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Title: Biomethanation of Syngas by Enriched Mixed Anaerobic Consortia in Trickle Bed Reactors

Konstantinos Asimakopoulos, Hariklia N. Gavala, Ioannis V. Skiadas*

*Department of Chemical and Biochemical Engineering, Technical University of Denmark, Søtofts
Plads 229, 2800 Lyngby, Denmark*

*Corresponding author e-mail address: ivsk@kt.dtu.dk / phone number: [+45 45 25 27 29](tel:+4545252729)

Authors ORCID numbers

Konstantinos Asimakopoulos: 0000-0003-2786-8603

Hariklia N. Gavala: 0000-0002-3924-5334

Ioannis V. Skiadas: 0000-0001-6183-4592

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Conflict of interests

The authors declare that there is no conflict of interest

Abstract

Two identical Trickle-Bed Reactor setups were designed and operated under mesophilic conditions and atmospheric pressure, without pH control, for the biomethanation of syngas consisted of 45% H₂, 25% CO₂, 20% CO and 10% CH₄. The reactors were inoculated with mixed methanogenic microbial consortia formerly

1 adapted to the gaseous mixture. During the start-up of the reactors acetic acid accumulation in the liquid
2 broth resulted in a pH decrease to levels unfavorable for methanogenic activity. This was corrected by
3 introducing a strong phosphate buffer in the medium (K_2HPO_4/KH_2PO_4 : 87 mM/13 mM). Channeling
4 phenomena observed across the trickle bed were eliminated by setting a high liquid recirculation rate
5 ($1600\text{ l}/l_{\text{bed}}/\text{d}$). The reactors were operated for 294 days presenting minor deviations between them at the
6 24 extracted steady states and high cells retention even at a hydraulic retention time of 3.7 days. At a gas
7 residence time of 2.31 hours and a hydraulic retention time of 5.5 days the achieved CH_4 productivity was 2
8 $\text{mmol}/l_{\text{bed}}/\text{h}$ with 93% H_2 and 90% CO conversion efficiency and a 78% electron yield to CH_4 . The conducted
9 study verified that an enriched methanogenic microbial consortium can effectively convert syngas to CH_4 in
10 a trickle bed reactor under appropriate operational conditions.

11 **Statement of Novelty**

12 Syngas can be produced from biomass gasification and also it is a byproduct of several industrial processes.
13 It is carrier of a high amount of energy and carbon that cannot be stored (due to high content of H_2 and CO)
14 unless it is processed. The presented work introduces a potential solution by fixing the carbon and energy
15 content of syngas into CH_4 , a compound that can be easily stored in the natural gas grid. The proposed
16 biological process was, to our knowledge, performed for the first time by enriched mixed microbial
17 consortia in a trickle bed reactor and sheds light on the effects of operational parameters, such as the
18 composition of the mineral medium, the liquid recirculation rate, the hydraulic retention time and the gas
19 retention time.

20 **1. Introduction**

21 The total population of the world is rising very fast, projected by the United Nations to surpass 9.5 billion by
22 2040 [1]. In the same time frame, the global energy consumption is expected to increase by 28% [2],
23 indicating the urgency of a shift from the dependency on fossil fuels, which are limited [3], towards
24 renewable sources of energy. Establishing a biobased economy is also essential due to the tangible effects

1 of climate change on our planet [4]. First-generation biofuels ignited a lot of bickering and controversy
2 targeted over the “food vs fuel” deliberation and, therefore, opened the path leading to second-generation
3 (2G) biofuels production which is more sustainable and attractive to the policymakers [5]. A feedstock for
4 2G biofuels that has recently drawn a lot of attention [6–9] is synthesis gas or syngas, a gas mixture
5 consisting mainly of CO, H₂ and CO₂.

6 Syngas is a product of the gasification of biomass [10] and a byproduct of many industrial processes such as
7 steel milling, petroleum refining and waste incineration [11, 12]. Its abundance and its content in CO and H₂
8 render it, though, an important source of energy to be exploited. Yet, the vast storage capacity of the
9 natural gas grid cannot be used for syngas due to its content in CO, H₂ and CO₂ and, as a result, it has to be
10 processed either chemically or biologically to form storable products. Furthermore, biological syngas
11 conversion presents many advantages compared to the chemical catalysis alternative such as mild
12 temperature and pressure, no demand for fixed H₂/CO in syngas, high product specificity and high
13 tolerance to poisoning from impurities [13, 14]. However, production of 2G biofuels through syngas
14 fermentation has not reached a commercial stage yet, mainly because of the low mass transfer rate of CO
15 and H₂ to the water-based microbial cultures and the relatively low growth rate of the anaerobic
16 microorganisms [15, 16]. Recently, the progress of Lanzatech in its efforts for commercial on site syngas
17 fermentation of CO rich gases from steel mills to ethanol shows that the first fully operational commercial
18 scale plant is approaching [12].

19 The most studied products of syngas bioconversion are ethanol and acetic acid with a trend towards the
20 production of heterologous products springing up along with innovative techniques in metabolic
21 engineering [17]. Apart from liquid biofuels and commodity chemicals, biomethane is gathering more and
22 more attention over the last few years due to its high energy content that allows it to be used as a vehicle
23 fuel and its convenience to be introduced in the natural gas grid when it covers the specifications [15, 18]
24 thus resulting in also exploiting the large storage capacity of the gas grid. In addition, syngas

biomethanation has the advantage to be able to be performed by mixed microbial consortia without the need of sterilization expenses. These consortia are broadly available in waste and wastewater treatment anaerobic environments, hence they come at no cost [19] and they present high robustness since they grow under harsh environmental conditions [20].

The microbial groups that are principally involved in syngas biomethanation are carboxydutrophic hydrogenogens, methanogens and acetogens, hydrogenotrophic methanogens, homoacetogens and acetoclastic methanogens [21]. The biological reactions catalyzed by the aforementioned microbes are presented in Table 1. No matter which sequence of these reactions is followed for the production of CH₄, the maximum overall stoichiometric molar conversion of H₂ plus CO to CH₄ is 25%, independent of the ratio between H₂ and CO and the fraction of CO₂ in syngas. H₂ is an electron donor and therefore acts as an energy source, CO can act both as an energy source and a carbon source while CO₂ is a fully oxidized molecule and can only act as a carbon source. This allows a diverse consortium of microbes to grow syntrophically on this gaseous substrate depending on their preferred energy and carbon source and the occurring metabolites.

Table 1. Biological reactions corresponding to the different microbial groups involved in syngas biomethanation. The standard change of Gibbs free energy was adapted by Grimalt et al. [15]

Microbial Group	Biocatalytic reaction	ΔG° (kJ/mol)
Carboxydutrophic hydrogenogens	$\text{CO} + \text{H}_2\text{O} \rightarrow \text{CO}_2 + \text{H}_2$	-20
Carboxydutrophic methanogens	$4 \text{CO} + 2 \text{H}_2\text{O} \rightarrow 3 \text{CO}_2 + \text{CH}_4$	-210.9
Carboxydutrophic acetogens	$4 \text{CO} + 2 \text{H}_2\text{O} \rightarrow \text{CH}_3\text{COOH} + 2 \text{CO}_2$	-165.4
Hydrogenotrophic methanogens	$\text{CO}_2 + 4 \text{H}_2 \rightarrow \text{CH}_4 + 2 \text{H}_2\text{O}$	-135.6
Homoacetogens	$2 \text{CO}_2 + 4 \text{H}_2 \rightarrow \text{CH}_3\text{COOH} + 2 \text{H}_2\text{O}$	-104.6
Acetoclastic methanogens	$\text{CH}_3\text{COOH} \rightarrow \text{CH}_4 + \text{CO}_2$	-31

Besides the selection of the type of the biocatalysts, a crucial parameter that should be thoroughly considered in syngas fermentation processes is the bioreactor configuration employed since it is associated with the circumvention of the poor gas-to-liquid mass transfer [22]. Undoubtedly, the most studied and used bioreactor in gas fermentations is the stirred-tank due to its simplicity and the limited optimization of

1 parameters needed (impeller configuration, agitation speed, gas flow rate and gas injection module) [23].
2 In order to increase the mass transfer rate in this type of reactors, the goal is to decrease the size and the
3 retention of the gas bubbles and as a result provide higher gas-liquid interfacial area. Compared to stirred
4 tank reactors, trickle bed reactors have been reported to provide improved gas-to-liquid mass transfer
5 rates [24] due to their high surface per volume ratio available from the packing material, where biofilm
6 grows. This advantage comes at a lower cost because of the absence of mechanical mixing. Another major
7 advantage of trickle bed reactors is cell retention because of the inherent ability of a big diversity of
8 microbes to form biofilms [25]. Whereas, in many cases biofilm formation is connected with fouling
9 phenomena [26], this is not the case in trickle bed reactors for syngas fermentation where the organic
10 loading rate is low and does not allow for high microbial growth. The basic drawback is channeling [27],
11 that is the flow of the liquid through limited paths across the packed bed resulting in a non-homogenous
12 biofilm growth in the reactor and lower CH₄ productivity rates. The operational parameters that are
13 amenable to optimization are the liquid recirculation rate, the gas inflow rate and the hydraulic retention
14 time. In addition to these, principal design parameters are the diameter/height ratio of the column, the
15 type of the packing material and its geometry, the module of the introduction of the liquid and the gas in
16 the column as well as the orientation of the flow of the gas and the liquid stream (co-current or counter-
17 current) [28].

18 Trickle bed reactors have been studied for syngas fermentation and biogas upgrade due to the demand of a
19 high gas to liquid mass transfer for these processes. Kimmel et al. [29] and Klasson et al. [30] were the first
20 ones to report the potential of syngas biomethanation with trickle bed reactors and the improved methane
21 productivities achieved compared to bubble columns. In their research the biological conversion of syngas
22 was performed by a synergistic microbial triculture of *Rhodospirillum rubrum*, *Methanosarcina barkeri*, and
23 *Methanobacterium formicicum*. Rachbauer et al. [31] and Burkhardt et al. [32, 33] assessed the use of a
24 mesophilic hydrogenotrophic mixed microbial culture in a trickle bed reactor for the upgrade of biogas
25 produced from anaerobic digestion with the supply of H₂ and showed that the setup could produce a CH₄

rich gas following all the prerequisites to be injected in a natural gas grid. Improved methane productivity rates were reported by Strübing et al. [34, 35] when H₂/CO₂ methanation was performed under thermophilic conditions with mixed microbial consortia. Alternative reactor configurations reported in literature for syngas biomethanation are reverse membrane bioreactors [36, 37] and multi - orifice baffled bioreactors [38].

The purpose of this study was to assess the methanogenic potential of syngas bioconversion by enriched mixed microbial consortia in a trickle bed reactor and designate those operational parameters that affect predominantly the conversion efficiency of the syngas components to CH₄. Furthermore, we examined how the range of liquid byproducts is affected by specific changes in operational parameters. To our knowledge this is the first study of syngas biomethanation in trickle bed reactors by enriched mixed microbial consortia.

2. Materials and Methods

2.1 Inoculum

Anaerobic sludge was collected from the Lundtofte Wastewater Treatment plant (Lundtofte, Denmark) and from a lab-scale manure treating anaerobic digester (Chemical and Biochemical Engineering Department, Technical University of Denmark). The two anaerobic sludges were mixed in equal volumes (50/50 v/v) and then a batch enrichment process, which is meticulously described by Grimalt et al. [39], was applied. Briefly, the enrichments took place in anaerobic serum vials at 37 °C and pH 7 with 1.3 atm, 0.4 atm and 0.3 atm partial pressure of H₂, CO and CO₂, respectively, in the headspace.

The enriched cultures were stored in 15% w/w glycerol freeze stock cultures. Before their inoculation to the reactors, they were reactivated by incubation at 37 °C with 2 atm of synthetic gaseous substrate (45% H₂, 25% CO₂, 20% CO and 10% CH₄) in the headspace.

2.2 Growth medium composition

The medium used for the enrichment process and the reactivation of the microbes as well as for the initiation of the bioreactor experiments is a modified basic anaerobic medium (BA) consisting of a buffering

1 system, salts, vitamins, trace elements, a chelating and a reducing agent. The composition of the growth
2 media is outlined in Table 2. Solution A is an aqueous solution of NH_4Cl (100 g/L), NaCl (10 g/L), MgCl_2
3 hexahydrate (10 g/L) and CaCl_2 dihydrate (5 g/L). Solution B is an aqueous solution of anhydrous K_2HPO_4
4 (151.53 g/L). Solution D is an aqueous trace metals solution containing $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ 2000 mg/l, H_3BO_3 50
5 mg/l, ZnCl_2 50 mg/l, CuCl_2 30 mg/l, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ 50 mg/l, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ 50 mg/l, AlCl_3 , 50 mg/l,
6 $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 50 mg/l, NiCl_2 , 50 mg/l, $\text{Na}_2\text{SeO}_3 \cdot 5\text{H}_2\text{O}$ 100 mg/l, $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ 60 mg/l and chelating agent
7 (NTA 1 g/l). The aqueous Vitamin solution contains vitamins B7, B6, B2, B1, B12, folic acid, nicotinic acid, P-
8 aminobenzoic acid, lipoic, thiotic acid and DL-Pantothenic acid as described by Wolin et al. [40]. Na_2S was
9 injected in the medium at anaerobic conditions after flushing with an inert gas (N_2) and sealing the vial with
10 a rubber stopper.

11 Nevertheless, during the experimental procedures required changes to the medium were applied in order
12 to face observed disturbances from the desired conditions. These changes are described in the Results and
13 Discussion section along with their necessity and their effect on the experimental results.

14 **Table 2. Volume of the solutions added to 1 L of total medium volume**

Solution	Volume of solution per liter of medium
Solution A	10 ml
Solution B	2 ml
Solution D	1 ml
NaHCO_3 (619 mM)	50 ml
Vitamin Solution	10 ml
Na_2S (100 mM)	10 ml
H_2O	917 ml

2.3 Experimental configuration

2

3

4 **Figure 1. Bioreactor Process Flow Diagram.** The basic parts of the lab scale bioreactor setup are 1: Syngas cylinder, 2: Pressure
 5 regulator, 3: Mass Flow Controller, 4: Trickle Bed Column, 5: Liquid reservoir, 6: Liquid Sampling port, 7,11,13: Peristaltic pumps,
 6 8: Gas sampling port, 9: Gas flowmeter, 10: Gas exit, 12: Fresh Medium, 14: Liquid Effluent and 15: Magnetic Stirrer

7 The experimental configuration of the trickle bed bioreactor is presented in Fig.1. Artificial syngas with a

8 composition of 45% H₂, 25% CO₂, 20% CO and 10% CH₄ was supplied from a 50 L gas cylinder (1, AGA

9 Industrielle gasser) and was flowing through a pressure regulator (2, AGA Industrielle gasser) and a mass

10 flow controller (3, Insatech, Denmark) into the anaerobically sealed trickle bed column (4), which was filled

1 with polypropylene/polyethylene packing material (BioFLO 9 - Smoky Mountain Biomedia, USA) with a
2 surface area of $800 \text{ m}^2/\text{m}^3$ and a density of 1 g/cm^3 . The occurring gas mixture after flowing through the
3 trickle bed column was entering the headspace of a liquid reservoir (5) also anaerobically sealed and then it
4 was flowing through a gas sampling port (8) and a gas flowmeter (9, Ritter) before it exited the setup (10).
5 Liquid was continuously recirculated with a peristaltic pump (7, Cole Parmer) from the liquid reservoir to
6 the top of the trickle bed column flowing through a liquid sampling port (6). Continuous liquid inflow and
7 outflow was achieved with an anaerobically sealed bottle containing fresh medium (12) and a collector of
8 the effluent (14) along with two peristaltic pumps (11, 13) operated always at the same flow rate. The
9 experimental work was split in two Phases. During Phase 1, the feeding of fresh liquid medium and the
10 removal of equal volume of medium from the liquid reservoir was done manually (that is parts 11, 12, 13
11 and 14 were not installed). In Phase 1 equipment 1 – 10 and 15 was installed. Equipment 11-14 was
12 installed after the end of Phase 1 and before the start of Phase 2. In Phase 2 the feeding and the liquid
13 outflow was performed continuously with a peristaltic pump (Cole Parmer). During Phase 2, parts 11, 12, 13
14 and 14 were installed and the recirculation pump (7) was replaced with a peristaltic pump (Watson Marlow)
15 that could achieve higher flow rates. In short, in terms of the configuration of the reactor the principal
16 distinction between Phase 1 and Phase 2 was the manner in which fresh medium was added in the reactor
17 and liquid effluent was removed. In Phase 1 fresh medium was added manually on a daily basis while in
18 Phase 2 it was added continuously with a peristaltic pump.

19 The trickle bed column and the liquid reservoir vessel were made of borosilicate glass and had a double
20 wall which allowed temperature regulation via water recirculation from a water bath (Julabo). The liquid
21 reservoir was positioned on top of a magnetic stirrer (15, IKA) set at 150 rpm so as to avoid accumulation of
22 solids (cells or inorganic precipitates) in the liquid reservoir. The temperature was controlled at 37°C , the
23 pressure was atmospheric and. The volume of the bed was 180 ml (height to diameter ratio was 4.18) and
24 the liquid volume in the reservoir was 180 ml during Phase 1 and 220 ml during Phase 2. Due to the size of
25 the experimental setup a pH probe could not be fitted and thus the pH was not controlled. Two identical

1 setups were used in order to check the reproducibility of the process. The reactors were covered with black
2 tape so as to ensure that no photosynthetic growth will arise. Finally, in order to verify that the conversion
3 of the syngas was taking place only in the column and not in the reservoir, gas samples were occasionally
4 taken with the addition of a gas sampling port between the bed and the reservoir. The contribution of the
5 reservoir on the conversion of the substrate was negligible.

6 10 steady states were obtained during Phase 1 and 14 steady states were obtained during Phase 2. A
7 steady state was considered when in two consecutive days the deviations in the conversion efficiencies of
8 the syngas components and the methane production rate were less than 5%. The obtained 24 steady states
9 are presented in Tables 3 and 4.

10 **2.4 Analytical Methods**

11 Gas samples were analyzed for the determination of the molar composition of CO, H₂, CO₂ and CH₄ with an
12 SRI 8610C gas chromatograph. The chromatograph was equipped with a Molsieve 13X column and a silica
13 gel column with the orientation of the flow of the gaseous phase towards any of the two columns being
14 regulated by a rotating valve. 50 µl gas samples (model 1750 gas-tight syringe by Hamilton) were injected in
15 the chromatograph and the column temperature was held at 65 °C for 3 min. Then a temperature ramp of
16 10 °C/min was initiated until the temperature of 95 °C when the rotating valve was changing the direction
17 of the flow towards the silica gel column with a 24 °C/min ramp. CO, H₂ and CH₄ were separated by the
18 Molsieve 13X column before the rotation of the valve while CO₂ was separated by the silica gel column
19 after the rotation of the valve. The composition of liquid samples containing volatile fatty acids (VFAs)
20 (acetic acid, propionic acid, butyric acid, iso-butyric acid, valeric acid and caproic acid) and alcohols (ethanol
21 and 1-butanol) were determined with High Performance Liquid Chromatograph (Shimadzu, USA) equipped
22 with a refractive index detector and an Aminex HPX-87H column (Bio-Rad). The temperature of the column
23 was stable at 63 °C and the flow of the eluent (12 mM H₂SO₄) was constant at 0.6 ml/min. Each sample run
24 lasted 40 min. A spectrophotometer (DR2800, Hach Lange) was used to measure the optical density of
25 liquid samples at 600 nm (OD₆₀₀) in order to indicate changes in the microbial cells concentration.

2.5 Sampling from the Trickle Bed Reactors

Liquid samples (2 ml) were collected from the liquid sampling ports of the trickle bed reactors on a daily basis and this procedure was combined with equivalent addition of fresh medium in the reactors. The liquid samples were used for the measurement of the OD, the pH and the VFAs concentration. The composition of the gas phase was also measured on a daily basis offline. A gas-tight syringe was used to collect gaseous samples which were injected in the gas chromatograph for analysis, immediately after collection. During Phase 1 there was no continuous liquid effluent and influent since fresh medium was introduced manually as a pulse. Therefore, the term liquid replacement was used instead of the term hydraulic retention time (HRT).

2.6 Optical Assessment of Channeling Phenomena in the bed

After the end of Phase 1, when the positive effect of the liquid recirculation rate on the conversion efficiency of the substrate was observed, an optical test of the distribution of the liquid phase in the column was performed. Fluorescein (Dye content 95% - Sigma Aldrich) was dissolved in water at a concentration of 1 g/l and was inserted in a trickle bed reactor packed with Bioflo 9 packing material identical to the ones in operation. The liquid recirculation rate was gradually increased from 160 to 400 l/l_{bed}/d (step 80 l/l_{bed}/d) with the peristaltic pump (Cole Parmer) used in Phase 1 and from 800 to 2000 l/l_{bed}/d (step 80 l/l_{bed}/d) with the peristaltic pump (Watson Marlow) used in Phase 2. The solution of fluorescein was dying with a bright yellow color the paths from which it was trickling through across the bed. The effect of the liquid recirculation rate on the trickle flow regime was optically assessed.

2.7 Calculation formulas

The product yields were calculated as moles of electrons assimilated into product i per moles of e⁻ released from the consumption of the energy carriers of the gaseous substrate, CO and H₂. Each mole of H₂ carries 2 moles of e⁻ ($n_{e^{-}H_2} = 2 e^{-}mol$) and the same applies for CO ($n_{e^{-}CO} = 2 e^{-}mol$).

$$e^{-}yield_i = Y_i \left(\frac{e^{-}mol_{product}}{e^{-}mol_{substrate}} \right) = \frac{n_{(i)} \cdot n_{e^{-}(i)}}{n_{H_2} \cdot n_{e^{-}H_2} + n_{CO} \cdot n_{e^{-}CO}} \quad (1)$$

1 $n_{(i)}$: amount of product i produced in $[\frac{mmol}{l_{bed} \cdot h}]$

2 n_{H_2} : amount of H_2 consumed in $[\frac{mmol}{l_{bed} \cdot h}]$

3 n_{CO} : amount of CO consumed in $[\frac{mmol}{l_{bed} \cdot h}]$

4 $n_{e^-(i)}$: moles of electrons fixed per mol of i released $[e^- \cdot mol]$

5 For the calculations presented in this work $n_{e^-(i)}$ was equal to 8 for CH_4 and acetic acid, 14 for propionic
6 acid and 20 for butyric acid.

7 For example, assuming that 5 mmol/ l_{bed} /h of CH_4 were produced while 30 mmol/ l_{bed} /h of H_2 and 20
8 mmol/ l_{bed} /h of CO were converted, the yield would be $e^- yield_{CH_4} = \frac{5 \cdot 8}{30 \cdot 2 + 20 \cdot 2} = \frac{40}{100} = 40\%$.

9 The conversion efficiency of the gaseous substrates CO and H_2 was calculated as follows, similarly to the
10 removal efficiency in biotrickling filters:

11
$$C_i\% = \frac{c_{(i) inflow} - c_{(i) outflow}}{c_{(i) inflow}}\% \quad (2)$$

12 With $c_{(i)}$ expressed in $[\frac{mmol}{l_{bed} \cdot h}]$.

13 The composition of syngas used for substrate was recommended by an external partner as the outflow of a
14 pilot scale biomass gasifier. CO_2 is at a high stoichiometric excess and, as a result, its conversion efficiency is
15 not presented in this study.

16 Since CH_4 was both a product and a neutral gas in the inflow the amount of CH_4 flowing in the reactor was
17 subtracted from the total amount of CH_4 flowing out of the reactor in order to calculate the net CH_4
18 production.

1 Empty Bed Residence Time (EBRT) was defined as the amount of time that the gas would stay in the trickle
 2 bed of the reactor assuming an empty bed and no conversion along the bed.

3
$$EBRT = \frac{V_{bed}}{u_{gas}} \quad (3)$$

4 V_{bed} : Volume of the empty bed (ml)

5 u_{gas} : Gas inflow rate (ml/h)

6 2.8 Set of Experiments pefromed in Phase 1 and Phase 2

7 Steady state data were collected principally during the two Phases of operation of the bioreactors. The
 8 operational parameters for each steady state were chosen in a methodological way so that important
 9 information could be extracted regarding their effect on the performance of the bioreactors. In Phase 1 ten
 10 steady states were achieved (table 3) studying the consequences of the increase of the Liquid Recirculation
 11 Rate and the decrease of the EBRT under a steady liquid replacement of 10 ml/d (HRT = 18 d).

12 **Table 3. Operational conditions at which the different steady states were obtained during Phase 1**

Steady State Number	Days	Liquid Replacement (ml/d)	Liquid Recirculation (l/l _{bed} /d)	EBRT (h)
1	80-86	10	160	6
2	87-91	10	200	6
3	92-99	10	240	6
4	100-103	10	280	6
5	104-106	10	320	6
6	107-111	10	320	5
7	112-115	10	360	5
8	116-126	10	360	4.3
9	127-134	10	400	4.3
10	135-140	10	400	3.8

13
 14 In contrast to Phase 1, during Phase 2 the Liquid Recirculation Rate remained steady (except for steady
 15 state 24) at 1600 l/l_{bed}/d and the effect of the HRT and the EBRT were studied (table 4). During Phase 2 the

1 concentration of the trace metals in the medium was increased 2.5 times and continuous inflow and
 2 outflow of liquid was initiated.

3 **Table 4. Operational conditions at which the different steady states were obtained during Phase 2**

Steady State Number	Days	HRT (d)	Liquid Recirculation ($l/l_{bed}/d$)	EBRT (h)
11	166-176	5.5	1600	3.00
12	177-179	5.5	1600	2.73
13	180-181	5.5	1600	2.50
14	182-194	5.5	1600	2.31
15	195-201	5.5	1600	2.14
16	202-206	5.5	1600	2.00
17	207-211	5.5	1600	1.88
18	212-216	5.5	1600	3.25
19	217-225	11	1600	3.25
20	228-249	11	1600	1.83
21	250-270	5.5	1600	1.83
22	271-282	4.4	1600	1.46
23	283-290	3.7	1600	1.46
24	291-297	3.7	2000	1.46

4

5

6 **3. Results and Discussion**

7 **3.1 Results**

8 **3.1.1 Reactors start-up**

9 Before the initiation of the experiments, the two reactors were flushed with syngas for 4 hours in order to
 10 establish an anoxic environment. The reactors were then inoculated with enriched mixed mesophilic
 11 methanogenic anaerobic consortia in a total liquid volume of 180 ml (60 ml inoculum and 120 ml medium).
 12 The liquid recirculation rate was set at 20 ml/min or 160 $l/l_{bed}/d$ and the syngas flow rate at 10 ml/min (0.3
 13 h EBRT) in order to maintain a high amount of gas supply and thus to secure a fast growth of microbes and
 14 potentially a faster biofilm formation on the packing material. When microbial growth was observed in the
 15 liquid phase a 5 ml/d liquid replacement (manually drawn and injected) with fresh medium was initiated.
 16 The pH was not controlled. The results for the 44 days of operation after inoculation are presented in Fig. 2.

1 Initially a three days lag phase was observed which was succeeded by a fast rise of the concentration of the
2 cells in the liquid phase with a simultaneous production of acetic acid. This resulted in a fast drop of the pH
3 below 6 which is unfavorable for methanogenesis. Production of CH₄ could not be determined during this
4 startup phase due to the high gas inflow rate masking any differences of CH₄ composition between influent
5 and effluent gas streams.

6 On day 15, the liquid broth of the reactor was diluted 5 times with fresh medium, so as to increase the pH
7 and a manual 10 ml/d replacement of the liquid with fresh medium was continued. The syngas flow was
8 decreased to 5 ml/min. However, CH₄ production was still undetectable due to the high methane inflow
9 rate. It was obvious that the increase of the daily liquid replacement volume of the medium decreased the
10 rate of pH drop but it was not enough to maintain the pH at high enough levels (close to 7) for optimal
11 growth of methanogens.

12 The observed increase of acetic acid indicated that under these operating conditions acetogenic bacteria
13 produced it at higher rates than the methanogens could consume it. The formation of biofilm on the
14 packing material was visible after 20 days of operation but only on specific positions within the bed of the
15 bioreactors where liquid was flowing through. This phenomenon is known as channeling and is a challenge
16 with this kind of setups where liquid is not flowing uniformly through the packed bed due to the geometry
17 of the packing material.

Figure 2. Development of OD₆₀₀, acetic acid concentration and pH for the 44 first days of the 2 bioreactors' operation. Upper chart (a) refers to Reactor 1 and lower chart (b) refers to Reactor 2

3.1.2 Change of the buffering capacity of the medium

On day 44, the reactors were emptied of the liquid broth and they were reinoculated with fresh enriched anaerobic culture in a total liquid volume of 180 ml (120 ml medium and 60 ml inoculum) in order to secure growth of all the microbial groups including those that could have been inhibited and washed out during the period the pH was too low. The reactors were operated under batch mode in terms of the liquid but

1 under continuous gas supply at a flow of 0.5 ml/min for the next 15 days. On day 60, the reactors' total
2 VFAs concentration was 1.32 g/l for Reactor 1 and 1.43 g/l for Reactor 2 corresponding to pH values of 6.1
3 and 6.0 respectively. On the same day the low syngas inflow rate allowed us to document for the first time
4 CH₄ productivity at 0.15 mmol/l_{bed}/h and 0.19 mmol/l_{bed}/h respectively.

5 Between day 60 and day 80, it was attempted to establish a medium with a strong buffering capacity able
6 to withhold the pH close to a value of 7 without causing inhibitory effects to the microorganisms. Solution B
7 used for the medium preparation was finally modified to K₂HPO₄ and KH₂PO₄ concentrations of 87 mM and
8 13 mM in the medium, respectively. The medium with the stronger buffering capacity was applied on day
9 73. This resulted in a steady rise of the pH of the reactors to 6.7 – 6.8 after 7 days of operation.

10 **3.1.3 Operation of the reactors under steady state conditions – Phase 1**

11 From day 80 and until day 140 (the end of Phase 1), ten different steady states were reached (table 3)
12 under a constant daily liquid replacement of 10 ml. The two operational parameters that were manipulated
13 were the liquid recirculation rate and the EBRT (by changing the gas inflow rate).

14 Increasing the liquid recirculation rate from steady state 1 up to steady state 5 resulted in higher CH₄
15 productivity and higher conversion efficiencies of H₂ and CO in both reactors (Fig. 3). In Reactor 1, when the
16 liquid recirculation rate was doubled from 160 l/l_{bed}/d to 320 l/l_{bed}/d, the CH₄ productivity increased from
17 0.47 mmol/l_{bed}/h at steady state 1 to 0.62 mmol/l_{bed}/h at steady state 5, while the conversion efficiencies of
18 H₂ and CO increased from 69% and 63% to 80% and 71%, respectively. The same effect was observed when
19 the liquid recirculation rate was increased at lower EBRTs between steady state 6 and steady state 7 as well
20 as between steady state 8 and steady state 9. The increase of the liquid recirculation rate decreased the
21 magnitude of the channeling phenomena within the bed. Higher liquid recirculation rates resulted in a
22 more uniform trickling of the liquid through the packed column as well as a more uniform wetting of the
23 packing material and as a result the potential spots for biofilm formation were increased. Comparing steady
24 state 5 with 6, 7 with 8, and 9 with 10, it can be seen that an increase of the CH₄ productivity was observed

1 when the gas inflow rate increased. Nevertheless, this should also be seen from a conversion efficiency
2 point of view. The conversion efficiency of H₂ and CO decreased when the gas inflow rate increased, which
3 indicated that the biofilm was close to its maximum substrate uptake rate, allowing thus a higher
4 percentage of the gas influent to pass through the trickle bed unconverted. For example, in Reactor 2 a
5 decrease of the EBRT from 6 h to 5 h between steady state 5 and 6 led to an increase of the CH₄
6 productivity from 0.64 mmol/l_{bed}/h to 0.72 mmol/l_{bed}/h, while the conversion efficiencies of H₂ and CO
7 dropped from 82% and 72% to 75% and 63%, respectively. Therefore, the higher CH₄ productivity rate
8 occurred at a cost of a lower content of CH₄ in the outflow gas and a concomitant higher percentage of H₂
9 and CO.

Figure 3. Conversion efficiency of H₂ and CO and productivity of CH₄ for the first 10 steady states

By calculating the moles of e⁻ released from the conversion of CO and H₂ and how these e⁻ moles were distributed to the products, it may be observed in Fig. 4 that in both reactors more than 60% of the released moles of e⁻ were utilized for CH₄ production while 20% to 25% were used for the production of VFAs from acetogenic bacteria in each steady state. The maximum CH₄ e⁻ yield was 77% and was achieved in Reactor 1 at steady state 6. The *unidentified* part of the e⁻ moles fixation can be attributed to microbial growth and maintenance as well as other low concentration byproducts which were not detected.

Figure 4. Product yields expressed in terms of moles of electrons incorporated to the products per total moles of electrons released by the conversion of carbon monoxide and hydrogen as observed during experimental Phase 1

3.1.4 Operation of the reactors under steady state conditions - Phase 2

From Day 140 till Day 168 the reactors were in maintenance and important adjustments took place in order to further improve the substrate uptake potential of the microbes and to establish a continuous mode for the liquid phase. On Day 145 the peristaltic pump used for the recirculation of the liquid to the top of the trickle bed column was replaced with one that could achieve higher flow rates and the pumping speed was at 1600 l/l_{bed}/h so as to further limit the observed channeling phenomena. In addition, a pumping system was installed for the continuous inflow of fresh medium and outflow of liquid broth, as it is pointed out in

Fig. 1. (parts 11 – 14). Furthermore, it was observed that the concentration of the trace metals was a limiting factor for the growth of the microbial communities on the biofilm at higher gas inflow rates and as a result it was increased 2.5 times to the level that it was sufficient without causing any inhibitory effects. The tests for the effects of the trace metals' concentration in the medium were performed only in Reactor 1 so as to secure that the 2nd reactor could still operate in case of process failure from trace metal inhibition. The results showed a rise a of the conversion efficiency of H₂ and CO from 66% and 57% to 91% and 83%, respectively. This was accompanied by a significant increase of the microbial cells concentration in the liquid phase from 0.231 g/l to 0.326 g/ (indicating increased biofilm growth), an increase of the CH₄ productivity from 1.35 mmol/l_{bed}/h to 1.61 mmol/l_{bed}/h and a slight increase of the concentration of acids from 1.49 g/l to 1.61 g/l. When the positive effect was observed on Reactor 1 the same medium started being used for Reactor 2 which presented the same behavior as Reactor 1.

Figure 5. Effect of trace metals' concentration on the conversion efficiency of the substrate and the concentration of VSS in the liquid reservoir. The experiment was performed at 8 days HRT and 3 hours EBRT. TM: Concentration of Trace Metals, x1: initial concentration as described in Section 2.2

1 The reactors were operated from Day 168 till Day 297 (Phase 2) with the applied modifications. During this
2 time period 14 steady states were obtained and the values of the operational parameters are presented in
3 Table 4.

4 From steady state 11 till steady state 17 the HRT and the liquid recirculation rate remained stable while the
5 gas inflow rate was gradually increased so as to examine down to what level of EBRT the biofilm could
6 consume the available substrate. From the results presented in Fig. 6 it is deduced that down to an EBRT of
7 2.31 h the conversion efficiency of H₂ was constantly above 90% while the conversion efficiency of CO was
8 above 80%. From steady State 15 up to steady State 17 the conversion efficiency of the substrate dropped
9 with the EBRT due to the fact that the biofilm had reached its maximal uptake potential. On steady state 18
10 the increase of the EBRT to 3.33 h led to a conversion efficiency of H₂ and CO higher than 90% while CH₄
11 productivity dropped due to the low inflow rate of substrate (syngas). In addition, the percentage of CH₄ at
12 the exit of reactor 1 increased from 24% at steady state 17 to 38% and the percentage of H₂ and CO
13 dropped below 10% (table 5).

14 On steady state 19 the HRT was increased to 11 days and although no significant effect was noticed on the
15 conversion efficiency of the substrate, an important decrease on the e⁻ yield to VFAs was observed (Fig. 7)
16 in both reactors while the e⁻ yield to CH₄ remained unaffected. When the HRT was increased the rate of the
17 nutrients inflow was decreased proportionally and since the gas inflow rate remained the same it is
18 possible that specific nutrients necessary for the acidogenic metabolism in the already existing biofilm were
19 depleted. Moreover, the CH₄ content reached its maximum value at the exit of the reactor (38% for reactor
20 1 and 39% for reactor 2) while CO and H₂ had their minimum values from all the steady states tested. On
21 steady state 20 the gas inflow rate was increased to the levels of steady state 17 while the HRT remained at
22 11 days resulting in a slightly lower CO conversion efficiency and CH₄ productivity than steady state 17.
23 However, when on steady state 21 the HRT was drastically decreased to the level of steady state 17 the H₂
24 and CO conversion efficiencies dropped along with the CH₄ productivity. This is a consequence of the fact

1 that steep changes in the HRT of the trickle bed reactors cannot be followed by fast enough changes of the
2 biofilm structure and microbial composition potentially resulting in the opposite effects than expected. A
3 simultaneous decrease on the EBRT and the HRT was applied during steady state 22 and the efficiencies of
4 the two reactors recovered. However, the reactors behaved for the first time differently. While in both
5 reactors the conversion efficiency of the substrate was in the same levels, the distribution of the energy
6 released in the form of electrons differentiated; in Reactor 1 20% of the electrons were fixed into VFAs
7 while in Reactor 2 this was only 10%, thus allowing for more energy to be available for methanogenesis. On
8 steady state 24 though, when the liquid recirculation rate increased to 250 ml/min the two reactors
9 produced the same results showing that the deviation between them was just transient. Overall, the two
10 reactors run independently but under the same operational parameters for almost 300 days and they
11 presented almost identical behavior, pointing out an adequate reproducibility of the process. This is a
12 compelling element since use of mixed microbial consortia and reproducibility of results has been
13 questioned due to the complexity of their diverse nature [41].

14 **Table 5. Composition of the produced gas at the exit of the reactor during Phase 2**

Steady State Number	H ₂ (%)		CO (%)		CO ₂ (%)		CH ₄ (%)	
	Reactor 1	Reactor 2	Reactor 1	Reactor 2	Reactor 1	Reactor 2	Reactor 1	Reactor 2
11	9%	13%	8%	15%	50%	42%	33%	30%
12	9%	12%	8%	10%	51%	47%	32%	31%
13	10%	11%	8%	9%	52%	49%	31%	32%
14	13%	11%	10%	7%	49%	50%	28%	33%
15	14%	13%	10%	8%	48%	48%	28%	31%
16	16%	15%	11%	10%	47%	48%	26%	28%
17	19%	20%	13%	13%	44%	43%	24%	24%
18	7%	10%	7%	10%	48%	43%	38%	37%
19	6%	8%	6%	7%	50%	46%	38%	39%
20	18%	20%	13%	17%	45%	40%	24%	23%
21	25%	24%	21%	18%	34%	37%	20%	21%
22	22%	21%	15%	13%	41%	44%	22%	22%
23	21%	24%	14%	15%	43%	40%	22%	21%
24	22%	21%	15%	15%	41%	41%	22%	23%

15

1

2 Figure 6. Conversion efficiency of H₂ and CO and productivity of CH₄ for the steady states of Phase 2

Figure 7. Product yields expressed in terms of moles of electrons incorporated to the products per total moles of electrons released by the conversion of carbon monoxide and hydrogen during Phase 2.

3.2 Discussion

3.2.1 Production of VFAs and their effect on syngas biomethanation

The accumulation of acetic acid during the start-up of the reactors indicated that the acetoclastic

methanogens could not consume it fast enough in order to balance its production from the fast growing

1 acetogens. The same observation was made by Rachbauer et al. [31] when they initiated their experimental
2 trickle bed reactor for biogas upgrade. It is also well established that high partial pressures of CO reduces
3 the methanogenic activity [42] of mixed microbial consortia and that the CO dehydrogenase of acetogens
4 has higher affinity for CO than the one of methanogens [43]. Esquivel et al. [44] assessed the impact of CO
5 on methanogenesis under batch conditions on anaerobic sludge and at CO partial pressures ≥ 0.11 atm they
6 estimated partial inhibition of the methanogens with simultaneous accumulation of acetic acid. In addition,
7 adverse effects on methanogenesis of H_2/CO_2 have been reported by acetic acid concentrations as low as
8 1.2 g/l [45]. Therefore, it is of vital importance to maintain the acetic acid concentration at low levels during
9 syngas biomethanation.

10 For the examined process configurations in the present study, where no pH control was applied, the
11 accumulation of acids resulted in a fast pH drop to inhibitory levels for the methanogenic archaea. In
12 addition, it is well documented that changes on the pH of the microbial environment cause alterations on
13 the intracellular pH and the electrochemical gradient across the membrane of the microbes, thus,
14 influencing their metabolism and their bioenergetics [15]. Since the size of the reactors did not allow pH
15 control and in order to alleviate the negative effects of acetogenesis, it was necessary to increase the
16 buffering capacity in the medium. This was achieved by adding a K_2HPO_4/KH_2PO_4 (87 mM/13 mM) buffer in
17 the medium which resulted in maintaining the pH at high enough values for the growth of methanogens.
18 Nonetheless, the addition of a high phosphate concentration buffer is not recommended for full scale
19 plants. An additional measure, applied during Phase 2, was the use of low HRTs which was linked to the
20 sufficient provision of nutrients for microbial growth. The concentrations of acetate, propionate and
21 butyrate (which were the only VFAs detected at significant concentration) in the reactors are presented in
22 Fig. 8 along with the pH values. It is evident that the pH was rather stable during the experiments with
23 small fluctuations which could not have a serious effect on the performance of the methanogens.

1 VFAs production from mixed microbial consortia performing syngas biomethanation and biogas upgrade
2 has also been reported under thermophilic and extreme-thermophilic conditions [46, 47]. Strubing et al. [34]
3 performed biological methanation of CO₂ in a trickled bed reactor at 55 °C and experienced significant
4 reduction of H₂ conversion when no pH control was applied due to the activity of homoacetogenic bacteria.
5 Kougias et al. [48] further investigated the causes of the occurred acetate accumulation in thermophilic
6 anaerobic bioreactors performing 16S rRNA sequencing of the microbiome. They observed a high
7 population of homoacetogenic species and hydrogenotrophic methanogens but no presence of acetoclastic
8 methanogens.

Figure 8. Distribution of VFAs concentration and pH in the reactors for the second phase of the experiments. Upper chart (a) refers to Reactor 1 and lower chart (b) refers to Reactor 2

3.2.2 Effect of the Liquid Recirculation Rate on Biofilm Formation

An important factor affecting significantly the performance of the bioreactors during Phase 1 was the liquid recirculation rate. It was visible that when a low liquid recirculation rate was applied, the liquid was

1 trickling down the bed through specific narrow paths leaving a part of the bed dry. Thus, the available
2 surface area for biofilm formation was limited significantly. In order to evaluate the effect of the liquid
3 recirculation rate on the conversion efficiency of the substrate a progressive increase was applied from
4 steady state 1 till steady state 5 (Fig. 3). The results showed a linear increase of the substrate uptake and
5 finally at steady state 10 it could be clearly seen that the biofilm was covering the entire length of the bed.
6 Further increasing the liquid recirculation rate up to $1600 \text{ l/l}_{\text{bed}}/\text{d}$ on Phase 2 resulted in an even bigger
7 expansion of the biofilm, which was visually detected, compared to Phase 1. This observation was verified
8 also from the flow of 1 g/l fluorescein solution across the column at step-wise increased liquid recirculation
9 rates (section 2.6). As the flow was increasing from 160 to $1600 \text{ l/l}_{\text{bed}}/\text{d}$, a higher fraction of the bed was
10 dyed. Above $1600 \text{ l/l}_{\text{bed}}/\text{d}$ no significant increase of the dyed area in the bed was visually observed. Kimmel
11 et al. [29] also observed an increase in the conversion efficiency of CO when they operated their trickle bed
12 reactor at higher liquid recirculation rates. The effect of the liquid distribution on the trickle flow regime
13 has been addressed by Mederos et al [49] (in general and not for biomethanation of syngas though) and
14 several factors (such as the construction of the bed, the size and the geometry of the packing material and
15 the manner the liquid and the gas are introduced in the reactor) have been demonstrated to hinder an
16 ideal plug flow. A major bottleneck of trickle bed bioreactors or biotrickling filters is bioclogging, namely the
17 accumulation of biomass in the packed bed resulting in an undesired rise of the pressure drop across the
18 column [50]. This phenomenon was not present in this study since the organic loading rate introduced in
19 syngas fermentation is not high enough to establish a very thick biofilm while the concentration of VSS did
20 not surpass the value of 0.6 g/l .

21 The minimization of the negative effects of channeling phenomena is crucial when scaling up the setup
22 since $1600 \text{ l/l}_{\text{bed}}/\text{d}$ is not a feasible flow. A higher diameter and thus a larger bed volume is expected to
23 decrease the wall effect since most of the liquid flow would be able to trickle down through the bed
24 without coming in contact with the wall of the column. In addition, dedicated devices such as nozzles would

be employed for the introduction of the liquid in the top of the column so as to improve the distribution of the liquid amongst the packing material.

3.2.3 Effect of the Gas Inflow Rate on Syngas Biomethanation

The gas inflow rate constitutes an indication of the substrate uptake potential of the formed biofilm. High gas inflow rates usually lead a system to be mass transfer limited since there is not enough time for the liquid phase surrounding the biofilm to get saturated on the gaseous compounds [51]. On the other hand low gas inflow rates can designate kinetic limitations of the system if any exist [51]. Increasing the gas (substrate) inflow rate can indicate the point where the system has reached its maximum conversion capacity. That is, when the conversion efficiency of the available substrate has reached a maximum (for the applied operating conditions). Further increase of the gas inflow rate will result in a drop of the conversion efficiency and as a consequence, the CH₄ content of the gas exiting the reactor will decrease. This is what happened on the transition from steady state 14 to steady state 15 and up to steady state 17 (Fig. 6). Dependent on the desired outcome regarding the CH₄ content of the produced gas, the gas inflow rate can act as an optimization objective. Therefore for the case of the present study, if the target was set on maximizing the CH₄ content of the effluent gas the optimal operational choice would be steady state 14 or steady state 19. On the other hand, if the target was set on maximizing the CH₄ productivity (amount of methane produced per reactor volume and time) then the optimal choice would be steady state 24 for the first reactor and steady state 22 for the second. Compared to biotrickling filters that deal with volatile organic compounds under aerobic conditions and have EBRTs lower than two minutes [51], reported EBRTs for syngas fermentation surpass the two hours (table 6) indicating the adverse nature of this anaerobic process.

3.2.4 Comparison with literature

The biomethanation of biomass derived syngas has not been in the spotlight yet and, as a result, there are scarce reports of continuous bioreactors performing this process [23]. One of the first attempts for

1 methane production from a CO rich syngas mixture was demonstrated by Klasson et al. [30] who in contrast
2 to the current study used a defined tri-culture of microbes (the photosynthetic bacterium *R. rubrum*
3 performing the water-gas shift reaction and two hydrogenotrophic methanogens, *M. formicicum* and *M.*
4 *barkeri*). The researchers compared two setups, a trickle bed bioreactor and a packed bubble column
5 bioreactor and the maximum methane productivity rates achieved with each of the setups was 3.3
6 mmol/l_{bed}/h and 0.4 mmol/l_{bed}/h, respectively. It was reported that a 100% conversion of CO was possible
7 up to an EBRT of 2.45 h and the maximum CH₄ content in the exit of the trickle bed bioreactor was between
8 28% and 32%. At a similar EBRT of 2.5 h in the present study, 93% of the introduced H₂ and 88% of CO was
9 converted resulting to a final CH₄ content of 31%. Nevertheless, in the present study there was no need for
10 sterilization of the bioreactors due to the high diversity of the used enriched microbial consortia and no
11 need for a light source to ignite photosynthetic growth. Based on their results, they recognized the
12 advantage of the trickle bed reactor over the bubble column regarding the mass transfer rate of the
13 sparingly soluble syngas compounds. Later, the same research group compared the performance of two
14 trickle bed reactors with different volumes (1 l and 26 l) embodied with the aforementioned triculture and
15 the same syngas mixture as a scale-up scenario [29]. Nonetheless, the high-volume reactor reached to a
16 maximum CH₄ productivity rate of 0.45 mmol/l_{bed}/h at an EBRT of 8.7 h, which was 6 times lower compared
17 to the one reported for the low-volume reactor. The difference was attributed to the influence of the
18 channeling phenomena since it was observed that the radial liquid distribution was more limited in the
19 large reactor due to the selection of packing material with a 2 times bigger nominal diameter compared to
20 the small reactor. This phenomenon is not expected to occur if a smaller size of packing material is selected
21 so as to form narrower trickle paths in the bed and if the liquid is sprinkled in the bed through a nozzle in
22 order to expand its distribution across the top surface of the column.

23 Westman et al. [37] demonstrated an alternative approach in order to overcome the bottlenecks of mass-
24 transfer limitation and low cell retention during syngas fermentation. The researchers encased anaerobic
25 mixed microbial consortia from sewage sludge in porous hydrophilic polyvinylidene fluoride membranes

1 with a shape of sachets and then placed them in a reverse flow reactor where syngas with a composition of
2 55% CO, 20% H₂ and 10% CO₂ was introduced from the bottom of the vessel. The maximum CH₄
3 productivity achieved was 0.35 mmol/l_{reactor}/h with a 95% H₂ and an 88% CO conversion efficiency. They
4 also compared their results with an identical setup where the cells were not encased in the sachets
5 calculating the maximum methane productivity at 0.2 mmol/l_{reactor}/h, an observation that showed the
6 advantageous performance of the biofilm based system that was able to tolerate higher syngas inflow rates.
7 Compared to the present study a 10-fold lower maximum CH₄ productivity was achieved which can be
8 attributed to the high content of CO in the inlet and a potential lower mass transfer rate of the selected
9 bioreactor configuration. On a different perspective Guiot et al. [19] assessed the potential of a 30 l gas lift
10 reactor inoculated with granular sludge to convert a gas stream containing only CO as a carbon and energy
11 source in mesophilic conditions. When the gas recirculation rate was 29 l/l_{reactor}/d and the CO retention
12 time 8.6 h, the CH₄ productivity reached its maximum value of 0.126 mmol/g_{VSS}/h with a 67% CO
13 conversion efficiency and a 95% yield to CH₄. The researchers argued that the low mass transfer of CO to
14 the media was the reason that higher CO conversion efficiencies were not reached. In the present study,
15 the conversion efficiency of CO reached a value as high as 93% at an EBRT of 3.25 h. On the other hand Luo
16 et al. [52] combined CO fermentation to CH₄ with anaerobic digestion in a hollow fiber membrane
17 bioreactor so as to assess the versatility of the formed biofilm to elevated pressures of CO. The results
18 showed that the microbial communities were able to tolerate the inhibitory effects of CO even at a
19 pressure of 1.58 atm in the fibers and fully consumed it when the gas retention time was set at 4.8 h.

20 Besides syngas biomethanation, trickle bed bioreactors have been tested for similar biological processes
21 such as H₂/CO₂ methanation and biogas upgrade. Compared to the inlet gas composition tested in this
22 study, the biomethanation of H₂/CO₂ is occurring in a less adverse environment because of the absence of
23 CO from the gas phase. Burkhardt et al. [32] deployed a 26.8 l counter-current flow trickle bed reactor
24 inoculated with digested sewage sludge for the conversion of 80% H₂ and 20% CO₂. The maximum specific
25 CH₄ productivity at batch experiments was found to be 2.18 mmol/l_{bed}/h with a gas flow rate of 2.3 l/l_{bed}/d

1 and a liquid recirculation rate of 10.7 l/l_{bed}/d. Based on these experiments the same research group
2 designed a continuous process with a scaled-up version of the reactor at 61 l bed volume and the substrate
3 was a gas mixture of 79% H₂ and 21% CO₂ [33]. At an EBRT of 3.16 h and a liquid recirculation rate of 3.1
4 l/l_{bed}/d the CH₄ productivity was 2.78 mmol/l_{bed}/h and 98% CH₄ concentration in the outflow. Finally, with
5 the same bioreactor configuration in a lower scale (5.8 l) Rachbauer et al. [31] managed to fully convert 4:1
6 H₂/CO₂ to CH₄ with a productivity of 3.4 mmol/l_{bed}/h at an EBRT of 2.3 h and a liquid recirculation rate of 62
7 l/l_{bed}/d. Compared to the present work similar maximum methane productivities were achieved in the
8 aforementioned studies. The high concentration of CH₄ in the outflow is a result of the perfect
9 stoichiometric ratio of H₂/CO₂ in the inflow whereas in the present study the composition of syngas in the
10 inflow is far from the ideal stoichiometry which would demand an additional supply of pure H₂ with an
11 inflow of 1.15 times the inflow rate of syngas.

12 A comparison between the efficiencies achieved during the present study with those from similar
13 configurations already reported in the literature (Table 6) clearly shows that the used setup (reactor and
14 microbial culture) is among the most efficient. It is anticipated that further optimization (composition of
15 the mineral medium, choice of packing material and manner of gas and liquid injection in the reactor) can
16 result in even higher methane productivities. In systems like those studied in this work, the size can play a
17 significant role affecting their efficiency (see section 3.2.2 about wall effect and liquid introduction).
18 Therefore, it is expected that scaling up of the setup should be done with cautiousness taking into account
19 all the challenges presented so far in the literature.

20
21 **Table 6. Syngas biomethanation technologies reported in literature at their optimal conditions**

Reactor Type	Volume (ml)	Temperature (°C)	Inoculum	Molar Syngas Composition	CH ₄ Productivity (mmol/l _{bed} /h)	Gas Retention time (h)	Reference
Packed bubble column	3260	34	Triculture (<i>R. rubrum</i> , <i>M. formicicum</i> ,	15 % Ar, 9.6 % CO ₂ , 55% CO,	0.4	7.24	[30]

			<i>M. barkeri</i>)	20.4% H ₂			
Trickle bed reactor	736	37	Triculture (<i>R. rubrum</i> , <i>M. formicicum</i> , <i>M. barkeri</i>)	14.8 % Ar, 9.9 % CO ₂ , 55.6% CO, 20.4% H ₂	3.3	2.45	[30]
Trickle bed reactor	26052	37	Triculture (<i>R. rubrum</i> , <i>M. formicicum</i> , <i>M. barkeri</i>)	14.74 % Ar, 9.72 % CO ₂ , 54.42% CO, 21.11% H ₂	0.45	8.70	[29]
Trickle bed reactor	1051	37	Triculture (<i>R. rubrum</i> , <i>M. formicicum</i> , <i>M. barkeri</i>)	14.82 % Ar, 9.67 % CO ₂ , 55.62% CO, 19.68% H ₂	2.8	2.10	[29]
Reverse membrane bioreactor	600	55	Anaerobic culture from municipal solid sludge digester	55% CO , 20% H ₂ , 10% CO ₂	0.35	Not mentioned	[37]
Trickle bed reactor	61000	37	Anaerobic sludge from sewage plant	79% H ₂ , 21% CO ₂	2.78	3.2	[32]
Trickle bed reactor	5800	37	Anaerobic culture from a digester	80% H ₂ , 20% CO ₂	3.40	2.3	[31]
Trickle bed reactor	180	37	Enriched anaerobic consortia	45% H ₂ , 25% CO ₂ , 20% CO, 10% CH ₄	2.03	2.31	This study (maximum conversion)
Trickle bed reactor	180	37	Enriched anaerobic consortia	45% H ₂ , 25% CO ₂ , 20% CO, 10% CH ₄	3.34	1.46	This study (maximum productivity)

1

2 4. Conclusions

3 The current study assessed the potential of enriched mixed microbial consortia derived from anaerobic
4 digesters to form rigid biofilms within two trickle bed bioreactors running in continuous mode for 297 days
5 and efficiently convert syngas to CH₄. Moreover, the provided data highlight the key bottlenecks of the
6 process such as pH drop from the accumulation of acids and channeling phenomena in the bed.
7 Maintaining a steady pH (by pH control in case of full scale) in the reactors and guaranteeing a homogenous

biofilm growth across the bed was vital for the improvement of the process. The results showed that CH₄ productivity rates of 2 mmol/l_{bed}/h can be achieved with a 93% H₂ and 90% CO conversion efficiency and an e⁻ yield of 78% at an EBRT of 2.31 hours, an HRT of 5.5 days and a liquid recirculation rate of 1600 l/l_{bed}/d. Higher CH₄ productivities were also possible at a cost of the conversion efficiency of the substrate with the maximum value being 3.34 mmol/l_{bed}/h on the second reactor. It was also demonstrated that, despite the tremendous microbial diversity in these communities and the very long term parallel operation under the same operational parameters, the two reactors presented great reproducibility deviating rarely from one another.

Overall, this work sheds more light on the sparsely researched topic of syngas biomethanation and addresses a new concept that enables the on-site conversion of biomass derived syngas to storable energy in the form of CH₄. The acquired data constitute a strong basis for the further optimization of the process and they could be used as a background for the scaling-up of the set-up.

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