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CO₂ valorization by a new microbiological process

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ABSTRACT

Carbon dioxide constitutes one of the largest carbon feedstocks for fuel production. This work demonstrates that an environmental bacterial consortium grown on methane is able to directly and selectively reduce CO₂ into formate. The conversion was carried out at 30°C and atmospheric pressure, without any the addition of organic molecules (such as cofactor), photons or H₂ to the reaction medium. When exposed to a CO₂:air (1:1 v/v) mixture, the consortium was able to produce 280 ± 10 mg g_{dry cell}⁻¹ of formate in 15 days. A control experiment performed solely with air coupled to NMR analysis suggested that at least 17% of the formate was produced from direct CO₂ reduction. The formate produced could be used as a precursor for methanol production from methane by methanotrophic bacteria or it could be acidified to feed Direct Formic Acid Fuel Cells.

1. Introduction

Carbon dioxide (CO₂) accounts for more than 75% of global greenhouse gas (GHG) emissions, with annual anthropogenic emissions of about 25 to 35 Gt [1]. These emissions, which today represent around 65% of GHG emissions in CO₂ equivalents [1], derive mainly from heavy and energy producing industries (cement plants, aluminum and steel production sites, coal and oil-fired power plants), as well as transport [1,2]. Use of CO₂ as a raw material at the industrial level is currently being studied as a possible solution to reduce levels of this gas in the atmosphere. However, today, industrially, CO₂ is mainly used as a fluid rather than a reactant and it originates mainly from natural sources or fermentation plants, its industrial use representing less than 1% (i.e. 100 Mt/yr) of annual emissions [2]. Indeed, industrial use of the gas as a chemical reactant is currently very limited and based on the affinity of some nucleophilic molecules for CO₂ to form urea, carbamates and cyclic organic carbonates [3].

In this context, over the last fifty years direct conversion of anthropogenic CO₂ into alternative fuels (such as methane, C₁–C₅ alcohols or formic acid) has become a major research topic because it could help solve two major future challenges, namely climate change and fossil fuel shortages. Nevertheless, activation of CO₂ molecules is very difficult because dissociation of the C=O bond requires a large amount of energy (i.e. 795 kJ mol⁻¹). Activation of CO₂ therefore requires efficient and selective catalysts but also, quite often, relatively high temperatures and pressures.

Both homogeneous and heterogeneous catalysts have been reported

to catalyze hydrogenation reactions [2,4,5], artificial photosynthesis [6–10] and electrochemical reduction [3,11,12], giving rise to fuels and molecule platforms from CO₂. However, despite recent progress, these chemical conversion routes still exhibit low durability and/or selectivity [6,13–15].

The biological processes for CO₂ activation are interesting because they are usually selective and require mild conditions. These biological processes are considered sustainable as they are low-energy-consuming and reagent use and/or by-product release that could have a negative environmental impact is generally limited. Currently, only processes implementing photosynthetic microorganisms such as microalgae or cyanobacteria have been developed at the industrial scale. The main products recovered from CO₂ valorization by these processes are lipids which can be then converted into biodiesel [16,17]. Open raceway technology has allowed the scaling-up of microalgae processes but their development is still limited due to (i) nutrients and mass transfer limitations, (ii) the need for good sunlight capture with special bioreactors requiring very large areas and low depths, (iii) the need to maintain a basic pH (8–10), and (iv) the need to harvest the microorganisms in order to extract the compounds of interest. All these constraints limit productivity and generate relatively high costs [18]. At the laboratory scale, cyanobacteria have been genetically modified to produce alcohols directly from CO₂ [18–21]. Very recently, the carbon assimilation pathway of the cyanobacterium *Synechococcus elongatus* PCC 7942 has been directed towards the production of 2,3-butanediol from CO₂ and glucose in the dark [22]. Such an advance overcomes the limitations related to the need for sunlight, but the need to add glucose still makes

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the bioprocess unattractive from an economic point of view. In addition, the biological stability of the modified cyanobacteria has yet to be improved for industrialization purposes [18–22].

Among the biological catalysts, Formate Dehydrogenase (FDH) enzymes are known to be reversible and several works have focused on the selective reduction reaction of CO₂ into formate by extracted, purified and improved FDH [23,24]. Recently, the enzyme nitrogenase, a well-known N₂-reducing enzyme, has also been reported to be able to catalyze the conversion of CO₂ into formate and methane [25,26]. However, the scaling-up of this bio-conversion process is difficult because it requires the addition of costly cofactors to provide the essential electrons and protons for CO₂ bio-reduction [27,28]. Electrochemically assisted reactors (also known as bio-electrolyzers), inoculated with electro-active litho-autotrophic bacteria, constitute another biological means of CO₂ conversion [16,29–34]. Two different configurations are possible with these systems: (1) CO₂ reduction may be catalyzed by the biotic cathode and the required electrons are supplied by the polarized cathode while the protons derive from water oxidation at the anode or (2), in the case of use of hydrogenotrophic bacteria, the cathode electrons and the protons arising from the anode can be used *in situ* to generate dihydrogen (H₂) that will then be assimilated by the bacteria. Recently, a methylotrophic bacterium, *Methylobacterium extorquens* AM1, was reported to have produced formate from CO₂ with the first bio-electrolyzer configuration, but no cathodic current driven by the presence of CO₂ was evidenced and the CO₂ reduction reaction was not demonstrated [31]. When hydrogenotrophic bacteria were used with the second configuration, different products such as methane [31], acetate [32], PolyHydroxyButyrate (PHB), biomass and C3-C5 alcohols [33] were obtained from CO₂ and H₂. However, the fact that hydrogen (H₂) is an expensive energy vector and its solubility in water is low constitute major drawbacks for the scaling-up of this process that produces molecules with a lower energy potential than H₂.

The hydrogenotrophic bacterium *Ralstonia eutropha* H16 has been genetically modified to use formate as a source of carbon and protons instead of CO₂ and H₂ to produce isobutanol and 1-methyl-butanol [34]. Bio-electrolyzer tests were carried out with the bacterium to try and generate formate *in situ* at the cathode by reduction of CO₂ [34], but electrochemical reduction of CO₂ still remains difficult.

Finally, methanotrophic bacteria (*Methylosinus trichosporium* IMV 3011 and *Methylosinus sporium*) have been reported to selectively convert CO₂ into methanol with their enzymes (including FDH first that can reduce CO₂ into formate) and their intracellular PHB stock being used as a source of electrons and protons [35–37]. Contrary to the other biocatalysts, with these bacteria, there is no need to add costly chemicals such as cofactors or H₂ to the reaction medium, or to provide the reaction system with light, or to genetically modify the metabolic pathway of the bacteria to recover products directly from CO₂. Moreover, methanotrophic bacteria use methane (CH₄) as a carbon and energy source to grow, meaning the global carbon balance of the CO₂ valorization bioprocess is positive as our city and agricultural wastes are a renewable source of methane. Methanotrophic bacteria are thus interesting biocatalysts for CO₂ reduction. However, the methanol production obtained was very low (3.6 μmol_{methanol} g_{dry cell}⁻¹).

In this work, an environmental bacterial consortium rich in methanotrophic bacteria was shown to directly and selectively catalyze the reduction of CO₂ into formate, a chemical which could be considered as a future energetic vector since its acidic form, i.e. formic acid, can be used as fuel in Direct Formic Acid Fuel Cells (DFAFCs) or as an H₂-storage molecule. Formate could also be employed as an electron donor for oxidation of CH₄ into methanol by methanotrophic bacteria [38,39]. Significant amounts of formate were able to be produced (1.06 mmol_{formate} g_{dry cell}⁻¹) without the addition of costly chemicals to the reaction medium or *in situ* generating compounds for CO₂ reduction. In addition, the native consortium was grown on methane, a renewable source of carbon. This new CO₂ valorization route appears very promising and a patent application was therefore submitted [40], justifying

the composition of the bacterial consortium remaining confidential until publication of said patent.

2. Materials and methods

2.1. Bacteria and culture conditions

A bacterial consortium [40] containing methanotrophic bacteria, the composition of which remains confidential, was grown in a modified nitrate mineral salts (NMS) medium enriched with copper and iron. The bacteria were grown in sealed vials incubated at 30 °C on a rotary shaker (Unimax 1010, Heidolph) operated at 160 rpm. The headspace of the vials was filled with a mixture of CH₄ and air (1:1 v/v) that was used as the source of carbon and energy; the gas volume was 3-fold that of the liquid. The NMS medium was composed of 1.06 g/L KH₂PO₄, 4.34 g/L Na₂HPO₄·12H₂O, 1.7 g/L NaNO₃, 0.34 g/L K₂SO₄ and 0.074 g/L MgSO₄·7H₂O; the pH of NMS medium was adjusted to 7.0 ± 0.1 with NaOH, 0.1 M or HCl, 0.1 M and the medium was then autoclaved. Mineral, copper and iron solutions were prepared independently and sterilized by filtration on a 0.2-μm acetate cellulose membrane (Sartorius) before being added to the NMS medium. The final concentrations of the mineral solutions were 0.57 mg/L ZnSO₄·7H₂O, 0.446 mg/L MnSO₄·H₂O, 0.124 mg/L H₃BO₃, 0.096 mg/L Na₂MoO₄·2H₂O, 0.096 mg/L KI, 7.00 mg/L CaCl₂·2H₂O, that of the copper solution 0.798 mg/L CuSO₄, and that of the iron solution 11.20 mg/L FeSO₄·7H₂O.

2.2. Preparation of the bacterial suspension

At the beginning of the stationary phase, the cultured cells were harvested by centrifugation at 4000 g for 20 min at 4 °C and then washed once with phosphate buffer 20 mM at pH 7.0. A reaction medium containing 20 mM of phosphate buffer at pH 7.0 and 5 mM of MgCl₂ was used to re-suspend the cells to reach a final optical density at 600 nm (OD₆₀₀) of about 10. Part of the resulting suspension was stored at –20 °C with glycerol (20% w/w) for further biochemical analysis.

2.3. CO₂-reduction tests in batch mode

The batch reactors were 60 mL-glass vials sealed with aluminium caps. The bacterial suspension was distributed into the batch reactors, with 6 mL being added per reactor. To assess the ability of the consortium to reduce CO₂, several experiments were conducted with different headspace atmospheres (Table 1) that were first sterilized by filtration on 0.2-μm PTFE filters (Sartorius). For experiments A, B and C, a large set of vials was filled with the same bacterial suspension and incubated at 30 °C under constant rotation (160 rpm). Each experiment was monitored for 20 days and at each sampling point, a vial was taken for analytical measurements. Different measurements were performed: optical density at 600 nm (OD₆₀₀), pH, salts analysis by ionic chromatography (IC) and quantification of the volatile organic compounds (VOC) contained in the liquid samples by headspace GC–MS.

Each experiment was conducted in triplicate. CO₂-reduction tests were also carried out with ¹³C₂O₂ to confirm the origin of the products arising from CO₂ reduction by carbon NMR analyses.

Air was taken from the atmosphere while CO₂ (99.995%) and N₂

Table 1
Experimental conditions for the CO₂ reduction tests.

Experiment	Presence of bacteria	Headspace atmosphere
A	Yes	CO ₂ :Air (1:1 v/v)
B	Yes	Only Air (100 %)
C	Yes	Only N ₂ (100%)
D	No	CO ₂ :Air (1:1 v/v)

(99.995%) were obtained from Air Liquide and $^{13}\text{CO}_2$ (99.9%) was obtained from Sigma Aldrich.

2.4. Sample processing

At various reaction times, the bacterial suspensions in the reaction vials (i.e. 6 mL) were recovered and divided into different fractions. A first fraction was used to measure pH and OD_{600} . A second fraction was centrifuged at 10 000 g at 4 °C for 10 min; the supernatant was then recovered to be immediately analyzed (by GC–MS or ionic chromatography). In case of NMR characterization, a portion of the freshly sampled suspension (0.5 mL) was transferred into NMR tubes for direct analysis.

2.5. Analytical methods

Optical density (OD_{600}) was measured at 600 nm with a UV-2400 spectrophotometer (Shimadzu, Japan) and pH was measured with a C831 pH-meter (Consort, Belgium).

Volatile organic compounds, including methanol, ethanol, formaldehyde and acetaldehyde, were quantified by gas chromatography (Clarus 580, Perkin Elmer), coupled to mass spectrometry (Clarus SQ-8-MS, Perkin Elmer). A headspace system (Turbomatrix 16S, Perkin Elmer) was used for sample injection. Prior to analysis, acetonitrile was used as an internal standard at a final concentration of $39.3 \pm 0.2 \text{ mg L}^{-1}$. The method included a 15-min sample heating step to 75 °C followed by injection into the GC system. Separation was carried out using a capillary column (Rt-Q-Bond Plot, Restek) under isothermal conditions (150 °C for 25 min). Calibration curves were obtained independently for each VOC.

Salt quantification of supernatants was carried out by ionic chromatography (IC). Sample portions of 25 μL were injected into a Dionex ICS-1000 system (Thermo Scientific™) with an IonPac AS19 (0.4 x 250 mm) capillary column and elution was carried out under the following conditions: 10 mM KOH (0–10 min), then 10–45 mM KOH (10–30 min).

For NMR analysis, 450 μL of the sample (complete suspension) was poured into a 5 mm NMR tube with 50 μL of D_2O . The analyses were performed using a NMR BRUKER Avance III – 500 MHz spectrometer–CryoProbe Helium system for four hours. Different standards prepared in the reaction medium were analyzed independently by NMR to obtain the carbon spectra of these molecules.

3. Results and discussion

As detailed in Table 1, different experimental conditions were used to check the ability of the consortium to reduce CO_2 into formate. The conditions of experiment A correspond to the reaction conditions for formate production, i.e. bacterial consortium in presence of a CO_2 :air mixture (1:1 v/v). Experiments B, C and D, performed without adding CO_2 (B, C) or without bacteria (D), were blanks or control tests. Experiments B and C aimed to assess whether there was any possible microbial by-product release when the bacteria were placed in presence of air (B) or N_2 (C). Experiment D was performed without bacteria and in presence of CO_2 to check that spontaneous CO_2 reduction could not occur.

Fig. 1 presents the results obtained in the batch reactors where formate appeared in the reaction medium, i.e. experiments A and B (Table 1); for each experiment, three independent runs were performed in parallel and each point represents the mean values obtained.

Regarding OD_{600} , a nearly 25% decrease in bacterial concentration was observed during the first few days which then remained constant (from day 5), with no differences being observed between headspace atmospheres A and B (Fig. 1). As the initial OD_{600} was high (7.6 ± 0.6), the biomass concentration still remained significant during the experiments despite the decrease. The observations

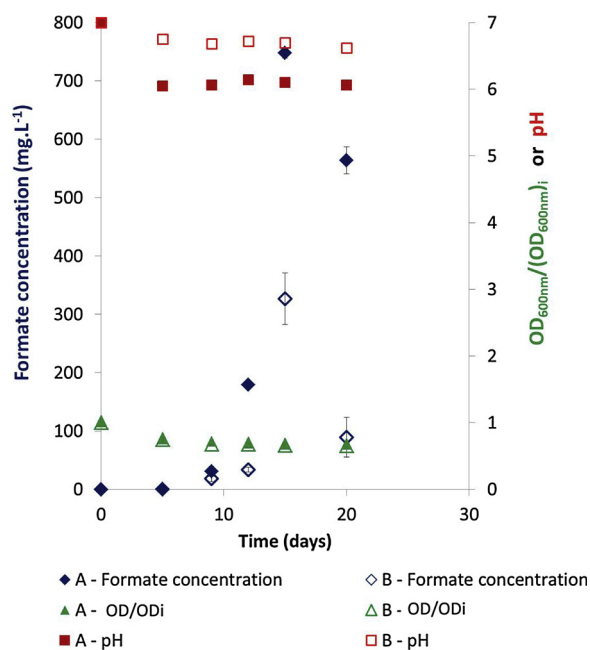


Fig. 1. Reaction profiles of formate production, pH and $\text{OD}_{600}/(\text{OD}_{600})_i$ obtained for experiments A and B. A: bacterial consortium with an equimolar CO_2 :air mixture and B: bacterial consortium with only air; $(\text{OD}_{600})_i$ means initial OD_{600} ; $(\text{OD}_{600})_f = 7.6 \pm 0.6$ corresponding to $2.45 \text{ g dry cell L}^{-1}$.

regarding OD_{600} were similar for experiment C (data not shown). The fact that methanotrophic bacteria have been reported to be very resistant and able to form spores in stressing conditions [41] could also explain the preservation of OD_{600} over 20 days despite the fact that the bacteria were exposed to unusual conditions (i.e. absence of their substrate CH_4 and high CO_2 content).

In the reactors where CO_2 was added (experiment A), pH decreased from 7.0 to 6.4 ± 0.2 within 5 days and then remained at that value which corresponds to the first acid dissociation constant (pKa) of CO_2 in water. A similar change in pH was observed in the reactors of experiment D as they also contained high levels of CO_2 (data not shown). In the vials containing only air (experiment B), pH decreased slightly to 6.8 ± 0.1 due to the presence of traces of CO_2 (i.e. 400 ppmv). When the headspace contained N_2 (experiment C), pH remained constant at its initial value, i.e. 7.0 ± 0.1 (data not shown).

The control tests carried out with the consortium and N_2 (experiment C) showed no evidence of microbial by-product release or formation by GC–MS or ionic chromatography throughout the duration of the experiment. No products were detected either in the control tests performed without bacteria but with an equimolar mixture of CO_2 and air (experiment D), which confirmed that spontaneous CO_2 reduction did not occur.

In experiments A and B (consortium exposed to an equimolar mixture of CO_2 and air or to air), no VOC were detected by GC–MS. Nevertheless, ionic chromatography analyses demonstrated the appearance of formate after 8 days in both of the experiments (Fig. 1). The consortium was able to produce up to 750 mg L^{-1} of formate in the CO_2 :air mixture (1:1) at day 15 of the reaction, corresponding to a formate production of $280 \pm 10 \text{ mg g}_{\text{dry-cell}}^{-1}$ (taking into account the bacterial mass initially introduced into the reactors).

When the consortium was exposed to air (experiment B), which contains a small fraction of CO_2 (400 ppmv), formate production was lower than that of experiment A (Fig. 1). In fact, 330 mg L^{-1} of formate were produced corresponding to a production of $145 \pm 15 \text{ mg g}_{\text{dry-cell}}^{-1}$. These results show that formate production is CO_2 - and bacteria-dependant and suggest that formate could be formed from CO_2 present in the atmosphere.

Nevertheless, the amount of formate obtained (i.e. $330 \text{ mg L}^{-1} \times 6 \cdot 10^{-3} \text{ mL} = 1.98 \text{ mg}$) in experiment B (only air) was much higher than the expected theoretical quantity taking into account the CO_2 content of air (54 mL of air with 400 ppmv of CO_2 that can be transformed to a maximal formate mass of 0.04 mg). A possible explanation for the appearance of formate in excess in the reaction medium could be mixed acid fermentation. This bacterial phenomenon occurs under oxygen limitation and could give rise to products such as acetate and formate by cleavage of intracellular pyruvate by pyruvate formate lyase [42]. In this work, anoxic conditions could have occurred due to a rapid depletion of the oxygen initially present in the reactors since oxygen is the usual final electron acceptor for the consortium; the bacterial consortium is indeed first grown under aerobic conditions before being exposed to CO_2 (Section 2.1).

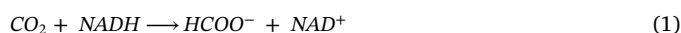
In order to confirm the actual ability of the consortium to reduce CO_2 into formate, a new set of experiments was carried out using a mixture of $^{13}\text{CO}_2$:air (1:1). The reaction was then monitored by ^{13}C NMR. Nuclear magnetic resonance spectroscopy (NMR) is only sensitive to ^{13}C since it has a spin number of $\frac{1}{2}$. $^{13}\text{CO}_2$ -labelling was consequently used to monitor any potential ^{13}C -containing product. In addition, the technique can be used to detect products irrespective of their localization (i.e. inside or outside the cells) as the NMR magnetic field can penetrate bacterial cells. The NMR spectrum obtained after 8 days of reaction is shown in Fig. 2.

Resonance peaks for $^{13}\text{CO}_2$ ($\delta = 124.6 \text{ ppm}$) and its corresponding ionized form $\text{H}^{13}\text{CO}_3^-$ ($\delta = 160.2 \text{ ppm}$) were visible at the beginning. After 8 days of reaction, a new peak appeared ($\delta = 170.9 \text{ ppm}$), corresponding to the standard fingerprint of ^{13}C -labelled sodium formate. Since no other labelled compound was detected in the reaction medium, the labelled formate is thus likely to result from direct $^{13}\text{CO}_2$ reduction. This result demonstrates that the consortium is thus able to reduce CO_2 into formate. However, in this experiment, it was not possible to recover semi-quantitative information about the labelled formate concentration produced since the $^{13}\text{CO}_2$ and $\text{H}^{13}\text{CO}_3^-$ signals were saturated. The only available information was that the formate concentration on day 8 was higher than the low NMR threshold for detection of labelled formate (i.e. 6 mg L^{-1}). If it is considered that bacteria exposed to CO_2 :air (Fig. 1, run A) were able to produce $35 \pm 2 \text{ mg L}^{-1}$ of formate on day 9, it can be assumed that at least 17% ($= 6 / 35 \times 100\%$) of the formate produced resulted from CO_2

reduction.

Reduction of CO_2 by the consortium may be explained by several possible enzymatic cycles inside the bacteria. Among the bacteria of the consortium, methanotrophic bacteria are supposed to be of CO_2 reduction catalysts. These bacteria use CH_4 as an energy and carbon source. As described in Fig. 3A, the first step involves methane oxidation to methanol by methanol monoxygenase (MMO); methanol is then successively converted into formaldehyde and then formate by methanol dehydrogenase (MDH) and formaldehyde dehydrogenase (FADH), respectively. Finally, formate dehydrogenase (FDH) catalyzes the oxidation of formate to CO_2 . The methane consumed is fixed in the carbon cycle for protein and biomass production, and the CO_2 produced from CH_4 oxidation is reported to be also mainly fixed in the carbon cycle through carboxylase enzymes incorporating CO_2 into organic molecules already present inside the cells [43]. In addition, formate can also enter the carbon cycle for the production of bacterial material, and PolyHydroxyButyrate (PHB) in particular. PolyHydroxyButyrate is an energy storage polymer accumulated during the culture phase under oxygen and nitrogen limitation [44,45].

A potential route for CO_2 assimilation by the bacteria is described in Fig. 3B. The CO_2 could indeed be reduced into formate by the direct action of FDH and in that case, the equation balance of the reaction would be:



where NADH is the reduced form of the Nicotinamide Adenine Dinucleotide (NAD) cofactor present in living cells. However, the stock of endogenous NAD is finite. For the CO_2 reduction reaction to occur (Eq. (1)), the oxidized form of NAD (i.e. NAD^+) has to also be continuously reduced into NADH (Fig. 3B). Some intracellular enzymes such as β -hydroxybutyrate dehydrogenase are able to recycle NAD^+ into NADH by simultaneously oxidizing an electron and proton donor source (Fig. 3B).

Compared to the work of Xin (2007) [36], no methanol traces were evidenced in the samples even if the compound could have been potentially present in the reaction medium as a product of successive formate reductions catalyzed by FADH and then MDH (Fig. 3B). One possible explanation for this phenomenon could be that formate production was so high that it led to inhibition of FADH.

Furthermore, formate was assayed in the supernatant of the

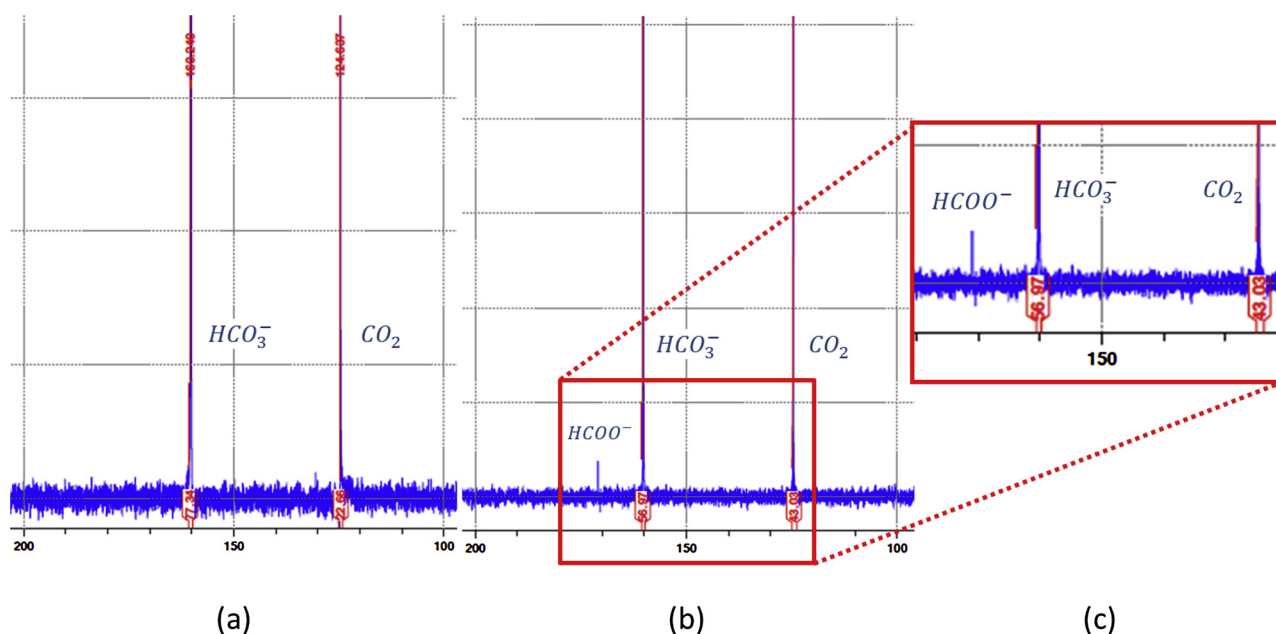


Fig. 2. NMR spectrum at (a) $t = 0$ days, where CO_2 and HCO_3^- are presents; (b) $t = 8$ days showing a new compound with a chemical shift corresponding to formate, which is enlarged (c).

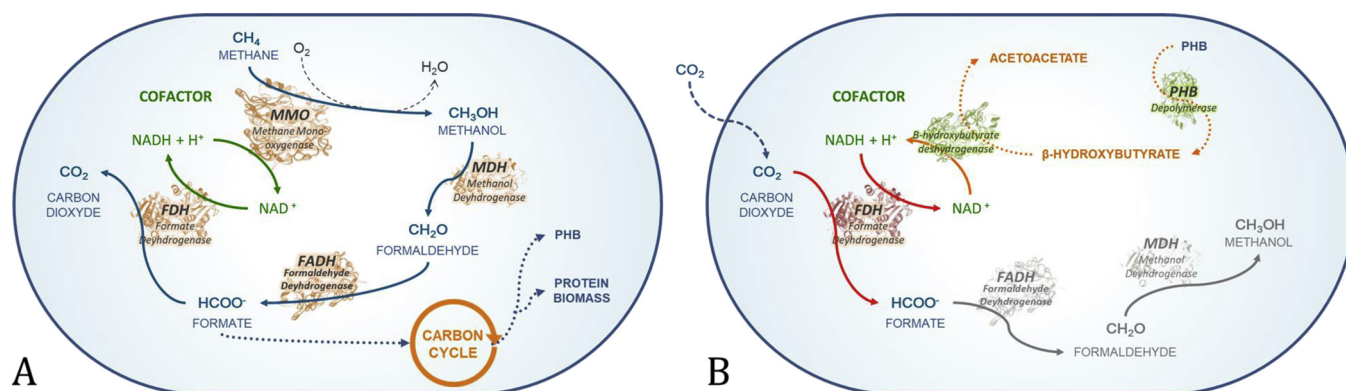


Fig. 3. Methanotrophic bacteria: (A) pathway for CH₄ assimilation and (B) suggested pathway for CO₂ reduction into formate.

samples, meaning that the formate was external to the cells. At this stage, it was assumed that the formate was produced inside the cells by the enzymatic machinery required for both CO₂ and NAD⁺ reductions and then released out of the cells. The probability that these reactions were catalyzed by free enzymes resulting from cell lysis is low since the meeting probability between FDH, CO₂, NAD⁺, and the enzyme regenerating NAD is low. Regarding formate excretion from the cells, two phenomena could be assumed: (1) formate release could be due to a bacterial regulation process when intracellular formate concentrations become too high for cell viability as formate does indeed have antimicrobial properties [46] and its intracellular accumulation could lead to cell death, and (2) population differentiation could occur among the bacteria meaning that some bacteria could play the role of formate factories for the survival of other bacteria consuming the formate. Formate is effectively reported in the literature as a precursor used by methanotrophic bacteria for cell maintenance reactions [47,48].

A 5-day period seems to correspond to the adaptation phase for bacteria and their enzymes to their new carbon substrate, i.e. CO₂ instead of CH₄, before formate production occurs. A decrease in formate concentration (by 27 ± 1%) was then observed after 15 days (Fig. 1). This could be due to formate consumption. Bacterial regulation or changes in bacterial population interactions or substrate limitation could be at the origin of this decrease. However, the theoretical amount of CO₂ available in the initial CO₂:air mixture of the headspace was calculated according to the gas volume inside the vial (i.e. 54 mL) and the ideal gas equation. The calculated CO₂ available (i.e. 1.09 mmol) was nearly 10 times higher than the maximum theoretical amount of CO₂ consumed for formate production (i.e. 0.10 mmol) assessed using Eq. (1). This means that there was no CO₂ limitation and that the bacteria had an unlimited capacity for exogenous formate production; the result therefore indicates that the limiting factor was rather the source of electrons and protons (i.e. the internal PHB) required for NAD recycling and thus for CO₂ reduction into formate (Fig. 3B).

The initial PHB dry mass content of the bacterial suspension was found to be about 15 ± 5% (data not shown). A theoretical calculation, considering that PHB was composed of 1 000 monomer units, showed that a PHB mass content of approximately 10% would indeed make it possible to reach the maximum concentration of formate produced (i.e. 750 mg L⁻¹). However, the exact number of units making up intracellular PHB is unknown and PHB could also be used in other pathways such as mixed acid fermentation. This hypothesis therefore needs further investigation. Much more experimental work is also required to clarify the explanations provided above and to specify the metabolic ways of this interesting CO₂ activation bioprocess.

4. Conclusions

A bacterial consortium containing methanotrophs was able to reduce CO₂ into formate with a positive carbon balance as bacterial

biocatalysts were grown on methane, which is also a greenhouse gas. This bioprocess thus constitutes a new promising way to valorize CO₂. The role of each bacterium composing the consortium will be further investigated to both fully understand the mechanism and optimize formate production from CO₂. In addition, formate production was shown to be enhanced by another phenomenon that is likely to be mixed acid fermentation. At this stage, it is assumed that 17% of the total formate produced resulted from CO₂ reduction; however, this content needs to be precisely quantified. The impact of operating conditions should also be studied in the future to control the contribution of CO₂ reduction on mixed acid fermentation. Finally, another way to optimize the process will focus on continuous formate recovery from the reaction medium and bipolar electro dialysis will also be tested.

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