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ORIGINAL PAPER

Biological Methanation of H_2 and CO_2 with Mixed Cultures: Current Advances, Hurdles and Challenges

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Abstract

In order to take action against global warming and ensure a greater energy independence, countries around the world are expected to drastically increase the proportion of renewable energy in their energy mix. However, the intermittent production of energy explains why energy supply and demand do not match. In this context, biomethanation, coupled with anaerobic digestion, could be an interesting approach to transform the extra amount of produced electricity by converting hydrogen (produced by electrolysis) and carbon dioxide (present in biogas) into methane. In this review, we have summarized several recently published results which involve biological methanation processes performed by mixed cultures, with an emphasis on microbiological as well as process aspects. In particular, the different microorganisms involved in the process, as well as the used metabolic pathways, along with their kinetic and thermodynamic specificities, are described. Furthermore, the influence of process parameters such as the type of reactor, the type of diffuser and the choice of H_2 injection (in situ or ex situ) or the different operating conditions are presented. Explanations of the different performances observed in literature are assumed, technical bottlenecks are listed, and possible solutions to overcome these issues are presented. Finally, the current commercial deployment of this technology is discussed through the example of three companies offering different biomethanation solutions.

Yan Rafrafi and Léa Laguillaumie have Shared co-first authorship. The two authors have worked together on this publication and contributed equally.

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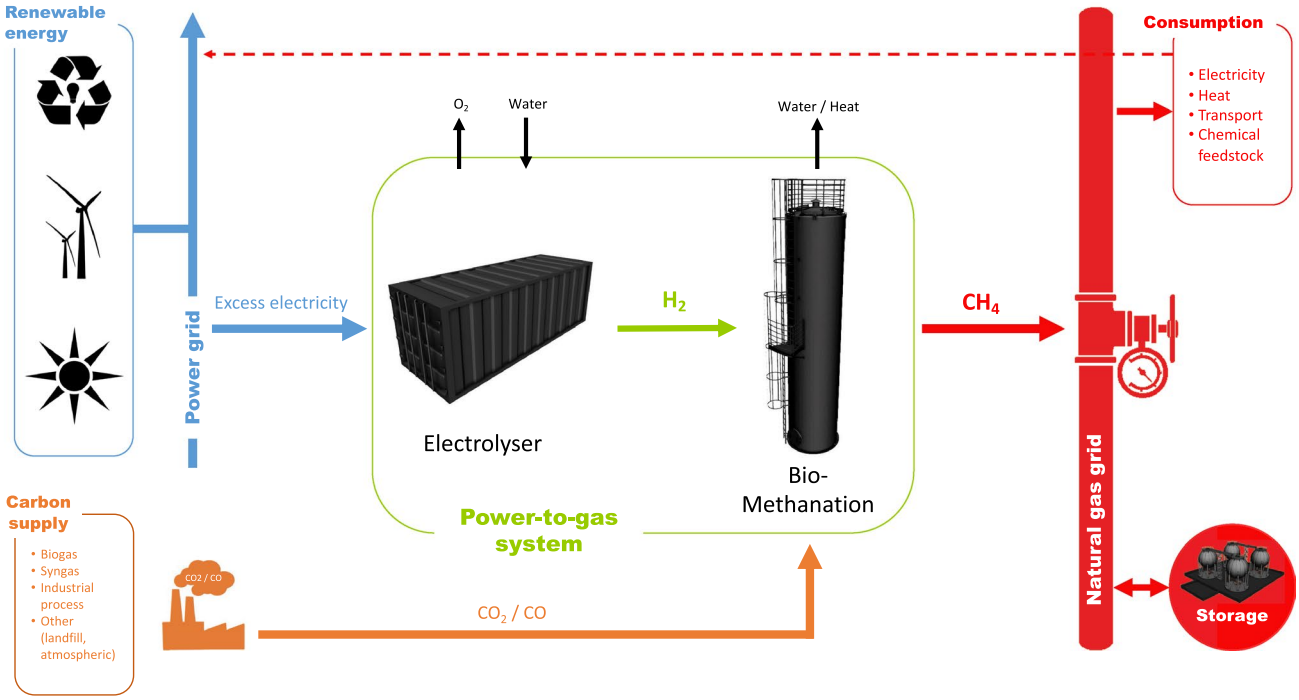
Graphic Abstract



Keywords Biomethanation · Biogas upgrading · Mixed cultures · Hydrogen · Carbon dioxide reduction

Statement of Novelty

This review article highlights the current great interest for biological methanation. We have considered the numerous results from recent literature on biological methanation processes performed by mixed cultures, with an emphasis on microbiological as well as processes aspects. In particular, the different microorganisms involved in the process as well as the metabolic pathways used with their kinetic and thermodynamics specificities are detailed. Then, the influence of process parameters or the different operating conditions are presented. Finally, the actual commercial deployment of this technology is discussed through the example of three companies offering different biomethanation solutions.

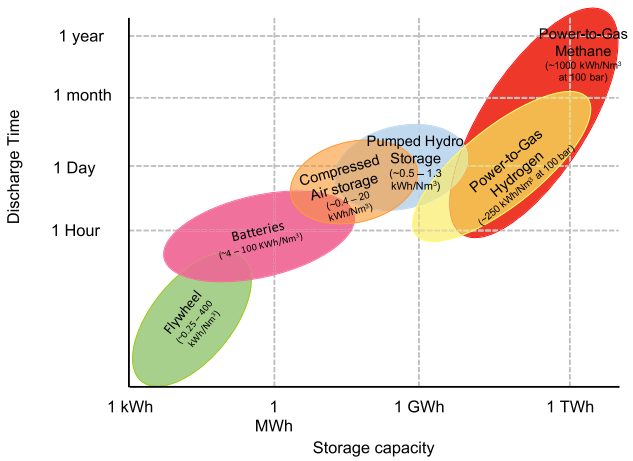


Fig. 1 Comparison of the discharge time, storage capacity and energy density (kWh/m^3) of different energy storage technologies. Increasing discharge time and storage capacities are observed for respectively, flywheel, batteries, compressed air storage, pumped hydro-storage and power to gas methane are plotted Adapted from Schaaf et al. [108]

Introduction

The European commission has set the target for reaching 27% of renewable energy in the energy mix by 2030. In this context, the development of wind and solar energy is increasing [1]. The production of renewable energies fluctuates on a daily and seasonal basis, which increases the gap between electricity supply and demand. It is important to estimate this gap when implementing emerging renewable energies, since the electricity grid cannot always integrate such discrepancies. In the case of over-production, converting extra electricity into heat is not likely since this electricity often occurs during warm seasons, i.e. when heat is not

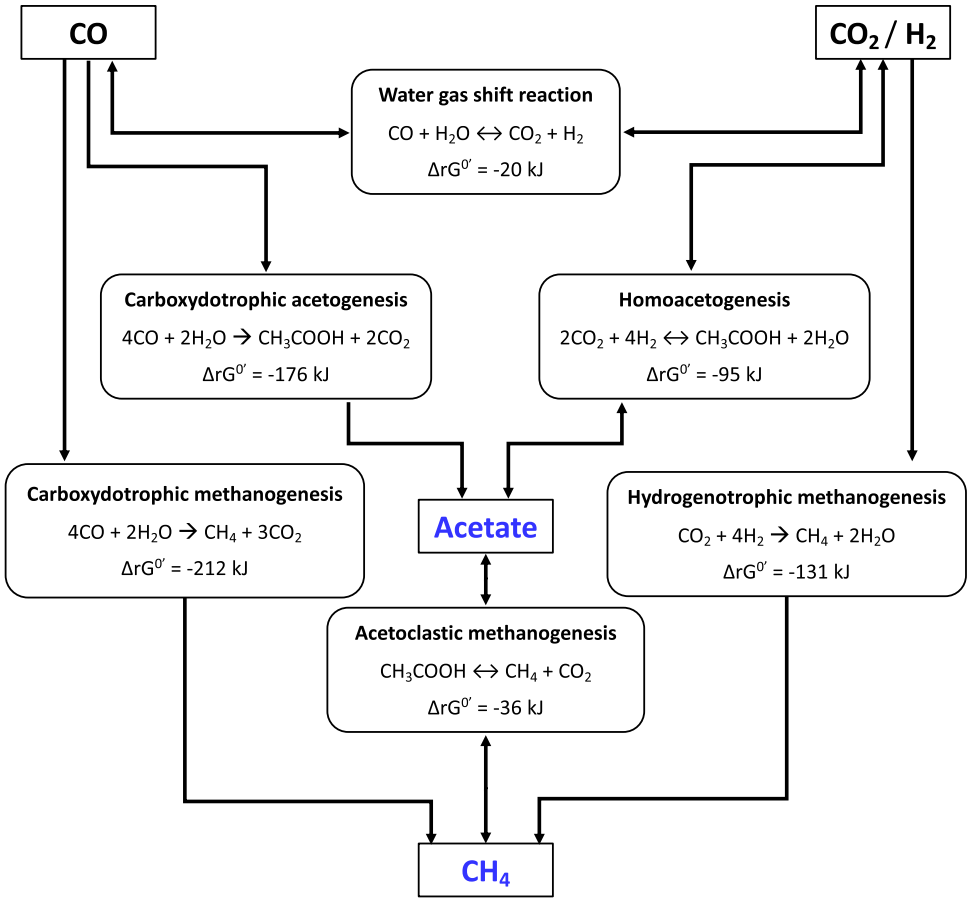


Fig. 2 Different routes and their Gibbs free energy of reactions for biological methane production according to gas substrates availability. The CO can be transformed through water gas shift, carboxydotrophic acetogenesis or carboxydotrophic methanogenesis to respectively, hydrogen and CO_2; acetate, and methane. The CO_2 and H_2 can be transformed through homoacetogenesis, hydrogenotrophic methanogenesis to respectively acetate and methane. Acetate can be assimilated to methane via acetoclastic methanogenesis. The $\Delta rG^{0\prime}$ are mentioned for the reaction from left to right

needed. Several other solutions exist for storing the energy such as flywheels, batteries, compressed air energy storage and pumped hydro energy storage (Fig. 1). However, the conversion of power into hydrogen and/or into methane is the only solution capable of providing sufficient storage capacity and long enough discharge times to be in line with future energy demands at the global energy network scale. Therefore, transforming over-produced electricity into hydrogen or methane seems to be a good option. The possibility of injecting gas into the gas network gives direct access to its very large transport and storage capacities: for instance, in France, natural gas storage capacities are 300 times larger than those of the electricity network (137 TWh versus 0.4) [2]. Not only does gas present an advantageous flexibility in terms of storage capacities, but also the possibility of being used in vehicles. The conversion of electricity into methane could be performed through two successive stages, firstly from electricity to hydrogen and then hydrogen to methane, *i.e.* the methanation reaction (Eq. 1). The process also exists for syngas valorisation which uses carbon monoxide (CO). Figure 2 presents the different possible reactions which lead to the final production of methane, depending on the considered gas substrates.

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad (1)$$

At the European level, Denmark, Germany and Italy, have supported biogas technologies and particularly their biomethane potential. In 2009, biomethane production boomed thanks to incentive bonuses for upgrading biogas. Although a downward trend is now observed due to the withdrawal in 2014 of substrate bonuses dedicated to energy crops and to biogas upgrading, the development of biogas upgrading technologies remains interesting for future perspectives since economies of scale can be done by connecting several biogas plants to a joint biogas upgrading facility [3]. Different scenarios for the power to gas development capacity were studied in numerous countries. For example in France, the residual surpluses of electricity should account for 13 TWh in 2030 and between 34 and 67 TWh in 2050 [2]. Considering technical and economic constraints, a realistic development scenario by 2030 can range between 1200 and 1400 MWe of installed power to gas capacity, able to convert between 2.5 and 3 TWh of electricity, and produce between 1.8 and 2 TWh of synthetic gas. In 2050, the installed capacity could reach between 7700 and 24,000 MWe, converting between 21 and 72 TWh of electricity, i.e. between 6 and 24% of the

national power consumption, and producing between 14 and 46 TWh HHV[1] of synthetic gas. Whichever the presented scenario, the study shows that the carbon (CO_2, CO) needed for methane production could be entirely covered by renewable sources through anaerobic digestion and gasification of biomass. Finally, these power to gas facilities would also be able to produce altogether between 5 and 18 TWh of valuable heat, and between 3400 and 11,700 ktons of oxygen which can be used by the industrial or health sectors.

When considering the power to methane concept, two main types of methanation technologies can be developed: thermochemical catalytic methanation or biological methanation. The thermochemical catalytic methanation was discovered during the 19th century, during Paul Sabatier's studies on catalysis. This technique is now widespread in different chemical industries (ammonia synthesis, purification of hydrogen, gasification). Production units are large scale and operate continuously at high temperatures (between 300 and 600 °C) and pressures (around 100 bar). Catalysts, often based on rare metals (Ni, Pt), are generally sensitive to impurities which may be present in certain input gases (for example biogas) and must be often regenerated. Due to high pressure and temperature conditions, this process is rather developed at large scale. Therefore, such installations should be located close to massive electricity production sites (wind turbine and photovoltaic parks, power plants) where the produced methane can be directly injected in gas grids or stored to be used for vehicle transportation. New catalysts and reactors need to be developed in order to enable the development of the technology at intermediate scales. A new process has recently been discovered, which consists in exciting the catalyst by magnetic induction and not thermochemically. This process presents the advantage to operate at low temperature and at ambient pressure. This technology can operate intermittently, can be located in decentralised sites and is well adapted to small installations [4]. Yet its effectiveness in real conditions still needs to be proven.

Another process currently under progress, and which is very promising, is progressively gaining interest within the research field. This new process is biological methanation or biomethanation. Biomethanation process can either use a single microbial species (pure culture) or a microbial consortium (mixed culture). Even if pure cultures can be very effective in this process [5], they are sensitive to contamination and are therefore difficult to be implemented. On the other hand, under optimal operating conditions, mixed culture processes can also be efficient and above all be very robust [6].

In this review, the focus will be on the biological methanation of H_2 and CO_2 gas mixtures where catalysts are microbial consortia (i.e. mixed cultures). Indeed, despite the lower reaction rate, due to a lower volumetric mass transfer coefficient, biomethanation has several advantages over catalytic methanation: (i) It is easier to operate with milder operating conditions (temperatures between 35 and 70 °C and pressures between 1 and 10 bars); (ii) It is less sensitive to impurities since compounds such as H_2S and NH_3 can serve as nutrients for microorganisms; (iii) Catalyst renewal is carried out continuously thanks to microbial growth; (iv) The operating costs are lower [2]. In addition to these benefits, and mainly because of similar reactor conditions and operation modes, biological methanation is usually complementary to anaerobic digestion technologies for biogas upgrading.

Biological methanation occurs naturally during the last step of anaerobic digestion, by converting hydrogen and carbon dioxide into methane (Eq. 1). This step can be intensified in order to produce more CH_4 by injecting H_2 and/or CO_2 in an anaerobic bioreactor. In this case, the microbial community is rapidly dominated by a hydrogenotrophic archaea population. Biomethanation enables biogas upgrade, and can hence stand for a CO_2 removal system. As presented in this review article, methanation is able to reach a proportion of methane in the biogas between 90 and 98%, i.e. levels which can reduce the use of post-processing purification technologies before injection in the gas grid.

Two different types of biomethanation strategies are observed: either the methanation reaction is performed directly in the anaerobic digester named in situ biomethanation process, or performed in a separate unit named ex situ biomethanation process. In situ methanation offers the possibility to reduce the investment costs since only one reactor is necessary. Nevertheless, a dedicated separate biomethanation reactor offers the possibility to better control and command the digester unit and to maintain higher H_2 loads enabling higher methane productivities. Whatever the system (ex- or in situ), microorganisms and metabolic pathways, involved in either process, are quite similar.

Besides reaching higher proportions of methane in the biogas, biomethanation can also increase the productivity of methane. Indeed, as mentioned previously, combining both anaerobic digestion and methanation represents a good option for upgrading the biogas and this value chain will be the focus of the present review paper. The syngas methanation will be mentioned but not detailed. Biological conversion of CO involves specific metabolic pathways (Fig. 2) which, to our point of view, need to be specifically discussed in an independent study, as already detailed by Grimalt-Alemany et al. [7].

The remainder of this review will first address the microbial feature before focusing more specifically on the process and performances of in situ and ex situ methanation. Finally, particular attention will be given to overall bottlenecks of biomethanation before focusing on a few companies offering large-scale solutions.

[1] Higher heating value: HHV.

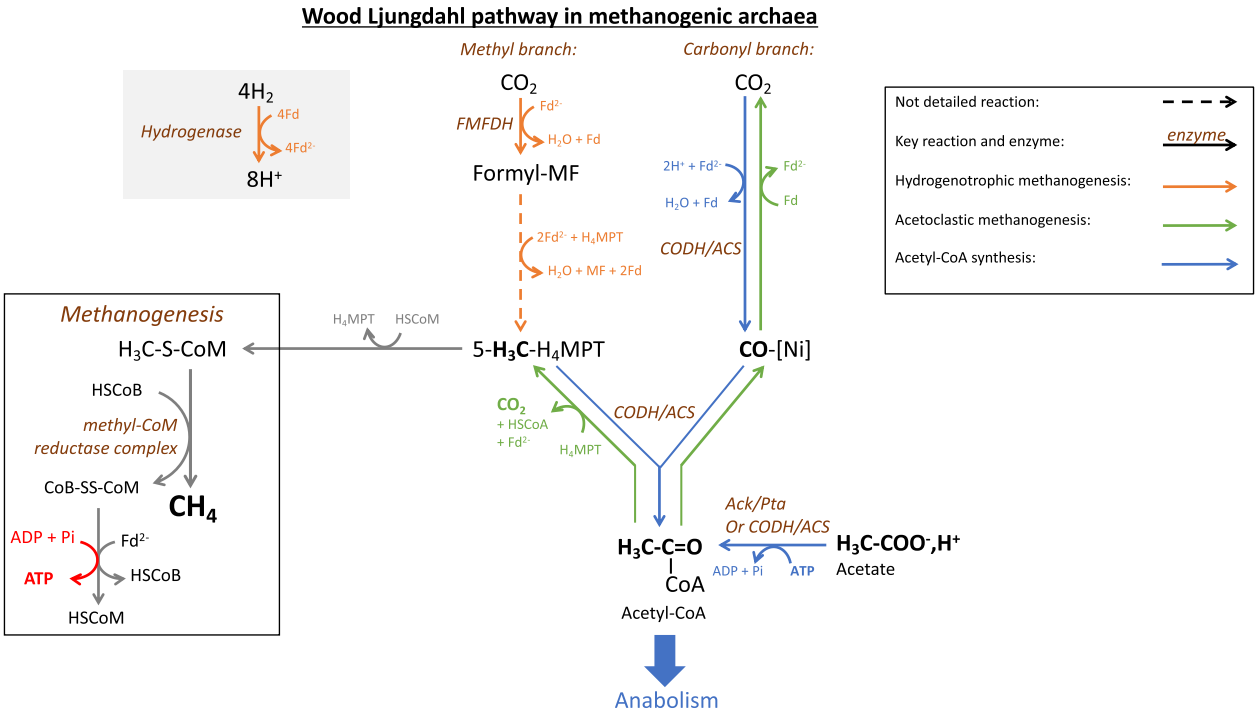


Fig. 3 Wood-Ljungdahl pathway in methanogenic archaea, hydrogenotrophic and acetoclastic methanogenesis, and CO_2 fixation for biomass synthesis. *MF* methanofuran, *H_4MPT* tetrahydromethanopterin, *FMFDH* formylmethanofuran dehydrogenase, *CODH/ACS* carbon monoxide dehydrogenase-acetyl-CoA-synthase complex, *Pta* phosphotransacetylase, *Ack* acetate kinase

The Methanogenesis: Microbial Aspects

Methanogenesis Metabolic Routes

Microorganisms involved in methanogenesis reactions are methanogenic archaea. Considering biomethanation systems, two types of methanogenic communities are prevalent: hydrogenotrophic and acetoclastic. Hydrogenotrophic methanogens use H_2 and CO_2 as sole energy and carbon sources, which reverts to a chemolithoautotrophic metabolism. On the other hand, acetoclastic methanogens use acetate as a source of energy and carbon, and this metabolism is defined as chemoorganoheterotrophic. The genera able to use acetate for a methanogenic metabolism are identified as *Methanosarcina* and *Methanothrix*. However, acetoclastic methanogens can be flexible, as some species such as *Methanosarcina barkeri* have been found to use either H_2 or acetate according to their respective availability [8]. Other methanogenic archaea use C1 compounds (carboxydotrophs) and methyl compounds (methylotrophs) such as CO, methanol, methanthiol and methylamine. These substrates are specific, so these microorganisms are rarely found in H_2/CO_2 adapted consortia. Despite this range of substrates, methanogens use a common metabolic pathway for carbon fixation and energy production as shown in Fig. 3. The acetyl-CoA reducing pathway, also known as the Wood-Ljungdahl pathway (WLP), is used by methanogens, but also homoacetogenic bacteria. This pathway allows autotrophic microorganisms to fix carbon from CO_2 and/or CO and also provides the energy needed for cell growth. The WLP is a linear path composed of two branches. The methyl branch, synthesises a methyl group (CH_3). This latter compound is used either for the generation of methane and ATP through hydrogenotrophic archaea, or for acetyl-CoA synthesis and anabolism. The carbonyl branch, provides the carbonyl group (C=O) of acetyl-CoA. Acetoclastic methanogenesis consists in firstly producing acetyl-CoA from acetate on the expense of ATP (activation), and secondly hydrolysing the activated acetate (acetyl-CoA) to produce CO_2 and a methyl group for methane formation (Fig. 3). In *Methanosarcina,* acetate activation is undertaken with acetate kinase and phosphotransacetylase, whilst in *Methanothrix,* activation is performed by CODH/ACS (carbon monoxide dehydrogenase-acetyl-CoA-synthase complex) [9]. CODH/ACS complex also enables the use of CO by carboxydotrophic methanogens. Moreover, this enzyme is highly oxygen-sensitive due to the presence of a metallic core composed of nickel, and Fe-S clusters (ferredoxins) [10, 11]. This enables to carry out low energy electron exchanges, which was impossible with the conventional NAD(P)H/NAD(P)$^+$ couple ($E_0' = -320$ mV). Indeed, the redox potentials of the $CO_2/HCOO^-$ and H_2/H^+ couples are −432 and −414 mV respectively [12]. For these reasons,

Table 1 kinetic growth parameters of main competing populations in methanation processes with microbial consortia

Population	μmax (day^{-1})	Ks (mgCOD/L)	ATP yield (ATP/reaction)	$\Delta rG^{0\prime}$ (kJ)	Stoichiometric reaction	References
Hydrogenotrophic methanogens	0.02–2.6 (33-35 °C) 8–12 (55–60 °C)	H_2 4.8×10^{-5}–0.6	0.5	− 131	$4H_2+CO_2\rightarrow CH_4+2H_2O$	[103–107]
Acetotrophic methanogens (mixed culture on acetate at 35 °C)	0.08–0.36	Acetate 165–185	0.5	− 36	$Acetate^-+H^+\rightarrow CO_2+CH_4$	
Homoacetogens (*C. ljungdahlii* on $CO/CO_2/H_2$ at 37 °C)	1.20–4.68	H_2 1.9–2.5	0.33	− 95	$4H_2+2CO_2\rightarrow Acetate^-+H^++2H_2O$	
Syntrophic acetate oxidizing bacteria (*Thermacetogenium phaeum* 58 °C co-cultured with *Methanothermobacter thermoautotrophicus*)	0.73	NA	0.33	+ 95	$Acetate^-+H^++2H_2O\rightarrow 4H_2+2CO_2$	

NA not available

dissolved oxygen levels and redox potential of the medium are hence important process parameters to be controlled. This explains why culture media often contain reductive agents such as Na_2S, which also serves as a sulphur source for methanogens [13].

The Energetic and Kinetic Aspects of Microbial Biomethanation

Mixed cultures are characterized by the ability to perform a large range of biochemical reactions, and inter-species interactions. With H_2 and CO_2 as sole substrates, different metabolic groups can coexist in the culture media. Homoacetogenic bacteria and hydrogenotrophic methanogens convert directly H_2/CO_2 into acetate or methane respectively. Acetoclastic methanogens and syntrophic acetate oxidizing bacteria (SAOB) consume acetate for the production of methane or H_2/CO_2 respectively. Since homoacetogenic and methanogenic microorganisms are both able to reduce CO_2 by using H_2 through the WLP (see "Methanogenesis Metabolic Routes" section), these two microbial groups, grown under mixed culture conditions, are hence in competition for the same substrates: H_2 and CO_2. In most cases, the limiting substrate is H_2 because of its low solubility in water compared to CO_2.

In anaerobic systems, terminal electron acceptors have weak energy potentials. This means that in such systems, there is a low energy harvesting mechanism deployed by the cells, and thus low growth rates compared to aerobic metabolisms. Moreover, anaerobic microorganisms have efficient metabolic routes for energy harvesting from a large range of substrates. Acetogenic bacteria for example are highly flexible microorganisms. The latter grow on different organic substrates such as sugars and alcohols, but also in autotrophy by using CO_2 or CO, if no organic substrate is available [14, 15]. Lee et Zinder reported the ability of an acetogen, *Thermacetogenium phaeum*, to change the direction of the WLP catabolic route according to substrate availability, i.e. (i) if H_2 concentration is significant then the bacteria reduce the CO_2 to acetate through reductive WLP; (ii) however, if H_2 is limiting, for example due to its syntrophic consumption by methanogens, the bacteria will oxidize acetate [16]. This dynamic behaviour has been observed in in situ biomethanation reactors undergoing variations of the H_2 partial pressure [17]. It is now well established that the synthesis or the oxidation of acetate is governed by H_2 concentration, due to thermodynamic constraints [18–20]. Anaerobic systems are energy limited because reactions undergo close to thermodynamic equilibrium, meaning that changes in substrate and product concentrations can reverse the direction of reactions (ΔrG) to suppress [19, 21]. Anaerobic systems are rather driven by thermodynamic constraints than by kinetic parameters, and growth rates are more relevant when defined as a function of the free energy change of catabolic routes [20–22]. Substrate threshold concentrations exist for each bioreaction, and depends on the terminal electron acceptor used. Indeed, there is a substrate concentration below which the Gibbs free energy turns positive, thereby preventing the reaction [23]. There is also a concentration below which the released energy is not sufficient for synthesising ATP, which is called the energy threshold. In this case, cells turn from growth state to survival state, as described by Karadagli et Rittmann in their mathematical model of methanogenic growth based on H_2 concentration thresholds [18].

Table 1 presents kinetic, bioenergetic and thermodynamic parameters of different metabolic groups of interest. These kinetic values diverge in literature, with differences which

sometimes reach one order of magnitude. However, it is possible to provide some differentiating trends between species. The maximum specific growth rate refers to the biomass production rate during exponential growth phase of a microorganism. It is specific to the physico-chemical parameters of the culture and to the used substrates, as well as depends on the strain. These specificities can explain the different kinetics observed within the same population. This analysis of parameters describing population dynamic can help understanding some behaviours in biological reactors. Considering this, it should be possible to predict competition resulting in specific experimental conditions according to thermodynamics and kinetics.

Hydrogentotrophic methanogens growth rates increase with temperature, from 33–35 to 55–60 °C. This explains that there is more competition between homoacetogens and methanogens under mesophilic conditions, whilst methanogens often outcompete under thermophilic conditions [24]. Acetoclastic methanogens have lower growth rates when grown at 35 °C compared to other communities. However in anaerobic digestion systems, and thus in in situ methanation processes, acetoclastic methanogens are highly represented in the consortia compared to ex situ processes, due to acidogenesis and acetogenesis steps which provide acetate [25]. The growth of hydrogenotrophic methanogens is limited by H_2 provided by hydrolysis and acidogenesis steps. Indeed, in the case of in situ methanation, the addition of H_2 is liable to unbalance this equilibrium by promoting the growth of hydrogenotrophic methanogens which consume the CO_2 produced during previous steps, and hence enrich the output gas in methane which can reach 80–90% depending on experimental conditions (Table 2).

For microorganisms, the half-saturation coefficient for a limiting substrate (Ks) represents the concentration of this substrate when the growth rate (μ) reaches half of μmax. The lower the Ks value, the more the microorganism is able to grow on the substrate even at low concentrations. This parameter also describes the affinity of a microorganism for the substrate. In a biomethanation system, two main competitions for substrate are identified (i) between hydrogenotrophic methanogens and homoacetogens for H_2 substrate (ii) between acetoclastic methanogens and syntrophic acetate oxidizing bacteria for acetate substrate. According to Table 1, hydrogenotrophic methanogenic archaea have lower Ks compared to homoacetogens. This difference of affinity explains why such archaea are dominant under low P_{H2} and thermophilic conditions, which is the case in ex situ methanation processes. Indeed, these archaea will consume all the available substrate, thereby leaving none for the growth of other communities such as homoacetogenic bacteria. This is due to the fact that the substrate concentration threshold is higher, in other words a lower affinity for the substrate. Another competition for the substrate occurs in in situ methanation processes between acetoclastic methanogenesis and syntrophic acetate oxidizing bacteria (SAOB). According to Table 1, both involved communities have the same order of magnitude for Ks and μmax, with a slightly higher growth rate for heterotrophic bacteria such as SAOB. This means that the competition is probably strong in in situ processes. Whether acetoclastic methanogens or heterotrophic bacteria consume the acetate, this substrate will nevertheless finally converge to methane, either directly from acetate to methane, or through intermediates such as H_2 and CO_2 which are further transformed into methane by hydrogenotrophic methanogens.

However, under mesophilic conditions, not only should Ks be considered, but also μmax. Indeed, under low temperatures, the μmax of homoacetogens can be higher compared to archaea. Therefore, the divergence of μmax can compensate the difference observed for Ks and finally lead to a high apparent growth rate of homoacetogens competing with archaea for the same substrate in certain conditions. Some process parameters such as hydraulic retention time (HRT) can be determinant in microbial selection. Slow growing microorganisms are most of the time Ks strategists: they will have higher affinity for the substrate and thus outcompete fast growing microorganisms at high HRT. Fast growing microorgansisms have higher growth rate and will survive to short HRT whereas low growing microorganisms will be washed out.

Impacts of Process Parameters on Methanogenesis

Microbial consortia engineering [26] is a way to enable the selection of specific microbial functions with the modification of process parameters such as HRT, gaz retention time (GRT), temperature and pH. In such consortia, competition and synthrophy between microorganisms are observed (see "The Energetic and Kinetic Aspects of Microbial Biomethanation" section). Synthrophy is defined as metabolic interactions between different microorganisms e.g. heterotrophic acetogenic bacteria and methanogenic archaea in anaerobic digestion which are mutually dependent [21, 27].

In mixed cultures, the competition and synthrophy between microorganisms will define the predominance of one species compared to another (competition) and the presence of several species, taking advantage of one from another (synthrophy).

Therefore, by selecting specific process conditions, inherent population diversity and consortia functions are likely to shift. The influence of such parameters on methanogen growth and performances are discussed in the following paragraphs.

Considering the three populations: homoacetogens, acetoclastic and hydrogenotrophic methanogens, temperature might be the most important parameter since hydrogenotrophic methanogens seem to be the only population consuming H_2/CO_2 at 55–60 °C. Indeed, under thermophilic conditions only few homoacetogens such as *M.*

Table 2 Operational conditions and performances of in situ biomethanation reactors with mixed cultures

Refs.	Inoculum	Substrate	Injected gas/injection mode	Reactor configuration	Volume (L)	HRT Substrate (days)	HRT gas (days)	recirculation	pH	T(°C)	Loading rate H_2 (m^3/m^3/d)	Hydrogen consumption rate (m^3/m^3/day)	Methane productivity (m^3/m^3/day)	%CH4	agitation (rpm)
[44]	Cattle manure	Manure	H_2 through 2 ceramic gas diffusers	CSTR	3.5	14	NA	NA	8.3	55	0.68	0.228	0.4536	65	65 100 300
[75]	Digested manure	Manure & whey	H_2 through column diffuser	CSTR column	0.6	15	0.39–0.66	No	7.74	55	1.5–1.7	1.214	0.757	53	150
								NA	7.84			1.413	0.839	68	300
			H_2 through ceramic diffuser					NA	7.89			1.482	0.885	75	150
[49]	Digested Sewage sludge obtained from a mesophilic anaerobic reactor	Sewage sludge	Coke gas (CO. H_2,CO = 92/8) through hollow fibre (bubbleless) polyurethane	Hollow membrane fibres reactor	2	10	NA	No	7	37	0	NA	0.277	64.4	200
							1.54		7.5		1.3	NA	0.4365	89.9	
							0.69		9		2.88	NA	0.6525	98.8	
[59]	Mesophilic industrial biogas treating plant maize sweet sorghum silage pig manure	Cellulose	H_2: headspace	Glass serum bottle	0.16	unlimited (batch)	NA	NA	9.38	37	0.54	0.022	0.163	79.8	NA
									7.89		0.50	0.021	0.14,904	95.5	0 to 250
[52]	Wastewater treatment plant granules	potato starch wastewater	H_2: bottom of reactor Packing = rashig rings, inert alumina ceramic sponge Injection = bottom	UASB reactor + separate injection chamber	1.4 + 0.2	5	NA	NA	7.92	55	3.5	1.769	1.52	40.4	NA
									7.9		2.6	1.412	1.49	44.9	
									7.93		2.6	1.756	1.47	52	
[17]	Mesophilic manure based digested sludge	Straw	$H_2 + CO_2$: CO_2 in the headspace and H_2 pulse injection every 2–3 days Injection = bottom	CSTR	0.3	20	2.86	No	7.92	38	1.3	NA	0.46	94	**450**
									8.33			NA	0.22	71	
									8.34			NA	0.2905	83	
[43]	Grass sillage digested inoculum	Grass sillage	H_2 through fish stone diffuser (low G/L transfer)	CSTR	9.5	46	1	Yes	7.97 (7.81 in control)	55	5.05	NA	1.82 (1.5 in control)	32.1	NA
			H_2 through ceramic diffuser (High G/L transfer)						8.37 (7.89 in control)		5.29	NA	2.52	60.3	
			H_2 through ceramic diffuser (High G/L transfer)						7.93 (7.89 in control)		5.29	NA	0.33	6.1	
[57]	Anaerobic granular sludge	Synthetic wastewater	$H_2/CO_2/CO/CH_4$: P1–P5 (100/0/0/0/0); P6 = 85.8/1.3/5.1/7.8; P7:84.9/1.3/10.0/3.8; Injection = `bottom	2 successives UASB	0.7 (×2)	1	NA	NA	1st UASB = 7.5; 2nd UASB = 8	35	P1 = 1.456 P2 = 2.576 P3 = 3.92 P4 = 5.15 P5:6.496 P6 = 7.056 P7:7.056	NA	0.331-1.272	P1 = 94.9 P2 = 93 P3 = 92.9 P4 = 92.7 P5 = 92.9 P6 = 92.8 P7 = 92.2	NA

NA not available, *P1—P7* period 1 to period 7

thermoacetica with a μmax (60 °C) = 0.7 day^{-1} are capable of achieving optimal growth [28]. However, this temperature remains below the optimum temperature for the growth of thermophilic hydrogenotrophic methanogens (Table 1).

Therefore, methanogens have a higher growth rate under thermophilic conditions, compared to homoacetogens, which provides them with an advantage for growth and enables them to dominate the mixed cultures in these conditions. Under lower temperatures, which are typical for in situ methanation processes, competition with homoacetogens for example can arise. This is probably the reason why ex situ methanation is able to provide a higher proportion of methane in the output gas, up to 98%, and this by increasing the temperature of operation compared to in situ methanation systems [29–31].

The main temperatures found in literature were 35 °C and 55 °C. Kotsyurbenko et al. [32] showed that the temperature-dependence of μmax was lower for homoacetogen than for methanogens. Enrichment studies confirmed that the selection of populations was different between mesophilic (37 °C) and thermophilic (55 °C) conditions. First, mesophilic enrichments led to a higher α-diversity compared to thermophilic enrichments [24, 30]. In addition, mesophilic assays (25–35 °C) enriched the microbial consortium with *Clostridium* spp., *Anaerobacterium* spp. and *Methanospirillum* spp. whereas thermophilic (55 °C) assays enriched the consortium with *Thermoanaerobacterium spp*. and *Methanothermobacter* spp. [24, 33–35]. In other studies, the archaeal selection remained resilient in selecting *Methanothermobacter* and *Methanobacterium* under thermophilic conditions, and only *Methanobacterium* under mesophilic conditions [36, 37].

Moreover, temperature has not only an influence on microbial kinetic parameters, but also on thermodynamic equilibriums and gas transfer. For instance, the solubility of H_2 and CO_2 will decrease with increasing temperatures.

The study of transfer rates for different gases showed that temperature had variable effects on the maximum gas to liquid (G/L) mass transfer. In biological methanation systems, as considered here, gases of interest are H_2 and CO_2, while the k_La is often determined with O_2 and in water. The maximum transfer rates of these three gases in a perfectly mixed and aerated reactor, which k_La (O_2) is 51 h^{-1}, were compared (Eq. 2). As expected, the increase of temperature had a positive effect on k_La [38] (decrease in viscosity, increase in diffusion), with values reaching 59 and 64 h^{-1} at 45 °C and 55 °C respectively. On the other hand, the gas solubility in water decreased with increasing temperatures [39], and this by facilitating the diffusion between phases. The global mass transfer rate depends on both k_La and gas solubility (Eq. 2). Calculations show that an increase of temperature, from 35 to 55 °C, leads to a significant decrease of O_2 and CO_2 maximum mass transfer rate by 6.8% and 20.4% respectively. However, when it comes to H_2, a decrease of only 0.2% was observed. Therefore, in this case, the increase of temperature had a 100 times stronger effect on CO_2 mass transfer (and 200 times for O_2) compared to H_2. This could be of great importance in biological methanation processes, because a lower CO_2 transfer triggered by an increase in temperature could induce a limitation in the system. As H_2 solubility is always very low, temperature variation has only a very small impact (1.93 mg/L at 0 °C and 0.76 mg/L at 80 °C) [40]. H_2 transfer rate thereby remains constant, and could even increase with temperature in specific systems exposing a high k_La sensitivity towards temperature. Regarding the kinetics of hydrogenotrophic methanogens at high temperature, as previously described, such effects coincide with additional beneficial effects of increasing temperature for biomethanation.

The liquid Hydraulic Retention Time (HRT) is an important parameter for intensifying the biological processes. However, when producing methane with a gaseous substrate, HRT mostly impacts the concentration of biomass which is the catalyst of the reaction, and of nutrients. By referring to the growth rate (Table 1) measured in continuously stirred tank reactor (CSTR) systems, a decrease of HRT is liable to wash out slow growing methanogenic archaea and hence favour homoacetogens, and this particularly under mesophilic conditions as discussed previously. Since better performances are reported in biofilm reactors, such as hollow fibre membrane biofilm reactors (HfMBR), biofilm plug-flow reactors and trickle-bed reactors (see "The Biomethanation Types of Reactors and Performances" section), several studies compared the microbial composition between these biofilms and suspended biomass. Using biofilm reactors will have an important influence on selecting the HRT, but also the SRT (solid residence time) since both parameters can be uncoupled. Asimakopoulos et al. showed that biofilms contained a higher proportion of methanogenic archaea in a trickle-bed reactor compared to suspended free cells, and this whichever the temperature (six fold more at 37 °C and two-fold at 60 °C) [41]. It is assumed that the majority of methanogenic activity is undertaken within the biofilm attached to membrane modules. On the other hand, genus belonging to *Firmicutes* family are more abundant in the liquid phase. Microbiological analysis identified these populations as carboxydoacetogens and homocetogens. Considering these results, it should be possible to lower the HRT in order to wash out the suspended microorganisms (rich in acetogens) and hence retain the desired methanogenic activity (concentrated in biofilm). Therefore, reducing the HRT will increase the performance and selectivity of the reaction, for instance avoiding electron loss during the production of volatile fatty acids (VFAs). This could be an advantage to perform the reaction in an attached biomass reactor dedicated to population selection.

Regarding the pH, the optimal pH range for methanogenesis is usually studied in anaerobic digesters. In this context,

neutral pH values between 7 and 7.5 are considered as optimal conditions. In in situ methanation systems, pH is chosen to favour the entire anaerobic digestion process involving hydrolysis, acidogenesis, acetogenesis and methanogenesis reactions, whilst the pH range in ex situ systems is applied to favour the methanogenesis reaction only. Since CO_2 in the media is constantly consumed, the pH is often higher than neutrality, and usually close to 8. However, the methanation reaction can still continue as long as the pH does not exceed 9 [29, 42, 43]. Therefore, as shown in Tables 2 and 3, the range of pH in ex situ systems are wider than in in situ systems.

The gas partial pressure and particularly the P_{H2} has an important influence on microbial kinetics, thermodynamics, and physico-chemical parameters. Increasing gas partial pressures will increase the G/L mass transfer. This has a positive effect on the growth of hydrogenotrophic microorganisms, because substrates become available for microorganisms. Controlling the P_{H2} favours the specification of microbial consortia towards a dedicated function of biomethanation. Methanogens and homoacetogens compete for available H_2 in anoxic environments. Since the Ks of methanogens is lower than for homoacetogens, at high HRT, low partial pressure of H_2 will be favourable for the growth of methanogenic archaeal populations which will hence outcompete homoacetogens (Table 1). The increase of HRT will also enable an increase of the microbial biomass concentration and therefore, for an identical specific microbial activity, should increase the global reaction of methanogenesis. In the case of CO_2, an increase of soluble CO_2 in the liquid phase will acidify the medium, i.e. decrease the pH. Even if carbon dioxide is continuously consumed by the methanation reaction, the decrease of pH should be carefully controlled in order to avoid inhibition of methanogens.

Furthermore, CO_2 solubility is high enough and allows CO_2 partial pressure to remain low while providing enough substrate for autotrophic metabolisms.

As a conclusion to this section and according to literature data, we wish to highlight that, in order to obtain a higher hydrogenotrophic methanogen activity than homoacetogen activity, a process can be designed as the following: a long HRT with low hydrogen partial pressure, a pH value of 8, and under thermophilic conditions. The benefit of applying high HRTs is based on the increase of biomass concentration, and on higher gas entry rate conversions. However, using systems with fixed biomass gives the possibility to reduce the HRT in order to wash out non-attached biomass which is assumed not to be specific for the desired reaction. The growth of attached cells, exposing the specific activity will not be affected and will hence continue ensuring and amplifying the methanogenic reaction.

The Biomethanation Types of Reactors and Performances

Several types of methanation reactors are referenced, starting from small batch studies to higher continuous pilots. The easiest way to operate the reactors is the batch test in flasks [44]. However, such processes cannot be directly transferred to real situations nor could they give exploitable results for scale up. Despite these drawbacks, batch tests give interesting information to understand the biological processes as well as limitations, inhibitions or competitions.

In biomethanation, the main obstacle to be tackled is the transfer of hydrogen to microorganisms in the liquid phase. Another issue which needs to be addressed is how to maintain the slow growing archaea inside the reactor (Table 1). In order to counteract these limitations, an important part of the studies (either in situ or ex situ has tested various types of G/L transfer strategies or biomass supports. Mixing is an important parameter that can increase the G/L transfer in the reactor, by liquid recirculation (between 80 and 700 mL/min,) or mechanically (from 65 to 800 rpm) with an impeller or magnetic stirrer. However, a recent study on ex situ methanation has shown that a too high stirring speed (> 1000 rpm) could have negative effects on the production of methane, probably because of the shear force stress [45]. Even if authors quote an in situ biomethanation study where the gas mass transfer is improved with a stirring intensity as high as 1000 rpm [46]. G/L mass transfer can also be improved by varying or de-coupling gas and liquid residence time. For example, increasing GRT by recirculation enables hydrogen to be better consumed. On the other hand, increasing the HRT will be favourable for maintaining slow growing archaeal microbial biomass and therefore prevent any leaching out.

The reactors consist either in a continuous phase of liquid with dispersed gas, such as a bubble column or an immersed membrane bioreactor (hollow fibre, bubbleless [47–49] or reverse membrane bioreactor (PVDF) [50]), or in a continuous gas phase where liquid is percolated (trickle-bed reactors) [51]. Membranes have the advantage not only to increase G/L mass transfer but also to favour biofilm formation, thereby increasing the biomass residence time [47]. Moreover, fixed bed reactors were developed in order to maintain the biomass inside the reactor [52, 53]. All these points will be discussed more precisely later in the review.

The following ("The In Situ Biomethanation" and "The Ex Situ Biomethanation" sections) will present alternatively the main in situ and ex situ biomethanation studies reported in literature. Performances for both in situ and ex situ reactors will be compared and results presented in "Comparison Between In- and Ex Situ Biomethanation Processes" section.

Table 3 Operational conditions and performances of ex situ reactors

Refs.	Reactor configuration	Injected gas/injection mode/recirculation	Tests performed	*T* (°C)	pH	Retention time (h)	Loading rate H_2 ($m^3/m^3/d$)	Methane productivity ($m^3/m^3/d$)	Ratio H_2/CH_4	$\%CH_4$ max (corresponding productivity)	Max productivity (corresponding $\%CH_4$)
[48]	Hollow-fibre membrane biofilm reactor: Hf-MBR (volume 0.33 L;working volume 0.19 5L)	H_2/CO_2 (4:1) through membrane (without bubbles)	Effect of pH. Test at 4.2–5.5 and 6.5–7.5 ≥ mineral medium ajusted at pH 5 or pH 7	37	4.2–4.5 and 6.5–7.5 (nr) pH of the medium ajusted before injection in the reactor	NA	NA	1.13 to 3.95	NA	90% (1.13)	3.95 (60%)
[51]	Trickle-bed reactor (26.8 L) with bioflow	H_2/CO_2 (4:1) through bottom of reactor	Test of gas flow rate (2.3 to 11.6 $m^3/m^3FB/d$)	37	NA	NA	1.84 to 9.28	0.82 to 1.17	3.84 to 4.29	97.9% (0.65)	1.17 (94%)
[31]	Trickle-bed reactor (88 L) with bioflow (61 L)	H_2/CO_2 (4:1) through bottom of reactor	Test of different loading rate of H_2	37	NA	4	0.4 to 6	0.2 to 1.49	NA	98% (0.4)	1.49 (92%)
[47]	Hf-MBR (40 L; 31 L useful)	H_2/CO_2 (4:1) through membrane made up 232 polymeric fibres	Test of different recirculation rates (0.1–4.83 m^3/d)	55	NA	NA	10.1 to 40.2	8,84	NA	NA	8.84(NA)
[65]	Fixed bed reactor packed with vermiculite shales (7.2L) and granular perlite (1.8L) material +1.7 L of water	H_2 and CO_2 controlled separately	Nutrient recirculation test; acid pH with the addition of nutrients containing NH_4CONH_2	53–55	6.6 to 6.94 (nr)	144	7.2 to 36	1.73 to 4.03	NA	90%* (1.73)	4.03 (50%*)
[63]	Trickle-bed reactor: column 1.5 m height diametre de 0.08 m. polypropylene carrier for fixed biomass, packed volume = 0.00578 m^3	Biogas + H_2	Optimization of the H_2/CO_2 ratio: from 6.7 to 3.7– > optimum found between 3.67 and 4.15	37	6.8 to 7.0 (nr)	1.3 to 3.3	4.98 to 11.16	1.2 to 2.52	NA	96% (1.3*)	2.52 (84%)
[29]	Two upflow reactors in serie (working volume 1.4 L)	CH_4:CO_2:H_2 (23:15:62%)	Test of two different gas recirculation rates	52	8.2* to 8.8* (nr)	16	1,86	NA	NA	66%	NA

Table 3 (continued)

Refs.	Reactor configuration	Injected gas/injection mode/recirculation	Tests performed	*T* (°C)	pH	Retention time (h)	Loading rate H_2 ($m^3/m^3/d$)	Methane productivity ($m^3/m^3/d$)	Ratio H_2/CH_4	$\%CH_4$ max (corresponding productivity)	Max productivity (corresponding $\%CH_4$)
[29]	CSTR (working volume 1.4 L) with magnetic stirrer (300 rpm)	CH_4:CO_2:H_2 (23:15:62%)	Test of two different gas recirculation rates	52	8.0* to 8.6* (nr)	8	1,86	NA	NA	79%	NA
[29]	Bubble column (working volume 1.4 L)	CH_4:CO_2:H_2 (23:15:62%)	Test of two different gas recirculation rates	52	8.1* to 8.7* (nr)	8	1,86	NA	NA	98%	NA
[30]	CSTR (2 L) mix with magnetic stirrers	Biogas + H_2	Test of 2 different temperatures: thermophilic and mesophilic	35 and 55	8.17 at 35 °C (nr) 8.49 at 55 °C (nr)	NA	0.192 to 0.510	0.1 to 0.36	NA	89% (0.1) at 55 °C	0.36 (85%) at 55 °C
[37]	CSTR of 1 L with 0.6 L of working volume with magnetic stirrer; injection of the gas through ceramic diffuser in the bottom of reactor	H_2, CH_4, and CO_2 with the ratio 60:25:15	Different mixing speed (500 and 800 rpm) and different gas injection rate	55	~7.8 (nr)	1 to 8	1.8 to 14.4	0.9 to 5.3	3.2 to 4.34	95.4% (1.5)	5.3 (90.8%)
[43]	Bubble column (9.5 L).	H_2/CO_2 and $H_2/CO_2/CH_4$ using a ceramic diffuser	Injection of H_2/CO_2 and $H_2/CO_2/CH_4$	55	7.1 to 8.5 (nr)	0.33 to 24	7.3 to 73.3	0.85 to 9.1	NA	85% (0.85)	9.1 (15%)
[68]	3 trickle-bed reactor in series (3 × 22.5 L)	H_2:CO_2 (1:3.9)	Influence of liquid flow modulation at 5 bar	40	7.3 to 7.4 (nr)	4,5	4,8	5,62	NA	97% (5.62)	5.62 (97%)
[71]	Biotrickling filter (BTF); paking: polyurethane foam; working volume of 291 mL	H_2:CO_2:N_2 (17.5:17.5:65)	Use of cattle manure as nutrient media	52	7.5 to 8.0 (nr)	1.32 to 4	3.91 to 11.9	0.69 to 2.08	NA	67% (2.08)	2.08 (67%)

Table 3 (continued)

Refs.	Reactor configuration	Injected gas/injection mode/recirculation	Tests performed	*T* (°C)	pH	Retention time (h)	Loading rate H_2 ($m^3/m^3/d$)	Methane productivity ($m^3/m^3/d$)	Ratio H_2/CH_4	$\%CH_4$ max (corresponding productivity)	Max productivity (corresponding $\%CH_4$)
[53]	Biotrickling filter (BTF); paking: Glass rings; working volume of 1 L	H_2:CO_2:CH_4 (62:15:23)	Gas input was directed in concurrent or countercurrent flow of the trickling media.	54	8.12 to 8.63 (liquid media were neutralised twice per week using HCl 1 M)	14 to 2.1	1.1 to 7.2	0.26 to 1.74	NA	99.1 (0.88)	1.74 (95.1%)
[72]	Trickle-bed reactor 72.6 L; Vpacking 58.1 L	H_2:CO_2 (3.5:1 to 4:1)	Test of different H_2 loading rate	55	6.2 to 7 –> pH regulated with first with NaOH and then with K_2HPO_4 after day 66	0NA	1.7 to 62.1	1.3 to 15.4	NA	99.1 (1.7)	15.4 (98.1)
[62]	Trickle-bed reactor (90 L) with bioflow (61 L)	H_2/CO_2 (4:1) and biogas: H_2 through bottom of reactor	Test with CO_2:H_2 at atmospheric pressure and 1–25 bar in 2 reactors and with biogas:H_2 at atmospheric pressur in a third reactor	38	7.4 to 5 (nr) –> decrease in pH as pressure increases	NA	0.8 to 13.1	5,75	4,13	99.7 (3.1) at 1 bar	5.75 (97.7) at 5 bar

NA not available, *nr* not regulated

*Estimated from a figure

The In Situ Biomethanation

The in situ methanation process consists of injecting H_2 directly into the reactor digesting organic matter in order to upgrade the biogas (through CO_2 consumption) and increase CH_4 content in the produced biogas. The effect of injecting H_2, and sometimes CO_2 have some effects on methane productivity, methane percentage and on biogas upgrading.

However, in in situ biomethanation, it is quite difficult to differentiate the efficiency of the biomethanation itself from the efficiency of anaerobic digestion, and this is due to the degradation of organic matter and the lack of information regarding the performances of anaerobic digestion. Therefore, it is important to compare such a process to a controlled reactor, without H_2 injection. Moreover, methane productivity and H_2 conversion efficiency both depend on the initial performance of anaerobic digester (before H_2 injection).

Among the benefits of in situ methanation, is the possibility to reduce investment costs, since only one reactor is needed and the purification system is reduced [54]. However, in such processes, the amount of H_2 introduced in the digester must be carefully controlled. From a thermodynamical point of view, a high partial pressure of H_2 might cause direct inhibition of hydrolysis and acetogenesis, since hydrogen is a product of the above-mentioned reactions. Moreover, high H_2 partial pressure can also lead to indirect inhibition of methanogenesis because this step of anaerobic digestion depends on the previous ones. Such impacts will respectively reduce digestion performances or accumulate VFAs, thereby decreasing the pH (see "The Bottlenecks and Strategies for Improvement of Biomethanation" section). The negative impact of elevated P_{H2} on hydrolysis is well referenced [55]. Also, high P_{H2} tends to be more favourable for the growth of homoacetogens compared to methanogens [56].

For these main reasons, in situ methanation processes are seldomly developed at an industrial scale compared to ex situ processes (see "Large-Scale Pilot Plant of Biomethanation" section).

The Table 2 gathers the main articles referring to in situ methanation studies. As stated, various gases were injected in the digesters: pure H_2 [44], coke gas composed of H_2/CO: 92–8% [49], or even syngas with various ratio conditions of $CO/H_2/CO_2$ [57]. In order to reach the stoichiometric ratio of 4 between H_2, and CO_2, and to increase the hydrogen conversion efficiency to CH_4, the biogas can at times be supplemented with CO_2. This additional injection helps to balance the decrease of carbonate ions in the liquid due to the consumption of CO_2 by the methanation reaction [58, 59].

In literature, relatively simple reactors are developed for in situ configurations such as CSTR or UASB (Upflow Anaerobic Sludge Blanket) reactors. UASB reactors have the advantage to involve microorganisms in the form of granules, which are known to be more resistant to potential inhibitions (CO, H_2 etc.) [60]. A supposedly innovative reactor designed by Xu et al. presents two successive UASB reactors treating synthetic wastewater [57]. However, the substrates (organic wastewater and H_2) are only fed in the first stage of the process. Therefore, this design will probably have the same effect as a single UASB, twice the size in height and equipped with a recirculating gas system. However, one innovative point is the recirculation of gas in each UASB reactor.

In order to improve the G/L mass transfer, which is the main limiting factor in biomethanation, different technologies are developed for injecting the gas: simply at the bottom of the reactor or in the headspace [44] and with a high mixing speed, through ceramic diffusers at the bottom of the reactor [44, 58], or hollow-fibre bubbleless modules [49]. The use of packed beds (raschig rings, inert alumina ceramic sponge) is also tested in order to increase the G/L mass transfer [52] or the microorganism/liquid (containing nutrients and soluble gas substrate) interface.

The variety of inocula is large and similar to various microbial consortia found in anaerobic digesters, such as sewage sludge, cattle manure, primary or secondary sludge, granules from wastewater treatment plants etc. These consortia contain a large diversity of microorganisms, each able to ensure either hydrolytic, acidogenic, acetogenic or methanogenic functions. In in situ biomethanation, the objective is to increase the hydrogenotrophic methanogenic activity while maintaining hydrolysis and fermentation inside the digesters.

Both mesophilic and thermophilic conditions were investigated, but to our knowledge, no specific studies have yet been undertaken on the effect of temperature on in situ methanation performance. Even if thermophilic studies seem to be favourable to perform biomethanation, in in situ processes, anaerobic digestion also needs to be effective and resilient, which is mostly the case under mesophilic conditions [61]. Indeed, as shown in Table 2, the maximum CH_4 percentage is obtained in mesophilic conditions.

The hydrogen organic charges studied at lab scale range between 0.5 and 7 m^3/m^3/day depending on the operating conditions. With this charge, the hydrogen conversion efficiency range is generally high and the ratio between consumed H_2 and produced CH_4 is close to the stoichiometric value of 4.

Figure 4 represents the methane percentage versus methane productivities for in situ studies. A linear trend is observed between both parameters showing a decrease of the percentage of methane with increasing methane productivities. Maximum productivities vary between 0.15 m^3/m^3/day and 2.52 m^3/m^3/day but globally the median value is close to 0.45 m^3/m^3/day. The value of 2.52 is quite an exception and is obtained in CSTR reactors by injecting the gas

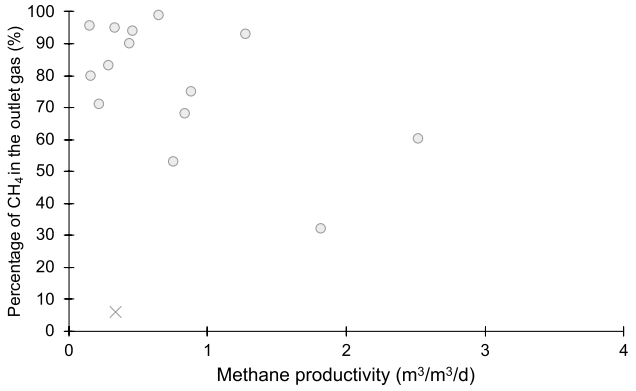


Fig. 4 Evolution of the percentage of CH_4 (in %) as a function of the methane productivity ($m^3/m^3/day$) in in situ technologies. The cross represents the study of Voelklein et al. [43] with 6.1% of CH_4 and 0.33 $m^3/m^3/day$ where the system failed to transform this H_2 and methanogenesis was inhibited by VFA accumulation to suppress (11.8 g/L)

through ceramic diffusers which provides a high G/L transfer rate [43]. The lower productivity value (0.14 $m^3/m^3/day$) is obtained in a classical glass serum flask where the G/L transfer system is not optimised. In in situ studies, the methane percentages vary between 6.1 and 98.8% (median value at 79%). The higher value is obtained in hollow fibre reactors with a H_2 organic charge of 2.88 $m^3/m^3/day$. The value of 6.1% of CH_4 is outside this trend. Indeed, such poor performances were obtained in a study where authors attempted to intensify the process by applying a high H_2 charge [43]. The system failed to transform this excess of H_2 and methanogenesis was inhibited which led to the accumulation of VFAs (11.8 g/L with mainly acetate, propionate and a small proportion of isovalerate).

The Ex Situ Biomethanation

Although ex situ methanation processes can be used to treat CO and/or industrial CO_2, such systems are quite often coupled to anaerobic biogas digesters. This involves the injection of hydrogen and biogas in an independent reactor. In opposition to in situ methanation, this technique is quite interesting since it offers the possibility to dissociate process conditions, such as temperature and pressure, applied separately to the digester and to the methanation reactor. By doing so, optimal conditions can be applied to satisfy hydrogenotrophic methanogens, i.e. high temperature and high P_{H2}. Indeed, the hydrogen partial pressure is not an issue since hydrogenotrophic methanogens handle these conditions.

The injected gas is generally either a mixture of hydrogen and biogas [30, 62, 63], or syngas mainly composed of CO, H_2, CO_2 [50]. However, in laboratory experiments, a mixture of H_2 and CO_2 [31, 37, 47, 48, 51, 62, 64–68] or H_2, CO_2 and CH_4 can also be used. Different types of reactors can be implemented, namely CSTR, membrane, trickle-bed, bubble column, biofilm, plug-flow or a hybrid technology.

Different types of devices may be used for injecting the gas: two ceramic diffusers [37], hollow fibre membranes, bubbleless membranes [47, 48], stainless steel diffusers or Al_2O_3 ceramic membranes [69] either connected at the bottom, at the top [65] or eventually in the headspace of the reactor [37].

Regarding the inoculum, studies which focus on pure cultures are not part of this review. Indeed, as explained in the introduction, this review focuses on studies involving mixed cultures only. In this case, two main types of inocula are considered: an inoculum already acclimated to H_2 and CO_2 as sole substrates [37, 53, 65, 69] or an inoculum from an anaerobic digester [31, 36, 47, 48, 63, 67].

In most cases, thermophilic (more than 50 °C) and mesophilic (around 37 °C) conditions were studied. However, the other parameters (types of reactor, diffuser, pressure, etc.) have such a significant effect that it is difficult to compare performances of the different studies in terms of productivity and percentage of CH_4 based on the temperature criterion alone. However, some studies have specifically investigated the effect of temperature on biomethanation performances [36, 37, 69]. In these studies, the increase of temperature seems to have a positive effect on the methanation reaction. Indeed, some results obtained in batch show a better conversion rate (> 60%) of CO_2 and H_2 under thermophilic conditions than mesophilic conditions [37]. Likewise Guneratnam et al. [70] showed that their system was more efficient at 65 °C than at 55 °C. These results are in agreement with "Impacts of Process Parameters on Methanogenesis" section which described that temperatures above 50 °C were more favourable for the growth of methanogenic archaea than for homoacetogenic bacteria, and that an increase in temperature does not significantly impact the transfer of H_2.

Table 3 lists the operating conditions and performances of different ex situ reactors on a laboratory scale. A wide range of H_2 loading rates have been tested, starting at 0,19 m^3/m^3reactor/day [30] up to 230 m^3/m^3reactor/day [67]. However, most of the studies focused on loading rates between 2 and 10 m^3/m^3reactor/day [37, 51, 53, 63, 69, 71]. The theoretical ratio of H_2 to CH_4 given by the stoichiometry is 4. Most of the ratios found in literature are in the close range of the stoichiometry (between 3.6 m^3 H_2/m^3 CH_4 and 4.4 m^3 H_2/m^3 CH_4) [37, 49, 51, 62]. A value of 11.1 m^3 H_2/m^3 CH_4 was determined by Bassani et al. [69]. This value is quite different from the theoretical value and was only measured at the beginning of the experiment. According to the authors, results were obtained at a stage where hydrogen was used for maintenance and microbial growth, i.e. before methane production takes place. However, this ratio could still be questionable since anaerobic microorganisms need

to perform catabolic reactions in order to gain energy for anabolic reactions and maintenance.

Regardless of the type of reactor tested, the maximum percentage of methane reached is above 90% and even beyond 95% in most cases, which makes direct injection of the produced gas into the natural gas network possible in most countries. However, for this methane percentage depending on the selected technology, the range of achieved methane productivities is variable. Regarding the hollow fibre membrane reactors, performances reported range from 0.9 to 8.84 m^3/m^3/day [47, 48, 64]. For CSTR reactors, methane productivities range from 0.9 to 5.3 m^3/m^3/day [29, 30, 37]. In fix-bed reactors, or trickle-bed reactors productivity values were reported between 0.82 and 15.4 m^3/m^3/day [51, 62, 63, 65, 68, 72]. Bubble column studies show a methane productivity between 0.85 and 9.1 m^3/m^3/day [29, 43]. The highest productivity reported for a mixed culture was recorded by Savvas et al. [67] using a biofilm plug-flow reactor. Authors reached a maximum value of 40 m^3CH_4/m^3/day. Therefore, trickle-bed and biofilm plug-flow reactors seem to be the best configurations for achieving high methane productivities.

Figure 5 represents the evolution of the quality of the produced gas (expressed as a percentage of methane content) as a function of the methane productivity (in m^3/m^3/day) for all studies listed in Table 3. The graph has been drawn with both the maximum percentage of methane and associated productivity and the maximum productivity with the associated percentage of methane. The results show, in most studies, a strong compromise between the quality of the gas produced and productivity (black squares). This is probably due to the low mass transfer of hydrogen in the liquid medium, in which the biological methanation reaction takes place. Indeed, many authors working on biomethanation describe the transfer of hydrogen in the liquid medium as the most limiting step of the process [37, 47, 51, 63–65, 69]. However, according to Fig. 5, five studies (represented by grey squares) stand out with a much lower compromise between quality and productivity of the outgoing methane. The common feature of these five studies is that authors have found a way to improve the G/L mass transfer of H_2. Indeed, studies noted 1, 2 and 3 in Fig. 5 use a specific type of reactor: the trickle-bed [62, 68, 72]. This type of reactor, which is not filled with liquid but with a packed bed, seems to have many advantages in the context of biomethanation: firstly, the trickle-bed reactor does not need additional mechanical energy (e.g. no agitation required) to increase the transfer of gas into the liquid [63], and secondly, the use of packing material enables to (i) fix the biomass which serves as catalyst for the reaction in the form of a biofilm [31] (ii) provide a high surface-area per reactor volume which increases the contact surface serving as an interface for hydrogen mass transfer [72]. It seems obvious that these last two aspects can

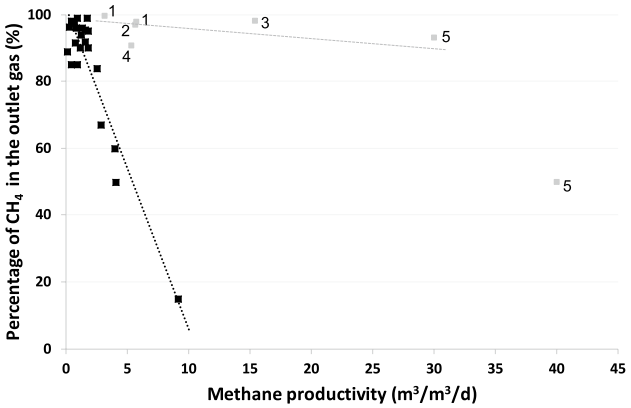


Fig. 5 Evolution of the percentage of CH_4 (in %) as a function of the methane productivity (m^3/m^3/day). The grey squares represent the results of studies that stand out with 1: [62]; 2: [68]; 3: [72] 4: [37]; 5: [67]. Studies noted 1, 2 and 3 are performed in trickle-bed. The square noted 4, corresponds to a study carried out in CSTR. The study noted 5, was carried out in a biofilm plug-flow reactor consisting of a single tube 13 mm in diameter and 7 m long. These studies have, all 5, improved the G/L transfer rate through design and utilisation of specific reactor or the use of mechanical energy

explain that three out of the five studies which stand out in Fig. 5, were carried out on trickle-beds. In Fig. 5, the square noted 4, illustrates a study carried out in a CSTR by Luo et Angelidaki [37]. Authors used an intensive stirring speed (800 rpm) to decrease the size of gas bubbles and hence to improve the gas distribution which led to an enhanced G/L mass transfer. This technique, although effective for increasing G/L mass transfer, will be difficult to apply on an industrial scale due to the large amount of energy required for mixing [63, 67, 73]. The last study, noted 5, was carried out by Savvas et al. using a biofilm plug-flow reactor consisting of a single tube with the following dimensions: 13 mm in diameter and 7 m long [67]. According to the authors, this design enables to (i) reduce the amount of liquid surrounding the biofilm to a minimum layer (ii) maximize the gas residence time by increasing the traveling distance of gas molecules through the reactor. Despite the good performances, plug-flow reactors seem difficult to set up on an industrial scale (problem of gas supply, plugging…). However, this study shows the importance of the desin of the reactor in biomethanation, notably the dimension in terms of diameter to height ratio. Finally, it is interesting to note that even in this study, a compromise between quality and productivity arose when the authors aimed at treating a very high H_2 loading rate.

Comparison Between In- and Ex Situ Biomethanation Processes

The performances of both in situ and ex situ systems are plotted in Fig. 6. As previously presented in Figs. 4 and 5,

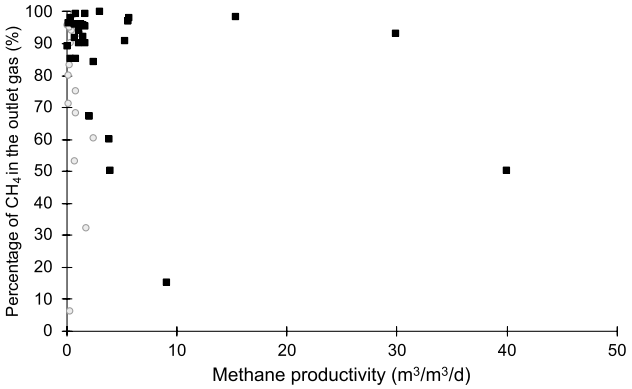


Fig. 6 The percentage of methane obtained in in situ (grey circle) and ex situ (black squares) processes are plotted against the corresponding methane productivity

the higher the CH_4 percentage, the lower the productivities, and this independently of the considered technology. Although maximum percentages of methane, above 95% have been recorded in both processes, such values were nevertheless less frequent in in situ methanation. Indeed, a significant part of in situ studies report methane percentages between 70 and 80%. In addition, it clearly appears that lower methane productivities are reached in in situ methanation systems with some studies achieving a maximum of 2.52 m^3CH_4/m^3/day whilst ex situ systems presented more than 10-fold productivity values with 30 or 40 m^3/m^3/day. It seems that the intensification of processes was not tested in in situ technologies probably because of the risk of H_2 inhibition. The example of Voelkein et al. shows that increasing H_2 can lead to the inhibition of methanogens and to the accumulation of VFA [43].

The Bottlenecks and Strategies for Improvement of Biomethanation

Gas to Liquid (G/L) Mass Transfer

In biological methanation, substrates involved in the reaction (H_2 and CO_2) are injected in the reactor in a gaseous form, whilst the biocatalyst is in the aqueous phase and can only metabolise soluble substrates. However, the solubility of the gas in the liquid is low, especially for hydrogen. In fact, hydrogen is 500 times less soluble in water at 60 °C compared to CO_2 [54]. Knowing this, it is easy to understand that the main challenge in biological methanation is the transfer of gases and particularly of H_2 from the gas to liquid phase and this is confirmed by many research groups [52, 53, 58, 64, 71, 72, 74]. The H_2 G/L mass transfer is described by the following equation:

$$Transfer\ of\ H_2 = k_L a * (P.X_{H2}.H_{eH2} - S_{H2}) \quad (2)$$

where k_L: Overall Coefficient of Material Transfer (length.time^{-1}), a: Exchange surface (area.volume^{-1}), $P.X_{H2}$: H_2 partial pressure in equilibrium with the liquid phase, He_{H2}: Hydrogen constant of Henry's Law (mass.volume^{-1}.pressure^{-1}), S_{H2}: H_2 concentration in the liquid (mass.volume^{-1}).

This equation shows that optimisation of the H_2 mass transfer can be achieved by (i) increasing the partial pressure of the compound of interest (here H_2) (ii) increasing the volumetric mass transfer coefficient (k_La), which is inherent to the operational conditions, the reactor design and the geometry/type of gas diffuser used.

Regarding the k_La, some studies have tried to increase this coefficient by designing specific devices such as hollow fibre membrane [75] or bubbleless membrane [47, 64]. Other authors have applied intensive stirring in order to increase the reaction kinetics and the quality of the gas in the outlet [37, 76]. The gas recirculation flow is also another parameter able to increase the k_La [29, 77]. Finally, k_La values are specific to the type of reactor and actually should be one of the criteria used for choosing the bioreactor dedicated to biomethanation [78, 79].

Another way to increase the transfer of H_2 is to increase the operating pressure of the bioreactors. This allows acting on both the hydrogen partial pressure (P_{H2}) and the size of bubbles, which is directly linked to the exchange surface (a) between the gas and the liquid. For example Martin et al. showed a higher conversion efficiency by increasing the reactor pressure [80]. Similarly, Burkhardt et al. found better performances by applying a 5 bar pressure compared to atmospheric pressure [62]. Moreover, the majority of pre-industrial scale reactors work under pressure in order to promote the G/L mass transfer of hydrogen. This is the case for the Electrochaea biocat project and the Viessman reactor in Allendorf (see "Large-scale Pilot Plant of Biomethanation" section) which are both able to apply working pressures reaching 10 bars [81, 82].

Impurities of the Inlet Gas

Another aspect to consider is the quality of the injected gas. Indeed, the composition of the incoming gas (biogas or syngas) will depend on the type of process used before biomethanation, the type of substrate and the operating parameters used to produce this gas. In addition to the main compounds used during the biomethanation reaction (CO_2 for biogas and H_2, CO_2, CO for syngas), minor compounds (N_2, H_2O, H_2S, NH_3, benzene, siloxanes, volatile organic compounds, phenols, cyanide…), which are considered as impurities, are produced and can influence the performance of the whole process [83–85]. Of course, this influence depends on the type of molecules and their concentration. N_2 or H_2O found

in biogas and syngas, are inert meaning that they do not directly affect the performance of microorganisms, but can dilute the output gas and thus reduce its calorific value [86]. Since these molecules do not interact with microorganisms, it might be appropriate, depending on their concentration, to remove them physicochemically before injecting the gas into the gas grid.

The influence of NH_3 and H_2S is more difficult to determine. Indeed, depending on their concentration and the form in which they are present in the culture medium (which depends on pH value), they can either inhibit microorganisms or be a source of nutrients. Indeed, NH_4 is used as a source of nitrogen in biomethanation culture media [30, 37, 72] while the free NH_3 form has been identified as a strong inhibitor of methanogenesis [87]. Likewise, H_2S can be used as a source of sulfur by methanogens [72, 88, 89] even if in the liquid medium it can be found under different forms (HS^-, S_2^- and H_2S) depending on the pH of the culture media. Nevertheless, the H_2S form is a potential inhibitor for methanogens [90, 91]. The other minor compounds (benzene, siloxanes, volatile organic compounds, phenols, cyanide…) are more specific when using syngas and can cause problems related to the inhibition of microbial consortia [7]. Despite the fact that microorganisms involved in biological methanation seem to be more tolerant to impurities than chemical catalysts [6, 63], specific studies undertaken on their influence in the context of biomethanation are few. Further research in this area is needed in order to establish whether or not these impurities need to be removed from entering gas, which could hence impact the overall cost of biomethanation processes.

Methanogens Sensitivities

Another important feature when considering a continuous reactor mode, is that methanogens are very sensitive to process conditions, and particularly to short retention times. Indeed, these microorganisms have a slow growth rate and therefore need long retention times to remain in the reactor. In order to tackle the issue of methanogens being washed out, several authors suggested to use sludge enclosed in bags in order to retain the biomass during the whole process [50]. Others designed a three phase trickle-bed reactor with biofilms (grown on a Bioflow support), a liquid and a gas phase [31, 51, 63]. CO and several impurities, such as tars, NOx and NH_3, can also inhibit the activity of methanogens. However, the latter elements will not be discussed since this present review focuses on H_2 and CO_2 biomethanation. Nevertheless, more information on this topic can be found in recent reviews such as Grimalt-Alemany et al. [7].

Production of Water

In addition to methane, the methanation reaction produces water (Eq. 1) and this can be an issue that is poorly mentioned in literature. Indeed, water production could affect the management of digesters and/or methanation reactors whether in situ or ex situ methanation processes are used, respectively. According to Strübing et al. this problem is particularly important for systems where the volume of liquid is significantly smaller than the reaction volume, such as trickle-beds, and especially after the gas load is significantly increased [72]. In these cases, the microbial biomass and the liquid medium can be rapidly diluted, which can affect the microbial productivity, the buffering capacity and concentration of nutrients and trace elements. Therefore, in the case of important gas loads, which involve a high amount of produced water, specific solutions will have to be designed to retain the biomass, and also to control the buffer, nutrients and trace elements with highly sensitive systems. To overcome this issue, authors suggest to apply membrane techniques in order to separate and extract the water produced by metabolic reactions without washing out the biomass, trace elements and nutrients. However, this solution has not yet been tested. Another study using a reactor with a larger liquid phase than the gas phase, succeeded in maintaining biological methanation activity for a 6 months period without adding any micro or macro nutrients [92]. Nevertheless, at the end, authors recognised that on a longer term, the water produced during methanogenesis might be problematic. Indeed, for these authors, when considering closed systems, dilution cannot be avoided and will eventually lead to a lack of nutrients. Therefore, authors suggested to use a biofilm reactor in order to separate the culture from any liquid processing route and defined it as the best option to overcome dilution, because it does not consume too much energy nor does it disrupt the methanation process. In any case, more studies should be carried out to better understand this problem and suggest appropriate solutions.

When considering suspended biomass reactors, another possible impact of water production is the decrease of the HRT, by removing the water in order to maintain a constant liquid volume, and hence the loss of methanogen biomass. To our knowledge, this feature is not discussed in literature, probably because productivity levels achieved in suspended biomass reactors at laboratory scale are low. However, the problem may arise on an industrial scale with pressurised reactors and for which the productivity will probably be much higher.

Heat Production

According to Eq. 1, the methanation reaction results in a significant reduction in the overall number of molecules in

the process: i.e. from 4 mol H_2 and 1 mol CO_2 to roughly 1 mol CH_4 and 2 mol H_2O. This decrease in entropy leads, de facto, to the production of heat. Nevertheless, on a laboratory scale this phenomenon is difficult to measure, because of the low productivity observed and the heat dissipation through the walls of the reactors, and to maintain a sufficiently negative Gibbs free energy defined as a driving force for hydrogenotrophic methanogenesis [91, 93, 94]. This explains why, in most studies, authors need to heat the reactor in order to maintain a constant temperature. Burkhardt et al. even claim that unlike Sabatier catalytic processes, it would not be necessary to cool the biological methanation reactors because the enthalpy of the reaction is much lower [62].

On the other hand, Schill et al. used a calorimetric reactor set-up to determine the heat production generated during exothermic growth of *Methanothermobacter thermoautotrophicus* [94]. The authors calculated a significant heat production by the microbes reaching 16.3 $W.L^{-1}.h^{-1}$ at a flow rate of 576 m^3H_2/m^3/day. Based on these results it could be assumed that with a well-insulated reactor, the heat generated by the reaction could at least compensate the heat lost through dissipation. Depending on the methane productivity, it might even be necessary to cool the reactor during operation to keep a constant temperature [94] and a heat flow could even be recovered and used [90]. Nevertheless, Götz et al. estimate that for systems with an operating temperature below 100 °C, such as for biological methanation reactors, the potentially generated heat could be difficult to valorise [95]. The use of heat for district heating is suggested if the methanation facility is close to inhabited areas.

Partial Pressure of Hydrogen

Previously, "The Energetic and Kinetic Aspects of Microbial Biomethanation" and "Impacts of Process Parameters on Methanogenesis" sectiona explained that the microorganisms performing anaerobic digestion have more or less affinity for hydrogen. Nevertheless, above a certain partial pressure, hydrogen can also inhibit certain stages of anaerobic digestion, and this can be problematic, particularly in the case of in situ methanation. Indeed, in this case, the increase of the hydrogen partial pressure (> 10 Pa) can inhibit the degradation of VFAs and particularly of propionate and butyrate [96, 97]. Similarly, Agneessens et al. have shown that the injection of several pulses of H_2 in an in situ biomethanation process leads to the accumulation of acetate [46]. This accumulation may be due to an overexpression of the homoacetogenic pathwPay [56] and/or an inhibition of acetoclastic methanogenesis. However, the increase of the hydrogenotrophic population can locally reduce the hydrogen partial pressure and thus remove these inhibitions, but this is only possible if the right amount of hydrogen is introduced in the reactor in relation to the amount of available CO_2 [55].

pH

Independently of the selected system, whether ex- or in situ biomethanation, particular attention should be given to the variation of pH since the injection of gas (H_2/CO_2), and particularly CO_2 can disturb the carbonate/carbon dioxide equilibrium, thereby affecting the pH and reducing the biological methanation reaction yield [44]. In most studies, whether in situ or ex situ, the pH is not regulated, which can be problematic. For example, in the case of in situ biomethanation, carbonate consumption by methanogenic archaea can lead to a pH increase in the digester which can inhibit acetoclastic methanogenesis [52, 75]. This pH increase is also reported in ex situ methanation systems but seems to be less problematic [29, 30]. As a precaution, studies suggest the use of a nutrient medium supplemented with HCl in order to adjust the pH [53, 69]. Conversely, studies (often ex situ systems) have shown a decrease in pH during methanation which can lead to a decrease in process performances [62, 72]. This decline of pH may be due to (i) the production of VFAs (and especially acetate) via homoacetogenesis (Fig. 2) (ii) the acidification of the medium caused by the continuous supply of CO_2 (and notably after an increase of the gas feeding rate or decrease of activity) (iii) the decrease of the buffer capacity of the medium due to the dilution with water produced during the reaction (as explained above) (iv) the increase of CO_2 solubility when the reactor is under high pressure (above 5 bars) [62].

In all cases, when the pH is disturbed and significantly reduces the performances of the system, a regulation of the pH should be applied. However, pH regulation can be costly and limit the economic viability of the process. One option would be to adapt introduced feedstocks [37, 44], or to add liquid digestate which not only contains all the nutrients required for undergoing methanation, but is also able to increase the buffering property of the medium thanks to inherent compounds such as carbonates [71].

Renewable Energy and Electricity Intermittence

Renewable power to gas systems imply that the gas injected in the grid is produced by using electricity from renewable resources (e.g. wind turbines, photovoltaic panels). This electricity, necessary for the electrolysis of water to produce H_2 is intermittent since only the surplus of electricity is interesting to valorise. Therefore, the electricity is available only a few hours per day and above all during summer. The flexibility of biological methanation systems facing intermittence is not well referenced. Nevertheless, some studies have

shown that after a more or less long period of shut downs (e.g. due to technical modifications) as well as after changes in H_2 loading rates, performances in terms of productivity and percentage of methane were quickly restored [31, 63, 93]. When using a trickle-bed reactor, Strübing et al. [98] demonstrated that after a 30-min operational off-cycle it was almost immediately possible to recover a normal full loading rate (62.1 m^3H_2/m^3trickle-bed/d), while after 24 h of interruption, normal conditions were recovered with a 60 min stepwise load increase. Moreover, another study with the same authors observed a better restart of performances after maintaining the reactor a certain period at 25 °C compared to 55 °C [93]. Burkhardt and Buch [51], also studied a trickle-bed and showed that the production of methane for energy storage can be controlled to the minute. These types of results are encouraging and seem to show the possibility to conduct flexible operations within biomethanation processes.

Large-Scale Pilot Plant of Biomethanation

Few large-scale biomethanation projects are referenced in the literature, illustrating that the technology is only at the beginning of its commercial application. However, some examples are starting to emerge through different companies.

MicrobEnergy (Viessmann)

The Viessmann group, belonging to the MicrobEnergy company, has carried out large-scale tests with both in situ and ex situ reactors.

A 100 m^3 in situ pilot was tested in Allendorf (Germany). Without H_2 injection in the digester, biogas coming out from the anaerobic digester contained 53% CH_4 and 47% CO_2 with a flow rate of 330 m^3/h, which represented 175Nm^3/h of biomethane. With the injection of H_2 at a rate of 20 m^3 H_2/h in the digester, the produced biogas also reached 330 m^3/h but was enriched in methane (60% CH_4, 38% CO_2) which increased the biomethane production to 198 m^3/h [99].

A 5 m^3 ex situ demonstration plant was also installed in Allendorf (Germany). The reactor could operate under temperatures ranging from 50 to 80 °C and pressures ranging from 5 to 15 bars. Biological methanation is performed using an aeration stirrer operating at 400 rpm. The use of two electrolysers of 150 kW each provided a maximum hydrogen flow rate of 60 m^3/h. Tests were carried out with H_2 at an incoming flow rate of 15 m^3/h and results showed a biogas flow rate of 5.5 m^3/h with 96% of CH_4 [82, 100].

In 2020, Microbenergy plans to install an ex situ methanation reactor in Dietikon in Switzerland. On the long-term, the plan would be to convert 450 m^3/h of hydrogen to biomethane with CO_2 provided from residual gases from treatment plants (https://www.microbenergy.de/aktuelles).

Krajete

Krajete is an Austrian company founded in 2012 and which offers ex situ methanation systems based on CSTR. The reactor is under pressure up to 15 bars and with stirring speeds reaching 800 rpm. These conditions are able to provide a methane purity above 95%. This company claims a robust process towards impurities, poisons and intermittent operations and the ability to use different sources of CO_2 such as combustion gas from petrol, diesel as fuel, syngas from the steel industry, syngas from waste incineration, raw biogas, purified biogas, CO_2 from biogas (https://www.krajete.com).

During laboratory scale tests (at 15 bars), Krajete obtained interesting results with productivities reaching 33 m^3CH_4/m^3reactor/h. Further tests were undertaken in a "customized" 450 L reactor in order to assess the performance of the process in larger volumes [101]. However, to our knowledge, no results from these tests are available to date.

Electrochaea GmbH

Electrochaea GmbH was founded in 2010 in Chicago in the United States. This company offers an ex situ single culture bioprocess with *Methanothermobacter thermautotrophicus*, which is protected by a patent. The reactor operates at a temperature of 65 °C and at pressures from 1 to 10 bars.

A first test was carried out in 2011 on a brewery digester in Saint Louis. This test demonstrated the possibility of using untreated industrial biogas as a carbon source [80]. In 2013, a first pre-commercial test was carried out in Foulum in Denmark, with a non-optimized 10 m^3 CSTR reactor and using raw biogas as a CO_2 source. The main objective of the Foulum Project was to demonstrate the efficiency, productivity, robustness, and responsiveness of the technology (www.electrochaea.com/technology). In 2016 the BioCat project started at the Avedøre wastewater treatment plant in Denmark. The 3.5 m^3 reactor was operated at 62 °C and under 9 bars. The plant was fed with raw biogas, a mixture of CO_2 and methane. Results showed a stable conversion rate of CO_2 to methane with values higher than 98%. Methane productivity reached 14.3 m^3CH_4/m^3reactor/h alongside with the production of 320 kW/h of heat. After drying and cleaning the outlet gas by using a small post-processing unit, the composition of the gas reached 98% of CH_4, 2% of H_2, 1% of CO_2 and <40 ppm of H_2O. In addition, the reactor was shut-down for several time intervals and the instantaneous response following the start-up was independent of the duration of the shut-down [81, 102]. By 2025, the company

plans to open factories with a capacity reaching more than one gigawatt.

Conclusion

The numerous studies analysed in this bibliographic review demonstrate that biological methanation is a promising way to produce power from methane. Associating anaerobic digestion and biological methanation seems to be particularly interesting due to the complementarity of these two processes. However, the use of microbial consortia requires operating parameters, which have to be selected and controlled with great caution in order to select the microorganisms of interest. The comparison between in situ and ex situ biomethanation processes showed that higher methane productivities and higher methane percentages are reached with ex situ systems. In addition, independently of the chosen system (in situ or ex situ), bottlenecks have been identified and the solutions to overcome these barriers are now being suggested. Thus, although at its beginning, the commercialisation of the first industrial processes of biomethanation is emerging and solutions suggested to improve these processes will probably intensify over the coming years.

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