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# Inhibitions from syngas impurities: impact of common tar components on a consortium adapted for syngas biomethanation

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## Abstract

In a circular economy approach, syngas biomethanation is a promising technology for waste to energy conversion. However, syngas can contain impurities, notably tar, that can limit the processes upgrading syngas downstream gasification. The effect of tar on syngas biomethanation is unknown. Therefore, for the first time, common tar components were tested on a consortium adapted for syngas biomethanation to assess the resistance of the microorganisms. Four light tar components (benzene, toluene, styrene and phenol) commonly found in syngas were selected and tested at different concentrations in batch tests. Adding pollutant up to inhibitory concentrations affected both the lag phase of microbial growth and the rates of the bioreactions. Hydrogenotrophic methanogens were found to be more sensitive than carboxydrotrophs. Amongst the four tested pollutants, phenol appears to be the most problematic, due not only to its high inhibitory effect but also to its high solubility, allowing phenol in the syngas to reach high inhibitory concentrations. This study paves the way for further research on the resistance of syngas biomethanation to impurities contained in the syngas.

## Keywords

Syngas biomethanation ; syngas impurities inhibition ; syngas fermentation ; tar inhibition

## Highlights

- The inhibitory impact of tar pollutants from syngas on biomethanation was assessed.
- Hydrogenotrophic methanogens appear to be more sensitive than carboxydrotrophs.
- Adding pollutants both impacted the rate and the lag time of microbial growth.
- Phenol was the most inhibitory component.

## 1. Introduction

The increasing energy demand in the world, coupled with future limitation of available fossil fuels [1] brings the necessity to find more sustainable modes of energy production. Using renewable feedstocks such as biomass residues or waste is a promising approach to develop new energy sources [2]. Indeed, using waste to produce energy is an interesting way to reduce their environmental impact and to produce cleaner energy. In this regard, gasification is an increasingly studied solution to upgrade waste or biomass to energy in the form of syngas, which is a mixture of  $H_2$ ,  $CO$ ,  $CO_2$ ,  $N_2$  and  $CH_4$ . Gasification is a versatile process that allows quick disposal of wastes, can convert a wide variety of inputs and can lead to a great diversity of end products in the form of heat, power,  $H_2$ , and second generation fuels [2, 3].

However, gasification of biomass faces a few issues, one of which being the production of undesirable products such as polycyclic aromatic hydrocarbons (PAH), nitrogen compounds ( $HCN$ ,  $NH_3$ ), sulfur compounds ( $H_2S$ ,  $COS$ ,  $CS_2$ ), halogenated compounds ( $HCl$ ,  $HBr$ ,  $HF$ ,  $HI$ ,  $HIO_3$ ) and tars. The definition of tars varies according to the studies, but a consensus defines them as all hydrocarbon with molecules weight superior to benzene [4]. They can be classified in various ways, one of which is according to molecular weight with the heavy tars undetectable by GC (class 1), heterocyclic aromatics such as phenol, cresol...(class 2), light aromatics such as benzene, toluene...(class 3), light PAH compounds such as indene, naphthalene (class 4) and heavy PAH compounds such as fluoranthene, pyrene (class 5) [5]. Tar have a negative impact on the gasification process, for instance tar condensation can lead to pipe clogging [6] and to process deficiencies. Tar can also limit the technologies downstream of the gasification process: gas engines require tar levels not higher than  $50 \text{ mg/Nm}^3$ , gas turbine  $5 \text{ mg/Nm}^3$ , and fuel cells  $1 \text{ mg/Nm}^3$  while tar levels can range from  $100 \text{ mg/Nm}^3$  to  $100 \text{ g/Nm}^3$  depending on the gasifier technology [5].

In addition to being converted to heat or power, syngas can be upgraded to biofuels. In this regard, syngas fermentation is a well-studied technology at commercial stage [7]. Bioprocess can also be impacted by tar, as tar can induce cell dormancy and product redistribution [8]. However, the studies assessing the impact of tar on fermentation usually use pure culture to generate ethanol and acetic acid [9–11]. It is possible that using adapted mixed cultures would allow for better resistance to tar components, since some microorganisms could tolerate or degrade some compounds inhibitory to other microbial groups [12, 13].

Another promising option for syngas upgrading is to convert syngas to methane. The benefits are to take advantage of the existing infrastructures for natural gas and to be able to mix the renewable methane with natural gas for a progressive energy transition.

Syngas conversion to methane can be carried out either via catalytic methanation or biological methanation. Even though the former is a better established technology [14], biological methanation is a promising technology that displays several advantages, such as milder operating conditions in terms of temperature and pressure, conversion of syngas independently of the ratio  $H_2/CO$ , and a potential higher resistance to syngas impurities. Especially, biomethanation is expected to have a high resistance to  $NH_3$  and  $H_2S$  compared to catalytic methanation, since these components are known to be necessary for methanogenic growth [15].

In a biomethanation process, the reactions are facilitated by microorganisms that act as catalysts while supporting their growth [16]. Usually, biomethanation processes use adapted mixed cultures [17–21]. In this case, the conversion of syngas is the result of complex syntrophic or competitive interactions, and the main metabolic routes involved are dependent of the temperature [22, 23]. In thermophilic conditions, studies have shown that the system can be simplified to two main reactions:



With CO being converted by carboxydrotrophic microorganisms with  $H_2/CO_2$  production and concurrent hydrogenotrophic methanogenesis [19, 23–26]. To our knowledge, only one study experimented biomethanation with a real syngas from a fluidized bed gasifier using steam as gasification agent and wood pellet as intransit [27]. Tar was cleaned through tar cracking and carbon filter. No apparent inhibition of the biomethanation process was observed, indicating either a good resistance of the biological consortium or a good efficiency of the cleaning process. This result is very promising for the syngas biomethanation technology. However, the tar content of the syngas was not disclosed. Therefore, the potential impact of tar and tar type on a syngas biomethanation consortium is today unknown. To our knowledge, there is no inhibition data available for a consortium adapted for syngas biomethanation. Thus, there is a need to quantify the impact of syngas impurities on biomethanation to dimension adapted cleaning processes. For this study, the attention was focused on tar, particularly on lighter tars that are less likely to condense in a cleaning process.

To select the studied tar, common compositions of tar in syngas were considered. According to Coll et al. [28] and Gautam et al. [29], the main tar components are aromatics (37-66%), PAHs (21-27%) and phenols (5-7%). Consequently, we researched inhibition data for components from these main groups. Inhibition data were scarce for these types of components and were only found for acetoclastic methanogens [30–33]. Four components were selected, that are at the same time found in high amounts in syngas and have available inhibitory data in the literature: benzene, toluene, styrene and phenol. They are commonly found in syngas from biomass gasification [29, 34–37]. The impact of the

four selected light tars at different concentrations on a consortium adapted for syngas biomethanation were evaluated in batch test and compared to inhibitory concentrations from the literature.

## 2. Material and Method

The tests were performed in 305 mL flasks, with a liquid volume of 85 mL. The temperature was adjusted at 55°C with a thermoregulated orbital incubator, that also enable gas-liquid mass transfer (agitation at 120 rpm). The flasks were kept in the dark to avoid light exposure of the sensitive methanogens [38]. The consortium was collected from a pressurized continuous reactor performing synthetic syngas biomethanation [19]. The collected medium was diluted two times to reach a total solid concentration of 4.2 g/L in the flasks. The artificial syngas was produced with H<sub>2</sub> (>99.9999%) being generated using an electrolyser (Claind srl, Italy) and CO (>99%) and CO<sub>2</sub> (>99.7%) being supplied with gas bottles (Air Liquide, France). No synthetic medium was added to the flasks, as it was considered that the reactor consortium was sufficiently concentrated in nutrients. The pollutant was then added to the flask, except for the control. 1 mL of Na<sub>2</sub>S (16.2 g/L) was then added to each flask to ensure anaerobic conditions.

Each flask was flushed for 2 mins with an artificial syngas mixture (40% CO, 40% H<sub>2</sub>, 20% CO<sub>2</sub>) to ensure anaerobic conditions and saturate the liquid with syngas. As the gas mixture was obtained from three mass flow controllers, the initial gas concentrations in the flasks were measured using gas chromatography (Fusion GC, Inficon, Switzerland) to lower the uncertainties on this value. Then the flasks were closed using a rubber seal and the pressure was increased up to 2 bar<sub>55°C</sub> by injecting the same syngas mixture through a needle perforating the rubber seal.

Gas sampling was then performed manually, using a syringe connected to a needle, 2 or 3 times per day and were not sampled at night. 5 mL of gas at room temperature were sampled each time and analysed with gas chromatography. The pressure in the flasks was measured before and after sampling.

As stated earlier, the pollutant tested were benzene (99.5%), toluene (99.8%), styrene (99.9%) and phenol (99.5%). The concentrations tested corresponded either to the solubility of the component at 55°C to expose the microorganisms to the highest concentrations they could experience, or to a concentration that could lead to 50% of inhibition (IC<sub>50</sub>). Due to a lack of quantified inhibitory concentrations for carboxydrotrophic or hydrogenotrophic methanogens in the literature, the IC<sub>50</sub> concentrations were obtained from the literature data for mesophilic acetoclastic methanogens. They are presented in Table 1, as well as the references used to determine the solubilities.

Table 1. Solubilities and IC50 found in literature for the four pollutants.

Pollutant	Solubility at 55°C (g/L)	Ref	IC50 (g/L)	Ref
Benzene	2.02	[39]	1.2 – 1.5	[30, 31, 33]
Toluene	0.7	[40]	0.58	[30, 31]
Styrene	0.5	[41]	0.01 – 0.15	[30, 33]
Phenol	83 g/L (25°C)	[42]	2.4 - 2.1 - 1.1	[31–33]

For benzene and toluene, since the IC50 concentrations found in the literature were closed to the maximum solubility at 55°C, only the concentration at solubility was tested. For styrene, concentrations at solubility and at IC50 were tested. For phenol, since the concentration at solubility was much higher than the IC50 concentrations found in the literature, it was hypothesized that it would completely inhibit the microorganisms and that it was irrelevant to inject that much pollutant. Therefore, three concentrations were tested : the IC50 concentration found in literature (2 g/L), a concentration ten time higher and a concentration ten times smaller. The tested concentrations are displayed in Table 2. All experiment were performed in duplicate for each concentration.

Table 2. Tested concentrations in the inhibition tests. All concentrations were tested in duplicate.

Pollutant	Concentrations tested (g/L)
Benzene	2.0
Toluene	0.7
Styrene	0.01
	0.5
Phenol	20
	2
	0.2

Gas-liquid mass transfer coefficient ( $k_La$ ) was characterized for oxygen using an a EasySense O<sub>2</sub> 21 probe (Mettler Toledo, Switzerland) to measure dissolved oxygen concentration. The reoxygenation method in clean water was used according to He et al. [43]. The  $k_La$  for O<sub>2</sub> was found to be 13 h<sup>-1</sup> at 1 atm. It must be noted that the introduction of the oxygen probe in the system created flow perturbations that could increase the mass transfer coefficient .

In a biological process, the rate of substrate uptake can be estimated from the Monod equation:

$$r = \frac{dS}{dt} = -\frac{\mu_{max}X}{Y} \times \frac{S}{K_S+S} \quad (3)$$

With  $\mu_{max}$  the maximum specific growth rate (1/time) for the culture,  $S$  the substrate concentration (mass/volume),  $K_S$  the half-saturation constant (mass/volume),  $X$  the biomass concentration and  $Y$  the growth yield (mass/mass).

The system is considered to be mass transfer limited. Indeed, the flasks had both lower pressure (2 bar initially) and lesser  $k_La$  (13 h<sup>-1</sup> for O<sub>2</sub>) than the continuous gas fed reactor (4 bar – 28 h<sup>-1</sup>), which is considered transfer limited [19]. Therefore, mass transfer limitation implies that the substrate concentration  $S$  is low and  $S \ll K_S$ . In this case, according to Eq. 3, the reaction can be considered to be first order with regards to substrate concentration [44]. Therefore, the apparent rate constants  $k_i$  (h<sup>-1</sup>) for the substrate uptake and methane production were computed by fitting a first order model on the curve of the evolution of the molar composition of the substrate (CO or H<sub>2</sub>) in the gas phase. For some experiments, CO conversion started before H<sub>2</sub> conversion, leading to H<sub>2</sub> accumulation in the gas phase according to Eq. 1. In this case, the apparent rate constant  $k_{H_2}$  was calculated by fitting a first order model after the start of H<sub>2</sub> conversion. All experimental results, fittings as well as the quality of the fitting ( $R^2$ ) are available in Supplementary Material.

It should be noted that in a transfer limited system, the apparent rate constant corresponds to the rate at which the substrate is transferred to the liquid phase. The inhibition factor was then calculated in comparison with the substrate rate uptake of the control and the one of the flask with pollutant:

$$IC = \frac{k_{i,control} - k_{i,pollutant}}{k_{i,control}} [\%] \quad (4)$$

An inhibition factor of 0 means no inhibition by the pollutant, whereas an inhibition factor of 100 corresponds to full inhibition. It was calculated that the purges made for gas sampling had a low impact (<5%) on the substrate uptake rate calculations and on the evolution of moles of gaseous components in the headspace; they were therefore neglected. The measured pressures were however corrected for the water content. All experiments were performed in duplicate, and the apparent rate constants were calculated by fitting the model for each replicate. Then, they were used to calculate the mean  $\bar{k}_i$  and standard deviations, which were used to calculate the inhibition factors and their uncertainties.

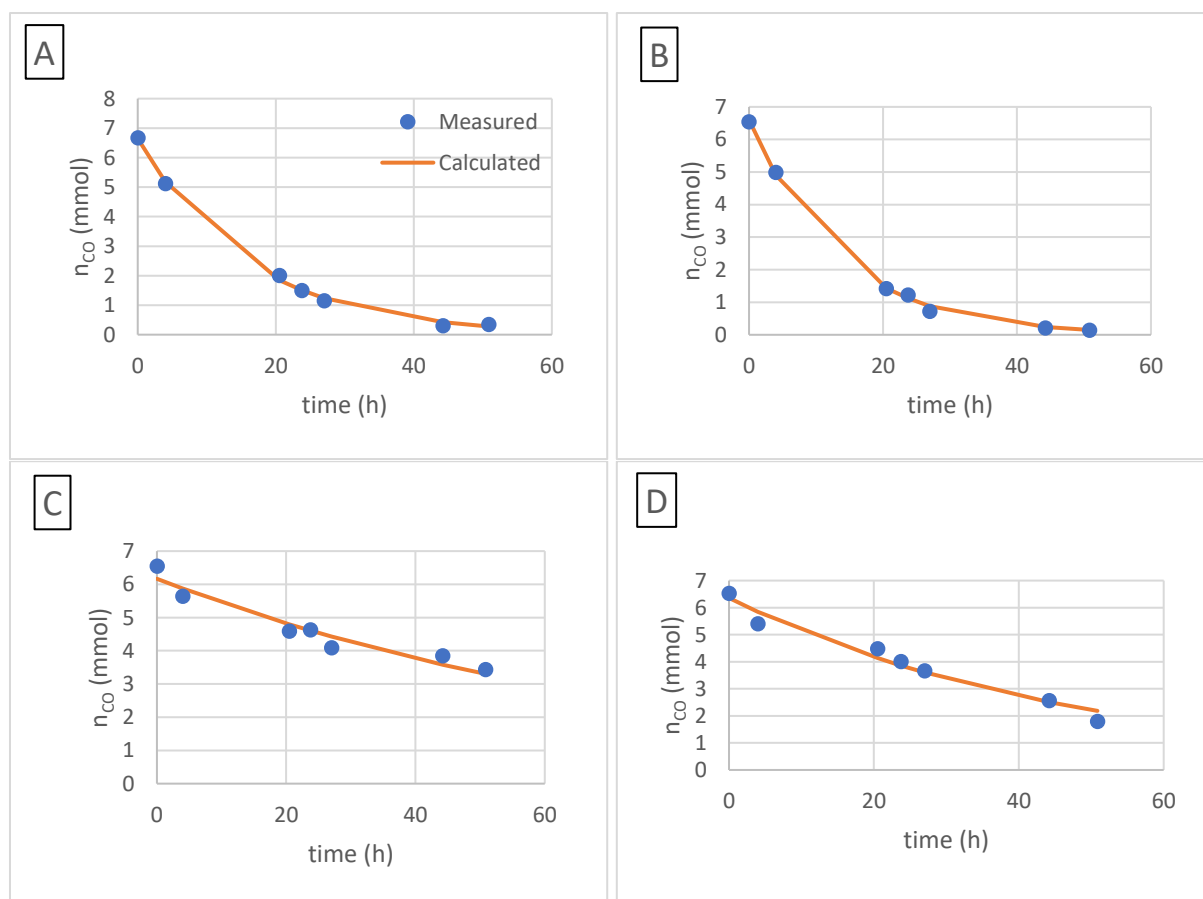
### 3. Results

#### 1.1 First order model fitting: benefits and limitations

To be able to calculate the apparent kinetic constant  $k$ , a first order model was considered as mentioned in Material and Methods. For many experiments, this model seemed appropriate. For instance, regarding CO conversion, the model was able to accurately predict the evolution of CO amount in the absence of pollutant (**Fig. 1-a** and **Fig. 1-b**) with  $R^2$  of 1.0.

In the presence of 0.5 g/L of styrene (**Fig. 1-c** and **Fig. 1-d**), an apparent inhibition of  $76 \pm 9\%$  was observed compared to the control. In the presence of an inhibition, the reaction rate is lowered and could become inferior to the mass transfer rate, leading to CO accumulation in the liquid. In this case, the hypothesis made above that concentration  $S$  is low and  $S \ll K_s$ , leading to consider a first order model, could become inaccurate. However, even in the presence of a strong inhibition at 0.5 g/L of styrene, the first order model was still able to correctly predict the evolution of CO uptake, with  $R^2$  of 0.94 and 0.97 for the first and second replicate respectively.

The calculated  $k_{CO}$  were similar between the two replicates for the control, with relative uncertainty of 9% (Table 3). The uncertainty was higher with 0.5 g/L of styrene (26%). This could be due to the lesser quality of the fitting.

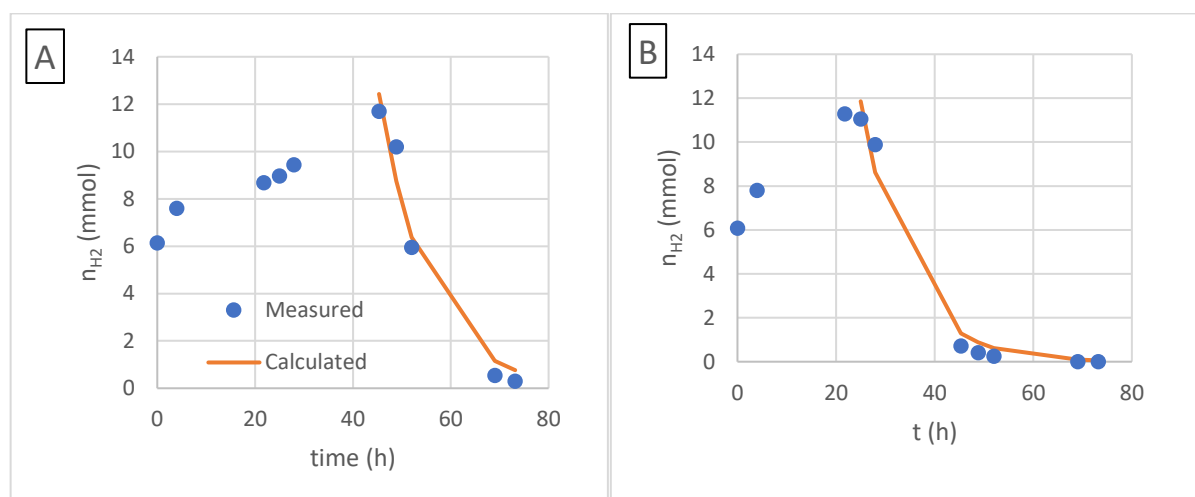


**Fig. 1** Evolution of the amount of CO (mmoles) in the absence of pollutant, first replicate (A) and second replicate (B), and in the presence of 0.5 g/L of styrene, first replicate (C) and second replicate (D). Apparent kinetic constants  $k_{CO}$  were calculated for each replicate by fitting the model. The mean apparent kinetic constants were then calculated:  $(6.9 \pm 0.6) \cdot 10^{-2} h^{-1}$  for the control,  $(1.7 \pm 0.4) \cdot 10^{-2} h^{-1}$  with 0.5 g/L of styrene)

A main limitation of this fitting was the discrepancy between two replicates regarding the start of the reactions for some experiments. For instance, in the experiment with 0.2 g/L of phenol (**Fig. 2**), there was a difference in the lag phase:  $H_2$  conversion started after 48 hours in the first replicate, whereas it started after 22 hours in the second replicate. As mentioned in the Material and Methods section,



the first-order model was fitted after the start of H<sub>2</sub> uptake. The fitting was considered satisfactory, with R<sup>2</sup> values above 0.97 in both cases. Moreover, the calculated k<sub>H<sub>2</sub></sub> values were similar, resulting in a mean value of 0.1 h<sup>-1</sup> with a relative uncertainty of only 9%. It remains unclear why an identical concentration of phenol would have a different impact on the lag phase of the reaction but not on the apparent rate constant. Another limitation of the fitting was the low number of experimental points. Due to the delay before the start of methanogenesis, for some experiments the model was only fitted to three points (Supplementary Material), which is an issue regarding the relevance of the calculated apparent conversion rate. Still, the use of the first order model was considered satisfactory as it allowed to compare the apparent conversion rates between various experiments. It would be interesting to model the behaviour of the system using more complex biochemical models as proposed by Grimalt-Alemany et al. [45], by extending the model with inhibitory effects [46].



**Fig. 2** Evolution of the amount of H<sub>2</sub> (mmoles) in the presence of 0.2 g/L of phenol, for the first (A) and second replicate (B).

### 1.2 Inhibition: impact on the rate constants and on the lag time microbial growth

The evolution of the amount of each gas for one experiment are displayed in **Fig. 3** for the control, for toluene and styrene at their maximal solubility and for phenol at 20 g/L. The same general trends were observed for the other pollutants and concentrations (Supplementary Material). The impact of the pollutants has been studied on the apparent kinetic constant k of CO and H<sub>2</sub> conversion, as well as CH<sub>4</sub> production.

It can be seen in **Fig. 3-a** that CO conversion was impacted by the pollutant addition, at different extent depending on the pollutant. We can see by the shape of the curves that considering a first order reaction in regard to substrate is a good estimate of the system behaviour. We observed that adding these pollutants at these concentrations had an impact on the reaction rates, for example, the apparent rate constant relative to CO uptake (**Fig. 3-a**). The control had an apparent rate constant of  $(6.9 \pm 0.6) \cdot 10^{-2} \text{ h}^{-1}$ , whereas with the presence of pollutants, the apparent rate constants were smaller

:  $(5.2 \pm 0.5) \cdot 10^{-2} \text{ h}^{-1}$  for toluene (0.7 g/L),  $(1.7 \pm 0.4) \cdot 10^{-3} \text{ h}^{-1}$  for styrene (0.5 g/L) and  $(4.8 \pm 0.7) \cdot 10^{-3} \text{ h}^{-1}$  for phenol (20 g/L). The presence of pollutant also impacts the  $\text{H}_2$  uptake rate (as can be seen by the shape of the curves in **Fig. 3-b**, as well as by the calculated values in Table 3), that had for example an apparent rate constant of  $(1.0 \pm 0.2) \cdot 10^{-1} \text{ h}^{-1}$  for the control and of  $(9.5 \pm 1.6) \cdot 10^{-2} \text{ h}^{-1}$  with toluene. Methane production (**Fig. 3-c**) was also slowed down with apparent rate constants  $(8.4 \pm 0.3) \cdot 10^{-2} \text{ h}^{-1}$  for the control and  $(3.6 \pm 1.6) \cdot 10^{-2} \text{ h}^{-1}$  for the flask with toluene. The data are summarized in Table 3.

*Table 3. Apparent rate constants and inhibition factors for one experiment testing high concentrations of toluene, styrene and phenol. The apparent rate constants for a given concentration presented are mean values of rate constants obtained from two replicates.*

	Control	Toluene (0.7 g/L)	Styrene (0.5 g/L)	Phenol (20 g/L)
$\overline{k_{CO}} \text{ (h}^{-1}\text{)}$	$(6.9 \pm 0.6) \cdot 10^{-2}$	$(5.2 \pm 0.5) \cdot 10^{-2}$	$(1.7 \pm 0.4) \cdot 10^{-3}$	$(4.8 \pm 0.7) \cdot 10^{-3}$
$\overline{k_{H_2}} \text{ (h}^{-1}\text{)}$	$(1.0 \pm 0.2) \cdot 10^{-1}$	$(9.5 \pm 1.6) \cdot 10^{-2}$	$(8 \pm 0) \cdot 10^{-3}$	$(4.8 \pm 0.1) \cdot 10^{-3}$
$\overline{k_{CH_4}} \text{ (h}^{-1}\text{)}$	$(8.4 \pm 0.3) \cdot 10^{-2}$	$(3.6 \pm 1.6) \cdot 10^{-2}$	$(5 \pm 5) \cdot 10^{-5}$	$0 \pm 0$
$IC_{CO} \text{ (\%)}$		$24 \pm 14 \%$	$76 \pm 9 \%$	$93 \pm 2 \%$
$IC_{H_2} \text{ (\%)}$		$9 \pm 34 \%$	$93 \pm 2 \%$	$95 \pm 1 \%$
$IC_{CH_4} \text{ (\%)}$		$58 \pm 21 \%$	$100 \pm 0 \%$	$100 \pm 0 \%$

Moreover, regarding  $\text{H}_2$  conversion and  $\text{CH}_4$  production, a higher lag time before the start of the conversion was also observed.  $\text{H}_2$  conversion began only at day 24 (**Fig. 3-b**) for the flask containing toluene, whereas it had already started at day 4 for the control. The same trend can be observed in **Fig. 3-c**, with methane production starting after day 20 for the flask containing toluene whereas there was no lag time for the control. It is likely that  $\text{H}_2$  conversion began also at day 20 with methane production, but at a much smaller rate than  $\text{H}_2$  production from CO, leading to  $\text{H}_2$  accumulation in the headspace until day 24. Therefore, we can conclude that the pollutants had two possible impacts on the biological reactions: it could increase the delay at which the microbial growth would start, and it could slow down the reaction rates.

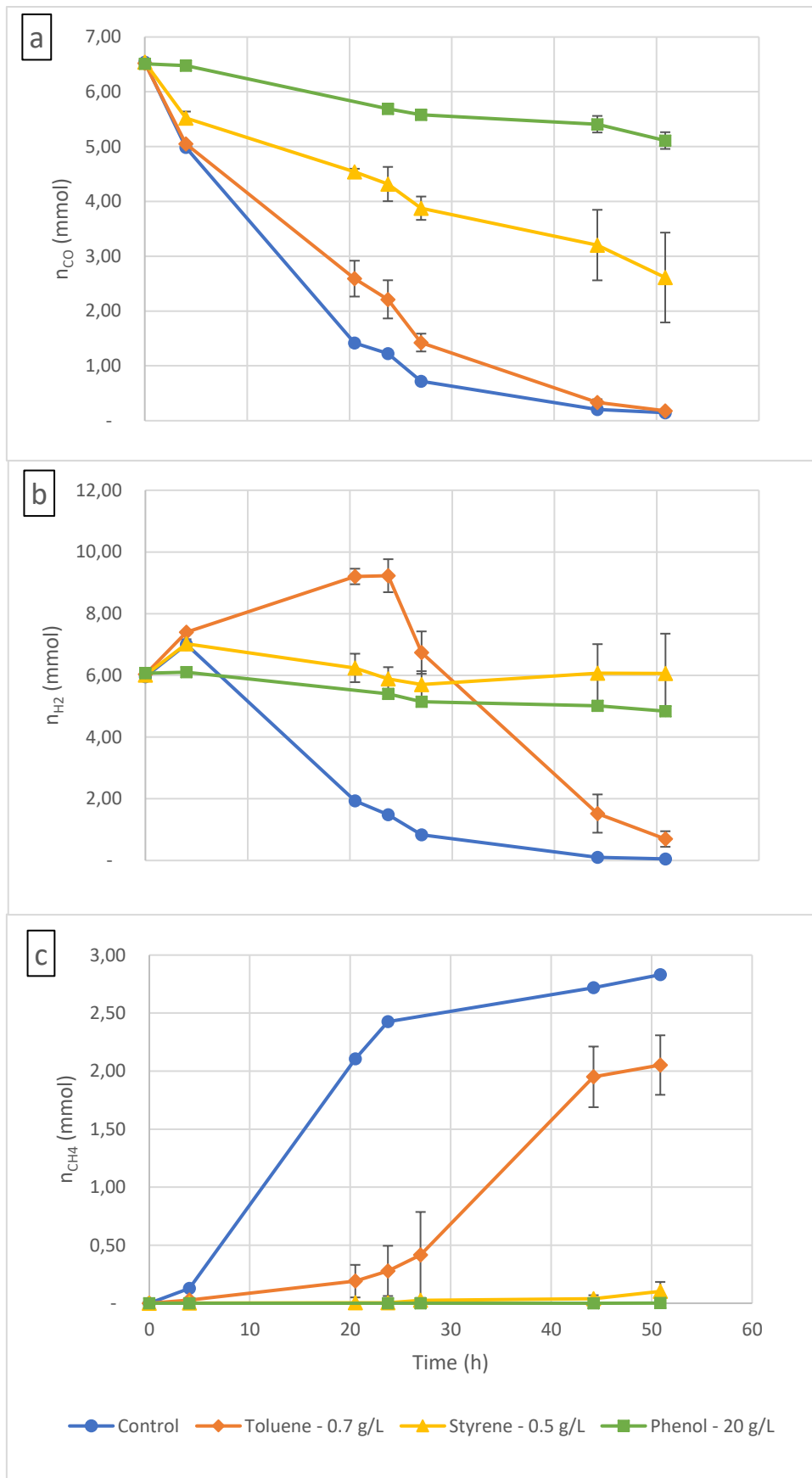
### 1.3 Highlighting of the metabolic routes

The metabolic routes are highlighted by these experiments. Indeed, two steps can be distinguished. First, CO conversion with  $\text{H}_2$  accumulations (day 1-4 for the control and day 1-24 in the presence of toluene), then  $\text{H}_2$  conversion with methane production (**Fig. 3**). This is coherent with the already

observed metabolic routes in thermophilic conditions, where CO is preferentially converted to H<sub>2</sub>/CO<sub>2</sub> and H<sub>2</sub>/CO<sub>2</sub> to methane by hydrogenotrophic methanogens [19, 23–26].

In the control, there is a difference of four days between the start of CO conversion (**Fig. 3-a**) and the start of H<sub>2</sub> conversion (**Fig. 3-b**). This is likely due to the higher sensitivity of methanogens to oxygen [47] or light [38], that they have been exposed to during the preparation of the flasks. It is unlikely that it is due to an inhibition lift on methanogens due to lower CO exposure as CO is converted. Indeed, the consortium has been exposed in the pressurized continuous reactor to CO partial pressure up to 1.6 bar without apparent CO inhibition [19], and the initial CO partial pressure in the batch flasks is two times smaller (0.8 bar). Even though CO conversion to H<sub>2</sub>/CO<sub>2</sub> is an intermediate of methanogenesis, in continuous experiment no lag phase were observed between CO and H<sub>2</sub> conversion [19].

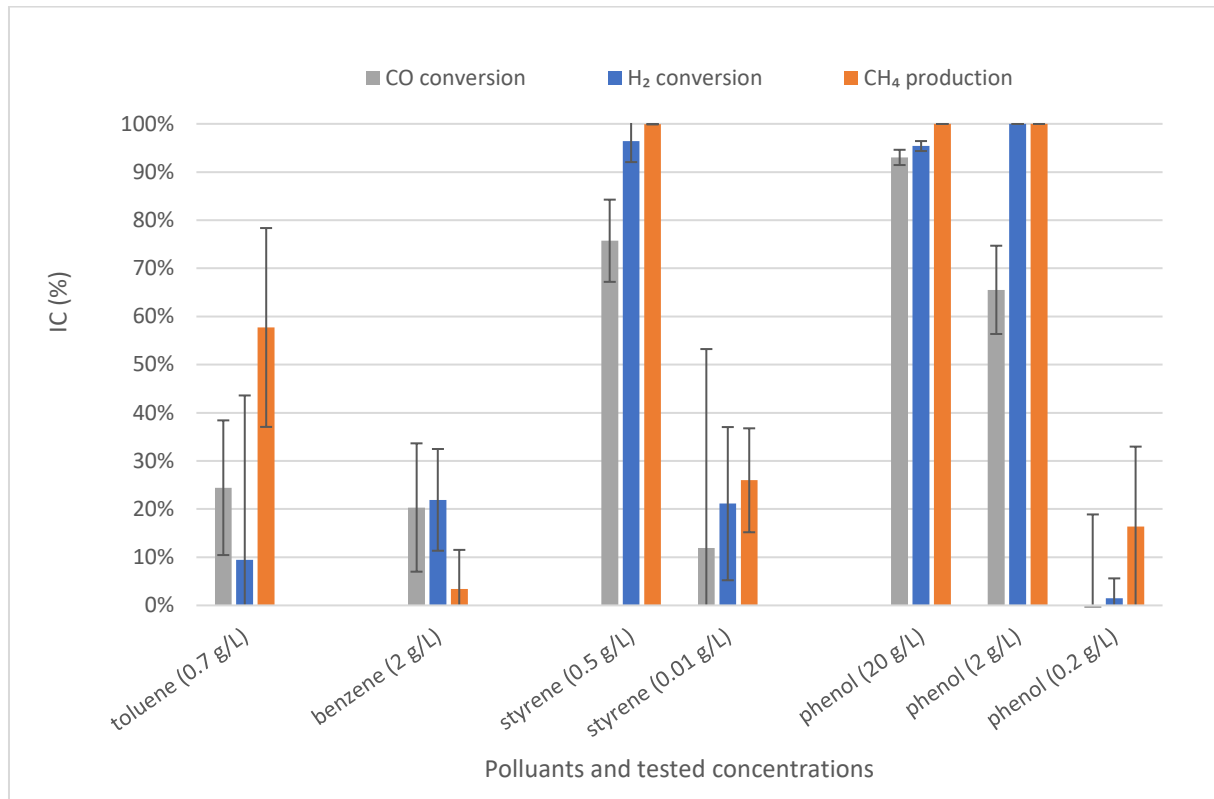
It appears that adding pollutant impacts differently the two main conversion routes. Regarding toluene (at 0.7 g/L), CO conversion was affected by the pollutant ( $24 \pm 14\%$  of inhibition according to Eq. 4) as well as H<sub>2</sub> conversion ( $9 \pm 34\%$  of inhibition, the high uncertainty could be due to the quality of the fitting and the low number of experimental points as mentioned above. Fitting displayed in Supplementary Material. ). However, inhibition of hydrogenotrophic methanogenesis was also expressed by a higher delay before the start of the conversion compared to the control. Regarding styrene (0.5 g/L), CO conversion was able to occur even though with a visible inhibition (**Fig. 3-a**), but H<sub>2</sub> conversion to methane did not occur after 50 days (**Fig. 3-b and C**). For phenol (20 g/L), since the decrease in CO moles is very small, it is not possible to determine if it is due to a slight carboxydrotrophic activity or to gas leaks or uncertainty of measure.



**Fig. 3** Evolution of the amount of CO (mmoles) (A), H<sub>2</sub> (B) and CH<sub>4</sub> (C) in the headspace of the reactor. Purges made for gas sampling were neglected.

#### 1.4 Carboxydrotrophs are more resistant than hydrogenotrophic methanogens

**Fig. 4** shows the inhibition factor calculated according to Eq. 4, for the different pollutants and concentrations tested.



**Fig. 4** Inhibition results for the tested pollutants and concentrations. IC were computed according to Eq. 4

Carboxydrotrophic microorganisms were more resistant to inhibition and were the first to be activated when the concentration of the pollutant decreased. For example, almost all microbial activity was inhibited for a phenol concentration of 20 g/L (95 ± 1 %, 100 ± 0 % and 93 ± 2 % of inhibition for H<sub>2</sub> conversion, CH<sub>4</sub> production and CO conversion, respectively). Lowering the concentration to 2 g/L, H<sub>2</sub> conversion and CH<sub>4</sub> production were still strongly inhibited (100 ± 0 % for both), but CO conversion inhibition was lowered to 66 ± 9 %. Decreasing the concentration to 0.2 g/L once again reduced all inhibition to a negligible level. The same phenomenon was observed for styrene at 0.5 g/L, where CO uptake inhibition (76 ± 9%) was less significant compared to H<sub>2</sub> conversion inhibition (93 ± 2%) and CH<sub>4</sub> production (100 ± 0%). When the styrene concentration was further decreased to 0.01 g/L, CO inhibition was lower (12 ± 41%), indicating a significant trend despite the strong uncertainties, but a remaining inhibition of around 20% was still observed for hydrogenotrophic methanogens. This trend, where the two main metabolic routes are affected differently by the pollutant, was consistent across all experiments. Therefore, we can conclude that the hydrogenotrophic methanogens are more sensitive to the pollutants tested than the carboxydrotrophic microorganisms.

As can be observed in **Fig. 4**, the pollutants impacted differently H<sub>2</sub> conversion and CH<sub>4</sub> production. For example, for toluene, H<sub>2</sub> conversion was inhibited by  $9 \pm 34$  %, whereas CH<sub>4</sub> production was inhibited by  $58 \pm 21$  %. As mentioned earlier, H<sub>2</sub> conversion to CH<sub>4</sub> is assumed to be the main metabolic route. The gap between the inhibition values for H<sub>2</sub> conversion and CH<sub>4</sub> production can be explained by the uncertainties. Moreover, other minor metabolic routes for H<sub>2</sub> conversion and CH<sub>4</sub> production could be involved, mainly with acetate production or consumption [16], which could explained the gap between the values.

### *1.5 Comparison with the literature data*

The results were compared to the literature data presented in Table 1. For benzene and toluene, the concentration tested were higher than the reported IC<sub>50</sub> and corresponded to the maximum solubility at 55°C of the components (**Fig. 4**). The observed inhibition was much lower than 50% for benzene ( $3 \pm 8$  % of inhibition). For toluene, the observed inhibition of methane production was  $58 \pm 21$  %, however at a concentration (0.7 g/L) higher than the reported IC<sub>50</sub> (0.58 g/L). Considering the high uncertainty of the results, it is hard to conclude when comparing to the literature data. For styrene, methane production was only inhibited by  $26 \pm 11$  % at the IC<sub>50</sub> concentration reported for acetoclastic methanogens. Therefore, it appears that for benzene and styrene, thermophilic hydrogenotrophic methanogens adapted for syngas biomethanation are more resistant than mesophilic acetoclastic methanogens. However, for phenol, the concentration corresponding to an IC<sub>50</sub> for acetoclastic methanogens (2 g/L) strongly inhibited methanogenesis ( $100 \pm 0$  %), indicating a higher sensitivity.

These results confirm that using inhibition data for acetoclastic methanogenesis is not accurate enough to predict the behaviour of a thermophilic consortium adapted for syngas biomethanation. This reinforces the necessity to develop specific quantification methods to investigate the inhibition of tar components on biomethanation consortia.

Moreover, the data should be compared at equivalent biomass concentrations, as there are some indications that higher biomass concentration could reduce the impact of an inhibitory concentration [48, 49]. The microorganisms can be partially inhibited but still perform bioreactions at a lower rate. In this case, increasing the biomass concentration can compensate for the inhibition and lead to higher reaction rates. For instance, Araya et al. [36] found an IC<sub>50</sub> for styrene of 0.15 g/L for a volatile suspended solid concentration (VSS) of 65.4 g/L, whereas Sierra-Alvarez and Lettinga [38] found an IC<sub>50</sub> of 0.01 g/L at 1 g<sub>VSS</sub>/L. The difficulty in a mixed consortium adapted for syngas biomethanation is to know the biomass concentration for a specific group, as VSS concentration does not give the concentration of biomass related to methanogens or carboxydotrophs. Moreover, the structure of the

biomass (suspended, granular...) also impact the resistance of the microbial population to the pollutants [50]. Therefore, comparing inhibitory concentration on syngas biomethanation consortium should be done taking this into consideration.

Furthermore, the results presented in this study must be interpreted considering gas-liquid mass transfer limitations. Indeed, as explained in the Material and Method, the system is considered transfer limited. Therefore, the inhibition results presented represent the impact of inhibition on rates that are already transfer limited. In other words, the absence of observed inhibition does not mean that there is no inhibition, as long as the biological substrate uptake rates remain over the mass transfer rate. Indeed, high biological uptake rates have been observed. For instance, in a former study that examined the intensification of syngas biomethanation, a CO specific activity of  $2.5 \text{ L}_{\text{STP}}/\text{g}_{\text{biomass}}/\text{d}$  was calculated [49]. With a biomass concentration of 4.2 g/L in the flasks, this would result in a CO biological conversion rate of  $10.5 \text{ L}_{\text{STP}}/\text{L}/\text{d}$  in the batch tests of this study. However, considering a  $k_L a$  of  $13 \text{ h}^{-1}$  as mentioned in the Materials and Methods section, the maximum mass transfer rate at the beginning of the experiment, with a CO partial pressure of 0.8 bar, can be estimated to be  $0.01 \text{ L}_{\text{STP}}/\text{L}/\text{d}$ . This value is significantly lower than the estimated biological uptake rate. Therefore, when comparing inhibition data from the literature, it is important to keep in mind that a low inhibitory concentration measured with a system with poor mass transfer could have a much more significant inhibitory effect in a system with better mass transfer performance.

In addition, the results are only to consider on a non-acclimated sludge. Indeed, some anaerobic microorganisms are able to degrade pollutants such as benzene [51], toluene [52], phenol [53] and styrene [54]. It can be hypothesised that a consortium adapted for syngas-biomethanation could be adapted to degrade some of the tar components present in syngas. Such a consortium would be even more tolerant to syngas impurities, as it would be enriched in microorganisms degrading the pollutants and protecting the more sensitive methanogens and carboxydrotrophs.

All these parameters (biomass concentration, mass transfer limitations, adaptation of the consortium to the pollutant) are to consider when designing syngas cleaning process. More tests need to be done in systems not limited by transfer, for instance by lowering the biomass concentration to allow for the biological rates to be inferior to the mass transfer rates. In such system, for fixed biomass concentration, IC50 concentrations should be defined for tar components present in syngas. We recommend this methodology to ensure the comparison of literature data. This would allow the establishment of a database of inhibitory concentrations on syngas biomethanation consortia, which would help in designing an adapted syngas cleaning process.

### 1.6 Comparison with syngas compositions

The range of concentrations tested in this study is wide. The worst case was considered (maximum solubility of the considered components) and as well as a reference inhibitory concentration from the literature. As an example, the tested concentrations were compared to the syngas composition from Gautam et al. [29], who characterized tar formation during the gasification of wood pellet, using a stratified downdraft gasifier.

Using Henry's law, we calculated the concentrations in pollutant that their syngas would induce in a biomethanation reactor operated at 55°C and 4 bar. Henry's coefficients were corrected for temperature according to Sander [53]. The results are presented in Table 4. For instance, injecting in a biomethanation reactor a syngas with a composition similar to that of Gautam et al. [29] of 2.4 mg/Nm<sup>3</sup> of benzene would provoke a dissolved concentration of benzene in the liquid of 1.1 x 10<sup>-5</sup> g/L. In this study, a concentration of 2 g/L of benzene was tested on a non-acclimated sludge and only 20% of inhibition was observed on carboxydrotrophic and hydrogenotrophic activity. Therefore, the benzene composition of a syngas with composition similar to that of Gautam et al. [29] should not induce inhibition on syngas biomethanation.

Table 4. Dissolved concentrations in pollutant that syngas from Gautam et al.[29] would induce in a biomethanation reactor operated at 55°C and 4 bar.

Component	Syngas concentration		Concentration in the biomethanation reactor	
	Minimum concentration (mg/Nm <sup>3</sup> )	Maximum concentration (mg/Nm <sup>3</sup> )	Minimum dissolved concentration (g/L)	Maximum dissolved concentration (g/L)
Benzene	1.4	2.4	6.6 x 10 <sup>-06</sup>	1.1 x 10 <sup>-05</sup>
Toluene	76.8	198.3	2.8 x 10 <sup>-04</sup>	7.1 x 10 <sup>-04</sup>
Styrene	21	65.1	1.3 x 10 <sup>-04</sup>	3.9 x 10 <sup>-04</sup>
Phenol	6.9	67.2	7.6 x 10 <sup>-01</sup>	<b>7.4</b>

The same can be said for toluene and styrene, the tested concentrations that induced mild inhibition (around 20%) are 10<sup>4</sup> times higher than what a syngas with a composition similar to that of Gautam et al. [29] would induce in a biomethanation reactor, therefore these components would likely not induce inhibition in these concentrations.

However, in the case of phenol, a syngas with a composition similar to that of Gautam et al. [29] would induce a dissolved phenol concentration of 7.4 g/L in a biomethanation reactor. Yet, phenol composition of 2 g/L induced full inhibition of hydrogenotrophic activity and methanogenesis, and 66 ± 9 % inhibition of carboxydrotrophic activity. Therefore, we can consider that 7.4 g/L of phenol would



have a high inhibitory impact on non-adapted microorganisms. An adapted syngas cleaning technology in order to remove phenol from syngas should be considered when working with syngas with a composition similar to that of Gautam et al. [29].

In addition, when working in continuous mode, the accumulation of the contaminants in the biomethanation reactor must be considered. In the end, the concentration of pollutant experienced by the microorganisms will be an equilibrium between the injected quantities in the syngas, the part consumed by acclimated microorganisms and the liquid purge.

### Conclusion

For the first time, the impact of four common light tar components of syngas on a syngas-biomethanation consortium were investigated. The highest concentration (solubility at 55°C) as well as IC50 values from the anaerobic digestion literature were tested. Adding pollutant both impacted the rate and the lag time of microbial growth. Methanogens were more impacted by the pollutant than carboxydrotrophic microorganisms. Due to its high solubility and inhibitory effect, phenol was identified as a key component to clean from the syngas. The need for a specific database regarding inhibition of syngas biomethanation consortia was highlighted and a methodology was proposed to allow consistency between different studies.

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## Statements and Declarations

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### **Competing Interests**

The authors have no relevant financial or non-financial interests to disclose.

### **Author Contributions**

All authors contributed to the study conception and design. Material preparation and data collection were performed by Julie Figueras. Data analysis was performed by all authors. The first draft of the manuscript was written by Julie Figueras and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

### **Data Availability**

The datasets generated during and/or analysed during the current study are not publicly available due to confidentiality.