CEST 2023

18th International Conference on Environmental Science and Technology

Athens, Greece, 30 August to 2 September 2023

Enrichment of mesophilic syngas-converting consortia for methane production

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Abstract

The use of waste biomass as raw material for syngas production has emerged as a sustainable and cost-effective alternative to the conventional gasifiable feedstock. In addition, syngas bioconversion into value-added compounds could be an interesting approach for carbon fixation and syngas valorization, but some limitations must be overcome. One of the most important issues is to establish a robust microbial culture able to use CO as a carbon source. In the present study, an anaerobic consortium was enriched with a synthetic syngas mixture with a composition similar to that obtained from biomass gasification. Four syngas dilutions (25, 50, 75 and 100%) were tested to determine the CO tolerance of the mixed culture and evaluate its growth kinetics. The results showed the successful enrichment of a mesophilic syngas-converting consortium able to thrive in concentrations up to 30% CO. Volatile fatty acids (mostly acetate) and CH_4 were the main products of its metabolism, reaching up to 54.27±1.04 mM of VFAs concentration and a CH_4 production of 15.41±0.15 $mmol \cdot d^{-1}$. The accumulation of organic acids in the liquid phase appeared as a key factor for the development of the community, triggering its inhibition.

Keywords: *carbon monoxide, mesophilic consortia, methane, syngas, volatile fatty acids.*

1. Introduction

Synthesis gas, commonly known as syngas, is a gaseous mixture composed mainly of carbon monoxide (CO), hydrogen (H_2) and carbon dioxide (CO_2), with lower concentrations of other compounds such as methane (CH_4). Traditionally, this mixture has been generated via gasification of non-renewable sources such as natural gas, petroleum coke or coal (Wilhelm et al., 2001). However, there is an increasing interest in replacing these conventional raw materials with more sustainable feedstocks. In this context, biomass gasification emerges as a feasible alternative for syngas production (Molino et al., 2016), where several types of crops and industrial or urban wastes could be used.

Once the synthesis gas is obtained, it is used for fuels and chemicals production through different physical-chemical processes, the most common being the Fischer-Tropsch process (FT). It is characterized by its high temperature and pressure conditions and the requirement of a specific H_2:CO ratio (Liew et al., 2016). This makes necessary the development of alternative processes where these limitations could be solved, such as the gas fermentation. The biological conversion of CO and CO_2 emerges as a sustainable and cost-effective alternative due to the wide range of CO and H_2 concentrations that microorganisms can tolerate (thus minimizing gas conditioning) and to the high specificity of the obtained products (Molitor et al., 2016). In addition, although the fermentation of CO, CO_2 and H_2 allows to obtain acetate as the major product, it is possible to synthesize value-added compounds such as ethanol or 2,3-butanediol (Bae et al., 2022). Nevertheless, the investigation of this novel biotechnology must be optimized for its implementation at industrial level. Among others, one of the principal limitations is the low growth and productivity rate of the syngas-converting microorganisms (Asimakopoulos et al., 2018). Therefore, the selection of an adequate pure culture or consortium plays a key role in process optimization.

Despite pure cultures are commonly used as inoculum in gas fermentation processes, the use of enriched mixed cultures has recently arisen as a promising alternative (Parera at al., 2022). More precisely, anaerobic sludge from wastewater treatment digesters represents a suitable source of bacteria capable of using CO as a source of energy and carbon (Sipma et al., 2003). This type of cultures is characterized by its ability to synthesize a wide range of products (fatty acids, alcohols and CH_4) and by presenting a high substrate utilization and product yields compared to pure strains. However, changes in population dynamics over time and the complexity of microbial interactions between the species hinder the studio of this type of consortia (Liew et al., 2016). The objective of the present study was to evaluate the growth kinetics of a novel open mixed culture. The consortium

was enriched with a synthetic syngas mixture with a composition similar to that obtained from biomass gasification. The production of CH_4 and volatile fatty acids (VFAs) was evaluated, among other parameters, under mesophilic conditions at four different syngas dilutions (25, 50, 75 and 100%).

2. Materials and methods

2.1. Materials

The mineral salt medium used for the enrichment and kinetic study was composed of ($g \cdot L^{-1}$): 0.5 yeast extract, 30.0 PIPES, 0.0005 resazurin, 0.3 NH_4Cl, 0.3 NaCl, 0.1 $MgCl_2 \cdot 6H_2O$, 1 mL of acid trace elements solution ($g \cdot L^{-1}$: 1.8 HCl, 0.06 H_3BO_3, 0.06 $MnCl_2$. 0.9 $FeCl_2$, 0.06 $CoCl_2$, 0.01 $NiCl_2$, 0.07 $ZnCl_2$) and 1 mL of alkaline trace elements solution ($g \cdot L^{-1}$: 0.4 NaOH, 0.02 Na_2SeO_3, 0.03 Na_2WO_4, 0.02 Na_2MoO_4). A NaOH 6M solution was added until the pH was adjusted to 7.0. 50 and 200 mL of the medium was dispensed into the 120 and 1200 mL bottles, respectively, and the desired gas mixture was added to the headspace. The bottles were then autoclaved (121ºC, 21 min) and then a stock solution ($0.01\ mL \cdot mL^{-1}_{medium}$) consisting of a vitamin solution ($g \cdot L^{-1}$: 0.02 biotin, 0.2 nicotinate, 0.1 p-aminobenzoate, 0.2 thiamine, 0.1 pantothenate, 0.5 pyridoxin, 0.05 thioctic acid, 0.1 riboflavin, 0.1 cobalamin, 0.05 folate, 0.05 lipoate) and a solution of $CaCl_2 \cdot 2H_2O$ (11 $g \cdot L^{-1}$) at a ratio 10:1, respectively, was added. Finally, the medium was reduced by adding 0.04 $mL \cdot mL^{-1}_{medium}$ of L-cysteine (17.5 $g \cdot L^{-1}$). A 0.22 µm filter was used to sterilize the solutions added after the bottles were autoclaved.

A synthetic syngas mixture composed of 35:30:25:10 %v/v of H_2, CO, CO_2 and CH_4, respectively, was used in the different experiments. The dilution of this mixture was carried out using N_2 as a make-up gas.

2.2. Enrichment and kinetics procedure

Anaerobic sludge from the municipal wastewater treatment plant of Valladolid (Spain) was used as inoculum in the enrichment experiment. For the enrichment, two 120 mL serum bottles containing 50 ml of mineral medium and an initial headspace composition of 25% v/v of syngas in a nitrogen atmosphere were inoculated to a final concentration of 5% v/v. The headspace atmosphere was replaced upon CO and H_2 depletion. After three replacements, one of the bottles was used as the inoculum for a new set of duplicates of bottles under the same conditions and headspace composition. Following three additional headspace replacements, one of the duplicates was used to inoculate the enrichment bottles fed with the subsequent syngas concentration as described in Figure 1. In total, four different headspace atmospheres (25, 50, 75 and 100% of syngas) were tested and eight transfers were performed. Under all tested conditions, the bottles were incubated at 37ºC and 150 rpm with a total gas pressure of 1.2-1.3 bar.

The first duplicate of each tested condition was used as the inoculum for the kinetics test. Once the headspace atmosphere was replaced 12 times, 2.5 mL were inoculated into a new bottle. Upon complete depletion of CO and H_2, the headspace atmosphere was replaced 3 times. This culture was used as inoculum (5%v/v) of 1200 mL bottles, that contained 200 mL of mineral medium. Four bottles were measured twice per day. The gas phase composition, pH, optical density at 650 nm (OD_{650}) and the volatile fatty acids (VFAs) concentration were monitored. In each kinetic studio, all the parameters were monitored during three cycles of CO and H_2 consumption. As in the enrichment test, the bottles were incubated at 37ºC and 150 rpm with a total gas pressure of 1.2-1.3 bar.

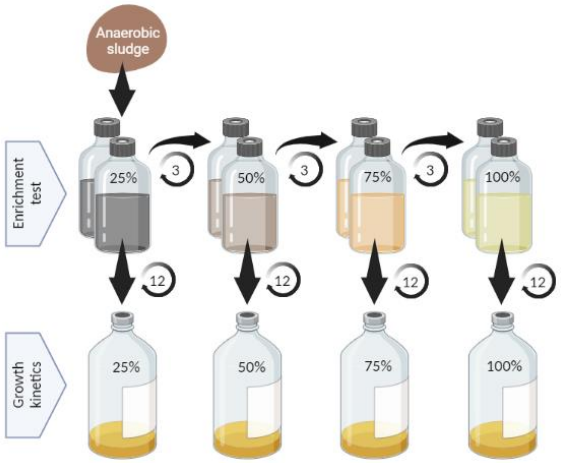

Figure 1. Schematic representation of the enrichment and kinetic studio procedure. The percentage inside the bottles represents the syngas dilution in the gas phase and the number inside the loops indicates the number of headspace atmosphere replacements before each transfer (drawn with BioRender®).

3. Results and discussion

3.1. Enrichment of syngas-converting bacteria

Eight transfers were performed to obtain an enriched microbial culture adapted to the synthetic gas mixture. To prevent growth inhibition due to CO concentration, the enrichment was carried out with an initial syngas concentration of 25% (equivalent to 0.22 mmol CO). Within five days after inoculation, complete depletion of CO and H_2 and a slightly CH_4 production (≈0.03 mmol) was observed. This rapid consumption was maintained in the following stages, where gas phase replacement was performed every 2-3 days. An increase in the CH_4 production was observed in subsequent stages until 0.82±0.03 mmol CH_4 was reached. A similar trend was observed in the other three gas dilutions tested. When the headspace was composed of 50, 75 and 100% of syngas, a rapid CO and H_2 consumption was also observed. These results showed that higher CO concentrations in the gas phase did not inhibit the mixed culture. Similar findings have been reported by other authors (Navarro et

al. (2016) at CO partial pressures of 0.3 atm (similar to the highest composition here tested of 100% of syngas). On the other hand, only when the gas phase was composed of a 50% of syngas a different pattern of CH_4 production was observed. While working under 75 and 100% of syngas resulted in a gradual increase in the CH_4 production from the first stage (equivalent to the trend described above for the 25% syngas dilution), it was not until the fourth stage when a significant CH_4 production was obtained under 50% syngas. This temporary inhibition of the methanogenic activity could suggest a change in the microbial community due the new gas phase conditions implemented.

The evaluation of the culture performance in the long term showed interesting results regarding CH_4 production, finally reaching a stationary value for each syngas dilution tested. In particular, 19±0.02, 0.29±0.07, 0.50±0.07 and 0.70±0.07 mmol CH_4 were reached in the last seven stages under 25, 50, 75 and 100% of syngas, respectively. These results contrast with those obtained in other enrichment tests. For instance, Esquivel-Elizondo et al. (2017) observed a variable CH_4 production using 30%v/v CO along three transfers.

3.1. Kinetic tests

After enriching an open mixed culture able to grow under all the gas dilution tested, a comprehensive evaluation of its performance was carried out. When a 25% of syngas was employed, the consumption rates of CO and H_2 were 1.81±0.18 and 4.35±0.73 mmol·d^{-1}, respectively, in the first stage. During this time, the highest increase in biomass concentration was observed, corresponding to a maximum growth rate (μ_{max}) of 2.32 d^{-1} (R^2=0.999). This rapid biomass generation probably is due to the high concentration of yeast extract in the mineral medium. On the other hand, a slight CH_4 production was observed in the last days of this stage (0.27±0.09 mmol·d^{-1}) in contrast with the rapid increase in VFAs concentration, which reached a final concentration of 18.29±0.36 mM (Figure 2). However, in the next two stages this trend changed, and the carbon was mainly allocated for CH_4 production, with values of 1.64±0.88 mmol CH_4·d^{-1}. The consumption rate of CO and H_2 also increased up to 4.23±1.79 mmol and 5.09±1.58 mmol·d^{-1}, respectively. Despite the biomass concentration increased at the beginning of the second stage, its value stabilized at 916.79±70.31 mg·L^{-1}. The VFAs concentration also reached a stable value of 19.12±0.30 mM. Acetate was the main organic acid present in the liquid phase (68.58±2.58%). During these three stages evaluated, just a slightly decrease in the pH value was observed (the test started and finished with a pH value of 6.85±0.00 and 6.63±0.03, respectively).

A similar behavior was observed when the growth kinetics was evaluated under 50% syngas. The first stage showed a CO and H_2 consumption rate of 4.86±0.41 and 5.90±0.71 mmol·d^{-1}, respectively. A slightly lower μ_{max} was recorded under these conditions (1.98 d^{-1}, R^2=0.996). These results suggested that more carbon was allocated for by-products synthesis, which was confirmed by the CH_4 and VFAs production values. In this sense, the production rate of CH_4 was ten times higher than that observed in the previous gas dilution tested (2.65±0.68 mmol·d^{-1}) and the final VFAs concentration at the end of this first stage was 25.69±0.65 mM. In the second and third stages, the CH_4 production rate reached 3.46±0.97 mmol·d^{-1}, while the CO and H_2 consumption rates increased up to 8.21±0.31 and 10.72±0.23 mmol·d^{-1}, respectively. The biomass and VFAs concentration experienced a progressive increase during these two last stages, achieving a final value of 986.18±11.08 mg·L^{-1} and 33.49±1.09 mM, respectively. Acetic acid was once again the main VFA produced, whose concentration represented 85.70±2.99%. The increase in the VFAs production also affected the pH of the liquid phase, decreasing to 6.39±0.00.

Surprisingly, a partial or total inhibition of the mixed consortia was observed when higher syngas concentrations were used. When a 75% of syngas was employed, the behavior during the first stage was similar to that described in previously tests. The CO and H_2 consumption rates were 5.72±0.71 and 8.50±0.25 mmol·d^{-1}, respectively, and CH_4 production only occurred during the last days of this stage, reaching a maximum value of 2.41±0.34 mmol CH_4·d^{-1}. The VFAs concentration increased up to 36.97±2.24 mM and acetate was the main organic acid (85.30±2.91%). However, during the second stage, the H_2 consumption and the CH_4 production became irregular, while the CO consumption was not affected (9.77±0.74 mmol·d^{-1}). Despite H_2 was finally consumed, the results suggested a partial inhibition of the hydrogenotrophic activity. It is worth noting that this inhibition occurred concomitantly with a high VFAs accumulation (52.65±4.59 mM) and, subsequently, a decrease of the pH to 6.04±0.06. This trend was also observed when a 100% of syngas was supplemented. In the first stage, the CO and H_2 consumption rates were 6.93±1.60 and 8.96±1.16 mmol·d^{-1}, respectively. In the following stage, the CO consumption remained similar (7.43±0.26 mmol·d^{-1}) whereas an irregular H_2 consumption and CH_4 production was again observed. In addition, VFAs accumulation (54.27±1.04 mM) and a decrease in pH (5.83±0.00) were also observed. These results are consistent with the observations of previous authors on the importance of pH for the microbial community. For instance, Parera et al. (2022) reported that this parameter is crucial in the regulation of acetogenic activity. In the case of 75% of syngas, a progressive VFAs consumption was observed along the third stage to a final concentration of 46.46±2.78 mM. This triggered a decrease in the pH value to 6.19±0.06, allowing for a recovery of the hydrogenotrophic activity (3.36±0.14 mmol·d^{-1}) and the CH_4 production (2.42±0.16 mmol·d^{-1}). In contrast, no consumption of VFAs was observed when 100% of syngas was used, which led to total inhibition of the consortium.

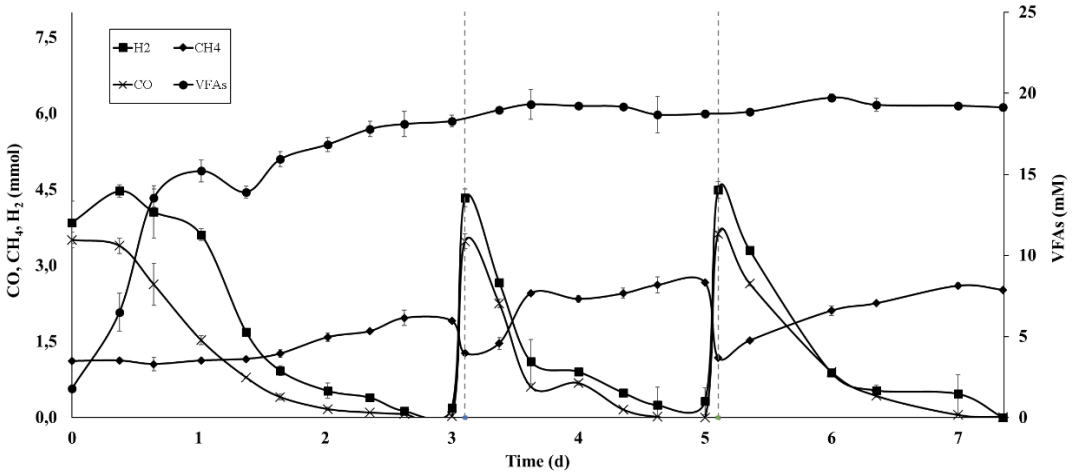

Figure 2. Evolution of gas phase composition and VFAs production when a 25% of syngas was used

4. Conclusion

In this work, a novel open mixed culture was enriched using a synthetic syngas with a composition similar to that obtained from biomass gasification. The enrichment test showed that the mesophilic consortia was able to use the CO and H_2 present in the gas mixture as a carbon and energy source, while producing CH_4 in the long term. On the other hand, the exhaustive evaluation of four syngas dilutions (25, 50, 75 and 100%) revealed changes in the population dynamics. An equilibrium between the acetogenesis and methanogenesis seems to be crucial to ensure the stability of the mixed culture. The results suggest that high concentrations of carbon promote the accumulation of VFAs and, subsequently, a pH drop. This decrease in the pH value could be a key factor in the inhibition of the culture. Future work should study the optimal pH and gas-liquid ratio to ensure the performance of this open mixed culture.

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