

Continuous Hydrogen Production from Syngas via the Biological Water-Gas Shift Reaction in a Trickle Bed Reactor: Effect of Nutrient Management, Inhibitors, and Hydraulic Retention Time

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Abstract

Background

Continuous hydrogen (H₂) production from synthesis gas (syngas), generated from thermochemical processes (e.g. gasification and pyrolysis) or industrial off-gas emissions (e.g. steel mills), remains underexplored despite the potential of the biological water-gas shift (BWGS) reaction. This study investigates H₂ production from CO using a natural microbial consortium derived from food waste in a trickle-bed reactor (TBR) with polyethylene sponge packing.

Results

The experiment was conducted for more than 100 days across three phases: (i) startup with concentrated medium addition, (ii) inhibitor testing with volatile fatty acids (VFAs) and ethanol, and (iii) operation under hydraulic retention time (HRT) control. During phase I, repeated concentrated medium additions coincided with elevated measured trace-metal concentrations (Cu ~ 35,000×, Fe ~ 26×, Mn ~ 107× above medium-formulated levels), correlating with increased VFA production (up to 12g/L) and reduced CO-to-H₂ conversion. Phase II demonstrated that VFA addition was associated with a greater decline in CO conversion than ethanol addition, with a peak VFA concentration of 21.2 g/L, and the consortium showed limited recovery after prolonged inhibitor exposure. In phase III, HRT control of 4 days sustained 100% CO conversion with an H₂ yield of 0.66 ± 0.04 mol/mol CO and an H₂ productivity of 3.2 ± 0.2 L/L/d at 12 mL/min of gas flow. No CH₄ was detected throughout the experiment, consistent with suppressed methanogenesis during BES-treated operation. A gas flow ramp test identified 26 mL/min as the highest flow meeting the outlet-CO criterion during the short-term ramp test. 16S rRNA sequencing revealed a community shift from Bacillota/Coprothermobacterota dominance in phases I and II to Pseudomonadota dominance in phase III, with low-abundance taxa such as *Neomoorella* and *Clostridium* representing possible, unconfirmed contributors to BWGS activity, suggesting functional specialisation in the system.

Conclusions

This study demonstrates continuous H₂ production *via* the BWGS reaction using a waste-derived microbial consortium in a TBR. The findings highlight the importance of controlling nutrient addition and VFA accumulation to maintain stable H₂ production. Although trace-metal accumulation and VFA buildup coincided with declining H₂ production, the underlying metabolic shifts require further investigation. The observed changes in microbial community composition across operational phases further suggest that reactor operation conditions are important in determining the community during continuous BWGS in syngas fermentation.

Highlights

- Continuous H production from syngas was achieved in a trickle-bed reactor.

- A waste-derived microbial consortium sustained 100% CO conversion for 12 days.
- At 4-day HRT, H₂ yield was 0.66 mol/mol CO with 3.2 L/L/d productivity.
- VFA accumulation was associated with declining CO conversion and H₂ production.
- Full CO conversion was maintained at gas flow rates up to 26 mL/min.

1. Background

Hydrogen (H₂) is a versatile energy carrier and chemical feedstock with broad applications in the chemical, material, and food industries [1]. The demand for H₂ in Europe alone is projected to reach up to 1785 terawatt-hours (TWh) by 2050 to support the industrial transition to climate neutrality [2]. However, nearly all the current H₂ production relies on fossil fuels (e.g. coal, oil, natural gas), undermining its sustainability [3]. To achieve climate neutrality, H₂ production must increasingly rely on low-carbon energy and renewable resources. The most established low-carbon route is water electrolysis powered by renewable electricity, such as solar and wind energy [1]. Alternatively, H₂ can be biologically produced from renewable sources, including biomass, water, and organic matter, through a process collectively referred to as biohydrogen production [4–6]. There are different biological routes to produce H₂, such as photofermentation, biophotolysis, microbial electrolysis cells, and dark fermentation [5], with photofermentation and dark fermentation achieving a Technology Readiness Level (TRL) up to 5 [6]. A comparatively underexplored route is synthesis gas (syngas) fermentation via the biological water-gas shift (BWGS) reaction, in which carboxydophilic microorganisms convert carbon monoxide (CO) into H₂ [7]. While syngas fermentation has been commercialised for ethanol production, most notably by LanzaTech, converting steel mill off-gas up to 125,000 t/year capacity to ethanol [8], H₂ as the target product remains considerably less developed, particularly at industrial scale. From a sustainability perspective, syngas fermentation offers potential advantages as a flexible platform for biofuels and chemicals production, using waste-derived feedstocks that would otherwise contribute to emissions, with a possible integration with existing gasification infrastructure [9].

The syngas mixture is mainly composed of H₂, CO, carbon dioxide (CO₂), and methane (CH₄) generated from diverse feedstocks through thermochemical processes (e.g. gasification) [10] or from heavy industry emissions, such as steel mills' off-gases [11]. Several microorganisms are known to catalyse the BWGS reaction, including *Carboxydothermus hydrogenoformans*, *Thermosinus carboxydivorans*, and *Citrobacter* sp Y19 [12]. To date, most of the syngas fermentation studies have focused on pure cultures. However, open mixed cultures, such as natural microbial consortia, represent a viable alternative, showing advantages such as microbial resilience, higher adaptability to environmental changes, and the non-requirement for sterile conditions, making them attractive for industrial implementation [13].

While batch systems can achieve high CO conversion efficiencies [14, 15], continuous operation is essential for industrial viability, offering improved space-time yields, consistent product quality, and

reduced downtime [16]. In syngas fermentation, process efficiency is generally limited by gas-liquid mass transfer, as CO and H₂ have very low solubility [17]. Continuous stirred tank reactors (CSTRs), the reactor type dominant in the literature for syngas fermentation, require energy-intensive mechanical stirring to achieve adequate mass transfer [18], whereas bubble column reactors (BCRs), which are cost-effective, suffer from limited mixing [19]. Trickle-bed reactors (TBRs) address these bottlenecks by promoting thin-film gas-liquid contact with packing material, which simultaneously enhances mass transfer and provides a stable surface for microbial growth, supporting high cell concentration [20].

Syngas fermentation for liquid products, such as ethanol and acetate, has progressed to high TRLs and commercial scales using pure cultures, as previously reported [8, 9]. Reactor configurations for syngas fermentation have also been extensively investigated, with TBRs demonstrating potential for continuous gas conversion [18, 20]. For mixed microbial consortia, a TRL 4–5 has been demonstrated for CO conversion into CH₄ in a TBR, highlighting the potential for scale-up of continuous syngas biomethanation [21]. In contrast, continuous H₂ production from CO-rich syngas using mixed cultures in TBRs remains comparatively understudied [8, 13]. Important challenges associated with continuous operation include the management of nutrient accumulation in the culture medium [15], the potential inhibitory effects of fermentation products on syngas fermentation, as demonstrated for acetate in mixed-culture systems [22], and the identification of operational conditions that sustain continuous H₂ production, which remains to be established.

This study addresses these gaps by presenting, to our knowledge, the first demonstration of continuous H₂ production via BWGS reaction using a natural microbial consortium derived from food waste sludge in a TBR. The reactor was operated across three sequential phases designed to test whether process instability was driven by nutrient accumulation, metabolite inhibition, or insufficient liquid-phase renewal. We hypothesised that (i) concentrated medium addition would progressively disturb the metabolic balance of the consortium through trace-metal accumulation and VFA formation, (ii) VFAs would exert stronger inhibition than ethanol on CO conversion, and (iii) hydraulic retention time (HRT)-controlled operation would restore process stability by continuously removing inhibitory metabolites. In addition, microbial community dynamics were characterised across phases to link operational conditions with shifts in community composition and process performance.

2. Materials and Methods

2.1. Inoculum and nutrient media

2.1.1. Inoculum

The natural microbial consortium used in this study was collected from a 3000-m³ biogas reactor (Sobacken, Borås, Sweden), which operates at a temperature of 58 °C, with a digestion mixture of food and industrial biodegradable waste. The natural microbial consortium was incubated at the original

temperature before a centrifugation step at 10,155 g for 5 minutes to concentrate the solid content. In phase III, a washing pre-treatment step was performed to reduce the presence of micro- and macronutrients in the natural microbial consortium, as previously applied in a previous study[15]. The washing pre-treatment was composed of a centrifugation step at 10,115 g force for 5 min to recover the precipitate, a washing step of the precipitate with ultrapure water in a 250 µm sieve until the conductivity of the wastewater was lower than 0.1 mS/cm, and lastly, another centrifugation step at 10,115 g force to recover the precipitate. The total suspended solids (TSS) and volatile suspended solids (VSS) content of the inoculum for phases I and II were: $11.42 \pm 0.12\%$ and $7.89 \pm 0.14\%$, and for phase III were: $10.62 \pm 0.34\%$ and $8.16 \pm 0.08\%$, respectively. The reactor was inoculated with a VSS concentration of approximately 20 g/L for all phases.

2.1.2. Nutrient media

For this experiment, the medium DSMZ 507: *Carboxydotherrus* medium, formulated by DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Germany), was selected. The medium was modified with the formulation composed of (g/L): KCl (0.33), $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (0.52), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.29), NH_4Cl (0.33), KH_2PO_4 (0.33), NaHCO_3 (1.0) as the basal nutrients, an addition of 1 ml/L of a trace element solution composed (g/L) of $\text{Na}_2\text{-EDTA} \cdot 2\text{H}_2\text{O}$ (5.2), $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (1.5), ZnCl_2 (0.07), $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (0.1), H_3BO_3 (0.006), $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (0.19), $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (0.002), $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (0.024), $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (0.036), 1 ml/L of a vitamins solution composed (mg/L) of biotin (20), folic acid (20), pyridoxine hydrochloride (100), thiamine HCl (50), riboflavin (50), nicotinic acid (50), calcium D-(+)-pantothenate (50), vitamin B₁₂ (1), p-aminobenzoic acid (50), (DL)-alpha-lipoic acid (50), and an addition of 20 mL/L of a neutralized sulphide solution 3% (w/v). In addition to the modified medium, 2-bromoethanesulfonate (BES) was added at a 0.5 g/L concentration at the beginning of phase I and phase III to inhibit methanogens when new inoculum was added.

2.2. Trickle bed reactor (TBR) configuration and operation

The TBR used was designed and custom-built by RISE (Research Institutes of Sweden, Sweden) specifically for this study, with a configuration illustrated in Fig. 1. The reactor has a total volume of 3.5 L, with 1.2 L used as a nutrient medium container and 2.3 L corresponding to the gas bed where the packing material is settled. The gas bed was filled with polyethene sponge as packing material, cut into 2 cm squares. The selection of polyethene sponge as a packing material was based on its higher surface area, which improves gas distribution and mass transfer and, consequently, microbial attachment and conversion efficiency [23]. The nutrient medium was recirculated once per hour for one minute at a pump speed of 400 mL/min, yielding a total recirculation flow of 9.6 L/d ($400 \text{ mL/min} \times 1 \text{ min} \times 24 \text{ cycles/day}$). Liquid was distributed over the packing material via a perforated plate at the top of the gas bed. For phase HRT was defined based on the 1.2 L liquid reservoir volume and controlled by two peristaltic pumps (Watson Marlow, UK) programmed with a programmable logic controller (PLC) (Schneider Electric SE, France) in the same way as the medium recirculation pump.

In this study, to maximise the driving force for mass transfer and for the ease of product separation, a counter-current operation was selected, where the medium was recirculated from the top downwards, and the gas from the bottom upwards. The synthetic syngas was composed of (%) CO: CO₂: N₂ (65: 15: 20), and the gas flow was controlled by a mass flow controller (Bronkhorst EL-FLOW, The Netherlands). This composition was selected to represent a typical CO-rich syngas steel mill emission from a blast oxygen furnace gas, where high CO concentrations are commonly achieved [24]. After the gas passes through the gas bed, the gas flows through a water trap and then to a gas flow meter (μ Flow unit, BPC Instruments, Sweden). A pH controller system (B. Braun Biotech International, Germany) was connected to the reactor, consisting of a pH probe (Hamilton Germany GmbH, Germany), a main controller system, and a set of peristaltic pumps to maintain approximately pH 6.0 with the addition of acid (1.0 M HCl) and base (1.0 M NaOH) solutions, which were pumped into the nutrient medium container as needed. The temperature of this study was set to 55°C and maintained by a water bath (Grant Instruments, England) through the reactor jacket. More detailed instrumentation information is provided in **Table S1**.

2.3. Experimental Phases

Phase I: Startup operation with a 50 mL medium removal and a 50 mL concentrated medium addition every seven days, with a total duration of 44 days.

Phase II: Inhibitor testing and recovery period. This phase starts after a medium replacement, after phase I. After the recovery period from phase I, phase II systematically tested the effect of external inhibitors, which can also be metabolite products from the process. Synthetic VFA was one of the external inhibitors, composed of (g/L): acetic acid (12.8); propionic acid (0.30); isobutyric acid (0.20); butyric acid (1.0); and isovaleric acid (0.20), with a total VFA concentration of 14.5 g/L representing the highest VFA accumulation achieved before starting phase II. Then, a combination of the previous VFA concentration with ethanol at 1.8 g/L was applied, representing the ethanol concentration observed in phase I. A recovery phase was also evaluated, with the total duration of phase II representing 52 days.

Phase III: operation with HRT controls (2 and 4 days), a gas flow ramp protocol from 12 to 28 mL/min with an increase every 24 h after $\leq 5\%$ CO concentration was observed. In this phase, the whole reactor was set up again, changing the inoculum and the packing material, to not have any influence from the tests performed during phase II. The total duration of phase III was 36 days.

2.4. Analytical methods

The gaseous composition of the outflow and the liquid samples for VFA analysis were evaluated by a gas chromatograph (GC) (Perkin-Elmer, Clarus 500 and 590, USA) following the methods previously described [14]. The liquid samples for ethanol were analysed by high-performance liquid chromatography (HPLC) (Waters Corporation, USA) equipped with a hydrogen-based column (Animex, HPX87-H; Bio-RAD, USA) and Ultraviolet (UV-Vis) and RI detectors at 240 nm (Waters Corporation, USA) operated at 60°C with an eluent (0.5 mM H₂SO₄) flow rate of 0.6 mL/min. To evaluate if the concentrated medium addition caused a nutrient accumulation, the process wastewater was analysed in triplicate,

using a previously described method for elemental analysis [25]. TSS and VSS were determined according to standard methods for the examination of water and wastewater [26].

2.5. Data analysis

Gas concentrations were measured on a dry bases, as a water trap was installed after the outlet stream and prior to the μ Flow unit. Gas volumes were normalised to standard conditions (25°C, 1 atm). Since the inlet gas was synthetic with a fixed, defined composition and controlled flow rate, inlet concentrations were treated as know constants. Outlet gas composition was determined from the volumetric flow rate measured by the μ Flow unit combined with dry-basis GC concentrations. Then, CO conversion (%), H_2 productivity (L H_2 /L /d), H_2 yield (mol H_2 /mol CO), and the empty gas residence time (EGRT) were calculated using the following equations:

$$CO \text{ conversion } (\%) = \frac{(CO_{in} - CO_{out})}{(CO_{in} * 100)}$$

1

$$H_2 \text{ productivity} = \frac{(H_{2out} (L) * 24)}{V_{gasbed} (L)}$$

2

$$H_2 \text{ yield} = \frac{H_{2out}}{(CO_{in} - CO_{out})}$$

3

$$EGRT (h) = \frac{V_{gasbed} (mL)}{(Inlet_{flow} (mL/min) * 60)}$$

4

Performance metrics (CO conversion, H_2 yield, and H_2 productivity) were calculated for each gas measurement point and reported as mean $\pm$ standard deviation of temporal data points within each defined operation period (phase I: n = 153 over 68 days; phase II: n = 74 over 50 days; phase III: n = 55 over 40 days). These values represent process variability over time within a single continuous reactor and are not derived from independent biological replicates, which is standard practice in long-term continuous studies. Phase comparisons are based on observed trends and mean values across periods. Within phase III, a one-way analysis of variance (ANOVA) was applied to compare performance metrics between the two HRT conditions (2-day and 4-day HRT). ANOVA was used to test the significance of the results, with $p < 0.05$ considered statistically significant. All the analyses were made using Minitab® ver. 22.2.2 (Minitab, LLC).

2.6. Microbial analysis

Samples for microbial community analysis were collected from the original natural microbial consortium before starting the process, and after phase II and phase III, and stored at -20°C for subsequent lyophilisation using a freeze-dryer (Labconco, USA) for 72 h. The sealed tubes with the lyophilised samples were sent to MacroGen Europe BV (The Netherlands) for full-service 16S rRNA gene amplicon sequencing and bioinformatics analysis, comprising genomic DNA extraction (Qiagen kit), PCR amplification of the V3-V4 regions (primers 341 F and 805 R), library preparation, pair-end sequencing on an Illumina MiSeq platform, and downstream processing as described below.

Adapter and primer sequences were removed from the raw reads using Cutadapt v3.2 [27], and forward and reverse reads were trimmed to 250 bp and 200 bp, respectively. Amplicon sequence variants (ASVs) were generated using the DADA2 pipeline v1.18.0, performing error-correction, merging, and denoising, in sequences with an expected error of 2 or less, and chimeric sequences were removed using the DADA2 function `removeBimeraDenovo` [28]. ASVs shorter than 350 bp were filtered out using R v4.0.3. Read counts were normalised by subsampling to the lowest read count across all samples using QIIME v1.9.0 [29]. Taxonomy was assigned to each ASV against the NCBI 16S reference database using algorithms such as the Bayesian classifier [30]. Alpha diversity was assessed using the Shannon index, the Gini-Simpson index, phylogenetic diversity, and observed ASVs. Multiple sequence alignment was performed with MAFFT v7.475 [31]. Beta diversity was evaluated based on Bray-Curtis dissimilarity, weighted UniFrac, and unweighted UniFrac distances, and visualised through principal coordinate analysis and UPGMA clustering.

3. Results

Continuous conversion of syngas into H_2 offers a route to upgrade CO-rich gas streams from biomass, waste, or industrial processes into a clean energy carrier, but stable operation remains challenging because of the microbial activity, nutrient supply, metabolite accumulation, and poor gas–liquid mass transfer. In this study, a TBR packed with polyethylene sponge was used to support a food-waste-derived microbial consortium for continuous H_2 production *via* the BWGS reaction. The process was evaluated through three successive operational phases addressing medium supplementation, exposure to VFAs and ethanol, HRT-controlled operation, and microbial community development. The following sections first show how concentrated medium addition affected CO conversion and metabolite formation, then examine the inhibitory role of VFAs and ethanol, before demonstrating how HRT control improved process stability and how these operational changes shaped the reactor microbial community. No CH_4 was detected in the outlet gas throughout the whole experiment, consistent with methanogenesis suppression in the BES-treated reactor.

3.1. Effect of concentrated medium addition on H_2 production

Phase I was dedicated to examining the hypothesis that periodic concentrated medium addition could maintain microbial activity during startup without disturbing CO-to-H₂ conversion. Concentrated medium (50 mL) was added every seven days, and initially, CO was totally converted from day 2 to 10, with a corresponding H₂ yield of 0.60 ± 0.03 mol/mol CO (Fig. 2A). After three days of the first concentrated medium addition, the CO conversion has reduced to $72.3 \pm 6.04\%$, together with H₂ yield (0.44 ± 0.03 mol/mol CO). CO conversion and H₂ yield have shown some recovery from day 17 to 20, achieving 94.89 ± 4.81 (%) and 0.56 ± 0.02 mol H₂/ mol CO. The most impacted period was after the second addition, where H₂ yield was reduced to 0.31 ± 0.23 mol/mol CO. However, from day 34, the CO conversion into H₂ started to be unstable, not sustaining CO conversion close to 100%, while the VFA concentration achieved the highest values during this phase (Fig. 2B). Total VFA rose from less than 1 g/L after the second sampling point (day 6) to 10.06 ± 0.22 g/L on day 34, with acetic acid consistently representing > 85% of total VFA during the whole period of phase I. Even after the addition of concentrated medium, the CO-to-H₂ conversion did not achieve the same rates as at the beginning.

Elemental analysis confirmed that metabolite accumulation was accompanied by a pronounced imbalance in nutrient composition. The elevated trace-metal concentrations measured in the liquid phase (Table 1) coincided with the repeated concentrated medium additions, suggesting accumulation as a plausible driver, although direct causation cannot be established from elemental analysis alone. Cu concentrations were approximately 35000x higher than the levels expected from the DSMZ 507 formulation. All measurements were performed in triplicate by MP-AES, and the low standard deviation contradict random contamination, though possible sources beyond medium additions, including leaching from reactor materials, cannot be excluded, requiring further investigation in future work. Macronutrients, such as Ca, K, and Mg, were measured at 4–50% of the levels expected from DSMZ 507 formulation. This reduction could reflect microbial uptake, precipitation, sorption, or other removal mechanisms that was not independently confirmed. Ethanol was also detected during phase I at a concentration of 1.8 g/L. The concurrent presence of ethanol and VFA suggests that a fraction of the microbial community was redirecting carbon flux through alcohol dehydrogenase pathways under stress conditions. Overall, phase I has presented a CO conversion (%) of $67.8 \pm 31.6\%$ (ranging from 0–100%), an H₂ productivity of 2.7 ± 1.5 L H₂/ L/ d, and an H₂ yield (mol/mol CO) of 0.74 ± 0.29 .

Table 1
Comparison of expected (DSMZ 507) and measured elemental concentrations in the liquid phase.

Element	DSMZ 507 (ppm)	Measured (ppm)	Ratio	Interpretation
Fe	0.42	11 ± 0.5	26.2×	Accumulated
Cu	0.75 × 10 ⁻³	26.2 ± 0.6	34933.3×	Highly accumulated
Mn	0.03	3.2 ± 0.2	106.7×	Accumulated
Na	268	698 ± 2.9	2.6×	Na from NaHCO ₃ /BES
Ca	77.4	42 ± 0.6	0.5×	Lower than formulation level
K	262.2	50.5 ± 0.7	0.2×	Lower than formulation level
Mg	60.9	2.4 ± 0.2	0.04×	Lower than formulation level

All measurements were performed in triplicate by MP-AES (mean ± SD, ppm = mg/L). DSMZ 507 reference values represent elemental concentrations calculated from the formulation. “Lower than formulation level” indicates measured concentrations below the expected formulation value, the removal mechanism (microbial uptake, precipitation, sorption, or removal losses) was not independently confirmed.

3.2. Inhibitor testing and process recovery

Phase II was designed to examine whether the process deterioration observed in phase I was primarily caused by accumulated VFAs, ethanol, or their combined effect. As acetic acid dominated the VFA profile in phase I, we hypothesised that VFA accumulation, rather than ethanol, would be the main driver of reduced CO conversion and incomplete recovery. Therefore, at the start of phase II, a total medium replacement was carried out, which reduced VFA concentration from 16.6 g/L to 3 g/L, providing clean data on CO to H₂ conversion and VFA production (Fig. 3A and B). After 14 days, the CO-to-H₂ conversion was stable, and the VFA concentration reached 11.25 g/L. In the startup phase, the overall CO conversion reached 77.9 ± 24.3% with an H₂ productivity of 2.1 ± 0.9 L H₂/L/d and an H₂ yield of 0.70 ± 0.21 mol/mol CO.

To examine the potential inhibitory effect of VFAs, a new medium with synthetic VFA at 14.5 g/L was introduced. The addition of synthetic VFA at 14.5 g/L was correlated with a decrease in CO conversion, which dropped from 77.9 ± 24.3% in the overall start-up period to 24.8 ± 47.3% in the overall VFA inhibition test. After seven days, H₂ reached 33% in the outlet gas composition, while CO remained at 25%, indicating incomplete conversion. Moreover, the addition of synthetic VFA pushed the accumulated concentration to 18 g/L. In the end, the VFA test presented a CO conversion of 24.8 ± 47.3%, a productivity of 1.1 ± 0.9 L H₂/L/d, and an H₂ yield of 0.67 ± 0.22 mol/mol CO.

As ethanol was also registered during phase I with VFA, a synthetic composition of VFA, at the same concentration as before, with ethanol at 1.8 g/L, was added to the system with a fresh medium. The combined addition of VFA with ethanol caused further VFA accumulation (21.2 g/L) (Fig. 3B), the highest level observed during the entire experiment. The H_2 concentration was nearly stable for seven days, keeping the concentration at 30–40%. CO concentration was kept low after ethanol addition, going down to 5%. However, an increase in CO concentration started to be observed after five days, reaching 17% after the seven-day period of the inhibitory test, which can be correlated with VFA accumulation in the system, as described in the inhibitory test with VFA alone. Even after the VFA consumption observed at the end of the experiment, it was not enough to sustain H_2 production. After the test with VFA and ethanol, the overall CO conversion was $86.6 \pm 3.9\%$, the H_2 productivity was 2.9 ± 0.1 L H_2 /L/d, and the H_2 yield was 0.84 ± 0.03 mol/mol CO.

During the ethanol-only test, CO sharply increased to almost the original concentration. Although ethanol did not have as much of an impact as VFA alone, CO dropped quickly after ~ 48 hours, contrasting with VFA-only, which did not reach a CO level lower than 5%. In the present study, after four days of experiment, the CO concentration had started to increase, and at the end of the experiment has reached 20% of the gas outlet composition. VFA accumulation was lower than that of VFA alone and the combination of VFA with ethanol. At the end of the ethanol test, the overall CO conversion in this phase was $84.7 \pm 18.8\%$ (ranging from 0–98%), the H_2 productivity (H_2 /L/d) was 2.9 ± 0.5 , and the H_2 yield (mol/mol CO) was 0.83 ± 0.13 .

To evaluate whether the microbial consortium could recover from inhibitor exposure, new medium additions were applied after the VFA with ethanol test and after ethanol alone. After the synthetic VFA and ethanol inhibitory test, the new medium added was effective for the recovery capacity of the natural microbial consortium. After seven days, the CO-to- H_2 conversion reached a similar rate as at the beginning of the inhibitory test, presenting 7% CO in the gas outlet. On the contrary, as illustrated in Fig. 3A, after the ethanol-only test, CO-to- H_2 conversion has not fully recovered, at $58.0 \pm 17.5\%$ compared to $77.9 \pm 24.3\%$ during the pre-inhibitor startup, with CO concentration in the gas outlet not reducing below 20% for over a week. These results demonstrate that the recovery capacity of the microbial community was exceeded, which motivated a complete replacement of the microbial consortium, medium, and packing material for phase III.

3.3. Hydraulic retention time control evaluation for process stability

Phase III was designed to examine the hypothesis that continuous liquid renewal through HRT control could stabilise BWGS performance by preventing VFA accumulation and potentially limiting the accumulation of soluble fermentation products. Unlike the periodic medium replacement strategy used in phases I and II, HRT control continuously removed soluble metabolites while maintaining biofilm-associated biomass in the packed bed. The reactor was therefore restarted with fresh medium, a new

microbial consortium, and new polyethene sponge packing material to avoid carry-over effects from phase II. Under HRT of 4 days (days 0–14 of phase III), the system stabilised with a VFA concentration < 4g/L. During these first 12 days under HRT 4, gas flow was also systematically increased from 12 to 28 mL/min (Fig. 4C) to test the reactor gas conversion capacity at higher loading rates. For this period, to test the gas conversion capacity, the flow was increased after 24 hours if the CO concentration was $\leq$ 5%. Low outlet CO was maintained at tested gas flows up to 26 mL/min; after that, at 28 mL/min, CO sharply increased and did not return to concentrations lower than 5%. Higher gas flows increased the CO loading rate but reduced gas residence time, creating a trade-off between substrate supply and conversion efficiency. Even with different gas flow schemes, a high overall CO conversion was registered in this test ($85.0 \pm 25.2\%$, ranging from 6–100%) with an H_2 productivity of $6.7 \pm 1.7 H_2/L/d$, and an H_2 yield of $0.76 \pm 0.14 \text{ mol/mol CO}$.

To investigate whether faster liquid renewal would limit VFA accumulation and support BWGS activity, an HRT of 2 days was applied. This shorter HRT maintained VFA below 5 g/L and restored CO conversion to H_2 (Fig. 4A). An overall CO conversion rate of 93.3 ± 11.0 (ranging from 68–100%) was registered under HRT 2, together with an H_2 productivity of $4.2 \pm 1.3 H_2/L/d$, and an H_2 yield of $0.76 \pm 0.18 \text{ mol/mol CO}$. During HRT 2, the most stable gas flow at which complete CO conversion was sustained was 12 mL/min (0.31 volume per hour – VVH). A gas flow ramp test (12–28 mL/min; 0.31–0.73 VVH) revealed a threshold at 26 mL/min (0.68 VVH), above which CO conversion declined. VFA accumulation was compared between HRT conditions using a Tukey pairwise comparison (first HRT 4: $n = 25$; HRT 2: $n = 17$). No statistically significant difference in VFA accumulation was detected between the first HRT 4 and HRT 2. It should be noticed that this comparison is limited by the different gas-flow applied on both HRTs, including the ramp test conducted during HRT 2, which may have influenced VFA dynamics. HRT 4 was subsequently re-applied to evaluate process performance under longer retention time and more conservative liquid use. Even achieving a CO conversion of 100% for the whole period (12 days), the H_2 productivity and H_2 yield were smaller than under HRT 2 (Table 2). Therefore, HRT control is essential for long-term operation, not only to maintain high CO-to- H_2 conversion, but also to provide a practical control strategy based on real-time monitoring of soluble metabolites.

Table 2
 Process performance metrics for phase III. HRT: hydraulic retention time. EGRT: empty gas residence time.

HRT (days)	CO conversion (%)	H_2 productivity (L H_2 /L/d)	H_2 yield (mol H_2 /mol CO)	EGRT (h)
4 (14 days)	85.0 ± 25.2	6.7 ± 1.7	0.76 ± 0.14	1.59
2 (8 days)	93.3 ± 11.0	4.2 ± 1.3	0.76 ± 0.18	2.87
4 (12 days)	100.0 ± 0.0	3.2 ± 0.2	0.66 ± 0.04	3.19

3.4. Microbial community dynamics

The microbial sequencing has revealed distinct community structures across the experimental phases (Fig. 5). The original inoculum (FW – I & II) was dominated by Bacillota (~ 70%), and after operation during phases I and II (P – I & II), the microbial community maintained this dominance but also showed a substantial enrichment of Coprothermobacterota (~ 25%).

After restarting with a new inoculum (FW – III) and operating under HRT control during phase III (P – III), a large community shift occurred. Pseudomonadota became the dominant phylum (~ 65%), while Coprothermobacterota has almost disappeared (< 1%). This shift reflects the strong selection pressure imposed by the HRT control. At the genus level (Fig. 5B), the community extracted from the packing material in phase III (P – III) was dominated by *Stenotrophomonas* (~ 45%) and *Pseudomonas* (~ 30%). Minor populations were observed, such as *Thermicanus* (3.2%), *Clostridium* (~ 0.2%), *Neomoorella* (1.2%), Clostridia (~ 0.3%), and Bacillota (~ 5%), which may potentially have played a functional role in the system.

4. Discussion

This study investigated continuous H₂ production through the BWGS reaction using a food-waste-derived microbial consortium in a TBR. Three operational phases were examined to understand how nutrient addition, metabolic-product accumulation, and HRT control influenced reactor performance. The results showed that repeated concentrated medium addition was associated with trace-metal accumulation, VFA accumulation, and a decline in H₂ production. In contrast, HRT-controlled operation enabled stable CO conversion, achieving 100% CO conversion and an H₂ yield of 0.66 ± 0.04 mol/mol CO. The following discussion explores these observations in relation to previous research on trace-metal effects, inhibition during biological syngas conversion, TBR performance, and microbial community dynamics.

The trace metal accumulation observed during phase I (Table 1) suggests that repeated concentrated medium addition impacted the nutrient balance of the system. Cu showed the most prominent accumulation relative to the original medium formulation, although its measured concentration was below the acute toxicity reported for anaerobic systems [32]. Nevertheless, metal concentrations alone may not fully describe their biological effects, as the balance between individual trace elements can influence microbial growth and product formation. In syngas fermentation with *Clostridium ljungdahlii*, Cu was identified as the most significant trace metal affecting cell growth, with excess Cu potentially causing enzyme inactivation and decreasing carbon flow towards growth and metabolites [33]. Similarly, trace metal optimisation experiments with *Clostridium carboxidivorans* P7 demonstrated that adjusting the trace metal combination shifted product yields by 10-fold, indicating that the ratio between trace metals is more important than their absolute concentrations [34]. In the present study, trace-metal accumulation combined with macronutrient depletion (Table 1) may have created an imbalanced nutrient environment, which may explain the shift from H₂ production towards acetogenesis. The simultaneous accumulation of VFAs and ethanol also indicates a change in the distribution of fermentation products. Such changes are consistent with the sensitivity of syngas fermentation pathways to pH and acid concentrations, which can influence organic acid and solvent production [35].

More generally, the energetic and metabolic constraints of anaerobic CO conversion can influence the electron flow between H₂ production and other reduced metabolites, such as acetate [36]. However, the present study does not establish whether the electron flow was redirected from the BWGS reaction towards acetogenesis or solventogenesis, which requires further investigation. The association between product accumulation and the decrease in H₂ production therefore motivated the targeted inhibition experiments conducted in phase II.

The phase II experiments support the interpretation that VFAs, particularly acetic acid, were associated with a larger decline in reactor performance than ethanol under the tested conditions. This finding is consistent with previous work from biological gas-conversion systems. In syngas biomethanation, acetate concentrations above 2 g/L were reported to inhibit syngas conversion [22]. Although it was a biomethanation system rather than the BWGS reaction, it provides relevant evidence that acetate accumulation can affect biological syngas conversion. In addition, a dark fermentation study reported decreases in H₂ production of 50 and 90% at undissociated acetic acid concentrations of 73.3 and 686.7 mg/L, respectively [37]. At the acetic acid concentrations and pH values observed in this study (**Section 3.2**), the estimated concentration of undissociated acetic acid (~ 800 mg/L) substantially exceeded the thresholds associated with H₂ inhibition in dark fermentation (686.7 mg/L) [37]. This comparison supports the potential of acetate-mediated inhibition, but the threshold cannot be transferred directly to the present system because dark fermentation and the BWGS reaction system differ in their microbial communities, metabolic pathways, and operating conditions.

The results also suggest that acetate dynamics may have involved processes other than net accumulation. Previous studies have described alternative VFA-consuming pathways in anaerobic systems, including pathways observed under BES inhibition [38], conversion of acetate into medium-chain fatty acids (MCFA) by chain-elongating communities [39], and syntrophic acetate oxidation by thermophilic microbial populations [40]. These pathways provide possible explanations for VFA conversion in mixed cultures, but they cannot be confirmed in the present reactor because the relevant intermediate products and metabolic activities were not measured. Likewise, the absence of ethanol consumption during the combined VFA-ethanol test (**Section 3.2**) does not establish whether ethanol-to-acetate conversion occurred, because simultaneous production and consumption could mask changes in the final concentration. The weaker inhibitory effect of ethanol alone nevertheless indicates that ethanol was not the primary inhibitor under the conditions tested. Its potential indirect effects on microbial community metabolism remain unclear.

Following exposure to inhibitory metabolites, medium replacement allowed partial recovery, suggesting that BWGS activity was not immediately or completely lost. However, repeated exposure to elevated VFA and ethanol was followed by incomplete recovery of CO conversion. This suggests that the consortium's recovery can depend on the extent and duration of inhibitor compounds exposure, although the experiments do not establish a precise recovery threshold. The incomplete recovery motivated the transition to phase III, in which HRT control was used to improve liquid-phase replacement and limit the accumulation of soluble products.

Continuous operation with HRT control enabled sustained BWGS activity and provided a contrast to the VFA accumulation and declining H₂ production observed in phases I and II. At an HRT of 4 days and a gas flow rate of 12 mL/min, the reactor achieved 100% CO conversion and an H₂ yield of 0.66 ± 0.04 mol/mol CO, with an empty gas residence time (EGRT) of 3.2 h. This performance can be compared with a thermophilic TBR study which achieved full CO and H₂ conversion during syngas biomethanation at an EGRT of 0.75 h [21]. However, the two systems differed in their metabolic objective, feed-gas composition, and microbial process, so the difference in EGRT should not be interpreted as a direct comparison of intrinsic reactor efficiency. The present system operated with a CO inlet concentration of 65%, compared with 20% in the biomethanation system, and produced H₂ rather than CH₄. The different operating requirements may therefore reflect differences in both reactor conditions and microbial conversion processes.

The energetic constraints of the BWGS reaction provide additional context for this difference. The standard Gibbs energy change reported for the BWGS reactions is approximately – 20 kJ/mol, compared with approximately – 211 kJ/mol for methanogenesis [36]. These values indicate a substantially smaller energy yield for the BWGS reaction on the stated reaction basis, although the values should be compared when the reaction stoichiometry and reference conditions are consistent. The thermodynamic difference may influence the energy available for microbial growth, but the present study did not directly measure growth rates or biomass-specific conversion kinetics. Reactor-scale performance is also affected by gas-liquid mass transfer and biomass activity. Modelling of TBR systems for biological CO conversion at lab and pilot scale has evaluated the interaction between mass transfer and microbial conversion capacity as a function of liquid Reynolds number and gas loading [20]. In the present study, CO conversion declined above a gas flow of 26 mL/min (0.68 VVH), as observed under 24-h step changes in gas flow. Whether this threshold reflects mass transfer limitation, microbial conversion capacity, or a combination of both factors was not experimentally distinguished, and further experiments would be required to separate these contributions. Comparing TBR configurations across syngas fermentation studies reveals substantial differences in reactor design and operating conditions. One study operated a semi-pilot-scale TBR (i.e. 5 L packed bed) with a plastic (i.e. polypropylene/polyethylene) carrier, achieving full CO and H₂ conversion for biomethanation at an EGRT of 0.75 h under thermophilic conditions [21]. Another study used a smaller volume, 1 L packed bed, with polyethylene packing material for syngas biomethanation in batch mode [41]. In contrast, the present study used a 2.3 L packed bed with polyethylene sponge packing at a substantially higher CO inlet concentration (65% versus 20%) and achieved 100% CO conversion for H₂ production at an EGRT of 3.2 h. The longer EGRT required for H₂ production may reflect the lower energy yield of the BWGS reaction compared to methanogenesis, as discussed above, though reactor geometry and operating conditions also differ substantially between studies. Modelling of CO conversion in laboratory-scale TBR (220 mL) and semi-pilot TBR (5 L) identified a transition from mass transfer limitation to microbial growth limitation at higher gas loading rates [42]. The decline in CO conversion observed above 26 mL/min in the present study is consistent with such type of transition, but the specific limiting factor (e.g. mass transfer or microbial capacity) was not

experimentally established and should not be generalised as a universal threshold beyond the conditions tested in the present study.

The increase in VFA concentrations during the gas-flow ramp (**Section 3.3**) suggests that the higher gas loading altered the balance between CO conversion and product accumulation. One possible hypothesis is that a greater portion of the available carbon was directed towards acetate and other reduced products rather than H₂ production. However, the present analysis does not establish the distribution of CO conversion between hydrogenogenic and acetogenic populations. Thermodynamic analysis of mixed-culture syngas fermentation has shown that acetogenic and methanogenic pathways can be energetically favoured over hydrogenogenic due to the higher energy yield of their respective reactions [36, 43]. This provides a possible explanation for the observed product shift, although the extent to which these energetic differences determine the reactor response remains uncertain.

The improved stability under HRT control is consistent with the interpretation that soluble-product accumulation contributed to the instability observed during the earlier phases. By renewing the liquid phase while retaining immobilised biomass, the TBR configuration allowed accumulated products to be removed. The results therefore support HRT controls as a practical strategy for maintaining conversion under the conditions tested. Further work should determine whether the benefit arises primarily from VFA removal, improved nutrient renewal, or other changes in the reactor environment.

The steady-state H₂ yield of 0.66 ± 0.04 mol/mol CO places the present system within the range of continuous BWGS reaction studies using different microorganisms and reactor configurations (Table 3). Higher yields have been reported with pure cultures under distinct conditions. For example, *Carboxydotherrmus hydrogenoformans* achieved an H₂ yield of 0.92 mol/mol CO in a hollow fibre membrane reactor (HFMBR) [44], while the photosynthetic bacteria *Rhodospirillum rubrum* and *Rhodopseudomonas palustris* P4 achieved yields of 0.88 and 0.94 mol/mol CO, respectively, in a continuous stirred tank reactor (CSTR) [45, 46]. The hyperthermophilic archaeon *Thermococcus onnurineus* NA1 has also been investigated for CO conversion in a pressurised bubble column reactor, with a reported H₂ productivity of 242 L/L/d [47]. In addition, *Rubrivivax gelatinosus* CBS demonstrated CO conversion in a TBR, with reported conversion of approximately 75% [48]. These studies differ in organism, temperature, reactor design, pressure, light input, and reported performance metrics. Therefore, the yield values alone do not establish a definitive ranking of process performance. The contribution of the present study is the demonstration of continuous H₂ production at ambient pressure using a non-sterile, food-waste-derived microbial consortium in a relatively simple TBR configuration. This approach avoids the need for a defined pure culture and, unlike photosynthetic systems, does not require light input. These features may offer practical advantages for waste-derived syngas applications, although potential reductions in capital and operating costs would need to be demonstrated through techno-economic assessment. Therefore, the results provide a basis for further development of mixed-culture BWGS reaction systems, with improvements in H₂ yield, volumetric productivity, and process stability.

Table 3
Comparison of biological water-gas shift reaction studies.

Organism	Reactor	Temp. (°C)	H ₂ yield (mol/mol CO)	CO conversion (%)	H ₂ productivity (L/L/d)	Reference
Mixed consortium	TBR ^a	55	0.66 ± 0.04	100	3.2	Present study
<i>T. onnurineus</i> NA1	Pressurised BCR ^b	80	–	–	242	[47]
<i>C. hydrogenoformans</i>	HFMBR ^c	70	0.92 ± 0.04	97.6	67	[44]
<i>R. rubrum</i>	CSTR ^d	30	0.88	58	2.5	[45]
<i>R. palustris</i> P4	CSTR ^d	30	0.94	100	22.1	[46]
<i>R. gelatinosus</i> CBS	TBR ^a	25	~ 1	~ 75	–	[48]
^a Trickle bed reactor (TBR); ^b bubble column reactor (BCR); ^c hollow fibre membrane reactor (HFMBR); ^d continuous stirred tank reactor (CSTR)						

Microbial community analysis revealed differences in community composition across the operational phases, suggesting that reactor conditions were associated with community restructuring. Bacillota dominated the original inoculum used for phases I and II (FW – I & II). This phylum includes Clostridia and several thermophilic carboxydophilic microorganisms associated with CO conversion and the BWGS reaction [7, 49, 50]. During phases I and II (Fig. 5), the relative abundance of Coprothermobacterota increased as Bacillota became less dominant. Members of Coprothermobacterota, recently reclassified from the former phylum Firmicutes, have been associated with acetic acid production under thermophilic conditions [51], but their enrichment alone does not demonstrate that they caused the observed VFA accumulation. It can be hypothesised that the enrichment of Coprothermobacterota influenced VFA accumulation during phases I and II, although this community shift occurred alongside changes in reactor chemistry and performance, and its functional significance remains unsolved.

During phase III, Pseudomonadota become dominant, while Coprothermobacterota declined to low relative abundance (Fig. 5B). The genera *Stenotrophomonas* and *Pseudomonas* were dominant in this phase. Although *Stenotrophomonas* are known for its environmental versatility and can produce exopolysaccharide (EPS) [52], *Pseudomonas* has been observed in microbial communities from anaerobic digesters and biofilms [53, 54]. However, their potential roles in biofilm structure or community stability in the present system remain a hypothesis. Similarly, low-abundance taxa related to known hydrogenogenic carboxydrophs, including *Thermicanus* [55], Clostridia [49, 56], and *Neomoorella* [50, 57], provide plausible candidates for H₂ production, but cannot be assigned as responsible for the

measured H_2 based on 16S rRNA gene sequencing alone. The BWGS reaction involves CO dehydrogenase (CODH) and hydrogenase activity [58, 59], but the presence and expression of the corresponding genes were not measured in this study. The BWGS reaction proceeds intracellularly via CODH, which oxidises CO to CO_2 , generating reduced ferredoxin, and a hydrogenase re-oxidises the ferredoxin by reducing protons to H_2 [36, 59]. In the present study, these enzymatic steps were carried out by the native whole-cell consortium immobilised on the polyethene sponge packing; no enzymatic activity assays, CODH protein quantification, or hydrogenase gene-expression analyses were performed. Therefore, the reactor metrics reported here reflect integrated whole-cell catalytic activity under the applied operating conditions, with individual microbial contributions hypothesised from community composition analysis rather than directly measured enzyme levels. Consequently, the observed community composition is consistent with the observed results, but does not prove functional specialisation within the present microbial community. Metagenomic or metatranscriptomic analysis, together with targeted CODH and hydrogenase measurements, would be needed to determine which populations contributed to CO conversion and H_2 production.

The strategy of washing the food waste sludge showed a lower relative abundance of Thermotogota, while Bacillota remained detectable. These differences may reflect changes in microbial composition associated with the washing procedure, but relative-abundance data cannot determine whether microorganisms were physically removed or retained. In our previous study using the same pre-treatment approach, washing substantially reduced the residual concentrations of several macro- and trace elements, including Fe, K, Mg, Mn, Al, and Na, while Ca was comparatively retained [15]. Changes in nutrient availability and trace elements may have contributed to the shifts in microbial community observed in the present study in phase III, although the previous study did not directly assess the effect of washing on microbial community composition. Moreover, exposure to stress conditions, such as heat and oxygen, can also trigger sporulation in some Clostridia [60], but the present data do not establish whether sporulation contributed to the observed pattern. The subsequent enrichment of Pseudomonadota, a phylum that was minor in both original inocula, is consistent with an influence of phase-III conditions, but the effects of HRT control, inoculum washing, and reactor restart cannot be separated, consistent with previous results that process parameters rather than the inoculum source determine microbial community in anaerobic systems [61].

The Principal Coordinate Analysis (PCoA) (**Figure S1**) showed separation between inoculum and post-process samples along principal component (PC) 1 (34.93% of the variation) and between phases I and II along PC2 (23.31%). These patterns describe differences among the four sequenced sample profiles but do not establish significant differences between phases. The separation of phase III samples is consistent with an association between HRT-controlled operation and community restructuring. However, because inoculum pretreatment, reactor conditions, and process data were not independently controlled in the community comparison, the relative contribution of each factor cannot be quantified from the PCoA alone. Overall, the results indicate that both the starting inoculum and operational data should be considered when interpreting the microbial community dynamics of this system.

The results identify several priorities for further development of continuous BWGS in TBRs. It should be noted that all observations reported here derive from a single 2.3 L packed-bed reactor operated with a defined synthetic gas mixture, and the findings cannot be directly extrapolated to larger scales or real gasification-derived syngas without independent validation. Under the conditions tested, HRT-controlled operation provided stable CO conversion, whereas repeated concentrated medium addition was associated with nutrient imbalance and VFA accumulation. The observed gas flow threshold of 26 mL/min (0.68 VVH) provides a reactor-specific operational boundary under the conditions tested, rather than a universal limit for scale-up. Further work should independently investigate gas and liquid loading, nutrient delivery, and VFA removal to determine their effects on CO conversion, H₂ yield, and productivity. At pilot scale, additional factors will require attention, including hydraulic effects on liquid distribution, gas-liquid mass transfer coefficient at increased bed dimensions, the impact of real syngas impurities (e.g. H₂S, tars, NH₃) on microbial activity, and the potential for foam or biofilm fouling. Testing alternative packing materials and liquid-distribution strategies may also help establish whether mass transfer can be improved without compromising microbial retention. The potential advantages of non-sterile operation and waste-derived inocula, including reduced capital and operating cost, are conceptually promising but would need to be quantified through techno-economic analysis before conclusions can be drawn.

5. Conclusions

This study demonstrated continuous H₂ production from CO-rich syngas through the BWGS reaction using a non-sterile, food-waste-derived microbial consortium immobilised in a TBR. Repeated concentrated medium addition was associated with trace-metal accumulation, VFA buildup, and declining H₂ production, while inhibitor tests suggested a stronger adverse association for VFAs than for ethanol under the tested conditions. Under HRT-controlled operation, the reactor achieved 100% CO conversion and an H₂ yield of 0.66 ± 0.04 mol/mol CO at an HRT of 4 days, a gas flow of 12 mL/min, and an EGRT of 3.2 h. CO conversion declined at 28 mL/min during the flow-ramp experiment, identifying an operational limitation under the conditions investigated. Microbial community composition also differed between operational phases, although the functional roles of individual taxa remain to be confirmed. Overall, these results highlight the importance of liquid-phase renewal and controlling inhibitory metabolite accumulation for stable continuous BWGS operation. Further work should optimise gas and liquid loading, validate performance at pilot scale with real syngas, and assess strategies to improve H₂ yield and productivity.

Declarations

Competing interests

The authors declare that they have no competing interests.

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Author Contribution

AN: Conceptualisation, investigation, experimental design, performance and analysis, writing the original draft. KC: supervision in experimental performance, resources, review and editing. MT: Conceptualisation, experimental design, supervision, funding acquisition, project administration, review and editing.

Data Availability

All data supporting the findings of this study are available within the paper and its Supplementary Information.

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Figures

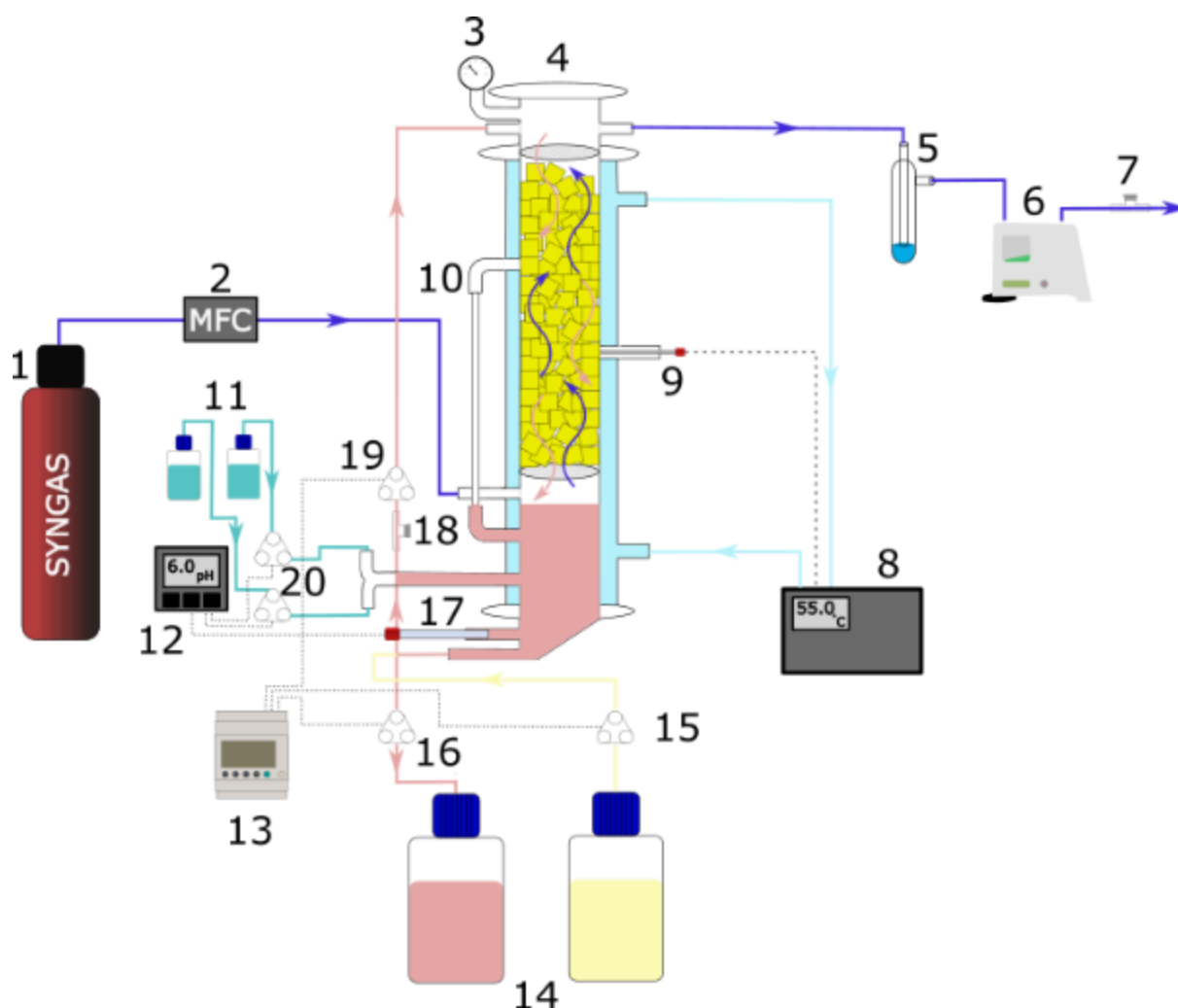


Figure 1

Illustration of the TBR set-up designed, configured, and operated in this study. The main parts of the configuration are: 1) Syngas flask, 2) mass flow controller (MFC), 3) pressure gauge, 4) TBR, 5) water trap, 6) μ Flow unit, 7) gas sampling unit, 8) water bath for water jacket recirculation, 9) temperature probe, 10) liquid level indicator, 11) acid and base solutions, 12) pH meter/control, 13) programmable logic controller (PLC), 14) effluent media and new media flasks, 15) peristaltic pump for addition of new media, 16) peristaltic pump for discard effluent media, 17) pH probe, 18) liquid sampling unit, 19) peristaltic pump for liquid recirculation, 20) peristaltic pumps for pH control.

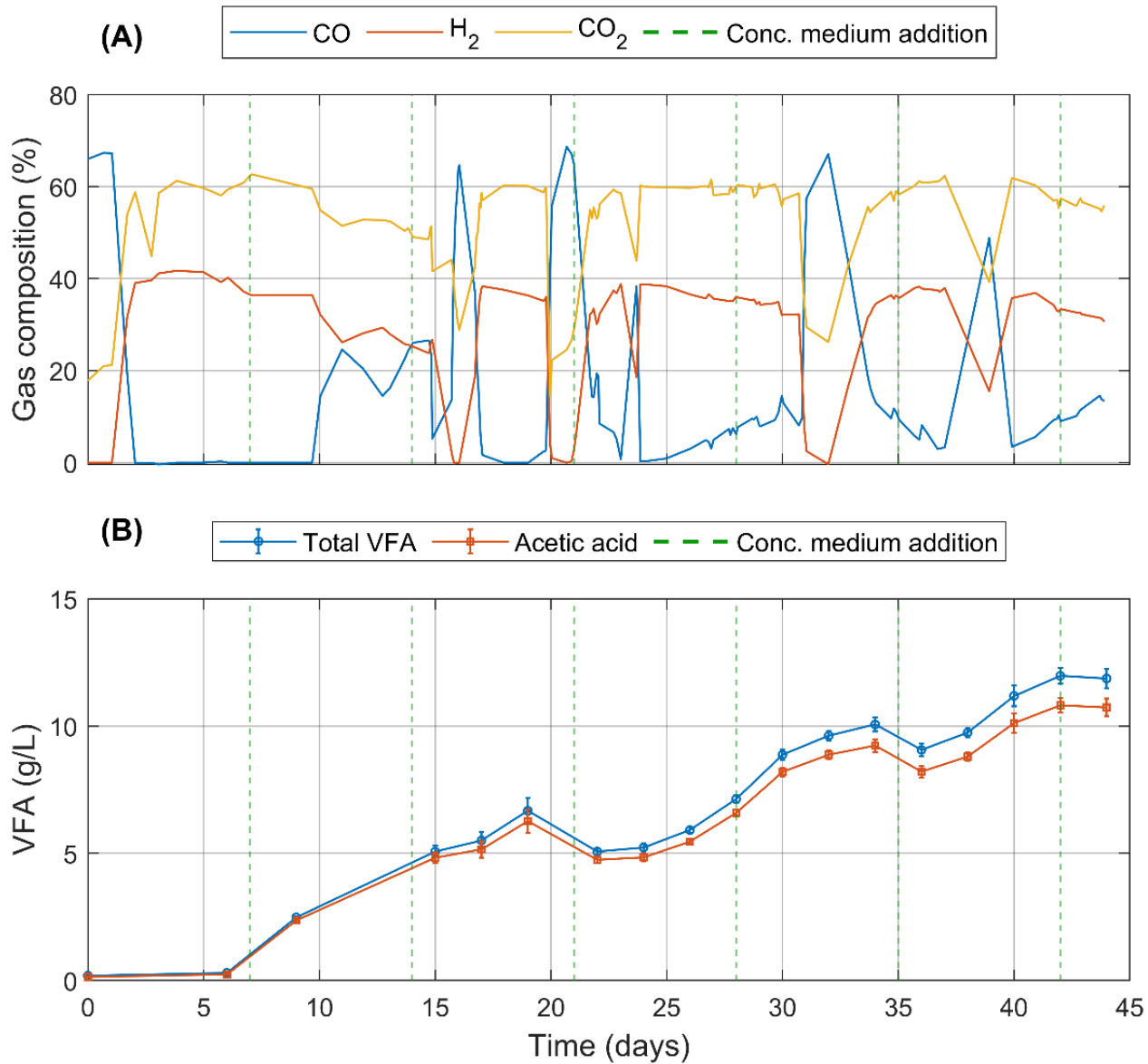


Figure 2

Process data from phase I for: (A) gas outlet composition and (B) total VFA and acetic acid concentration.

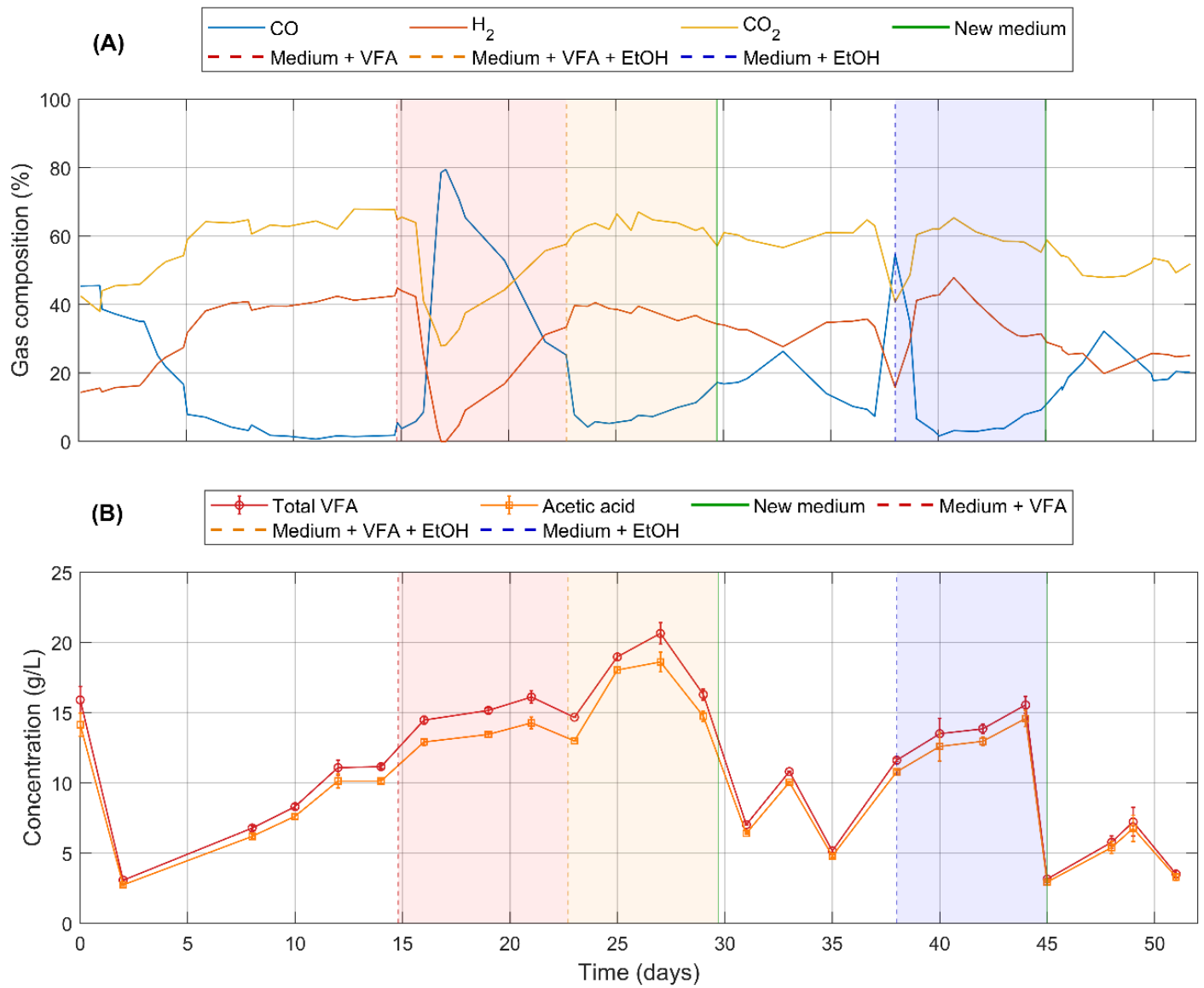


Figure 3

Process data from phase II for: (A) gas outlet composition and (B) total VFA and acetic acid concentration.

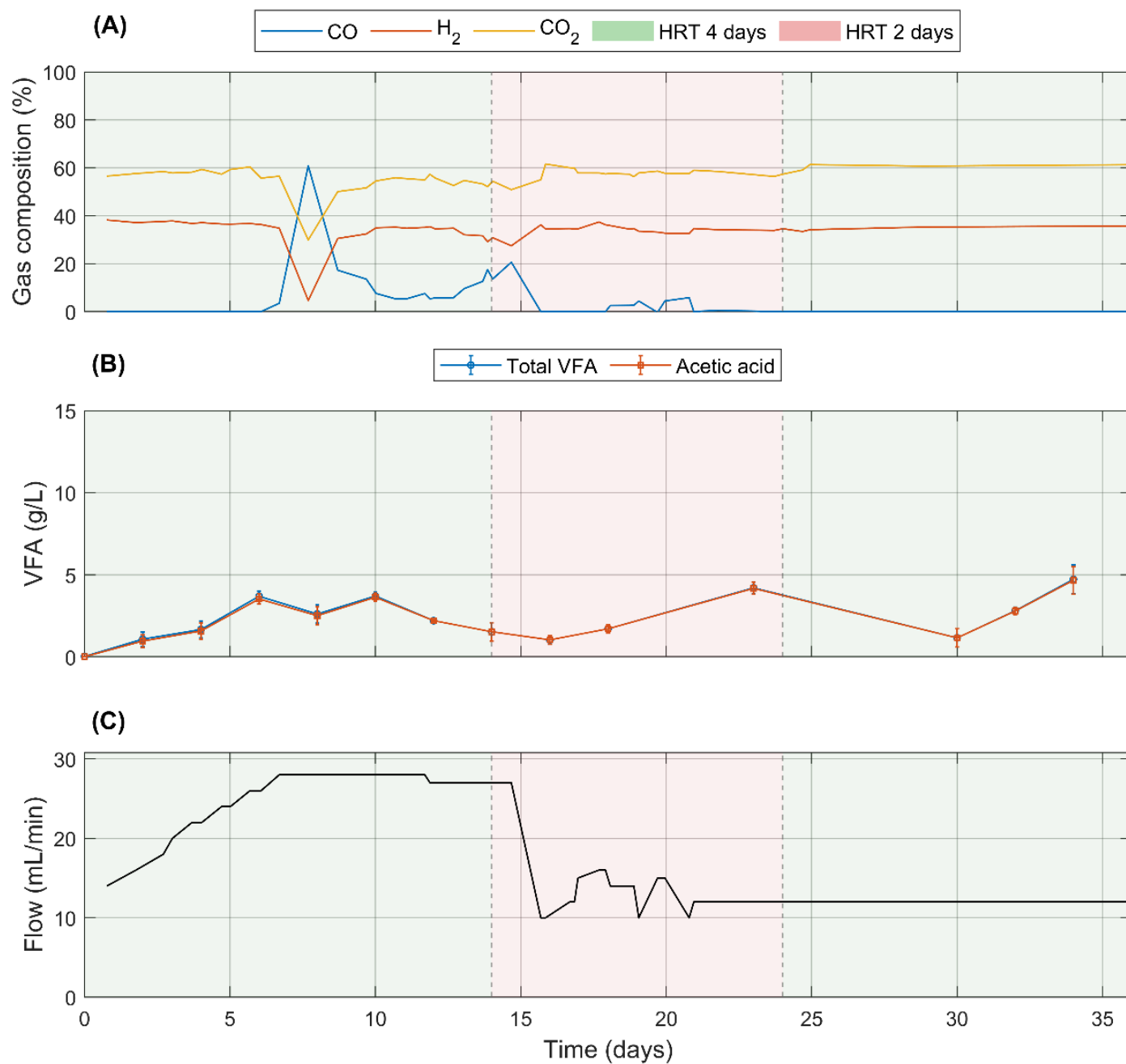


Figure 4

Process data from phase III for: (A) gas outlet composition; (B) total VFA and acetic acid concentration; and (C) gas flow inlet.

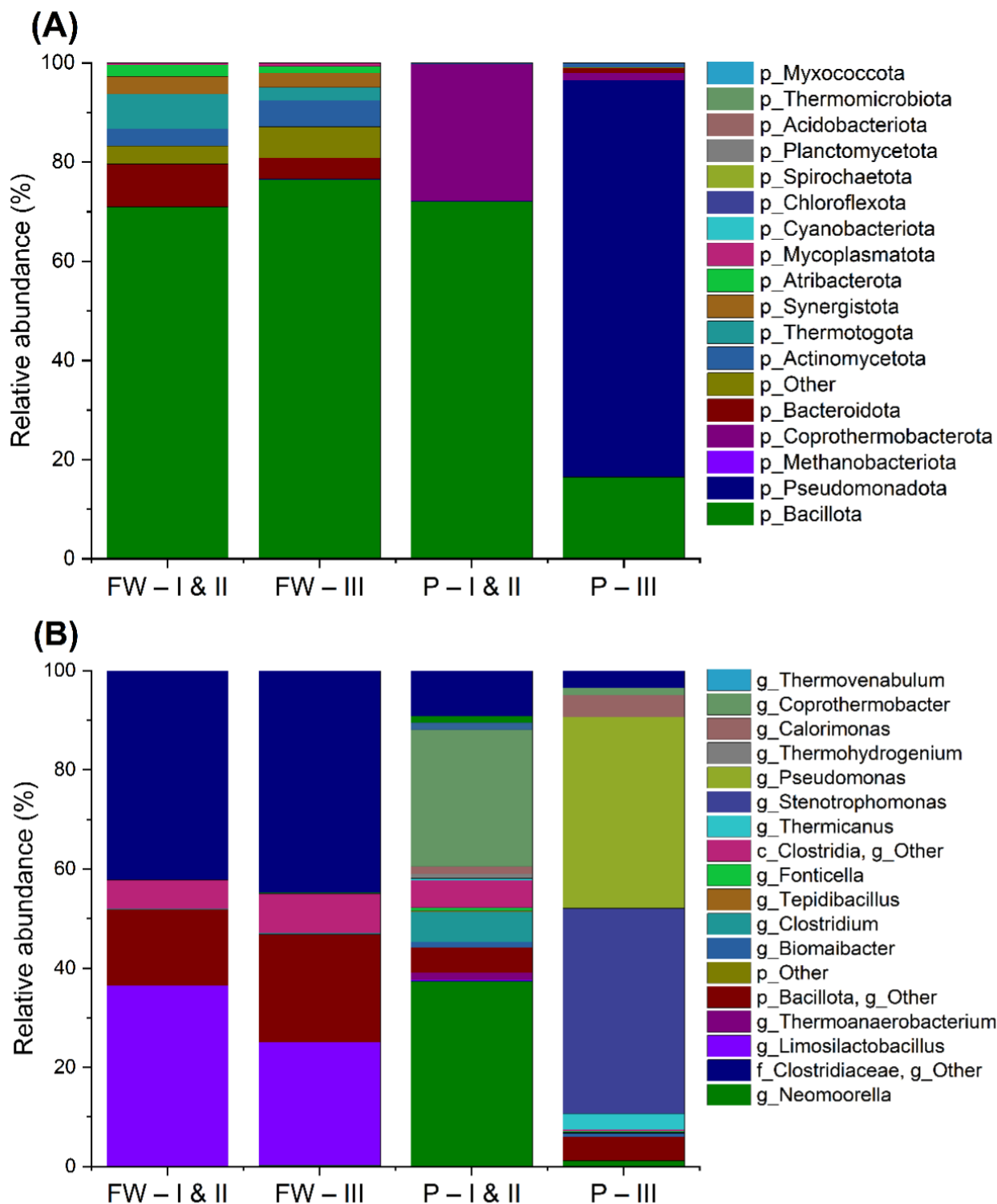


Figure 5

Change in the microbial community at different phases(A) at the phylum level, (B) at the genus level. (FW - I & II) inoculum after the centrifugation step used in phase I and phase II; (FW - III) inoculum after the washing step used in phase III; (P - I & II) microbial community after the end of phase II; (P - III) microbial community after the end of phase III.

Supplementary Files

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