H2020-LC-SC3-2018-2019-2020
EUROPEAN COMMISSION
European Climate, Infrastructure and Environment Executive Agency (CINEA)

Biofuels production from **S**yngas
Fermentation for **A**viation and maritime use

Grant Agreement No 884208

Deliverable 4.4

Results of the pilot run

Document Details

Due date	31st December 2023 (M45)
Actual delivery date	21st December 2023 (M45)
Lead Contractor	BBEPP
Version	Final
Prepared by	BBEPP
Input from	VTT
Reviewed by	CERTH

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Abbreviations

AA	Acetic acid
BBEPP	Bio Base Europe Pilot Plant (BioSFerA partner, Belgium)
BBMPP	Bio Base Mobile Pilot Plant (gas fermentation pilot plant from BBEPP)
BFB	Bubbling fluidized bed
CERTH	Centre for Research and Technology Hellas (BioSFerA partner, Greece)
CO	Carbon monoxide
CO_2	Carbon dioxide
COS	Carbonyl sulfide
EFT	Effective fermentation time
FID	Flame ionization detector (Gas chromatography)
FPD	Flame photometric detector
GC	Gas chromatography
HCl	Hydrogen chloride
HCN	Hydrogen cyanide
H_2S	Hydrogen sulfide
i.d.	Internal diameter
LHV	Lower heating value
LoD	Limit of detection
n.a.	Not analyzed
NaOH	Sodium hydroxide
NH_3	Ammonia

NH_4^+	Ammonium
O_2	Oxygen
OD	Optical density
ppm_v	Parts per million by volume
TCD	Thermal conductivity detector (Gas chromatography)
TRL	Technology readiness level
WP	Work package

Contents

1 Executive Summary

This deliverable compiles the results of the scale-up of a syngas fermentation process for the production of acetic acid (AA) at technology readiness level 5 (TRL5). Within the scope of BioSFerA work package 4 (WP4), and more specifically task 4.3, the mobile gas fermentation unit of Bio Base Europe Pilot Plant (BBEPP) was integrated with the biomass gasification units of VTT (Bioruukii facility) in order to perform 24 L runs for the production of AA from **real biomass-derived syngas**. The syngas fermentation process involves the growth of *Moorella thermoacetica*, an acetogenic bacterium capable of producing AA alongside growing biomass. The lab scale development of the syngas fermentation process within BioSFerA WP3, task 3.3, was utilized as a starting point to bring the technology to TRL5.

First, a 24 L run (BioSFerA-T4.3-F01) was conducted with synthetic gases as substrate to set a benchmark for the process at this scale before testing real biomass-derived syngas. Afterwards, two runs (BioSFerA-T4.3-F02 and BioSFerA-T4.3-F03) were performed with **ultra-cleaned syngas** derived from the gasification of **bark** by VTT. To meet one of the goals of task 4.3, certain purification steps of the syngas cleaning unit were bypassed to obtain a higher impurity syngas (**acid-scrubbed syngas**) from bark, with an increased amount of several impurities (e.g., H_2S, HCN, COS, and benzene) compared to the ultra-cleaned syngas. Another two runs (BioSFerA-T4.3-F04 and BioSFerA-T4.3-F05) were performed utilizing acid-scrubbed syngas as feedstock for the gas fermentation process. Due to the prominent inhibition observed in BioSFerA-T4.3-F05, further adjustments were implemented in the syngas cleaning unit to obtain a slightly higher purity gas (**alkaline-scrubbed syngas**) from gasified bark, with an increased amount of NH_3, but a reduced amount of the other impurities (H_2S, HCN, COS, and benzene) compared to acid-scrubbed syngas. Also, two runs (BioSFerA-T4.3-F06 and BioSFerA-T4.3-F07) were carried out with alkaline-scrubbed syngas as substrate.

Additionally, a 24 L run (BioSFerA-T4.3-F08) was performed with alkaline-scrubbed syngas derived from the gasification of **straw**, based on the target of widening the feedstock range for gasification-syngas fermentation processes envisioned in BioSFerA task 4.3. Furthermore, three additional tests at 24 L scale (BioSFerA-T4.3-F09, BioSFerA-T4.3-F10, and BioSFerA-T4.3-F11) were run with synthetic gases, ultra-cleaned, and alkaline-scrubbed syngas derived from gasified **bark**, respectively, with the goal of closing the gap towards the **target AA productivity** of 0.5 g/L.h. This was done by improving the gas transfer rate of the syngas components through an increase in the agitation speed. Finally, a **continuous fermentation with cell recycling** at 24 L scale (BioSFerA-T4.3-F12) was performed both on synthetic gases and alkaline-scrubbed syngas derived from gasified **bark**. This report describes the results obtained in all these fermentations, as well as the gasification and syngas cleaning processes preceding the syngas fermentation.

2 Biomass gasification and hot gas cleaning (VTT)

2.1 Test facility and test procedure

The syngas for the ultra-cleaning process was produced by an atmospheric-pressure bench-scale bubbling fluidized-bed gasifier (BFB100) illustrated in Figure 1. The bed diameter of the reactor is 100 mm and the freeboard diameter, 150 mm. The test facility was heated with external electrical heaters to compensate heat losses, and fluidizing gases were preheated electrically to about 310°C below the grid.

The start-up bed was added into the reactor as a batch before the fuel feeding was started. A mixture of silica sand and dolomite was used as a bed material, and it was also fed during the test runs as a make-up in order to compensate the removed and elutriated bed material.

The feedstock was fed from the live-bottom fuel tank equipped with the screw feeder. The feedstock and bed material streams were combined into one screw feeder before entering the gasifier. The dosing screws were calibrated, and all loaded fuel and bed material batches were weighed in advance as well as their remaining amounts after the test run in order to calculate their actual consumptions.

The filter dust was removed by filtration, and the metallic filter elements were cleaned by pulsing them with nitrogen as needed. Bottom ash was removed from the gasifier during the test runs in order to prevent any accumulation of harmful ash compound into the bed.

The BFB gasifier was operated at atmospheric pressure and at 800 °C temperature with bark, and at about 720 °C with straw. A mixture of steam and oxygen was used as the gasification agents in bark test runs, whereas only steam was used in straw gasification. A small amount of nitrogen was used to purge fuel feeding system and the pressure drop measuring lines. Oxygen and nitrogen were fed into the reformer, and their feed rates were adjusted according to the temperatures in the reformer.

Gas composition after the filter and after the reformer were measured by gas analyzers and offline samples. In addition to the solid input streams (feedstock and bed material), also solid output streams (filter dust and bottom ash) were measured and analyzed. More information can be found in previous publications[1,2]. The gasification process is illustrated in Figure 1.

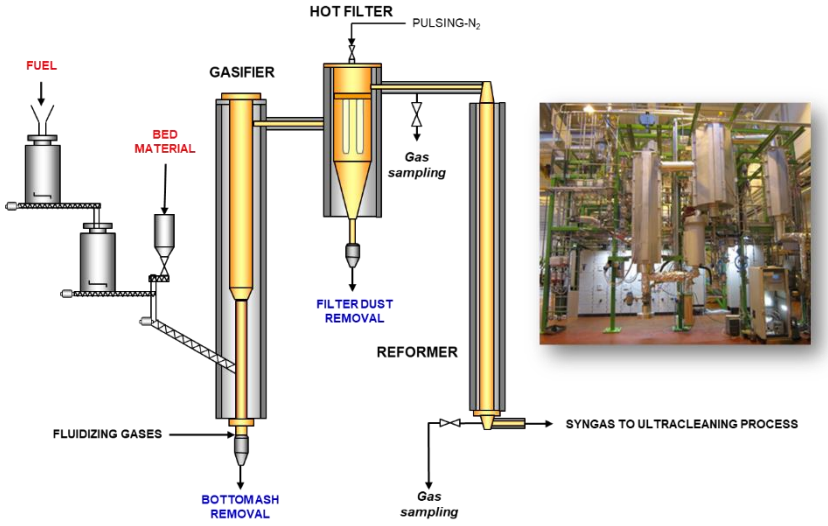

Figure 1. Bench-scale atmospheric-pressure bubbling fluidized-bed gasifier (BFB100).

In total, six day-long campaigns to generate syngas for the fermentation process were performed during the project with different process configurations including feedstock and gas cleaning configuration.

2.2 Feedstocks

Based on the bench-scale gasification results obtained within BioSFerA task 3.1 and its conclusions, crushed bark, was used as gasifier feedstock in the first five test campaigns in order to minimize the operational risks in the piloting operation. Additionally, straw pellets were used as gasifier feedstock in the final sixth campaign. Their compositions are presented in Table 1.

Table 1. Feedstock analyses.

Feedstock	**Bark**	**Straw**
Gasification campaign	**23/21,23/22,23/24,23/33,23/37**	**23/40**
Moisture, wt-%	7.7	7.5
In dry matter, wt-%		
Ash	3.5	6.8
C	49.0	46.2
H	6.0	5.6
N	0.4	1.3
S	0.06	0.06
LHV, MJ/kg (dry)	19.4	17.3

A mixture of silica sand and Myanit-dolomite was used as bed material. Main composition of Myanit was the following: 30.2 wt-% CaO, 20.6 wt-% MgO, 3.6 wt-% SiO_2, 0.5 wt-% Fe_2O_3 and 44.0 wt-% released CO_2.

2.3 Operation conditions and gas composition after the reformer

Most of the test runs were carried out with bark in almost identical gasification conditions, and one test run with straw. Table 2 summarizes the average operation conditions and measured gas syngas composition after the reformer with respect to the feedstock.

Table 2. Summary of the average operation conditions and gas measurements in the gasification campaigns.

Gasification campaign	23/21, 23/22, 23/24, 23/33, 23/37	23/40
Feedstock	Bark	Straw
Gasification conditions		
T(bed-average), °C	804	722
T(freeboard-average), °C	794	715
Fluidizing velocity, m/s	0.57	0.57
Steam-to-fuel, kg/kg-daf	0.9	1.1
O_2 feed, % of stoichiometric combustion	8.0	0.0
O_2, wt-% of fluidizing gases	11.9	0.0
Hot gas cleaning conditions		
T(filter), °C	559	552

T(reformer), °C	930	943
C conversion to gas and tars (from C-losses), %	79.0	73.2
Product gas after the reformer		
Measured dry gas composition, vol-%		
CO	19.2	15.3
CO_2	17.7	18.3
H_2	38.9	36.3
N_2 (calculated as difference)	23.4	28.2
CH_4	0.8	1.9
C2Hy	0	0
C3-C4Hy	0	0
NH_3*	0.01	0.05
Gas contaminants (measured from dry gas)		
Benzene, mg/m^{3}n	6	169
Sum of tars, mg/m^{3}n	21	47
H_2O in wet gas, vol-%	32.6	37.4
Calculated conversions in reformer, %		
Benzene	99.9	97.2
Tars	99.7	99.8
CH_4	79.4	63.1
NH_3	96.0	93.5

* From previous test run with same feedstock.

3 Final syngas cleaning (VTT)

3.1 Test facility and test procedure

The final syngas cleaning, or ultra-cleaning process, "UC5" is illustrated in full in Figure 2. The process is designed for a nominal feed gas flow rate of 5-10 m^3n/h dry gas. In the BioSFerA tests, the utilization of the scrubber units and fixed bed reactors involved bypassing the existing pressurized water scrubbing unit, which is not illustrated in the figure but is typically used for CO_2 removal. The process was operated at the pressure of the BFB gasifier, corresponding to 0-100 mbar above atmospheric pressure.

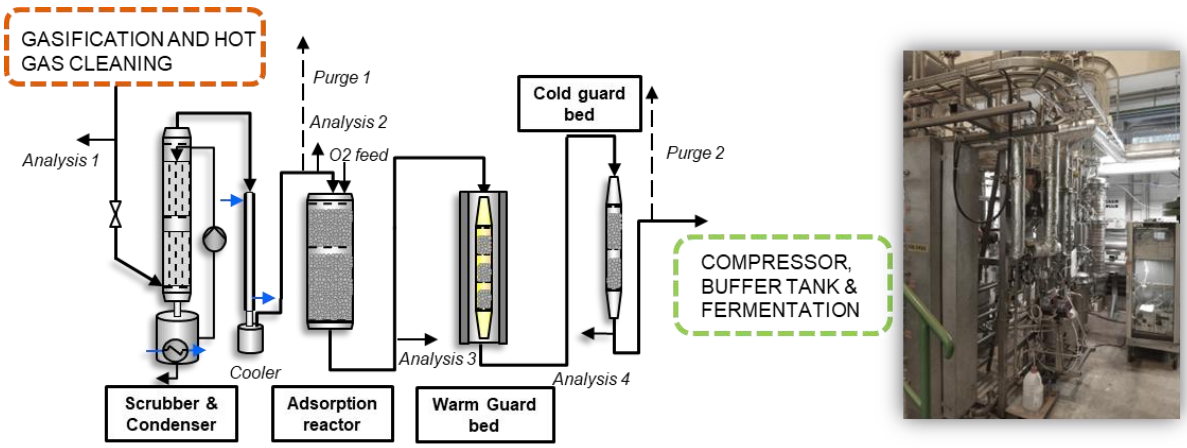

Figure 2. Left: Schematic illustration of the full ultra-cleaning process UC5 (Configuration I). Right: Picture of UC5.

The complete process configuration depicted in Figure 2 was initially employed to generate high-purity (ultra-cleaned) syngas, following a similar approach as previously reported[2,3]. In the subsequent campaigns, modifications were made to the UC5 process to generate lower-quality syngas for the fermentation tests in order to study the effects of real syngas impurities on the fermentation process.

3.1.1 Description of unit operations

3.1.1.1 Scrubber&Condenser with acid or caustic injection

The aqueous scrubber&condenser serves as the initial unit operation in the final syngas cleaning process, comprising a counter-current column with an inner diameter (i.d.) of 0.164 m. This column is filled with Pall rings, and the bed height is 1.3 m. The syngas temperature at the outlet of the scrubber was maintained within the range of 25-35 °C. Notably, the system operated with an acidic water circulation during campaigns 23/21, 23/22, and 23/24, while the subsequent campaigns utilized a caustic water circulation. This choice in water circulation pH played a crucial role in determining the gas impurities in the cleaned syngas.

When operated in acidic mode, NH_3 is captured since aqueous ammonia dissociates according to Equation 1:

$$NH_3(aq) + H_3O^+(l) \rightleftarrows NH_4^+(aq) + H_2O \quad \text{(Equation 1)}$$

Consequently, acidic gas capture is generally diminished when the scrubber is operated in acidic mode. In acidic mode, the pH of the circulating water was set to pH 3 using formic acid injection.

The scrubber was modified in the BioSFerA project to be able to be operated in NaOH feeding mode. When operated in caustic mode, acid gas capture is improved (including H_2S, HCN, and CO_2). H_2S reacts according to Equations 2 and 3[4]:

$$H_2S(aq) + NaOH(aq) \rightleftarrows NaHS(aq) + H_2O \quad \text{(Equation 2)}$$

$$NaHS(aq) + NaOH(aq) \rightleftarrows Na_2S(aq) + H_2O \quad \text{(Equation 3)}$$

The continuation from the first reaction according to the second reaction to sodium sulfide product is favored when the pH is higher, typically over 12.

Likewise, the reactive absorption for CO_2 proceeds similarly, as shown in Equations 4 and 5[4]:

$$CO_2(aq) + NaOH\ (\text{aq}) \rightleftarrows NaHCO_3(aq) + H_2O \quad \text{(Equation 4)}$$

$$NaHCO_3(aq) + NaOH(aq) \rightleftarrows Na_2CO_3(aq) + H_2O \quad \text{(Equation 5)}$$

Maintaining a higher pH, typically above 10, promotes carbonate formation as a continuation of the bicarbonate reaction. It is also crucial to recognize the impact of absorption kinetics on the gas cleaning process. Generally, the reactions involving CO_2 are slower compared to those involving H_2S. This difference in reaction rates allows for a more selective removal of H_2S from gas streams containing both H_2S and CO_2. This selectivity is achieved by employing absorption columns designed to minimize gas-liquid contact time.[4]

Furthermore, prevention of solid formation is required since sodium salts have limited solubility in water. Sodium carbonate has a solubility of 30.7 g/100 ml water and sodium sulfide 18.6 g/100 ml water at room temperature. An increase in the temperature increases solubility[5,6]. Given its role as a condenser for the steam-rich syngas, the current column is less susceptible to concerns related to solid formation.

3.1.1.2 Adsorption reactor

The adsorption reactor, with an i.d. of 0.25 m, has two beds filled with activated carbon adsorbents. The top bed is loaded with non-impregnated carbon designed for the adsorption of residual tars and benzene. Meanwhile, the second bed, a larger one with the same non-impregnated activated carbon, primarily targets H_2S or other trace component removal. To further enhance H_2S removal, a smaller bed containing highly efficient caustic-impregnated carbon is employed as a final precautionary step.

Optimal conditions for H_2S removal are maintained by adjusting the reaction parameters. The relative humidity of the gas is set to approximately 55-70%, and the reactor temperature is maintained at around 25 °C. Additionally, a constant air feed of 0.1 Ndm^3/min is introduced into the reactor during testing. This air feed serves to induce oxidative H_2S removal, a technique demonstrated in previous research[7]. The combined design and operating conditions of the adsorption reactor contribute to its effectiveness in removing targeted impurities from the syngas.

3.1.1.3 Guard beds

The warm guard bed is a fixed bed reactor with an i.d. of 0.085 m. The reactor is mounted inside a furnace and is equipped with a preheater. The two uppermost beds were packed with commercial ZnO adsorbent with Al_2O_3 support. The purpose of this material is to remove especially COS through the COS hydrolysis reaction, as well as removal of trace H_2S formed in the hydrolysis reaction. The material is also active in catalyzing HCN hydrolysis into NH_3.

A Cu-based catalyst was used for deoxygenation to remove any oxygen left from the activated carbon bed air feeding. The deoxygenation step was operated at slightly higher temperature, at ca. 200 – 230 °C, while the top ZnO bed was maintained at ca. 180-200 °C.

The cold guard bed reactor (i.d. 0.085 m) was operated at room temperature and was filled with acidic and caustic impregnated activated carbons.

3.1.2 Summary of gas cleaning process configuration in the test campaigns

The BioSFerA campaigns incorporated various UC5 process configurations to intentionally produce syngas with distinct impurity concentrations. The overarching strategy involved selectively eliminating specific process steps to streamline the gas cleaning process, aligning it with the requirements of the fermentation tests and the targets of BioSFerA project. Three distinct process configurations were tested:

- **Configuration I (Ultra-cleaned syngas):** Implemented in campaigns 23/21 and 23/22, this configuration aimed to generate high-purity syngas. It encompassed the entire process chain, including acidic scrubbing, adsorption reactor, warm guard bed, and cold guard bed.
- **Configuration II (Acid-scrubbed syngas):** Deployed in campaign 23/24, this configuration targeted higher impurity concentrations in the syngas. In this setup, only the scrubber system was utilized with bypass of the subsequent cleaning steps from Configuration I. The scrubber was operated in acidic mode.
- **Configuration III (Alkaline-scrubbed syngas):** Deployed in campaign 23/33, 23/37 and 23/40, this setup also only featured the scrubber, but operated in caustic mode.

Table 3 provides a summary of the syngas cleaning unit configuration for each campaign, outlining the modifications made to the UC5 process in each instance. This approach allowed for the generation of syngas with tailored impurity profiles, facilitating the study of their effects on the subsequent fermentation process.

Table 3. Process configuration by campaign for the final syngas cleaning process UC5 (X: in use; –: not in use).

UC5 configuration	**I**		**II**	**III**		
Gasification campaign	**23/21**	**23/22**	**23/24**	**23/33**	**23/37**	**23/40**
Scrubber&Condenser	Acidic	Acidic	Acidic	Caustic	Caustic	Caustic
Adsorption reactor	X	X	–	–	–	–
Warm guard bed	X	X	–	–	–	–
Cold guard bed	X	X	–	–	–	–

Table 4 summarizes the adsorbent and catalyst materials respective bed masses for the fixed bed reactors for configuration I. The beds were fresh packed before the first campaign 23/21 and were not changed for campaign 23/22.

Table 4. UC5 configuration I adsorbent/catalyst loadings in gasification campaigns 23/21 and 23/22.

	Position	**Mass, kg**
Adsorption Reactor		
Non-impregnated AC 1	Bed 1	1.5
Non-impregnated AC 1	Bed 2: Top	6.6
Impregnated AC 1	Bed 2: Bottom	1.6
Warm guard bed		
ZnO adsorbent	Bed 1 and 2	2.6
Cu/Zn catalyst	Bed 3	1.8
Cold guard bed		
Impregnated AC 2 (acidic)	Bed 1	0.3
Impregnated AC 1 (caustic)	Bed 2: Bottom	0.3

ZnO adsorbent was Actisorb S2 by Clariant. Cu/Zn catalyst was Research Catalysts Inc GetterMax® 133. Non-impregnated AC 1 was Jacobi Carbons Sulfox. Impregnated AC 1 was Jacobi Carbons VA4, and Impregnated AC2 was Jacobi Carbons VB1.

3.1.3 Process control

In the BioSFerA test campaigns, a distinct approach was adopted for the downstream process coupling. The downstream fermentation pilot plant was equipped with a pressurized-air driven gas compressor and a sizable buffer tank with a capacity of 1.8 m^3. During the day-long gasification and syngas cleaning campaigns, the buffer tank was filled with syngas, which was later utilized in the fermentation process.

The gas compressor served as the driving force to transport the gas from the gasifier through the syngas purification process and into the buffer tank, up to pressures of 12 bar_g. A simplified representation of the final gas cleaning process is illustrated in Figure 3, detailing the control mechanisms implemented through the gas compressor and purge flows.

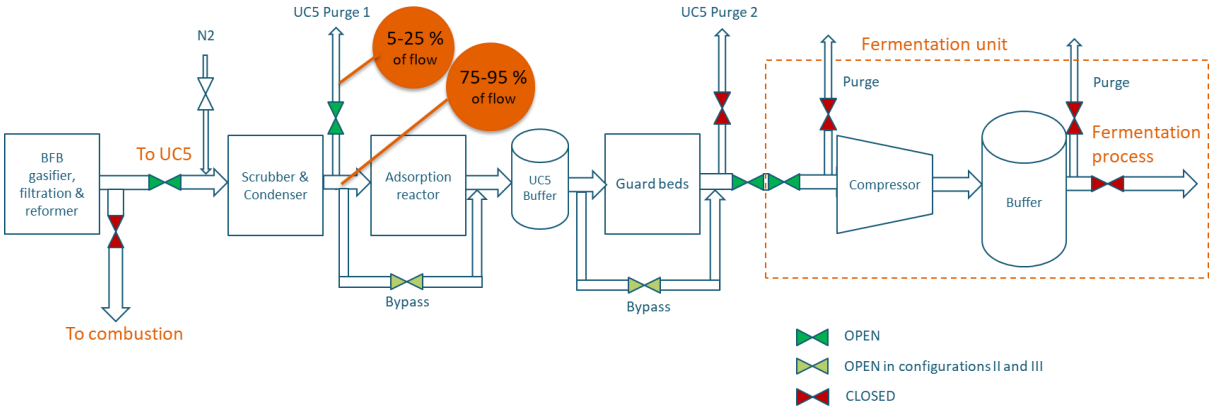

Figure 3. Simplified final gas cleaning flowsheet. The picture depicts flow configuration during operation (filling of the buffer tank).

Before each campaign, the system was inertized with N_2, including the interfaces such as gasifier-UC5 and UC5-mobile gas fermentation unit in order to eliminate possible sources of air (O_2). Similar to the gasifier, the UC5 process was leak-tested prior to each campaign at 200 mbar. Since the compressor causes an underpressure in the suction side, an oxygen sensor was continuously monitoring the gas quality. The startup procedure of the coupled process was initialized by the gasifier with the syngas directed to the combustor, while the UC5 valve was closed. Meanwhile, UC5 was flushed with N_2 to UC5 purge 2. UC5 was then coupled to the gasifier by closing the combustion line valve and opening the UC5 valve while simultaneously ending the N_2 feeding to UC5. Minutes later the compressor inside the mobile gas fermentation unit was started and the UC5 purge 2 was closed.

In order to scale-match the compressor input and the gasification output, UC5 gas purge 1 was in use during the campaigns, acting as a type of relief-valve. The gasifier was operated at an excess capacity relative to the compressor input, and the gasifier full syngas output was directed to UC5. The excess syngas was vented from UC5 purge 1 after the scrubber&condenser, thus ensuring a relatively stable gasifier pressure regardless of compressor capacity fluctuations.

The pressure fluctuations were further stabilized by the UC5 buffer of 0.1 m^3 volume. The flow rate through the system likely varied as a function of time due to the changing compressor output pressure (buffer filling). However, as the gasifier was operated at relatively stable conditions, the flow rate to the scrubber&condenser unit remained relatively stable.

Safety features in the system included automatic separation of process steps (gasifier-UC5 and UC5-mobile gas fermentation unit) in case UC5 process measurement limits were exceeded. Additionally, each section was equipped with mechanical pressure safety valves.

3.1.4 Gas analysis

Table 5 shows the analytical methods employed in the gas analysis in UC5. The analysis was conducted intermittently through manual sampling, employing colorimetric tubes or gas bag samples. Key impurities identified in syngas during prior tests with the same gasifier and feedstocks have included H_2S, COS, HCN, NH_3, HCl, and benzene & tars. Other components, such as organic sulfurs have previously not been detected (limit of detection 0.1 ppm). Other impurities such as metal carbonyls, NO_x, and SO_x compounds have not been analyzed.

Table 5. Major syngas impurities and the analytical techniques employed in UC5 (before and after final gas cleaning).

Impurity	Analytical method	Limit of detection (LoD)
H_2S	Colorimetric tubes	0.1 ppm
	FPD-GC (gas bag)	0.1 ppm
COS	FPD-GC (gas bag)	0.1-0.5 ppm
HCN	Colorimetric tubes	0.1 ppm
NH_3	Not analyzed	-
HCl	Colorimetric tubes	<0.1 ppm
Benzene & Tars*	Not analyzed	-

* Analyzed after the reformer.

FPD-GC calibration was performed using 15000 mol ppm H_2S and 151 ppm COS gas with relative error of ±2%. For calibration, a Pierburg gas diluter was used with N_2 as dilutant. The FPD-GC results are the average of 3 injection results from two parallel sample bags. For campaigns with FPD-GC samples, typically one or two samples were taken.

The colorimetric tubes were of type Dräger H_2S and HCN with relative standard deviation of 10-15%. H_2S colorimetric tube samples were compared to FPD-GC results with high degree of agreement. The colorimetric tube results are an average of samples taken during the campaign at a sample size of n = 1-7.

3.2 Operation conditions and impurities concentration after final syngas cleaning

Campaign 23/21 was the first test with gasifier generated syngas, and the second campaign 23/22 was configurationally a repeat of 23/21. For campaign 23/24 onwards, UC5 was modified to configuration II and III and the catalyst/adsorbent beds were either bypassed or emptied. The average UC5 process measurements and the contaminant analysis results are presented in Table 6.

Table 6. Average operation conditions and impurities concentrations after UC5 by campaign.

	23/21	23/22	23/24	23/33	23/37	23/40
Feedstock	**Bark**	**Bark**	**Bark**	**Bark**	**Bark**	**Straw**
UC5 configuration	**I**	**I**	**II**	**III**	**III**	**III**
UC5 TOS, h	7.1	3.7	6.0	4.5	2.9	2.9
UC5 gas P (feed), mbar	55	49	45	73	88	57

UC5 gas feed flow rate, m^3n/h dry[c]	8.1	7.9	8.1	8.6	10.1	4.4
Scrubber&Condenser						
Water additive	Formic acid	Formic acid	Formic acid	NaOH 1M	NaOH 2M	NaOH 5M
Gas T (feed), °C	179	162	176	183	156	161
Gas T (out), °C	27	26	25	29	37	27
Water pH	2.3	2.6	3.0	9.7	n.a.	10.8
Water circulation (dm^3/h)	260	260	290	270	180	180
Water T (feed), °C	30	28	26	31	40	30
Adsorbent bed						
Beds T, °C	29	32	-	-	-	-
Warm guard bed						
Top beds T, °C	183	200	-	-	-	-
Bottom bed T, °C	202	224	-	-	-	-
Gas contaminants (dry basis) after reformer, ppm_v[a]						
H_2S	100	65	n.a.	40	16	210
COS	n.a.	4	n.a.	n.a.	n.a.	n.a.
NH_3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
HCN	2	1.2	n.a.	1	0/LoD	8.5
HCl	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Gas contaminants (dry basis) after UC5, ppm_v[b]						
H_2S	0/LoD	0/LoD	65	17.5	2	2.8
COS	n.a.	0/LoD	5.3	n.a.	3.6[d]	18.5
NH_3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
HCN	0/LoD	0/LoD	1	0.5	0/LoD	0/LoD
HCl	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Benzene & Tars	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

[a] For campaigns 23/21 and 23/22, the sampling point was after the UC5 scrubber&condenser. For the rest of the campaigns, analysis was performed after the reformer.

[b] For campaigns 23/21, 23/22 and 23/24, the sampling point was after the full UC5 process. For the rest of the campaigns, analysis was performed after the UC5 scrubber&condenser.

[c] Based on gasifier mass balances.

[d] Sampling point: mobile gas fermentation unit reactor off-gas.

The final syngas cleaning section underwent operation for a duration ranging from 3 to 7 hours, sufficient to fill the buffer tank of the mobile gas fermentation unit. During the bark feedstock campaigns, the dry gas flow rate to UC5 averaged between 8 to 10 m^3n/h. With a moisture content of approximately 30 vol%, the condensation rate was estimated to be around 2.5 to 3.5 kg/h. With the straw feedstock, the gasifier was operated at a lower output and thus the flow rate to UC5 was 4.4 m^3n/h.

In the initial three campaigns employing an acidic wash (configurations I and II), the sampling point for analyzing acid gas impurities in the post-reformer syngas was positioned after the scrubber&condenser. This decision was grounded on the assumption that H_2S and HCN are not effectively removed by the acid wash. During campaign

23/21, the syngas H_2S concentration before UC5 was measured at 100 ppm_v. However, subsequent campaigns demonstrated a reduction in H_2S concentration. Notably, in the final bark gasification campaign 23/37, the H_2S concentration was observed to be only 17 ppm_v.

In campaign 23/22, the concentration of bark-derived COS was measured at 4 ppm_v, and in campaign 23/24, it increased to 5.3 ppm_v. This observation aligns with previous gasification experiences, establishing COS/H_2S ratios at 0.02-0.15.

In the initial two campaigns featuring bark as feedstock, HCN concentrations in the raw syngas ranged between 1-2 ppm_v. As anticipated, the final campaign (23/40) with straw feedstock exhibited higher concentrations of impurities in the raw syngas, including 210 ppm_v H_2S, 8.5 ppm_v HCN, and at least 18.5 ppm_v COS (based on results after UC5). However, concentrations of benzene and tars were not measured after UC5. In campaign 23/40 the gas bag samples from the mobile gas fermentation unit reactor off-gas showed that C2-C5 and C6+ hydrocarbons at above 0.01 %vol concentrations could not be detected with a FID-GC. In addition, the off-gas analysis revealed similar H_2S and HCN concentrations as reported in Table 6 after UC5.

Post-UC5 gas analysis results from campaign 23/22 demonstrated complete removal of H_2S, COS, and HCN when UC5 operated under the full process configuration I. In contrast, with UC5 configuration II, H_2S, COS, and HCN were present in the product gas. Under UC5 configuration III, H_2S was mostly removed, remaining in the product gas at ppm-level quantities, while COS was fully or almost fully retained, as observed in campaign 23/37 at 3.6 ppm_v and campaign 23/40 at 18.5 ppm_v. HCN appeared to be effectively removed in UC5 configuration III, as indicated by analysis results from campaigns 23/37 and 23/40. The varying pH conditions in campaign 23/33 led to varying HCN analysis results, with values either recorded at pH 9.4 and approximately 0.5-1 ppm_v concentrations or undetected (0 ppm/LoD) at pH 9.8. Figure 4 shows examples of the UC5 process conditions as a function of time.

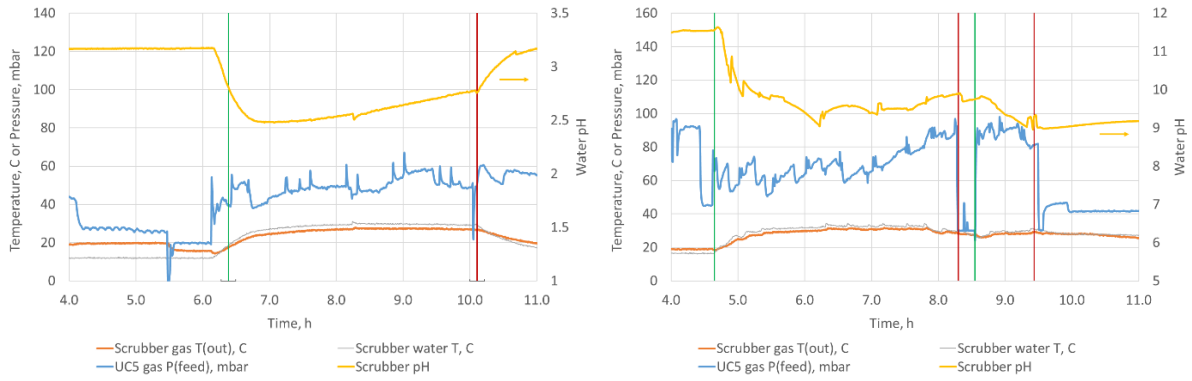

Figure 4. UC5 scrubber&condenser process conditions. Left: Campaign 23/22 Right: Campaign 23/33. Vertical green line represents syngas feed start to UC5 and red line syngas feed stop to UC5.

The figure presented illustrates key dynamics within the process when the system is operated in coupled mode. Notably, campaigns 23/33 and 23/24 encountered process shutdowns during buffer filling. Shutdown events during coupled operation were attributed to either pressure levels surpassing limits (underpressure) or equipment malfunctions. This led to unavoidable dilution of product gas with additional nitrogen.

Figure 4 indicates that maintaining basic conditions at the scrubber posed more challenges compared to acidic conditions. This is partially attributed to the substantially higher NaOH consumption on a relative basis to acid feeding, driven by reactions with CO_2. To address these challenges, adjustments were made during subsequent campaigns. Water circulation rates were intentionally decreased during specific instances, as outlined in Table 6 water circulation, to reduce gas-liquid contact. Furthermore, in later campaigns, the NaOH feed concentration was elevated, in an attempt to improve the control of the caustic scrubbing process. In the inaugural campaign with

caustic scrubbing, 23/33, an investigation into the effect of water pH on H_2S removal was undertaken, as illustrated in Figure 5.

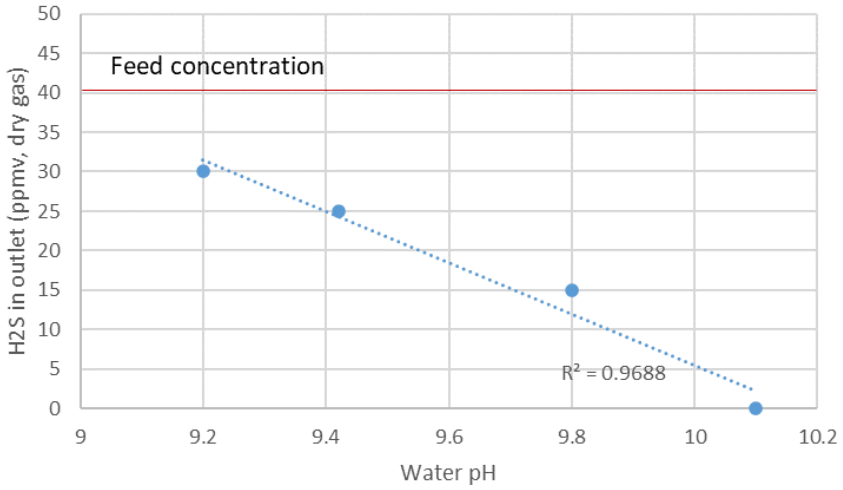

Figure 5. H_2S concentration after the scrubber&condenser as a function of scrubber water pH. Water temperature 27-31°C, NaOH addition, water circulation rate 150-290 dm³/h. Feed avg: 8.1 m³n/h, 18 vol-% CO_2 and 40 ppmᵥ H_2S.

The findings reveal a trend even in the presence of multiple variable conditions (e.g., changes in water circulation rates). The results indicate that a pH of over 10 is sufficient to remove a H_2S feed concentration of 40 ppm to below 5 ppmᵥ in the UC5 scrubber with liquid-to-gas ratio of 19-34 kg H_2O/m^3n syngas.

Given the limitations in analyzing each impurity in the cleaned syngas across all campaigns, Table 7 provides the authors' best estimations of the purification capabilities associated with different process configurations. This table serves as an overview of the purification performance, despite the analytical constraints.

Table 7. Estimated gas purification performance of the different UC5 gas cleaning configurations.

Impurity	I (Ultra-cleaned syngas)	II (Acid-scrubbed syngas)	III (Alkaline-scrubbed syngas)
H_2S (ppmᵥ)	Removal to <0.1 ppm	Low/no removal	Removal to <0.5-10 ppm
COS (ppmᵥ)	Removal to <0.1 ppm	Low/no removal	Low/no removal
NH_3 (ppmᵥ)	Removal to <1 ppm[a]	Removal to <1-5 ppm[a]	Low/no removal
HCN (ppmᵥ)	Removal to <0.1 ppm	Low/no removal	Removal to <0.1 ppm
HCl (ppmᵥ)	Removal to <0.1 ppm[a]	Low/no removal[a]	Removal to <0.1 ppm
Benzene (mg/m³n)	Removal to <1 mg/m³n	Low/no removal[a]	Low/no removal[a]
Tars (mg/m³n)	Removal to <0.1 mg/m³n	Low/no removal[a]	Low/no removal[a]
Organic S (ppmᵥ)	<0.1 ppm	Unknown	Unknown

[a] Not verified in this work, based on previous results.

Configuration I, previously tested with both bark and straw feedstocks[2], has been analytically verified to effectively remove impurities to sub-ppm concentrations. The results presented in this work corroborate these findings. Additionally, prior research[3] has independently investigated the standalone performance of the acidic scrubber&condenser. Under acidic conditions (pH < 4), NH_3 is estimated to be effectively removed, but there is little to no removal of acid gases such as H_2S, HCN, or HCl. COS, benzene, and non-condensable tars are known not to readily absorb under any aqueous conditions.

In contrast, when the scrubber&condenser water is caustic (pH > 9), acid gases are removed to a significant, but perhaps not complete, degree. Importantly, high CO_2 concentrations may hinder the removal of acid gas impurities. However, under caustic excess conditions, assuming sufficient gas-liquid contact, acid gas impurities are readily removed to low-ppm_v levels. In a caustic scrubber&condenser, ammonia is not expected to be captured. Table 8 provides estimates of the campaign-specific cleaned syngas impurity concentrations, taking into account the different process configurations.

Table 8. Gasification impurities concentrations in cleaned syngas based on analytical results and authors' estimations (red represents high concentration, yellow and orange medium concentration and green low concentration).

Gasification campaign	**23/21**	**23/22**	**23/24**	**23/33**	**23/37**	**23/40**
Feedstock	**Bark**	**Bark**	**Bark**	**Bark**	**Bark**	**Straw**
Type of syngas	**Ultra-cleaned syngas**		**Acid-scrubbed syngas**	**Alkaline-scrubbed syngas**		
H_2S	None/LoD	None /LoD	Tens of ppm_v's	Few to tens of ppm_v's	Few ppm_v's	Few ppm_v's
COS	None /LoD	None /LoD	Few ppm_v's	Few ppm_v's*	Few ppm_v's	Few to tens of ppm_v's
NH_3	None/few ppm_v's*	None/few ppm_v's*	None/few ppm_v's*	Tens to hundreds of ppm_v's*	Tens to hundreds of ppm_v's*	Hundreds of ppm_v's*
HCN	None /LoD	None /LoD	ppm_v level	None to ppm_v level	None /LoD	None /LoD
HCl	None /LoD	None/sub-ppm_v*	None/sub-ppm_v*	None/sub-ppm_v*	None/sub-ppm_v*	None/sub-ppm_v*
Benzene & Tars	None/few ppm_v's*	None/few ppm_v's*	Few to tens of ppm_v's*	Few to tens of ppm_v's*	Few to tens of ppm_v's*	Tens of ppm_v's*

* Estimation.

The estimated complete table of impurities for each campaign shows that campaign 23/21 and 23/22 product gases (ultra-cleaned syngas) both were likely very pure and the impurities that were measured could not be detected with the analytical methods employed (detection limit 0.1 ppm_v). Campaign 23/24 (acid-scrubbed syngas) was the opposite, with likely pass-through of all acid gases amounting to tens of ppm's of H_2S, a few ppm of COS, likely at least tens of ppm's of NH_3 and ppm level of HCN. Also, benzene and tars could be expected to be present in this product gas. With process configuration III (alkaline-scrubbed syngas) and bark feedstock, the expected purified syngas H_2S concentration in the few ppm_v range (except campaign 23/33 where scrubber conditions were not always optimal), with likely few ppm_v COS pass-through. Ammonia pass-through is expected, meaning tens to hundreds of ppm's in the syngas. HCN is not expected in the syngas, but benzene and tars will likely exist in the tens of ppm_v's range. With straw feedstock and UC5 configuration III (alkaline-scrubbed syngas), the syngas contained relatively little H_2S, a few ppm_v's, but the COS concentration was in the few tens of ppm_v's. Compared to the bark feedstock campaigns, this purified gas can be assumed to contain higher amounts of benzene and tars and ammonia, but none or very little HCN (<0.1 ppm_v).

4 Syngas fermentation to acetate at TRL5 (BBEPP)

4.1 Test facility and test procedure

As described in deliverable D3.4, the commercially available wild-type strain *M. thermoacetica* DSM2955 shows adequate performance for acetate production, and therefore, it was the chosen acetogenic bacterium to bring this technology to TRL5. *M. thermoacetica* is a homoacetogen, meaning that its metabolic end-product is only acetate (and not ethanol, lactate, etc.), which is produced alongside biomass growth. These two characteristics are desirable for a syngas fermentation process aiming to produce solely acetate as a feedstock for a second stage fermentation.

The main parameters influencing the syngas fermentation process performance were investigated at bench scale in BioSFerA task 3.3. In the 24 L runs described in this deliverable, parameters such as agitation speed, syngas flow rate, pressure, etc. were adjusted based on the resulting optimal conditions from the lab tests. In terms of mode of operation, batch with partial harvest and refill strategy, together with continuous fermentation with cell recycling, were investigated.

The BioSFerA-T4.3 runs were performed at the Bio Base Mobile Pilot Plant (BBMPP), **a gas fermentation mobile pilot plant manufactured to be moved close to the point-source emissions**. All the preparatory activities and safety considerations related to the transportation and installation of the BBMPP at VTT Bioruukki premises were successfully accomplished in the preceding BioSFerA tasks 4.1 and 4.2 (Figure 6). The fermentations were carried out at 24 L scale, one of the continuous stirred-tank reactor (CSTR) installed in the BBMPP (Figure 7). All online parameters (temperature, DO, agitation speed, syngas flow rate (in/out), pH, base addition) were continuously monitored (data not shown). The temperature was set at 60 °C and the pH was controlled via addition of sodium hydroxide (NaOH; no acid needed as it is already produced in the fermentation). Antifoam was added when excessive foaming was observed.

Figure 6. The Bio Base Mobile Pilot Plant (BBMPP) in operation at VTT Bioruukki premises.

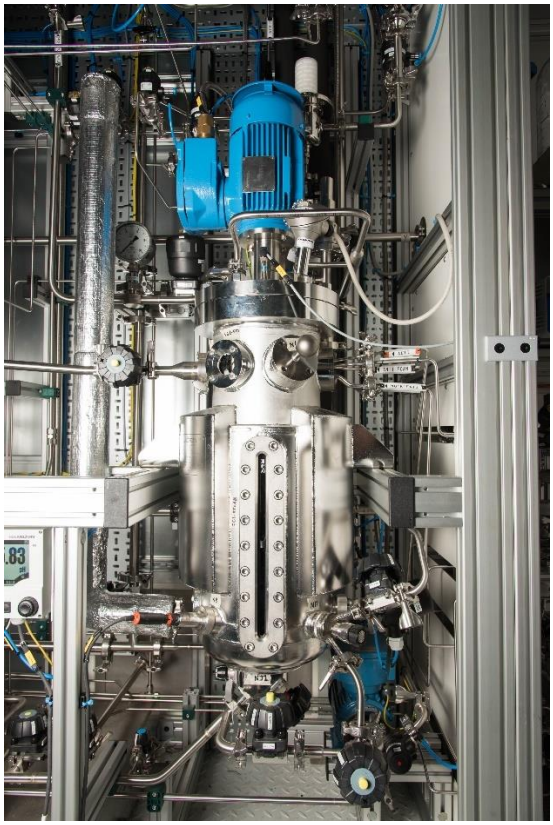

Figure 7. 24 L continuous stirred-tank reactor (CSTR) installed in the Bio Base Mobile Pilot Plant (BBMPP).

The seed train to inoculate the bioreactor was started from *M. thermoacetica* stock cultures preserved at – 80 °C and consisted of two steps. The second seed step was utilized to inoculate the 24 L reactor. Nonetheless, this seed train was only performed to start up the cultivation in the fermentor. To avoid preparing seed trains continuously, the harvest and refill strategy was followed: once the acetic acid concentration was around 20-25 g/L or the base addition trend started to flatten (meaning that acetate was barely being produced), 90% of the fermentation broth was harvested and the reactor was filled up to 10 L volume with fresh culture medium.

Based on the work carried out in BioSFerA tasks 3.1 and 3.3 and the preparatory activities of tasks 4.1 and 4.2, the coupling of gasification and syngas cleaning processes together with syngas fermentation to acetate became feasible, which later happened within the scope of task 4.3 and the results are presented below.

4.2 Syngas fermentation results

4.2.1 Benchmark with synthetic gases

The fermentation conditions for the benchmark run with synthetic gases at 24 L scale (BioSFerA-T4.3-F01) were chosen based on the optimizations done at 10 L scale at BBEPP facilities within the scope of BioSFerA task 3.3. The optical density measured at a wavelength of 600 nm (OD_{600}), and ammonium (NH_4^+) and AA concentrations are presented in Figure 8.

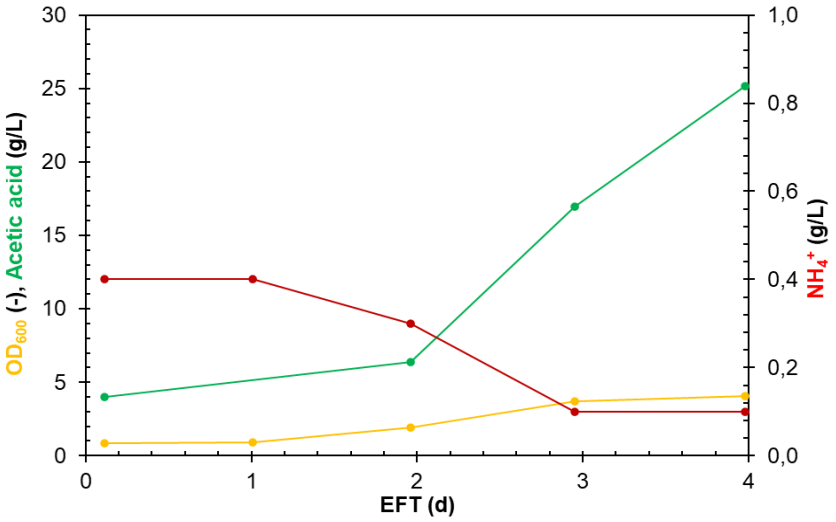

Figure 8. Offline data BioSFerA-T4.3-F01 run.

After almost 4 d (95.5 h) of fermentation, an OD_{600} of 4.0 (equivalent to 1.2 g/L CDW) was reached. Meanwhile, the AA titer was 23.3 g/L, which means the overall AA productivity of the fermentation was 0.23 g/L.h. Additionally, the maximum AA productivity was also calculated from the base addition trend (not shown), given that the neutralization reaction of AA and NaOH is equimolar (1:1). The max. AA productivity achieved in the benchmark fermentation was 0.46 g/L.h.

The target AA titer of 30 g/L was not met due to the implementation of the partial harvest and refill strategy. This benchmark fermentation was stopped before its total completion to avoid having a long lag phase in the next batch due to the inhibition exerted by high acetate concentrations on *M. thermoacetica*. However, the target AA titer was achieved and exceeded in the lab scale fermentations performed and reported in deliverable D3.4. On the same trend, the target AA productivity of 0.50 g/L.h was not reached in the benchmark run, but the max. AA productivity obtained in this run slightly improves the results reported in deliverable D3.4 (max. AA productivity obtained was 0.42 g/L.h).

4.2.2 Testing different-quality syngas from gasified bark

After setting the benchmark on synthetic gases, the tests with real biomass-derived syngas started, proving the *in situ* connection of biomass gasification, syngas cleaning, and syngas fermentation processes. To evaluate the feasibility of connecting both pilot plants, it was decided to start the tests with the purest gas (**ultra-cleaned syngas**) that VTT can produce from bark. As explained in section *3.2 Operation conditions and impurities concentration after final syngas cleaning*, all the major contaminants are below 0.1 ppm in the ultra-cleaned syngas. The OD_{600}, NH_4^+ and AA concentrations of the two runs on ultra-cleaned syngas are presented in Figures 9 and 10.

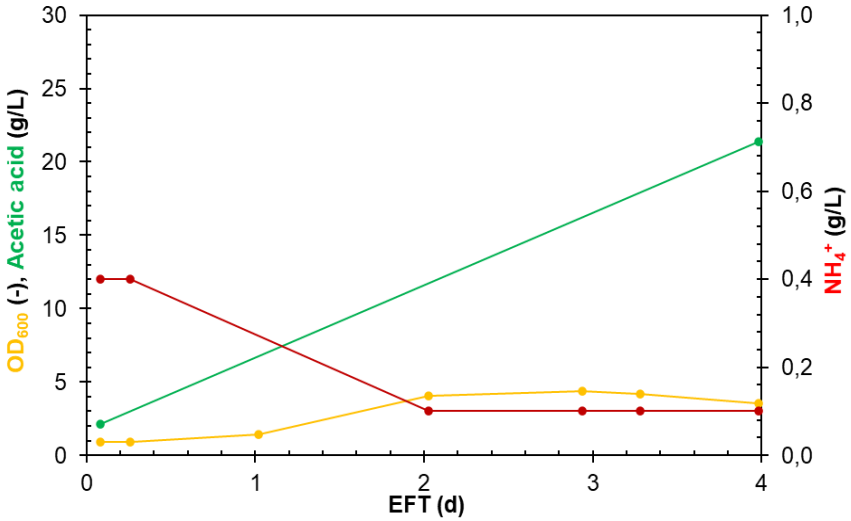

Figure 9. Offline data BioSFerA-T4.3-F02 run.

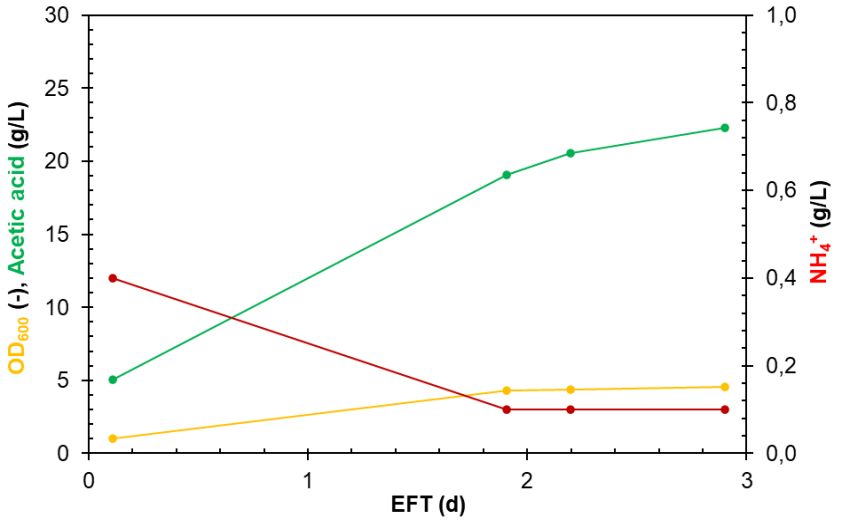

Figure 10. Offline data BioSFerA-T4.3-F03 run.

Both runs achieved a similar biomass growth (OD_{600}): 4.4 (F02) and 4.6 (F03). In terms of AA titer, both fermentations also yielded similar results: 21.4 (F02) and 22.3 (F03) g/L. Even though the biomass concentration slightly decayed in F02, it is interesting to observe that *M. thermoacetica* was able to produce a considerable amount of AA, comparable to that of F03. The overall AA productivity amounts to 0.21 (F02) and 0.26 (F03) g/L.h, in line with what was observed in the benchmark. This also occurs when looking at the max. AA productivity achieved: 0.45 (F02) and 0.49 (F03) g/L.h. Surprisingly, the second test on ultra-cleaned syngas even yielded a higher max. AA productivity compared to the benchmark. All in all, it seems like *M. thermoacetica* is able to perform similarly on synthetic gases and ultra-cleaned syngas.

In the search for ways of reducing the OPEX and CAPEX costs of an industrial scale plant of the BioSFerA concept, some purification steps of the cleaning unit were eliminated to obtain a cheaper (but more crude) syngas. Subsequently, the tolerance of the microorganism to the more crude syngas was tested. In this way, **two consecutive** tests were performed on a lower quality syngas, namely **acid-scrubbed syngas**. Compared to the ultra-cleaned, the acid-scrubbed syngas contains a higher amount of **H_2S**, **HCN**, **COS**, and **benzene** (Tables 6 and

8). The OD_{600}, NH_4^+ and AA concentrations of the two runs on acid-scrubbed syngas are presented in Figures 11 and 12.

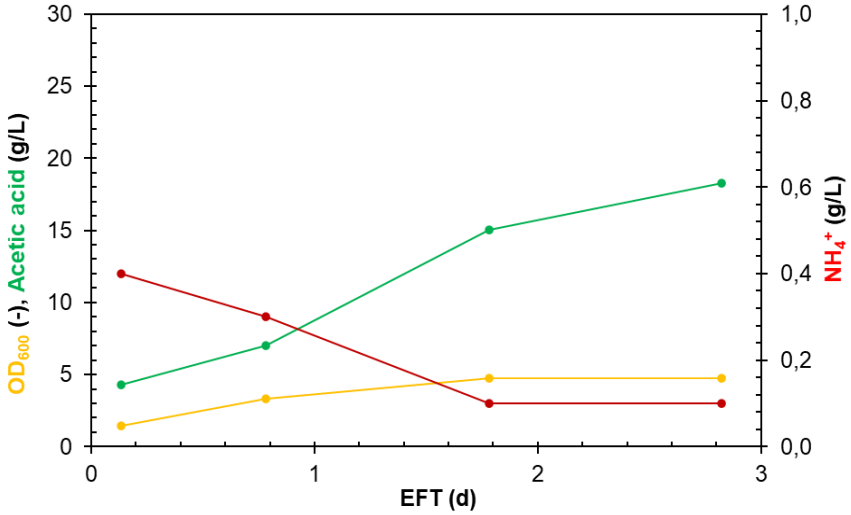

Figure 11. Offline data BioSFerA-T4.3-F04 run.

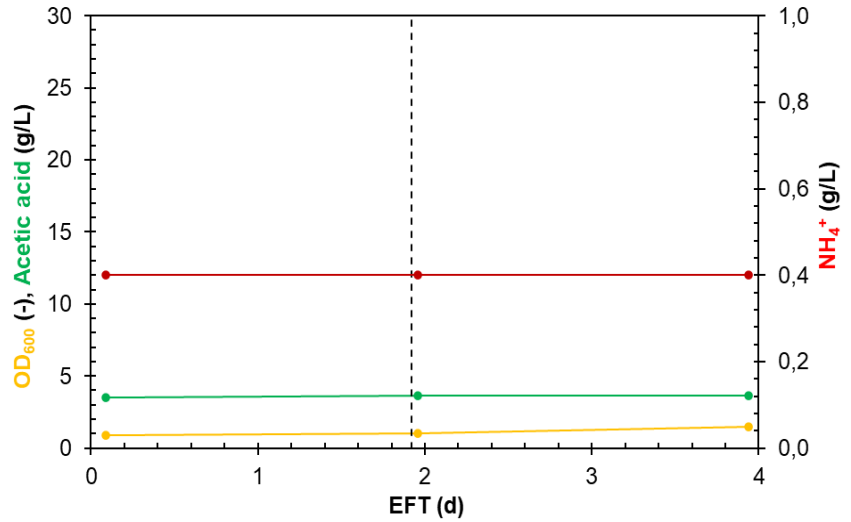

Figure 12. Offline data BioSFerA-T4.3-F05 run. The dashed (- - -) line indicates the switch from acid-scrubbed syngas to synthetic gases.

The first test on acid-scrubbed syngas yielded comparable results to the benchmark and the fermentations on ultra-cleaned syngas: an OD_{600} of 4.8, an AA titer of 18.3 g/L, an overall AA productivity of 0.22 g/L.h, and a max. AA productivity of 0.45 g/L.h were achieved. Nonetheless, when performing a consecutive test on acid-scrubbed syngas, *M. thermoacetica* was not able to grow nor produce AA. It seems in this case that the higher amount of toxic contaminants (mainly H_2S and HCN) and its accumulation in the fermentation broth impair the performance of the microorganism. These results match one of the conclusions of deliverable D3.2: *'Considering these experiments, we can conclude that the concentration of H_2S is the most critical contaminant factor for bacterial growth"*. Because *M. thermoacetica* was not able to grow nor produce AA for almost 2 d in the second test with acid-scrubbed syngas, it was decided to switch to synthetic gases as feedstock to evaluate the impact of the inhibition. Almost 2 d after the switch, *M. thermoacetica* was able to slowly restart growth and AA production,

meaning that the inhibition exerted by the acid-scrubbed syngas was strong, but not lethal. Due to time constraints, the fermentation had to be stopped after 4 days.

To tackle the inhibition by the contaminants but with the goal of producing a cheaper, more crude syngas to reduce both CAPEX and OPEX, the syngas cleaning process was again modified to obtain a different type of syngas, namely **alkaline-scrubbed syngas**. Compared to the acid-scrubbed, the alkaline-scrubbed syngas contains a higher amount of **NH_3**, but a lower amount of **H_2S** and **HCN** (Tables 6 and 8). The OD_{600}, NH_4^+ and AA concentrations of the two **consecutive** runs on alkaline-scrubbed syngas are presented in Figures 13 and 14.

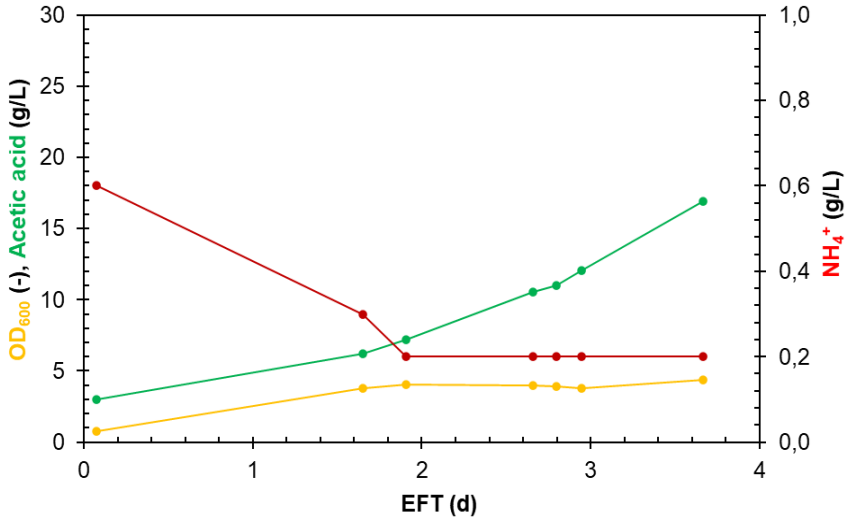

Figure 13. Offline data BioSFerA-T4.3-F06 run.

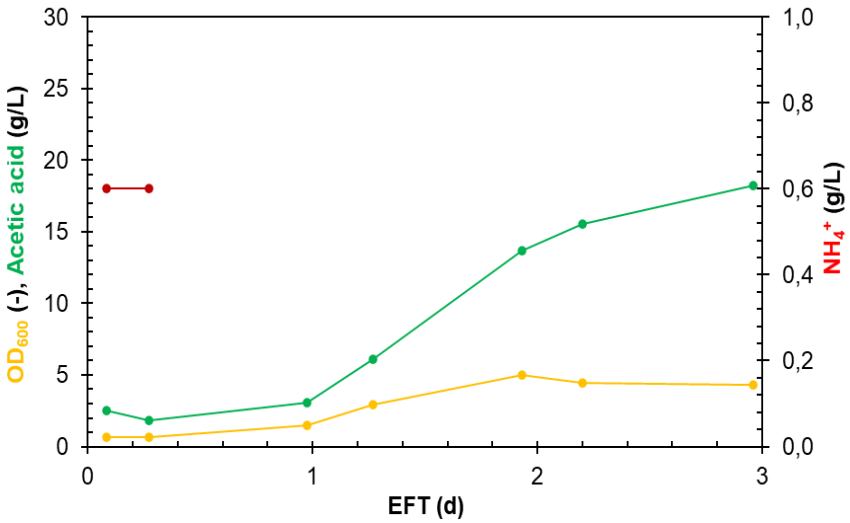

Figure 14. Offline data BioSFerA-T4.3-F07 run.

Opposite to what was observed when performing consecutive tests on acid-scrubbed syngas, the alkaline-scrubbed syngas did not impair the performance of *M. thermoacetica* in the second consecutive fermentation. This means that the configuration of the syngas cleaning unit to produce alkaline-scrubbed syngas (configuration III) is more favorable for the microorganism compared to the one that yields acid-scrubbed syngas (configuration II). Analyzing the results in detail, both runs yielded similar biomass growth (OD_{600}): 4.40 (F06) and 4.97 (F07), although a slight decay of the biomass concentration was again observed in the second test. Interestingly, the first run on alkaline-scrubbed syngas yielded a low AA titer of 16.7 g/L; during this process, a positive change was observed indirectly

in the acetate production rate (directly in the base addition trend; data not shown). Proof of this change is the noticeable difference in the max. AA productivity: 0.16 g/L.h before, and 0.33 g/L.h after the change. This could be derived from the fact that *M. thermoacetica* needs some time to adapt to the contaminants levels of alkaline-scrubbed syngas.

In the same trend, the second test on alkaline-scrubbed syngas yielded even better results. The acetate titer was 18.3 g/L, and the overall AA productivity of the process was 0.26 g/L.h. Furthermore, the max. AA productivity achieved in F07 amounts to 0.52 g/L.h, which goes beyond the BioSFerA target of 0.5 g/L.h. At first sight, it seems the alkaline-scrubbed syngas yields results comparable to those set by the benchmark in the fermentation conditions tested so far, and therefore the syngas cleaning process could be simplified and the CAPEX and OPEX reduced.

4.2.3 Broadening the range of feedstocks for gasification-syngas fermentation processes

Once the suitable setup for the syngas cleaning unit was found, it was decided to test the performance of *M. thermoacetica* on alkaline-scrubbed syngas derived from more complex feedstocks, such as straw. As stated in BioSFerA public deliverable D3.1, straw has a higher sulfur and nitrogen content when compared to bark, and therefore, it can lead towards a higher number of impurities and consequently, a major impairment of the microorganism's performance. The OD_{600}, NH_4^+ and AA concentrations of the run on alkaline-scrubbed syngas derived from gasified straw are presented in Figure 15.

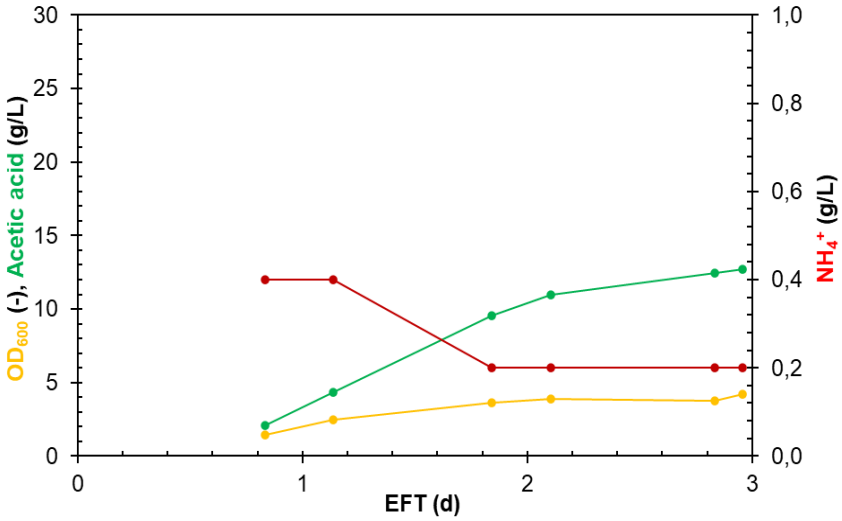

Figure 15. Offline data BioSFerA-T4.3-F08 run.

After almost 3 d (71 h) of fermentation, an OD_{600} of 4.2 was reached. Meanwhile, the AA titer was the lowest obtained so far, 12.7 g/L, with an overall AA productivity of 0.21 g/L.h. It has to be noted that in the beginning of this fermentation there was a power blackout lasting for 4 h, which caused the temperature inside the reactor to drop to 37 °C and *M. thermoacetica* not being fed syngas for that amount of time. However, the bacterium was able to recover, grow, and produce AA. The max. AA productivity achieved in this test was 0.32 g/L.h, also the lowest one obtained so far if the second fermentation with acid-scrubbed syngas is not taken into account. Due to the lower performance of *M. thermoacetica* on the alkaline-scrubbed syngas derived from gasified straw, it was decided to carry out the process intensification tests on syngas obtained from a cleaner feedstock, namely bark.

4.2.4 Process optimization on real biomass-derived syngas

4.2.4.1 Parameter intensification

Based on the goals on WP4 related to the syngas fermentation process, some tests were performed at higher gas transfer rates aiming to achieve the target AA titer (30 g/L) and productivity (0.5 g/L.h) in order to reduce the CAPEX and OPEX of the integrated full-scale plant of BioSFerA concept. The gas transfer rate was increased by performing the fermentations at a greater agitation speed, which allows a better mixing and solubilization of the gaseous substrates in the fermentation broth, and avoids potential substrate limitations. In this case, a similar strategy was followed compared to the tests on different-quality syngas: first, a benchmark run was carried out on synthetic gases at increased agitation speed, and afterwards, the same increased agitation speed was applied in tests with ultra-cleaned and alkaline-scrubbed syngas. Due to the inhibition observed in the second test with acid-scrubbed syngas at lower gas transfer rates, this syngas was not included in the process intensification. The OD_{600}, NH_4^+ and AA concentrations of the run at increased agitation speed on synthetic gases are presented in Figure 16.

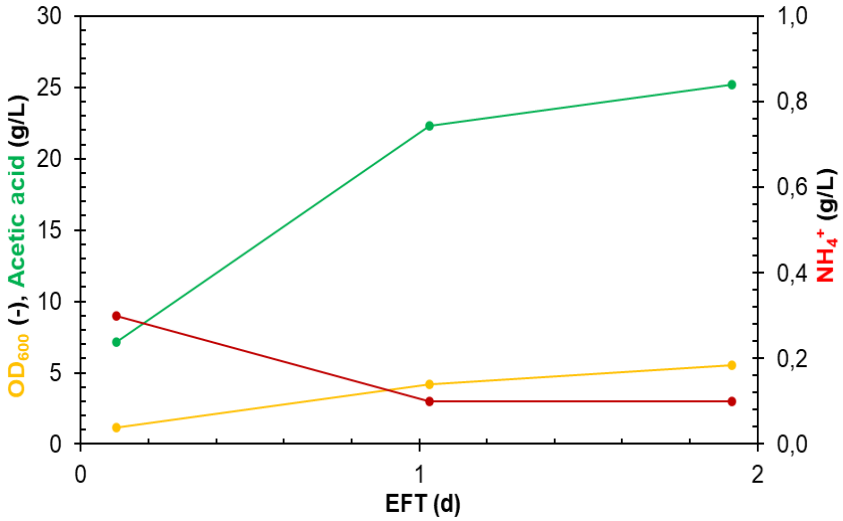

Figure 16. Offline data BioSFerA-T4.3-F09 run.

As it can be seen from the graph, the first noticeable effect of increasing the agitation speed is a great reduction in the effective fermentation time (EFT), which also brings along an increased overall AA productivity. After almost 2 d (46.1 h) of fermentation, an OD_{600} of 5.54 (equivalent to 1.6 g/L CDW) was reached, higher than all the previous tests. At the same time, an AA titer of 25.2 g/L was achieved, with an overall AA productivity of 0.41 g/L.h. Interestingly, *M. thermoacetica* was able to produce AA at a max. productivity of 0.64 g/L.h during this fermentation. This means the max. AA productivity was increased 40% compared to the benchmark at a lower agitation speed, showcasing the potential of this technology when fully optimized at high gas transfer rates.

After setting the benchmark on synthetic gases with increased agitation speed, the process optimization with biomass-derived syngas followed. First, a run on ultra-cleaned syngas with increased agitation speed was performed. The OD_{600}, NH_4^+ and AA concentrations of the run at increased agitation speed on ultra-cleaned syngas are presented in Figure 17.

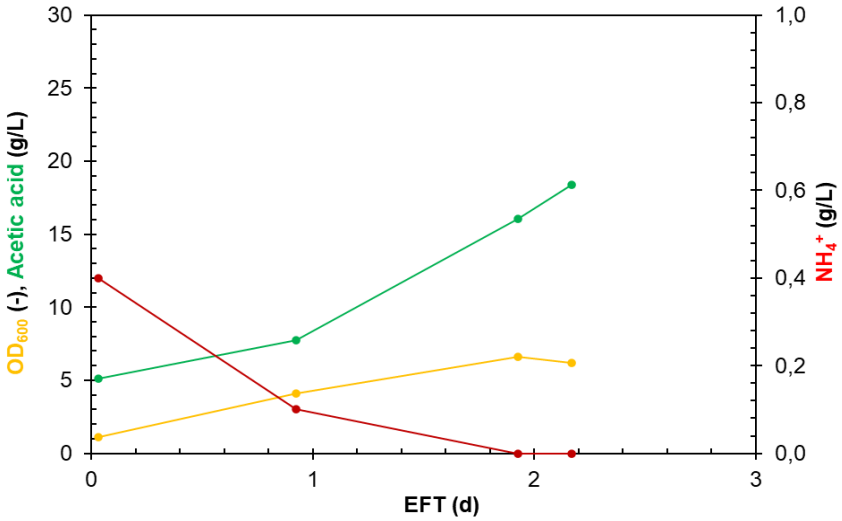

Figure 17. Offline data BioSFerA-T4.3-F10 run.

As observed in the run F09, this test was also shorter compared to the previous fermentations. However, taking a closer look at the results, it seems that increasing the agitation speed does not have the same positive effect on the outcome of the fermentation when working with ultra-cleaned syngas. When increasing the agitation speed, the gas transfer rate is enhanced, meaning that more molecules of gases (CO, CO_2, and H_2) will be solubilized and available for the microorganism. However, the increased agitation speed also increments the solubilization of the contaminants, which can have a negative effect on the fermentation. In this sense, after 2.2 d (52.8 h) of fermentation in F10, an OD_{600} of 6.6 was reached (although there was a slight decay in biomass concentration towards the end of the run). An AA titer of 18.3 g/L was obtained, with an overall AA productivity of 0.26 g/L.h. However, the max. AA productivity achieved during this fermentation was 0.40 g/L.h. Comparing these results to the ones obtained in runs F02 and F03 (ultra-cleaned syngas at lower agitation speed), there was a higher biomass production, but both the AA titer and max. productivity were lower. It seems the process optimization with increased agitation speed on ultra-cleaned syngas did not have the expected positive results as observed when doing such an optimization on synthetic gases. As stated, this could be attributed to a higher solubilization of small amount of impurities present in the ultra-cleaned syngas.

The OD_{600}, NH_4^+ and AA concentrations of the run at increased agitation speed on alkaline-scrubbed syngas are presented in Figure 18. The results obtained on this syngas with increased agitation speed followed the same trend as the results just reported above.

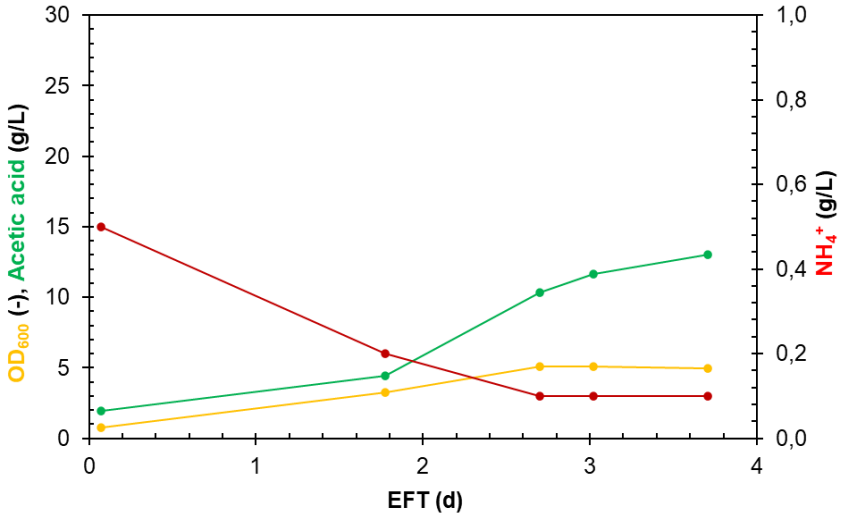

Figure 18. Offline data BioSFerA-T4.3-F11 run.

Interestingly, F11 lasted almost 4 d (89 h), a big difference compared to the previous tests with increased agitation speed. This is already indicative of the negative effect of a higher gas transfer rate on the outcome of the fermentation when working with a more crude syngas. After 89 h, an OD_{600} of 5.1 was reached, but the AA produced only amounted to 13.0 g/L, similar to the titer obtained in F08 (alkaline-scrubbed syngas from gasified straw). The max. AA productivity in F11 was 0.31 g/L.h, also in line with F08 results. It seems alkaline-scrubbed syngas derived from gasified bark and alkaline-scrubbed syngas from gasified straw (more contaminants) yield similar results in terms of AA production when the agitation speed is increased with the first of the two syngas. Even though the syngas from bark contains lower amount of contaminants, the increase in the agitation speed increments their solubility, hampering the performance of *M. thermoacetica* at a similar level as the second of the two syngas.

4.2.4.2 Mode of operation: Continuous with cell recycling

Building on the work performed within BioSFerA task 3.3, a cell recycle system was installed in the BBMPP to perform continuous syngas fermentation, with constant withdrawal of a rich AA stream from the reactor while concentrating the biomass. This continuous mode of operation with the cell recycle set-up could avoid the inhibition of AA on the microorganism and boost the AA productivity, due to the higher amount of cells in the vessel. More importantly, the reactor could be ran for several days or weeks.

Due to time constraints and technical problems, only one continuous run could be performed with this set-up. The latter was identical to the set-up utilized in task 3.3 and presented in deliverable 3.4, consisting of an external ceramic membrane to retain and recirculate the cells back to the reactor, and three high-pressure resistant pumps: 1) feeding of fresh medium, 2) pumping of filtrate into the filtrate vessel, and 3) recirculation of fermentation broth over the external loop (Figure 19). The continuous run (BioSFerA-T4.3-F12) was started on synthetic gases and batch mode; after 1 d, the recirculation over the cell recycle system was initiated, as well as the continuous mode. The aim was to hold the feeding and filtrate rates at similar levels to keep the fermentation volume inside the reactor constant. After 1 d of recirculation, the gas feed was changed from synthetic gases to alkaline-scrubbed syngas to evaluate the behavior of *M. thermoacetica* on real biomass-derived syngas with this set-up. Finally, 1 d later, the agitation speed was increased to see whether this parameter had the same inhibitory effect in continuous mode as it was previously seen on batch mode. The OD_{600}, NH_4^+ and AA concentrations of the continuous run with cell recycle are presented in Figure 20.

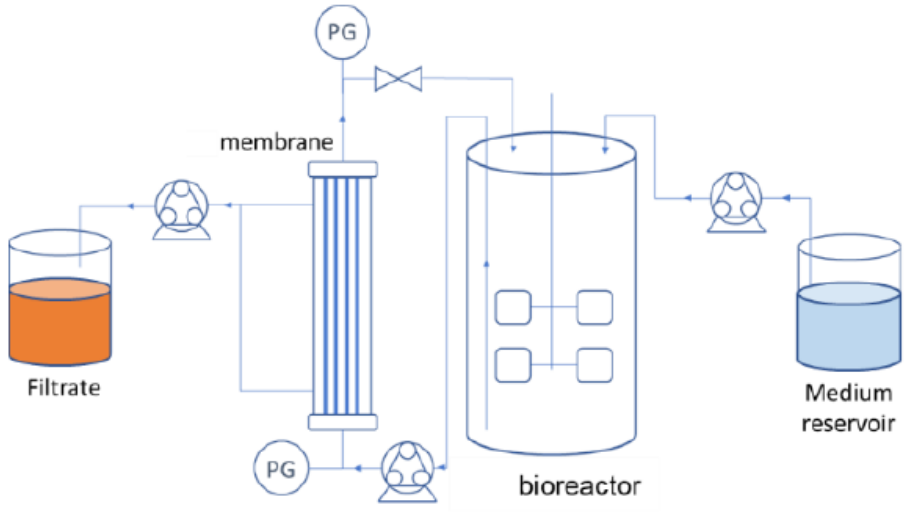

Figure 19. Scheme of a cell recycle set-up with an external ceramic membrane for a continuous syngas fermentation process. PG: Pressure gauge.

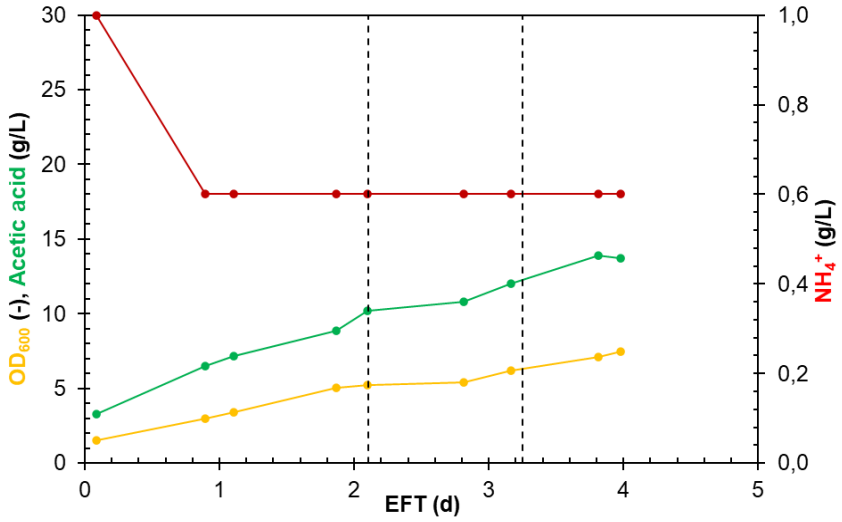

Figure 20. Offline data BioSFerA-T4.3-F12 run. The first dashed (- - -) line indicates the switch from synthetic gases to alkaline scrubbed syngas; the second dashed (- - -) line indicates an increase in the agitation speed.

As mentioned before, some technical issues (clogging of the ceramic membrane) only allowed for one, short continuous syngas fermentation. The continuous mode was started on synthetic gases at t = 1.1 d, allowing the OD_{600} to increase from 3.4 to 5.2. The AA titer and productivity were kept around 10 g/L and 0.41 g/L.h, respectively, in this stage. At t = 2.1 d, the gas feed was switched to alkaline-scrubbed syngas, and *M. thermoacetica* was able to keep growing and producing AA at a decent rate. At the end of this stage, the OD_{600} was increased to 6.2, whereas the AA titer and productivity were kept around 12 g/L and 0.32 g/L.h, respectively. Finally, an increment of the agitation speed was performed at t = 3.2 d, while still running the continuous process on alkaline-scrubbed syngas. The bacterium was able to keep growing, as it reached an OD_{600} of 7.5 by the end of the fermentation, but the AA productivity was negatively affected, dropping to 0.09 g/L.h. Both the greater agitation speed while working on alkaline-scrubbed syngas and the AA titer (around 14 g/L) could be the cause of this reduced AA productivity in the last stage compared to the previous two.

To circumvent the technical issues experienced with the cell recycle system, different strategies could be applied to avoid the clogging of the membrane and unlock the true potential of continuous operation mode with cell recycling. One option would be to try different kind of pumps that allow the obtention of a higher cross-flow velocity, which would yield a better performance of the filtration process and reduce or completely avoid the clogging of the membrane. Another strategy could involve the use of other type of membranes rather than ceramic membranes. Hollow-fiber or spiral wound membranes could be tested in the search for reducing the number and frequency of clogging issues.

5 Conclusions and future prospects

The two pilot plants, namely VTT's gasification and gas cleaning plant and BBEPP's mobile gas fermentation plant (BBMPP), were successfully coupled within the scope of BioSFerA WP4 (tasks 4.1 and 4.2), and fermentation runs were also successfully performed on real syngas derived from the gasification of bark and straw, bringing the technology to TRL5 (task 4.3). The main conclusions resulting from the syngas fermentation tests as well as suggestions for future developments are listed below.

Biomass gasification and cleaning section

Syngas was generated using a BFB gasifier with two distinct biomass feedstocks, namely bark and straw. The syngas underwent purification in a dedicated gas cleaning process, where modifications to the gas process configuration were implemented to produce syngas with diverse impurity profiles and concentrations, tailored for use in fermentation tests.

The initial tests aimed at generating ultra-cleaned syngas proved successful, with key impurities such as H_2S, COS, and HCN not being detected in the cleaned syngas. This was achieved through a multi-step process involving both aqueous scrubbing and adsorption/catalysts. Subsequent test campaigns deliberately introduced more impurities into the syngas. This was achieved either by reducing the number of gas cleaning steps, retaining only the aqueous scrubbing process, or by utilizing a higher impurity content gasification feedstock, namely straw.

Using only acidic water scrubbing, syngas with HCN and H_2S and COS but low NH_3 content could be generated (acid-scrubbed syngas). Conversely, employing caustic water scrubbing allowed the production of syngas with low H_2S and HCN but elevated COS and NH_3 content (alkaline-scrubbed syngas).

Conclusions from syngas fermentation tests with real biomass-derived syngas

- The parameter settings resulted from BioSFerA task 3.3, concerning the lab scale optimization of the syngas fermentation, could be applied at a higher scale, smoothening the start-up of the TRL5 tests.
- *M. thermoacetica* has shown strong resilience towards biomass-derived syngas contaminants, being able to grow and produce AA similarly on synthetic gases, ultra-cleaned, and alkaline-scrubbed syngas derived from gasified bark in low gas transfer rate conditions.
- The syngas cleaning unit set-up to obtain acid-scrubbed syngas (with higher concentrations of acidic contaminants compared to ultra-cleaned or alkaline-scrubbed syngas) does not seem to be an effective cleaning strategy to perform syngas fermentation, leading to microbial inhibition on consecutive tests.
- Higher impurity gasification feedstocks would potentially require a more targeted cleaning strategy, since alkaline-scrubbed syngas derived from gasified straw causes a considerable reduction of *M. thermoacetica* performance in low gas transfer rate conditions, compared to alkaline-scrubbed syngas derived from gasified bark.
- Process intensification through the increment of agitation speed does not seem a good strategy to boost the AA titer and productivity of the process, since a greater agitation speed could also potentially lead to a higher solubility of contaminants, which hampers the outcome of the fermentation.
- When optimized, a cell recycle system to perform continuous syngas fermentation is a set-up with great potential to achieve higher biomass concentrations, and therefore, higher AA titer and productivities, and to operate the fermentation continuously for several days or weeks.

- Overall, the target AA productivity of 0.50 g/L.h was achieved both on synthetic gases (BioSFerA-T4.3-F09: 0.64 g/L.h) and alkaline-scrubbed syngas (BioSFerA-T4.3-F07: 0.52 g/L.h), and it was nearly achieved on ultra-cleaned syngas (BioSFerA-T4.3-F03: 0.49 g/L.h). In terms of AA titer, the target of 30 g/L was not reached in any of the fermentations due to the application of the harvest & refill strategy.

Future prospects for biomass-derived syngas fermentation

- Though promising results were obtained using real biomass-derived syngas (ultra-cleaned and (alkaline/acid)-scrubbed syngas), more experiments are needed to unravel whether intermediate-quality syngas could be used to strike the balance between economics and performance of the process.
- Feeding ultra-cleaned and alkaline-scrubbed syngas resulted in similar outcomes as the one obtained on synthetic gases in low gas transfer conditions, but not when this transfer rate was increased through an increment of the agitation speed. In this regard:
    a. Strain development at lab scale is needed to obtain microorganisms capable of tolerating biomass-derived syngas contaminants. This could be done through adaptive laboratory evolution (ALE) experiments or via a metabolic engineering approach.
    b. Process optimization involving other parameters rather than agitation speed, such as syngas flow rate, pressure, or temperature, could lead to the obtention of better results.
- Solving the technical issues regarding the cell recycle set-up could yield a breakthrough in the syngas fermentation process with *M. thermoacetica*, allowing for higher biomass concentrations, but also greater AA titers and productivities. Additionally, running the syngas fermentation process in a continuous mode could also reduce the impact of the contaminants in the fermentation broth, as they would be constantly diluted by the fresh feed and washed-out through the membrane.

Next steps in other parts of BioSFerA project from this work:

- The results derived from these TRL5 runs have been or will be used as input for other BioSFerA WPs, such as WP6: Scaling-up the BioSFerA concept, and WP7: Sustainability and Health & Safety issues. More specifically, the operational aspects of the fermentation and the syngas cleaning requirements have been shared.

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