetyl-CoA, which subsequently enters the RBO cycle for chain elongation to butyrate (Liu et al., 2024). However, acetyl CoA does not exclusively enter the RBO cycle, but is partially converted into acetate via substrate-level phosphorylation to meet the cell's energy requirements (Wu et al., 2019). It is expected that only about one-sixth of the acetyl-CoA molecules are directed toward acetate production, as higher proportions lead to excessive ethanol oxidation (EEO), a competing pathway that diverts carbon and electrons away from chain elongation (Shrestha et al., 2023). EEO results in a loss of ethanol molecules, modifying the optimal 4:6 acetate:ethanol molar ratio required for efficient chain elongation (Liu et al., 2024). This undesired pathway is usually controlled through H_2 partial pressure, as high pressure values makes EEO thermodynamically unfeasible (Wu et al., 2019; Wu et al., 2023). H_2 plays also a central role in several competing pathways. For instance, hydrogenotrophic methanogenesis involves H_2 consumption, thereby promoting EEO, while acetoclastic methanogenesis reduces the availability of both acetate and H_2. Additional competing processes include anaerobic carboxylate oxidation, which entails a loss of fatty acids as CO_2, and the use of ethanol, acetate and H_2 as e^- donors by sulphate reducing bacteria (Baleeiro et al., 2019).

$$C_2H_5OH + CH_3COOH \rightarrow C_3H_7COOH + H_2O \tag{9}$$

Since acetate and ethanol are the primary products in gas fermentation, chain elongation emerges as an interesting process for butyrate production, where two different strategies can be applied. One approach is based on the use of a single bioreactor for both syngas fermentation and chain elongation. This method has been mainly evaluated with co-cultures, where one species is responsible for acetate and ethanol production, and another carries out the elongation process. While no studies were found with bioreactors operating in continuous mode, several works have explored this concept in batch experiments. For example, Fernández-Blanco et al. (2022) operated a 2-L STR with a co-culture of *Clostridium aceticum* and *Clostridium kluyveri* fed with 10 mL min^{-1} of syngas. The reactor was first inoculated with *C. aceticum* to promote the accumulation of acetate and ethanol in the liquid medium, and then *C. kluyveri* was added to start the elongation. The butyrate concentration achieved was 7.0 $g_{butyrate}$ L^{-1} but at the expenses of ethanol addition to promote acetate elongation. The presence of butyrate has also been observed in continuous bioreactors using open mixed cultures. Quintela et al. (2024) detected butyrate under all conditions tested but, unlike acetate production, its synthesis was maximized at pH 6.0. Under these conditions, the genus *Clostridium* was predominant, which is associated with the production of acetate and ethanol, then favouring the presence of chain elongating strains from *Rummeliibacillus* genus. In this assay, a productivity of 1.17 $g_{butyrate}$ L^{-1} d^{-1} and an accumulation of 3.5 $g_{butyrate}$ L^{-1} was observed, representing 24 % of the total VFAs. Butyrate was also detected by Shen et al. (2018) in a HFMBR, but at considerably lower productivities and concentrations (0.17 $g_{butyrate}$ L^{-1} d^{-1} and 0.25 $g_{butyrate}$ L^{-1}, respectively). The authors also associated butyrate production with the presence of *Clostridium* in the microbial community.

Despite the possibility of synthesizing butyrate in one single bioreactor, this process implies the generation of an effluent composed of a mix of short-chain compounds, where butyrate typically presents a low titer. Thus, an alternative approach consisting of a two-stage process has also been evaluated, where gas fermentation and chain elongation are physically separated. This configuration is particularly interesting, as it not only enhanced the concentration of the desired product in the effluent, but also boosted the production of each individual compound. Several works have assessed this strategy for gas fermentation to produce medium- and long-chain compounds using open mixed cultures. However, to our knowledge, only the study of Gildemyn et al. (2017) reported the elongation of acetate generated in a syngas fermenter using the pure culture *C. kluyveri*. The continuous experiment was conducted in a STR, achieving a butyrate productivity of 5.0 $g_{butyrate}$ L^{-1} d^{-1} when a real syngas effluent was used. Vasudevan et al. (2014) reported significantly higher productivities using an open mixed culture as biocatalyst in an anaerobic biofilter (BF). The inoculum was obtained from a chain-elongating reactor, with the effluent from a syngas fermenter serving as the substrate. During start-up, a high CH_4 production was observed when the pH was near 6.5, with CH_4 concentrations in the gaseous effluent reaching up to 80 % v/v, likely due to aceticlastic methanogenesis. To prevent CH_4 production, the pH was decreased to 5.5, which enhanced chain elongation activity. The highest butyrate productivity and concentration achieved were 20.0 $g_{butyrate}$ L^{-1} d^{-1} and 19.4 $g_{butyrate}$ L^{-1}, respectively.

Caproate production through gas fermentation is similar. The direct conversion of syngas to caproate (Reactions (10) and (11)) has been reported using the strain *Clostridium carboxidivorans* P7 by Phillips et al. (2015), who evaluated different mineral media and culture techniques. The maximum caproate concentration reached in serum bottles was 0.36 $g_{caproate}$ L^{-1}. Although some studies operated with continuous bioreactors fed with syngas using this strain, the production of caproate has not been either detected or reported (Shen et al., 2014, 2017). Thus, caproate production by pure cultures is quite limited, making the use of open mixed cultures essential, as caproate formation has been observed in several enriched syngas-converting consortia (Fernández-Naveira et al., 2019; Grimalt-Alemany et al., 2018b). As mentioned, chain elongation is the main mechanism to produce medium-chain compounds from mixed culture. In this case, caproate is synthesized by the elongation of butyrate, also employing ethanol as e^- donor according to Reaction (12).

$$16CO + 6H_2O \rightarrow C_5H_{11}COOH + 10CO_2 \tag{10}$$

$$6CO_2 + 16H_2 \rightarrow C_5H_{11}COOH + 10H_2O \tag{11}$$

$$C_2H_5OH + C_3H_7COOH \rightarrow C_5H_{11}COOH + H_2O \tag{12}$$

In contrast to butyrate production, caproate purification is less expensive than short-chain compounds due to its hydrophobic characteristics (Steinbusch et al., 2011). Therefore, integrating gas fermentation and chain elongation in the same bioreactor appears more feasible, but no promising results have been obtained so far. For example, Quintela et al. (2024) observed a maximum caproate productivity of 1.0 $g_{caproate}$ L^{-1} d^{-1}, while Wang et al. (2018) reported even lower values of 0.1 $g_{caproate}$ L^{-1} d^{-1}. These lower productivities could be associated to the toxicity of caproate derived from its accumulation in the aqueous medium. Both studies associated the presence of *Clostridium* with caproate production.

Consequently, two-stage systems have also been implemented for caproate production. When using a real syngas fermentation effluent, Vasudevan et al. (2014) reached a maximum caproate productivity of 1.7 $g_{caproate}$ L^{-1} d^{-1}. The best results to date have been obtained by Kucek et al. (2016) in a BF packed with Kaldnes K1 rings. The BF inoculum was obtained from an ethanol-rich yeast fermenter, and the substrate consisted of a synthetic mixture of acetate and ethanol, designed to mimic the composition typically obtained from syngas fermentation processes. Different effluent flowrates and ethanol/acetate

Table 3
Overview of bioreactor configurations and conditions for gas fermentation performed by mixed cultures.

Reactor type Working volume	Conditions	Configuration		Syngas composition (%v/v)	Max products concentration (g L^{-1})	Max products productivity (g L^{-1} d^{-1})	Ref.
		Liquid phase	Gas Phase				
TBR 400 mL	37°C pH 4.5-7.5	Cont. 133.3 mL d^{-1}	Cont. 7.5 mL min^{-1}	20:45:25:10 $CO:H_2:CO_2:N_2$	**Acetate: 17.2** Butyrate: 3.5 Caproate: 2.9 Ethanol: 3.1	**Acetate: 5.8** Butyrate: 1.2 Caproate: 1.0 Ethanol: 1.0	Quintela et al. (2024)
HFMR 400 mL	55°C pH 5.5-6.5	Cont. 260 mL d^{-1}	Cont. 0.1–0.15 atm	40:60 $CO:H_2$	**Acetate: 24.6** Butyrate: 0.25	**Acetate: 16.4** Butyrate: 0.17	Shen et al. (2018)
BF 400 mL	30°C pH 5.5-6.5	Cont. 0.5 mL min^{-1}	-	-	**Butyrate: 19.4** Caproate: 1.0	**Butyrate: 20.0** Caproate: 1.7	Vasudevan et al. (2014)
BF 700 mL	30°C pH 5.2	Cont. 0.2–0.5 L d^{-1}	-	-	Butyrate: 0.6 Caproate: 0.5 **Caprylate: 0.3**	Butyrate: 0.4 Caproate: 3.4 **Caprylate: 8.0**	Kucek et al. (2016)
Bottles 800 mL	37°C pH 7.0-6.0	Batch	Cont. 20 mL min^{-1}	15:40:20:25 $CO:H_2:CO_2:N_2$	Acetate: 6.4 Ethanol: 5.9	-	Guo et al. (2024)
STR 2.5 L	37°C No pH control	Cont. 0.33 mL min^{-1}	Cont. 10–199 mL min^{-1}	23.1:17.2:10.6:8.8:21.5 $CO:H_2:CO_2:CH_4:N_2$	**Ethanol: 29.4**	**Ethanol: 5.7**	Manna et al. (2024)
HFMR ultrafiltration 400 mL	35°C pH 6.0	Cont. 76.4–420 mL d^{-1}	Cont. Pressure control	40:60 $CO:H_2$	-	Acetate: 0.6 Butyrate: 0.2 Caproate: 0.1 Ethanol: 0.02 Butanol: 0.02	Wang et al. (2018)
HFMR microfiltration 400 mL	35°C pH 6.0	Cont. 76.4 mL d^{-1}	Cont. Pressure control	40:60 $CO:H_2$	-	Acetate: 0.1 Butyrate: 0.05 Caproate: 0.06	Wang et al. (2018)
BCB 1.0 L	20–28°C pH 4.5–5.5	Batch	Cont. 0.9–2.74 L d^{-1}	30:45:25 $CO:H_2:CO_2$	Acetate: 1.0 Propionate: 0.06 Butyrate: 0.05	-	Andreides et al. (2024)
STR 2.0 L	37°C pH 4.5-7.5	Cont. 400–800 mL d^{-1}	Cont. 10–25 mL min^{-1} Gas recirculation	20:45:25:10 $CO:H_2:CO_2:N_2$	**Acetate: 7.4** Butyrate: 0.5 Ethanol: 1.9	**Acetate: 1.5** Butyrate: 0.1 Ethanol: 0.4	Grimalt-Alemany et al. (2020)
STR 3.3 L	37°C pH 6.5	Cont. 0.6−1.8 mL min^{-1} Cell recycle	Cont. 200 mL min^{-1}	39:27:24:10 $CO:H_2:CO_2:N_2$	**Acetate: 3.4** Ethanol: 2.0	**Acetate: 2.7** Ethanol: 1.0	
STR 3.3 L	37°C pH 6.5-7.0	Cont. 0.6 mL min^{-1} Cell recycle	Cont. 200 mL min^{-1}	20:43:25:12 $CO:H_2:CO_2:N_2$	**Acetate: 2.7** Ethanol: 0.3	**Acetate: 0.7** Ethanol: 0.08	Liu et al. (2014a)
STR 3.3 L	37°C pH 7.0	Cont. 0.6 mL min^{-1} Cell recycle	Cont. 200 mL min^{-1}	28:60:12 $CO:H_2:N_2$	Acetate: 4.1 **Ethanol: 6.0** Propanol:0.2 Butanol:0.1	Acetate: 1.1 **Ethanol: 1.6** Propanol: 0.05 Butanol: 0.03	
STR 2.5 L	37°C pH > 6.1	Batch	Cont. 18 mL min^{-1}	38:28.5:28.5:5 $CO:H_2:CO_2:N_2$	**Ethanol: 2.4** Propanol: 1.0 Butanol: 0.1	-	Liu et al. (2014b)

BF: Biofilter, HFRM: Hollow Fiber Membrane Reactor

ratios were tested. The highest caproate productivity (3.4 $g_{caproate}$ L^{-1} d^{-1}) was observed at 0.5 L d^{-1} with an ethanol/acetate ratio of 15. In addition, this work revealed the possibility of producing caprylate from the syngas fermentation effluent, with a maximum productivity of 8.0 $g_{caprylate}$ L^{-1} d^{-1}. The authors highlighted the absence of *Clostridium* in the microbial community, so a different chain elongator was enriched.

The studies compiled in this review article highlight the potential of open mixed cultures for producing VFAs from syngas fermentation (Table 3). A systematic comparison with monocultures suggests that similar or even higher productivity and concentrations of VFA can be achieved. However, some factors must be considered for successful bioreactor implementation. For instance, since the inoculum source often exhibits methanogenic activity, it must be inhibited to promote carbon utilization for VFAs production instead of CH_4. This can be achieved through various approaches, such as the use of methanogenic inhibitors (e.g BES, heat-shock pretreatment, etc.) or inoculum pretreatment. In addition, environmental conditions, particularly pH, should be carefully controlled to enhance the selectivity and titer of a particular carboxylic acid, as these conditions largely determine the final product distribution. This is probably the main challenge when implementing open mixed cultures, as the simultaneous synthesis of a wide range of products compromises downstream processes related to VFA purification. Therefore, bioreactor configuration is crucial, with a two-stage system offering a promising strategy to improve productivity, particularly for medium-chain VFAs.

3.3. Alcohols

Several alcohols can be synthesised in the WLP via alcohol dehydrogenases (ADH) (Fig. 2). Due to their use as biofuels, this application of gas fermentation is highly developed, even at large-scale. In particular, ethanol production from syngas (Reactions (13) and (14)) has been extensively studied and the process has been implemented at large scale by INEOS Bio, Coskata and LanzaTech. These three companies use pure cultures as biocatalyst, such as *Clostridium ljungdahlii, "Clostridium ragsdalei"* and *C. carboxidivorans* (Daniell et al., 2012). The potential of these strains as ethanol producers is well-known and their implementation in continuous bioreactors has been demonstrated in several studies. For instance, Phillips et al. (1993) observed a maximum ethanol concentration of 48 $g_{ethanol}$ L^{-1}, corresponding to a productivity of 4.0 $g_{ethanol}$ L^{-1} d^{-1} by the strain *C. ljungdahlii* in a 1-L STR. In the case of *C. carboxidivorans*, productivities of 6.7 $g_{ethanol}$ L^{-1} d^{-1} have been achieved by Shen et al. (2017) in a continuous horizontally oriented rotating packed bed.

$$6CO + 3H_2O \rightarrow CH_3CH_2OH + 4CO_2 \quad (13)$$

$$2CO_2 + 6H_2 \rightarrow CH_3CH_2OH + 3H_2O \quad (14)$$

The use of microbial consortia to produce alcohols has also been explored, with several enrichments detecting ethanol formation during gas fermentation (Chakraborty et al., 2019; García-Casado et al., 2024; Grimalt-Alemany et al., 2018a). However, few studies have focused on their implementation in continuous bioreactors and the resulting productivities were lower compared to those obtained with monocultures. For example, Grimalt-Alemany et al. (2020) enriched an open mixed culture from a heat-treated mixture of anaerobic sludges in a continuous STR, under low pH values (4.5) to enhance ethanol production and suppress chain elongation. Acetate remained the main product generated (1.5 $g_{acetate}$ L^{-1} d^{-1}), with low concentrations of butyrate and reduced ethanol productivities of ~0.4 $g_{ethanol}$ L^{-1} d^{-1}. Better results were obtained by Quintela et al. (2024) when using a pH below 6.0, and by Liu et al. (2014a), with maximum ethanol productivities of 1.0 and 1.7 $g_{ethanol}$ L^{-1} d^{-1}, respectively. However, it is important to note that this productivity values were obtained at low CO and H_2 conversion efficiencies. These and several works summarized in Table 3 reported ethanol production by open mixed cultures consisting mainly of *Clostridium* spp., but no promising productivities have been observed.

After ethanol, 2,3-butanediol (2,3-BDO) has emerged as one of the most common alcohols synthetized by acetogenic bacteria (Reactions (15) and (16)). In contrast to other alcohols, pyruvate acts as a precursor in the synthesis of 2,3-BDO via 2,3-butyrate dehydrogenase (23BDH), instead of acetyl-CoA (Schiel-Bengelsdorf and Dürre, 2012). Several species from *Clostridium* genus can use syngas for 2,3-BDO production, such as "*C. autoethanogenum*", *C. ljungdahlii,* and "*C. ragsdalei*" (Jack et al., 2019; Köpke et al., 2011; Ricci et al., 2021). Its production has been already implemented at large scale by the company LanzaTech employing "*C. autoethanogenum*" as biocatalyst (Daniell et al., 2012). Several works have evaluated the potential of this strain in batch bioreactors at lab scale, but just employing CO as carbon source, instead of syngas. For example, Abubackar et al. (2016) worked with "*C. autoethanogenum*" in a 1.2-L STR fed with pure CO at a flowrate of 10 mL min^{-1}. Cycles at different pH values (5.75–4.75) were used to improve alcohols production, reaching a maximum 2,3-BDO concentration of 2.77 $g_{2,3\text{-}DBO}$ L^{-1}. However, to the best of our knowledge, this alcohol has not been detected in any syngas fermentation using mixed cultures.

$$11CO + 5H_2O \rightarrow CH_3CH(OH)CH(OH)CH_3 + 7CO_2 \quad (15)$$

$$4CO_2 + 11H_2 \rightarrow CH_3CH(OH)CH(OH)CH_3 + 6H_2O \quad (16)$$

On the other hand, the presence of complex alcohols has been detected in several studies, despite their synthesis limited to few strains. For instance, the direct conversion of syngas to butanol (Reaction (17) and (18)) and hexanol (Reaction (19) and (20)) has been only reported with *C. carboxidivorans* P7, while "*B. methylotrophicum*" is also capable of transforming synthesis gas into butanol (Grethlein et al., 1991; Phillips et al., 2015). Both experiments were carried out in serum bottles and the only assay performed in a bioreactor was the one reported by Fernández-Naveira et al. (2019), where fed-batch experiments with *C. carboxidivorans* P7 were carried out in a 1.2-L STR. The influence of trace metals and pH was evaluated. The highest butanol concentration (2.13 $g_{butanol}$ L^{-1}) was obtained without pH control, resulting in a natural acidification from 6.2 to 5.0, in the presence of selenium and tungsten. Conversely, the absence of these trace elements facilitated hexanol synthesis, yielding a maximum concentration of 0.39 $g_{hexanol}$ L^{-1} under similar pH conditions.

$$12CO + 5H_2O \rightarrow CH_3(CH_2)_3OH + 8CO_2 \quad (17)$$

$$4CO_2 + 12H_2 \rightarrow CH_3(CH_2)_3OH + 7H_2O \quad (18)$$

$$18CO + 7H_2O \rightarrow CH_3(CH_2)_5OH + 12CO_2 \quad (19)$$

$$6CO_2 + 18H_2 \rightarrow CH_3(CH_2)_5OH + 11H_2O \quad (20)$$

The limited availability of efficient butanol and hexanol producers from syngas presents an opportunity for the application of mixed cultures, although the synthesis of both alcohols has been rarely reported. To our knowledge, butanol has only been produced at significant concentrations during the enrichment experiments conducted by He et al. (2022), using anaerobic sludge after a heat-shock pretreatment. The culture, which exhibited a high abundance of the genus *Clostridium*, was enriched with CO and, when a pH below 5 was reached due to acetate and butyrate accumulation, butanol was detected at a maximum concentration of 6.8 $g_{butanol}$ L^{-1}. An additional experiment was conducted to specifically evaluate the ability of this open mixed culture to convert syngas (10:20:20:50 % v/v H_2:CO:CO_2:N_2) into butanol. Butanol accumulation was slightly higher when compared to a gas phase composed only of CO (0.33 vs. 0.30 $g_{butanol}$ L^{-1}). However, these results require validation in continuous assays, as only Liu et al. (2014a) have reported butanol production by an open mixed culture in a continuous bioreactor. In their study, a maximum butanol concentration of 1.11 $g_{butanol}$ L^{-1} was observed with a productivity of ≈0.03 $g_{butanol}$ L^{-1} d^{-1}, and its production was also associated to the presence of *Clostridium* spp. in the microbial community.

Finally, hexanol biosynthesis via gas fermentation constitutes a novel approach, with only few studies reporting its production. For instance, Liu et al. (2014b) tested a culture previously obtained to produce complex alcohols. When operating in batch mode, butanol was the main alcohol observed (0.09 $g_{butanol}$ L^{-1}), with ethanol and propanol also detected. In a second experiment conducted in serum bottles, 1.5 g L^{-1} of different VFAs were added in the liquid medium to boost their transformation into the respective alcohols. When caproate was used, it was almost completely transformed (90.7 %) into hexanol (1.0 $g_{hexanol}$ L^{-1}), demonstrating the potential of the consortia to produce this alcohol. However, hexanol production from syngas has primarily been studied using co-cultures. Diender et al. (2016) evaluated the potential of "*C. autoethanogenum*" and *C. kluyveri* as a co-culture to produce higher alcohols and medium-chain fatty acids. The effect of different VFAs was evaluated in serum bottles, achieving a hexanol concentration of 2.5 mM (0.26 $g_{hexanol}$ L^{-1}) when 12 mM of caproate was added to the liquid medium. Nevertheless, higher butanol concentrations of 6 mM (0.44 $g_{butanol}$ L^{-1}) were detected by adding 45 mM of butyric acid. This low hexanol production was associated to the inhibitory effect of caproate, therefore continuous systems may offer a solution to prevent its accumulation. In this sense, Richter et al. (2016) used a 1-L STR with a co-culture of *C. ljungdahlii* and *C. kluyveri*. The best hexanol productivity observed was 17.7 mmol-C L^{-1} d^{-1} (0.02 $g_{hexanol}$ L^{-1} d^{-1}), corresponding to a concentration of 2.93 mM in the liquid broth (0.30 $g_{hexanol}$ L^{-1}), when working at a low dilution rate and a pH value of 6.0.

Overall, the studies presented in this section raise questions regarding the real potential of mixed cultures for alcohols production via syngas fermentation. While ethanol is the only compound detected at significant concentrations, its levels are notably lower compared to those observed with pure cultures. However, complex alcohols such as butanol have been detected in several studies and their synthesis is currently not feasible with monocultures. Therefore, future research should focus on exploring strategies to enhance the production and selectivity of these alcohols in open mixed culture systems.

3.4. Added-value compounds

Previous sections have explored the potential of syngas bioconversion as a platform for the synthesis of different bio-based compounds, with a focus on energy and chemical production. However, C1 gaseous substrates can also be employed to produce added-value compounds, such as biopolymers, single-cell protein, and cosmetic products. As a result, syngas-converting microorganisms have also been investigated for these applications. For instance, the strain *Hydrogenbacillus schlegelii* has recently been tested as a novel hydroxyectoine-producer microorganism from syngas, reaching a maximum accumulation of 0.05 $g_{hydroxyectoine}\ g_{biomass}^{-1}$ (Marcos-Rodrigo et al., 2025). On the other hand, the proteobacteria *Cupriavidus necator* stands out for its ability to produce poly(3-hydroxybutyrate) (PHB) from CO_2 and H_2 under aerobic conditions (Lee et al., 2021; Wang et al., 2024). This strain has even been tested using flue gas from a cogeneration plant by Langsdorf et al. (2024), where a PHB accumulation of 43±3 % was observed.

However, the production of added-valuable compounds from synthesis gas has often relied on the genetic modification of several acetogenic strains. In this regard, *C. necator* has been engineered to oxidized CO to CO_2, enabling syngas to serve as a novel carbon source and increasing PHB production by this strain (Vlaeminck et al., 2022). Under anaerobic conditions, the successful production of PHB from a syngas mixture was achieved by recombinant *"Clostridium coskatii"* and *C. ljungdahlii* strains (Flüchter et al., 2019). In fact, *Clostridium* strains have been widely used as model acetogen for genetic engineering (Jun-Zhe et al., 2025; Poulalier-Delavelle et al., 2023; Wilding-Steele et al., 2021), making them particularly attractive for the biosynthesis of novel products from syngas. For example, an engineered *"C. autoethanogenum"* strain was capable of producing ethyl acetate from CO as the sole carbon source, representing one of the first reported cases of autotrophic ester production (Dykstra et al., 2022). Genetic tools have also been employed to maximize the production of a specific compound. For example, *C. ljungdahlii* has been genetically modified to enhance acetate yield or redirect its metabolic pathways by suppressing acetate synthesis in favour of ethanol, acetone and other compounds (Berzin et al., 2012a, 2012b; Leang et al., 2013). However, the field of genetic modifications remains under development, with several critical aspects to be addressed, particularly concerning growth energetics and energy conservation (Latif et al., 2014).

In the case of open mixed cultures, several works have demonstrated their potential to transform C1 gaseous substrates into commercial bioproducts. For example, open mixed cultures have recently been employed for the conversion of CH_4 into polyhydroxyalkanoates (PHAs) under nitrogen-limiting conditions (Pérez et al., 2024) or into ectoine under saline stress conditions (Rodero et al., 2024). Therefore, it can be expected that CO and CO_2 could also be metabolised for this purpose, although this possibility has been scarcely explored. In general, the production of added-value compounds via syngas bioconversion entails a two-stage process that separates syngas fermentation from the synthesis of the target compound. This strategy has been widely used for biopolymer production, based on the use of acetogens to obtain an acetate or formate rich effluent in a first stage that is subsequently used as substrate for PHA synthesis (Hwang et al., 2020; Lagoa-Costa et al., 2017). PHB contents up to 41.5 % have been reported using this process (Portela-Grandío et al., 2021). Two-stage systems have also been implemented for pigments and lipids production, where syngas fermenters are coupled to a second bioreactor commonly inoculated with *Yarrowia lipolytica* strain (Naveira-Pazos et al., 2022; Robles-Iglesias et al., 2024). This approach has achieved lipids contents of up to 36.2 % ($g\ g^{-1}$) and a β-carotene concentration of 0.8 $g\ L^{-1}$ (Robles-Iglesias et al., 2023). Notably, the effluents used contained a diverse mix of carboxylic acids, a typical feature of gas fermentation processes mediated by open mixed cultures. Therefore, the use of microbial consortia could be interesting if the VFA ratios obtained support a significant production of these high-value compounds. However, this potential application remains largely unexplored and requires further investigation.

4. Key technological challenges in syngas fermentation

The literature reviewed in this article confirms the successful application of mixed microbial cultures at laboratory scale for syngas conversion, particularly to produce CH_4 and VFAs. These studies also illustrate how different bioreactor configurations and operational strategies can be considered to optimize syngas fermentation by mixed cultures. However, several challenges should be overcome to ensure a successful scale-up, mainly related to gas-liquid mass transfer limitations, syngas impurities, accumulation of toxic metabolites, or the economic feasibility of downstream process. Therefore, this section addresses these barriers and provides an overview of strategies aimed at ensuring the viability and long-term stability of the process.

4.1. Bioreactor design to overcome gas-liquid mass transfer limitations

The limited solubility of the main compounds present in syngas has consistently been identified as one of the main challenges in gas fermentation. At 25°C, Henry's law constants for CO and H_2 are 1.78×10^{-5} and 1.43×10^{-5} atm^{-1}, respectively, significantly lower compared to those of CO_2 (6.23×10^{-4} atm^{-1}) (Sander, 2023). Therefore, efforts in the development of syngas bioconversion technologies have been focused on boosting the mass transfer from gas to liquid phase. The volumetric mass transfer coefficient (k_La) is the reference parameter used to compare technologies and strategies in terms of mass transfer. It depends on several factors, including gas diffusivity and liquid medium characteristics (k_L), as well as the interfacial contact area between the two phases (a) (Ho et al., 2020). Overall, the most common strategy to enhance k_La is optimizing reactor design rather than increasing gas solubility through operating conditions (Gavala et al., 2021). For instance, only a few studies have explored improving gas solubility using pressurized bioreactors (Tables 2 and 3). In contrast, most research focuses on evaluating the impact of several parameters (such as internal gas recirculation, agitation speed, bubble-fine diffusers, etc.), or reactor type on the mass transfer. In this context, the following section presents a compilation of conventional reactor configurations used in gas fermentation, along with an evaluation of novel technologies.

The STR is the most common bioreactor, due to its simple operation, perfect mixing conditions and easy scalability. For this configuration, agitation speed is a crucial parameter that allows to break the large syngas bubbles leaving the spargers into fine bubbles, then increasing the gas−liquid interfacial area (Ungerman and Heindel, 2007). Considering different gas flowrates and impeller velocities, the reported CO volumetric mass transfer coefficients range between 11 to 155 h^{-1} (Riggs and Heindel, 2006). Despite several studies summarized above having demonstrated efficient performance at the laboratory scale, large-scale implementation for gas fermentation remains problematic. Achieving the highest k_La values requires elevated agitation speeds, which would result in excessive power costs and pose a risk of damaging the microbial culture as a result of shear stress (Bredwell et al., 1999; Bredwell and Worden, 1998). In fact, given its widespread implementation at industrial scale, the effect of mechanical agitation on microorganisms has been studied extensively. For example, Rahimzadeh et al. (2024) evaluated the impact of different radial-flow and axial-flow impellers on mass transfer and shear stress in large-scale systems. The study revealed that the use of axial-flow impellers (e.g. pitched blade impeller, and elephant ear impeller) resulted in a lower shear environment while achieving higher mass transfer coefficients. However, the authors also highlighted that shear stress tends to increase at larger scales, as higher agitation speeds are required to maintain constant mass transfer rates. BCBs and gas-lift reactors represent interesting alternatives, where gas is supplied at the bottom of a cylindrical vessel and turbulence is generated directly by rising bubble flow. In gas-lift reactors, this turbulence is further increased by a concentric tube (Asimakopoulos et al., 2018). Comparative studies indicate that gas-lift reactors outperform BCBs in mass transfer efficiency due to the longer gas residence times supported by the downcomer. For example, higher k_La coefficients were recorded in a gas-lift reactor (130 h^{-1}) in comparison with those of a similar BCB (40 h^{-1}) for CO transfer, at the same gas inflows (Munasinghe and Khanal, 2010, 2014). In addition, both configurations generate lower shear stress compared to STRs. Although bubble-induced friction can also damage cell membranes, its impact is generally less significant and can be mitigated by optimizing the type of aeration used (Moresi, 2025). Therefore, the configuration of the gas sparger plays a critical role, not only in influencing culture growth, but also in determining the mass transfer efficiency. Different types of fine bubbles diffusers have been implemented to minimize bubble size and maximize the gas-liquid interface area (*a*). The impact of bubble size is evidenced in the study of Munasinghe and Khanal (2010), where the difference between 0.5–1.0 mm and 20 μm diffusers was evaluated. Results showed that the highest k_La coefficient achieved for 0.5–1.0 mm diffusers was almost half that of the smaller pore sizes of 20 μm (40 vs. 71 h^{-1}) in a CO-water system. In this context, nanobubble spargers emerge as an innovative approach where bubble sizes are reduced to below 100 nm, thereby maximizing the *a* term. This size reduction also alters the physicochemical properties compared to those of larger dimensions. Ulatowski and Sobieszuk (2020) presented a comprehensive overview of the different methods available for generating fine bubbles, such as ejectors, venturi tubes, and ultrasonication, and discussed their potential applications in environmental fields, particularly wastewater treatment. However, the use of nanobubbles to enhance gas-liquid mass transfer in bioreactors remains largely underexplored. Finally, it is important to highlight that the linear relationship between the k_La coefficient and the gas flow rate in these reactors underscores the need for balancing these two parameters. Excessive gas flow increases aeration costs, making it crucial to optimize gas flow rates to achieve efficient mass transfer without incurring prohibitive operational expenses (Yasin et al., 2015).

As these k_La values are not particularly promising, several studies have focused on exploring alternative bioreactor configurations, with membrane and packed bed reactors showing the best results (Asimakopoulos et al., 2018) (Fig. 3). They differ from the previous configurations in the fact that microorganisms are not suspended in the liquid medium but contained in a biofilm formed on the membrane module or the packing material (Elisiário et al., 2022). In syngas biomethanation, packed bed reactors are predominantly used (see Table 2). Among these, the TBR is the most common configuration, where gas and liquid are distributed co-currently or counter-currently over the packing material that supports the biofilm, with the liquid phase being continuously recirculated to supply nutrients, maintain pH and washout metabolites (Feickert Fenske et al., 2023). In both configurations, the packing media is commonly composed of inorganic materials such as plastic rings, polyurethane foam or activated carbon. However, novel and recycled materials are currently being explored to improve mass transfer performance. For instance, the use of 3D foam-printed packing was evaluated by Flagiello et al. (2022) due to its design versatility and their results revealed superior mass transfer performance compared to conventional commercial packings. Similarly, Sáez-Orviz et al. (2024) addressed the effectiveness of recycled plastic as packing material. These authors also highlighted the potential of 3-D printed extruded recycled plastics for customizable designs. Additionally, clay-based materials have garnered interest due to their low pressure drop and efficiency, as reported by Lebrero et al. (2021). The mass transfer coefficients observed in these packed bed reactors are slightly higher than those reported for suspended biomass bioreactors. For instance, Goonesekera et al. (2024) studied mass transfer in a TBR at different liquid recirculation rates (20 and 280 mL min^{-1}), reporting k_La values ranging from 1.4 to 190 for H_2 and from 2.9 to 124 h^{-1} for CO, when a syngas mixture composed of 65:17:13:5 % v/v H_2:CO:CO_2:N_2 was supplied. On the other hand, hollow-fiber membranes are typically implemented for gas fermentation processes aimed at producing VFAs and alcohols (see Table 3). These systems are characterized by a lower energy consumption and the absence of gas losses due to bubble formation (Shen et al., 2018; Zhang et al., 2013). Membrane module is usually made of hydrophobic material and recent studies have explored novel compounds to enhance their performance. For instance, the use of non-porous polydimethylsiloxane resulted in a higher mass transfer rate compared to other hydrophilic yet porous materials (Orgill et al., 2013). Other non-porous materials and configurations have also been successfully implemented in HFBRs, such as ultra-thin composite hollow fibers and systems in which the bioreactor and the gas diffuser are physically separated (Lee et al., 2012; Munasinghe and Khanal, 2012). The CO mass transfer has been evaluated in HFMBRs, reaching k_La coefficients between 86 and 947 h^{-1} depending on the gas inlet pressure (5–30 psig) and liquid recirculation rate (0.3–1.5 L min^{-1}) (Munasinghe and Khanal, 2012).

In addition to these configurations with improved mass transfer coefficients, novel technologies have also been investigated to address the mass transfer limitations inherent to syngas fed systems. For example, Taylor flow reactors could be an interesting alternative, as this configuration exhibits k_La coefficients up to 500 h^{-1} for O_2 (Kraakman et al., 2023). Another alternative that has been implemented for gas fermentation is based on the bulk-gas-to-atomized-liquid concept (BGAL), where the liquid phase is atomized into small droplets before being dispersed into the gas phase. Sathish et al. (2019) coupled BGAL to a

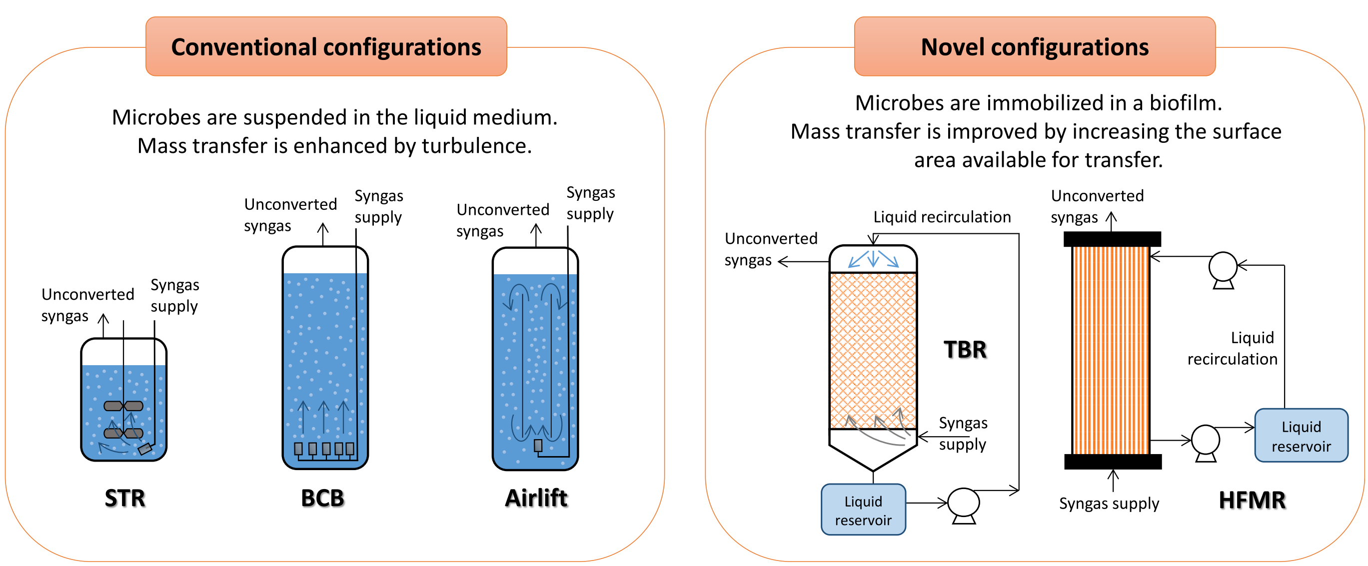

Fig. 3. Main bioreactor configurations used in syngas bioconversion processes. STR: Stirred Tank Reactor, TBR: Trickle Bed Reactor, BCB: Bubble Column Bioreactor, HFRM: Hollow Fiber Membrane Reactor.

packed bed reactor, achieving overall k_La coefficients of around 1620 h^{-1} for O_2. Similarly, external loop gas-lift reactors can support high k_La coefficients for CO (650–750 h^{-1}) (Puiman et al., 2022). Nevertheless, these novel technologies should be tested in real gas fermenters to determine their actual potential, as both mass transfer and the development of the microbial community must be considered.

4.2. Scale-up bottlenecks and process integration

With the aim of coupling biomass gasification to syngas fermentation mediated by mixed cultures, several key factors must be considered to ensure the feasibility of the process scale-up. Focus on the syngas employed, one of the primary challenges is its inherent composition variability, which implies the development of a robust process capable of buffering such fluctuations. As mentioned in Section 3.2, CO and H_2 concentrations have a significant impact on productivity due to potential process inhibition or the promotion of competing metabolic pathways, respectively. However, the use of microbial consortia offers the advantage of easily buffering these fluctuations more effectively than pure cultures. As noted by Parera Olm and Sousa (2022), open mixed cultures exhibit greater resilience to syngas streams with varying compositions and to the inhibitory effects of syngas impurities, whereas pure cultures are generally more sensitive to environmental fluctuations. This enhanced tolerance is mainly attributed to the presence of CO-tolerant microorganisms that reduce dissolved CO levels, creating favourable conditions for CO-sensitive microbes to thrive.

The presence of impurities in syngas must also be considered, as all syngas-converting processes require gas conditioning and cleaning. In fact, gasification and gas cleaning are among the main contributors to the overall cost, accounting for approximately 50–65 % of the total process cost (Martinelli et al., 2020). When syngas is intended for biological conversion, the impact of impurities such as tars, hydrogen sulphide (H_2S), ammonia (NH_3), and nitric oxide (NOx) on the culture broth must be carefully evaluated. For instance, Ahmed et al. (2006) observed that the presence of tars partially inhibited the growth of *C. carboxidivorans*, also affecting products distribution. Rückel et al. (2021) further tested the effects of H_2S, NOx and NH_3 on the same strain. Interestingly, the presence of low concentration of H_2S and NH_3 enhanced culture growth and alcohol production. However, higher concentrations resulted in a prolonged lag phase, although final products concentrations remained similar. In contrast, when *C. carboxidivorans* was exposed to NOx, its growth was negatively affected and an undesired acidic by-products accumulation was observed. Overall, microbial communities can gradually adapt to trace impurities in syngas up to a maximum tolerance value, beyond which complete inhibition of microbial activity can occur. Therefore, pre-acclimation to these compounds may reduce the need for extensive gas conditioning in fermentation processes. Minimizing gas conditioning requirements allows for the use of more cost-effective cleaning techniques compared to those needed for high-purity syngas. For instance, wet scrubbing with water can remove up to 90 % of H_2S and 99 % of NH_3, making more expensive alternatives such as acid scrubbing unnecessary (Rey et al., 2024). Nonetheless, gas cleaning remains a critical step to ensure process viability and long-term stability.

Once the challenges related to syngas variability and impurities have been addressed, the next major barrier is improving process productivity. Acetate and ethanol are known to yield the highest productivities in syngas fermentation. However, the current challenge lies in achieving an efficient and economically viable process for synthetising medium- and long-chain acids or complex alcohols, which hold significantly higher commercial value. For instance, in 2021, the market prices for acetic, butyrate and caproate acid were approximately 500–800, 1600–5000 and 3000–5200 USD per tonne, respectively (Sukphun et al., 2021). The conclusions drawn in Section 3 revealed the potential of mixed cultures for syngas conversion; however, several key factors should be considered. First, it is essential to optimize operating conditions to favour the synthesis of the targeted product, minimizing carbon loss to undesirable by-products and promoting the enrichment of the appropriate microbial populations. For instance, based on the studies reviewed in this article, syngas biomethanation is primarily mediated by hydrogenotrophic methanogens of the genus *Methanothermobacter* under thermophilic conditions (55–60°C). Respect to VFA production, a high abundance of *Acetobacterium* spp. is associated with increased acetate production. To support its growth, neutral pH values in the range of 7–8 are recommended. In contrast, *Clostridium* spp., commonly associated with chain elongation processes for the production of butyrate and caproate, thrives under slightly acidic conditions (pH 6–5). Although solventogenesis mediated by microbial communities is also largely driven by *Clostridium* spp. at pH values close to 4, their productivities remain low and are not yet competitive with those achieved using pure cultures for industrial applications.

Once the desired mixed culture is enriched, then an appropriate bioreactor configuration must be selected. In Sections 3.2 and 3.3 has been highlighted that the use of one single bioreactor is feasible for synthetize several compounds (e. g. acetate or ethanol), but the use of two-stage bioreactors systems could be key to enhance the production of complex compounds. In fact, their low productivity is mainly attributed to their toxicity. The undissociated medium- and long-chain VFAs can easily diffuse across the cell membrane, leading to intracellular acidification and causing detrimental effects on the microbial culture (Liu et al., 2024). In the case of alcohols, their inhibitory effect is related to an increase in membrane fluidity, which disrupts cellular homeostasis (Fernández-Naveira et al., 2016). The inhibitory effects of both VFAs and alcohols also increases with the chain length. For instance, Zhang et al. (2016) evaluated the effect of several compounds on the strain *C. carboxidivorans* P7 and found that caproate was toxic at lower concentrations than acetate or butyrate, and that butanol was more toxic than ethanol. Therefore, two-stage systems offer and advantage by allowing control of product concentration in each bioreactor, thereby improving overall system productivity and mitigating inhibitory effects.

Finally, one of the major challenges associated with syngas fermentation processes mediated by mixed cultures is the purification of the fermentation effluent. Several works highlight the difficulty of separating short-chain acids diluted in water and the high cost of the purification step (40–50 % of the total costs) (Menon and Lyng, 2021). Despite the aforementioned strategies can help to minimize microbial and product diversity in the fermentation broth, the growth of certain species that synthesize undesired by-products is often inevitable. Consequently, the final effluent consists of a complex mixture of compounds, reducing the titer of the target product, which is typically present at low concentrations (Neto et al., 2024). When the fermentation process is aimed at producing VFAs, conventional in-line separation techniques include adsorption, distillation, or liquid-liquid extraction, with the acidic conditions of the effluent facilitating their recovery (Ramos-Suarez et al., 2021). However, these separation techniques are characterised by a high energy demand and capital investment, thereby increasing the overall cost of the process. For example, the downstream cost associated with lactic acid production was evaluated by Phanthumchinda et al. (2018), who reported that traditional processes involving distillation entail significant higher costs (1.4−1.7 USD kg^{-1} lactic acid produced) compared to novel technologies such as membranes systems (approximately 0.8 USD kg^{-1} lactic acid produced). The membrane-based recovery has emerged as an interesting and cost-effective alternative, as it enables not only the selective recovery of VFAs but also the separation and recirculation of biomass back into the bioreactor (Sukphun et al., 2021). The study carried out by Bóna et al. (2020) compared the efficiency of several membrane configurations, highlighting that reverse osmosis resulted in the highest VFA rejection, whereas nanofiltration provided better purification of individual compounds. It is also important to note that feed characteristics, such as pH or acid concentration, must be also considered during process design.

Similar technologies are proposed for alcohols purification, most notably the membrane-based systems utilizing pervaporation. In this technique, vacuum is applied to generate a permeate vapor stream that is further condensed to obtain an enriched alcohol stream (Vane, 2008). Chovau et al. (2011) investigated the efficiency of this technique for ethanol recovery from an effluent containing diverse fermentation products. Authors concluded that ethanol flux and permeate concentration increased in the presence of sugars or salts, while carboxylic acids interact with the membrane, increasing water flux and thereby diluting the ethanol concentration. Nevertheless, this membrane-based technology is still in its early stages of development. Further research is required to optimize materials and solvents to enhance its efficiency, particularly for complex fermentation effluents (Zacharof and Lovitt, 2013).

Overall, this section outlines the main challenges associated to the use of mixed cultures as syngas-converting platforms, from syngas generation to final product purification. Although various strategies have been proposed, their implementation has predominantly been limited to laboratory-scale testing. As mentioned in Section 3.1, several pilot plants for syngas biomethanation have been tested, achieving productivities comparable to those of conventional anaerobic processes. However, there is a notable lack of information on pilot- or larger-scale gas fermentation processes mediated by mixed cultures. To our knowledge, the largest reactor was a 100-L STR employed by Kundiyana et al. (2010), who coupled a biomass gasifier with a syngas fermenter inoculated with *Clostridium* spp. This solventogenic bioreactor operated in batch mode and primarily produced ethanol (25.3 g L^{-1}) and 2-propanol (9.3 g L^{-1}), with lower concentrations of acetate (4.8 g L^{-1}) and trace amounts of 1-butanol (0.5 g L^{-1}) also detected. The authors identified mass transfer limitation and low cell density (1.1 g L^{-1}) as major process bottlenecks. An extended lag phase, attributed to the presence of syngas impurities, was also observed; however, no in-depth evaluation of the specific impact of these impurities was conducted. Acetate production by *M. thermoacetica* through gas fermentation was also tested at pilot scale (24 L), reaching a maximum acetate concentration of 25.2 g L^{-1} (Acuña López et al., 2024). Therefore, future studies should prioritize evaluating syngas fermentation at larger scales to validate the promising results obtained at laboratory scale, and to gain a more comprehensive understanding of both syngas preconditioning requirements and final product purification strategies.

5. Conclusions

The works compiled in this review showed the potential of open mixed cultures as biocatalyst in gas fermentation processes coupled to biomass gasification. The conversion of syngas to CH_4 through microbial communities has been widely studied and the productivities reported are similar to those obtained for conventional anaerobic digestion. Promising results have also been obtained in the synthesis of VFAs, with acetate typically being the predominant carboxylic acid. Its elongation to butyrate and caproate via two-stage systems has been demonstrated as a feasible approach. In contrast, the synthesis of alcohols and high-value compounds still needs further investigation. To ensure process scalability, several key aspects must be addressed, including syngas conditioning, bioreactor configuration and effluent purification. The tolerance of microbial consortia to various impurities can significantly reduce the need for extensive syngas purification, while their robustness helps mitigate fluctuations in syngas composition. Mass transfer remains a critical factor in gas fermentation processes, highlighting the need to explore novel reactor configurations. Finally, the downstream stage must enable an efficient and economically viable product separation, with membrane-based systems emerging as a promising solution.

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.

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Data availability

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

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