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Bioproduction of acetic acid from carbon dioxide as single substrate and zero valent iron (ZVI) by clostridia

Büşra Bayar, María C. Veiga, Christian Kennes *

Chemical Engineering Laboratory, Faculty of Sciences and Center for Advanced Scientific Research (CICA), BIOENGIN group, University of La Coruña, E-15008, La Coruña, Spain

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ABSTRACT

Developing innovative bioprocess strategies for carbon capture and utilization has been a major focus of research over the past decade as a way of creating a more resilient world. In this study, zero-valent iron (ZVI) was utilized to generate hydrogen, which was then utilized as an electron donor for CO_2 reduction by either *Clostridium aceticum* or *Clostridium carboxidivorans*. For this bioprocess, an early acidic condition in the reactors facilitated more hydrogen production and thereby more acetic acid accumulation, in contrast to later acidification, elucidating the role of pH in ZVI-CO_2 bioconversion. Acetic acid was produced as single end product of the process in both batch bottle studies with no pH control as well as in reactor studies with pH regulation. This is, to the best of our knowledge, the first report on selective pure CO_2 bioconversion to acetic acid by clostridia, with ZVI. In order to minimize costs, scrap iron rather than pure ZVI can be used for scaling-up. By lowering the pH to 6.6 for *C. aceticum*, in reactor studies with 75 g/L ZVI, some ethanol production (125 mg/L) was also observed, besides acetic acid. In reactor studies, a maximum acetic acid concentration of about 2 g/L was obtained at ZVI concentrations of 50 and 75 g/L. Thus, ZVI dosage and medium pH have a major effect on the metabolites generated during CO_2 utilization.

1. Introduction

As a result of the urbanization and industrialization activities of most developing countries, the concentration of greenhouse gases (GHG) such as carbon dioxide (CO_2) has significantly increased in the atmosphere. This anthropogenic pollution causes global warming and thus climate change and leads to negative effects on humans and on the environment, such as inefficient agriculture, rising sea levels, and desertification [1]. The Paris agreement aims to limit the average global temperature rise to 2 °C above pre-industrial level. Reducing this level to 1.5 °C is one of the other aims of this agreement. The International Panel on Climate Change (IPCC) has reported that net zero emissions must be achieved by 2050 in order to keep the global temperature rise below 1.5 °C [2]. Despite these global plans for the future, US-Environmental Protection Agency (EPA) reports indicate that the atmospheric carbon dioxide concentration has increased from 280 ppm in the pre-industrial period (1750) to 404 ppm in 2016 [3]. Conversion of CO_2 to value added products such as biofuels and biochemicals through carbon capture and utilization (CCU) technologies plays a pivotal role in sustainable development by contributing to the mitigation of atmospheric CO_2 and reducing the dependency on fossil fuels. Biological processes, such as gas fermentation, are CCU technologies that have attracted considerable interest. In gas fermentation, a group of bacteria known as homoacetogens is capable of reducing CO_2 as their only carbon source, following the Wood Ljungdahl pathway (WLP), to produce acetate and, sometimes, other products like ethanol, butanol, and butyric acid, among others. This is a promising CCU technology, but an external supply of energy, generally in the form of H_2 gas, needs to be provided to these microorganisms when CO_2 is used as electron acceptor. The hydrogenase enzyme catalyses the electron transfer from H_2 to ferredoxin, coupled with reduction of NAD(P). The electron required for the reduction of CO_2 to formate in the first step of the methyl branch of the WLP is either provided by H_2, reduced ferredoxin, or NAD(P)H [4]. For the carbonyl branch of the WLP, CO_2 is reduced to CO using electrons provided by reduced ferredoxin [4].

External H_2 addition may reduce the sustainability and cost-efficiency of this bioprocess. Given that Zero Valent Iron (ZVI) could generate H_2 under anaerobic condition opens the possibility to exploit this as potential electron donor for CO_2 bioconversion. ZVI is mentioned in the literature mostly for its possible role in the anaerobic digestion of waste or wastewater for the production of methane or biogas [5]. ZVI

* Corresponding author.
E-mail address: Kennes@udc.es (C. Kennes).

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has also been used to remove arsenic, chlorinated organic compounds, nitrate, nitroaromatic compounds, heavy metals, phenol and dyes in processes such as groundwater remediation and wastewater treatment [6]. It is non-toxic, abundant, and rather cheap, depending on its origin. Generated hydroxide ions increase the overall pH of the solution, act as an acid buffer, and therefore the pH generally proceeds to neutral values (Eq.1).

$$Fe^0 + 2H_2O \rightarrow Fe^{2+} + H_2 + 2OH^- \quad (1)$$

The use of ZVI as a reductant in anoxic environments not only produces hydrogen gas when it reacts with water, but it also decreases the oxidation-reduction potential (ORP), which both promote the growth of homoacetogens. ZVI was also used to prevent propionic acid fermentation in an acidic environment, which is undesirable in anaerobic digestion studies, thanks to its ability to reduce the ORP while preventing a decrease in pH [7,8]. At the same time, ZVI added to anaerobic digestion media has been reported to increase the amount of acetic acid obtained as a result of the process by increasing the activity of enzymes effective in the conversion of propionic acid to acetic acid: dehydrogenase (DH), pyruvate-ferredoxin oxidoreductase (POR), Phosphate acetyltransferase (PTA), acetate kinase (AK) [8].

Although the enhancing effect of ZVI on anaerobic digestion has been studied, bioconversion studies with anaerobic cultures have mainly focused on methanogenesis; besides literature reporting that ZVI improves methane production in anaerobic sludge by playing the role of electron donor [9–14]. This chemical has also been used to enhance CO_2 to CH_4 conversion with pure methanogenic cultures [15–17]. The study reported by Daniel et al. [16] was the first one demonstrating the chemoautotrophic conversion to methane using ZVI as an energy source, instead of supplying H_2 gas to the system externally, and using pure methanogenic strains [16]. Recently, another study [18], reported methane production from CO_2 as sole carbon source with ZVI addition to anaerobic granular sludge. In those studies, ZVI concentrations ranging between 0 and 200 g/L were generally tested and a pH value of 6 was often determined as optimum.

Research on acetogenesis with ZVI as electron source is still in its infancy. In a study showing that hydrogen oxidizing bacteria maintain their metabolism using the cathodic hydrogen released after the anaerobic corrosion of mild steel, it was reported that the acetogen *Acetobacterium woodii* used externally fed CO_2 as a carbon source and cathodic hydrogen as an energy source [19]. In the recent study cited above [18], the accumulation of some VFA was observed with anaerobic granular sludge, using bromoethanesulfonate (BES) as an inhibitor of methanogens, in presence of CO_2 and with the addition of ZVI. The same research group then concentrated more on CO_2 conversion to VFA by adding ZVI to the anaerobic granular sludge [20]. It was aimed to enhance homoacetogenesis and inhibiting methanogens by using BES as in previous studies with other strategies, such as adjusting the pH of anaerobic granular sludge to low values, short-time exposure of anaerobic sludge to heat and high salinity exposure (30–90 g NaCl/L) of anaerobic granular sludge. The highest performance was observed when anaerobic granular sludge was exposed to 50 mM BES, with 100 g/L ZVI, and at pH 6–6.5. In another recent study, with CO_2 as sole carbon source, ZVI was used as electron donor and enhanced methanogenesis and acetogenesis simultaneously, with a total CO_2 conversion to CH_4 and acetate of 81.67% [21].

The present research focused on the bioconversion of pure CO_2, in presence of ZVI, with the acetogenic bacteria *Clostridium aceticum* and *Clostridium carboxidivorans*, to valuable commodities. To investigate the effect of ZVI concentrations on hydrogen generation, several batch bottle tests were conducted with the fermentation medium of each bacterial strain, without the presence of microorganisms. Findings from the aforementioned abiotic studies were used to conduct bioconversion assays of CO_2 employing the bacterial strains at various initial pHs in batch bottles. As a scaling up approach and to understand metabolites production at different ZVI concentrations and constant pHs, experiments were also conducted in 1.2 L custom-built bioreactors with automated pH control systems. To the best of our knowledge, aiming high metabolites production and acetogenesis with CO_2 conversion to VFA and the use of ZVI as sole electron donor by pure homoacetogenic cultures has not been reported so far.

2. Material and Methods

2.1. Bacterial strains and cultivation in serum bottles

The acetogenic strains *Clostridium aceticum* (DSM 1496) and *Clostridium carboxidivorans* (DSM 15243), used in the experiments, were obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany. The composition of the liquid medium for growth of *C. aceticum* was as follows (per liter distilled water): NH_4Cl, 0.20 g; yeast extract, 3 g; KH_2PO_4, 1.76 g; K_2HPO_4, 8.44 g; $MgSO_4.7\ H_2O$, 0.33 g; $NaHCO_3$, 10 g; L-Cysteine-HCl, 0.30 g; $Na_2S{\cdot}9\ H_2O$, 0.92 g; rezasurin (stock solution concentration, 10 g/L), 1 mL, 2 mL of vitamins solution and 2 mL of trace metals solution. The vitamins stock solution was composed of (per liter distilled water): biotin, 40 μg; folic acid, 40 μg; pyridoxine-HCl, 200 μg; thiamine-HCl, 100 μg; riboflavin, 100 μg; nicotinic acid, 100 μg; D-Ca-pantothenate, 100 μg; vitamin B12, 10 μg; p-aminobenzoic acid, 100 μg; lipoic acid, 100 μg. The composition of the trace metals solution for *C. aceticum* was as follows (per liter distilled water): $N(CH_2COOH)_3$, 30 mg; $MnSO_4{\cdot}H_2O$, 10 mg; NaCl, 20 mg; $FeSO_4.7\ H_2O$, 2 mg; $CoSO_4.7\ H_2O$, 3.6 mg; $CaCl_2.2\ H_2O$, 2 mg; $ZnSO_4.7\ H_2O$, 3.6 mg; $CuSO_4.5\ H_2O$, 200 μg; $KAl(SO_4)_2.12\ H_2O$, 400 μg; H_3BO_3, 200 μg; $Na_2MoO_4.2\ H_2O$, 200 μg; $NiCl_2.6\ H_2O$, 500 μg; $Na_2SeO_3.5\ H_2O$, 6 μg; $Na_2WO_4.2\ H_2O$, 8 μg.

The medium for growing *C. carboxidivorans* was composed of the following compounds (per liter distilled water): yeast extract, 1 g; NaCl, 2 g; NH_4Cl, 2.5 g; KCl, 0.25 g; KH_2PO_4, 0.25 g; $MgSO_4.7\ H_2O$, 0.5 g, $CaCl_2.2\ H_2O$, 0.1 g; trace metals solution, 10 mL; vitamin solution, 10 mL; resazurin, 1 mL; and cysteine-HCl, 0.60 g. The vitamins stock solution per liter distilled water contained pyridoxine, 10 mg; thiamine, 5 mg; riboflavin, 5 mg; D-Ca-pantothenate, 5 mg; thioctic acid, 5 mg; paraamino benzoic acid, 5 mg; nicotinic acid, 5 mg; vitamin B12, 5 mg and D-biotin, 2 mg; folic acid, 2 mg; and 2-mercaptoethanesulfonic acid, 2 mg. The composition of the trace metals solution for *C. carboxidivorans* (per liter distilled water) was as follows: 2 g $N(CH_2COOH)_3$, 1 g $MnSO_4{\cdot}H_2O$, 0.80 g $Fe(NH_4)_2(SO_4)_2.6\ H_2O$, 0.20 g $CoCl_2.6\ H_2O$, 0.20 g $ZnSO_4.7\ H_2O$, and 20 mg each of $CuCl_2.2\ H_2O$, $NiCl_2.6\ H_2O$, $Na_2MoO_4.2\ H_2O$, Na_2SeO_4, and Na_2WO_4.

To prepare the nutrient medium for *C. aceticum* and *C. carboxidivorans*, the afore mentioned chemicals for each strain, except vitamins, were dissolved in distilled water. The solution was distributed in 120 mL serum bottles, each with a working volume of 40 mL. In order to remove oxygen from the bottles, they were flushed with pure N_2 for at least 5 min. Then, CO gas was introduced into the bottles and the pH of the medium was adjusted to 7.5 for *C. aceticum* and 6.0 for *C. carboxidivorans*, by adding 2 M HCl or 2 M NaOH during the flushing period. Each bottle was then immediately closed with a Viton stopper and sealed tightly with an aluminum crimp to ensure anaerobic conditions. For sterilization, the bottles were autoclaved at 121 °C for 20 min and then allowed to cool down. After adding the filter sterilized and oxygen-free vitamins solution to the medium, 12.5% (5 mL) of active seed culture was inoculated into each bottle. The bottles were kept with constant agitation at 150 RPM inside a thermostated orbital incubator (Infors HT, Bottmingen, Sweden) at 30 °C.

2.2. Abiotic batch experiments with CO_2 + ZVI

Two sets of batch experiments, at various ZVI concentrations (0, 25, 50, 75 g/L), were conducted in serum bottles pressurized with 1.5 bar CO_2, using either the *C. aceticum* or the *C. carboxidivirans* medium

described in Section 2.1 to determine the amount of H_2 gas produced and the pH variation caused by generated OH^- from anaerobic corrosion of ZVI in water. This study utilized a working volume of 60 mL medium in 120 mL serum bottles. The bottles were then autoclaved for 20 min at 121 °C. After CO_2 addition, the pH of the batch experiment with *C. aceticum* medium was initially 7.5, whereas the batch experiment with *C. carboxidivirans* media was initially 6.0, based on the near optimal pH values for each strain. Gas samples were collected daily to determine the H_2 gas generation, and liquid samples were taken to measure the pH.

2.3. Biotic batch experiments with pure homoacetogens using CO_2 + ZVI

Two set of biotic batch experiments were performed in serum bottles with a working volume of 60 mL and with addition of 25 g/L ZVI. In the first set, *C. aceticum* was tested with different initial pHs of 6.5, 7.25 and 8, in duplicates. The pH was adjusted before CO_2 addition. All the experimental procedure was similar to the abiotic study explained in Section 2.2. The bottles were pressurized with 1.5 bar CO_2, and 12.5% (v/v) (7.5 mL) inoculum (*C. aceticum*) was added.

In the second set of experiments, each bottle was pressurized with 1 bar CO_2 four times instead of only once to 1.5 bar as in the first set of experiments. Each bacterium, *C. aceticum* and *C. carboxidivorans*, was tested separately in this second set. During preparation of the experiments, the pHs of the media, for both *C. aceticum* and *C. carboxidivorans*, were adjusted to 8.5 while purging with N_2. A pH value of 8.5 was chosen considering the decrease that would take place after CO_2 dissolution and taking into account a more significant decrease in pH with the *C. carboxidivorans* medium compared to the *C. aceticum* medium. CO_2 was pressurized inside the bottles up to 1 bar**,** after the bottles were autoclaved and cooled down. The pH of the medium was measured and confirmed for the desired growth conditions of the microorganism used. The pure bacterial strains *C. aceticum* or *C. carboxidivorans* (12.5%, 7.5 mL) were inoculated in the respective bottles under sterile conditions.

For all the experiments, the bottles were maintained inside an orbital shaker at 150 RPM in a 30 °C incubation chamber. All the biotic batch experiments were carried out in duplicate. During the experimental run, gas samples were periodically collected for analysing H_2 production and CO_2 reduction. Simultaneously, liquid samples were also collected for analysing the production of metabolites.

2.4. Bioreactor experiments with pH control

For bioreactor experiments with pH control, pH electrodes were installed in 1.2 L custom made reactors with 300 mL working volume. Growth conditions and culture media preparation were the same for all experiments, as explained below. The liquid medium without cysteine-HCl, $Na_2S{\cdot}9\ H_2O$, and vitamins solution was prepared and added to the reactors containing varying concentrations of ZVI. The reactors were then autoclaved at 121 °C for 20 min. Right after autoclaving, the reactor containing the medium was flushed with pure N_2 gas for around 1 h in order to create anaerobic conditions. The reactors were pressurized with 0.5 bar pure CO_2 and they were placed in an orbital shaker at 150 RPM and room temperature. After one day, the head pressure was measured to be sure that pressurized CO_2 in the headspace is transferred to the liquid medium. Cysteine-HCl, $Na_2S{\cdot}9\ H_2O$ and the vitamins solution were introduced in the medium. If the pH was outside the optimal range for the microorganism, it was adjusted by using 2 M HCl and/or 2 M NaOH. *C. aceticum* was inoculated under sterile conditions with 12.5% (37.5 mL) active seed culture. The reactors were then placed in a water bath (Julabo SW22, Germany) with 150 RPM agitation and a constant temperature of 30 °C throughout the experimental run. Three different sets of reactor experiments were performed with the conditions summarized in Table 1.

Table 1
Experimental conditions used in the various studies using CO_2 + ZVI.

	Abiotic batch study	1st set of biotic batch study	2nd set of biotic batch study	Abiotic reactor study	1st set of biotic reactor study	2nd set of biotic reactor study
Parameters examined	ZVI concentration (0, 25, 50, 75 g/L)	pH (6.5, 7.25, 8.0)	Strain (*C. aceticum*, *C. carboxidivorans*)	ZVI concentration (25, 50 g/L) (*C. aceticum* medium)	Acidification at different times (20th day and 8th day)	ZVI concentration (50 and 75 g/L) and acidification on 8th day
Strain	–	*C. aceticum*	*C. aceticum* or *C. carboxidivorans*	–	*C. aceticum*	*C. aceticum*
Initial pH	7.5 (for *C. aceticum* medium) 6.0 (for *C. carboxidivorans* medium)	6.5, 7.25 and 8.0 (before pressurizing with CO_2)	8.5 for (*C. aceticum* and *C. carboxidivorans*) (before pressurizing with CO_2)	7.5	7.5	7.5
Working /Total volume	60 mL/120 mL	60 mL/120 mL	60 mL/120 mL	600 mL/1200 mL	300 mL/1200 mL	300 mL/1200 mL
ZVI concentration	0, 25, 50, 75 g/L	25 g/L	25 g/L	25, 50 g/L	25 g/L	50, 75 g/L
Gas (CO_2) pressure	1.5 bar (pressurized once at the beginning)	1.5 bar (pressurized once at the beginning)	1 bar (pressurized periodically / 4 times)	0.5 bar (pressurized once at the beginning)	0.5 bar (pressurized once at the beginning)	0.5 bar (pressurized once at the beginning)
General conditions	Assays in all batch experiments were kept on an orbital shaker with 150 RPM and 30 °C.			Assays in all reactor experiments were kept in a water bath with 150 RPM agitation and 30 °C.		

* all the batch biotic bottle studies were performed in duplicate

2.5. Abiotic reactor experiments with CO_2 + ZVI

These experiments were conducted in the same way as the biotic reactor experiments described in Section 2.4, using a 600 mL working volume of *C. aceticum* medium in a 1.2 L reactor. Two experiments were conducted, one with a ZVI concentration of 25 g/L and another one with a ZVI concentration of 50 g/L. Each reactor was pressurized to 0.5 bar with pure CO_2 and maintained at a constant pH of 7.5 throughout the experimental runs. Gas samples were taken daily to assess the amount H_2 gas generated.

2.6. Analytical methods

For all the batch and reactor studies, gas samples were taken from the headspace of the serum bottles and reactors, respectively, using a gas syringe to analyze the H_2 and CO_2 concentrations. For measuring the H_2 gas-phase concentrations, a gas chromatograph (GC) (Agilent Technologies, Madrid, Spain) was used, which was fitted with a 15-m HP-PLOT Molecular Sieve 5A column (ID – 0.53 mm; film thickness – 50 μm) and equipped with a thermal conductivity detector (TCD). The temperatures of the oven and the injection port of the GC, which used helium as the carrier gas, were 50 °C and 150 °C, respectively. The oven temperature was initially kept constant at 50 °C, for 5 min. Afterwards, to reach a final temperature of 90 °C, it was raised by 20 °C·min^{-1} for 2 min. Likewise, CO_2 was measured on another HP 5890 gas chromatograph (GC, Agilent Technologies, Madrid, Spain) with a Porapak Q 80/100 (inox) column (2 m × 1/8′) connected to a TCD. The injection, oven and detection temperatures were maintained at 90, 25 and 100 °C, respectively, and helium was used as carrier gas.

During all experiments, 1.5 mL liquid sample was withdrawn from bottles and reactors periodically to analyze the water soluble products, acetic acid and ethanol, in the culture broth by using an high-performance liquid chromatograph (HPLC) (HP1100, Agilent Co., USA) having an Agilent Hi-Plex H Column (300 × 7.7 mm) and equipped with both a refractive index detector (RID) and a diode array detector (DAD) at 50 °C. A 0.005 M H_2SO_4 solution was used as the mobile phase for the HPLC, and was fed at a flow rate of 0.8 mL/min. The amount of sample injected into the HPLC was 20 μL at it was analyzed at 45 °C. First, liquid samples taken from the experimental system were centrifuged at 7000 RPM for 5 min using a centrifuge (ELMI Skyline Ltd. CM 70M07). Then, the supernatant was filtered through a 0.22 μm PTFE filter (Labbox, Barcelona, Spain) before placing the sample in the HPLC system. A pH sensor (Knick, Elektronische Messgerate GmbH & Co. KG, Germany) was used to measure the value on-line in the reactor experiments with in-build pH control system to regulate the pH. The pH was regulated through the addition of a 2 M HCl solution, fed automatically to the reactors by means of a peristaltic pump, and/or a 2 M NaOH solution by manual addition using a syringe.

3. Results and discussion

3.1. Abiotic batch and reactor studies to determine the ZVI amount for biotic experiments

Abiotic batch experiments with various ZVI concentrations (0, 25, 50, and 75 g/L) were conducted to decide on the amount of ZVI to employ in further biotic experiments. During the experiment, an increase in the pH of all bottles was detected as a result of the reaction described in Eq. 1, except the control trials that did not contain ZVI (Fig. 1a). The highest production of H_2, which may be the process limiting substrate, was found in bottles supplemented with 75 g/L ZVI for both *C. aceticum* and *C. carboxidivorans* media (Figs. 1b and 2b). Although the highest ZVI concentration tested, 75 g/L, seemed to be the most beneficial in terms of H_2 generation, those bottles also had the largest pH increase with both media. Considering the inclusion of bacteria and the absence of pH control in the biotic batch studies, a medium

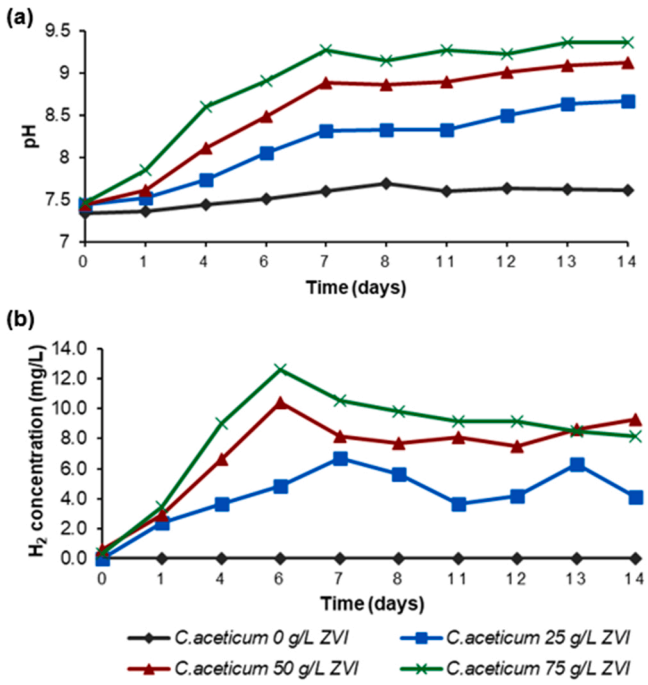

Fig. 1. Profiles of abiotic bottle studies with different concentrations of ZVI using *C. aceticum* medium; (a) pH, and (b) H_2 measured in the headspace of the bottles.

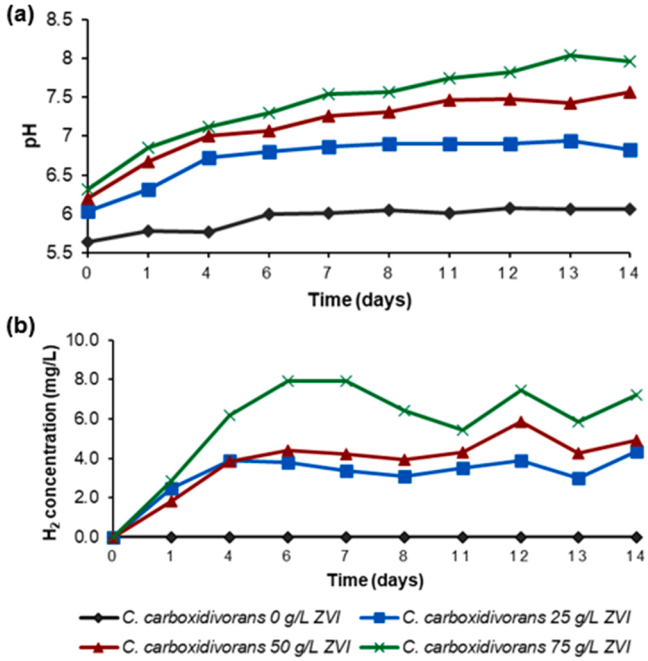

Fig. 2. Profile of abiotic bottle studies with different concentrations of ZVI using *C. carboxidivorans* medium; (a) pH, and (b) H_2 measured in the headspace of the bottles.

pH beyond the bacterial optimum range may be detrimental to bacterial growth and their ability to synthesize metabolites. As can be seen in Fig. 1a, the pH of the experimental bottles containing 25 g/L ZVI gradually increased from its initial value to approximately 8.5 on day 14, an optimum pH range for growth of *C. aceticum*. As a result, a concentration of 25 g/L ZVI was considered to be suitable for non-pH controlled batch studies under the aforementioned conditions.

The experiments were also conducted in reactors to determine the

amount of H_2 produced at various ZVI concentrations (25 and 50 g/L) and a constant pH of 7.5. As shown in Fig. 3b, the hydrogen profile measured in the headspace of both reactor experiments was quite comparable; hence, subsequent biotic investigations were conducted using 25 g/L of ZVI.

3.2. Iron dissolution in C. carboxidivorans medium

During the batch bottle experiments with *C. carboxidivorans*, samples were collected from the bottles and centrifuged at 7000 RPM for 5 min before being filtered into 2 mL HPLC vials through 0.22 μm PTFE filters. Within 10 min after performing the aforementioned sample preparation procedure, the color of the filtered liquid sample changed to yellow, and a precipitate of the same color was also seen at the bottom of the HPLC vial. However, this phenomenon was not observed with samples from bottles containing *C. aceticum* medium. The change in color and precipitation of the filtered sample could well be attributed to iron particles being oxidized, resulting in a size reduction smaller than the filter pore size, allowing them to pass through the filter. This sample preparation procedure was repeated using three different kinds of filters, PTFE, nylon, and cellulose acetate, to confirm that the filter material had no effect on the iron size reduction.

Additionally, subsequent trials with and without *C. carboxidivorans* in the *C. carboxidivorans* medium showed that size reduction was not a biological reaction but rather a chemical reaction involving chemicals of the *C. carboxidivorans* medium. Besides, we did not observe this phenomenon in the *C. aceticum* medium nor in the presence of *C. aceticum*, which prompted us to examine the chemicals responsible for the chemical reaction by comparing the compositions of *C. aceticum* and *C. carboxidivorans* media. After examination, it was noticed that three compounds were present in *C. aceticum* medium but not in *C. carboxidivorans* medium: K_2HPO_4, $Na_2S{\cdot}9\,H_2O$, $NaHCO_3$. After examining the effect of each of these chemicals individually by different trials, we found that the chemicals $NaHCO_3$ and K_2HPO_4 are the ones that stop the size reduction of iron.

The dissolution of passivated oxide layers on the surface of ZVI is pH dependent as a consequence of anaerobic corrosion with water. A low pH starts and accelerates the dissolution of the passive Fe^{2+} external layer [22]. It is possible that the dissolution of the iron surface can be seen in the *C. carboxidivorans* medium, which has lower pH values compared to the *C. aceticum* medium. In addition, it was stated that the anions (HCO_3^-, SO_4^{2-} and HPO_4^{2-}) in the medium can also prevent the degradation of the ZVI particles and the dissolution of their passivated outer surface. In our study, it could be observed that $NaHCO_3$ and K_2HPO_4, used as buffers in the *C. aceticum* medium, might have prevented the dissolution of the iron particles. In a study by Bae and Hanna (2015) on the removal of the contaminant nitrophenol, nano ZVI was used in an unbuffered system and the effect of different pH values on nano ZVI dissolution was investigated [23]. In accordance with our findings, it was reported that low pH values between 6 and 7 create a better environment for iron dissolution compared to higher values between 8 and 9.

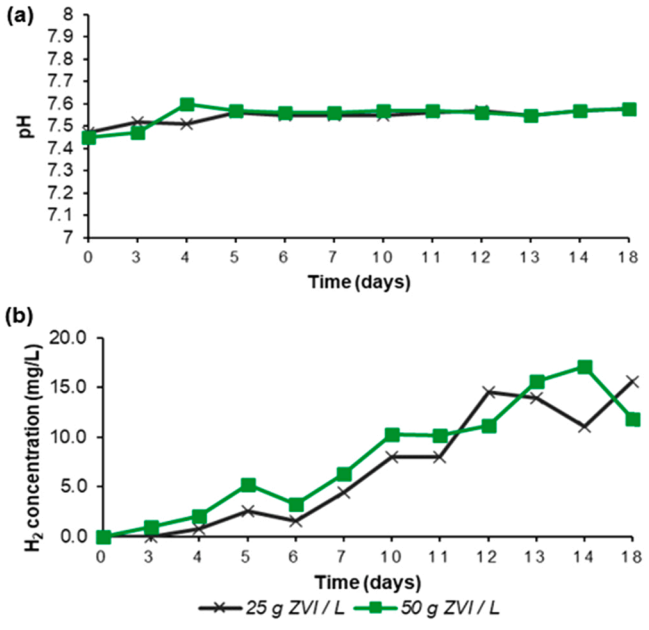

Fig. 3. Profile of abiotic reactor studies with concentrations of 25 and 50 g/L ZVI using *C. aceticum* medium; (a) pH, and (b) H_2 measured in the headspace of the bottles.

3.3. Biotic bottle experiments with pure homoacetogens using CO_2 + ZVI

Based on the results of the abiotic bottle experiments (Section 3.1), it was determined that the optimal dosage of ZVI for the batch experiment is 25 g/L. This dosage would not only generate H_2 at adequate amount, but would also provide an appropriate pH range of the medium for the microorganisms being studied in research where pH control is difficult to perform, such as the serum bottle experiments.

The first biotic bottle experiment was conducted with *C. aceticum* to determine the effect of different pHs (6.5, 7.25, and 8) on its growth and metabolites production with CO_2 (pressurized to 1.5 bar CO_2) and 25 g/L ZVI. It was observed that the highest amount of H_2 produced was in bottles with low initial pHs, while the lowest amount of H_2 produced was in bottles with the highest initial pH (Fig. 4b). Because of the hydroxyl ions released due to anaerobic corrosion with water, the pH of each bottle steadily increased from the first day. An acetic acid concentration of up to 1178 mg/L was achieved in bottles with a higher initial pH (Fig. 4c). As a result of this observation, it can be concluded that although the lowest amount of hydrogen was detected in the headspace of the bottles with the highest initial pH tested, the bottles with the highest initial pH provided *C. aceticum* with the optimal pH condition for maximum metabolite accumulation. No H_2 production occurred in the control bottles and thus no acetic acid was observed in those bottles. It is most likely that H_2 generation and consumption occurred concurrently, resulting in the detection of a negligible quantity of H_2 in the headspace. One approach to validate this is to measure the amount of metabolites produced, in this case acetic acid, during the time period considered. For example, on the sixth day, the H_2 concentration in the bottles, that had an initial high pH (pH 8), declined significantly and the acetic acid production increased steadily. A significant rise in acetic acid production and a reduction in H_2 detected in each bottle towards the end of the experimental run might be ascribed to possible adaptation of the *C. aceticum* strain to the ZVI environment and the absence of deteriorating pH at that point of the experimental run. The pH of the bottles ranged between 7.5 and 8.5 towards the end of the experiment. The pH buffering effect of ZVI was also observed as the bottles whose pH was adjusted to different values at the beginning converged to neutral values within the range of 6.6 – 7.0.

In the second set of experiments, instead of pressurizing above 1.5 bar at the beginning of the experiment, which might result in a larger pH decrease, deviating from the bacterial optimal pH range, the bottles were pressured to 1 bar CO_2 at four distinct times, on day 8, 16, and 23, during the 30-day trials, as explained in Material and Methods. For both *C. aceticum* (Fig. 5) and *C. carboxidivorans* (Fig. 6) experiments, there was a major drop in pH when CO_2 was added, followed by a rise in pH for the rest of the period until the next CO_2 pressurization. For this set of studies, a pH of 8.5 was chosen during medium preparation, which also eliminates deviations from the bacteria's ideal growth pHs during CO_2 additions. This increased initial pH also has an effect on the amount of H_2 generated, since the lower the pH, the more H_2 is generated by anaerobic oxidation of ZVI. This can also be seen by observing that the first set of experiments, which started at a lower pH, recorded a greater

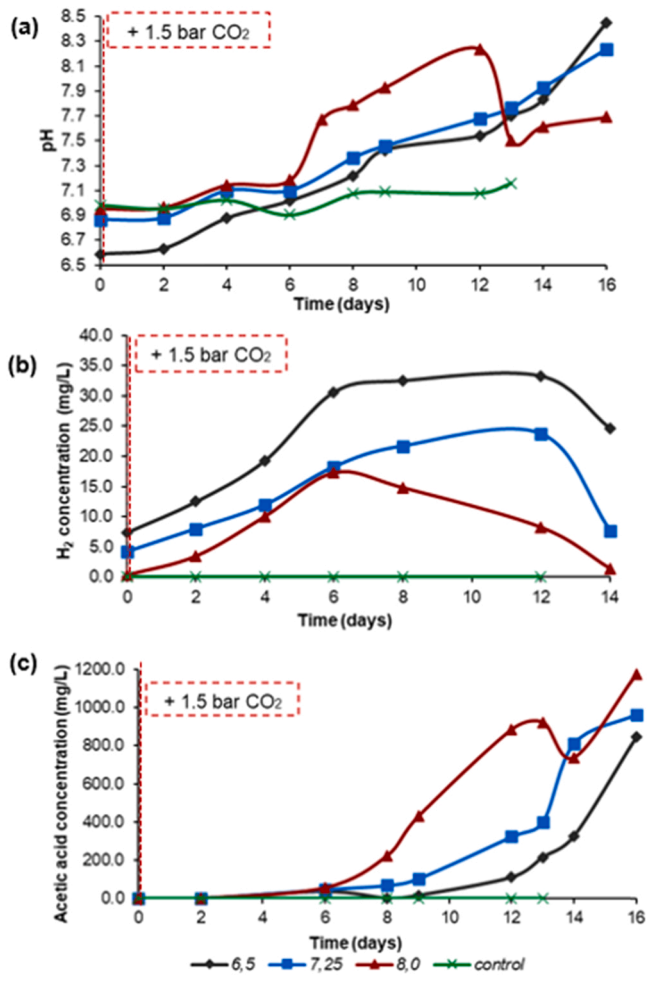

Fig. 4. Optimization of acetic acid production in the first set of batch studies; (a) pH, (b) H_2 concentration produced by ZVI-water reaction, (c) acetic acid produced. (The initial pH values are written as labels, control without iron at pH 8).

quantity of H_2 in the headspace than the second set of experiments. However, the quantity of acetic acid produced must also be taken into account since the bacteria use part of the generated H_2 for their metabolism. The amount acetic acid generated throughout the 30-day experiment with *C. aceticum* was 1400 mg/L in the second set of tests with four times pressurization of the bottles with 1 bar CO_2 (Fig. 5d), while 1178 mg/L acetic acid production was observed in 16 days in the first set of experiments, which used a single initial pressurization of 1.5 bar CO_2 (Fig. 4c). By comparing the first and second set of experiments with *C. aceticum*, it can be concluded that H_2 was the limiting substrate that significantly affects the metabolites produced. For instance, in the experiment performed at an initial pH of 8.0 and with a single CO_2 pressurization to 1.5 bar (first set of experiments with *C. aceticum*), only a minimal amount of H_2 was detected at the end of the experimental run (Fig. 4b), while achieving the maximum acetic acid production of 1178 mg/L in 16 days (Fig. 4c). Comparing with the data shown in Fig. 5c, which was conducted at a higher initial pH of 8.5 and with two CO_2 pressurizations to 1 bar, less hydrogen was detected in the headspace than in the first set of experiments, and the acetic acid production reached only 780 mg/L.

The second set of experiments with sequential pressurization of the bottles to 1 bar CO_2 was also tested by using a different strain, *C. carboxidivirans* (Fig. 6). As the medium for this strain is less buffered, starting at a higher pH of 8.5 helped maintaining the pH within an appropriate range for growth of this strain even after CO_2 addition. Throughout the 30-day trial, the pH was within the range of 7.1–5.5. At this lower pH, more H_2 was detected than in the experiment that used the *C. aceticum* medium, under the same sequential pressurization conditions of CO_2 to 1 bar. The environment to generate H_2 and maintaining the medium's optimal pH range all influenced the product formation by *C. carboxidivirans*, which produced around 2000 mg/L acetic acid by the end of the 30 days experiment. Similarly as observed during the abiotic study with *C. carboxidivirans* medium, iron size reduction was also observed in this set of experiments.

3.4. Bioreactor studies with pH adjustments

Contrary to the preliminary studies conducted in serum bottles, the subsequent experiments carried out in bioreactors were performed under controlled pH. The pH of the medium is considered to be one of

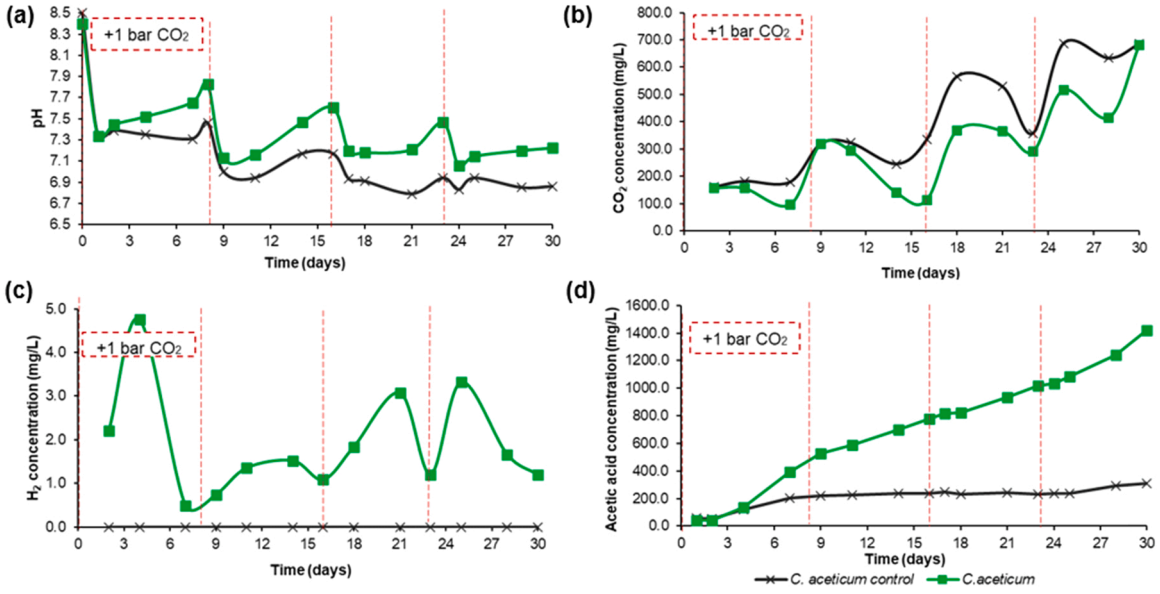

Fig. 5. Optimization of acetic acid (HAc) production in the second set of batch studies with *C. aceticum;* (a) pH, (b) CO_2 amount consumed, (c) H_2 produced by ZVI-water reaction, (d) acetic acid concentration produced. (*C. aceticum control*: *Clostridium aceticum* control bottle without iron, *C. aceticum*: *Clostridium aceticum* with iron).

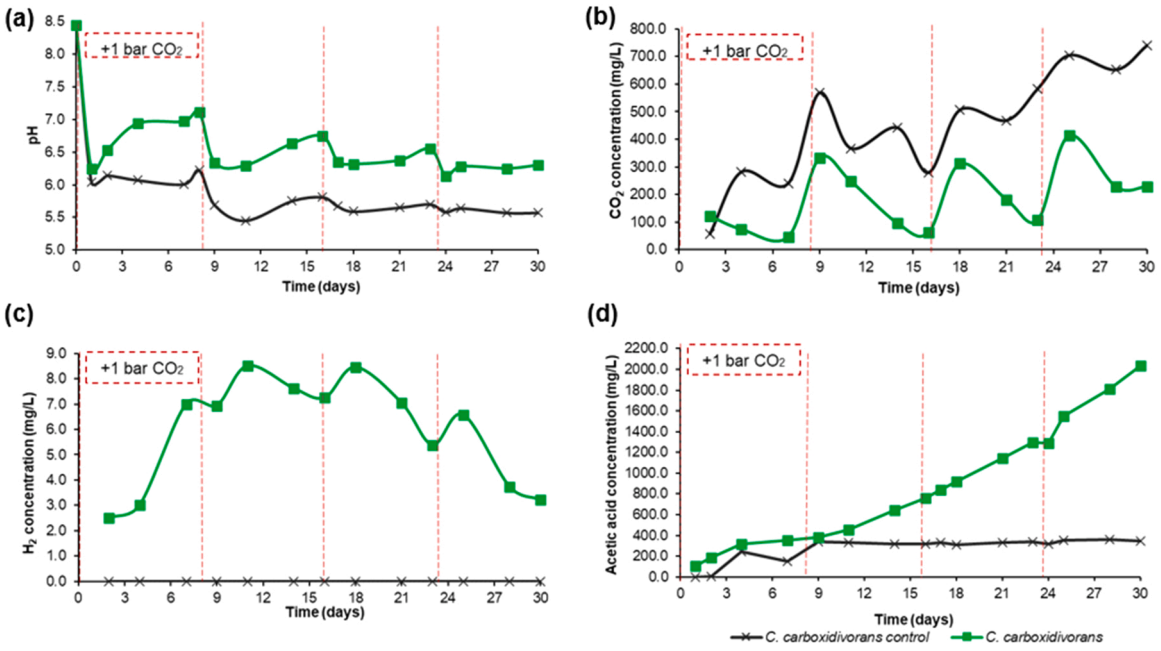

Fig. 6. Optimization of acetic acid (HAc) production in the second set of batch studies with *C. carboxidivorans;* (a) pH, (b) CO_2 amount consumed, (c) H_2 produced by ZVI-water reaction, (d) acetic acid concentration produced. (*C. carboxidivorans* control: *Clostridium carboxidivorans* control bottle without iron, *C. carboxidivorans*: *Clostridium carboxidivorans* with iron).

the most influencial parameters determining the end metabolites produced during anaerobic C1 gas fermentation [24,25]. Simultaneous bacterial growth and acetic acid generation occur at a higher rate during fermentation at optimum growth pH (close to neutral pH). When the fermentation pH is decreased, the produced acetic acid is protonated and transferred across the membrane into the cytoplasm, where it is deprotonated, disrupting cellular homeostasis [26]. Bacteria overcome this stress by converting the accumulated carboxylic acids into the corresponding alcohols. Thus, it is also one of the parameters that can be easily manipulated to stimulate homoacetogens, including the strain under investigation, *C. aceticum*, to produce their growth-related carboxylic acids and corresponding alcohols at the desired concentrations in order to avoid product inhibition during the process where product recovery is not applied [27].

Acetic acid was the predominant metabolite in all four reactor experiments, which were performed under similar fermentation conditions, with the exception of the day on which artificial pH adjustment to a lower value was initiated and maintained. Additionally, the effect of ZVI at 50 and 75 g/L was tested in the last two experiments (Table 1). The objective of the first two reactor studies was to optimize the fermentation conditions that could maximize the overall metabolites production by *C. aceticum* when grown under CO_2 as the sole carbon source and using ZVI as the electron donor. Given that the production of ethanol has been reported to occur when the pH decreases below the optimal growth pH, these studies were conducted at a constant initial pH value between 7.5 and 8, which is favorable for growth and production of acetic acid by *C. aceticum* [27]. Following this first stage of acetogenesis, the pH was adjusted to a lower value of 6.9 on different experimental days in the first two reactor studies and it was maintained around that lower pH value to stimulate solventogenesis, the process by which ethanol is produced from accumulated acetic acid. The initial pH of the experiment is critical because it must be in a range that is optimal for bacterial growth while also being ideal for the generation of H_2 gas from the anaerobic oxidation of ZVI through its reaction with water. Although lower pH values generate hydrogen more rapidly than a higher pH value, it has a detrimental effect on the growth of *C. aceticum* when it falls below a value of 7. For these aforementioned reasons, 7.5 was chosen as the starting pH value for all experiments. While the accumulation of metabolites such as acetic acid resulted in natural acidification of the fermentation medium, in this study, artificial pH adjustment was necessary due to the involvement of multiple reactions that affect the medium's pH. A decrease in pH may occur as a result of CO_2 dissolution, that leads to release of H^+ ions, or, as mentioned earlier, due to product formation (i.e., acetic acid production) by the bacterium. Additionally, anaerobic corrosion of ZVI in water occurs with the release of OH^- ions, which results in a rise in the medium's pH. The pH was automatically regulated by using a pH controller and by adding 2 M HCl and/or 2 M NaOH.

3.4.1. *Effect of pH adjustment in bioreactors to improve the final product concentration*

The pH of the first reactor experiment was reduced and maintained at 6.9 on the twentieth day after starting the experiment, and that of the second reactor experiment was adjusted on the eighth day, in order to understand the effect of the moment of pH adjustment on metabolites production from CO_2 when ZVI was used as electron donor. Both experiments lasted 40 days. The first reactor experiment produced less acetic acid than the second one, reaching 1367 and 1612 mg/L, respectively (Fig. 7). However, lowering the pH to create optimal conditions for ethanol production was hardly conducive as no ethanol production was observed. The increased acetic acid production in the second study may be attributed to the presence of an early acidic environment, which stimulates the anaerobic ZVI-water interaction and generates more H_2.

3.4.2. *Metabolites production with different concentrations of ZVI in bioreactors*

To increase the amount of electrons available for CO_2 reduction, reactors were loaded with different concentrations of ZVI, 50 g/L and 75 g/L, and the experiments were performed under the same conditions as the previous two reactor studies; however, with the pH adjusted on the eighth day, it was previously observed that increasing acetic acid production is possible by creating an acidic environment earlier, which facilitates the production of more H_2. On the eighth day of the experiment, prior to lowering the pH to 6.9, the reactor containing 75 g/L ZVI had produced 1423.69 mg/L acetic acid, while the reactor containing

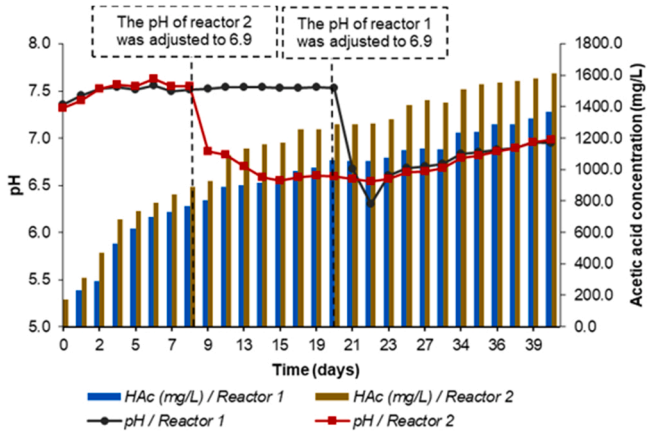

Fig. 7. pH and acetic acid (HAc) production profiles in reactor experiments carried out to optimize the pH adjustment.

50 g/L ZVI had produced 1231.85 g/L acetic acid. On the twelfth day, the production of acetic acid reached 1689.69 and 1556.3 mg/L, respectively (Fig. 8). Based on prior research with *C. aceticum,* it was determined that the bacterium could survive on syngas at a pH range of 6.6–6.7 [28]. The possibility of accelerating solventogenesis and increasing ethanol productivity at a lower pH prompted us to further decrease the experimental pH from 6.9 to 6.6 on the twelfth day. After 36 days of operation, the concentration of acetic acid was almost the same in both reactors, reaching 2113 and 2050 mg/L for 50 g/L and 75 g/L ZVI-containing reactors, respectively. Given the difference in maximum acetic acid production between the two reactors and the cost of ZVI, it would be more prudent to utilize less ZVI if the goal is to produce more acetic acid. On the other hand, some ethanol production was detected in the reactor with 75 g/L ZVI; and the maximum concentration of ethanol measured was 125 mg/L at the end of the experiment (Fig. 9).

Samanides et al. [20] obtained the highest acetic acid production of 2020 mg/L from CO_2 by inoculating anaerobic granular sludge and with 100 g/L ZVI, at a pH of 6–6.5 [20]. This is an acetic acid concentration similar to ours. The additional cost of the chemical BES or any inoculum pretreatment essential to suppress methanogens and thereby limit carbon flow towards methane is avoided by using pure cultures, as demonstrated in our study. However, while assessing the economics of this process, the necessity for a sterile working environment for maintaining pure cultures must also be considered. The best performance is obtained by selecting an ideal microbial candidate that is well adapted to the fermentation operating conditions. In this study, two strains of Clostridia were tested for their ability to produce metabolites from CO_2 as sole carbon source and in the presence of ZVI. Owing to the fact that

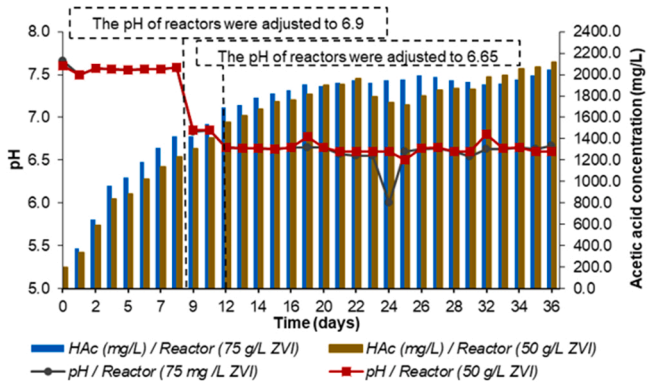

Fig. 8. pH and acetic acid production profiles in reactor experiments carried out at different concentrations of ZVI (75 and 50 g/L) using *C. aceticum.*

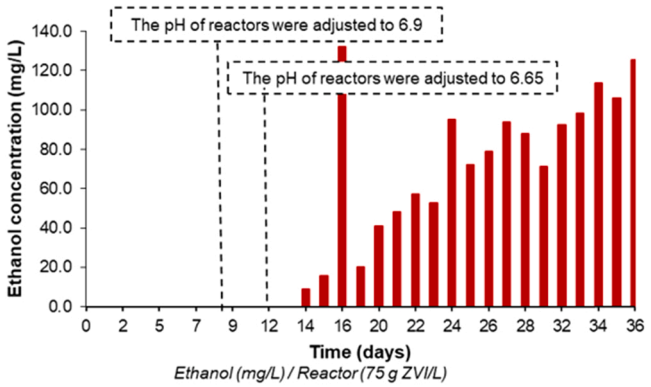

Fig. 9. Ethanol concentration (mg/L) produced by *C. aceticum* in experiments carried out at 75 g/L ZVI.

dissolving CO_2 in water results in a reduction in pH due to the generation of carbonic acid, and the rate of hydrogen production from anaerobic corrosion increases as the pH decreases, microorganisms with a lower pH as growth optimal are ideal candidates for CO_2 + ZVI bioconversion. Additionally, fermenting at a lower pH promotes solventogenesis, which results in the production of alcohols from the accumulated carboxylic acids. In this respect, *C. carboxydivorans* was chosen for this investigation because its optimum growth pH ranges from 5.0 to 7.0 [29]. On the other hand, *Clostridium aceticum* has been extensively employed as a very competent homoacetogen capable of producing large amounts of acetic acid and ethanol either autrophically (CO or CO_2 +H_2) or hetrotrophically (using sugars such as fructose) (Table 2). The supply of ZVI favors these obligatory anaerobes by lowering the ORP of the system, creating a more favorable environment for the bioprocessing [30]. However, their effect was significantly shown during anaerobic digestion of complex substrates by syntrophic microbial cultures through acetoclastic methanogenesis and hydrogenotrophic methanogenesis. Specifically, their involvement was clear in regulating the ethanol-, butyric- and propionic-type fermentation through maintaining certain ORP levels and thereby preventing excessive acidification [30,31]. Our work demonstrates the feasibility of employing ZVI as a source of hydrogen, which must typically be supplied externally during waste gas or syngas fermentation [32]. Additionally, by using waste iron scraps instead of ZVI powder, the additional costs associated with ZVI

Table 2
Different production yields of *C. aceticum* with different substrates and operating systems.

Substrates	Maximum metabolite production	Operation system	Reference
4% H_2: 18% Argon: 78% CO	2.27 g acetate / L (1.40 atm CO partial pressure and at 48 h)	Batch system	[34]
4% H_2:18% Argon:78% CO	1.28 g acetate / L (0.30 g/L cysteine-HCl·H_2O and sodium sulfide respectively)	Batch system	[35]
CO_2:H_2:N_2 3:12:10	14 mmol acetate L^{-1} h^{-1} (Trickle bed biofilm reactor)	Packed-bed and Trickle-bed biofilm reactor	[36]
CO:CO_2:H_2:N_2 30:5:15:50	9.4 g acetate / L, 5.6 g / L ethanol (Natural acidification + pH shifting + medium renewal)	Stirred tank reactor	[27]
Fructose, Pure CO	18 g acetate / L (CO fermentation at pH 8) 4.6 g ethanol / L (CO fermentation without pH control)	Stirred tank reactor	[28]
Pure CO_2, ZVI (25,50,75 g/L)	2 g acetate / L 125 mg ethanol / L (CO_2 and 75 g ZVI / L)	Stirred tank reactor	This study

chemicals can be avoided, while also increasing the process's sustainability. The influence of bulky form ZVI, on the other hand, should be examined, since particle size has been shown to have variable effects on microbial bioprocesses [33].

4. Conclusions

H_2 was generated by anaerobic interaction of ZVI with water in the growth medium and acted as an electron donor for CO_2 reduction by *C. aceticum* and *C. carboxidivorans* and for the production of acetic acid and sometimes ethanol. Among the three ZVI concentrations tested (25, 50, and 75 g/L), 25 g/L ZVI was shown to be the most effective at maintaining the fermentation pH within the range of the bacterial optimum pH during the H_2 evolution reaction in abiotic batch studies. In addition, the acetogenesis process originally evaluated in uncontrolled batch studies demonstrated the importance of optimizing a combination of multiple factors, including fermentation pH, CO_2 addition strategy, and ZVI dosage, for the CO_2 + ZVI bioconversion process. The addition of buffering agents such as $NaHCO_3$ or K_2HPO_4/KH_2PO_4 in fermentation medium is crucial to prevent iron dissolution and a steep increase in pH. The more sophisticated pH-adjusted reactor system was optimized to test the induction of solventogenesis, or the conversion of acetic acid to ethanol by decreasing the pH. The highest acetic acid production across all experiments was 2113 mg/L in the reactor study using 75 g ZVI/L, with *C. aceticum*. Ethanol synthesis was also observed, reaching 125 mg/L, in the same reactor.

CRediT authorship contribution statement

Büsra Bayar: Investigation, Data curation, Writing and Reviewing; **María C. Veiga**: Supervision, Resources, Funding aquisition, Writing and Reviewing; **Christian Kennes**: Conceptualization, Supervision, Funding acquisition, Project administration, Methodology, Data curation, Writing and Reviewing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] K.O. Yoro, M.O. Daramola, Co_2 emission sources, greenhouse gases, and the global warming effect, in: M.R. Rahimpour, M. Farsi, M.A. Makarem (Eds.), Advances in Carbon Capture, Woodhead Publishing, 2020, pp. 3–28.
[2] H. Kanaboshi, F. Sano, J. Oda, K. Akimoto, N. Onishi, Cost-efficient measures in the oil refinery and petrochemical sectors for the reduction of CO_2 emissions under the Paris Agreement and air pollution under the MARPOL Convention, Energy Clim. Change 2 (2021), 100027.
[3] EPA 430-P-17–001; Inventory of US Greenhouse Gas Emissions and Sinks. https://www.epa.gov/sites/default/files/2017-02/documents/2017_complete_report.pdf. [Accessed on 11/Nov/2021].
[4] K. Schuchmann, V. Müller, Autotrophy at the thermodynamic limit of life: a model for energy conservation in acetogenic bacteria, Nat. Rev. Microbiol. 12 (2014) 809–821.
[5] Y. Hwang, P. Sivagurunathan, M.K. Lee, Y.M. Yun, Y.C. Song, D.H. Kim, Enhanced hydrogen fermentation by zero valent iron addition, Int. J. Hydrog. Energy 44 (2019) 3387–3394.
[6] F. Fu, D.D. Dionysiou, H. Liu, The use of zero-valent iron for groundwater remediation and wastewater treatment: a review, J. Hazard. Mater. 267 (2014) 194–205.
[7] Y. Liu, Y. Zhang, X. Quan, Y. Li, Z. Zhao, X. Meng, S. Chen, Optimization of anaerobic acidogenesis by adding Fe^0 powder to enhance anaerobic wastewater treatment, Chem. Eng. J. 192 (2012) 179–185.
[8] X. Meng, Y. Zhang, Q. Li, X. Quan, Adding Fe^0 powder to enhance the anaerobic conversion of propionate to acetate, Biochem. Eng. J. 73 (2013) 80–85.
[9] Y. Feng, Y. Zhang, X. Quan, S. Chen, Enhanced anaerobic digestion of waste activated sludge digestion by the addition of zero valent iron, Water Res. 52 (2014) 242–250.
[10] X. Hao, J. Wei, M.C. van Loosdrecht, D. Cao, Analysing the mechanisms of sludge digestion enhanced by iron, Water Res. 117 (2017) 58–67.
[11] Y. Hu, X. Hao, D. Zhao, K. Fu, Enhancing the CH_4 yield of anaerobic digestion via endogenous CO_2 fixation by exogenous H_2, Chemosphere 140 (2015) 34–39.
[12] S. Karri, R. Sierra-Alvarez, J.A. Field, Zero valent iron as an electron-donor for methanogenesis and sulfate reduction in anaerobic sludge, Biotechnol. Bioeng. 92 (2005) 810–819.
[13] Y. Liu, Y. Zhang, B.J. Ni, Zero valent iron simultaneously enhances methane production and sulfate reduction in anaerobic granular sludge reactors, Water Res. 75 (2015) 292–300.
[14] G. Zhen, X. Lu, Y.Y. Li, Y. Liu, Y. Zhao, Influence of zero valent scrap iron (ZVSI) supply on methane production from waste activated sludge, Chem. Eng. J. 263 (2015) 461–470.
[15] N. Belay, L. Daniels, Elemental metals as electron sources for biological methane formation from CO_2, Anton. Van. leeuwenhoek 57 (1990) 1–7.
[16] L. Daniels, N. Belay, B.S. Rajagopal, P.J. Weimer, Bacterial methanogenesis and growth from CO_2 with elemental iron as the sole source of electrons, Science 237 (1987) 509–511.
[17] L.E. Mayhew, G.E. Lau, A.S. Templeton, Distinct geochemistries of water–basalt–Fe^0 reactions in the presence versus absence of CO_2-driven microbial methanogenesis, Chem. Geol. 428 (2016) 92–105.
[18] I. Vyrides, M. Andronikou, A. Kyprianou, A. Modic, A. Filippeti, C. Yiakoumis, C. G. Samanides, CO_2 conversion to CH_4 using Zero Valent Iron (ZVI) and anaerobic granular sludge: optimum batch conditions and microbial pathways, J. CO_2 Util. 27 (2018) 415–422.
[19] B.S. Rajagopal, J. LeGall, Utilization of cathodic hydrogen by hydrogen-oxidizing bacteria, Appl. Microbiol. Biotechnol. 31 (1989) 406–412.
[20] C.G. Samanides, L. Koutsokeras, G. Constantinides, I. Vyrides, Methanogenesis inhibition in anaerobic granular sludge for the generation of volatile fatty acids from CO_2 and zero valent iron, Front. Energy Res. 8 (2020) 37.
[21] L. Ma, L. Zhou, M.Y. Ruan, J.D. Gu, B.Z. Mu, Simultaneous methanogenesis and acetogenesis from the greenhouse carbon dioxide by an enrichment culture supplemented with zero-valent iron, Renew. Energy 132 (2019) 861–870.
[22] Y. Sun, J. Li, T. Huang, X. Guan, The influences of iron characteristics, operating conditions and solution chemistry on contaminants removal by zero-valent iron: a review, Water Res. 100 (2016) 277–295.
[23] S. Bae, K. Hanna, Reactivity of nanoscale zero-valent iron in unbuffered systems: effect of pH and Fe (II) dissolution, Environ. Sci. Technol. 49 (2015) 10536–10543.
[24] H.N. Abubackar, Á. Fernández-Naveira, M.C. Veiga, C. Kennes, Impact of cyclic pH shifts on carbon monoxide fermentation to ethanol by *Clostridium autoethanogenum*, Fuel 178 (2016) 56–62.
[25] Á. Fernández-Naveira, M.C. Veiga, C. Kennes, Effect of pH control on the anaerobic H-B-E fermentation of syngas in bioreactors, J. Chem. Technol. Biotechnol. 92 (2017) 1178–1185.
[26] J.L. Slonczewski, M. Fujisawa, M. Dopson, T.A. Krulwich, Cytoplasmic pH measurement and homeostasis in bacteria and archaea, Adv. Microb. Physiol. 55 (2009) 1–317.
[27] K. Arslan, B. Bayar, H.N. Abubackar, M.C. Veiga, C. Kennes, Solventogenesis in *Clostridium aceticum* producing high concentrations of ethanol from syngas, Bioresour. Technol. 292 (2019), 121941.
[28] K. Arslan, M.C. Veiga, C. Kennes, Autotrophic (C1-gas) versus heterotrophic (fructose) accumulation of acetic acid and ethanol in *Clostridium aceticum*, Bioresour. Technol. 337 (2021), 125485.
[29] J.S.-C. Liou, D.L. Balkwill, G.R. Drake, R.S. Tanner, *Clostridium carboxidivorans* sp. nov., a solvent-producing *clostridium* isolated from an agricultural settling lagoon, and reclassification of the acetogen *Clostridium scatologenes* strain SL1 as *Clostridium drakei* sp. nov, Int. J. Syst. Evol. Micr 55 (2005) 2085–2091.
[30] Y. Yang, F. Yang, W. Huang, W. Huang, F. Li, Z. Lei, Z. Zhang, Enhanced anaerobic digestion of ammonia-rich swine manure by zero-valent iron: With special focus on the enhancement effect on hydrogenotrophic methanogenesis activity, Bioresour. Technol. 270 (2018) 172–179.
[31] X. Kong, Y. Wei, S. Xu, J. Liu, H. Li, Y. Liu, S. Yu, Inhibiting excessive acidification using zero-valent iron in anaerobic digestion of food waste at high organic load rates, Bioresour. Technol. 211 (2016) 65–71.
[32] Á. Fernández-Naveira, M.C. Veiga, C. Kennes, H-B-E (hexanol-butanol-ethanol) fermentation for the production of higher alcohols from syngas/waste gas, J. Chem. Technol. Biotechnol. 92 (2017) 712–731.
[33] J. Zhang, Y. Qu, Q. Qi, P. Zhang, Y. Zhang, Y.W. Tong, Y. He, The bio-chemical cycle of iron and the function induced by ZVI addition in anaerobic digestion: a review, Water Res. 186 (2020), 116405.

[34] J.H. Sim, A.H. Kamaruddin, W.S. Long, G. Najafpour, *Clostridium aceticum*—a potential organism in catalyzing carbon monoxide to acetic acid: application of response surface methodology, Enzym. Microb. Technol. 40 (2007) 1234–1243.
[35] J.H. Sim, A.H. Kamaruddin, Optimization of acetic acid production from synthesis gas by chemolithotrophic bacterium–*Clostridium aceticum* using statistical approach, Bioresour. Technol. 99 (2008) 2724–2735.
[36] P. Riegler, E. Bieringer, T. Chrusciel, M. Stärz, H. Löwe, D. Weuster-Botz, Continuous conversion of CO_2/H_2 with *Clostridium aceticum* in biofilm reactors, Bioresour. Technol. 291 (2019), 121760.