Bilag 3.2 (1/16)

Dan F. Christiansen

Fra: Michael Vedel Wegener Kofoed <mvk@eng.au.dk>
Sendt: 22. oktober 2019 17:49
Til: Dan F. Christiansen
Cc: 'Anders Elkjær Tønnesen'; Lars Ditlev Mørck Ottosen; Nabin Aryal
Emne: SV: Forgasningsgas i biogasreaktor.

Hej Dan,

Det lyder som en spændende teknologi som kunne være interessant at sætte sammen med vores metaniseringsteknologi – specielt hvis den ikke opbygger problematiske tjærestoffer. Det ville vores agrofolk nok også være interesseret i.

Vi har selv haft fokus på teknologien som en nøgle til omsætning af sværtnedbrydelige restfraktioner, så det kunne jo være noget vi skulle sætte os sammen og diskutere mulighederne for. Hvor bor I henne?

Vores laboratoriereaktorer kører pt på syntetisk gas, da vores forbrug er større end det gas vi har tilgængeligt. Vi har dog tidligere kørt på forgasset halm.

Mvh

Michael

Fra: Dan F. Christiansen <dfc@DanskEnergiRaadgivning.dk>
Sendt: 22. oktober 2019 09:34
Til: Michael Vedel Wegener Kofoed <mvk@eng.au.dk>
Cc: 'Anders Elkjær Tønnesen' <aet@biowaste2gas.dk>; Lars Ditlev Mørck Ottosen <ldmo@eng.au.dk>; Nabin Aryal <aryalnabin@eng.au.dk>
Emne: SV: Forgasningsgas i biogasreaktor.

Hej Michael,

Takker for svar.

Prototypen har vi selv udviklet.
(testen på Risø blev muligt takket være en Innobooster-bevilling)

Vores termiske konvertering (forgasning) foregår ved en konstant temperatur og alle processer er 100 % styret. Derved er det muligt af fremstille en konstant gaskvalitet uden tjæreforbindelser.
(Samtidig bevares gødningsværdien i vores biokoks)

Vores forgasser er specielt designet til de såkaldte restprodukter, da det er vores overbevisning at teknologi netop til udnyttelse af energien i "restsegmentet" vil blive efterspurgt.

Gassens indhold af nitrogen kan reduceres ved ændringer i processen, men ikke helt elimineres, da noget af kvælstoffet sikkert stammer fra infeed.

Hvor får i jeres syngas fra ?

Med venlig hilsen
Dan
GGC-Tech / B2G

Tlf. 2437 5063

Fra: Michael Vedel Wegener Kofoed <mvk@eng.au.dk>
Sendt: 21. oktober 2019 20:41
Til: Dan F. Christiansen <dfc@DanskEnergiRaadgivning.dk>
Cc: Lars Ditlev Mørck Ottosen <ldmo@eng.au.dk>; Nabin Aryal <aryalnabin@eng.au.dk>
Emne: SV: Forgasningsgas i biogasreaktor.

Hej Dan,

Vi arbejder pt med udviklingen af bioteknologi til omdannelsen af syngas (H2, CO, CO2) til metan i projektet FutureGas, støttet af Innovationsfonden. Her udvikler vi reaktorsystemer til formålet og undersøger lige nu flere af de ting du spørger om. Svarene foreligger nok om et par måneder☺
Kan dog sige at N2 er et problem for jer, hvis I skal nå naturgaskvalitet, hvis det er det i sigter efter?

Har I udviklet prototypen med DTU Risø?

Mvh

Michael

Michael Vedel Wegener Kofoed
Project Director, APPLAUSE
Researcher
mvk@eng.au.dk
Mobile: +45 9352 1051

Aarhus University
Department of Engineering
Biological and Chemical Engineering
Hangøvej 2
8200 Aarhus N

AARHUS UNIVERSITET

From: "Dan F. Christiansen" <dfc@DanskEnergiRaadgivning.dk>
Date: Monday, 21 October 2019 at 15.50
To: Lars Ditlev Mørck Ottosen <ldmo@eng.au.dk>
Subject: Forgasningsgas i biogasreaktor.

Hej Lars,

Tak for snakken.
Hermed et par ord vedr. vores projekt med termisk forgasning af restprodukter.

Status kort;
Prototype bygget og testet på DTU Risø.
Infeed; pelleteret fiberfraktion fra biogasproces - granuleret spildevandsslam – pelleteret kyllingemøg
Gassammensætning typisk; 22 % H2 – 22 % CO – 2 % CH4. (derudover N og CO2)

Vi leger som sagt med tanken om på et tidspunkt at injicere vores gas i en biogasproces (forsøgsreaktor) for at se om det over tid vil være muligt at booste produktionen af biogas.

Spørgsmål:

1. Hvordan iblandes gassen bedst?

(3/16)

2. Hvor lang tid vil der typisk gå før den mikrobielle population er tilvænnet den nye sammensætning?
3. Er forgasningsgassens indhold af nitrogen et problem? (ca. 30 - 40 vol. %)
4. Skal forgasningsgassen tilføres brint for at få det maksimale udbytte?

På forhånd mange tak for svar.

Med venlig hilsen

Dan F. Christiansen
GGC-Tech / B2G
Tlf. 2437 5063

(4/16)

Dan F. Christiansen

Fra: Maja Nielsen <majn@seges.dk>
Sendt: 15. oktober 2019 15:09
Til: Dan F. Christiansen
Cc: 'Anders Elkjær Tønnesen'
Emne: SV: Vedr. forgasningsgas injiceret i en biogasproces.
Vedhæftede filer: Microbial Biogas enrichment-artikel.pdf

Hej Dan,

Hermed lige et par ord omkring forsøget. Jeg må indrømme at de eksakte tal og den eksakte effekt kan jeg ikke huske. Data fra forsøget må være i en folder på drevet et sted og hedder nok noget omkring 'forgasning' eller 'metanisering'.

1. Jeg kan ikke huske de specifikke tal her. Jeg mener ikke at vi kunne se en balance i tilførsel og produktion, men det er muligt at et kig på tallene vil kunne vise det. Dog, jeg mener at effekten var lavere end hvad vi havde forventet.

2. Gassen blev tilført direkte til reaktoren. Så vidt jeg husker så fik vi modificeret låget på reaktorerne og påsat et langt metalrør som nåede bunden af reaktoren. Om gassen blev injiceret med en sprøjte eller pumpet over kan jeg ikke huske. Vi modtag gassen i en stor gaspose og muligvis blev studsen fra posen påsat et inlet på reaktorlåget, men her er jeg lige lidt i tvivl. Vi arbejdede noget med hvordan vi kunne pumpe gassen i reaktoren, men jeg kan ikke huske hvordan det endte her og hvor langt Carsten nåede med denne del. Det vil klart være en fordel med en kontinuert tilførsel men i starten nok bedre med en punktvis tilførsel for at den mikrobielle population gradvist tilvænnes.

   Et andet aspekt er tilvænningen. Hvis I anvender inokulum fra enten gylle- eller slambaserede biogasreaktorer, så vil jeg forvente at I, i starten, vil se en begrænset effekt men gradvist vil denne øges. Dog, det er samtidigt vigtigt at indholdet af eg. H2S ikke bliver for højt (kan ikke huske hvilket ppm niveau som er maksimalt – men NOO kender dette tal), da det så vil inhibere biogasprocessen. Jeg ved ikke om I har adgang til en GC eller noget svarende, men rimelig karaterisering af gasprøverne vil være en fordel – i hvert fald i forhold til H2S. Jeg er i tvivl om hvor høj koncentration af CO som processen kan tåle, så det er også en faktor som I må prøve at lege lidt med.

   Biogasrektorer er egentligt ret fleksible – mikroorganismerne skal blot have lidt tid til at vænne sig til ændrede forhold. Det er en yderst blandet population af bakterier og archaea som findes i en biogasreaktoer. Sekventerede vi en prøve fra en reaktor vil det da også kun være en brøkdel som vi har et navn på. Derfor er jeg også ret optimistisk omkring at det er muligt at få det til at virke for der er helt sikkert nogle organismer som kan omsætte stofferne.

- AU (Lars Ditlev Mørck Ottosen) har på det seneste lavet en del arbejde på tilførsel af brint til reaktorerne og udgivet i flere artikler med Laura Agneessens og Mads Jensen som førsteforfattere. De har også arbejdet lidt med effekten af størrelsen af gasbobler i forhold til optaget af mikroorg. I reaktoren. Jeg har også vedhæftet en artikel af en phd studerende ved dansk gasteknisk center.

  Hvis I skal lave et mindre set-up og har nogle penge at skyde i det så er 'bioproces control' – et bud på firma. De laver forsøgsreaktorer til biogasproduktion hvor produktionen så monitoreres kontinuert. Jeg har nogle gange lavet yderligere modifikationer på flaske til placering af rør og inlet/oulet hos glaspusteren på Kemisk Institut, AU hvis det er nødvendigt.

Jeg ved ikke om I bevæger jer inden for universitetets murer, men Michael Vedel Kofoed på Institut for Ingenirøvidenskab kunne måske være en potential partner at søge nogle penge med hvis det er. Han har tidligere været på Teknologisk Institut og nu i gang med at starte forskningsgruppe hvor Lars Ottosen – også tidligere TI konsulent og sektionsleder – nu er sektionsleder.

Og sig endelig til – ring eller skriv - hvis I har yderligere spørgsmål. Det er virkelig et spændende projekt I går med.

Venlig hilsen

Maja Nielsen
Anlæg & Miljø

(5/16)

D +45 3339 4325
M +45 3066 2313
E majn@seges.dk

Fra: Dan F. Christiansen <dfc@DanskEnergiRaadgivning.dk>
Sendt: 11. oktober 2019 09:43
Til: Maja Nielsen <majn@seges.dk>
Cc: 'Anders Elkjær Tønnesen' <aet@biowaste2gas.dk>
Emne: Vedr. forgasningsgas injiceret i en biogasproces.

Hej Maja,

Håber du trives i dit nye job ☺

Lige et par hurtige spørgsmål fra en gammel kollega.

Vedr. det forsøg du engang gennemført med injicering af forgasningsgas i en biogas-forsøgsreaktor.
Så vidt jeg husker der var evidens for at metan-produktionen blev stimuleret/forøget efter kort tid.
(mener du fortalte at metan-bakterierne tilsyneladende lige skulle vænne sig til den nye sammensætning i reaktoren)

1. Var der evidens for at alt H2 og CO blev omsat til metan? (var der nogenlunde balance imellem H2/CO ind og CH4 ud ?)
2. Hvor i processen blev gassen injiceret? (var det direkte i reaktoren?)
3. Hvordan blev gassen injiceret? (med perforeret slange i reaktorbunden eller i infeed ?)

Som du nok har gættet arbejder vi videre med vores forgasningsprojekt og har planer om at bygge et pilotanlæg i størrelsen
100 kW. Et rigtigt spændende projekt kunne være engang at forsøge at booste produktionen på en rigtigt biogasanlæg.

På forhånd tak for dine svar.
(du må have en god weekend)

Med venlig hilsen

Dan F. Christiansen.
Biowaste2gas.

Bioresource Technology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Review

An overview of microbial biogas enrichment

Nabin Aryal[a,b], Torben Kvist[b], Fariza Ammam[c], Deepak Pant[d], Lars D.M. Ottosen[a,*]

[a] Biological and Chemical Engineering, Aarhus University, Hangovej 2, DK-8200 Aarhus N, Denmark
[b] Danish Gas Technology Centre, Dr. Neergaards Vej 5B, DK-2970 Horsholm, Denmark
[c] Department of Engineering Science, University of Oxford, Parks Road, Oxford OX1 3PJ, United Kingdom
[d] Separation and Conversion Technology, Flemish Institute for Technological Research (VITO), Boeretang 200, Mol 2400, Belgium

ARTICLE INFO

Keywords:
Methane enrichment
Biogas upgrading
Methanation
Hydrogenation
In-situ
Ex-situ and Bioelectrochemical System

ABSTRACT

Biogas upgrading technologies have received widespread attention recently and are researched extensively. Microbial biogas upgrading (biomethanation) relies on the microbial performance in enriched H_2 and CO_2 environments. In this review, recent developments and applications of CH_4 enrichment in microbial methanation processes are systematically reviewed. During biological methanation, either H_2 can be injected directly inside the anaerobic digester to enrich CH_4 by a consortium of mixed microbial species or H_2 can be injected into a separate bioreactor, where CO_2 contained in biogas is coupled with H_2 and converted to CH_4, or a combination hereof. The available microbial technologies based on hydrogen-mediated CH_4 enrichment, in particular *ex-situ*, *in-situ* and bioelectrochemical, are compared and discussed. Moreover, gas-liquid mass transfer limitations, and dynamics of bacteria-archaea interactions shift after H_2 injection are thoroughly discussed. Finally, the summary of existing demonstration, pilot plants and commercial CH_4 enrichment plants based on microbial biomethanation are critically reviewed.

1. Introduction

Methanation is the production of methane (CH_4) by thermo-chemical, catalytic and/or biological processes. The catalytic process, referred to as Sabatier process, is already in commercial use and usually performed by reacting hydrogen (H_2) with either carbon monoxide (CO) or carbon dioxide (CO_2) applying predominately nickel catalysis at higher temperatures 500–600 °C (Muñoz et al., 2015). Biogas is a CH_4-rich mixture of gas produced anaerobically by breaking down organic matters, such as energy crops, plant biomass, animal manure, agricultural residues, waste water treatment sludge and other sources of organic waste, in a biological process called anaerobic digestion (AD). The process is mediated by both mesophilic and thermophilic methanogenic microorganisms. AD is a well-established and mature technology. There are several limitations like high operating cost, expensive feedstocks and especially upgrading expenses. AD usually requires significant financial incentives and subsidies to compete with traditional fossil fuel-based energy technologies (Benjaminsson et al., 2013). Nevertheless, it is a key energy source in the emerging market for global renewable energy resources, and considered a key enabling technology for the transition to fossil fuel independency. It is estimated that global commercial biogas facilities and its role as an alternative energy carrier will become progressively important. Currently biogas production in Europe asserts to about 14 billion m^3 in natural gas equivalent and is expected to increase up to 28 billion m^3 in natural gas equivalent (European Biogas Association, 2013).

Typically, biogas contains a mixture of 40–60% CH_4 and 60–40% CO_2, traces of hydrogen sulfide (H_2S), ammonia (NH_3), H_2, oxygen (O_2), nitrogen (N_2) carbon monoxide (CO), hydrocarbons, volatile organic compounds (VOC) and siloxanes (Börjesson and Mattiasson, 2008). In anaerobic organic carbon degradation processes, biogas is generated in a complex process which involves four phases: (i) hydrolysis, (ii) acidogenesis, (iii) acetogenesis/dehydrogenation, and (iv) methanogenesis, all accomplished by syntrophic interaction of different archaeal-bacterial consortia as shown in Fig. 1. Additionally, in acidogenesis, some facultative anaerobes, for example *Ruminococcus, Paenibacillus, Streptococci* etc. convert soluble monomers to various gaseous and soluble metabolic products, for example VFAs, alcohols, CO_2, and H_2 (Ziganshin et al., 2013). Likewise, in acidogenesis, some facultative anaerobes for example *Clostridium, Ruminococcus, Paenibacillus, Streptococci* etc. convert soluble monomers to various gaseous and soluble metabolic products, *viz* alcohol, VFA, CO_2, and H_2. Subsequently, in acidogenesis, microbes like *Aminobacterium, Acidaminococcus, Desulfovibrio* etc. convert monomers into acetic acid and H_2. Finally, in the methanogenesis step, CH_4 is produced from both hydrogenotrophic methanogenic archaea utilizing H_2 and CO_2 or by aceticlastic

* Corresponding author.
E-mail address: kdmo@eng.au.dk (L.D.M. Ottosen).

https://doi.org/10.1016/j.biortech.2018.06.013
Received 7 April 2018; Received in revised form 5 June 2018; Accepted 6 June 2018
0960-8524/

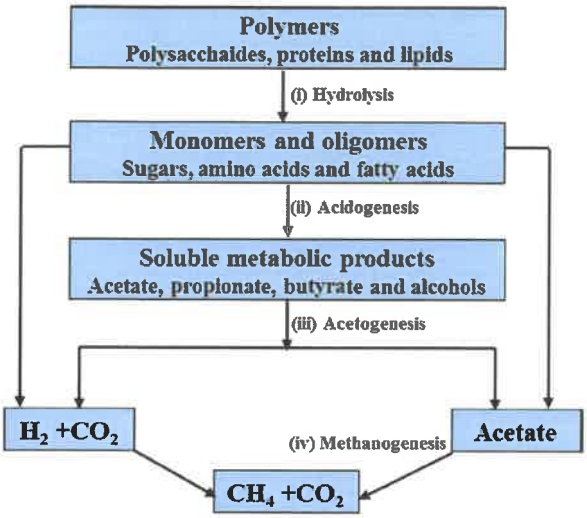

Fig. 1. The conventional approach of anaerobic degradation of organic matter to produce CH_4.

methanogenic archaea via consumption of acetic acid (Angelidaki et al., 1993).

Methanogenesis can be driven by three major pathways: (i) the CO_2 reduction (Wood Ljungdahl) pathway, (ii) the acetotrophic (aceticlastic) pathway and (iii) the methylotrophic pathway. These pathways are differentiated by the nature of the substrate and the energy source used for CH_4 production (Garcia et al., 2000; Liu and Whitman, 2008). The Wood Ljungdahl (WLP) also named hydrogenotrophic pathway is the most widespread and metabolically efficient pathway so far reported for energy generation and carbon fixation (Lever, 2016; Sousa et al., 2013). Although it is present in both archaea and bacteria, some metabolic differences were found in the methyl branch of the WLP in the archaeal phylum including enzymes and cofactors involved in the reduction of CO_2 to CH_4 (Borrel et al., 2016). Furthermore, the WLP in archaea can be involved in the reverse oxidation of organic compounds to regenerate reducing equivalents. For instance, *Thermacetogenium phaeum*, a syntrophically acetate oxidizing bacterium, was describe to use at least part of the WLP enzymes including CODH and tetrafolate-linked redox enzymes to oxidize acetate (Hattori et al., 2005). In the acetotrophic pathway, acetate is split into a methyl group and CO. The former one is used for CH_4 production while CO is oxidized to generate the required reducing power. The methylotrophic pathway involves C-1 compounds such as methyl-amines and/or methanol which could be used both as carbon and energy source. One molecule of C-1 compound is oxidized to generate electrons for the generation of three molecules of CH_4 (Costa and Leigh, 2014). The AD should have a balanced process in all four stages; otherwise it could lead to failure of methanation. For example, rapid acidogenesis stages might cause high acidity due to high accumulation of VFA, thus resulting in the inhibition of methanogenic microorganism due to reduced pH at high VFA concentration. Simultaneously, a rapid methanogenic process could be limited in the hydrolysis stage. (Luo and Angelidaki, 2013a).

Some impurities present in the biogas may have significant adverse impact on its utilization, e.g. making the gas corrosive and induce salt accumulation on process equipment, and increase emissions and hazards for human health (Song et al., 2017). Moreover, contaminants reduce the density, calorific value and wobbe index (WI) of biogas (Jin et al., 2017). Removing these contaminants is necessary in order to increase specific heat, minimize corrosion, and to assure quality required for injection in gas grid network systems. Currently, various biogas upgrading (understood as technologies to remove unwanted molecules from the gas) methods are commercialized, in particular water scrubbing, chemical adsorption, pressure swing absorption, membrane separation and cryogenic separation (Kadam and Panwar, 2017). Among these technologies, water scrubbing is the predominately applied technique accounting for almost 40% of the total upgrading plants (Angelidaki et al., 2018). In a water scrubber, CO_2 is absorbed in water leaving behind enriched CH_4, and the absorbed CO_2 is released back to the atmosphere. Nonetheless, similar to other upgrading technologies, water scrubbing is energy intensive and corrosive to equipment, thus adding extra operating costs to methanation processes. Another important drawback of conventional methods to upgrade biogas is the loss of CO_2 from the gas. Biogas constitutes an excellent source of quite concentrated CO_2 in a completely reduced atmosphere (no oxygen at all) and is therefore very well suited as a carbon source for CO_2 utilization, which will be a quite unique source in future energy systems without fossil fuels. Hence, biological methanation is an attractive alternative for biogas upgrading since as a biological method it is eco-friendly, cost-effective, low energy demanding (Luo and Angelidaki, 2013b; Roy et al., 2015), and makes use of a valuable CO_2 source instead of wasting it immediately. Furthermore, impurities, like H_2S, NH_3, H_2 and CO, may also be utilized by bacteria to upgrade biogas into CH_4 (Zeppilli et al., 2017).

Recently, an increasing number of academic research efforts have been dedicated towards the microbial CH_4 enrichment. In this review, a summary of microbial biogas enrichment is provided. Firstly, H_2 mediated biogas enrichment, in particular *in-situ* and *ex-situ* techniques are reviewed, followed by a brief summary of bioelectrochemical CH_4 enrichment processes from biogas. Finally, large-scale commercial biogas plants based on microbial technique and future research perspectives for advancing microbial enrichment approaches for biogas upgrading are discussed.

2. Biogas enrichment in anaerobic digestion

Biological CH_4 enrichment is an emerging concept for high volumetric CH_4 production combined with a conventional biogas plant. The enrichment process is carried out either with *in-situ* injection of H_2 inside the anaerobic digester or with H_2 injection in a separate reactor called *ex-situ*, where biogas is upgraded. H_2 might be produced on site by electrolysis, using renewable electricity from wind turbines or photovoltaic as power. Several European countries along with Denmark have periodically surplus of electricity produced from wind turbines. Storing excess of electricity in H_2 and use it to directly upgrade biogas constitutes an attractive approach for these countries (Sharman, 2005; Sovacool, 2013). Mixed microbial consortia, widely known as hydrogenotrophic methanogenic archaea, produces CH_4 utilizing CO_2 as a carbon source and externally supplied H_2 as an electron source (Muñoz et al., 2015). Additionally, H_2 mediated methanation would consume CO_2 from biogas plant and, therefore, improve energy density for further utilization, such as transport sector and gas grid injection. Importantly, non-converted 5–30% H_2 by volume with CH_4, would improve combustion properties of biogas as fuel without adverse impact on engines and other appliances (Akansu et al., 2004), but could on the other hand constitute a problem in relation to grid quality compliancy.

Fig. 2 *Ex-situ* and *In-situ* approach for biogas enrichment.

2.1. In-situ enrichment

In-situ biogas upgrading allows efficient use of AD avoiding extra infrastructure for post gas treatment. Nevertheless, direct H_2 injection may affect the performance of methanogens due to increasing H_2 partial pressure, which may result in inhibition of VFAs (propionate and butyrate) degradation (Agneessens et al., 2017; Fukuzaki et al., 1990). A recent study however showed that added H_2 only penetrates less than 1 mm from saturation into an active methanogenic substrate indicating that only a very small fraction of fermenting microorganisms will actually uptake H_2, even in the event of massive *in situ* H_2 addition inside the reactor (Garcia-Robledo et al., 2016). It appears that the

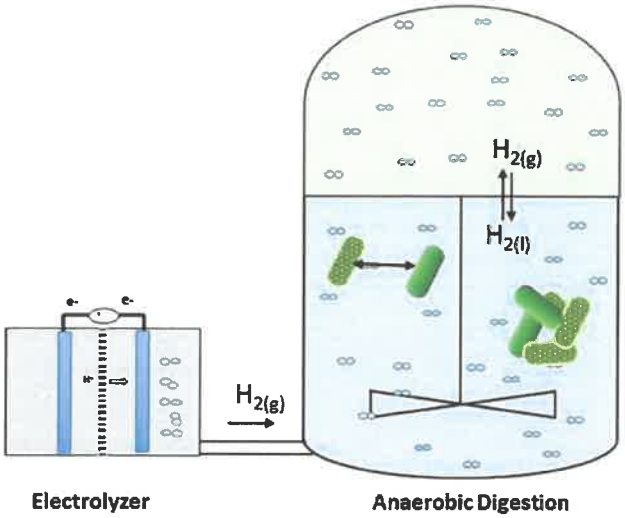

Fig. 2. Hydrogen (H_2) uptake in AD supplied from electrolyzer where "∞" is $H_{2(g)}$ represents in gaseous phase, and $H_{2(l)}$ in the liquid phase.

hydrogenotrophic methanogens under steady state, AD conditions are starved on H_2 (as also predicted by thermodynamics) and that these organisms under non limiting substrate conditions can increase their metabolic activity several order of magnitudes. Another point of attention is however that *In-situ* H_2 injection approaching a 1:4 CO_2:H_2 stoichiometric ratio will result in depletion of CO_2, due to hydrogenotrophic methanogenesis, possibly leading to an increase of pH which may in some cases limit methanogenic activity (Luo and Angelidaki, 2013a,b). It has however also been observed that homoacetogenesis is an important pathway for H_2 consumption, in particular when CO_2 concentrations becomes low (Agneessens et al., 2018). The production of acetate from CO_2 has a less dramatic influence on substrate pH since the removal of two carbonic acid are somewhat balanced by the production of one acetic acid. On the other hand, CO_2 depletion could cause C-source limitation for autotrophic hydrogenotrophic methanogens, which further confines CH_4 production (Liu and Whitman, 2008). In parallel, H_2 may accumulate in intermediate products, which might affect the partial pressure, pH and limit the mass transfer.

A research team investigated the *in-situ* biogas upgrading in the laboratory by alternating mixing rate and H_2 diffusion process. Alternative mixing speeds of 150 and 300 rpm and two different gas diffusers, particularly column diffuser with 0.5–1.0 mm in diameter, and a ceramic diffuser with 14–40 mm in diameter, were experimented. The biogas was upgraded up to 75% CH_4 when a ceramic H_2 gas diffuser was used at 150 rpm in order to improve H_2 mass transfer (Luo and Angelidaki, 2012). Also, 96.1% CH_4 enrichment was achieved when a hollow fiber membrane (HFM) module with 284 μm diameter of fiber for H_2 diffusion was used in (Luo and Angelidaki, 2013a). Notwithstanding, H_2 diffusion was limited due to biofilm formation in HFM (Luo and Angelidaki, 2013a). In another study, the same researchers further upgraded biogas by introducing H_2 into a 4.5 L Continuous Sterile Tank Reactor (CSTR) containing thermophilic anaerobic mix culture at 55 °C. The CH_4 production rate with H_2 injection was accelerated by 22% compared to the control reactor, and 80% of the injected H_2 was utilized by microbes. However, the H_2 consumption rate was affected by H_2 partial pressure and mixing rate (Luo and Angelidaki, 2013b). Likewise, 98–99% CH_4 enrichment was obtained by injecting simulated coke oven gas (92% H_2 and 8% CO) into an anaerobic digester through HFM. The CSTR was operated at 37 °C and pH 8.0. Martin et al. used a pure culture of *Methanothermobacter thermautotrophicus* and improved by 89% the conversion efficiency of H_2 for biogas upgrading (Wang et al., 2013) (Table 1).

Table 1
Comparison of different *in-situ* biogas upgrading in the laboratory scale reactors.

Reactor Configuration	Temperature (°C)	Inoculum Sources	H_2 diffusion method	Operation mode	Working volume (L)	Hydraulic Retention time (days)	Mixing rate (rpm)	CH_4 enrichment (%)	Reference
CSTR	55	Digested manure	ng	Continuous	3.5	14	300	65 + −3.3	Luo and Angelidaki (2012)
CSTR	55	AD sludge	Ceramic	Continuous	0.6	15	150	75 + −3.4	Luo and Angelidaki (2013a)
			Column	Continuous	0.6	15	150	53 ± 3	
			Column	Continuous	0.6	15	300	68 ± 2.5	
CSTR	55	AD sludge	HFM	Continuous	0.6	15	150	96.1 ± 1.1	Luo and Angelidaki (2013b)
CSTR	37	Sewage sludge	HFM with coke oven gas	Continuous	2	10	200	98.8 ± 0.3	Wang et al. (2013)
CSTR	60	*Methano-thermobacter thermautotrophicus*	Biogas H_2 + CO_2	Continuous	3–3.5	8	700	ng	Martin et al. (2013)
CSTR	35	Anaerobic granules,	80%–H_2 20% CO_2	Batch	0.05	ng	150	86%	Xu et al. (2015)
UASB	55	Anaerobic granulars	ng	Batch	0.2	5	6[c]	81.3 ± 0.6	Bassani et al. (2016)
				Batch	3.5	20	200	85.1 ± 3.7	
CSTR	38	Biogas Sludge	H_2 pulse injection	Batch	2	20	1000	100	Agneessens et al. (2017)

CSTR: Continuous Sterile Tank Reactor, AD: Anaerobic Digestion, ng: Not given, WWTP: Waste Water Treatment Plant, UASB: Upflow Anaerobic Sludge Blanket Digestion, HFM: Hollow fiber membrane, [c]Hour, [d]Recirculation in $mL.h^{-1}$.

Likewise, Xu et al. evaluated *ex-situ* and *in-situ* upgrading and the impact of anaerobic granular in batch mode where 86% CH_4 enrichment was achieved. The authors used acidified products consisting of acetate, propionate, and ethanol acclimated granules and glucose-acclimated granules. The authors claimed that interactions between the bacterial species in the glucose-acclimated granules was the key factor for the observed rapid consumption of CO_2 and H_2 (Xu et al., 2015). Similarly, packing materials, such as rushing rings and alumina ceramic sponge, were used in a UASB reactor in thermophilic conditions in an attempt to increase gas-liquid H_2 mass transfer, and the biogas was upgraded from 58 to 82% CH_4 (Bassani et al., 2016). Recently, Agneessens et al. experimented with kinetics of H_2 uptake applying pulse H_2 injection in *in-situ* biogas upgrading, which stimulated the adaptation of methagenogenic mixed culture towards H_2 environment and resulted in 100% CH_4 enrichment (Agneessens et al., 2017). In parallel, mass transfer limitation was also overcome. Although these experiments all demonstrate the biological potential for *in situ* methanation of H_2 added to AD exists, benefitting from already present microbial communities, all of them are laboratory studies in few liters volume. In addition to often being much less active per volume that industrial digesters, where thermophilic reactors produce 2–3 times their own volume in biogas d^{-1}, the laboratory experiments completely fail to address the mass transfer challenges of adding H_2 to full scale digesters, and truly full scale experiments are yet to be published in the literature. One exception for the scaling problem is the trickling bed like reactors which use a gas phase to distribute H_2 and CO_2 directly to a biofilm of methanogens. Here, the mass transfer barrier in the gas phase can be more or less neglected due to the possibility and high shear turbulence of the gas and a diffusion coefficient being about four orders of magnitude higher in gas compared to aqueous substrate. Therefore, scalability of the performance of smaller reactors can be expected in trickling bed like filters (Table 2).

2.2. Ex-situ enrichment

Ex-situ enrichment consists in using CO_2 from external sources, in particular from syngas, biogas, flue gas and H_2, from electrolyzers which are injected into an independent reactor, where enriched hydrogenotrophic cultures (pure or mix) use CO_2 as carbon source and electron acceptor and H_2 as reducing power to produce CH_4 and biomass . *Ex-situ* methanation required separate reactor thus demanding extra volume therefor volumetric CH_4 production rate is lower compared to *in-situ* CH_4 enrichment. To achieve *ex-situ* biogas upgrading a CSTR reactor was used and established a hydrogenotrophic methanogenic mixed culture, then increased CH_4 content up to 95.4%, where the reactor was operated at thermophilic conditions and contained H_2, CH_4, and CO_2 at 60:25:15 ratio at the start. H_2 mass transfer from headspace to reactor was the limiting parameter, thus increasing the mixing speed from 500 to 800 rpm improved the biomethanation process in 0.6 L volume reactor (Luo and Angelidaki, 2012). The same research group further used two-stage reactors coupling AD with *in-situ* H_2 generation both in mesophilic and thermophilic conditions. The biogas was upgraded up to 89% CH_4 in a mesophilic reactor and 85% in thermophilic reactor. Increasing the pH in the second reactor did not limit methanation, indicating the adaptation ability of microorganisms (Bassani et al., 2015). The same authors further evaluated the efficiency of up-flow reactors with stainless steel, alumina ceramic membranes diffusers and combination of both with different pore sizes. Interestingly, the device with larger pore size showed better output gas quality, up to 96.3% CH_4, achieving the best kinetics and better mixing properties (Bassani et al., 2017). Furthermore, an anaerobic trickle-bed reactor was operated with biofilm-bound methanogenic archaea where the biofilm formed by more selective species of microorganisms built a micro-climate and stimulated the metabolic process of H_2 conversion up to 99%, thus 97.9% CH_4 enrichment was achieved (Burkhardt and Busch, 2013; Strübing et al., 2017). Xu et al. evaluated the combined

Table 2
Comparison of different *Ex-situ* biogas upgrading in the laboratory scale reactors.

Reactor Configuration	Temperature (°C)	Inoculum source	Influent gas	Operation mode	Working volume (L)	Retention time (hr)	Mixing speed (rpm)	CH_4 enrichment (%)	Reference
CSTR	55	AD sludge	H_2, CH_4 and CO_2	Continuous	0:6	11–43	800	95.4 ± 2.8	Luo and Angelidaki (2012)
Trickle bed	37	Sludge from WWTP	$H_2 + CO_2$	Batch	26.8	2.25[£]	np	97.9	Burkhardt and Busch (2013)
Two-stage CSTR	35 ± 1	Biogas plants	Biogas from plant	Batch	2	33[£]	200	88.9 ± 2.4	Bassani et al. (2015)
	55 ± 1					20[£]	200	85.1 ± 3.7	
CSTR	35 ± 2	Anaerobic granules	H_2, CH_4 and CO_2 (60%, 25% and 15%)	Batch	0.05	ng	150	ng	Xu et al. (2015)
UASB	35 ± 2		H_2, CO_2 (20% and 80%)	Continuous	5	24	1.8–3[ε]	ng	
Trickle bed reactor	37 ± 2	Mixed anaerobic culture	Biogas, H_2 and CO_2	Continuous	5.8	3.5	na	96	Rachbauer et al. (2016)
CSTR	52 ± 1	Digested biogas plant	H_2, CH_4 and CO_2 (62%, 23% and 15%)	Continuous	1.2	35	300	79	Kougias et al. (2016)
Double UASB in placed in series				Batch	1.4	15	12[ε]	98	
Bubble column reactor				Continuous	1.2	35	12[ε]	97–98	
UASB	55 ± 1	Biogas plant	H_2, CH_4 and CO_2	Continuous	0.85	15	ng	96.3 ± 0.2	Bassani et al. (2017)

CSTR: Continuous Sterile Tank Reactor, UASB: Upflow Anaerobic Sludge Blanket Digestion, AD: Anaerobic Digestion, [£]Day, [ε]Recirculation in Lh^{-1}.

experiment of *ex-situ* and *in-situ* upgrading, using acidified products, in particular acetate, propionate and ethanol acclimated granules and glucose-acclimated granules to test hydrogenotrophic methanogenic activities (HMA) in UASB. The CH_4 upgrading in continuous cultivation was further enhanced due to improvement of hydrogenotrophic methanogenic activities. The author concluded anaerobic granules may be a good option for long-term operation of *ex-situ* CH_4 enrichment (Xu et al., 2015).

A designed anaerobic trickle-bed reactor containing immobilized microorganism packed bed operated at mesophilic temperatures and ambient pressure in a continuous process has significantly improved the mass transfer through three-phase interaction (biofilm–liquid phase–gas phase) and 98% of the CH_4 production was achieved by maintaining optimal liquid recirculation rate between 2.29 and 4.27 L per minutes and H_2 loading rate at 6.0 Nm^3/d in 26.8 L working volume lab scale reactor (Burkhardt et al., 2015). Similarly, Kougias et al. experimented with the importance of different reactor configuration systems and gas circulation rates. Three sets of reactors were designed, (i) double up-follow in series, (ii) CSTR and (iii) bubble column and tested for biogas upgrading. The bubble column reactor showed the best performance for CH_4 enrichment and H_2 utilization with the consumption of almost 80% of the injected H_2. When the liquid recirculation was increased from 4 Lh^{-1} to 12 Lh^{-1}, 98% CH_4 enrichment was achieved in both the bubble column reactor and the double up-flow reactor in series. Unfortunately, the CSTR reactor has shown only 79% CH_4 due to mass transfer limitation in 1.2 L working volume lab scale reactor. The stainless steel diffuser was not robust enough to dissolve fed gas to overcome mass transfer limitations (Kougias et al., 2016). Rachbauer et al. directly injected biogas from a pilot-scale biogas plant and H_2 from an electrolyzer into the trickle-bed reactor with an immobilized hydrogenotrophic culture and achieved 96% CH_4 enrichment by maintaining CO_2 and H_2 ration between 3.67 and 4.15. Thus CO_2 conversion rate highly depends on H_2 loading rate, H_2 and CO_2 ration and retention time inside the reactor. (Rachbauer et al., 2016). Furthermore, Hydrogen mediated biogas upgrading can be done by coupling with *ex-situ* and *in-situ* technology. Recently hybrid concept has been under discussion; nevertheless extra reactor volume and economic biogas upgrading are the main bottlenecks for hybrid technology (Kougias et al., 2017; Xu et al., 2015).

2.3. Hydrogen uptake

H_2 is a key intermediate in methanogenic degradation of organic matter and serves as reducing power for methanogenic archaea to produce CH_4. Although it is not an abundant element in the biosphere, H_2 plays an essential role in anaerobic microbial metabolism. Indeed, H_2 acts as an electron donor under anoxic conditions (Kleerebezem et al., 1999; Lovley and Ferry, 1985; Nedwell and Banat, 1981). In an active thermophilic AD, up to 5 m^3 of H_2 is produced and consumed per m^3 reactor volume per day, although the actual H_2 concentration must be extremely low for thermodynamic reasons, allowing an average lifetime of each H_2 molecule in the millisecond range. Methane production from H_2 is accomplished by H_2 producing syntrophic bacteria and H_2 consuming methanogenic archaea. It is well established that syntrophic microbial communities produce and consume H_2 by utilizing hydrogenases enzymes, which catalyze reversible conversion of H_2 into protons and electrons. For 1 molecule of CH_4 produced, 4 H_2 are consumed, while only 1 molecule of acetate is required to achieve the same yield of CH_4 (Stams and Plugge, 2009). The first reported intraspecific electron transfer was in an co-culture system, where *Methanobacterium ruminantium* consumed H_2 produced by microbes to reduce CO_2 to CH_4 (Bryant et al., 1967; Rotaru and Shrestha, 2016) in AD systems. The syntrophic stage is sensitive to inhibition by H_2 for thermodynamic reasons. For instance, at higher H_2 concentration, methanogenic metabolism is stimulated, and that of H_2 producer is inhibited, and vice versa (Stams and Plugge, 2009). Moreover, H_2 transfer between microorganisms via diffusion can be a rate-limiting step for methanation, which is described by the following Fick's law equation (1):

$$J = D.a.[(C_2 - C_1) * d^{-1}] \quad (1)$$

where, 'D' stands for diffusion coefficient in water, 'a' for the surface of the producers, 'c' for H_2 concentration, 'd' for the distance between two microorganisms and 'J' for the flux of H_2 metabolite. It has been established that the rate of H_2 transfer between species is significantly enhanced when cells aggregate to reduce the distance between microorganisms as shown in Fig. 2 (Stams and Plugge, 2009). In fact, thermodynamics and diffusion laws dictate an extreme intimacy between fermenting and methanogenic microorganisms in order for the methanogenic process to run at a reasonable rate) (Sørensen et al., 2001). The H_2 gas-liquid mass transfer rate can be described by the following Eq. (2)

$$R_t = 22.4\,kLa\,(H_{2gTh} - H_{2l}) \quad (2)$$

where, R_t is H_2 gas–liquid mass transfer rate, 1 mol gas corresponds to 22.4 L at Standard Temperature and Pressure (STP), kLa is gas transfer coefficient, H_2gTh is H_2 concentration in gas phase, while H_2l (mol/L) is dissolved H_2 in liquid phase. Mathematically, H_2 gas-liquid mass transfer rate can be increased by increasing kLa, which depends on reactor configuration and operational conditions (Pauss et al., 1990). Therefore, kLa can be optimized by changing parameters, for instance mixing speed and/or gas recirculation and H_2 diffuser devices (Díaz et al., 2015; Guiot et al., 2011; Luo and Angelidaki, 2013b, 2012). Other than mass transfer, critical parameters, such as H_2 partial pressure, temperature, concentration of microorganisms, type of substrate, organic loading rate, hydraulic retention time, reactor design, mechanical mixing rates have also impact on biological methanation (Lecker et al., 2017).

The stoichiometry of CO_2/H_2 gas ratio using hydrogenotrophic methanogens in a fixed bed reactor was evaluated and then 100% CO_2 conversion efficiency was achieved when a feeding ratio of CO_2/H_2 was maintained at 1:4 (Lee et al., 2012). Similarly, Ju et al. evaluated the impact of operating conditions, especially pH control on the methanation process in a hollow-fiber membrane biofilm reactor. When the reactor was maintained at pH 4.2–5.5, 80–90% CH_4 enrichment was achieved, whereas only 80% CH_4 was enriched when the pH was maintained between 6.5 and 7.5 (Ju et al., 2008). The reactor operated in acidic condition quickly became stable, and enhanced performance was associated with hydrogenotrophic methanogenesis. Kim et al. further evaluated a range of CO_2:H_2 ratios including, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, and 1:8 at controlled pH ≈ 7.1 to 7.3. The best ratio was found with stoichiometry ratio of CO_2:H_2 1:5 due to dissolution rates of H_2 in water (Kim et al., 2013). Similarly, effect of pressure was studied using pure methanogenic culture of *M. thermoautotrophicus* in CSTR and achieved up to 90% CH_4 enrichment by maintaining optimal gassing rate and optimal pressure (Seifert et al., 2014). Similarly, 95% CH_4 enrichment was achieved in a pilot hollow-fiber membrane bioreactor, when the gas was fed through a 0.4 μm membrane. The enhanced conversion efficiency was achieved due to enhanced mass transfer and loading rate (Díaz et al., 2015). Guneratnam et al. demonstrated that temperature has a significant role in the methanation process. Indeed, a 92% CH_4 enrichment was reached at two different thermophilic temperatures in an *ex-situ* methanation process. Interestingly, biological methanation was more efficient in 55 °C compared to 65 °C due to high acetic acid and other VFA production. The accumulated VFA subsequently converted to CH_4 and CO_2 by acetoclastic methanogens resulting in high CH_4 production (Guneratnam et al., 2017).

The partial pressure of gases inside the reactor is also crucial since the pressure can be altered to improve the solubility of gases, reduces the bubble size and increase the contact area between microorganism and gases (Díaz et al., 2015). It is stated that H_2 partial pressure below 0.001 and above 0.101 mbar inhibits the growth and propionate oxidizing bacteria as shown in equation (3)

$$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 3H_2 \quad (3)$$

Furthermore, Cazier et al., reported that H_2 partial pressure lower than 745 mbar inhibits the CH_4 production (Cazier et al., 2015). Another study reported no significant inhibition effect on CH_4 production when 745 mbar H_2 partial pressure was applied. Other factors, such as organic acids, especially lactate accumulation, were suggested to be responsible of pH inhibition of CH_4 production (Ghimire et al., 2018). Additionally, Deublein et al. reported that a partial pressure exceeding 10^{-3} mbar causes an accumulation of volatile fatty acids (VFA) (Deublein and Steinhauser, 2010). Despite that, Luo et al. suggested there is no inhibition of the VFA degradation due to high partial pressure (Luo et al., 2014). Nevertheless, accumulation of intermediate products and effect of partial pressure can be achieved by efficient supply of H_2, in particular slow injection, and pulse injection of H_2 (Agneessens et al., 2017).

2.4. Microbial dynamics in hydrogen mediated biogas upgrading system

Anaerobic digestion involves a variety of metabolic pathways and syntrophic associations among plethora of anaerobic microorganisms. Bacteria hydrolyze polymers into monomers and then produces lactate, volatile fatty acids (VFA) and alcohols. Syntrophic bacteria additionally ferment to acetate, formate, H_2, and CO_2, which are utilized by methanogens as substrates in their metabolic process. Thus syntrophic interactions plays a significant role in maintaining metabolic reactions during AD (Stams and Plugge, 2009). The performance of a methanogens population, composed of acetoclastic and hydrogenotrophic, highly depended on dissolved H_2 concentrations (Angelidaki et al., 1999; Weiland, 2010; Winfrey et al., 1977) High concentration of H_2 ensures accumulation of VFA, whereas low concentration enhances CO_2 and CH_4 formation. Direct injection of H_2 inside anaerobic digester stimulates hydrogenotrophic methanogens, for example *Methanomicrobium*, *Methanoculleus* and *Methanobacterium* (Agneessens et al., 2018; Lovley, 1985; Luo and Angelidaki, 2013b; Winfrey et al., 1977) In normal conditions, approximately 70% CH_4 is produced from acetate, mainly by aceticlastic methanogenesis, such as Methanosarcinales and bacterial syntrophic acetateoxidation, and the remaining 30% is generated by hydrogenotrophic methanogens directly from H_2/CO_2 (Luo and Angelidaki, 2013a,b), ultimately determined by the chemical composition of the substrate fed to the reactor.

An investigation described that H_2 injection not only increased hydrogenotrophic methanogenic activity, but also modified the archaeal community structure with the domination of *Methanothermobacter thermautotrophicus* (Luo et al., 2012). The same research group further investigated simultaneous H_2 utilization in *in-situ* biogas upgrading reactor, where inhibition of acetoclastic methanogenesis was observed due to H_2. The acetate concentration was also increased, which was attributed to higher pH, due to bicarbonate consumption by hydrogenotrophic methanogens. The authors suggested in order to maintain microbial dynamics and H_2 utilization efficiency, pH and H_2 dispersion have to be controlled during the AD process (Luo and Angelidaki, 2013b). Furthermore, microbial dynamic communities were also changed with coke oven gas (SCOG) consisting of 92% H_2 and 8% CO, which contained 64.4% CH_4 (Wang et al., 2013). Prior to SCOG injection, *Firmicutes, Proteobacteria, Bacteroidetes* and *Spirochaetes* species were equally distributed, and played a significant role in hydrolysis and acetogenesis (Pervin et al., 2013; Rivière et al., 2009; Zhang et al., 2009). With injection of SCOG, genus *Treponema* within *Spirochaetes* phyla was significantly higher than in the control experiment (Zhang et al., 2009). The authors further summarized the changes in archaea community with *Methanosaeta* and *Smithella* genus, which were dominant before adding SCOG and drastically decreased due to dynamics shift related to H_2 and CO conversion. Whereas hydrogenotrophic genus *Methanoculleus* and aceticlastic genus *Methanosaeta* were also increased significantly. Thus the claimed methanation occurred due to both hydrogenotrophic methanogenesis (direct) and homoacetogenesis and aceticlastic methanogenesis partnership (indirect) pathways (St-Pierre and Wright, 2013; Wright et al., 2013). Similarly, Bassani et al. investigated hydrogenotrophic methanogenesis after H_2 addition. The bacterial diversity was decreased, which resulted in a more specialized community (Bassani et al., 2015). The same authors further investigated that hydrogenotrophic methanogenic was the dominating activity, which was enhanced with the addition of H_2 and gas recirculation due to conversion of CO_2 and H_2 in the thermophilic granular UASB reactor (Bassani et al., 2017).

Recently, the adaptation of methanogens with pulse H_2 injection in *in-situ* biogas upgrading reactor was researched to improve biomethanisation (Agneessens et al., 2017). The injected H_2 started to be uptaken immediately by hydrogenotrohic methanogens. Methanomicrobiales, methanobacteriales and methanosarcinales were found to be the dominating species. Interestingly, *methanobrevibacter* decreased, while other hydrogenotrophic methanogens of methanobacteriales, in particular, methanobacterium and methanobacteriales, activities increased after H_2 injection. Also, abundance of methanosarcinales decreased to 8.4% indicating a shift towards hydrogenotrophic methanogenesis in the reactor. Decline of hydrogenotrophic activity was observed when H_2 injection was stopped. Thus, the authors claimed that pulse injections of H_2 enhanced its uptake and provided adaptability to methanogenic population during transition periods of low and high concentrations of H_2 inside the reactor. Moreover, Luo et al. investigated microbial community analysis in *ex-situ* biogas upgrading reactor and showed that different archaeal species were involved in mesophilic and thermophilic enriched acetoclastic and hydrogenotrophic methanogenic culture. During experiments hydrogenotrophic methanogenic activities were increased to provide high efficiency for biogas enrichment. The change in microbial dynamics was not significant compared to *in-situ* upgrading (Luo and Angelidaki, 2012). Hydrogen and carbon dioxide gas fermentation was also performed to produce with a pure culture, for example *M. thermautotrophicus* strain. Multiple research concluded methane production was not only dependent on H_2:CO_2 (4:1) ratio but both influent gas rates and dilution rates needed to be taken into account. Furthermore, thermodynamic constraints in particular, (i) energy required for cell synthesis from carbon dioxide; and (ii) entropy drop due to formation of macromolecules from the small molecules of hydrogen and carbon dioxide also remain the main bottleneck for high methane yield (Martin et al., 2013) to enhance the methanogenesis.

2.5. Bioelectrochemical upgrading

Bioelectrochemical system (BES) is an emerging technology for a clean and efficient production of biochemicals and biofuels from low-value wastes including gases like CO_2, using energy derived from renewable sources, in particular solar and wind as shown in Fig. 3 (Aryal et al., 2017; Blasco-Gómez et al., 2017; Geppert et al., 2016; Nevin et al., 2010). In this technology, CO_2 can be metabolically reduced to CH_4 by electroactive methanogens using electrons or reducing equivalents, in particular H_2 derived from cathode (Blasco-Gómez et al., 2017; Geppert et al., 2016; Lovley and Nevin, 2011) Electromethanogens can either directly accept electrons from electrodes or H_2 produced bioelectrochemically for CH_4 production in Eqs. (4) and (5) (Cheng et al., 2009; Schievano et al., 2018). The first report on bioelectrochemical CO_2 conversion to CH_4 and acetate reported by Kuroda et al. dates back to the mid-1990s, and later CH_4 production from CO_2 using electrochemical active microorganisms in BES was further elaborated in 1999 by Park et al. (Kuroda and Watanabe, 1995; Park et al., 1999). Later, in a pioneering study, Clauwaert et al. (2008) used bioelectrochemistry to produce H_2, and then CH_4 combining anaerobic digestion as external reactor. Additionally, Cheng et al. demonstrated production of CH_4 in BESs using CO_2 as a solo carbon source (Cheng et al., 2009). In another study, an electrode was directly placed at the bottom of a UASB reactor and applied potentially to generate H_2, and 25% CH_4 incensement was

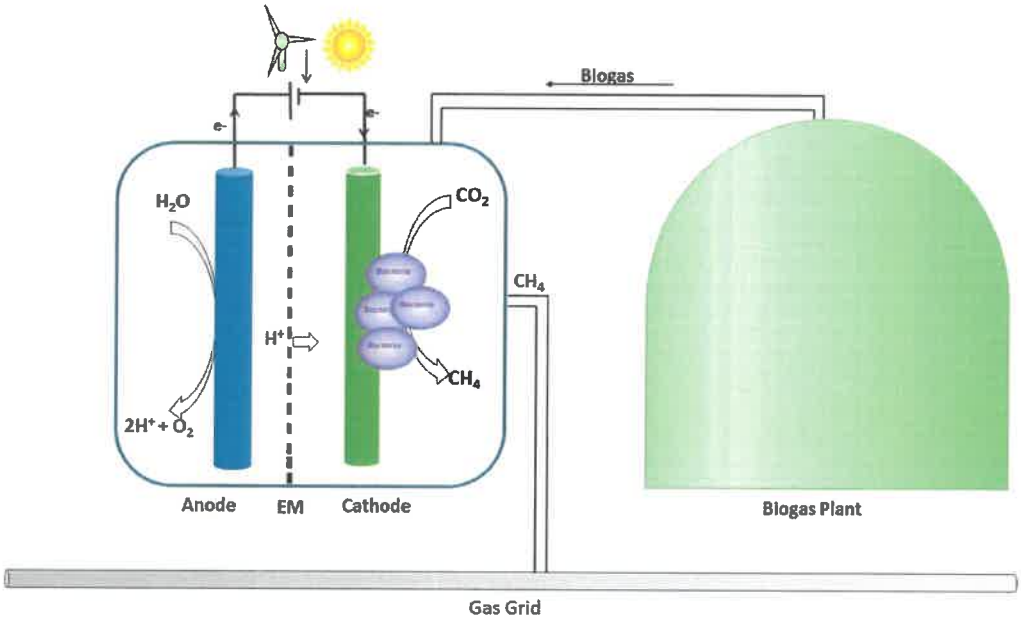

Fig. 3. Bioelectrochemical CH_4 enrichment phenomena discussed in this review, where EM represent Exchange membrane, electrochemical oxidation reaction takes place at the anode to generates O_2 and H^+ and electrochemically active microorganisms utilize the cathode as electron donor and CO_2 from biogas to produce CH_4.

reported due to enhanced hydrogenotrophic methanogenesis activity mediated by *in-situ* H_2 production (Tartakovsky et al., 2011) (Table 3).

$$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O \quad (4)$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (5)$$

$$2H_2O \rightarrow O_2 + 4H^+ + 4e^- \quad (6)$$

Recently, a biogas upgrading approach was proposed with the use of bioelectrochemical systems that allow microorganisms to produce CH_4 when water and electricity are provided in the system. Experimental proof of concept of bioelectrochemical biogas upgrading was described where electrodes were placed inside an anaerobic digester and CH_4 enrichment was achieved (Xu et al., 2014).

In a pioneering study, Xu et al. applied BESs to compare *in-situ* and *ex-situ* biogas upgrading. The assessment was done based on current density, which is equivalent to CO_2 reduction (Xu et al., 2014). The current density of *in-situ* was found almost 2.5 fold, (0.4 A/m^2 vs 1 A/m^2) higher than the one observed in *ex-situ*, indicating that the reactor design allowed better CO_2 mass transfer. Similarly, the operating mode was also found to play a significant role, with 3–4 fold higher CH_4 production obtained in a continuous system compared to batch mode. In the *in-situ* continuous mode, the current density was recorded as 3 A/m^2 when a single BES bioreactor was used. Importantly, 16S rRNA gene amplifications indicated that *Methanobacterium* was the most abundant genus indicating the electroactive phenomenon of CO_2 reduction. Similarly, Bo et al., proposed the coupling of microbial fuel cell (MFC) and anaerobic digestion (AD) to enhance the methane production. Such combination improve the cumulative CH_4 yield by 2.3 fold higher than anaerobic digestion alone (340.2 vs 147.1 mL) (Bo et al., 2014). In parallel, the CO_2 content was decreased significantly from 43.2% to 2.0%. Electrocatalytic production of H_2 due to stainless steel was utilized by bacteria to enrich CH_4 up to 98.1% Such phenomenon demonstrated that coupled MEC–AD is one of the best alternatives to upgrade biogas *via in-situ* H_2 production using a stainless steel BES reactor. The 16S rRNA gene amplifications indicated dominance of hydrogenotrophic methanogens including 5 genus, *Methanocorpusculum*, *Methanospirillum*, *Methanobacterium*, *Methanobrevibacter* and *Methanoculleus* responsible for CO_2 conversion (Bo et al., 2014).

The MEC-assisted AD systems was tested with co-cultivating *Geobacter* and *Methanosarcina* to evaluate co-culture conglomerate of microbial activities for biogas upgrading (Yin et al., 2016). CH_4 yield was increased by 24.1% due to combination of microbial consortium of *Geobacter* and *Methanosarcina*. It has been hypothesized that the presence of *Geobacter* mediated a direct electron transfer for the reduction of CO_2 contained in the biogas. In a recent study, a MEC-assisted AD system further demonstrated that CH_4 yield was 4 times faster than in an AD reactor due to the activity of exoelectrogenic bacteria and acetoclastic methanogen communities in the MFC-AD coupled reactor (Park et al., 2018). In another study, CH_4 yields were increased by 9.4% due to combination of two reactor set ups and electroactive activity of microbial consortium (Gajaraj et al., 2017). Likewise, in a similar reactor set up, 16S rRNA gene analysis showed that dominance of *Methanosarcina thermophile* performs acetoclastic methanogenesis to convert acetate and methanol into CH_4 and *Methanobacterium formicicum* performs hydrogenotrophic methanogenesis to produce CH_4 using formate, H_2, and CO_2 in an MFC coupled reactor with AD (Yin et al., 2016).

A different reactor set up was also tested to evaluate the impact of reactor configuration using two-chamber and single-chamber reactors and it was concluded that the two-chamber configuration enabled a higher cathodic current density production with 98% CH_4 achieved whereas only 56% CH_4 enrichment was obtained in the single-chamber reactor (Liu et al., 2017). The authors also highlighted that the efficiency difference observed between the two configurations may be attributed to oxygen evolution resulting from the water splitting in the single-chamber reactor as shown in Eq. (6). Whereas, the drastic increase in cathodic current in the two-chamber reactors was due to electrochemical H_2 evolution, which was utilized as reducing power by the biocatalysts. Jin et al. used an innovative microbial electrolytic capture, separation and regeneration cell, MESC, and upgraded biogas up to 97% in continuous mode. The authors claimed that this process does not require adding chemicals since acid and alkali are generated and utilized *in-situ* for upgrading. The separated CO_2 at the middle compartment may be further utilized in the methanation process. Notwithstanding, further scaling up is required and may be a major bottleneck for the commercialization of such technology (Jin et al., 2017). In another study, a coupled BES-MESC system was used for chemical absorption and regeneration of CO_2 to upgrade CH_4. The

Table 3
Summary of bioelectrochemical approach for biogas upgrading research as described in Fig. 3.

Reactor Configuration	Upgrading	Applied Potential vs SHE	Inoculum source	Operation mode	Working volume (L)	Current draw	Upgrading/Improvement	Reference
H-cell BES reactor combine with AD	*Ex-situ*	−0.7	Anaerobic granular sludge	Batch	0.8	0.4 A/m^2	CO_2 removal was significant	Xu et al. (2014)
	In-situ		synthetic brewery wastewater	Continuous	0.8	1 A/m^2	10% (v/v) CO_2 was removed	
Single BES cell	*In-situ*		sludge	Continuous	0.8	3 A/m^2	8% (v/v) CO_2 was observed	
Single Chamber BES coupled with MFC	*In-situ*	0.197	Culture from MFC	Batch	0.18	na	98.1% CH_4 enrichment	Bo et al. (2014)
single-chamber BES coupled with AD	*In-situ*	*0.197*	*Geobacter* with *Methanosarcina* sp.	Batch	0.25	304.3 A/m^3	24.1% CH_4 enrichment compare to control	Yin et al. (2016)
AD coupled with MFC	*In-situ*	0.6	WWTP	Batch	0.8	0.122 A/m^2	8–9% CH_4 enrichment	Gajaraj et al. (2017)
AD coupled with MFC	*In-situ*	0.3	FWTP	Batch	20	na	4 times faster than AD	Park et al. (2018)
Two-chamber BES	*In-situ*	−1	Thermophilic anaerobic sludge	Batch	0.5	−120 mA	98% CH_4 enrichment	Liu et al. (2017)
Single chamber BES					0.4	2 mA	56% CH_4 enrichment	
MESC	*In-situ*	1.4	Sludge WWTP	Batch	0.05 and 0.04	1.49 A/m^2	99–100% CH_4 enrichment	Kokkoli et al. (2018)
MESC	*In-situ*	1.5	Domestic WWTP	Continuous	0.05, 0.05, and 0.1	1.7 A/m^2	97.5% CH_4 enrichment	Jin et al. (2017)
Three compartment BES	*In-situ*	0.2	Activated sludge	Batch	0.81	87 mA	More than 90% CO_2 was removed	Zeppilli et al. (2017)

AD: Anaerobic digestion, WWTP: Waste Water Treatment Plant, MFC-Microbial Fuel Cell, BES: Bioelectrochemical System, MESC: Microbial electrolytic capture, separation and regeneration cell, FWTP: Food Waste Treatment Plant, SHE: Standard Hydrogen Electrode.

microbial electrochemical separation cell consisted of four successive chambers: anode, regeneration chamber, absorption chamber and cathode where the gas mixture contained 60% CH_4 and 40% CO_2 was used and then upgraded to 100% CH_4 (Kokkoli et al., 2018). Similarly, Zeppilli et al. tested a three-compartment BES system with anodic accumulation and cathodic chambers for biogas upgrading and nutrient recovery (Zeppilli et al., 2017). In this process, COD oxidation and CO_2 reduction occurred at anode and cathode, respectively, to produce CH_4. The applied potential promoted target ions species, in particular H^+, NH_4^+, OH^-, HCO_3^- and CH_3COO^- migration toward middle intermediate chambers thus separates nutrients while leaving enriched CH_4 gas. Nonetheless, the BES system for biogas upgrading still required to optimize the performances. The methane production and biogas upgrading via BES has not been economically assessed due to the primitive stage of technological development. However, a recent study concluded that acetate production via BES is rather expensive compared to anaerobic digestion due to low production yields, cost of membrane and electrodes in BES (Christodoulou and Velasquez-Orta, 2016). Furthermore, the hydrogen production applying electrolysis process powering with onshore wind energy has further shown a small reduction in production cost. Hence, BES system could be useful to produce the reducing equivalents and upgrade the biogas.

3. Large-scale plants

The scale of the gas upgrading plant profoundly influences the capital and operating costs of the biogas upgrading. The primary challenges for commercialization of microbial techniques are the performance improvement while maintaining low CH_4 enrichment costs. Several laboratory-scale research projects have been dedicated to overcoming problems related to the *in-situ*, *ex-situ* and bioelectrochemical technology. Nonetheless, laboratory-scale reactors are still upscaling while maintaining the highest CH_4 enrichment in biogas least focusing economical part. Microb2Energy, Electrochaea, EcoVolt, and Electrogas are the main microbial based large-scale plants until now.

3.1. MicrobEnergy – BioPower2Gas

BioPower2Gas (http://www.biopower2gas.de/) is the first commercial *in-situ* H_2 injection biogas plant based on biological methanation located in Allendorf, Germany. The biogas plant containing 15 Nm^3/h of CO_2 was used with 2 × 150 kW_e Polymer Electrolyte Membrane (PEM) electrolyzer for biogas upgrading. The community of methanogenic bacteria utilized H_2, which was supplied from the bottom to overcome mass transfer limitation. The resulting gas contained increased percentage of CH_4 from 50% to 75% (Bailera et al., 2017).

3.2. Electrochaea: Biological methanation in Avedøre, Denmark

A proof-of-concept *ex situ* biological CH_4 upgrading plant was constructed by Aarhus University at Aarhus University's facilities in Foulum, Denmark. After demonstration of the pilot plant at Foulum, a Biological Catalysis (BioCat) methanation plant was constructed by Electrochaea, in association with Audi, Hydrogenics, NEAS Energy, HMN Naturgas, Biofos and Insero located at Biofos waste water treatment plant, Avedøre, Denmark (Bailera et al., 2017). The commercial plant aims to operate biological CH_4 upgrading at 5 bars pressure by providing H_2 from electrolysis. The biogas from an anaerobic digester (60% CH_4 and 40% CO_2) or CO_2 separated from an amine scrubbing biogas upgrading process is used for biological methanation. The H_2 will be supplied from a 1 MW_e alkaline electrolyzer with energy supplied by excess wind power, and oxygen as a byproduct will be recycled into the wastewater treatment process. The product gas from an *ex-situ* methanation reactor contained 90–95% CH_4 and then upgraded gas is further purified through a membrane cleaning unit. The resulting gas composition with 98% CH_4, 2% H_2, 1% CO_2 and < 40 ppm H_2O is to be

injected into the 4 bar local gas distribution network of HMN Naturgas (http://www.electrochaea.com/).

3.3. Electrogas: Biological methanation in Foulum, Denmark

A full scale development of *in-situ* biomethanation is taking place at Aarhus University Denmark, applying a direct injection of H_2 into a 1200 m^3 thermophilic reactor utilizing agricultural waste. The mass transfer system applied based on customized venture injectors and is still under development to maximize mass transfer of H_2 and minimize H_2 break through to the produced gas (Jensen et al., 2018).

3.4. EcoVolt® reactor: cambrian innovation

Cambrian Innovation commercialized the EcoVolt® Reactor to converts industrial wastewater into clean water and renewable methane gas. Recently, Cambrian Innovation announced a partnership with the U.S. Army to demonstrate BioVolt™, a self-powered wastewater treatment system, however specific information was not disclosed yet. Company claimed that EcoVolt Reactors has efficiency to remove < 99.9% of contaminants in the spent brewing water, 15% of the brewery's electrical demand eliminating over 1600 metric tons of CO_2 per year. (http://cambrianinnovation.com/).

4. Future perspectives

Biogas can be used for various applications, e.g. in district heating systems, combined with heat and power and injection in the gas grid system for further utilization. Biogas is concentrated and compressed into cylinders and applied for industrial and household cooking proposes in developing countries. Recently, in Sweden, trains and buses have been operated using biogas as fuel with refueling stations on streets. It is expected that the demand for biogas (clean biomethane) driven vehicles will increase worldwide. Nevertheless, certain impurities, for instance water vapor, H_2S and siloxane which corrode parts of engines and pipes have to be removed from the biogas. Additionally, raw biogas exhibits a low heating value and Wobbe index due to the presence of CO_2. Thus, microbial biogas upgrading technologies have received widespread attention, particularly CO_2 perhaps utilized as a carbon source for microbes. H_2 mediated biogas upgrading technology is relatively expensive compared to other available technologies, for example chemical, physical and membrane due to additional cost of H_2 production. In addition, installation costs further increase the overall investment. Nonetheless, the state of the art of H_2 production, such as solid oxide electrolyzer cell (SOEC) and rapid decreases of production cost of renewable electricity in particular, wind power and photovoltaic add an opportunity to decrease the cost of H_2 production. With rapid cost reduction in renewable electricity production, microbial biogas upgrading is likely to be a cost-effective technology compared to physicochemical upgrading. In addition, by employing methanation, the amount of CH_4 from a given limited biomass resource can be increased with 70–100% only using electricity and technology. This is an opportunity which cannot be missed in the societal transition to a fossil independent, biomass driven carbon economy.

The *in-situ* H_2 upgrading design has the obvious advantage of simplicity, lower investments and also laboratory-scale reactor has shown 100% CH_4 enrichment. Although, it faces practical challenges related to low H_2 mass transfer and limited solubility of gases in water. Successful studies have shown the improved mass transfer when H_2 bubble size is minimized, combined with extensive stirring. Nonetheless, it requires extra electricity adding more cost for enrichment. Thus, further studies are needed on mass transfer limitation. H_2 is mixed applying various diffusors, for example metallic diffusor, ceramic sponge, gas permeable membranes and liquid recirculation to overcome mass transfer limitation, thus further research needs to be done for further optimization of *ex-situ* biogas upgrading. Recently, researchers have demonstrated that the hybrid technologies combining membrane pressurized water scrubbing and membrane-cryogenic benefited the techno-economics resulting in low operating costs, less energy consumption and high CO_2 and sulfur capture efficiency (Scholz et al., 2013; Song et al., 2017). Here, the knowledge gained in hybrid upgrading technology may be explored for further development of combining the technology with microbial biogas upgrading technology.

In the *in-situ* biogas upgrading, changes in microbial community is one of the bottlenecks for CH_4 enrichment. Thus further studies on microbial community analysis and investigation of the pathways by which the additional H_2 is consumed by homoacetogenesis are required. Investigations of potential CO_2 addition to form bicarbonate in digester might control the stoichiometrics of H_2:CO_2 ratio in *in-situ* H_2 injection, which might benefit the CH_4 enrichment. However, the overall conclusion of the results so far is that the microbial potential for *in situ* biomethanation is very good and can be managed.

Ex-situ methanation has superior volumetric H_2 consumption rates over *in-situ* technologies. However, *in-situ* biomethanation facilitates has low investment cost (CAPEX) compared to *ex-situ* technologies since existing anaerobic digestion can be used to upgrade the methane. Furthermore, rapid decrease in production cost of renewable electricity was experienced during the last decade, which ultimately lowers the operational cost (OPEX) for electrolyzer to produce hydrogen. Thus more study needs to be granted for economical assessment of *ex-situ* and *in-situ* methanation (Angelidaki et al., 2018; Jensen et al., 2018).

The BES system for biogas upgrading has been under development in a lab-scale reactor and is still at a very early stage of development requiring further scaling-up (Schievano et al., 2018). A major bottleneck for employing BES for biogas upgrading is the long-term functional stability of bio-electrodes, and the quite low current densities reported so far. Much denser and active biofilms must be seen for the technology to have industrial potential. Moreover, understanding of electron transfer mechanism and CH_4 formation on the surface of electrodes is still questionable (Rotaru and Shrestha, 2016; Rotaru and Thamdrup, 2016). In addition, investment costs, in particular those related to reactor design, electrodes and membrane, might further hinder the economical application of this technology. The biofouling of membrane and electrodes for long-term operation has also to be overcome and research further.

5. Conclusions

This review summarizes the CH_4 enrichment from biogas, which is a promising renewable energy option for biomethanation. Mostly, H_2 mediated *in-situ*, *ex-situ* and bioelectrochemical CH_4 enrichment has been summarized. The mass transfer limitation, reactor configuration, and microbial dynamics have to be explored in the future to optimize the microbial CH_4 enrichment. Undeniably, understanding the hybrid upgrading technology, it is further necessary to combine microbial technology for CH_4 enrichment. Furthermore, the knowledge gap between pilot tests and large-scale operations needs to be filled for commercialization of the microbial CH_4 enrichment technology.

Acknowledgement

This investigation was supported by a grant from FutureGas (ID 5160-00006A) project from Innovation Fund, Denmark – Innovationsfonden.

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