Microbial competition control between hydrogenotrophic methanogens and homoacetogens for selective production of acetate under hydrogen and carbon dioxide in successive batches

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Journal of Cleaner Production

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--Manuscript Draft--

Manuscript Number:	
Article Type:	Original article
Keywords:	Carbon dioxide; Methane; Acetate; Mixed Microbial Culture; Microbial selection; Successive batches
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Title:

Microbial competition control between hydrogenotrophic methanogens and homoacetogens for selective production of acetate under H_2/CO_2 in successive batches

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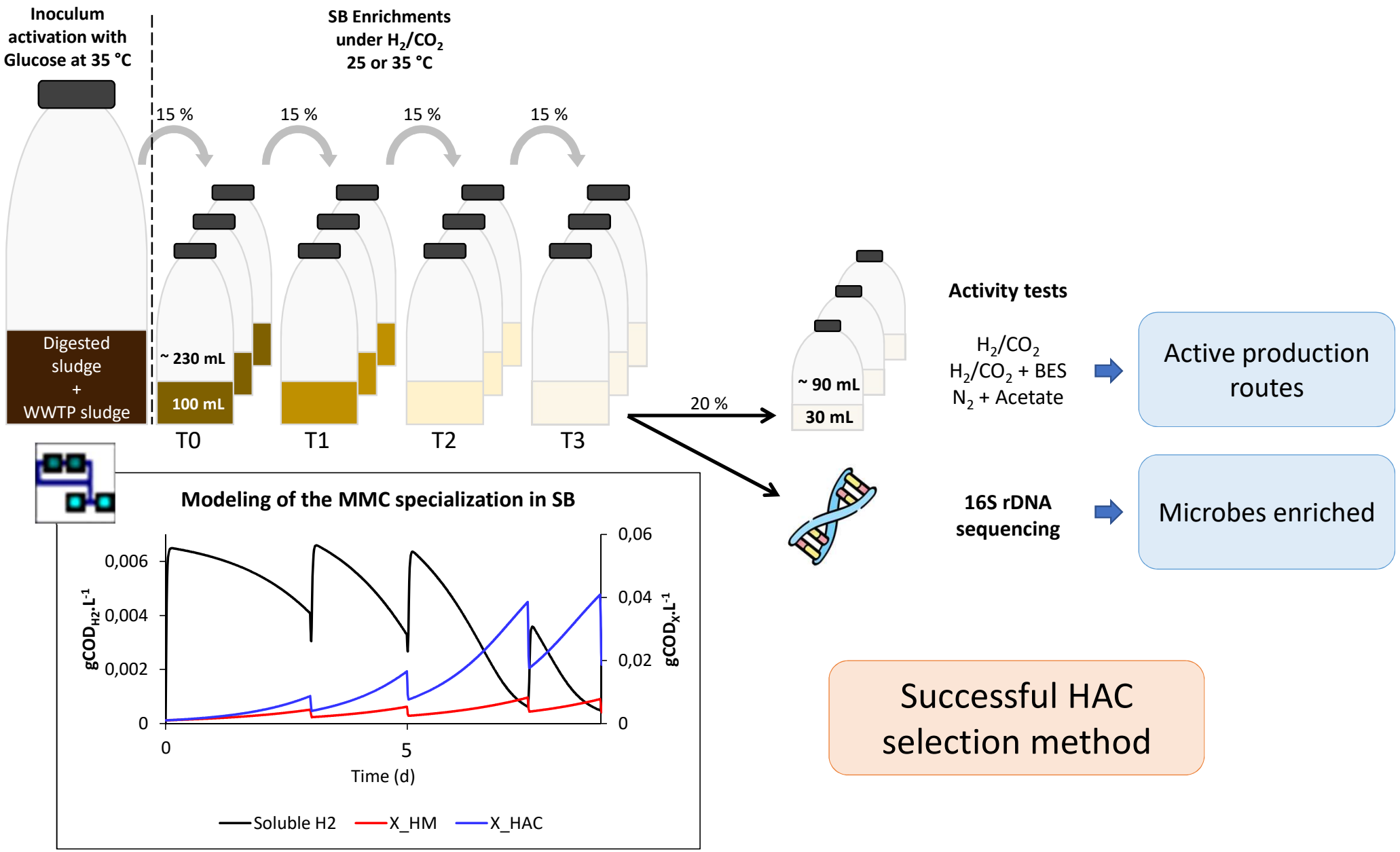
Declaration of interests

☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Graphical Abstract



Highlights:

- A homoacetogens selection method was developed from anaerobic mixed cultures
- Mass transfer limitation led to hydrogenotrophic methanogens selection
- Lower temperature from 35 °C to 25 °C benefited to homoacetogens growth

Figure 1: Main biological reactions considered in the system and discussed in this study.

Figure 1: Graphical explanation of the activities identified under the conditions of the activity tests carried out on the MMCs selected during the SB experiments. In green and black the feasible and unfeasible routes according to thermodynamics at initial state of the test respectively. Red cross represent the inhibitory effect of BES on methanogenesis. Adapted from (Navarro et al., 2016).

Figure 2: Electron balances considering acetate and methane produced over H_2 consumed during the four sets of SB carried out at 25 °C and 35 °C, under mass transfer limitation or not.

Figure 4: Results of the activity tests carried out with the MMCs enriched in SB at 35 °C under mass transfer limitation.

Figure 3: Results of the activity tests carried out with the MMCs enriched in SB at 25 °C A: under mass transfer limitation; B: avoiding mass transfer limitation.

Figure 6: Growth yields calculated during the activity tests of the enriched culture in SB at 25 °C with several gas injections and late transfer. In blue is represented the activity test with BES, corresponding to HAC growth. In red is represented the activity test without BES, corresponding to HM growth. A: growth yield as a function of time (HAC: n=3; HM: n=2). B: Growth yield according to PH2.

Figure 7: Relative abundance of taxa detected in the MMCs, at genus level, generated with rANOMALY package in R. MTL stands for mass transfer limitation.

Figure 8: Simulation of microbial competition during H2/CO2 fermentation in batch mode. A: Hydrogen and biomasses concentrations in the liquid phase; B: Total pressure and partial pressures of the different gases. Liquid volume: 0.5 L; Gas volume: 1.5 L; kLa: 10d-1; initial HM and HAC concentrations: 0.001 gCOD.L-1; KH2 (mol.L-1): 0.000001 for HM and 0.00052 for HAC; km (gCODH2.gCODX-1.d-1): 78.13 and 98.77 for HM and HAC respectively; growth yields (gCODX.gCODH2-1): 0.064 and 0.081 for HM and HAC respectively. C: ΔrG and D: FT were calculated as described in chapter2 section.

Figure 1

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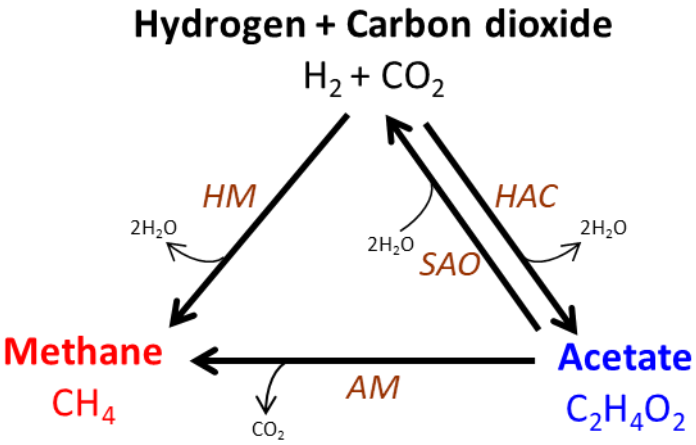

HM	$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$
HAC	$4H_2 + 2CO_2 \rightarrow C_2H_4O_2 + 2H_2O$
AM	$C_2H_4O_2 \rightarrow CH_4 + CO_2$
SAO	$C_2H_4O_2 + 2H_2O \rightarrow 4H_2 + 2CO_2$

Figure 2

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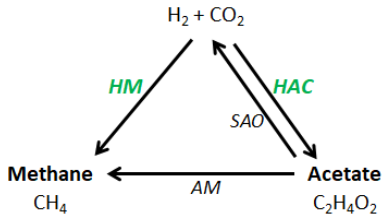
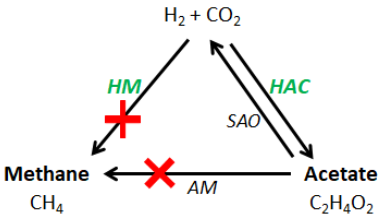
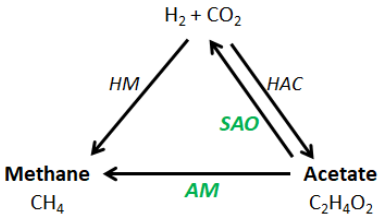
Hydrogenotrophic activity test	Homoacetogenic activity test	Acetotrophic activity test
$H_2 + CO_2$	$H_2 + CO_2 + BES$	N_2 + Acetate
		

Figure 3

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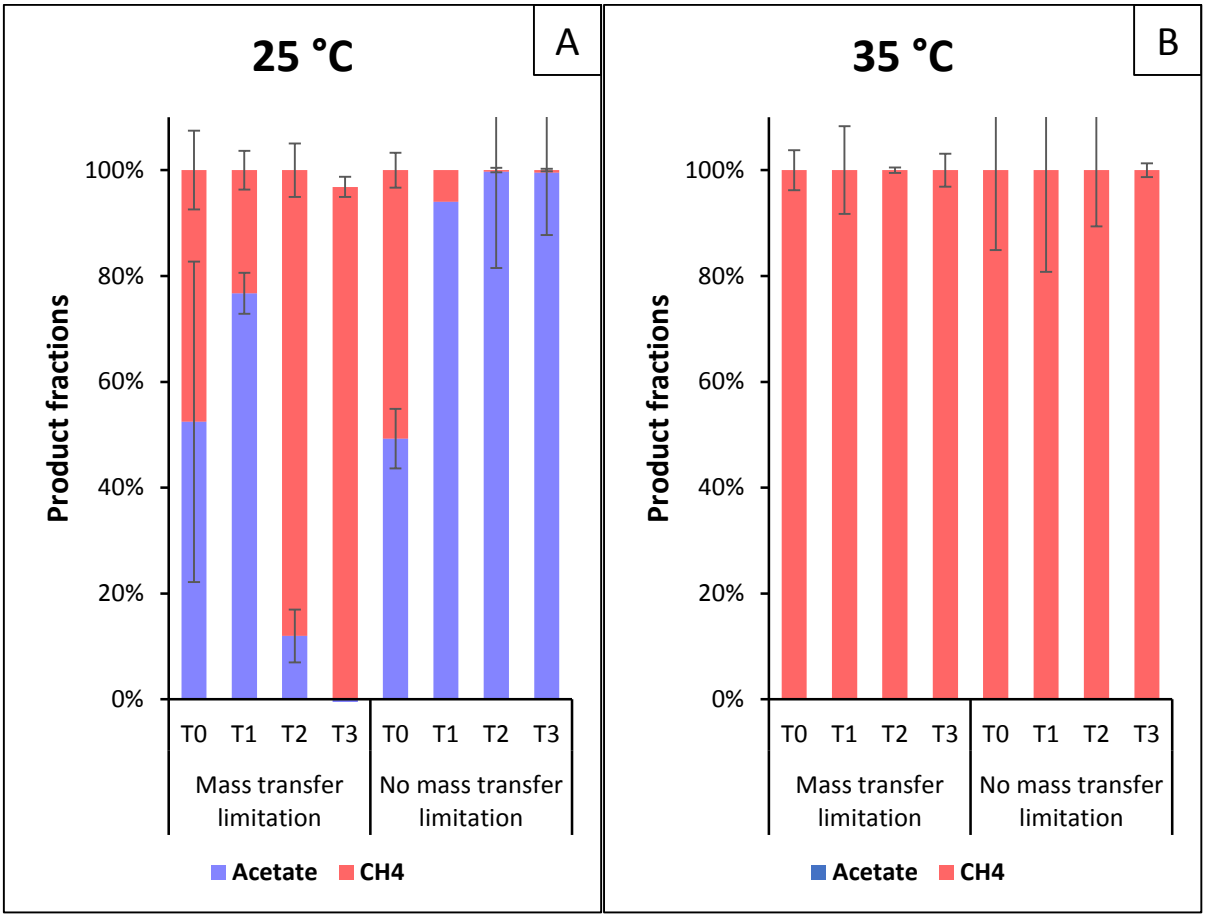


Figure 4

Activity tests on MMCs enriched in SB at 35 °C under mass transfer limitation:

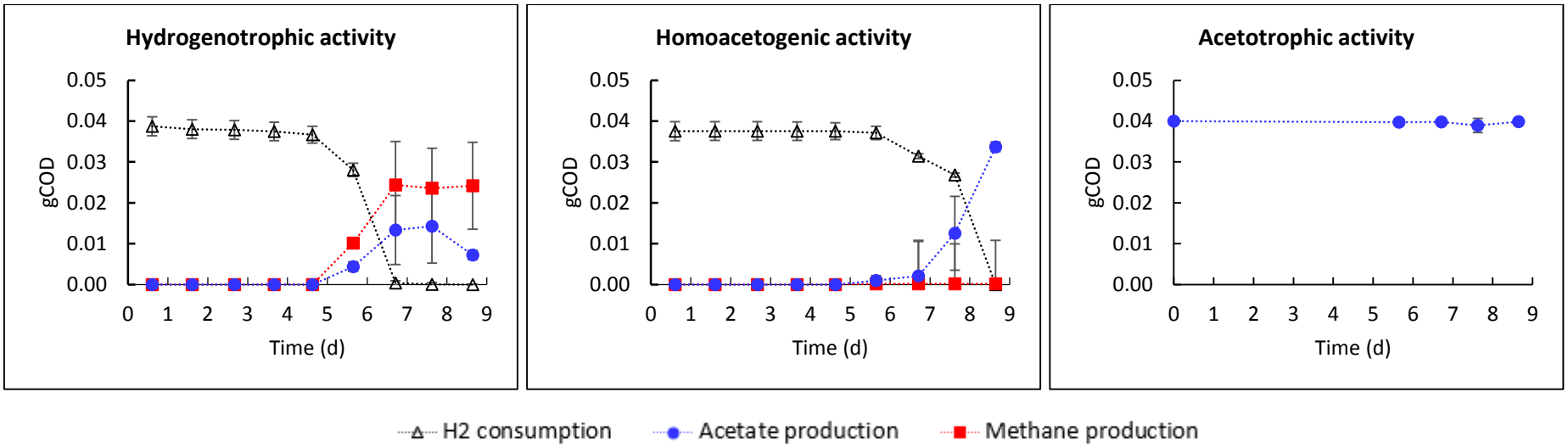
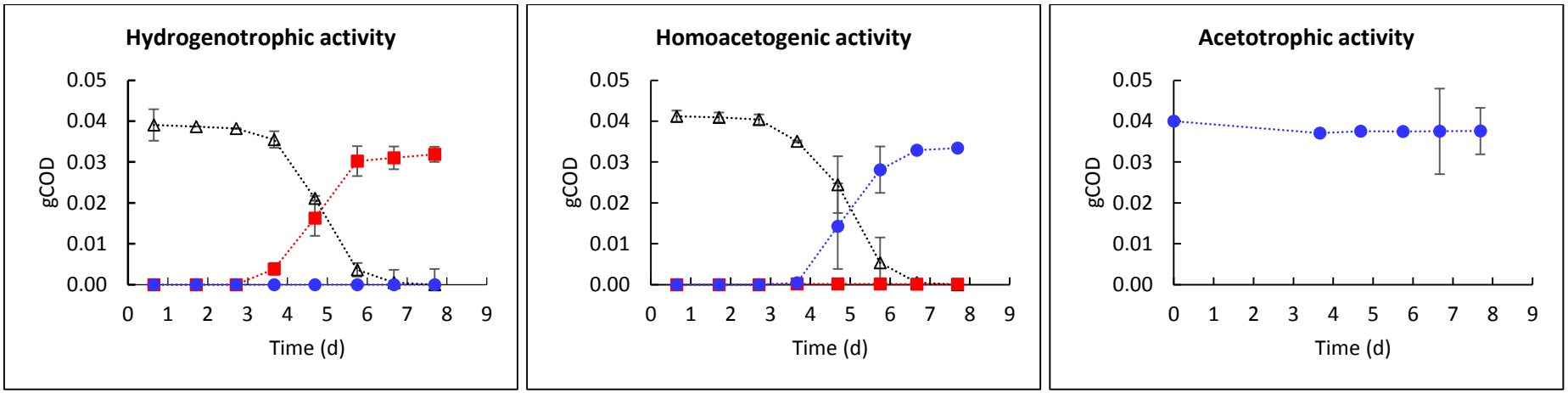


Figure 5

A: Activity tests on MMCs enriched in SB at 25 °C under mass transfer limitation:



B: Activity tests on MMCs enriched in SB at 25 °C avoiding mass transfer limitation:

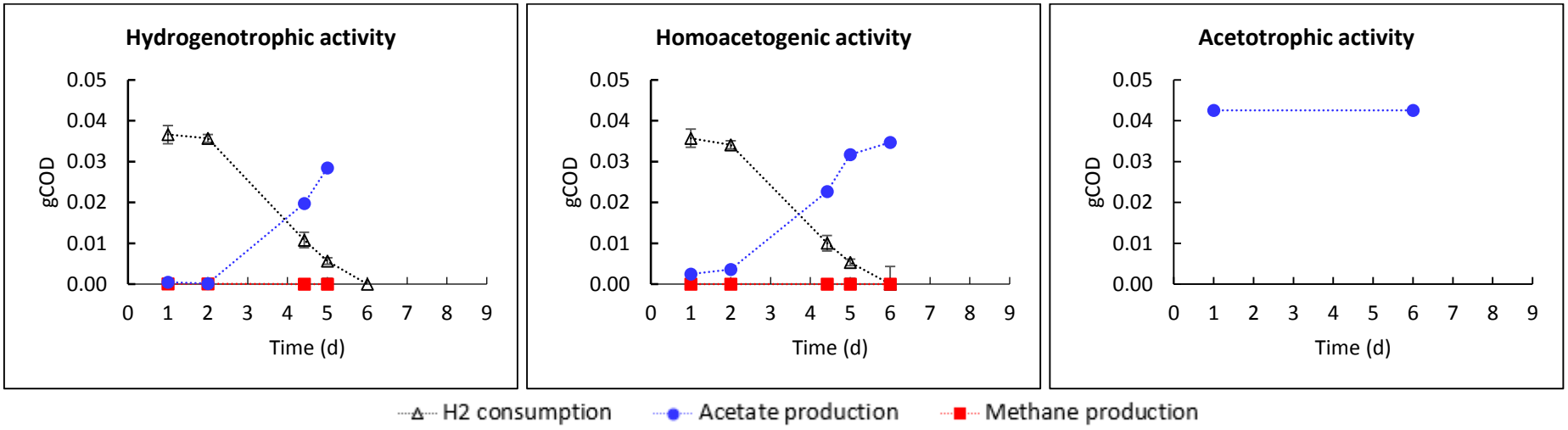


Figure 6

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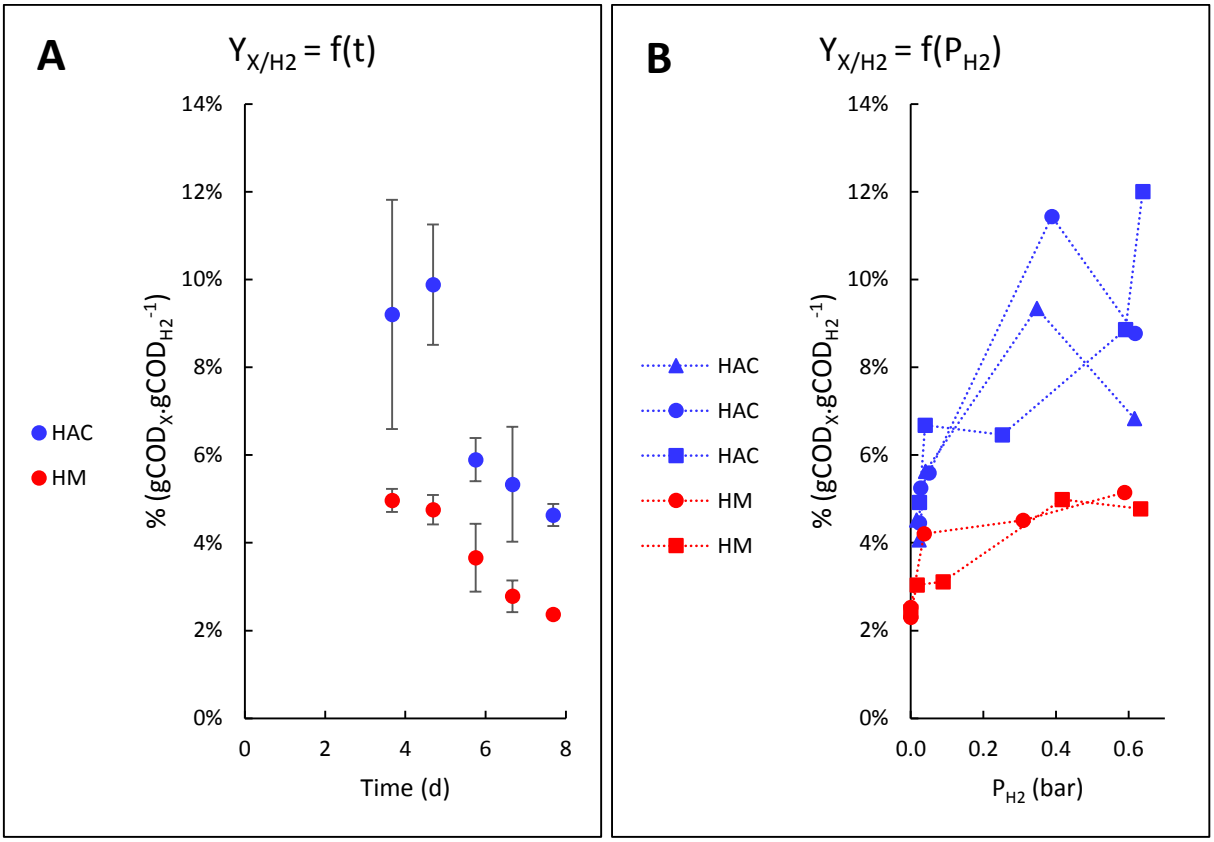

Figure 7

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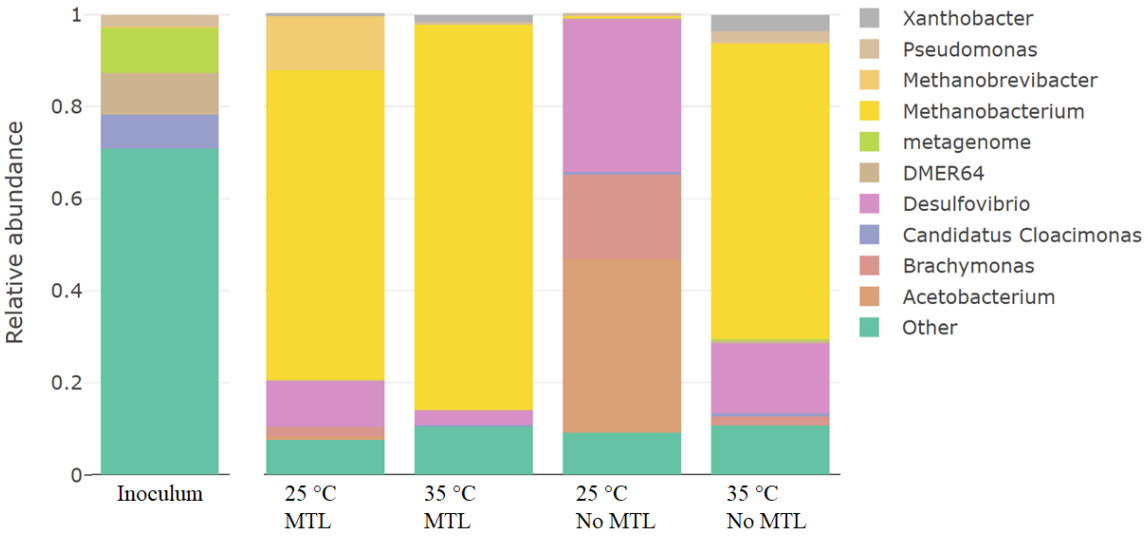

Figure 8

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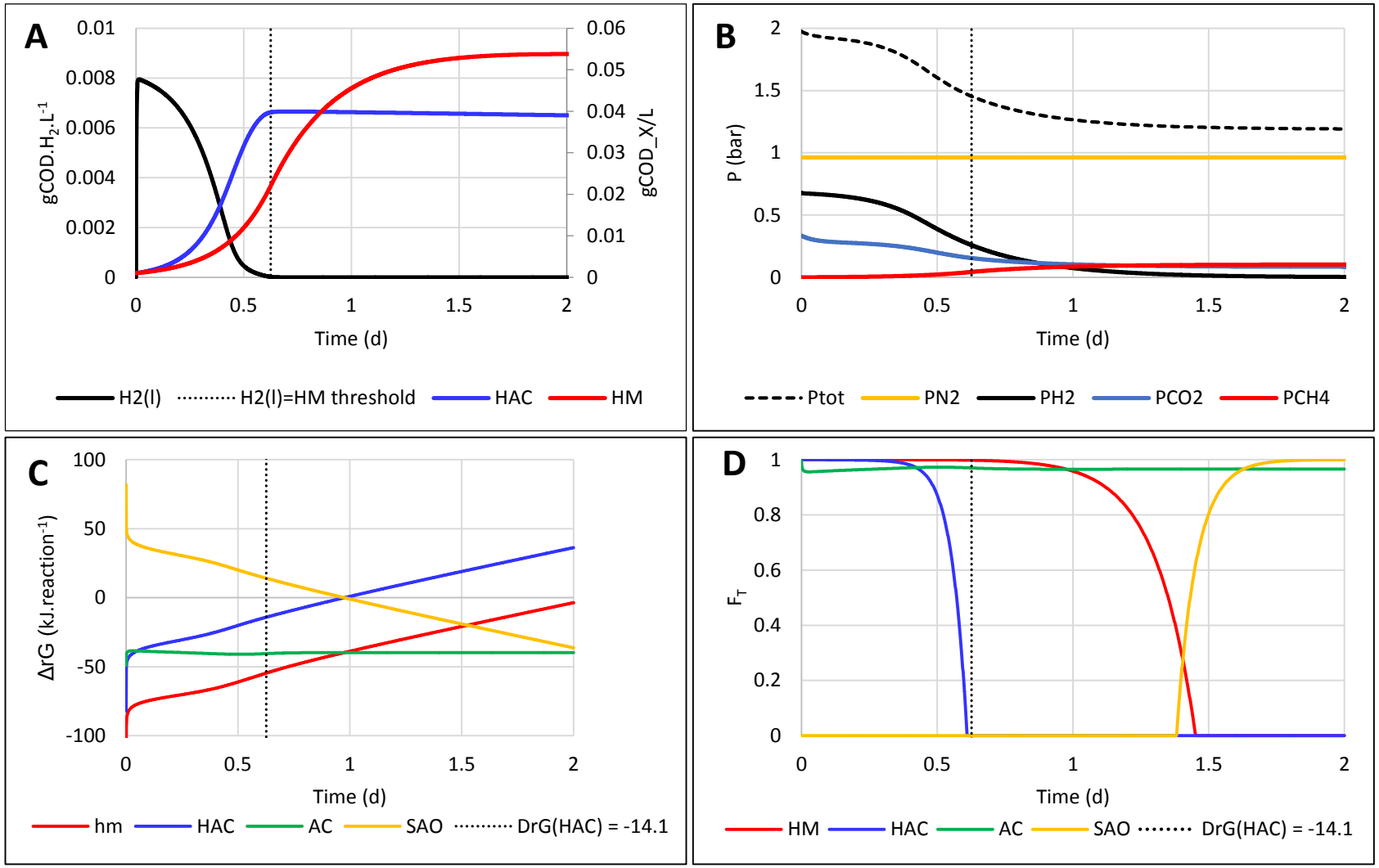

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This study contributes to bring new knowledge on the competition between HAC and HM. In this sense, mass transfer between the gas phase where the substrates are located, and the liquid phase which contains the microbial catalysts, as well as kinetic and thermodynamic aspects of biological reactions have been integrated in this work. The microbial competition between HM and HAC was studied in successive batches. The effect of temperature between 25 °C and 35 °C was investigated, as well as the time of each batch, leading to different states of mass transfer limitation in the system.

A clear effect of temperature between 25 °C and 35 °C on the outcome of the competition between HM and HAC was highlighted, as well as the effect of mass transfer limitation. This study contributes to identify specific process parameters influencing the selection of HAC over HM and should help in the design of experiments depending on the target product from H_2/CO_2.

In successive batches, lifting of mass transfer limitation, as well as lowering of the temperature to 25 °C made it possible to select HAC in mixed anaerobic cultures for acetate production by eliminating the methanogens, after four successive batches of 4 to 6 days each.

Keywords:

Carbon dioxide

Methane

Acetate

Mixed Microbial Culture

Microbial selection

Successive batches

Abbreviations:

AD: Anaerobic Digestion

AM: Acetoclastic Methanogens

HAC: Homoacetogens

HM: Hydrogenotrophic Methanogens

MMC: Mixed Microbial Culture

MTL: gas to liquid mass transfer limitation

SAO: Syntrophic Acetate Oxidisers

SB: Successive Batch

TS: Total solid

VS: volatile solid

WLP: Wood Ljungdahl Pathway

WWT: Waste Water Treatment

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1. Introduction

The development of a circular carbon economy has become a major area of research. Research efforts are on the rise to reuse organic agricultural and household residues as well as residual streams linked to industrial activity. These wastes constitute an interesting substrate for many biological processes aimed at the production of energy or high added value materials. New branches of the biorefinery are appearing to reuse solid wastes, which are more difficult to biodegrade. One of the ways of reusing this type of waste is gasification by thermochemical treatment. The solid carbonaceous material is thus transformed into synthesis gas, that is to say a mixture of gases such as CO, CO_2, H_2, and other minor gases such as H_2S or CH_4. Other sources of gas to be upgraded exist, such as the CO_2 co-produced in many industrial processes, and also the CO_2 contained in biogas from anaerobic digestion, which contains methane, but also around 30-50 % of CO_2 (Gavala et al., 2021; Angelidaki et al., 2018).

Homoacetogens (HAC) are interesting candidates for the biological reduction of CO_2 to chemicals using the Acetyl-CoA reductive pathway (or Wood Ljungdahl Pathway, WLP). The latter is a highly energetically efficient route fixing two CO_2 into acetyl-CoA, inherited from the earliest stages of life on earth. However, discovery of HAC dates from the 1930s, since when new homoacetogens keep being identified, isolated, and characterized. Interestingly, homoacetogenesis is not a phylogenetic trait, because HAC are found in different phyla. HAC can use a wide range of electron acceptors and donors, such as nitrate, but also organic reduced compounds such as ethanol. HAC use WLP as a catabolic route producing acetate during the generation of ATP, as well as for biosynthesis of cell components from acetyl-CoA. Other anaerobic microorganisms are encoding the WLP, including hydrogenotrophic methanogens (HM) and sulphate reducers. Hence, under anaerobic and autotrophic conditions and depending on the available substrates, different communities can coexist in a microbial mixed culture (MMC). Under H_2/CO_2 as sole energy and carbon sources, some interactions, in particular

competitive interactions, exist to fix the common substrates. If the medium doesn't contain other electron acceptors than CO_2 (*i.e.* Fe^{3+}, NO^{3-}, SO_4^{2-} or S^0), then the communities remaining in competition are HM and HAC. To favour HAC and acetate production over methane, it is necessary to find a way to eliminate or inhibit methanogens.

To inhibit methanogenesis, different strategies may work. It is possible to apply heat treatment to the microbial consortium with the aim of eliminating communities that are not able to sporulate, which is the case of methanogens. A revitalization work must then be done to exploit the metabolic potential of the sporulating microbial strains in MMC. This strategy has the advantage of efficiently eliminating methanogenic populations in most cases, even if some authors still observe the recovery of methane production after treatment (Liu et al., 2018). Some authors like Omar et al. (2018), were even able to observe a methane yield of 100 % after heat treatments at 70 or 90 °C for 30 min. In addition, this technique can also rule out non-sporulating hydrogenotrophic bacteria which may be of interest for the desired activity. In particular, *A. woodii*-like HAC are non-spore-forming bacteria (Schuchmann and Müller, 2014). This technique is also expensive, and energy consuming, and doesn't prevent contamination later during the operations. Another strategy, widely used in laboratory-scale studies, is the addition of chemical inhibitors of methanogenesis. The most used is 2-bromoethanesulfonate (BES), but there are others such as mercaptoethanesulfonate (MES) or lumazine (Liu et al., 2011). MES and BES mimic the methyl-CoM and thus block the enzyme of the last stage of methanogenesis (HM and AM). These inhibitors therefore make it possible to inhibit any type of methanogenesis. BES is used at different concentrations depending on the studies, from 10 to 50 mM and even 100 mM (Wang et al., 2017; Luo et al., 2018; Omar et al., 2018; Shen et al., 2018). It is interesting to note that the concentrations chosen do not seem to depend on the duration of the experiment nor on the temperature, and are rarely justified or discussed. However, during long cultivation times it is necessary to regularly add BES because

it is degraded over time in the system (Steinbusch et al., 2011; Luo et al., 2018). Chemical inhibitors are convenient for specific lab experiment, though they are not an economically viable solution either in the framework of the development of industrial production processes (Agler et al., 2011).

For this reason, the present study focuses on engineering MMC in order to eliminate methanogens' activity and selectively produce chemicals (*i.e.* acetate). Hence, the main challenge is to understand the competition between HM and HAC and be able to favour HAC rather than HM by applying specific process conditions. Different studies focused on the competition between HM and HAC because it is an undesirable reaction in dark fermentation for the production of H_2 for example (Fu et al., 2019; Regueira et al., 2018; Molenaar et al., 2017; Annie Modestra et al., 2015; Saady, 2013; Kotsyurbenko et al., 2001). Some researchers also have showed interest in HAC in the field of anaerobic digestion when it comes to operate at psychrophilic temperatures (Vavilin et al., 2000; Conrad et al., 1989). In gas fermentations though, when the substrate is either syngas or CO_2 and H_2, homoacetogenesis constitute a significant share of microbial activity responsible for acetic acid generation, directly competing with HM while it is greatly influenced by the temperature of the process (Grimalt-Alemany et al., 2019).

In this article, the competition between HAC and HM is investigated in batch mode under H_2/CO_2, by the implementation of successive batches (SB). SB were carried out at 25 °C or 35 °C, according to different community engineering strategies: with transfer during exponential phase or during stationary phase after several gas injections. In the first case, mass transfer limitation during the enrichment period was avoided, and the time to grow was shorter than in the second case that aimed to increase biomass production, implying mass transfer limitation. Activity tests were carried out on the enriched cultures to identify the metabolic functions selected. 16S rDNA high throughput sequencing was also undertaken on the enriched cultures.

2. Materials and methods

2.1. Mixed microbial culture origin and preparation

Two different sludges have been mixed to maximize the initial biodiversity for the enrichment experiments: 700 mL of a WWT plant sludge and 700 mL of a lab scale AD sludge. After mixing, the inoculum was placed in two serum bottles of 2L with 5 $g.L^{-1}$ of glucose, flushed with N_2 during 30 minutes and incubated at 35 C° without mixing. Active inoculum contained 19.7 $g.L^{-1}$ of total solids (TS) and 12.3 $g.L^{-1}$ of volatile solids (VS).

2.2. Growth medium

The growth medium was prepared from the four following solutions: salts (NH_4Cl, 50 $g.L^{-1}$; NaCl 10 $g.L^{-1}$; $MgCl_2.6H_2O$, 10 $g.L^{-1}$; $CaCl_2.2H_2O$, 5 $g.L^{-1}$), Na_2SO_4 50 $g.L^{-1}$, Vitamins (biotin, 2 $mg.L^{-1}$; folic acid, 2 $mg.L^{-1}$; pyridoxine–HCl, 10 $mg.L^{-1}$; riboflavin-HCl, 5 $mg.L^{-1}$; thiamine-HCl, 5 $mg.L^{-1}$; cyanocobalamin, 0.1 $mg.L^{-1}$; nicotinic acid, 5 $mg.L^{-1}$; p-aminobenzoic acid, 5 $mg.L^{-1}$; lipoic acid, 5 $mg.L^{-1}$; D-pantothenic acid hemicalcium salt, 5 $mg.L^{-1}$), Trace metals (Nitrilotriacetic acid, 2000 $mg.L^{-1}$; $Fe(SO_4)_2(NH_4)_2.6H_2O$ 800 $mg.L^{-1}$; H_3BO_3, 10 $mg.L^{-1}$; $ZnSO_4.7H_2O$, 200 $mg.L^{-1}$; $CuCl_2$, 20 $mg.L^{-1}$; $MnSO_4$, 1000 $mg.L^{-1}$; $Na_2MoO_4.2H_2O$, 20 $mg.L^{-1}$; $AlCl_3$, 10 $mg.L^{-1}$; $CoCl_2 \cdot 6H_2O$, 200 $mg.L^{-1}$; $NiCl_2$, 20 $mg.L^{-1}$; $Na_2SeO_4 \cdot 5H_2O$, 18 $mg.L^{-1}$; $Na_2WO_4 \cdot 2H_2O$, 22 $mg.L^{-1}$). Potassium dihydrogen phosphate (KH_2PO_4 1M) and dipotassium hydrogen phosphate (K_2HPO_4 1M) were used as buffering solutions. The medium was prepared by adding 20 $mL.L^{-1}$ of salts solutions, 1 $mL.L^{-1}$ of Na_2SO_4 solution, 10 $mL.L^{-1}$ of vitamins solutions, 10 $mL.L^{-1}$ of trace metals solution, 86 $mL.L^{-1}$ of KH_2PO_4 and 14 $mL.L^{-1}$ of K_2HPO_4. The pH of the medium under N_2 atmosphere was 5.98 ± 0.05.

2.3. Enrichment cultures in successive batches at 25 °C and 35 °C, with transfer during exponential or stationary phase of growth

SB were carried out in triplicates in 330 mL serum vials at 25 °C and 35 °C. The flasks were filled with 85 mL of growth medium, sealed with rubber stoppers and screw plugs and flushed 15 min with N_2 before the addition of 15 mL of active inoculum. 75 mL of CO_2 and 150 mL of H_2 were added in the flasks to reach a final relative pressure of 1 bar with H_2 and CO_2 partial pressures of 0.66 ± 0.03 and 0.33 ± 0.03 bar, respectively. The pH (measured externally) was 6.3 ± 0.1 after inoculation with raw activated inoculum (T0), and 6.1 ± 0.0 after inoculation with enriched cultures (from T1 to T3). Four successive batches were operated (T0-T3), selecting the most active culture at each step to inoculate the next triplicate of batches, similarly to Grimalt-Alemany et al. (2019).

2.4. Activity tests

To assess the enriched microbial activities, tests were carried out in 120 mL serum vials with 30 mL of liquid volume. Vials were filled with 24 mL of growth medium, sealed with rubber stoppers and screw plugs and flushed 10 min with N_2 before the addition of 6 mL of enriched culture T3 (20 % v/v). Triplicates of three different conditions were carried out (Figure 2). The first condition was H_2/CO_2, 30 mL of CO_2 and 60 mL of H_2 were injected in the vial. The second condition was H_2/CO_2 + BES, 15 mM of BES were added to the culture broth, 30 mL of CO_2 and 60 mL of H_2 were injected in the vials. The third condition was N_2/Acetate, 20 mM of acetate were added to the culture broth, without H_2/CO_2 injection. The vials were incubated at the temperature at which the inoculum (T3) had been grown (25 °C or 35 °C).

2.5. Analytical Methods

Gas composition in H_2, CO_2, CH_4, N_2, O_2 was analysed using a gas chromatograph (GC 8610C, SRI Instruments, USA) while liquid was analysed for volatile fatty acids and alcohols concentrations determination (formic acid, acetic acid, butyric acid, valeric acid, hexanoic acid, isobutyric acid, isovaleric acid, ethanol, butanol) using a High-Performance Liquid Chromatograph (HPLC Shimadzu, USA). Detailed conditions of the analytical methods are described in previous paper (Grimalt-Alemany et al., 2019). Microbial biomass was monitored with OD measurements at 600 nm using a spectrophotometer (DR2800, Hach Lange). A correlation between OD_{600} and total solids (TS) have been done with 50 mL samples of a CSTR culture inoculated with the same MMC at 25 °C under H_2/CO_2 (eq. K).

2.6. DNA extraction and 16S rDNA sequencing

Samples for genomic DNA extraction and 16S rDNA sequencing were collected from the activated inoculum, from the successive batch enrichments at the end of the third transfer at 25 and 35 °C, and from the CSTR on day 41, 62 and 68. DNeasy PowerSoil™ DNA Isolation Kit (Qiagen, Denmark) was used for the DNA extraction of the activated inoculum. DNeasy Blood & Tissue™ Kit (Qiagen, Denmark) was used for the DNA extraction of all other samples. DNA extracted were submitted to Macrogen Europe. Amplification of V4 and V5 regions of 16S rDNA was performed with 515F-Y 5'-GTGYCAGCMGCCGCGGTAA and 926R 5' CCGYCAATTYMTTTRAGTTT primers (Walters et al., 2015). Library preparation was done according to Illumina NGS workflow with Illumina Miseq instrument (Vigliar et al., 2015).

Raw reads containing primers were trimmed using cutadapt and untrimmed reads were discarded (Martin, 2011). Remaining reads were processed using the Qiime2 pipeline (Hall and Beiko, 2018). Specifically, reads were filtered and denoised using DADA2 and the taxonomic

assignment was performed with classify-sklearn using a classifier trained on the reference database SILVA132 using a naive-bayesian classifier (Callahan et al., 2016; Quast et al., 2013; Pedregosa et al., 2011). Data visualization was processed with rANOMALY package (Theil and Rifa, 2021).

2.7. Dynamic model on AQUASIM

A kinetic model has been developed on Aquasim 2.1g (Reichert, 1994) in order to study and simulate the microbial competition within the consortium, according to kinetics and thermodynamics features. The model includes dynamic microbial growth in accordance with Monod equation; dynamic physical-chemical processes of weak acid dissociation, acid-base equilibria (Musvoto et al., 2000), gas to liquid mass transfer, and thermodynamic calculations. H_2, CO_2 and CH_4 mass transfer rates are described in the model based on eq (1).

$$T_{ri} = K_L a_i \times \left(K_{Hi}^{cc} \times C_{i,aq} - C_{i,g}\right) \quad (1)$$

where $C_{i,aq}$ and $C_{i,g}$ the soluble aqueous and gaseous concentrations of compound i, $k_L a_i$ is the volumetric transfer coefficient and K_{Hi}^{cc} is dimensionless Henry's law volatility constant expressed as the ratio of concentrations in the gas over the liquid at equilibrium state. K_{Hi}^{cc} values were taken from Sander (2015), and were corrected for temperature based on eq. (2) and (3), $\Delta_{sol}H$ being the molar dissolution enthalpy, and H_i^{cp} the Henry's law solubility, values used for the correction are detailed in S1.

$$K_{Hi}^{cc} = \frac{1}{H_i^{cp}(T) \times RT} \quad (2)$$

$$H_i^{cp}(T) = H_i^{cp}\left(T^{\theta}\right) \times \exp\left(\frac{-\Delta_{sol}H}{R} \times \left(\frac{1}{T} - \frac{1}{T^{\theta}}\right)\right) \quad (3)$$

The model includes two growth processes describing hydrogenotrophic methanogenic archaea (X_{HM}) and homoacetogenic bacteria (X_{HAC}) production rates (Table 1), and cell biomass composition is considered as $C_5H_7NO_2$.

$$X_{hm}: \nu_{H_2}H_2 + \nu_{CO_2}CO_2 + \nu_{NH_4^+}NH_4^+ \rightarrow \nu_X C_5H_7NO_2 + \nu_{CH_4}CH_4 + \nu_{H_2O}H_2O + \nu_{H^+}H^+$$

$$X_{hac}: \nu_{H_2}H_2 + \nu_{CO_2}CO_2 + \nu_{NH_4^+}NH_4^+ \rightarrow \nu_X C_5H_7NO_2 + \nu_{C_2H_4O_2}C_2H_4O_2 + \nu_{H_2O}H_2O + \nu_{H^+}H^+$$

Hydrogen is considered as the limiting substrate in any case due firstly to its low solubility and secondly because CO_2 is supplied in excess compared to H_2 according to the stoichiometric ratio of the reactions. Thus, only Monod term for H_2 is included in growth rates and CO_2 substrate limitation is neglected. The model includes two decay processes describing X_{HM} and X_{HAC} biomass decomposition into composite materials X_C as it is described in ADM1 (Table 1), with k_{dec} set to 0.02 for HAC and HM. Note that in the framework of this project, the heterotrophic growth on composite material, as well as the volume variations due to water production, were considered as negligible and were not included. Table 2 shows the acid-base equilibria integrated in the model, values of dissociation constant used are found in S2.

Processes ↓	**State variables (mol.L^{-1})**										**Rates (mol.L^{-1}.d^{-1}) ↓**
	C_{Xc}	C_{Xh2}	C_{Xhac}	$C_{H2,liq}$	$C_{CH4,liq}$	C_{ac-}	$C_{H2O\ produced}$	$C_{IC,liq}$	$C_{IN,liq}$	C_{H+}	
Growth of X_{HM}		$Y_{XHM/H2}$		-1	1 - $Y_{XHM/H2}$		$\sum_{i=IC_{CO2},X_{HM},CH_4} O_i \nu_i, X_{HM}$	$-\sum_{i=X_{HM},CH_4} C_i \nu_i, X_{HM}$	- $Y_{XHM/H2}$	$Y_{XHM/H2}$	$k_{mol,HM} \times \frac{C_{H2}}{C_{H2}+K_{s,HM}} \times X_{HM}$
Growth of X_{HAC}			$Y_{XHAC/H2}$	-1		1 - $Y_{XHAC/H2}$	$\sum_{i=IC_{CO2},X_{HM},CH_4} O_i \nu_i, X_{HAC}$	$-\sum_{i=X_{HAC},C_2H_4O_2} C_i \nu_i, X_{HAC}$	- $Y_{XHAC/H2}$	$Y_{XHAC/H2}$	$k_{mol,HAC} \times \frac{C_{H2}}{C_{H2}+K_{s,HAC}} \times X_{HAC}$
Decay of X_{HM}	1	-1									$k_{dec,HM} \times X_{HM}$
Decay of X_{HAC}	1		-1								$k_{dec,HAC} \times X_{HAC}$

Table 1: Biochemical rate coefficients (ν_i) and kinetic rate equations for growth and decay processes included in the model.

Processes ↓	**State Variables (mol.L^{-1})**								**Rates (mol.L^{-1}d^{-1}) ↓**
	C_{acH}	C_{ac}	C_{IN}	C_{IC_co2}	C_{IC_hco3}	C_{IC_co3}	C_H	C_{oh}	
f_acH_ac	-1	1					1		$Ka_{acH} \times C_{acH}$
r_ac_acH	1	-1					-1		$C_{ac^-} \times C_{H^+}$
f_NH4_NH3			-1				1		$Ka_{NH_3} \times C_{NH_4^+}$
r_NH3_NH4			1				-1		$C_{NH_3} \times C_{H^+}$
f_CO2_HCO3				-1	1		1		$Ka_{CO_2} \times C_{CO_2}$
r_HCO3_CO2				1	-1		-1		$C_{HCO_3^-} \times C_{H^+}$
f_HCO3_CO3					-1	1	1		$Ka_{HCO_3^-} \times C_{HCO_3^-}$
r_CO3_HCO3					1	-1	-1		$C_{CO_3^{2-}} \times C_{H^+}$
f_H_H2O_OH							1	1	K_w
r_OH_H2O_H							-1	-1	$C_{OH^-} \times C_{H^+}$

Table 2: Rate coefficients and kinetic rate equations for acid-base reactions in the model.

Thermodynamic considerations were included into the model to evaluate the feasibility of the biological reactions. Especially the calculations of Gibbs free energy (ΔrG) and thermodynamic factor (F_T) as described by Jin et Bethke (2007). Table 3 shows the parameters sued for the calculations.

	HM	HAC	AM	SAO
ΔrG° (kJ/reaction)	-131	-82	-49	+82
Y_ATP (molATP/reaction)	0.5	0.3	0.5	0.3
ΔGp (kJ/reaction)	45	45	45	45
ΔGc (kJ/reaction)	22.50	14.85	22.50	14.85
X	2	1	2	1

Table 3: Thermodynamic parameters used for the calculation of the ΔrG of the different reactions considered in the experimental conditions (Grimalt-Alemany et al., 2020).

2.8. Theory/calculations

Experimental growth yields in g COD cells per g COD substrate (H_2) of HM and HAC were calculated from the activity tests in which only HM and HAC functions were detected.

$$Y_{\frac{X}{H_2}} = \left(\frac{\Delta OD_{600}+0.2807}{3.8255}\right) \times 1.4159 \times \frac{V_{liq}}{\Delta H_2}\ \text{gCOD}_X.\text{gCOD}_{H2}^{-1} \quad (4)$$

With 1.4159 = $gCOD_X.g_{VS}^{-1}$ if X = $C_5H_7O_2N$

3. ***Results and Discussion***

HAC is a promising route for biotechnology development of C1 compounds (CO, CO_2, methanol etc.) recovery into value added molecules. Studying the competition between HM and HAC could result in a method for inoculum development that presents advantages compared to addition of inhibitors or heat treatment. Grimalt-Alemany (2019) highlighted that the gas to liquid mass transfer rate was crucial in the outcome of the competition between HM and HAC. Especially, the author shows that HAC only grow under high H_2 availability and mesophilic conditions, due to the better capacity of HM to fix H_2 traces, and their much higher specific growth rates under thermophilic conditions. This indicates that the competition between HM and HAC follows the resource ratio competition model. Microorganisms can adopt two different strategies to have the advantage over their competitors. The first type of competitors are r-strategists, meaning that they have high specific growth rates, or high specific consumption rates. They will grow faster and become dominant. Conversely, K-strategists are microbes with a better substrate affinity, meaning that the bioavailability of the substrate is higher for them than for their competitors (Paul et al., 2021). Considering the low solubility of hydrogen, mass transfer coefficient appears as a determinant parameter during gas fermentation in batch mode, as the substrate is continuously delivered through gas to liquid mass transfer rate, rather than being totally available from the beginning of the fermentation, as it is the case in a classical single liquid phase fermentation. Indeed, mass transfer limitation will lead to a K-competition, benefiting HM in most cases. The exception could be psychrophilic conditions, under which HAC could have faster H_2 fixating capacities. Indeed, Conrad et al. (1989) observed that in MMC enrichments at 17 °C, HAC outcompeted HM, while at 30 °C HM outcompeted HAC. The authors also identified a critical temperature determining a switch in the outcome of the competition around 20-25 °C.

In this study, the microbial competition between HM and HAC under H_2/CO_2 was investigated in SB. The effect of temperature between 25 °C and 35 °C was investigated, as well as mass transfer limitation. A method for the enrichment of homoacetogens under H_2/CO_2 was developed.

3.1. Functional activities selected in SB

Error! Reference source not found. shows the electron balances between acetate and methane produced over H_2 consumed along the different SB carried out. Significant differences could be observed between the different SB. First, acetate was never detected in the liquid phase of SB conducted at 35 °C. On the other hand, at 25 °C, acetate could be detected, and the balance even moved towards acetate as the sole product when mass transfer limitation was overcome. In SB supposed to be mass transfer limited, acetate was detected in the first batches, but the balance progressively moved toward methane as the sole product by the end of the experiment. The fact that acetate was detected at the beginning of these SB may be due to mass transfer limitation being delayed because of higher gas solubility, and also lower HM specific growth rates at 25 °C than at 35 °C.

These results demonstrate a strong effect of temperature on the microbial competition between HM and HAC. Additionally, the way of implementing the SB with short batch times, and fast transfers might have contributed to favour HAC. A combination effect of avoiding mass transfer limitation with short growth times allowed to progressively eliminate methane production along the experiments. To characterize the MMCs functions selected by the end of the SB, hydrogenotrophic, homoacetogenic and acetotrophic activity tests were carried out (**Error! Reference source not found.**).

	$C_{H2(aq)}$ M	$C_{CO2(aq)}$ M	$C_{CH4(aq)}$ M	$C_{Acetate}$ M	$\Delta rG_{25\,°C}$ kJ/reaction	$\Delta rG_{35\,°C}$ kJ/reaction
Acetic acid → CO_2 + CH_4 (AM)	1.00E-10	1.00E-10	1.00E-10	0.023	-129	-132
Acetic acid + 2 H_2O → 2 CO_2 + 4 H_2 (SAO)	1.00E-10	1.00E-10	1.00E-10	0.023	-164	-175

Table 4: Gibbs free energy calculation of the acetate consuming reactions at the initial conditions of activity tests with N_2/Acetate. 1.00E-10 M is used instead of 0 to do the calculations. 0.023 ± 0.003 M was the average concentration measured at initial state over all the vials carried out with N_2/Acetate (n=18).

Error! Reference source not found. shows the results of activity tests carried out on the MMC enriched at 35 °C under mass transfer limitation. The results show that even if acetate was not detected during the SB period, HAC as well as HM were active in the activity tests. Hydrogenotrophic tests show that HAC and HM could grow, with methane being the main product. At 35 °C, HM specific growth rate was likely higher than that of HAC, and also higher than at 25 °C. Additionally, H_2 solubility is lower at 35 °C than at 25 °C, inducing that the mass transfer limitation was reached earlier, benefiting thus HM. Activity tests were also carried out on the two MMCs obtained at 25 °C. According to **Error! Reference source not found.**A, in MMC enriched at 25 °C under mass transfer limitation, HAC was active in the homoacetogenic tests, but couldn't grow in hydrogenotrophic tests in which HM outcompeted them. This demonstrates that HAC were still in the MMC even if acetate production was not detected anymore at the end of these SB. However, with HM being active, acetate production was not observed. Conversely, in the MMC enriched in SB at 25 °C, overcoming the mass transfer limitation, it was methane production that was not detected anymore at the end of the SB. But in this case, activity tests revealed that methanogenesis function was lost in the corresponding MMC. Indeed, only acetate production was detected during the hydrogenotrophic test.

These results demonstrate that methanogenic function could be irreversibly eliminated from the MMC by implementing a specific cultivation/enrichment method. This method consisted in lowering temperature to 25 °C, and avoid mass transfer limitation by transferring from a batch to another as soon as some substrate consumption was observed. In fact, the short time of each batch must have been another constraint for HM. Indeed, at 25 °C, longer lag phases were observed for HM than for HAC during the SB phase.

Acetate concentration and total pressure remained constant in all acetotrophic activity tests, indicating that the MMCs were not able to consume acetate in these conditions. The results of these activity tests show that AM and SAO were likely washed out from the MMCs along the SB, and could not be reactivated afterwards when placed under favourable conditions. AM have generally lower specific growth rates than HM (Batstone et al., 2002, p. 1), so if HM were washed out, it is consistent that AM were too. Regarding SAO, the reaction is only possible after acetate is produced, and thermodynamic calculations show that low H_2 and CO_2 partial pressures are necessary. In the case of the SB transferred rapidly to overcome mass transfer limitation, H_2 and CO_2 partial pressures were still significant in the gas phase, and acetate concentration was still low so SAO could not be active. A discussion about thermodynamic analysis of the SB is presented in section 3.3.

3.2. HM and HAC growth yields

The activity tests on the enriched culture in SB at 25 °C with several gas injections and late transfer were used for the calculation of growth yields.

According to Figure 6.A, HAC had higher growth yield than HM. For both HM and HAC, the growth yield was not constant along the batch and decreased. Growth yields calculated at the end of the batch were 2.4 % and 4.6 % ($gCOD_X.gCOD_{H2}^{-1}$) for HM and HAC respectively. However, the average growth yields were 3.7 % and 7.0 % ($gCOD_X.gCOD_{H2}^{-1}$) for HM and HAC respectively. This indicates that the production of biomass represented a higher part of H_2 fixed during the first stage of the batch. According to Figure 6.B, it is possible that HM and HAC growth yields are P_{H2} dependent and increase with P_{H2}. This would imply a variable stoichiometry, which has already been reported for the fermentation of glucose to acetate and butyrate (Rodríguez et al., 2006). Interestingly, Figure 6.B allows to identify minimal P_{H2} for which an activity is detected. In the case of HM, this P_{H2} was not quantifiable. However, for HAC, an average residual P_{H2} of 0.021 bar was identified in the vials, corresponding to 1.65.E-05 $mol \cdot L^{-1}$ of H_2 in the liquid phase at 25 °C. This confirms that HAC might have higher H_2 uptake thresholds than HM, and 25 °C was not low enough to reverse the substrate affinity of both communities.

3.3. Microbial compositions of the enriched MMCs

Figure 7 shows the different genera found in the enriched MMCs after the different SB carried out. HAC genus *Acetobacterium* was only selected at 25 °C and not at 35 °C, with highest abundance of 38 % in the MMC enriched avoiding mass transfer limitation (MTL). Additionally, genus *Methanobacterium* was selected in all enrichments except in this same MMC. However, *Methanobrevibacter* was found in all samples, with the highest abundance of 12 % in the enriched MMC at 25 °C under mass transfer limitation. This suggests that *Methanobrevibacter* was more adapted to 25 °C than *Methanobacterium*, and that temperature also affects the competition between different HMs. Finally, *Desulfovibrio* was found in all the samples, with particularly high abundance of 33 % in the SB at 25 °C without mass transfer limitation. The latter are sulphate reducing bacteria (SRB) capable of growing in chemolithoautotrophy on CO_2, but also in heterotrophy on acetate, using the WLP or the reductive glycine pathway (Sánchez-Andrea et al., 2020). Sulphate is the electron acceptor of the metabolism, and the electrons can come from different monomers such as sugars, amino acids, short chain fatty acids (including acetate), ethanol, and hydrogen. Consistently, they can be found in acetogenic and hydrogenogenic systems when sulphate is available (Sánchez-Andrea et al., 2020; Weijma et al., 2002; Cord-Ruwisch et al., 1988; Wood et al., 1986; Badziong et al., 1979). Since the mineral medium contained sulphates, these bacteria remained in competition with HAC at 25 °C when mass transfer limitation was overcome, demonstrating that conversely to HM, they had competitive specific growth rates with HAC in these conditions. SRB can use H_2 to reduce sulphate of the mineral growth medium as an anaerobic respiration, they can also oxidize acetate as an anaerobic fermentation, according to the following stoichiometries:

$$4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O \qquad \text{SRB}$$

344 $C_2H_4O_2 + SO_4^{2-} \rightarrow 2HCO_3^- + HS^-$ SAO-SRB

345 $H_2S + H_2O \leftrightarrow HS^- + H_3O^+$ pka = 7.04

346 The initial concentration of sulphate in the medium was 0.35 mM corresponding to a maximum of
347 H_2 consumed by SRB of 1.4 mM, or 0.022 $gCOD_{H2}.L^{-1}$. As the reactions produces reduced sulphur
348 in the forms of HS^-, it could explain that HM are enriched in most of the SB despite omitting
349 sulphide addition in the mineral medium (Muyzer and Stams, 2008).

350

351 ### *3.4. Modelling of the microbial competition between HM and HAC*

352 The biomasses profile (**Error! Reference source not found.**A) can be divided into two phases.
353 During the first phase, soluble H_2 concentration is much higher than H_2 thresholds of HM and
354 HAC. Both communities grow at their μ_{max}, which is the parameter that governs the competition
355 (r-competition). So, in this configuration, HAC dominate, but HM can also grow and remain in the
356 reactor, leading to a cohabitation of both microbial communities. During the second phase
357 delimited by vertical dot lines, soluble H_2 is limiting ($r_{H2} = T_{H2}$). Microorganisms enter in a phase
358 of competition for trace amounts of H_2, in which HM take the advantage due to their better affinity
359 to the substrate (K-competition). With these kinetic parameters, a k_La increase from 10 to 50 d^{-1}
360 allows to produce twice more HAC than HM. However, further increase of the k_La doesn't promote
361 anymore HAC. This is explained by the fact that increasing k_La delays the time when mass transfer
362 limitation is reached. Hence, above a certain k_La value, the final H_2 consumption rate remains
363 below the maximal mass transfer rate for the entire experiment, and the competition remains
364 governed by μ_{max}.

Error! Reference source not found.C and D show the calculations of ΔrG and F_T of HM, HAC, AM and SAO along the simulation. It is interesting to note that the mass transfer limitation is closely related to thermodynamic limitation for HAC. Indeed, when the system enters in mass transfer limitation (vertical lines), the ΔrG reaches -14 kJ/reaction, which is close to the value of ΔrGc found in literature for this community (-14.85 kJ.reaction^{-1}) (Grimalt-Alemany et al., 2020). Additionally, from 0.7 d, it shows that HM are also thermodynamically limited until the end. Hence, the limitation term of Monod, defined with K_{H2} is closely related to thermodynamic constraints. This demonstrates that empirical parameters such as K_{H2}, could be replaced by thermodynamic limitation terms. This also show that when substrate is not limiting, the system is mostly governed by kinetics, if products concentrations are not above the thermodynamic threshold. When the system is mass transfer limited, then the main limitation is thermodynamic. Although SAO and AM growth was not activated in the model, F_T calculations show that AM reaction is feasible all along the batch. A slight thermodynamic limitation of AM during the batch is due to P_{CO2} first, and then P_{CH4} at the end of the batch. SAO reaction is not feasible until acetate is produced and P_{H2} et P_{CO2} become low.

Dynamic simulation based on Monod kinetics is consistent with the F_T profiles calculated. Hence, both approaches represent well the substrate limitation phenomenon and are satisfying at a macroscopic scale for H_2/CO_2 fermentation in batch mode. However, the Monod kinetics predict a very low growth under H_2 limitation while F_T stops immediately the growth once the reaction is no more thermodynamically feasible. This implies that according to Monod growth kinetics, the competition between HM and HAC keeps going until the end of the batch. On the contrary, with F_T, the competition only occurs during the first stage of the batch. Furthermore, considering the difficulty to measure parameters such as Ks, the use of thermodynamic limitation terms is relevant

to describe anaerobic systems under other types of limitations, such as CO_2 limitation. Indeed, F_T not only considers substrate availability, but also product concentrations inhibition, temperature, and pH. Therefore, F_T should be more accurate to describe systems in dynamic state, or in new environmental conditions for which kinetic parameters are not available.

According to these simulations, it should be possible to favour HAC in batch mode, as long as the mass transfer rate (T_{H2}) is higher than H_2 consumption rates (r_{H2}). Hence, in batch mode, a key process parameter is the ratio between r_{H2} and T_{H2}. To decrease this ratio, two main options are the increase of the k_La of the reactor, and the decrease of the initial biomass concentration by diluting the MMC. Of course, a compromise must be found between the biomass dilution, and the amount of enriched MMC needed by the end of the experiment. The initial concentration of HM is also important and should be minimized, for example be choosing a MMC coming from anaerobic environment where methanogenic activity is low, like in psychrophilic environments. Considering this, batch mode appears as an interesting cultivation strategy for MMC specialization and enrichment, to provide inoculum. However, it seems not advisable for acetate production processes, as the substrate limitation will be an unavoidable obstacle.

In batch mode, biomass accumulates, and its concentration increases. This implies that r_{H2} reaches mass transfer limitation sooner or later, as long as substrate is available. This explains why batch mode in respect to the liquid phase is preferred for biological methanation, with a continuous gas supply. This way, higher biomass concentrations are reached, which increases methane production rate. Additionally, in the case of methanation, methane is continuously extracted in the gas phase, which makes it possible to operate over long periods without product accumulation issues.

In the case of acetate production though, the product accumulates in the liquid, which makes continuous mode on the liquid phase more interesting for this application to avoid product

411 concentration inhibition effects. Considering lab constraints to implement continuous reactors, it
412 can still be envisaged to carry out successive batches, diluting the biomasses at each new
413 inoculation step and therefore maintaining low rates and preventing mass transfer limitation. This
414 could be an interesting way to specialize and enrich a MMC with batch cultures. This strategy
415 could be a method of producing a specialized MMC and operate with higher k_La would allow to
416 reach higher HAC concentrations by the end of the experiment.

417 ## *4. Conclusions*

418 In this study, the microbial competitions were investigated in the framework of the fermentation
419 of H_2/CO_2 with MMCs. The objective was to favour the HAC and eliminate HM. SB were
420 implemented, at 25 °C and 35 °C, and with two different cultivation strategies. The first one waiting
421 for mass transfer limitation after multiple gas injections and consumption phases, and the second
422 not waiting for mass transfer limitation by transferring faster as soon as activity was detected after
423 a single gas injection.

424 First, the effect of temperature was observed, as acetate was never detected in the SB at 35 °C.
425 Then, at 25 °C and avoiding mass transfer limitation, homoacetogenesis remained as the sole
426 metabolic function identified in the activity tests. Hence, this cultivation method even allowed to
427 eliminate HM in the MMC. This was due to too low kinetic parameters values of HM, leading to
428 their loss along the SB set. In perspective of this study, it will be interesting to evaluate the
429 reproducibility of this cultivation method with different starting anaerobic MMCs to prove its
430 robustness and applicability to provide HAC specialized MMCs.

431 The temperature effect between 25 °C and 35 °C on the outcome of the competition was clearly
432 demonstrated, with *Methanobacterium* HM outcompeting HAC at 35 °C, while *Acetobacterium*

HAC coexisted with *Methanobacterium* and *Methanobrevibacter* HMs at 25 °C with simultaneous methane and acetate production observed. This behaviour indicates that the competition between HM and HAC follows the resource ratio model, and that under mass transfer limitation HM outcompete HAC because of their lower H_2 consumption threshold. Conversely, when mass transfer limitation is overcome, both HM and HAC can grow. As HAC have higher growth rates, HM can be washed out the system by implementing transfers before mass transfer limitation is reached.

AM and SAO were not observed in the activity tests. At 35 °C, this was mostly because of thermodynamic limitation of the reaction. However, at 25 °C, AM was feasible but still not detected. According to literature, AM have lower specific growth rates than HM, explaining that they were not capable of maintaining themselves in the SB, especially because their substrate, acetate, was produced along the batches, and so decreasing their potential time for growth. However, the SRB *Desulfovibrio* was detected at significant relative abundance in the MMC, because of the addition of sulphate instead of sulphide in the mineral medium. The promotion of SRB provided reduced sulphur in significant amounts as sulphate is the electron acceptor of these bacteria. Hence, HM were not sulphur limited during the tests. The consideration of SRB will be of interest for further optimizations of the microbial selection method developed in this work, to provide acetate and other VFA producing anaerobic MMCs.

Acknowledgements:

The authors want to acknowledge the department of chemical and biochemical engineering of Denmark Technical University for its collaboration in the framework of an international exchange.

Formatting and funding sources:

This work was supported by the french National Research Institute of Agriculture and Environment (INRAE) and the institute 3BCAR for financial support of an international mobility.

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Agler, M.T., Wrenn, B.A., Zinder, S.H., Angenent, L.T., 2011. Waste to bioproduct conversion with undefined mixed cultures: the carboxylate platform. Trends in Biotechnology 29, 70–78. https://doi.org/10.1016/j.tibtech.2010.11.006

Angelidaki, I., Treu, L., Tsapekos, P., Luo, G., Campanaro, S., Wenzel, H., Kougias, P.G., 2018. Biogas upgrading and utilization: Current status and perspectives. Biotechnology Advances 36, 452–466. https://doi.org/10.1016/j.biotechadv.2018.01.011

Annie Modestra, J., Navaneeth, B., Venkata Mohan, S., 2015. Bio-electrocatalytic reduction of CO2: Enrichment of homoacetogens and pH optimization towards enhancement of carboxylic acids biosynthesis. Journal of CO2 Utilization 10, 78–87. https://doi.org/10.1016/j.jcou.2015.04.001

Badziong, W., Ditter, B., Thauer, R.K., 1979. Acetate and carbon dioxide assimilation by Desulfovibrio vulgaris (Marburg), growing on hydrogen and sulfate as sole energy source. Arch. Microbiol. 123, 301–305. https://doi.org/10.1007/BF00406665

Batstone, D.J., Keller, J., Angelidaki, I., Kalyuzhnyi, S.V., Pavlostathis, S.G., Rozzi, A., Sanders, W.T.M., Siegrist, H., Vavilin, V.A., 2002. The IWA Anaerobic Digestion Model No 1 (ADM1). Water Sci Technol 45, 65–73. https://doi.org/10.2166/wst.2002.0292

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 13, 581–583. https://doi.org/10.1038/nmeth.3869

Conrad, R., Bak, F., Seitz, H.J., Thebrath, B., Mayer, H.P., Schütz, H., 1989. Hydrogen turnover by psychrotrophic homoacetogenic and mesophilic methanogenic bacteria in anoxic paddy soil and lake sediment. FEMS Microbiology Ecology 5, 285–293. https://doi.org/10.1111/j.1574-6968.1989.tb03382.x

Cord-Ruwisch, R., Seitz, H.-J., Conrad, R., 1988. The capacity of hydrogenotrophic anaerobic bacteria to compete for traces of hydrogen depends on the redox potential of the terminal electron acceptor. Arch Microbiol 149, 350–357. https://doi.org/10.1007/BF00411655

Fu, B., Jin, X., Conrad, R., Liu, Hongbo, Liu, He, 2019. Competition Between Chemolithotrophic Acetogenesis and Hydrogenotrophic Methanogenesis for Exogenous H2/CO2 in Anaerobically Digested Sludge: Impact of Temperature. Front. Microbiol. 10. https://doi.org/10.3389/fmicb.2019.02418

Gavala, H.N., Grimalt-Alemany, A., Asimakopoulos, K., Skiadas, I.V., 2021. Gas Biological Conversions: The Potential of Syngas and Carbon Dioxide as Production Platforms. Waste Biomass Valor. https://doi.org/10.1007/s12649-020-01332-7

Grimalt-Alemany, A., 2019. Syngas Fermentation to Biofuels: Evaluation of the Interplay of Kinetics and Thermodynamics for Directing Bioconversions based on Mixed Microbial Communities.

Grimalt-Alemany, A., Asimakopoulos, K., Skiadas, I.V., Gavala, H.N., 2020. Modeling of syngas biomethanation and catabolic route control in mesophilic and thermophilic mixed microbial consortia. Applied Energy 262, 114502. https://doi.org/10.1016/j.apenergy.2020.114502

Grimalt-Alemany, A., Łężyk, M., Kennes-Veiga, D.M., Skiadas, I.V., Gavala, H.N., 2019. Enrichment of Mesophilic and Thermophilic Mixed Microbial Consortia for Syngas Biomethanation: The Role of Kinetic and Thermodynamic Competition. Waste Biomass Valor. https://doi.org/10.1007/s12649-019-00595-z

Hall, M., Beiko, R.G., 2018. 16S rRNA Gene Analysis with QIIME2, in: Beiko, R.G., Hsiao, W., Parkinson, J. (Eds.), Microbiome Analysis: Methods and Protocols, Methods in Molecular Biology. Springer, New York, NY, pp. 113–129. https://doi.org/10.1007/978-1-4939-8728-3_8

Jin, Q., Bethke, C.M., 2007. The thermodynamics and kinetics of microbial metabolism. Am J Sci 307, 643–677. https://doi.org/10.2475/04.2007.01

Kotsyurbenko, O.R., Glagolev, M.V., Nozhevnikova, A.N., Conrad, R., 2001. Competition between homoacetogenic bacteria and methanogenic archaea for hydrogen at low temperature. FEMS Microbiol Ecol 38, 153–159. https://doi.org/10.1111/j.1574-6941.2001.tb00893.x

Liu, F., Monroe, E., Davis, R.W., 2018. Engineering Microbial Consortia for Bioconversion of Multisubstrate Biomass Streams to Biofuels. Biofuels - Challenges and opportunities. https://doi.org/10.5772/intechopen.80534

Liu, H., Wang, J., Wang, A., Chen, J., 2011. Chemical inhibitors of methanogenesis and putative applications. Appl Microbiol Biotechnol 89, 1333–1340. https://doi.org/10.1007/s00253-010-3066-5

Luo, G., Jing, Y., Lin, Y., Zhang, S., An, D., 2018. A novel concept for syngas biomethanation by two-stage process: Focusing on the selective conversion of syngas to acetate. Science of The Total Environment 645, 1194–1200. https://doi.org/10.1016/j.scitotenv.2018.07.263

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17, 10–12. https://doi.org/10.14806/ej.17.1.200

Molenaar, S.D., Saha, P., Mol, A.R., Sleutels, T.H.J.A., ter Heijne, A., Buisman, C.J.N., 2017. Competition between Methanogens and Acetogens in Biocathodes: A Comparison between Potentiostatic and Galvanostatic Control. Int J Mol Sci 18. https://doi.org/10.3390/ijms18010204

Musvoto, E.V., Wentzel, M.C., Loewenthal, R.E., Ekama, G.A., 2000. Integrated chemical–physical processes modelling—I. Development of a kinetic-based model for mixed weak acid/base systems. Water Research 34, 1857–1867. https://doi.org/10.1016/S0043-1354(99)00334-6

Muyzer, G., Stams, A.J.M., 2008. The ecology and biotechnology of sulphate-reducing bacteria. Nat Rev Microbiol 6, 441–454. https://doi.org/10.1038/nrmicro1892

Omar, B., Abou-Shanab, R., El-Gammal, M., Fotidis, I.A., Kougias, P.G., Zhang, Y., Angelidaki, I., 2018. Simultaneous biogas upgrading and biochemicals production using anaerobic bacterial mixed cultures. Water Research 142, 86–95. https://doi.org/10.1016/j.watres.2018.05.049

Paul, E., Bessière, Y., Dumas, C., Girbal-Neuhauser, E., 2021. Biopolymers Production from Wastes and Wastewaters by Mixed Microbial Cultures: Strategies for Microbial Selection. Waste Biomass Valor 12, 4213–4237. https://doi.org/10.1007/s12649-020-01252-6

Pedregosa, F., Varoquaux, G., Gramfort, A., Michel, V., Thirion, B., Grisel, O., Blondel, M., Prettenhofer, P., Weiss, R., Dubourg, V., Vanderplas, J., Passos, A., Cournapeau, D., 2011. Scikit-learn: Machine Learning in Python. MACHINE LEARNING IN PYTHON.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research 41, D590–D596. https://doi.org/10.1093/nar/gks1219

Regueira, A., González-Cabaleiro, R., Ofiţeru, I.D., Rodríguez, J., Lema, J.M., 2018. Electron bifurcation mechanism and homoacetogenesis explain products yields in mixed culture anaerobic fermentations. Water Research 141, 349–356. https://doi.org/10.1016/j.watres.2018.05.013

Reichert, P., 1994. AQUASIM – A TOOL FOR SIMULATION AND DATA ANALYSIS OF AQUATIC SYSTEMS. Water Science and Technology 30, 21–30. https://doi.org/10.2166/wst.1994.0025

Rodríguez, J., Kleerebezem, R., Lema, J.M., Loosdrecht, M.C.M. van, 2006. Modeling product formation in anaerobic mixed culture fermentations. Biotechnology and Bioengineering 93, 592–606. https://doi.org/10.1002/bit.20765

Saady, N.M.C., 2013. Homoacetogenesis during hydrogen production by mixed cultures dark fermentation: Unresolved challenge. International Journal of Hydrogen Energy 38, 13172–13191. https://doi.org/10.1016/j.ijhydene.2013.07.122

Sánchez-Andrea, I., Guedes, I.A., Hornung, B., Boeren, S., Lawson, C.E., Sousa, D.Z., Bar-Even, A., Claassens, N.J., Stams, A.J.M., 2020. The reductive glycine pathway allows autotrophic growth of Desulfovibrio desulfuricans. Nat Commun 11, 5090. https://doi.org/10.1038/s41467-020-18906-7

Sander, R., 2015. Compilation of Henry's law constants (version 4.0) for water as solvent. Atmos. Chem. Phys. 15, 4399–4981. https://doi.org/10.5194/acp-15-4399-2015

Schuchmann, K., Müller, V., 2014. Autotrophy at the thermodynamic limit of life: a model for energy conservation in acetogenic bacteria. Nature Reviews Microbiology 12, 809–821. https://doi.org/10.1038/nrmicro3365

Shen, N., Dai, K., Xia, X.-Y., Zeng, R.J., Zhang, F., 2018. Conversion of syngas (CO and H2) to biochemicals by mixed culture fermentation in mesophilic and thermophilic hollow-fiber membrane biofilm reactors. Journal of Cleaner Production 202, 536–542. https://doi.org/10.1016/j.jclepro.2018.08.162

Steinbusch, K.J.J., Hamelers, H.V.M., Plugge, C.M., Buisman, C.J.N., 2011. Biological formation of caproate and caprylate from acetate: fuel and chemical production from low grade biomass. Energy Environ. Sci. 4, 216–224. https://doi.org/10.1039/C0EE00282H

Theil, S., Rifa, E., 2021. rANOMALY: AmplicoN wOrkflow for Microbial community AnaLYsis. F1000Res 10, 7. https://doi.org/10.12688/f1000research.27268.1

Vavilin, V.A., Lokshina, L.Ya., Rytov, S.V., Kotsyurbenko, O.R., Nozhevnikova, A.N., 2000. Description of two-step kinetics in methane formation during psychrophilic H2/CO2 and mesophilic glucose conversions. Bioresource Technology 71, 195–209. https://doi.org/p

Vigliar, E., Malapelle, U., de Luca, C., Bellevicine, C., Troncone, G., 2015. Challenges and opportunities of next-generation sequencing: a cytopathologist's perspective. Cytopathology 26, 271–283. https://doi.org/10.1111/cyt.12265

Walters, W., Hyde, E.R., Berg-Lyons, D., Ackermann, G., Humphrey, G., Parada, A., Gilbert, J.A., Jansson, J.K., Caporaso, J.G., Fuhrman, J.A., Apprill, A., Knight, R., 2015. Improved Bacterial 16S rRNA Gene (V4 and V4-5) and Fungal Internal Transcribed Spacer Marker Gene

Primers for Microbial Community Surveys. mSystems 1, e00009-15. https://doi.org/10.1128/mSystems.00009-15

Wang, Y.-Q., Yu, S.-J., Zhang, F., Xia, X.-Y., Zeng, R.J., 2017. Enhancement of acetate productivity in a thermophilic (55 °C) hollow-fiber membrane biofilm reactor with mixed culture syngas (H2/CO2) fermentation. Appl Microbiol Biotechnol 101, 2619–2627. https://doi.org/10.1007/s00253-017-8124-9

Weijma, J., Gubbels, F., Hulshoff Pol, L.W., Stams, A.J.M., Lens, P., Lettinga, G., 2002. Competition for H2 between sulfate reducers, methanogens and homoacetogens in a gas-lift reactor. Water Sci Technol 45, 75–80. https://doi.org/10.2166/wst.2002.0294

Wood, H.G., Ragsdale, S.W., Pezacka, E., 1986. The acetyl-CoA pathway of autotrophic growth. FEMS Microbiology Reviews 2, 345–362. https://doi.org/10.1111/j.1574-6968.1986.tb01865.x