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Influence of acetate concentration on acetone production by a modified *Acetobacterium woodii*

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ABSTRACT

Global warming is the driving force for developing production processes of chemical compounds based on CO_2 reduction technologies. Bacteria can act as biological catalysts that reduce this gaseous substrate in added-value compounds. *Acetobacterium woodii* is one of the best-performing strains on H_2–CO_2 blends and naturally produces acetate. Acetone is a raw material deeply used in the chemical industry, and its global demand is increasing. Acetone-butanol-ethanol (ABE) fermentation is the oldest microbial production platform for acetone synthesis from organic substrates, and *Clostridium acetobutylicum* is the model strain for its production. In various wild-type acetogens and ABE-producing *Clostridium* species, acetate positively influences the synthesis of reduced products. In this work, a modified *A. woodii* strain expressing the enzymes of the acetone pathway from *C. acetobutylicum* was used to convert H_2–CO_2 streams into acetone. This study aims to assess the impact of acetate on acetone production catalyzed by such a modified A. *woodii*. Tests were carried out in serum bottles and a continuous stirred tank reactor up to a pressure of 10 bar, in gas-batch or in continuous gassing, providing different gas mixes. Outcomes indicated that acetone synthesis was stimulated when acetate concentration in the medium exceeded the threshold of 100–120 mM. Thus, acetic acid can affect acetone productivity in the modified *A. woodii* strain. This outcome should be considered in the design of fermentation processes, especially in setting up fermentations with the liquid continuous operative mode.

1. Introduction

Gas fermentation technology with acetogenic bacteria could be a sustainable alternative to the conventional fossil manufacturing industries. The synthesis of chemicals based on CO_2 serves the dual purpose of not extracting underground oil sources and removing a gaseous compound involved in global warming, contributing to achieving the 13th Sustainable Development Goal (SDG). Acetogens are versatile biocatalysts capable of naturally converting various waste gas streams rich in CO_2 and CO into valuable products, including short-chain organic acids, alcohols, and diols (Antonicelli et al., 2023; Sun et al., 2019). Running the Wood-Ljungdahl-Pathway (WPL) these bacteria can reduce CO or CO_2 to Acetyl-CoA (Schiel-Bengelsdorf and Dürre, 2012) (Fig. 1). Additionally, synthetic biol-

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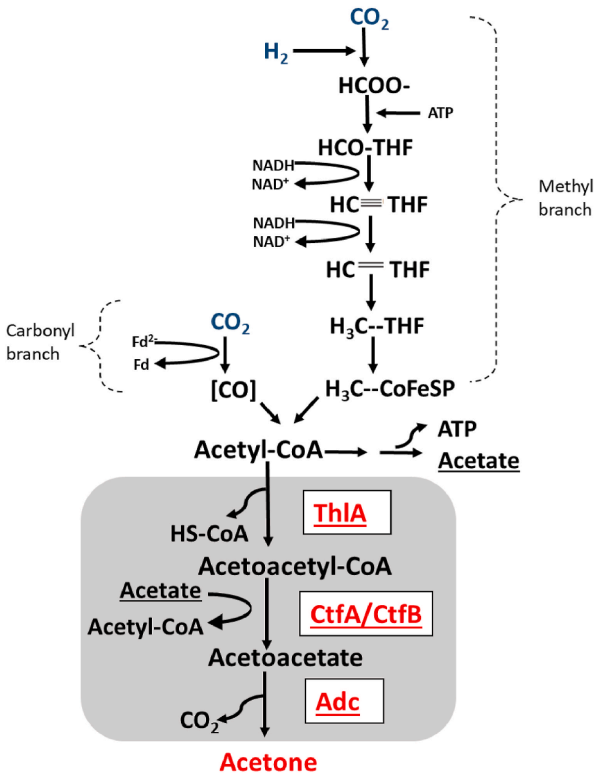

Fig. 1. Wood-Ljungdahl Pathway in *A. woodii* coupled with the acetone synthesis pathway of *Clostridium acetobutylicum* (grey square), based on (Hoffmeister et al., 2016). ThlA: thiolase A; CtfA/CtfB: acetoacetyl-CoA:acetate/butyrate CoA transferase; Adc: acetoacetate decarboxylase; THF: tetrahydrofolate, COFeSP: Corrinoid iron-sulfur protein.

ogy has enabled the production of various other compounds than the natural ones with these microorganisms (Köpke and Simpson 2020). Examples such as isopropanol and acetone have already been successfully scaled up to pilot production, utilizing mutants of *Clostridium autoethanogenum* and CO-based gaseous substrates (Liew et al., 2022). Although bioproduction from CO has been extensively researched in recent years, the valorization of CO_2-based wastes in conjunction with green H_2 has received comparatively less attention. This is primarily because growth with CO is energetically more favorable for many acetogens, especially Clostridium strains (Diender et al., 2015; Hu et al., 2011; Richter et al., 2016). In contrast, strains like *Acetobacterium woodii* demonstrate excellent potential as biocatalysts for H_2-assisted CO_2 valorization due to their high growth rate on this gaseous blend (Heffernan et al., 2020). Several metabolic engineering studies have focused on redirecting the metabolism of *A.woodii* away from natural acetate production, starting from CO_2 and H_2 substrate. *A. woodii* mutants capable of generating acetone are an example (Arslan et al., 2022; Hoffmeister et al., 2016; Tarraran et al., 2023). In all these modified strains, *Clostridium acetobutylicum*'s genes encoding enzymes involved in the pathway for acetone synthesis have been exploited. In fact, *C. acetobutylicum* is the model strain for acetone-butanol-ethanol (ABE) fermentation. ABE fermentation is the oldest microbial production platform for acetone synthesis from organic substrates, dating back to the early twentieth century. After the 1950s, with the spreading of low-cost petrochemical substrates, microbial acetone production was replaced by fossil-based processes (Sauer, 2016). Acetone is currently produced by the alkylation of benzene with propylene to make cumene, which is then subjected to an air oxidation reaction to provide phenol and acetone (Weber et al., 2014). Acetone has a wide range of applications. It is used as a chemical intermediate in manufacturing of bisphenol-A, methyl methacrylate, and aldol chemicals. It is also used as a direct solvent in various applications, including adhesives, paints and coatings, nail polish removers, printing inks, and other skincare products. Hence, advancing the development of gas fermentation processes based on CO_2 and H_2 for acetone production represents a crucial step towards establishing a sustainable circular bioeconomy. The highest reported acetone titer in the literature stands at 52 mM, which was achieved by Hoffmeister et al. (2016) by using an *A. woodii* mutant cultivated in a retentiostat bioreactor configuration. To scale up the process, it is imperative to establish a stable, steady-state acetone concentration exceeding this value by at least an order of magnitude (Liew et al., 2022). This enhancement can be achieved by acting both at the strain's molecular level and in the fermentation process's engineering. This study works on the fermentation process to assess the impact of acetate on enhancing acetone production catalyzed by a modified *A. woodii* strain expressing the heterologous pathway for acetone synthesis of *C. acetobutylicum.* Acetate positively influences the production of reduced products in various wild-type acetogens and ABE-producing *Clostridium* species. CO-based fermentation with acetate augmentation has resulted in higher ethanol production by *Clostridium autoethanogenum*, *Clostridium ljungdahlii*, and *Clostridium* sp. AWRP (Kwon et al., 2022; Schulz et al., 2023; Xu et al., 2020). Additionally, acetate addition has led to significant acetone–butanol–ethanol production in *Clostridium saccharoper-*

butylacetonicum N1-4 (Gao et al. 2015, 2016), *C. acetobutylicum* (Braun and Gottschalk, 1981), and *Clostridium beijerinckii* BA101 (Chen and Blaschek, 1999).

A.woodii can run the WLP starting from CO_2 and H_2. The pathway consists of a methyl and a carbonyl branch that converge in the synthesis of acetyl-CoA (Fig. 1). This intermediate is then converted to acetate, the main product of the WLP in the wild-type *A.woodii*. As mentioned above, the current work employs a modified *A.woodii* strain expressing the pathway for acetone synthesis from *C. acetobutylicum* (grey square in Fig. 1). This latter pathway exploits the acetyl-CoA from the WLP as the base compound to synthesize acetone. In particular, the heterologous genes inserted into the modified strain codes for the four sequential enzymes involved in the synthesis of acetone: i) Thiolase A (ThlA), which catalyzes the reaction of two acetyl-CoA into acetoacetyl-CoA; ii) CoA transferase A and B (CtfA/CtfB) that transfer the CoA moiety from the acetoacetyl-CoA to acetate; iii) Acetoacetate decarboxylase (Adc) that converts acetoacetate to acetone, by removing a C-atom as CO_2. Thus, unlike the wild-type strain, the modified *A. woodii* produces acetone besides acetate. As can be noticed, acetate is the final product of the WLP in *A. woodii* and serves as one of the essential inputs in the acetone synthesis pathway, specifically in the step catalyzed by the CoA-transferase (Lin et al., 2023; Wiesenborn et al., 1989).

The literature reports two other works in which *A. woodii* was modified by introducing the genes of the *C. acetobutylicum* acetone pathway (Arslan et al., 2022; Hoffmeister et al., 2016). Hoffmeister et al. developed a modified strain synthesizing acetone and described its metabolic performance in gas fermentations at atmospheric pressure. Working on the genetic development of the strain, Arslan and coworkers tested different combinations of genes to increase acetone production in *A. woodii* recombinant strains. The current work faces the enhancement of acetone synthesis by a modified strain working on the fermentation process rather than genetic optimization. Due to the low solubility of the gaseous substrates, the biocatalyst's activity could be limited by the low availability of dissolved substrates in the liquid medium, leading to low product yields (Van Hecke et al., 2019). Carrying on the gas fermentation at pressures higher than the atmospheric one allows more CO_2 and H_2 solubilization in the medium and should lead to higher metabolic product titer. Moreover, as described above, the acetate stimulates reduced product synthesis in clostridium species. Thus, as a first novelty, the current study grew a modified *A. woodii* strain expressing the heterologous pathway for acetone synthesis of *C. acetobutylicum* in a pressurized bioreactor (up to 10 bar) in high-pressure gas batch and continuous gassing fermentations to reach higher products concentration due to higher substrate availability. As a second novelty, the positive influence of 100 mM acetate on acetone production in the growing medium of such a modified strain was proved in batch cultures at atmospheric pressure.

Preliminary experiments to evaluate the biocatalyst performance and the pH influence were performed in serum bottles at atmospheric pressure, growing the bacteria on an H_2:CO_2 70:30 gas blend. Then, bacteria were cultured in the pressurized bioreactor at 1.1 bar (atmospheric pressure) and 10 bar. The gaseous substrates were provided in a high-pressure batch fermentation using an H_2:CO_2 70:30 mix or continuously providing the H_2:CO_2 70:30 or an H_2:N_2:CO_2 70:27:3 mixture. Finally, 100 mM of exogenous acetate was added in serum bottles to autotrophic cultures at different points of the growth curve to prove the effect induced on acetone synthesis.

2. Materials and methods

2.1. Microbial strain modification and plasmid

This work employed a genetically modified *A. woodii* strain for acetone synthesis developed as previously described (Tarraran et al., 2023). Briefly, the wild-type A. woodii strain was modified to express the TcdR sigma factor, allowing the expression of heterologous genes via the P_{tcdB} promoter. *A.woodii* DSM 1030 strain was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Germany). Applying a previously described allele exchange-based method, 300 bp at the 3′ end of *pyrE* was deleted (Baker et al., 2022). This deletion was an in-frame deletion and left the stop codon of *pyrE*. The mutant obtained grew thanks to uracil supplementation and was resistant to 5-Fluoroorotic acid. After that, *pyrE* was repaired using the pMTL-JPB23 plasmid (Baker et al., 2022) that allowed the concomitant insertion of the *tcdR* gene, coding for the sigma factor TcdR, at the 3' of the restored *pyrE*. TcdR enables the expression of heterologous genes under the control of the P_{tcdB} promoter. The *A.woodii* strain expressing the TdcR sigma factor was transformed with a plasmidic construction containing the operon composed of the genes coding for the acetone synthesis pathway of *C. acetobutylicum*. The plasmid was based on the pMTL84151 plasmid of the series pMTL80000 (Heap et al., 2009). It is a 9670 bp plasmid that contains a Gram-negative replicon and a Gram-positive replicon, ColE1 and pCD6, respectively. The selection marker is the *catP* gene from *Clostridium perfringens*, that lends resistance to thiamphenicol. Heterologous genes for acetone synthesis are controlled by the PtcB promoter, constitutively active when the plasmid is in a TcdR host. They are *cftA, cftB* (CA_P0163/CA_P0164) coding for the CoA transferase; *adc* (CA_P0165) coding for the acetoacetate decarboxylase; and *thl* (CA_C2873) coding for the thiolase (Tarraran et al., 2023). The plasmid was inserted into the cells through electroporation, and thiamphenicol was used as a selective agent in the culture medium of all the following experiments for its maintenance inside the transformed cells. The final *A.woodii* strain synthesizing acetone was named *A. woodii* $p_{acetone}$.

2.2. Cultivation media

The cultivation media used in this study were autotrophic and heterotrophic culture broths prepared and sterilized as previously described (Tarraran et al., 2023). 20 mM fructose was supplied into the heterotrophic cultures. The selective agent was thiamphenicol at a concentration of 15 mg/L.

2.3. Preparation of inocula for autotrophic fermentation

Frozen stocks (3 mL) of *A.woodii* $p_{acetone}$ grown in the heterotrophic medium added with 10% DMSO were stored at −80 °C. These bacteria were used as inoculum for the fresh culture medium prepared as follows. Serum bottles (total volume 160 ml) were filled with 30 mL of sterile heterotrophic medium and sealed with rubber caps. Subsequently, the bottles were gassed with N_2 to remove air and then 100 μL of H_2SO_4 1.5 M was added to adjust the pH to 7.2–7.3. Lastly, thiamphenicol and cysteine were added, and bottles were stored at room temperature for approximately 24 h before inoculation. The anaerobic medium was inoculated with bacteria and incubated at 30 °C (Balch et al., 1977) in an orbital shaker (Biosan, LV) until the culture reached an optical density at 600 nm (OD_{600nm}) ≈1.

2.4. Batch fermentation in autotrophic serum bottles

Serum bottles with a total volume of 160 mL were filled up with 30 mL sterile autotrophic medium and sealed with a rubber cup. After gassing with N_2 to create the anaerobic condition as described above, the feed gas blend was bubbled directly into the culture medium through a submerged needle at a rate of 14.7 L/h. Another needle was simultaneously inserted into the septum as a vent to avoid pressure build-up within the glass bottle. The gas mix supplied was 70% H_2 and 30% CO_2 (H_2:CO_2 2.33:1). Among the possible H_2:CO_2 ratios that could be provided to feed the bacterium, the current work chose this gas ratio because the first gas fermentation work that grew *A.woodii* in a pressurized bioreactor up to 3.5 bar in continuous gassing successfully provided this ratio in the feeding mix (Kantzow and Weuster-Botz, 2016). Moreover, the H_2:CO_2 2.33:1 blend was close to the ratio employed in a previous study that optimized acetic acid production exploiting *A. woodii* as the catalyst (H_2:CO_2 2.40:1) (Straub et al., 2014). After gassing, the pH self-stabilized at 7.2. Next, thiamphenicol and cysteine were added, and the bottles were stored at room temperature until use. For the inoculation, a heterotrophic culture volume was injected into the autotrophic bottle such that the final OD_{600nm} was around 0.2. Tests were conducted in a gas fed-batch mode to avoid feedstock depletion, preventing growth arrest. Gas was refilled daily. The refilling procedure was as follows: the headspace of the serum bottle was first washed with fresh 70% H_2 and 30% CO_2 mix at 14.7 L/h for 3 min. Then, the outlet needle was removed from the septum and an overpressure of ≈1.4 bar in the headspace was created by supplying the gas mixture through the inlet needle. Autotrophic cultures were incubated at 30 °C, upright, in the orbital shaker (200 rpm). During fermentations pH was checked and, when necessary, it was restored to 7–7.2 using NaOH 1 M.

2.4.1. Fermentation in serum bottles with exogenous acetate

For the fermentation with an augmented amount of exogenous acetate in the autotrophic growth, glacial acetic acid was first sterilized by filtration through a sterile PES syringe filter and then gassed with N_2. The anaerobic acetate was then added up to a concentration of approximately 100 mM to the standard autotrophic medium prepared as described in section 2.2. Adding acetic acid drops the pH of the medium, so a solution of sterile 3 M NaOH was used to raise the pH again to 7–7.2. Acetate was added to the serum bottle before inoculation or at the mid-exponential phase of the growth curve. In the first case, pH was adjusted before the inoculation. In the second case, the stock solution of acetic acid was injected gradually into the serum bottle and combined with the NaOH. This procedure allowed the maintenance of the pH of the growing culture between 6.5 and 7.2, avoiding cell stress due to fast pH changes during the growth phase. Throughout the experiments, the pH was controlled, and the gas was fed as detailed above (section 2.4). Experiments were performed in triplicates.

2.5. Fermentations in bioreactors

Atmospheric pressure fermentation (1.1 bar) and high-pressure fermentations (up to 10 bar) were conducted in a custom-adapted Continuous Stirred Tank Reactor (CSTR) manufactured by the H.E.L company (UK). The detailed description of the hardware was reported elsewhere (Tarraran et al., 2022). Briefly, the system consists of a jacketed 2 L stainless steel vessel. An oil bath (Julabo, DE) connected to the reactor jacket enables sterilization operations and control of the process temperature. Mass Flow Controllers (MFC) (Vögtlin Instruments, CH; Bronkhorst High-Tech BV, NL) allow control of the composition and in-flow rate of the gas mix provided. Gases are sparged in the medium through a micrometric sparger placed at the bottom of the vessel. Process parameters such as temperature, pH, redox, liquid level, and pressure are monitored by probes (Sentek, UK). Pression is controlled via a proportional Back Pressure Regulation (BPR) valve (Norgren, USA). pH control is carried out by a piston pump (Eldex, USA). The described reactor hardware can properly and safely work up to 11 bar pressure.

Autotropic experiments in liquid batch and continuous gas supply were performed at 1.1 bar, 1.7 bar, and at 10 bar. 0.8 L of the sterile autotrophic medium was pumped into the vessel and gassed with N_2 to remove air. Then, thiamphenicol and cysteine were added through a rubber septum. Before inoculation, a H_2:CO_2 70:30 mix was supplied at 4.5 L/h for 3 h at a constant pressure of 1.5 bar. The pH stabilized at 7.2–7.3. Inoculation of the reactor was performed by injecting a volume of a heterotrophic preculture of *A. woodii* $p_{acetone}$ such that the starting OD_{600nm} in the reactor was ≈0.2. Each test had a specific combination of feeding strategy, pressure, and gas mix supplied. Nevertheless, before setting the selected parameters, a first step of gas and liquid batch at 1.5 bar in a 70% H_2 and 30% CO_2 atmosphere was performed immediately after inoculation to allow bacteria to suit the autotrophic condition. When the OD_{600nm} inside the reactor rose to ≈0.5, parameters chosen for each test were applied. The gas feeding mode, the in-flow gas rate, the gas mix composition, and the pressure setpoint applied in each experiment are described in the dedicated results section. Fermentations were conducted at 30 °C and 400 rpm. pH was controlled at 7 with NaOH 3.5 M.

2.6. Analytical methods and calculation

Acetate, acetone, formate, and fructose were quantified using the high-performance liquid chromatography (HPLC). Samples of 2.5 mL, collected from the reactor's vessel or serum bottles, were filtered using a 0.22 μm PES syringe filter and placed in vials for

HPLC analysis. The analysis was performed using a Prominence HPLC System (Shimadzu, JP) equipped with a Refractive Index Detector and a Diode Array Detector. Compounds were separated applying 0.7 mL/min of H_2SO_4 0.005 M as the mobile phase in a Resez ROA-Organic Acid column (Phenomenex, USA) maintained at 50 °C.

Bacterial growth was assessed by measuring optical density at λ = 600 nm (OD_{600nm}) using either a V-730 Spectrophotometer (Jasco, JP) or a DH-5000 spectrophotometer (HACH, USA). Distilled water was used as a blank reference. To determine the dry cell weight (DCW) in autotrophic fermentation, a correlation factor of 0.38 (data not shown) was estimated.

The volumetric productivity (r) for a target metabolic product was calculated as follows:

$$r_p = \Delta c_p/\Delta t \quad (1)$$

where r_p is the volumetric productivity of the target product ($g_{product} * L^{-1} * h^{-1}$); t is the time (h) between two sampling points of the fermentation; c_p is the increase of product concentration ($g*L^{-1}$) between the two selected sampling points.

The specific productivity (q) for a target metabolic product was calculated as follows:

$$q_p = (1/c_{xav})*(\Delta c_p)/\Delta t \quad (2)$$

where q_p is the specific productivity of the target product ($g_{product} * g_{biomass\ (DCW)}{}^{-1} * h^{-1}$); t is the time (h) between two sampling points of the fermentation; c_{xav} is the biomass concentration average ($g*L^{-1}$) between the two selected sampling points; c_p is the increase of product concentration ($g*L^{-1}$) during the period considered.

Yields were calculated as follows:

$$Y_p\ (\%) = (g_p/\ g_{CO2inlet})*100 \quad (3)$$

where Yp is the yield of a specific product; g_p is the grams of the specific product synthetized; $g_{CO2inlet}$ is the total grams of the CO_2 provided throughout the experiments. For the experiments conducted in the bioreactor $g_{CO2inlet}$ consisted of the CO_2 supplied in the gas and liquid batch step at the beginning of the fermentation and the step with the continuous gassing.

Considering the reactor vessel or the serum bottle as the system, the CO_2 trapped in any form in it could be considered CO_2 converted because it is provided to the system and not lost from its outlet. Nevertheless, not all the CO_2 inside the system is effectively converted into metabolic products. Indeed, a variable part of it is still available in the reactor vessel or in the serum bottle, dissolved in the liquid (as CO_2 and as HCO_3^-) or in the headspace, ready to be consumed by bacteria, but still not consumed. If microorganisms don't immediately consume the CO_2 provided, the fraction of this gas available for metabolism but not already converted increases by increasing the pressure. For this reason, the conversion of the metabolized CO_2 into a specific metabolic product p was calculated. This parameter highlights the metabolic pathway in which the CO_2 is effectively addressed in the operating condition tested. The formula for the calculus is reported below:

$$(\text{mol } CO_{2\ \text{into p}} / \sum \text{mol } CO_{2\ \text{into acetate, formate, acetone, biomass}}) * 100 \quad (4)$$

where mol $CO_{2\ \text{into p}}$ is the mol of CO_2 embedded in a specific synthetized product p; $\sum$ mol $CO_{2\ \text{into acetate, formate, acetone, biomass}}$ is the sum of the moles of CO_2 converted in each main metabolic product (acetate, acetone, formate, and biomass).

3. Results and discussion

3.1. Modified A. woodii fermentation in serum bottles

This study employed a modified *A.woodii* strain harboring the acetone synthesis pathway (*A.woodii* $p_{acetone}$). The plasmidic construction is based on the pML84151 vector, and the genes inserted were from *C. acetobutylicum*. The P_{tcB} promoter from *C. difficile* controlled the acetone operon. *A.woodii* $p_{acetone}$ was grown autotrophically in batch fermentations in serum bottles to evaluate its growth and production performance in this setting with and without pH control. 30 ml of culture volume was fed with a H_2:CO_2 70:30 blend using a gas-fed batch strategy. The gas consumed was refilled up to around 1.4 bar daily to avoid cell starvation due to feedstock depletion. Fig. 2 displays the outcomes of the tests. In particular, Fig. 2A and B report the growth, measured as the optical density of the culture (OD_{600nm}), and the metabolic profile of *A.woodii* $p_{acetone}$ cultured without pH control. Fig. 2C and D report the growth and the metabolic profile of *A.woodii* $p_{acetone}$ cultured controlling the pH at ≈ 6.9 using NaOH.

As shown in Fig. 2A, without base control, the pH of the growing culture dropped from 7 to 5.7. Bacteria growth stopped when pH fell below ≈6. *A.woodii* $p_{acetone}$ main metabolic product was acetic acid (Fig. 2B). Its production started immediately after the inoculation and then quickly ceased after 45 h of fermentation when the pH dropped, stabilizing at a stable value around 60 mM (final concentration at 138.5 h = 63.1 ± 9.7 mM). Acetone synthesis started about 24 h after the inoculation and had a trend like acetate (Fig. 2B). It stabilized around 2.6 mM, reaching its maximum concentration of 3.1 ± 0.5 mM at 116 h of fermentation. Controlling the pH around 6.9 with NaOH during the fermentation, the measured OD_{600nm} value reached a stable value at around 1 (Fig. 2C). Nevertheless, when OD_{600nm} was >1 in serum bottles, biomass flocculation could be observed. Thus, it was not possible to get a reliable OD_{600nm} measurement. Nevertheless, the final acetate concentration was 2.5-fold higher (162.1 ± 4.7 mM) (Fig. 2D) than in culture without pH control (Fig. 2B). Bacteria started making acetone about 24 h after inoculation (Fig. 2D). However, this time, the production trend was particular. In fact, acetone synthesis began; when acetate concentration in the medium was about 90–100 mM (after 90 h from the beginning of the test), acetone stopped at 3.8 mM ± 0.12 mM and remained stable around this value (red square in Fig. 2D). Finally, acetate overcame 100–120 mM, and acetone production resumed. The final acetone concentration was

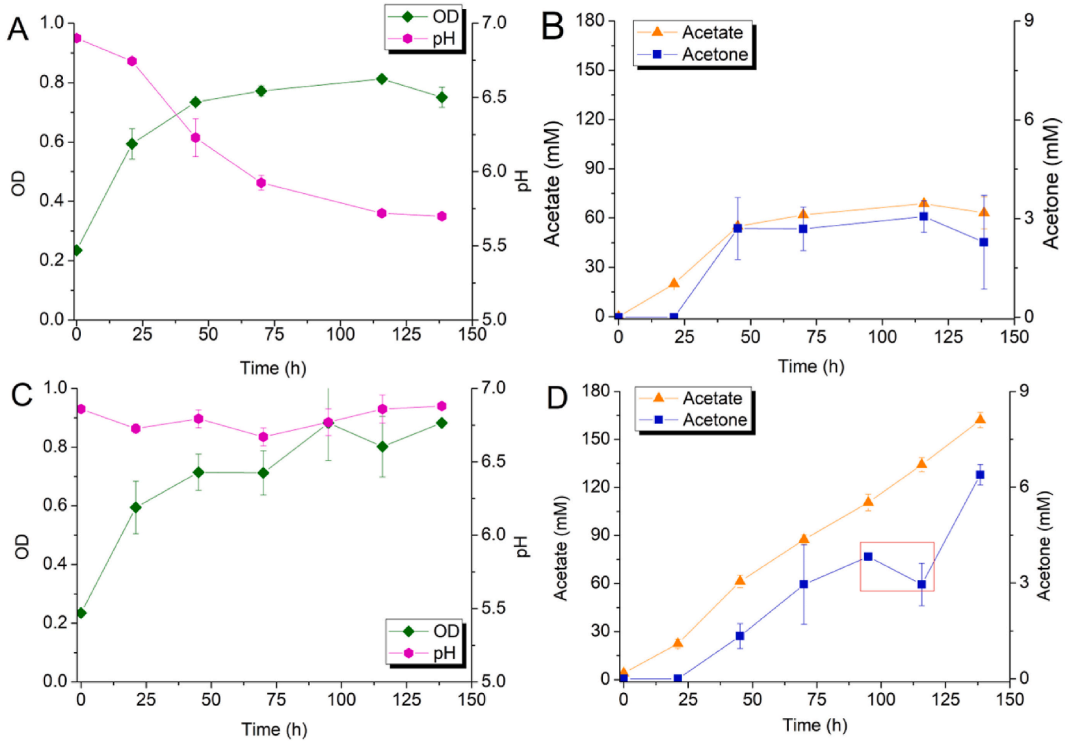

Fig. 2. *A.woodii* $p_{acetone}$ growth and main products synthesis profile in serum bottles in gas-fed batch. A) growth and pH trend of the culture grown without pH control; B) metabolic profile of the culture grown without pH control; C) growth and pH trend of the culture grown with pH control using NaOH; D) metabolic profile of the culture grown with pH control using NaOH.

6.4 ± 0.3 mM. Considering the fermentation conducted by controlling the pH, the acetone yield was 2.3 %, while the acetate yield was 60.9 %. 5.4 % of the CO_2 converted into metabolic products (see section 2.6) was addressed to synthetize acetone, and 91.7 % to synthetize acetate.

Demler and Weuster-Botz (2011) studied the influence of the pH on wild-type *A.woodii* grown in a bioreactor in liquid batch and continuous gassing operative mode. Authors reported that acetate production started immediately after inoculation but stopped when the culture medium pH dropped below 6. Tests performed in the current study showed a similar behavior of *A. woodii* $p_{acetone}$ in liquid batch and fed-batch gassing mode.

In a previous study of Hoffmeister et al. (2016), a modified *A.woodii* strain for acetone production was developed and grown in serum bottles. The backbone plasmid and the acetone pathway genes were from the same microorganism of the current study, but they were controlled by the thiolase A promoter region from *C. acetobutylicum*. The authors reported a max acetate concentration of 156.3 ± 3.3 mM and max acetone concentration of 13 ± 3.3 mM. Thus, the acetate concentration reached is similar to the one achieved by the strain in the current work, controlling the pH of the culture medium. Acetone concentration, instead, was higher. Nevertheless, the duration of the fermentation was longer than the current study (1000 h vs 138.5 h, respectively). More recently, Arslan and coworkers (2022) again modified *A.woodii* with *C. acetobutylicum*'s acetone pathway genes. They used the backbone plasmid pJIR750, and the thiolase A promoter region of *C. acetobutylicum* controlled the genes. Even with a different backbone plasmid than the current study, the final acetate concentration reached was 156.1 mM. Similarly to *A.woodii* $p_{acetone}$, the strain employed by Arslan et al. showed a lag phase in acetone production at the beginning of the growth curve. It reached a steady concentration of about 4.0 mM after about 100 h of fermentation.

3.2. Modified A. woodii fermentations in the bioreactor

Following gas-fed batch tests in serum bottles, the *A. woodii* $p_{acetone}$ strain was cultured in a bioreactor with a continuous gas supply. Experiments were carried out at atmospheric (≈1 bar) and high pressure, varying the gas mixture fed. Gas-fermentation processes at pressures above 1 bar leads to an increase in the concentration of the dissolved gaseous species in the liquid phase, avoiding cell starvation due to feedstock depletion (Kantzow and Weuster-Botz 2016; Tarraran et al., 2023). Specifically, the maximum pressure applied in this work was 10 bar, and the gas blends provided were H_2:CO_2 70:30 or H_2:CO_2:N_2 70:3:27.

3.2.1. Fermentation of A. woodii $p_{acetone}$ strain at atmospheric pressure in a CSTR

A. woodii $p_{acetone}$ was grown at 1.1 bar in a bioreactor in liquid batch and providing a 70 % H_2 and 30% CO_2 blend. As shown in Fig. 3, the first step of gas and liquid batch was performed for 24 h after inoculation to allow the cells suit to the new environment (see section 2.5). Then the reactor was continuously fed with 5.3 L/h of the 70 % H_2 and 30% CO_2 mix. The maximum OD_{600nm} was

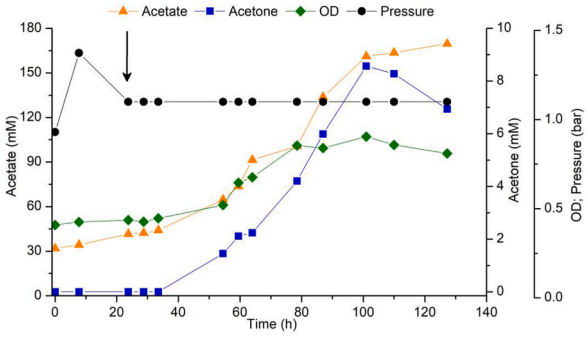

Fig. 3. Growth curve and main metabolic products of *A. woodii* $p_{acetone}$ in autotrophy at a pressure of 1.1 bar, in liquid batch and continuous gassing (70:30H_2:CO_2). The arrow indicates the initiation of the continuous gassing.

0.9 after 77.25 h. No biomass flocculation was observed inside the bioreactor. Acetic acid synthesis started slowly immediately after the inoculation and increased during the exponential phase, reaching a final concentration of 169.7 mM at the end of the fermentation. Acetone first appeared after 34 h and got a maximum concentration of 8.6 mM after 101 h. Then, it slightly decreased to 6.9 mM. Interestingly, the acetone production trend flattened in the middle of the exponential phase of growth (hours 59–64), when acetate concentration was ≈75 mM, and then rapidly resumed when the acetate concentration overcame 100 mM.

Before the stalemate (hours 59–64), the max acetone specific productivity (max $q_{acetone}$) was 34.4 $mg_{acetone}$* $g_{biomass}^{-1}$*h^{-1}. When the production resumed, the $q_{acetone}$ reached a higher value (37.7 $mg_{acetone}$* $g_{biomass}^{-1}$*h^{-1}). The maximum acetone volumetric productivity (max $r_{acetone}$) was 12.1 $mg_{acetone}$* L^{-1}*h^{-1} and was recorded after the stalemate. Considering the CO_2 metabolized by the bacteria (see section 2.6) 87.3 % was converted into acetate, 5.2 % in acetone. Nevertheless, according to the carbon balance, most of the CO_2 provided was not converted and lost from the reactor outlet. Acetone yield was 0.1 %, while acetate yield was 2.5%.

Prior to this work, Hoffmeister et al. (2016) and Arslan et al. (2022) addressed the topic of acetone production with modified *A. woodii* strains grown in a bioreactor at atmospheric pressure. Table 1 compares the main process parameters and outcomes of the mentioned studies and the current one. As shown in the table, Hoffmeister and colleagues reached the highest acetone concentration (16.5 mM). They did not mention a delay in acetone production at the beginning of the process. However, they reported that the highest acetone volumetric productivity (3.8 $mg_{acetone}$* L^{-1}*h^{-1}) was obtained when acetate in medium exceeded 1200 mM. The present study and the best-performing strain of Arslan et al. achieved similar maximum acetone concentrations (8.6 mM and 7.6 mM, respectively). Moreover, the authors of the latter study described that both strains listed in Table 1 had delayed acetone production. In *A. woodii* [pMTL84151_atc_{thlA}] culture, the compound was detected after 100 h of fermentation, when acetic acid concentration was 86 mM. In *A. woodii* [pJIR750_ac1t1], acetone was detected after 135 h of fermentation, when acetate in the culture medium was 165–200 mM (Arslan et al., 2022). Therefore, the modified strains developed by Arslan et al. displayed a common behavior with the strain of the current study. The acetate threshold found by Hoffmeister and coworkers was far higher than the current one (100 mM) or those described by Arslan et al. (86 mM, ≈180 mM). An *in vitro* study on the activity of the purified CoA transferase of *C. acetobutylicum* reported a k_m = 1200 mM for acetate (Wiesenborn et al., 1989). This result agrees well with the results of Hoffmeister and colleagues. However, outcomes of the latter study, the current work, and Arslan et al. referred to *in vivo* tests, in which the localization of the heterologous enzymes was intracellular, and the acetate concentration was unknown.

3.2.2. *Fermentation of A. woodii $p_{acetone}$ strain at 10 bar pressure in a CSTR*

After the atmospheric pressure tests, high-pressure experiments were performed. The gas-feed H_2:CO_2 70:30 was provided first in a batch mode up to 10 bar (high-pressure batch starts at t = 15.5 h in Fig. 4). Successively, when the pressure fell to 4 bar, it was restored at 10 bar, and a continuous gassing mode was applied at a flow rate of 4.95 L/h (t = 30.5 h in Fig. 4). At the beginning of the high-pressure gas batch, bacteria had a short lag phase of about 4 h (from t = 15.5 to t = 19.3 h in Fig. 4), stopping growth and acetate production. Then, the metabolic activity resumed, and biomass and acetate concentrations quickly increased. In the following 10 h of fermentation, pressure decreased from 10 bar to 4 bar. At the end of the gas-batch step, 30.5 h from the beginning of the experiment, acetate concentration was 101.1 mM and acetone 1.8 mM. Later on, the gaseous substrate was supplied continuously to avoid feedstock depletion, and from 33.5 h of fermentation on, the reactor pressurewas kept constant at 10 bar. The immediate consequence of the change in the feeding mode was an enhanced synthesis of formic acid. Its synthesis started at t = 33.5 h (Fig. 4) and kept an always increasing trend, reaching the final concentration of 127.0 mM (t = 58.3). As previously discussed (Kantzow and Weuster-Botz 2016; Tarraran et al., 2023), enhanced CO_2 availability in the liquid phase led to a metabolic impairment in *A.woodii* cells, resulting in formic acid production. Cells stopped growing. Nevertheless, besides formate, acetate production continued, arriving at 162.8 mM at the end of the test. Concerning acetone, the production trend followed the same behavior described above (sections 3.1 and 3.2.1). At the beginning of the fermentation, its production was delayed. It was detected after 30.5 h. Subsequently, from t = 33.5 h to t = 36 h, it had a block while acetate production was still going on, and then quickly resumed up to a final concentration of 8.5 mM (Fig. 4).

The final acetate and acetone concentrations obtained in the 10-bar pressure test were congruent to the experiment at atmospheric pressure (section 3.2.1). Nevertheless, in the high-pressure fermentation, the same amount of these products was reached in almost half time (127 h vs 58 h). The max $q_{acetone}$ was recorded when acetate concentration was between 120 and 140 mM and was about

Table 1
Comparison of parameters and results between studies employing modified *A. woodii* strains for acetone production in stirred tank bioreactors in continuous gas supply, at atmospheric pressure. r = volumetric productivity; q = cell-specific productivity; P_{tcdB} = TcdB promoter; P_{thlA} = ThlA promoter.

Study	Modified A. woodii strain name	Promoter; Backbone plasmid	Dealy in acetone synthesis	In-flow gas rate (L/h)	Stirring speed (rpm)	Gas mix H_2:CO_2:N_2	Initial OD	Max OD	Max acetate (mM)	Max acetone (mM)	Max $r_{acetone}$ ($mg*L^{-1}*h^{-1}$)	Max $q_{acetone}$ ($mg*g^{-1}*h^{-1}$)	Duration (h)
This study	*A. woodii* $p_{acetone}$	P_{tcdB}; pMTL84151	Yes	5.3	400	70:30:0	0.4	0.9	168	8.6	12.1	37.7	127
Hoffmeister et al. (2016)	*A.woodii* [pMTL84151_atc_{thlA}]	P_{thlA}; pMTL84151	–	30	800	20:80:0	–	2.2	1330	16.5	3.8	–	357
Arslan et al. (2022)	*A.woodii* [pMTL84151_atc_{thlA}]	P_{thlA}; pMTL84151	Yes	0.6	250	35:25:40	–	2.1	302	3.2	–	–	375
Arslan et al. (2022)	*A.woodii* [pJIR750_ac1t1]	P_{thlA}; pJIR750	Yes	0.6	250	35:25:40	–	2.5	438	7.6	–	–	500

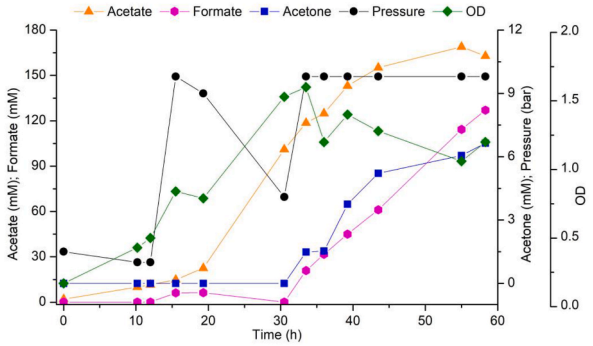

Fig. 4. Pressure trend, growth curve, and main metabolic products of *A. woodii* $p_{acetone}$ in autotrophy (70:30H_2:CO_2) at 10 bar, fed with gas-batch mode and subsequently in continuous gassing mode.

two-fold the one calculated in the experiment at atmospheric pressure (80.4 $mg_{acetone}$* $g_{biomass}^{-1}$*h^{-1} vs 37.7 $mg_{acetone}$* $g_{biomass}^{-1}$*h^{-1}, respectively). However, formic acid was also produced in a high amount in the high-pressure fermentation with continuous gas supply. The shift of the metabolic spectrum is highlighted by the distribution of the metabolized CO_2 in the gas and liquid batch step (before t = 30.5 h) with respect to the continuous gassing step (after t = 30.5 h). In the gas and liquid batch, the greater fraction of the metabolized CO_2 was addressed to acetate synthesis (93.8%) and, to lesser extent, to acetone (3.1%). In the high-pressure continuous gassing step, instead, the greater part of the metabolized CO_2 was split between the synthesis of acetic and formic acid (46% and 46.7%, respectively). Nevertheless, the metabolized CO_2 converted into acetone increased (7.4%). Acetone yield was 0.5% and acetate yield was 16.2%. Both values are at least five-fold higher than the yields obtained conducting the process at atmospheric pressure (0.1 % and 2.5%, respectively). Nevertheless, at the end of the fermentation at high pressure, formic acid was also detected: its yield was 3%.

To diminish formic acid production at high pressure, a second experiment was performed, reducing the CO_2 partial pressure (pP_{CO2}) by experimenting with the same maximum system's total pressure (10 bar) but partially replacing the CO_2 in the supplied mix with N_2. Fig. 5 shows the experiment. It was divided into two consecutive steps of continuous gassing at different total pressures: 10 bar and 1.7 bar. In the first step, from t = 24.7 h to t = 67 h, the gas blend H_2:CO_2:N_2 70:3:27 was provided at 5.3 L/h at 10 bar. Afterward, the same feeding mix was provided with the same in-flow gas rate but decreasing the system's total pressure to 1.7 bar (from t = 69.5 h to t = 114 h).

Differently from the outcomes of the previous high-pressure test conducted by providing the 70:30 H_2:CO_2 mix in continuous gassing, supplying continuously the 70:3:27 H_2:CO_2:N_2 blend at 10 bar (t = 24.7 h to t = 67 h), the biomass grew to OD_{600nm} up to 1.3. Formic acid was detected in a low amount after 40 h of high-pressure fermentation (t = 67 h). Moreover, it was wholly re-metabolized in a few hours at the beginning of the following 1.7-bar-step (from t = 69.5 h to t = 73.7 h). These results suggested that decreasing the pP_{CO2} allows for addressing the metabolite's spectrum away from formic acid production (Tarraran et al., 2023). Acetic acid synthesis started immediately after the inoculation and was boosted during the 10-bar-step with respect to the following 1.7-bar-step, probably thanks to the higher availability of the gaseous substrates in the liquid phase. The final concentration was 199.5 after 114 h of fermentation. Again, as previously seen, acetone production started about 24 h after the inoculation and displayed a short plateau that was overcome when acetate concentration was 130 mM (from t = 69.5 h to t = 73.7 h). Final acetone concentration was 14.9 mM. The max $q_{acetone}$ in the 10-bar-step was 66.3 $mg_{acetone}$* $g_{biomass}^{-1}$*h^{-1}, while in the 1.7-bar-step it was 25.7 $mg_{acetone}$* $g_{biomass}^{-1}$*h^{-1}. Nevertheless, the lowering of the acetone specific productivity was expected because the amount of dissolved gas in the liquid medium at 1.7 bar was lower than at 10 bar.

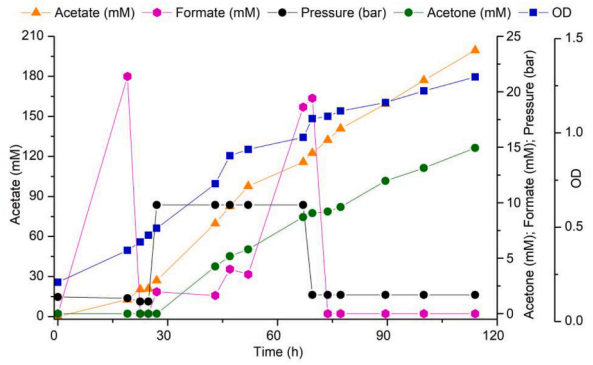

Fig. 5. Pressure trend, growth curve, and main metabolic products of *A. woodii* $p_{acetone}$ in autotrophy (70:3:27H_2:CO_2:N_2) at 10 bar and 1.7 bar, fed in continuous gassing mode.

As expected, lowering the amount of CO_2 in the gas mix led to addressing the metabolic spectrum towards acetic acid and acetone rather than formic acid synthesis. In the 10-bar-step, 86 % of the metabolized CO_2 was converted into acetic acid and 8.6 % into acetone. Only 6.9 % was embedded into formic acid, while at the same pressure in the previous experiment (70:30 H_2:CO_2 mix), it was 46.7 %. At the end of the experiment (t = 114 h), the acetone yield was 2.2 % and the acetate yield was 28.8 %. Formic acid was completely re-metabolized and not detected in the culture broth. To the best of the author's knowledge, the highest acetone concentration reported in the literature for a modified *A.woodii* strain for acetone production cultured in a bioreactor in liquid batch with continuous gassing was 16.5 mM (Hoffmeister et al., 2016). The maximum acetone concentration reached in the latter experimental setting was a close value: 14.9 mM. Nevertheless, in the work of Hoffmeister et al. (2016), the fermentation lasted 357 h, whereas in this case, it was faster: 114 h.

Results obtained in the current work in the bioreactor at high pressure and atmospheric pressure, besides literature data, suggest a link in *A. woodii* strains modified with genes of *C. acetobutylicum* for acetone synthesis between acetate concentration in the medium and acetone production, independently from the operative mode of the process, pressure, gas-feeding mode, and gas mix applied.

3.3. Tests of acetate influence on acetone production

Results from experiments described in sections 3.1 and 3.2 suggested that by overcoming the ≈100 mM acetate threshold in the medium, acetone synthesis was boosted. To confirm this observation, an experiment in serum bottles was set up, in which 100 mM of exogenous acetate was added to the autotrophic medium immediately before inoculum injection or when cells were in the exponential phase. Acetic acid addition leads to an acidification of the medium. Thus, the pH was raised again to 7 using NaOH to avoid a difference in cell growth due to the different pH of the culture. Moreover, pH was checked and adjusted at pH = 7 daily throughout the experiment. The gas mix fed was 70% H_2 and 30% CO_2. Every 24 h, the gas was refilled to avoid the block of the growth due to feedstock depletion.

The first test was carried on for 183 h and exogenous acetate was added before inoculation. Thus, ≈80 mM acetic acid was present in the culture broth from the beginning of the growth curve (Fig. 6). The concentrations of acetate and acetone at the end of the fermentation were 222.9 mM ± 4.3 mM and 9.8 mM ± 0.89 mM, respectively. As observed previously (section 3.1), and not expected this time, there was still a stalemate of acetone production between acetate concentrations ranging from 125 to 150 mM (from t = 41 to t = 67). Nevertheless, an interesting difference could be noted at the beginning of the growth curve: synthesis of acetone started immediately after the inoculation.

In a second experiment, exogenous acetate was added during the exponential phase of the growth curve (t = 23 h in Fig. 7). As the above-described one, this test was 183 h long. The final acetate concentration achieved 256.2 mM ± 35.1 mM, while the final acetone concentration was 21.5 mM ± 5.1 mM. Thus, adding acetate in the culture medium at the mid-exponential phase allowed the enhancement of acetone concentration two-fold than adding acetate at the beginning of the experiment. Interestingly, acetone synthesis didn't start immediately. It was detected after 41 h of fermentation. However, once it started, its production kept an increasing trend throughout the experiment (from t = 41 to t = 183).

Bacteria growth was not inhibited by adding ≈100 mM nor at the beginning of the growth curve neither at the middle exponential phase, showing superimposable growth trends (data not shown). The max acetone volumetric productivities adding acetate at the beginning of the growth curve was 5.1 $mg_{acetone} * L^{-1} * h^{-1}$ (acetate concentration ≈ 110 mM) while it was 9.4 $mg_{acetone} * L^{-1} * h^{-1}$ adding acetate in the mid-exponential phase (acetate concentration ≈ 250 mM). Considering the fermentation that achieved better results (exogenous acetate added in the mid-exponential phase), the acetone yield was 4.6 %, while the acetate yield was 52.1 %. 12.3 % of the metabolized CO_2 was embedded into acetone and 81.3 % into acetate. As discussed above (see section 3.1), when conducting the fermentation in serum bottles without adding exogenous acetate in the medium, 5.4 % and 91.7 % of the metabolized CO_2 were in acetone and acetate, respectively. Thus, the addiction of exogenous acetate addressed the metabolic flow within the cells toward the synthesis of acetone.

Among the heterologous genes for acetone production inserted in *A.woodii*, the CoA transferase of *C. acetobutylicum* is the enzyme with the higher K_m for its substrate (Ballongue et al., 1985; Wiesenborn et al. 1988, 1989) and could be the bottleneck of the pathway. Results obtained in the latter tests are similar to the behavior reported by Arslan and coworkers (2022), who brought acetone synthe-

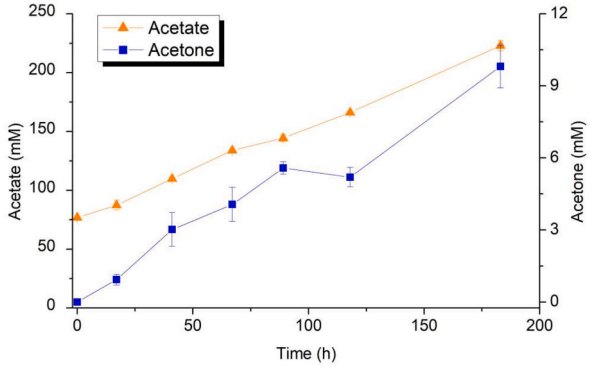

Fig. 6. Acetate and acetone production by *A.woodii* $p_{acetone}$ in serum bottles with exogenous acetate added before inoculation.

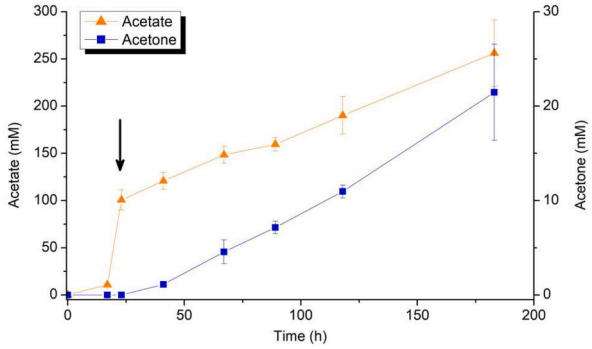

Fig. 7. Acetate and acetone production by *A.woodii* $p_{acetone}$ in serum bottles with exogenous acetate added in the exponential phase. The arrow indicates acetate addition.

sis forward in a modified *A.woodii* by replacing the *cftA/B* of *C. acetobutylicum* with the genes coding for the CoA transferase from *C. aceticum* in the genetic construction used to transform the host. The latter enzyme shows a lower k_m (≈40 mM) for acetate than CtfA/B from *C. acetobutylicum*. As a consequence, the authors reported that the delay in the acetone synthesis at the beginning of the fermentation was removed both in experiments in serum bottles and in the bioreactor. This result is consistent with the outcomes obtained in the current study adding 100 mM of exogenous acetate in the culture medium before inoculation. The present work, working on the process rather than on the genetic construct, suggests that is feasible to increase acetone synthesis by designing a process which allows the maintenance of the acetate concentration in the culture medium above ≈100 mM. This aspect should be especially considered in setting up fermentation with the liquid continuous operative mode. While changing the medium, the outflow rate of the spent culture broth from the vessel should be set so as not to fall below the threshold acetate concentration that enhances acetone production. Concerning *C. acetobutylicum's* genes, this concentration could be around 100 mM. Nonetheless, this should also be considered when *A. woodii* is modified with genes from other ABE species. For instance, in the aforementioned work by Arslan and coworkers exploiting *A. woodii* modified with *C. aceticum's* genes, the same phenomenon could occur at a lower acetate concentration (Arslan et al., 2022).

Despite the efforts and findings, the acetone yields reported in the present study were relatively low and suggest that significant genetic and process development still needs to be done to exploit *A. woodii* as a producer of this compound on H_2–CO_2 mixtures. Nevertheless, given the metabolic connection between the three molecules, this bacterium can be used for parallel acetate and formic acid production besides acetone. In this perspective, the results of the current work were the base for an environmental impact analysis and a business plan (available online: https://cordis.europa.eu/project/id/760994/results) of a process that considers acetone, acetate, and formate as products. This analysis pointed out that the scalability of the process is mainly related to the production of green hydrogen. While renewable electricity usage significantly improves environmental impact compared to traditional methods, a significant portion of the energy required for the bioprocess is linked to producing green hydrogen via electrolyzers. Thus, technological advancements that reduce the cost of green hydrogen production could significantly enhance the scalability and feasibility of the process. Challenges for scalability include high energy consumption of bioreactors and the need for economically sustainable approaches. Adoption of renewable energy sources is crucial but requires substantial investment and technological development. Therefore, while technology holds significant potential for CO_2 emission reduction, its large-scale development requires careful consideration of economic and environmental implications.

4. Conclusions

Utilizing CO_2 as a raw material to produce industrial compounds and avoiding its release into the atmosphere in gaseous form is a strategy to combat global warming (SDG 13). Acetone is a raw material deeply used in the chemical industry and its global demand is increasing. Acetogenic bacteria can produce short-chain organic acids, and alcohols using CO_2 and/or CO with H_2 as the carbon and energy source via the Wood-Ljungdahl pathway. In particular, the acetogenic bacterium *Acetobaterium woodii* is among the best performing bacteria on CO_2 and H_2 blends. The current study utilized a modified *A. woodii* expressing the acetone pathway enzymes from one of the best-known ABE strains: *Clostridium acetobutylicum*. The strain was named *A.woodii* $p_{acetone}$. Often low product concentrations are reported for gas fermentation, especially for compounds that are not naturally produced. In modified strains, target product synthesis can be enhanced by acting both at the strain's molecular level and in the fermentation process's engineering. This study focused on optimizing the process to improve acetone production from H_2–CO_2 catalyzed by *A.woodii* $p_{acetone}$. It assessed the positive effect of acetate concentration in the culture broth on the acetone production in such a modified strain.

Increasing the system's pressure leads to an increased concentration of gaseous substrates in the liquid medium and, consequently, it should allow higher synthesis of metabolic products. Thus, the current work experimented at atmospheric and high pressure (up to 10 bar). The catalyst's performance was first tested at atmospheric pressure in serum bottles using a gas-fed-batch strategy to prevent bacteria growth from ceasing due to feedstock depletion. Then, experiments in the bioreactor were conducted at atmospheric pressure and high pressure. Tests were performed with different gas feeding strategies: high-pressure gas batch and continuous gassing. Fermentations in continuous gassing were conducted by providing two diverse gas blends. In all experiments, results indicated that

acetone synthesis was stimulated when acetate concentration was above ≈100–120 mM, regardless of the setup (serum bottles or bioreactor), the operative mode applied (gas fed-batch, batch, or continuously supplied), the gas mix and the pressure.

To confirm this observation, an experiment in serum bottles was set up in which 100 mM of exogenous acetate was added to the autotrophic medium immediately before the inoculum injection or when cells were in the exponential phase. Results indicated that acetate synthesis started immediately when acetate was added before inoculation. A similar results was previously described in the literature using a modified *A.woodii* strain for acetone production in which the genes coding for the CoA transferase of *C. acetobutylicum* were replaced by genes from *C. aceticum* (Arslan et al., 2022), suggesting that the second enzyme of the acetone pathway could be responsible for this outcome. Nonetheless, when exogenous acetate was added to the medium in the serum bottles during the exponential phase of the growth curve, acetone volumetric productivity increased. The final concentration was 21.5 mM ± 5.1 mM, which is the highest concentration reported for a modified *A. woodii* strain for acetone production in serum bottles. Thus, acetate concentration in the medium can positively affect acetone productivity in the *A. woodii* $p_{acetone}$. This outcome should be considered in the fermentation process's engineering.

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CRediT authorship contribution statement

Loredana Tarraran: Conceptualization, Data curation, Investigation, Methodology, Resources, Visualization, Writing – original draft, Writing – review & editing. **Francesca Demichelis:** Data curation, Resources, Visualization, Writing – original draft, Writing – review & editing. **Valeria Agostino:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **Nicolò S. Vasile:** Data curation, Software. **Jonathan Baker:** Investigation, Writing – review & editing. **James Millard:** Investigation, Writing – review & editing. **Nigel P. Minton:** Funding acquisition, Supervision. **Candido F. Pirri:** Funding acquisition, Supervision. **Debora Fino:** Funding acquisition, Supervision. **Guido Saracco:** Funding acquisition, Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Debora Fino reports financial support was provided by Polytechnic of Turin. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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