Supplementary information for

Developement of a CO_2-biomethanation reactor for producing methane from green H_2

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Table S1 : Details of the bioreactor equipment

Mark	Apparatus	Features / Function
1, 8	**Top and bottom perforated flanges** **Béné inox** **Ref : 357182-65**	304L / 1.4307; DN65 / 2 $^{1/2}$"; thickness 18 mm Perforated flange for fluid circulation and bioreactor instrumentation
2	**ISO tube for double jacket** **Béné inox** **Ref : 372212-762**	304L / 1.4307; DN65 / 2 $^{1/2}$"; thickness 2 mm Double jacket for temperature control
3	**ISO tube for bubble column** **Béné inox** **Ref : 372212-1142**	304L / 1.4307; DN100 / 4"; thickness 2 mm Bubble column for the methanation reaction
4	**Borosilicate-viewing window** **Béné inox** **Ref: 261421-76**	304L ; DN76 / 3" ; PF = 10 bar. TF = 130°C Part of the bioreactor allowing visualization of the culture medium and correct operation of the gas bubbling system.
5	**Welded boss for instrumentation** **Béné inox** **Ref : 652344-10** **Sealed passage** **Ham-let** **Ref: 768LGSS12MMX3/8 & 760LST12MM**	316L ; 3/8" ; Internal diammeter 12.6 mm Connection for probe instrumentation up to 12 mm in diameter (e.g. pH probe). Used with "double ring" type fittings and Teflon rings.
6	**Porous metal filter** **Poral** **Grade 3**	304L SS; diameter 50 mm; mean pore size 10 µm Promotion of bubbly flow at the reactor inlet (gas supply)
7	**Porous metal filter** **Stem** **Ref. SPR1/8I**	316L SS; cylinder with surface 6 cm²; mean pore size 80 µm Promotion of bubbly flow at the reactor inlet (gas recirculation)

Figure S1 : CAD view of bioreactor with dimensions. Left: front cross-section; right: top view

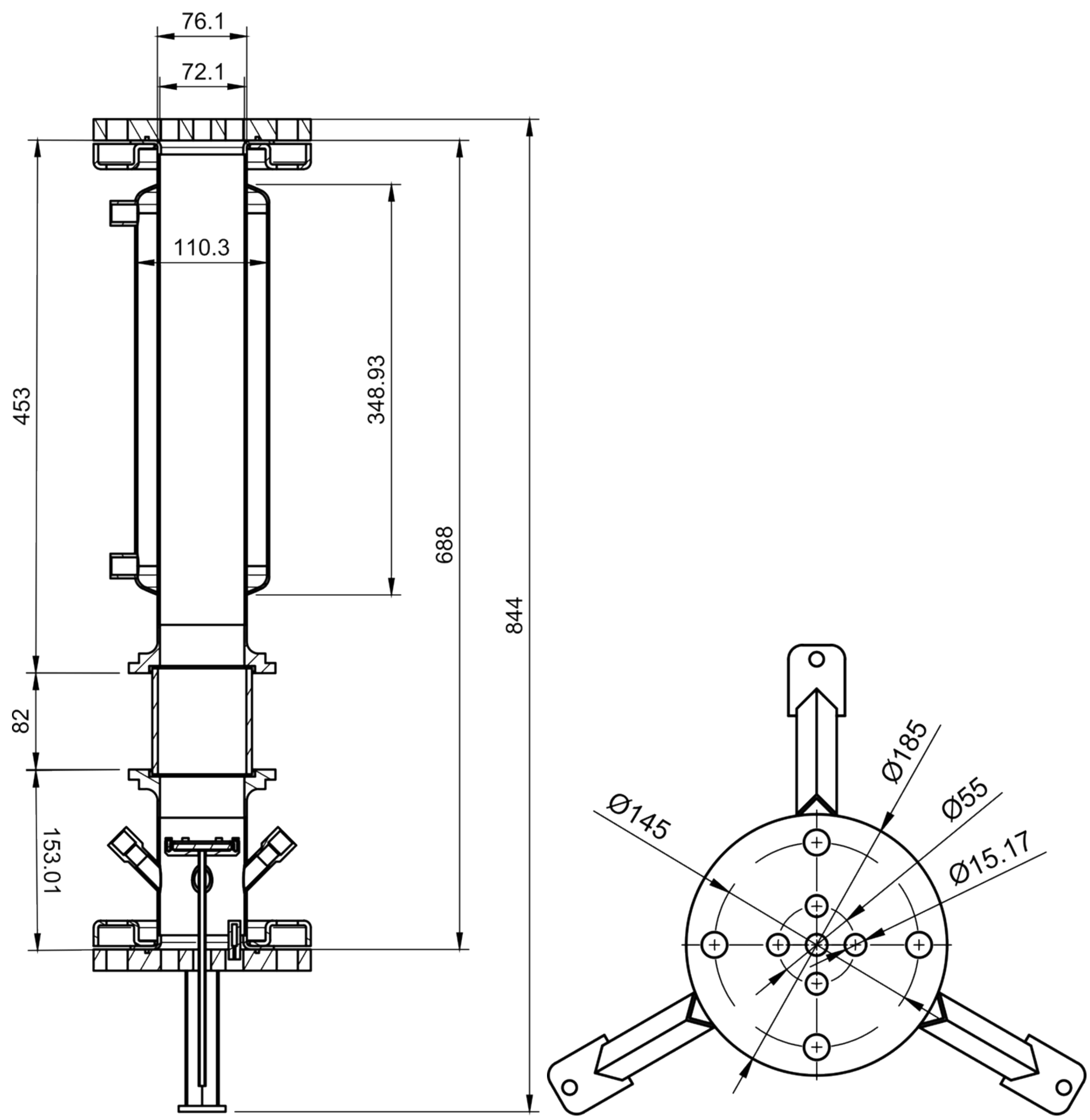


Table S2. Correlations, expressions and experimentally determined values used in the ADR model of the bubble column bioreactor

Dimension	Correlation / Expression / experimental value	Reference
Mass transfer	$\phi_i = k_{L,i}a \cdot \left(H_i^{cc} \cdot C_{G,i} - C_{L,i}\right)$	[1]
Coefficient de transfert de masse	$k_{l,i}^{d_b < 0.0025} = 0{,}31 \cdot \rho_l^{(2/3)} \cdot \left(\frac{g \cdot \rho_l}{\mu_l}\right)^{(1/3)}$ $k_{l,i}^{d_b > 0.0025} = 0{,}42 \cdot \rho_l^{(1/2)} \cdot \left(\frac{(g \cdot \rho_l)^{(1/2)}}{\mu_l^{(1/2)}}\right)^{(1/3)}$	[2]
Volume interfacial area	$a = \frac{6 \cdot \varepsilon_G}{d_b}$	[3]
Holdup	$\varepsilon_g = \frac{U_g}{u_b}$	[4]
Bubble rise velocity	$u_b = \frac{g \cdot \rho_l}{18\,\mu_l} d_b^2$	[4]
Superficial velocity	$U_G = \frac{Q_g}{S_{reac}} = \frac{Q_{n,g}}{S_{reac}} \cdot \frac{P_n}{P_{reac}} \cdot \frac{T_{reac}}{T_n}$	
Bubble size	$d_b = 0.17\ mm$	Experimentally measured in this work
Axial dispersion coefficient	$D_{ax} = K_r\, D_{reac}^{4/3}\, (U_g\, g)^{1/3}\ \ and\ \ K_r = 0.35$	[5]

Table S3: Details of experimental set-up equipment

Mark	Apparatus	Features / Function
A	**Manual pressure regulator Air Liquide** **Ref: BS 50-10-3.5**	Maximum pressure reduction: 10 bar (relative) Maximum flow rate: Q = 3.5 $Nm^3\ h^{-1}$ (from supplier N_2 calibration) Adjustment of the inlet pressure ($H_2 + CO_2$).
B	**Flow regulator** **Bronkhorst** **Ref. F-201CV**	Calibrated for H_2-CO_2 mixture in the range : 0.4 L h^{-1}< Q<2 L h^{-1} Measurement and regulation of the reactor feed rate
C	**Porous metal filter** **Poral** **Grade 3**	304L SS; diameter 50 mm; mean pore size 10 µm Promotion of bubbly flow at the reactor inlet (gas supply)
D	**Porous metal filter** **Stem** **Ref. SPR1/8I**	316L SS; cylinder with surface 6 cm²; mean pore size 80 µm Promotion of bubbly flow at the reactor inlet (gas recirculation)
E	**Pump** **Schwarzer Precision** **Ref : SP 622 EC-BL-DU-HR-DV (24 V DC)**	Positive displacement diaphragm pump Range: 3.39 L h^{-1}< Q < 11.13 L h^{-1} at 24°C and 1.14 bar Adjustment and measurement of the recirculation flow-rate (from top to bottom of the reactor)
F	**Hydraulic guard**	V = 12 mL Protection of the reactor against possible oxygen contamination
G	**Centrifugal Pump** **Ref : TD40G-1260B**	Voltage: 12V DC, Maximum liquid flow: 650 L h^{-1}, Power consumption: 1,6 A Circulation of the heat transfer fluid (DI water) in the reactor's double-jacket
H	**Flow regulator** **Bronkhorst** **Ref. F-211CV**	Calibrated for H_2-CO_2 mixture in the range 0.4 L h^{-1}< Q < 5 L h^{-1} Measurement and regulation of the reactor output flow
I	**Peltier thermal control unit** (See Table S	Cooling or heating for thermal regulation of the culture medium
J	**Bubble column bioreactor with double jacket thermal control**	V = 2.8 L, with double jacket (V = 1.7 L, exchange surface 833 cm²). Methanogen culture chamber for methanation reaction equipped with temperature control and gas dispersion system.
K	**Gas-liquid separator**	V = 108 mL. Separation of the liquid dragged in the upper part of the reactor (due to the possible creation of foam)
L	**Condenser**	V = 143 mL, exchange surface 132 cm² Water condensation of the outlet gas stream
M	**Safety valve** **Ham'let** **Ref. H-900-SS-L-6MM-SL-CE**	Adjustable Protection of the reactor in the event of overpressure
N	**Adjustable check valve** **Ham'let** **Ref. H-400A-SS-L-6MM-50**	Pressure adjustment of the reactor.
O	**Desiccator (alumina)**	V = 45ml Elimination of traces of water in the outlet gas before measuring the composition and the flow rate.

Table S4: Details of the experimental set-up on-line instrumentation.

Mark	Apparatus	Features / Function
1	**Thermal mass flowmeter** **Bronkhorst** **Ref. F-201CV**	See B in Table S3 Measurement of gas supply flow (unit of use L h^{-1}) Measurement uncertainty: ± 0.5 % (± 0.1 % of the full scale)
2	**Pressure sensor** **Keller** **Ref. PAA25**	Range 0 bar < P << 5 bars Measurement of the pressure of the feed gas Measurement uncertainty: ± 1.27 %
3,5, 6	**Temperature, pH and redox potential probe** **Mettler-Toledo** **Ref. NPRO3253i/SG/120**	Ranges 0 °C < T < 100°C ; 0 < pH < 12 ; -513 mV < ORP < 513 mV Measurement of the culture medium temperature (T), pH and redox potential (ORP) Measurement uncertainty: T ± 1.00°C ; pH ± 4.75 % ; ORP ± 5 mV
4	**Turbidity sensor** **DFRobot** **SEN0189**	Range 0 < OD_{600} < 2.5 (absorbance at 600 nm) Measurement of the culture medium turbidity representative of the cell concentration) Measurement uncertainty: ± 1.98 %
7	**Pressure sensor** **Keller** **Ref. PAA25**	Range 0 bar < P < 5 bars Measurement of the reactor's pressure Measurement uncertainty: ± 2.03 %
8	**Thermal mass flowmeter** **Bronkhorst** **Ref. F-211CV**	See H in Table S3 Measurement of outlet gas flow (unit of use L h^{-1}) Measurement uncertainty: ± 0.5 % (± 0.1 % of the full scale)
9	**Pressure sensor** **Wika** **Ref. A-10**	Range 0 < P < 3.2 bars Measurement of the pressure of the outlet gas Measurement uncertainty: ± 0.95 %
10	**Themocouple type K** **RS 397-1264**	Range -40 °C < T < 1100 °C Measurement of the temperature of the outlet gas Measurement uncertainty: ±- 1.5 °C
11	**NDIR gas sensor** **smartGAS** **Ref. F3-212507-05000**	Calibration range 0 < X_{CO2} < 50% Measurement of the CO_2 content in the outlet gas Measurement uncertainty: ± 1.24 %
12	**NDIR gas sensor** **smartGAS** **Ref. 042108-05000**	Calibration range 0 < X_{CH4} < 100% Measurement of the CH_4 content in the outlet gas Measurement uncertainty: ± 1.69 %
13	**Pump** **Schwarzer Precision** **Ref : SP 622 EC-BL-DU-HR-DV (24 V DC)**	See E in Table S3 Measurement of the recirculation flow-rate

Control module

A control module dedicated to temperature control and optical density measurement has been developed specifically for the needs of the experimental bench. The module is entirely based on Arduino® microcontrollers type Mega 2560 Rev3.

The microcontroller is used to regulate the temperature of a heat transfer fluid (water) using a heat exchange system (2). The system consists of a flat heat exchanger (3), Peltier thermoelectric modules (4) with a total output of 120 W, passive heat sinks (2) and fans (1). Temperature control is achieved by alternating the Seebeck and Peltier effects by reversing the thermoelement power supply. The temperature of the heat transfer fluid is controlled by a PT100 sensor and circulated by a centrifugal micropump. The components used are listed in table S5.

The microcontroller is also used to measure the concentration of microorganisms by measuring the turbidity of the medium (Figure S2). The system consists of a turbidity measurement cell (E) integrated in a culture medium recirculation loop using a volumetric pump (8). To obtain the concentration value in OD units, a calibration between the turbidity measurement and the absorbance measurement at 600 nm was carried out in the laboratory (Figure S3). The components used in the fabrication of the turbidity measurement module are listed in table S5.

Figure S2: Diagram of control module for temperature regulation and OD determination

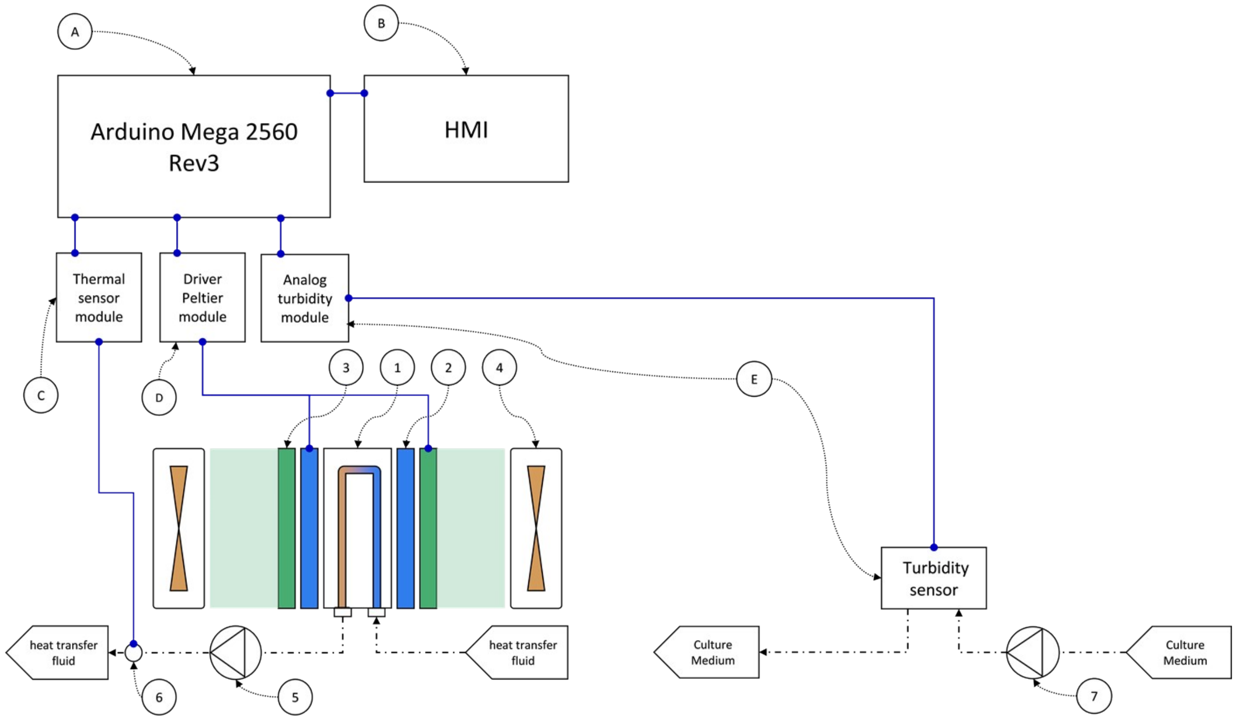


Figure S3: Correlation of optical density measurement at 600 nm in Escherichia coli by turbidity measurement using a DFRobot SEN0189 turbidimeter

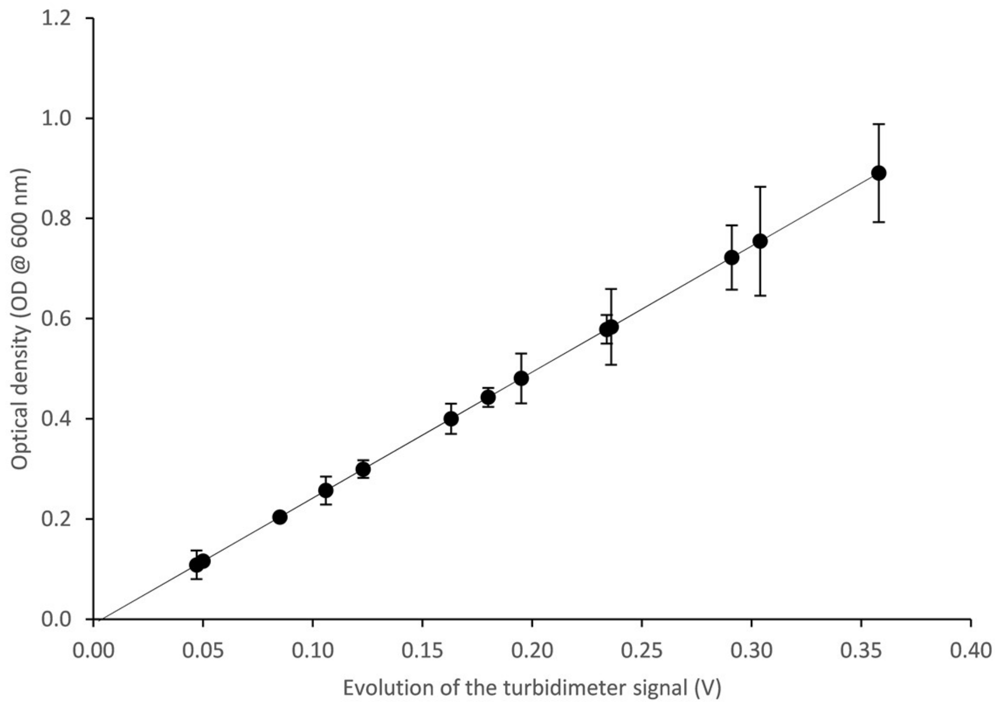
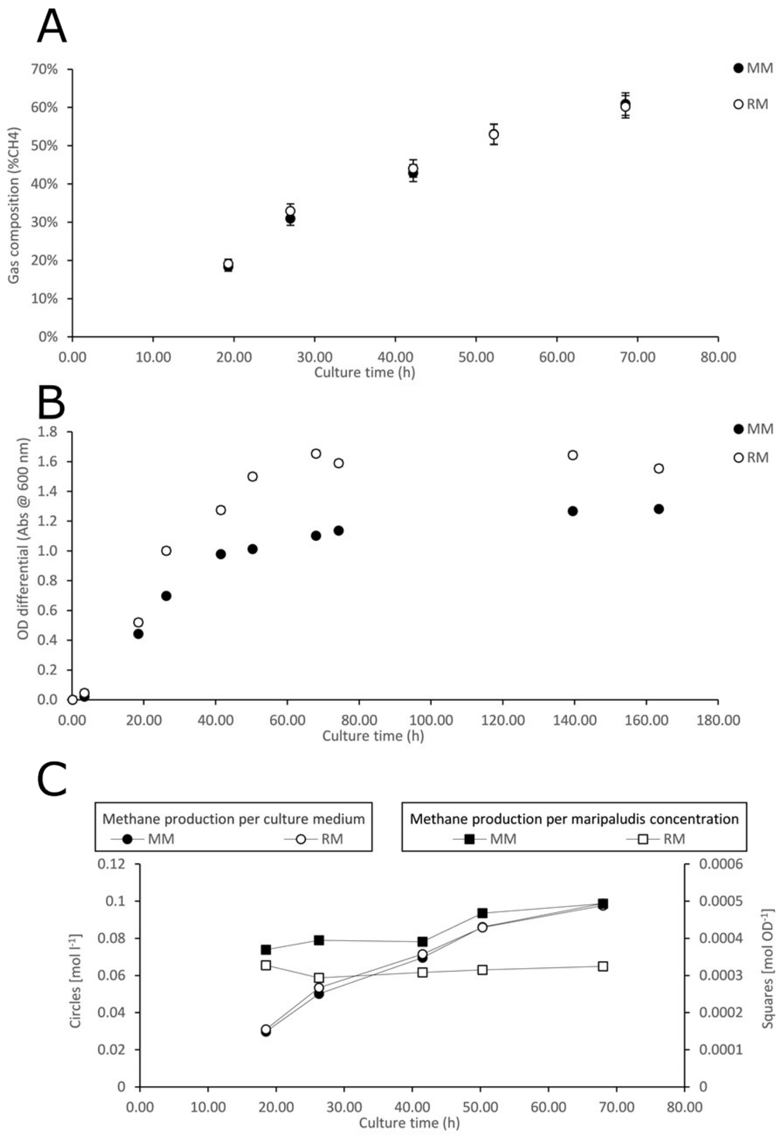


Table S5 Thermal and turbidity control module details

Mark	Apparatus	Features / Function
A	Microcontroller Arduino Ref : Mega 2560 Rev3	Voltage : 12 VDC
B	HMI Nextion Ref : NX4827T043	Voltage : 5 VDC ; Screen 4.3"
C	Thermal sensor module DFROBOT Ref: SEN0198	Functional detection range: 30 °C -350 °C Relative error: ±2%
D	Cytron Ref: Shield MD10	Voltage ranges: 7 – 30V Maximum current: 10 A
E	Analog turbidity sensor DFROBOT Ref: SEN0189	Operating Temperature: 5 °C – 90 °C Relative error: ±8% measured values
1	Heat exchanger Laird Technologies Ref: LI201	Material: Copper Size: 12.7 x 57 x 51mm
2	Peltier module Adaptive ET-199-14-15-E	Active Size: 40 x 40 mm Maximum cooling capacity: 94.3W Maximum temperature difference : +74K
3, 4	Fans and Passive heat sinks Xilence Ref: I200	Material: Aluminium Size: 95 x 95 x 70 mm Flow : 40.9 CFM Voltage alimentation : 12 VDC
5	Thermal water pump Ref: TD40G-1260B	Type: Centrifugal Voltage: 12V DC Maximum liquid flow: 650 L h^{-1}
6	Temperature sensor Jumo Ref: 902250/32-415-1001-1-3-200-04-2500/000	PT100 ; Ø 3 mm, length: 200 mm 3 wire ; classe B
7	Culture medium circulation pump RS Pro Ref : D250-03	Type: Diaphragm Flow Rate : 380 mL min^{-1} ; DP: 0.7 bars Voltage : 1.5 – 5 VDC Power consumption: 0.48 W

Figure S4: Evolution of the growth of M. maripaludis in solution (A) and of the volume fraction of CH_4 in the tube gas phase (B-C) in the two tested growth media.



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Gas chromatography

Gas chromatography (GC) measurements were used to determine the composition of the gaseous headspace in the cultures. They were carried out using a Perkin Elmer's Clarus 580 GC with a Porapak Q, 80/100, SS, 6' x 1/8" x 6" GC column, with N_2 as the carrier gas. FID Detector was used to analyse CH_4, CO_2 and CO. TCD Detector was used to analyse H_2 and O2. 50 µL of overhead gas were injected into the GC using a gas-tight glass Hamilton syringe. The quantity of gas injected is normalized with temperature and pressure, and compared with an extemporaneously produced calibration curve.

Figure S5: Monitoring of the temperature of the culture of M. maripaludis in the bioreactor

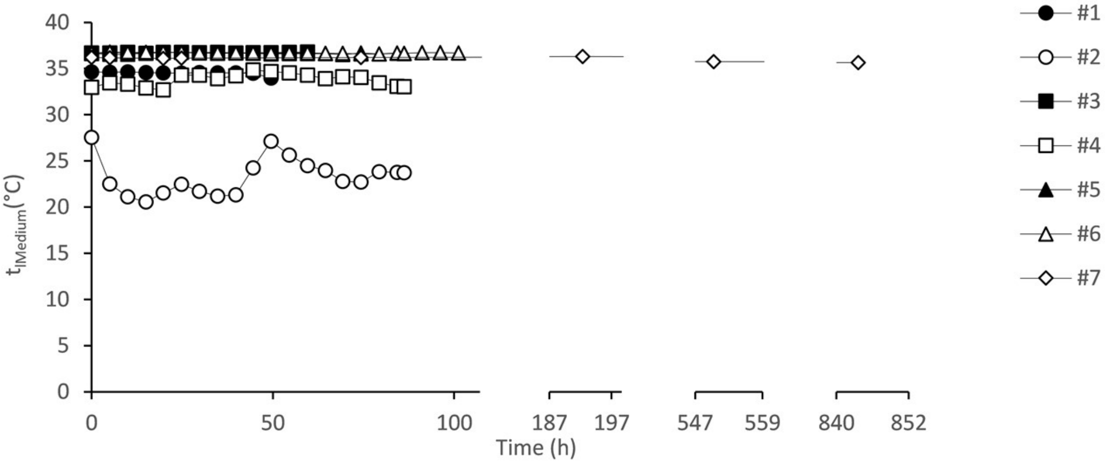


Figure S6: Monitoring of the $OD_{600\ nm}$ of the culture of M. maripaludis in the bioreactor

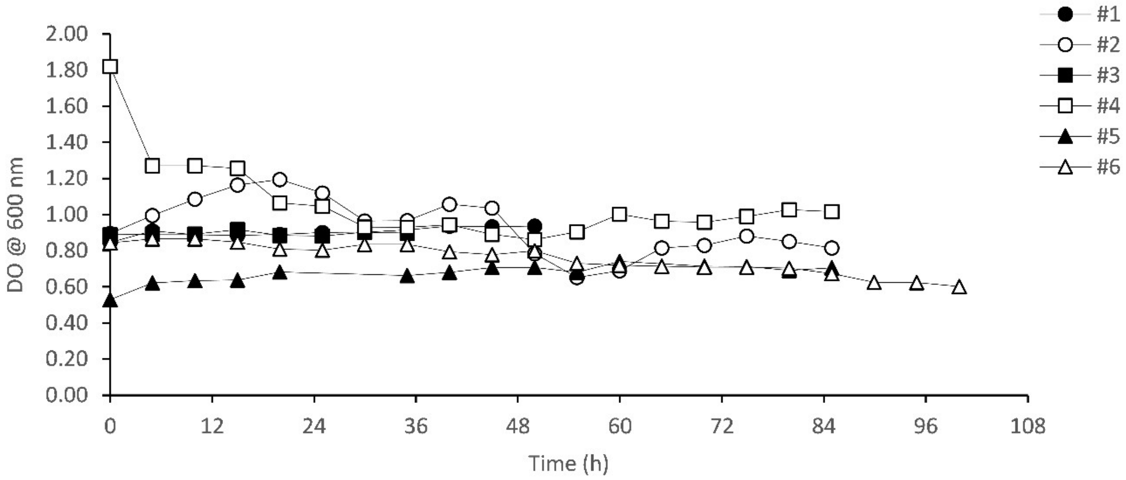


Figure S7: Monitoring of the redox potential of the culture of M. maripaludis in the bioreactor.

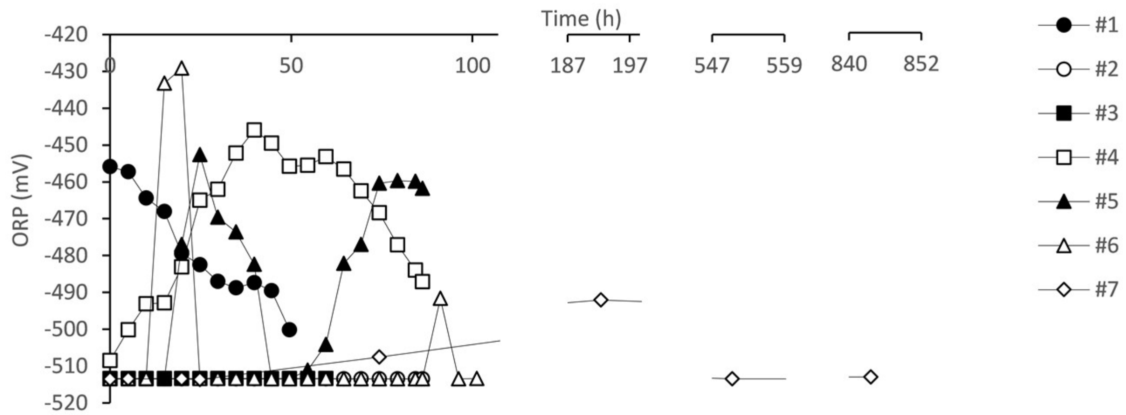


Figure S8: Monitoring of the pH of the culture of M. maripaludis in the bioreactor

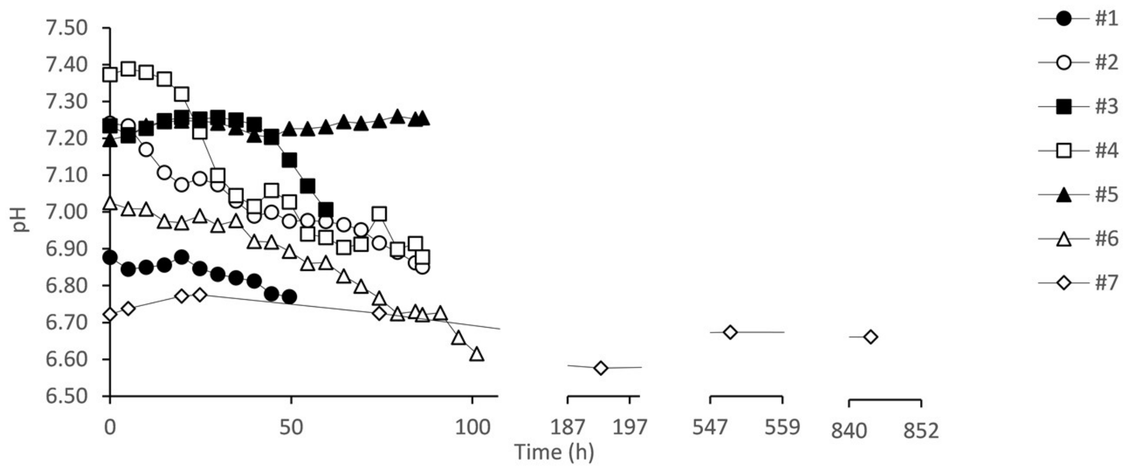


Figure S9: Pressure maintained in bioreactor

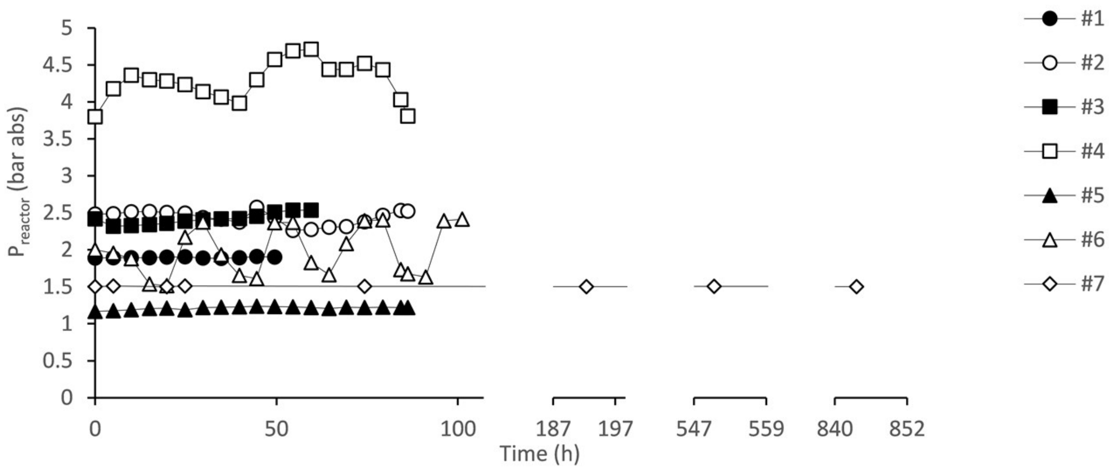


Figure S10: Hydrogen inlet flow rate in the bioreactor

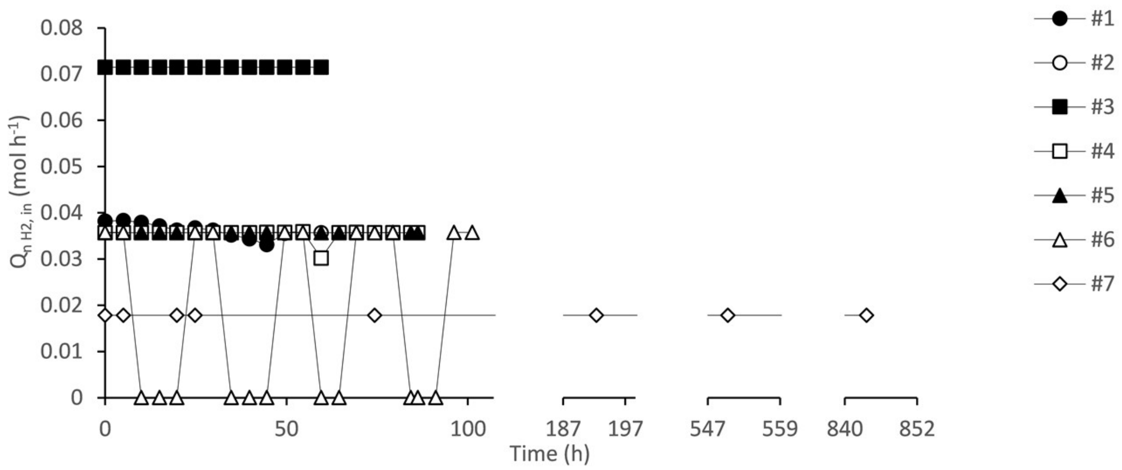
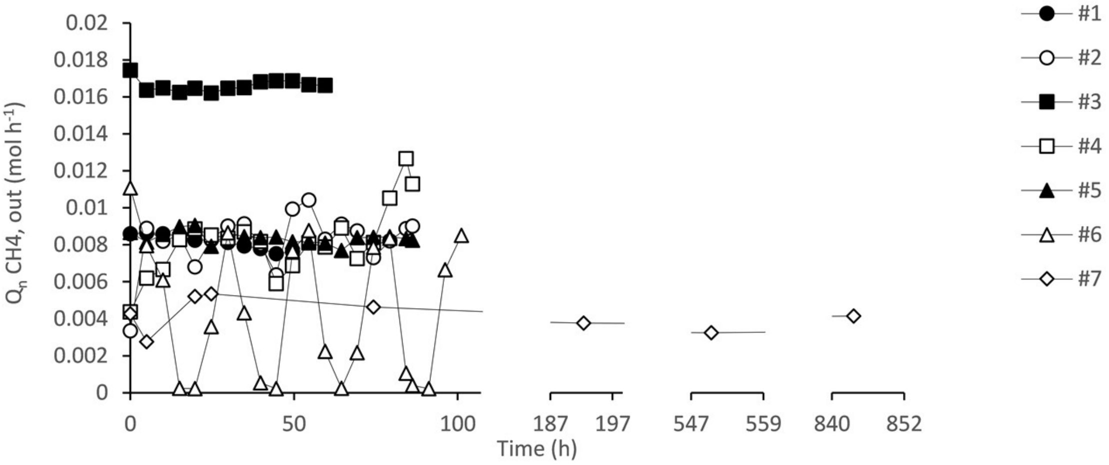


Figure S11: Methane outlet flow rate



List of Latin notations

a	interfacial area	$[m^2\,m^{-3}]$
$C_{\varphi,i}$	Molar concentration of « i » in the « φ » phase	$[mol\,m^{-3}]$
d_b	Diameter of gas bubbles in the column	$[m]$
D	Diameter	$[\,m\,]$
D_{ax}	Axial dispersion	$[m^2\,s^{-1}]$
ε	Gaz hold-up	$[-]$
g	Acceleration of gravity at the Earth's surface	$[m\,s^{-2}]$
H^{cc}	Henry's law coefficient expressed as a ratio of the concentrations of « i » between the liquid and gas phases	$[-]$
$k_{\varphi,i}$	Transfer coefficient of « i » in the « φ » phase	$[\,m\,s^{-1}]$
MER	Molar production of methane per reactor volume	$[mol\,m^{-3}\,s^{-1}]$
MP	Methane Production	$[mol\,m^{-3}\,m^{-3}]$
PVR	Power density per unit reactor volume	$[Wh\,m^{-3}]$
P	Pressure	$[Pa]$
Qv	Volume flow	$[m^3\,s^{-1}]$
Qn	Molar flow	$[mol\,s^{-1}]$
r_{app}	Apparent reaction rate	$[s^{-1}]$
S	Surface	$[m^2]$
T	Temperature	$[K]$
t	Time	$[s]$
U_g	Surface velocity of gas in the column	$[m\,s^{-1}]$
u_b	Rising speed of gas bubbles	$[m\,s^{-1}]$
χ	Stoichiometric conversion yield	$[-]$
Y_{CH_4}	Methane conversion yield	$[-]$
y_i	Gas volume fraction of compound « i »	$[-]$

List of Greek notations

ϕ_i	Volumetric molar flow of material « i » transferred	$[mol\,m^{-3}\,s^{-1}]$
η_i	Stoichiometric coefficient of the compound « i »	$[-]$
ρ	Density	$[kg\,m^{-3}]$

List of subscript

ax	Axial direction of flow
app	Relative to an apparent value
g	Relating to gas
i	Relative to the indefinite component
l	Relative to the liquid
n	Relative to normal conditions
$reac$	Reactor related
φ	Relative to a phase (liquid or gas)

List of superscript

°	Relative to a standard value
CC	Concentration ratio
d_b	Bubble diameter
in	Relative to a reactor inlet value
out	Relative to a reactor outlet value

List of abbreviations

ADM	Axial dispersion model
BCR	Bubble column reactor
BMP	Biological methane production
CAD	Computer-assisted design
CSTR	Continuous stirred-tank reactor
DCW	Dryed cell weight
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen
EnR	Renewable energy
FBR	Fixed bed reactor
FFKM	Perfluoroélastomères
HEPES	Biochemical buffer
LOHC	Liquid organic hydrogen carriers
MER	Methane evolution rate
MM	Minimum medium
MR	Membrane reactor
NDIR	Non-dispersive infrared sensor
OD	Optical Density
ORP	Oxydo Reduction Potential
RM	Rich medium

References

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