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Communication

Demonstrating Pilot-Scale Gas Fermentation for Acetate Production from Biomass-Derived Syngas Streams

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Abstract: Gas fermentation is gaining attention as a crucial technology for converting gaseous feedstocks into value-added chemicals. Despite numerous efforts over the past decade to investigate these innovative processes at a lab scale, to date, the evaluation of the technologies in relevant industrial environments is scarce. This study examines the fermentative production of acetate from biomass-derived syngas using *Moorella thermoacetica*. A mobile gas fermentation pilot plant was coupled to a bubbling fluidized-bed gasifier with syngas purification to convert crushed bark-derived syngas. The syngas purification steps included hot filtration, catalytic reforming, and final syngas cleaning. Different latter configurations were evaluated to enable a simplified syngas cleaning configuration for microbial syngas conversion compared to conventional catalytic synthesis. Fermentation tests using ultra-cleaned syngas showed comparable microbial growth (1.3 g/L) and acetate production (22.3 g/L) to the benchmark fermentation of synthetic gases (1.2 g/L of biomass and 25.2 g/L of acetate). Additional fermentation trials on partially purified syngas streams identified H_2S and HCN as the primary inhibitory compounds. They also indicated that caustic scrubbing is an adequate and simplified final gas cleaning step to facilitate extended microbial fermentation. Overall, this study shows the potential of gas fermentation to valorize crude gaseous feedstocks, such as industrial off-gases, into platform chemicals.

Keywords: syngas fermentation; *Moorella thermoacetica*; acetate; crushed bark gasification; syngas cleaning

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1. Introduction

Gas fermentation technologies have quickly captivated the attention of scientific research. They have the potential to contribute to the decarbonization of the economy through the conversion of inexpensive and gaseous C1 feedstocks, often regarded as waste, into value-added chemicals and biofuels [1–3]. One of the gas fermentation technologies rapidly advancing is synthesis gas (syngas) fermentation, a process that valorizes gas mixtures typically consisting of CO, CO_2, H_2, and N_2, harnessing the capabilities of gas-fermenting bacteria [4,5]. Gas mixtures containing the latter compounds can be derived from intensive industrial activities, such as steel mills or cement factories, or from the gasification of residual biomass or wastes, such as forestry residues, and agricultural, industrial, or municipal solid waste [3].

Acetogenic microorganisms can be used as biocatalysts to valorize syngas streams into chemicals, such as acetate. As syngas fermentation has been a target of scientific research in recent years, several acetogenic strains with great potential for use in this process have

been studied. One is *Moorella thermoacetica*, a thermophilic strain characterized by its ability to produce acetate as its main end-product (homoacetogen) and thrive in different syngas compositions [6–8]. The fact that *M. thermoacetica* is a homoacetogenic strain favors the direct usage of acetate-containing broth as feedstock for subsequent liquid fermentation. Indeed, syngas-derived acetate is considered a critical intermediate energy carrier that can be upgraded into value-added platform chemicals, such as succinate, 3-hydroxypropionate, and itaconic acid, or biofuels, paving the way toward a circular economy [9–12]. Acetate and other volatile fatty acids can also be derived from lignocellulosic biomass fermentation using rumen microorganisms, another sustainable pathway to obtain these intermediate energy carriers that is currently under intensive investigation [13].

Similar to syngas fermentation, gasification is gaining attention as a technology for converting solid low-value feedstocks into low molecular weight gas mixtures, i.e., syngas [14]. However, for syngas to become usable for downstream applications, the removal of impurities such as aromatic hydrocarbons (tars), S- and N-group compounds, halogens, and alkali compounds is often required [15,16]. Since syngas purification technologies can have a significant impact on the overall process costs, understanding the minimally required clean-up steps is crucial [17,18]. Although currently still in its infancy, hydrothermal gasification provides an interesting alternative, typically generating cleaner syngas streams that require less energy-intensive cleaning procedures [19].

The coupling of gasification and syngas fermentation for a hybrid thermal–biological route has emerged as an alternative to conventional thermal–catalytic processes to produce sustainable fuels or chemicals. The coupling of these two technologies brings several advantages, including (1) gasification converting low-value carbonaceous solids into syngas, (2) gasification maximizing the conversion of complex fractions (e.g., lignin) into syngas, improving the recovery efficiency compared to other technologies such as the enzymatic hydrolysis of lignocellulosic biomasses, and (3) syngas fermentation valorizing gaseous C1 feedstocks into valuable biofuels and chemicals [5,7,14,20]. Furthermore, biocatalysts are generally less susceptible to poisoning by contaminants in the syngas compared to metal-based catalysts, which often require syngas impurity removal to parts per billion (ppb) levels [21,22]. The complex interaction between microbes and the syngas impurities can yield effects that are advantageous in certain instances while being detrimental in others [23]. Additional advantages of conversion processes involving biocatalysts rather than chemical and metal-based catalysts include the lower toxicity of biocatalysts, the requirement of milder processing conditions in terms of pressure and temperature (lower energy input needed), and the higher specificity towards the desired end-products [7,24].

Several studies have described the coupling of biomass gasification and syngas fermentation to mainly produce ethanol and acetate. Indeed, *Clostridium ljungdahlii* was found to show comparable growth (0.6 g cell dry weight/L) and acetate production (around 15 g/L) in lignin- and beech wood-derived cleaned syngas [25]. Also, *Clostridium carboxidovorans* and *Clostridium autoethanogenum* obtained similar growth and product profiles in artificial syngas and torrefied wood-derived cleaned syngas [26]. Although the effect of biomass-derived syngas on the downstream fermentation process has been investigated, these streams were thoroughly cleaned to minimize the concentration of the typical syngas impurities (H_2S, NO_x, and HCN). Additionally, a few other studies evaluating the performance of biocatalysts in converting biomass-derived syngas streams containing low concentrations of pollutants have been reported [27–29]. Indeed, Infantes and collaborators evaluated the performance of *C. ljungdahlii* in different syngas streams containing part per million (ppm) concentrations of HCN and NH_3 and ppb concentrations of H_2S and COS. Despite these low impurity concentrations, longer lag phases and lower acetate titers were observed. In contrast, similar microbial growth rates, as compared to an impurity-free syngas stream, were achieved [28].

This study aims to evaluate the effect of partially purified syngas streams derived from gasified crushed bark on acetate production by *M. thermoacetica*. First, the coupling of the gasification and syngas fermentation pilot plants was established with ultra-cleaned syngas.

Next, different syngas purification configurations were assessed to optimize the gasification process for the syngas fermentation use case to achieve a simplified process configuration. This is one of the few studies investigating the impact of partially purified gasification syngas streams on the syngas-to-acetate fermentation process. Indeed, to the best of the authors' knowledge, this is the first report on biomass-derived syngas conversion to acetate using *M. thermoacetica*.

2. Materials and Methods

2.1. Gasification and Syngas Cleaning

2.1.1. Biomass Gasification

Crushed bark was used as feedstock in the test campaigns. Feedstock analysis indicated crushed bark had a moisture content of 7.7 wt%; in terms of dry matter, it was composed of the following (in wt%): 3.5 of ash, 49.0 of C, 6.0 of H, 0.4 of N, and 0.06 of S. Additionally, its lower heating value was 19.4 MJ/kg (dry).

The syngas was produced by steam–oxygen gasification of the crushed bark in an atmospheric-pressure bench-scale (up to 5 kg/h of feed) bubbling fluidized-bed gasifier (internal diameter, i.d., of 0.1 m with a freeboard i.d. of 0.15 m) at 800 °C; it had a steam-to-fuel ratio of 0.9 kg/kg dry ash free, with a hot gas cleaning section consisting of filtration and catalytic reforming. A mixture of silica sand and Myanit-dolomite was used as bed material. The test facility was partially heated with external electrical heaters to compensate for heat losses, and a small amount of nitrogen was used to purge the fuel feeding system and the pressure drop measuring lines. The process conditions and gas compositions after the reformer are given in Table 1. Hot gas filtration using metallic filters removed particles such as char, ash, and bed material from the raw syngas. Additionally, when the filtration temperature was sufficiently low (here operated at 560 °C), the condensation of volatile compounds, such as alkali metals (Na and K) and several heavy metals, occurred. After the hot filtration, the gas was catalytically (Ni-based catalyst) reformed at 930 °C in a fixed-bed reactor with additional oxygen feeding for tar and hydrocarbon conversion into syngas.

Table 1. Average process conditions and gas compositions in the gasification campaigns. daf: dry-ash free; ppm_v: parts per million by volume; nm^3: normal cubic meter.

Gasification Conditions	
Gasification temperature, °C	800
Fluidizing velocity, m/s	0.6
Steam-to-fuel, kg/kg daf	0.9
O_2 feed, % of stoichiometric combustion	8.0
Hot gas cleaning conditions	
Filtering temperature, °C	560
Reforming temperature, °C	930
Gas after the reformer	
Gas composition, vol % dry	
CO	19.2
CO_2	17.7
H_2	38.9
N_2	23.4
CH_4	0.8
H_2O in wet gas, vol %	32.6
Gas contaminants, dry	
H_2S, ppm_v	70
COS, ppm_v [1]	4
NH_3, ppm_v [1]	100
HCN, ppm_v	2
Benzene, mg/nm^3	6
Sum of tars, mg/nm^3	21

[1] Estimation: samples taken during different campaigns but with the same process configuration.

The reformed syngas contained ca. 39 vol % of H_2 and 19 vol % of CO and sulfur compounds, mainly in the form of H_2S, with a small amount of COS. NH_3 was present in the gas at around 100 parts per million by volume (ppm_v) concentration, while HCN was measured at 2 ppm_v. Small amounts of residual benzene and tars were detected in the reformed syngas.

2.1.2. Syngas Final Cleaning Unit

The final syngas cleaning process with a nominal syngas feed capacity of 5–10 nm^3/h was used to purify the filtered and reformed syngas further. Its configuration was modified to facilitate fermentation under varying syngas impurities and concentrations. The gasification and syngas cleaning train is illustrated in Figure 1.

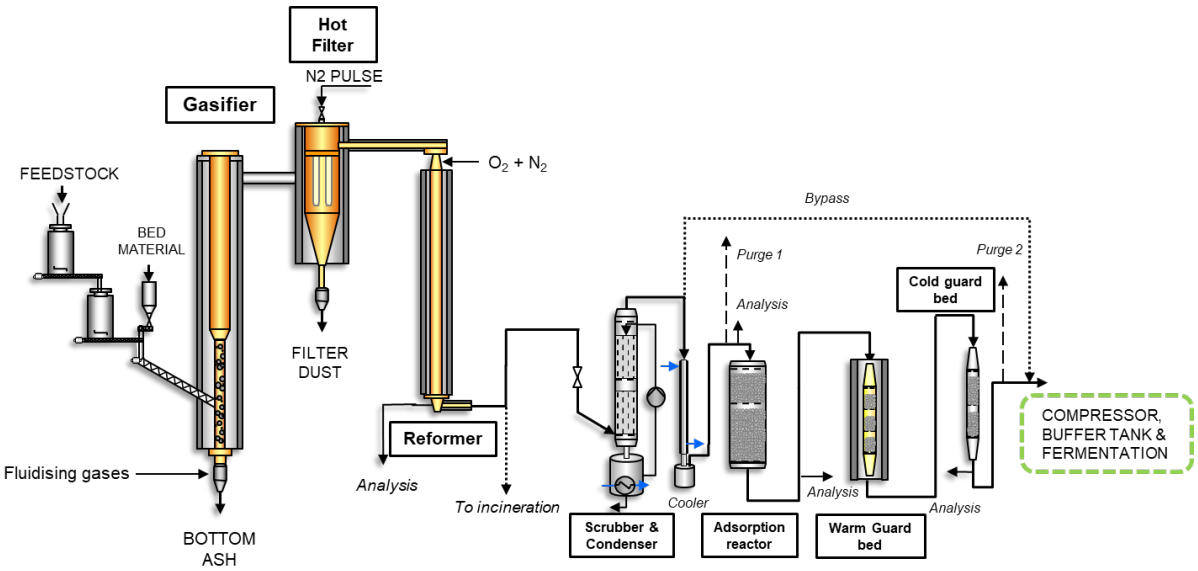


Figure 1. Schematic illustration of the atmospheric-pressure bubbling fluidized-bed gasifier coupled with the final gas cleaning process.

The final gas-cleaning process involved a water-scrubbing column (i.d. 0.16 m) filled with metallic random packings. The scrubbing column was operated at 30 °C in either acidic mode by injecting formic acid into the water circulation or caustic mode by injecting NaOH. Additionally, dry fixed beds were employed in the entire gas cleaning configuration for ultra-cleaned syngas. The adsorption reactor (i.d. 0.25 m) was filled with 6.6 kg of non-impregnated activated carbon and 1.6 kg of caustic-impregnated activated carbon. Guard beds included a heated reactor (i.d. 0.08 m) filled with 2.6 kg of ZnO/alumina, 1.6 kg of a Cu-based deoxygenation catalyst, and a 0.6 kg ambient temperature-impregnated activated carbon bed. The fixed beds were bypassed in the acid- or caustic-scrubbed syngas campaigns to achieve partially purified syngas streams. The process conditions from the test campaigns are presented in Table 2.

Table 2. Average final gas cleaning process conditions.

Configuration	Ultra-Cleaning	Acid-Scrubbing	Caustic-Scrubbing
Flow rate, nm^3/h	8.1	8.1	8.6
Pressure, bar	1.055	1.045	1.073
Scrubber water pH	2.3	3.0	9.7
Scrubber liquid/gas ratio, kg H_2O/nm^3 syngas	32	35	31
Adsorbent bed temperature, °C	29	-	-
Warm guard bed temperature, °C	202	-	-
Cold guard bed temperature, °C	24	-	-

The process was operated at slight overpressure, and the scrubber chemical addition in the ultra-cleaning and acid-scrubbing modes resulted in a water pH of 2.3 and 3.0, respectively. The average pH for the caustic scrubbing campaign was 9.7 (variance between 8.9 and 11).

2.1.3. Analytical Methods

Gas analyzers and off-line samples were used to measure gas composition after the filter and the reformer. In addition to the solid input streams (feedstock and bed material), solid output streams (filter dust and bottom ash) were also measured and analyzed. More information can be found in a prior publication [30].

During the campaign, the final gas-cleaning impurities analysis was conducted intermittently through manual sampling, employing colorimetric tubes or gas bag samples. Based on previous experiments conducted with the identical gasifier and feedstock, primary contaminants detected in the hot gas-cleaned syngas encompassed H_2S, COS, HCN, NH_3, benzenes, and tars.

COS was analyzed from gas bag samples using flame photometric detector-gas chromatography (FPD-GC), and the results were derived from an average of three injections from two parallel sample bags. For campaigns with COS analysis, typically one or two samples were taken.

H_2S and HCN analysis was performed using Dräger colorimetric tubes with a 10–15% relative standard deviation. The H_2S and HCN results were an average of samples taken during the campaign at a sample size of n = 1–7. The estimated limits of detection (LoD) using colorimetric tubes were 0.1 ppm_v and, with FPD-GC, 0.1–0.5 ppm_v.

Since the downstream syngas fermentation process is run with a strict anaerobe, the presence of O_2 in the syngas must be avoided. Therefore, an O_2 sensor was installed before the gas compressor to check for potential leaks that lead to contamination of the syngas.

2.2. Coupling of Gasification Facility and Mobile Gas Fermentation Pilot Plant

The mobile gas fermentation pilot plant (Bio Base Mobile Pilot Plant, BBMPP; MTSA Technopower B.V., Arnhem, The Netherlands, and Bio Base Europe Pilot Plant, Ghent, Belgium) was equipped with a pressurized air-driven gas compressor and a 1.8 m^3 buffer tank, where the biomass-derived syngas was stored for later usage in the fermentation process. With the compressor as the driving force, the buffer tank was filled up to 13 bar. Day-long gasification and syngas cleaning campaigns were sufficient to fill the buffer tank. Purge valves were utilized to release surplus gas and align the scale between the compressor input and the gasification output. This ensured a relatively stable gasifier pressure, irrespective of variations in compressor capacity.

2.3. Syngas Fermentation

2.3.1. Bacterial Strain

Moorella thermoacetica DSM 2955, acquired from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Brunswick, Germany), was used for all aseptic cultivations. Stock cultures of 5 mL of the late exponential culture in a complex and rich medium and a 100% CO_2 atmosphere with a 20% glycerol solution were prepared and stored at −80 °C in anaerobic Hungate tubes.

2.3.2. Media and Inoculum Preparation

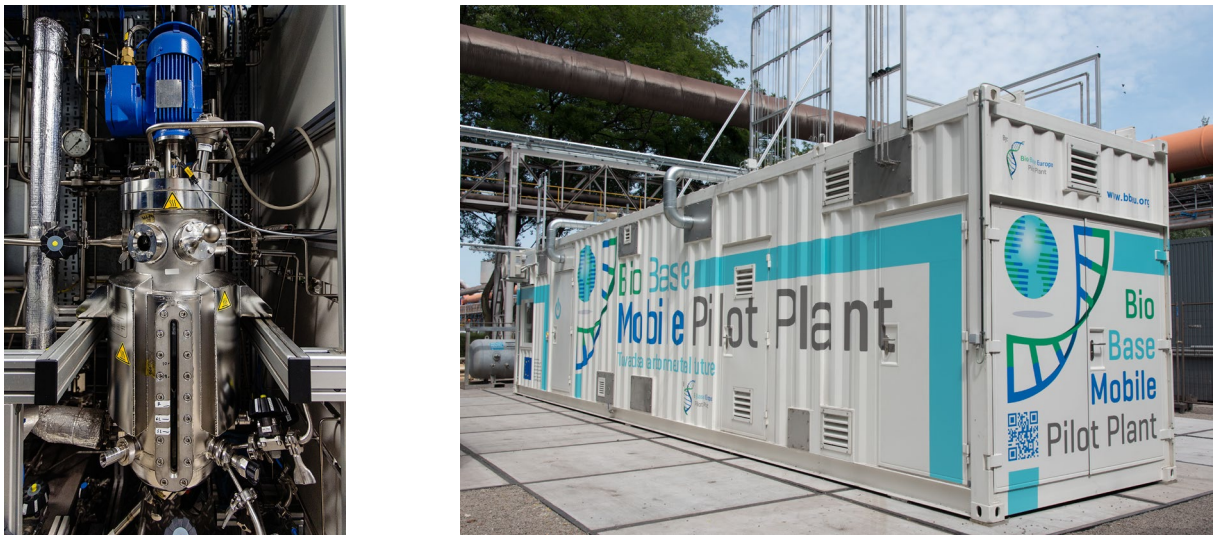
A complex and rich medium was used as the seed medium for the first preculture, which consisted of the following (per liter of distilled water): 18.0 g of glucose, 0.5 g of $(NH_4)_2SO_4$, 0.1 g of $MgSO_4 \cdot 7H_2O$, 0.055 g of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, 2.4 mg of $Na_2MoO_4 \cdot 2H_2O$, 0.01 mg of Na_2SeO_3, 5 g of tryptone, 5 g of yeast extract, 1 mg of resazurin sodium salt, 4.5 g of KH_2PO_4, 7.0 g of K_2HPO_4, 2 g of Na_2CO_3, and 0.3 g of cysteine-HCl·H_2O. For subsequent precultures, a modified version of the Demler medium was used [31], consisting of the following compounds (per liter of distilled water): 18.0 g

of glucose, 2.0 g of $(NH_4)_2SO_4$, 0.5 g of $MgSO_4 \cdot 7H_2O$, 2.0 g of yeast extract, 1 mg of resazurin sodium salt, 0.33 g of KH_2PO_4, 0.45 g of K_2HPO_4, 10.0 g of $NaHCO_3$, 0.3 g of cysteine-HCl·H_2O, 10 mL of a trace element solution, and 1 mL of a vitamin solution. The trace element solution consisted of the following (per liter of distilled water): 4.32 g of nitriloacetic acid trisodium salt, 1 g of $MnSO_4 \cdot H_2O$, 0.8 g of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, 0.36 g of $CoSO_4 \cdot 7H_2O$, 0.2 g of $CaCl_2 \cdot 2H_2O$, 0.36 g of $ZnSO_4 \cdot H_2O$, 0.02 g of $CuSO_4 \cdot 5H_2O$, 0.04 g of $KAl(SO_4)_2 \cdot 12H_2O$, 0.02 g of H_3BO_3, 0.02 g of $Na_2MoO_4 \cdot 2H_2O$, 0.05 g of $NiCl_2 \cdot 6H_2O$, 0.02 g of Na_2SeO_4, and 0.02 g of Na_2WO_4. The vitamin solution consisted of the following (per liter of distilled water): 0.04 mg of biotin, 0.04 mg of folic acid, 0.2 g of pyridoxine-HCl, 0.1 g of thiamine-HCl·$2H_2O$, 0.1 g of riboflavin, 0.1 g of nicotinic acid, 0.1 g of D-Ca-pantothenate, 0.002 mg of cyanocobalamine, 0.1 g of p-aminobenzoic acid, and of 0.1 g lipoic acid. The fermentation medium consisted of the following compounds (per liter of distilled water): 2.0 g of $(NH_4)_2SO_4$, 0.5 g of $MgSO_4 \cdot 7H_2O$, 4.0 g of a yeast extract, 1 mg of resazurin sodium salt, 0.33 g of KH_2PO_4, 0.45 g of K_2HPO_4, 0.3 g of cysteine-HCl·H_2O, 20 mL of a trace element solution, 2 mL of a vitamin solution, and 0.25 mL of antifoam 204.

Stock cultures were utilized to inoculate the first preculture by transferring 3.5 mL from a Hungate tube to 70 mL of anaerobic and complex medium (5% v/v inoculum) in 250 mL serum bottles (SBs), which were incubated in an orbital shaker at 200 rpm (orbit 1.9 cm) and 60 °C for 48 h. After inoculating the SBs, the anaerobic headspace was replaced by a 100% CO_2 atmosphere by flushing the SBs through a rubber septum. The first preculture was then utilized to inoculate the second preculture by transferring 10 mL to 200 mL of anaerobic and modified Demler medium (5% v/v inoculum) in 1 L SBs, which were incubated under the same conditions as the first preculture for 48 h. The anaerobic headspace of the second preculture SBs was also replaced by a 100% CO_2 atmosphere. Finally, 1 L of the second preculture was used to inoculate the fermenter (10% v/v inoculum).

2.3.3. Consecutive Batch Fermentations

Batch fermentations were carried out in a 24 L stainless steel, stirred tank gas bioreactor (Het Noorden BV, Gorredijk, The Netherlands) with a working volume of 10 L (Figure 2a), containerized in the BBMPP (Figure 2b). The reactor's agitator speed range is 220–1100 rpm, its maximum working pressure is 9 bar, and the gas feed flow rate range is 1–20 L/min.



(**a**) (**b**)

Figure 2. (**a**) 24 L stainless steel, stirred tank gas bioreactor used for the syngas fermentation tests. The working volume was 10 L. (**b**) The Bio Base Mobile Pilot Plant (BBMPP), a mobile fermentation unit intended to scale up gas fermentation processes within industrial environments.

All fermentation trials were performed as consecutive batch fermentations, meaning that when a batch process was completed, the reactor was partially harvested until 1 L of broth was left inside the vessel. The reactor was filled with a fresh and sterile fermentation medium up to 10 L to start the following fermentation. This process was intended to produce acetate, which acidifies the medium; therefore, the batch process was considered completed when the base addition was almost neglectable.

All fermentation runs were performed with continuous online monitoring and control of critical process parameters. The vessel's temperature was kept at 60 °C. The pH was set at 6.8 at the start of the fermentation, and then it was controlled at 6.0 by adding a 2 M NaOH solution. The stirrer speed was set at 530 rpm, and the total gas flow amounted to 1 L/min. The gas bioreactor was operated at 1.5 bar. Samples were collected daily and analyzed according to Section 2.3.4.

2.3.4. Analytical Methods

The biomass concentration, or cell dry weight (CDW) (g/L), was calculated through a CDW/optical density (OD) correlation factor obtained from previous experiments at a lab scale under comparable conditions (CDW/OD = 0.29 g/L/OD). The OD was measured at 600 nm, and the CDW was determined gravimetrically. To that end, 5 mL of the fermentation broth was centrifuged at 4700× *g* for 5 min. The cell pellet was washed with physiological water and resuspended in distilled water. Finally, the resuspended pellet was transferred to a moisture analyzer (MA37, Sartorius, Göttingen, Germany), which dried the sample until a constant weight was obtained. The acetate concentration was determined using a commercial spectrophotometric assay kit (K-ACETRM, Megazyme Ltd., Wicklow, Ireland). The ammonium (NH_4^+) concentration was measured with test strips (MQuant, VWR, Radnor, PA, USA). The maximum acetate productivity was calculated based on the online addition of a base solution, assuming the neutralization reaction of acetic acid and NaOH was equimolar: 1 mol of acetic acid is neutralized by 1 mol of NaOH.

3. Results and Discussion

3.1. Syngas Purification

The gasification and syngas cleaning processes were operated for a duration ranging from 3 to 7 h, sufficient to fill the buffer tank of the mobile gas fermentation unit. Three distinct process configurations were tested to produce syngas with varying profiles and concentrations of impurities for fermentation. The average contaminant analysis results, derived from the cleaned syngas from test campaigns with different gas-cleaning configurations, are showcased in Table 3.

Table 3. Average impurity concentrations after final gas-cleaning. LoD: limit of detection; n.a.: not analyzed.

Configuration	Ultra-Cleaning	Acid Scrubbing	Caustic Scrubbing
H_2S, ppm_v	0/LoD	65	17.5
COS, ppm_v	0/LoD [1]	5	4 [1,2]
NH_3, ppm_v	n.a.	n.a.	n.a.
HCN, ppm_v	0/LoD	1	0–0.5/LoD
Benzene and tars, g/nm^3	n.a.	n.a.	n.a.

[1] Estimation: sample taken during different campaigns but with the same process configuration. [2] Sampling point: mobile gas fermentation unit reactor off-gas.

The analysis of the ultra-cleaned syngas indicated a complete removal of H_2S, COS, and HCN. Conversely, the final gas-cleaning in the acid-scrubbing configuration resulted in H_2S, COS, and HCN in the gas product, partially purifying the primary contaminants. Similarly, under the caustic-scrubbing configuration, partial purification was achieved. H_2S was primarily eliminated, with residual quantities present in the gas product at low ppm levels, while COS remained entirely or almost fully in the syngas. HCN seemed to be effectively removed by caustic scrubbing. The varying pH conditions in the caustic-scrubbing

campaign led to differing HCN analysis results, with HCN detected at a scrubbing water pH of 9.4 and undetected (0 ppm/limit of detection, LoD) at a pH of 9.8 or higher. These process variations also impacted H_2S removal rates. In the initial campaign with caustic scrubbing, an investigation into the effect of water pH on H_2S removal demonstrated a linear decrease in H_2S in the outlet as a function of water pH, indicating that a pH of over 10 was sufficient to reduce an H_2S feed concentration of 40 ppm_v to below 5 ppm_v.

The ultra-cleaning configuration has been previously validated analytically to remove impurities to sub-ppm concentrations effectively [32]. The results presented in this study corroborated these findings, as the measured impurities could not be detected using the previously described analytical methods. However, this study encountered limitations in analyzing each significant impurity across all campaigns. Previous research has also examined the standalone performance of the acidic scrubber [33]. Under acidic conditions (pH < 4), NH_3 was estimated to be effectively removed, with little to no removal of acidic gases such as H_2S, HCN, and HCl. COS, benzenes, and non-condensable tars are known to not readily absorb under any aqueous conditions and are, therefore, likely to pass through a water scrubbing step. The partially purified syngas is expected to contain H_2S with caustic scrubbing, with the concentration correlating with the scrubbing water pH. Additionally, a pass-through of COS and NH_3 is anticipated, meaning few ppm and tens to hundreds of ppm are expected in the syngas, respectively. HCN is not likely present in the caustic-scrubbed syngas, but trace amounts of benzene and tars are expected.

3.2. Coupling of Gasification and Syngas Fermentation

The syngas fermentation process was run as a set of consecutive batch fermentation processes to minimize the preparation of various seed trains after the completion of each batch (Section 2.3.3). Figure 3 shows four consecutive batch processes on synthetic gas (B, D) or ultra-cleaned biomass-based syngas (A, C), demonstrating the consistency of *M. thermoacetica* aseptic cultures independently of the utilized gaseous feedstock.

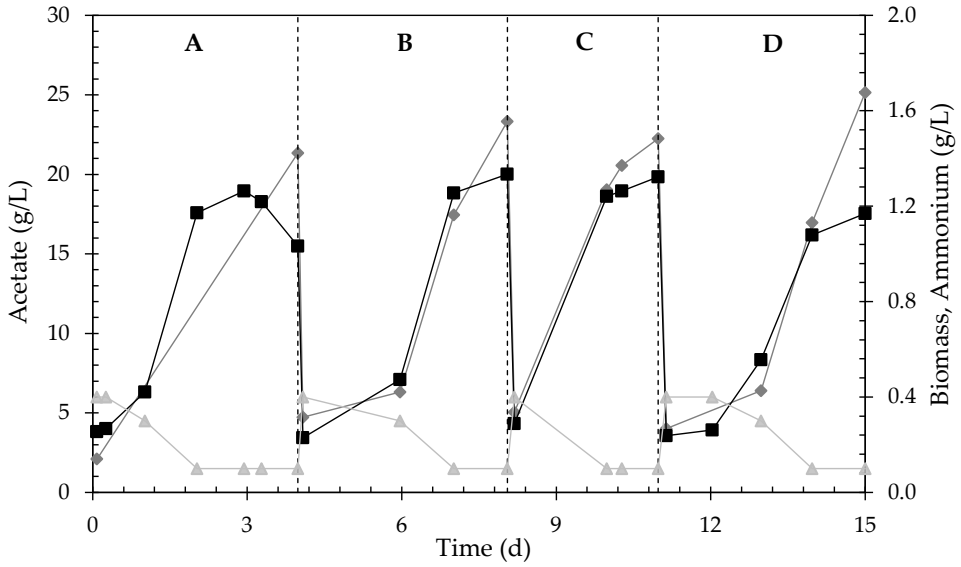


Figure 3. Biomass (■), acetate (◆), and ammonium (NH_4^+, ▲) concentrations of four consecutive batch runs. (**A**): ultra-cleaned syngas (vol-%: 34.3 H_2, 15.1 CO, 17.5 CO_2, 33.1 N_2); (**B**): synthetic gas (vol-%: 42.5 H_2, 22.2 CO, 23.3 CO_2, 12.0 N_2); (**C**): ultra-cleaned syngas, (**D**): synthetic gas (vol-%: 33.7 H_2, 15.5 CO, 17.6 CO_2, 33.2 N_2). The dashed line (---) indicates when the reactor was partially harvested and refilled with fresh medium.

Maximum biomass, acetate titers, and maximum acetate productivity are essential for assessing syngas-to-acetate fermentation performance. A trial carried out using synthetic gases as feedstock was used as a benchmark to evaluate the performance of *M. thermoacetica*

on ultra-cleaned syngas under the same experimental conditions (Figure 4). Interestingly, it was observed that *M. thermoacetica* could grow and produce acetate in ultra-cleaned syngas, yielding comparable results to the benchmark fermentation.

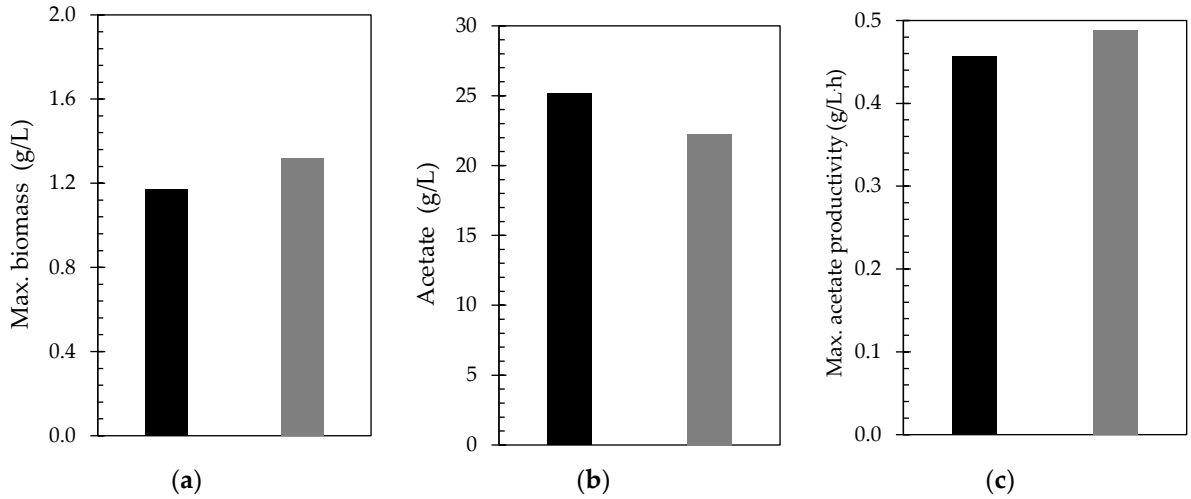


Figure 4. (**a**) Maximum biomass titer, (**b**) acetate titer, and (**c**) maximum acetate productivity of benchmark fermentation on synthetic gases (black; vol-%: 33.7 H_2, 15.5 CO, 17.6 CO_2, 33.2 N_2) compared to ultra-cleaned syngas fermentation (gray; vol-%: 34.3 H_2, 15.1 CO, 17.5 CO_2, 33.1 N_2).

Indeed, the microorganism reached a maximum biomass and acetate titer of 1.2 g/L and 25.2 g/L in synthetic gases and a comparable 1.3 g/L and 22.3 g/L in ultra-cleaned syngas, respectively. The maximum acetate productivity achieved throughout the syngas fermentation trials reached 0.49 and 0.46 g/L·h in ultra-cleaned syngas and synthetic gases, respectively. Since the final gas cleaning unit configuration utilized to obtain ultra-cleaned syngas targeted the removal of all impurities (Table 3), *M. thermoacetica* was able to yield equivalent performance in synthetic and ultra-cleaned syngas. These observations align with reports on the performance of several *Clostridia* species for the production of mainly ethanol and acetate in ultra-cleaned syngas [25,26,28,29]. Ultra-cleaned syngas can also be used as feedstock for Fischer–Tropsch synthesis or methanol synthesis for conversion into fuels and chemicals, as it meets the stringent purity requirements of synthesis catalysts [34]. However, it has been suggested that microbial catalysts are more tolerant to syngas impurities. This could lower the costs of syngas production and cleaning and the overall conversion process of waste biomass into chemicals such as acetate through gasification [24,35].

3.3. Effect of Partially Purified Syngas Streams on Prolonged Acetate Production

The conversion of less purified biomass-derived syngas streams into acetate by *M. thermoacetica* was subsequently assessed to evaluate its tolerance to syngas inhibitory compounds. By utilizing only hot filtration, reforming, and water scrubbing as purification steps, higher concentrations of impurities were present in the syngas (Table 3). *M. thermoacetica* reached a biomass and acetate titer of 1.4 g/L and 18.3 g/L, respectively, in the first batch fermentation in acid-scrubbed syngas (Figure 5). However, in contrast to the consistency observed on consecutive batches of synthetic or ultra-cleaned syngas (Figure 3), there were no signs of microbial growth nor acetate production in the second consecutive batch of acid-scrubbed syngas after the partial harvest and refill with fresh medium. Even after switching the gas feed from acid-scrubbed to synthetic syngas, no acetate production was observed, indicating *M. thermoacetica* was inhibited by the contaminants in acid-scrubbed syngas (Table 3).

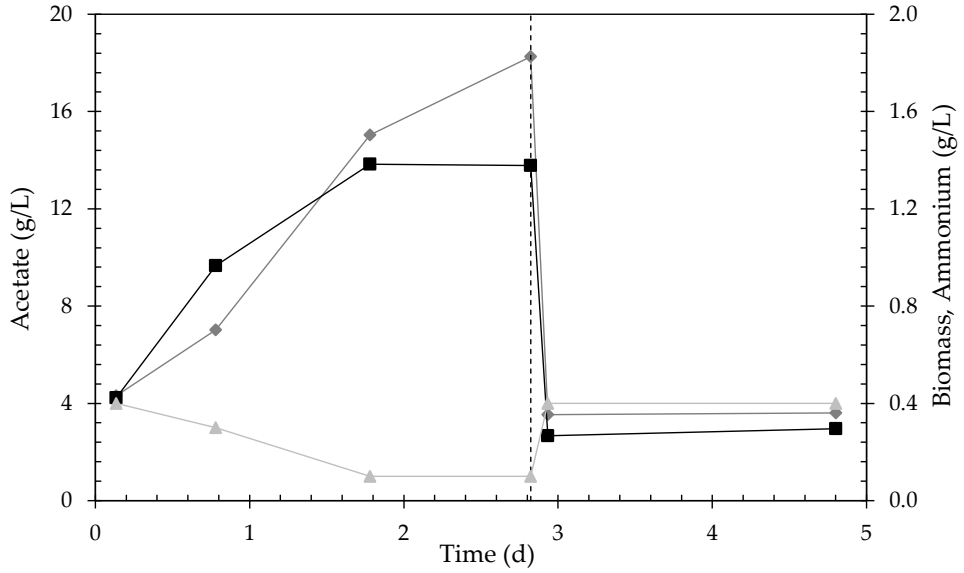


Figure 5. Biomass (■), acetate (◆), and ammonium (NH_4^+, ▲) concentrations of two consecutive batch runs on acid-scrubbed syngas (vol-%: 34.8 H_2, 16.5 CO, 16.6 CO_2, 32.1 N_2). The dashed line (---) indicates when the reactor was partially harvested and refilled with fresh medium.

Indeed, several impurities present in biomass-derived syngas can hinder the subsequent fermentation process [15,16,36,37]. Although H_2S was found to positively affect *Clostridium ragsdalei* in syngas fermentation in stirred-tank bioreactors, in the case of acid-scrubbed syngas, the H_2S concentration (65 ppm_v) was close to the upper limit of the acceptable range established by Oliveira and colleagues [36]. On the other hand, HCN has been identified as one of the most toxic contaminants in biomass-derived syngas. It has been demonstrated to inhibit *C. ljungdahlii* at a concentration of 0.027 g/L KCN, i.e., 27 ppm [38]. However, an HCN concentration of 0.91 ppm present in the straw-derived syngas was not fully inhibitory for acetate and ethanol production by the same microorganism, causing a more extended lag phase and a slight decrease in the acetate titer but enabling similar growth compared to impurity-free syngas [28]. Additionally, it has been reported that COS is a non-competitive inhibitor of the CO dehydrogenase in *Rhodospirillum rubrum* [39]. Hence, the observed microbial inhibition upon prolonged cultivation in acid-scrubbed syngas was most likely caused by elevated H_2S, HCN, or COS concentrations.

The acid gases H_2S and HCN were partially removed by switching the acidic scrubbing solution to a caustic one, as described in Section 3.1. The first batch process in caustic-scrubbed syngas reached a biomass and acetate titer of 1.3 g/L and 16.9 g/L in 3.7 days of cultivation. However, the second batch fermentation, preceded by a partial harvest and refill, yielded a biomass and acetate titer of 1.3 g/L and 18.3 g/L, respectively, in only 3 days (Figure 6). The maximum acetate productivity achieved during this set of caustic-scrubbed syngas trials was 0.52 g/L·h, the highest of all fermentation runs reported here.

The concentration of NH_3 in caustic-scrubbed syngas was expected to be higher than in acid-scrubbed syngas since it is a weak base. NH_3 has been identified as an inhibitor of hydrogenases in *C. ragsdalei* [40]. However, a NH_3 concentration of 150 ppm had no inhibitory effect on syngas fermentation by *C. ljungdahlii* [28]. Oliveira and collaborators have also demonstrated that a concentration of NH_3 up to 4500 ppm is acceptable for syngas fermentation processes with different *Clostridial* strains [36]. Therefore, the concentration of NH_3 in caustic-scrubbed syngas was not regarded as a potential inhibitor of the syngas fermentation process. The concentrations of H_2S and HCN were reduced in the caustic-scrubbed syngas, whereas the concentration of COS remained constant compared to acid-scrubbed syngas (Table 3). Thus, the impurities' concentration and the syngas fermentation results obtained upon prolonged cultivation in acid- or caustic-scrubbed syngas suggest that the most toxic compounds in partially purified syngas are H_2S and HCN.

In contrast, low ppm COS concentrations were tolerable. Likewise, the caustic-scrubbed syngas results indicate no observable effect of residual benzene and tars, which may have been present in the syngas. These findings suggest that a caustic solution is essential as a scrubbing agent to achieve partially purified syngas streams that enable sustained microbial fermentation by lowering the concentrations of H_2S and HCN. The results obtained in this study thus indicate that it is possible to simplify the syngas cleaning configuration, as *M. thermoacetica* could convert syngas even when it contained impurities, like NH_3, H_2S, and COS, which would typically hinder chemical synthesis. Hence, combined with milder operation conditions in terms of temperature and pressure, the thermal–biological route for producing sustainable chemicals or fuels could have a significant economic advantage over conventional thermal-catalytic processes.

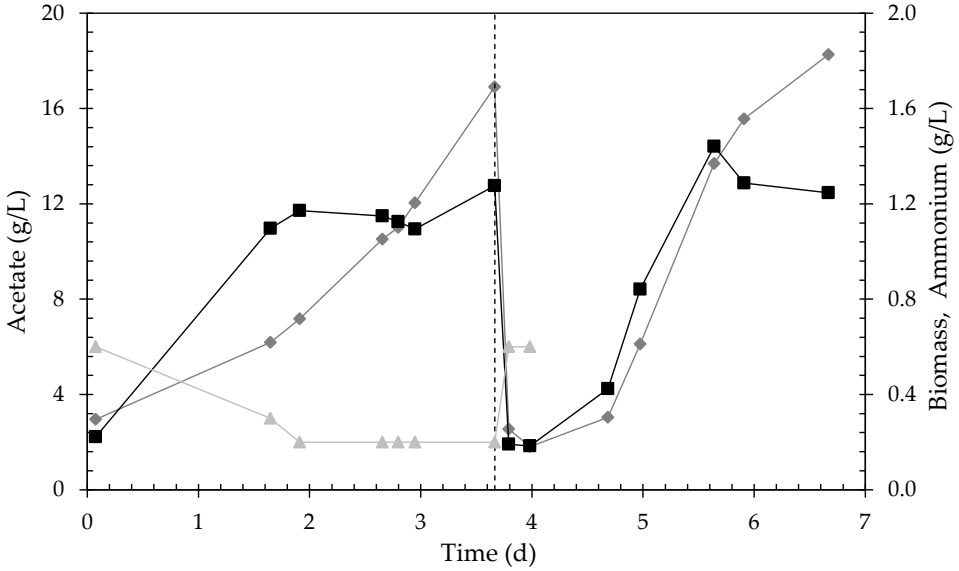


Figure 6. Biomass (■), acetate (◆), and ammonium (NH_4^+, ▲) concentrations of two consecutive batch runs on caustic-scrubbed syngas (vol-%: 34.7 H_2, 17.8 CO, 15.8 CO_2, 31.7 N_2). The dashed line (---) indicates when the reactor was partially harvested and refilled with fresh medium.

Despite the promising results obtained in this study, it is generally known that biomass properties can significantly impact the quality and composition of the syngas produced via gasification [7,41]. Therefore, future studies should evaluate the feasibility of simplifying syngas streams' cleaning process by gasifying more complex feedstocks, such as straw or municipal solid waste. The partially purified syngas streams derived from these feedstocks might adversely impact the downstream syngas-to-acetate fermentation. Additionally, a detailed techno-economic analysis and life cycle assessment would allow a more in-depth examination of whether the coupling of gasification and syngas-to-acetate fermentation has economic and environmental advantages compared to a standalone gas fermentation process converting industrial off-gases into acetate.

4. Conclusions

This study confirmed the feasibility of coupling biomass gasification and syngas fermentation at a pilot scale by utilizing *M. thermoacetica* as a biocatalyst. Furthermore, the resilience of this microorganism towards common syngas impurities was evaluated by streamlining the syngas cleaning process to produce partially purified syngas. Extended cultivation in partially purified syngas streams identified H_2S and HCN as the most detrimental compounds. Consequently, caustic scrubbing was recognized as an adequate final gas cleaning step for filtered and reformed syngas, facilitating extended microbial cultivation. This research demonstrates the resilience of biocatalysts against various impu-

rities, laying the groundwork for simplifying syngas cleaning processes and reducing the expenses associated with the valorization of industrial waste off-gas.

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