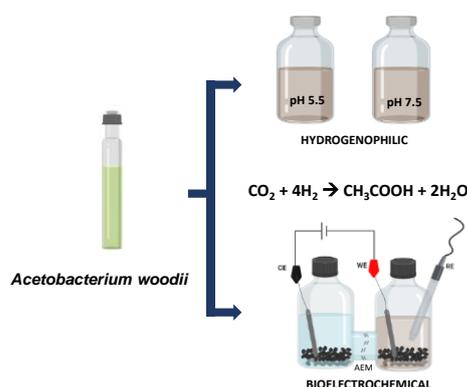


Hydrogenophilic and Bioelectrochemical Production of Acetate with a Pure Culture of *Acetobacterium Woodii*

Jacopo Ferretti, Marco Zeppilli, Lorenzo Cristiani, Mauro Majone, Marianna Villano

Department of Chemistry, University of Rome Sapienza, Piazzale Aldo Moro 5, Rome, 00185, Italy
marco.zeppilli@uniroma1.it

In recent years there has been a growing interest in the potential use of autotrophic acetogenic bacteria to produce compounds of interest through CO₂ fixation, representing an alternative solution to currently used CO₂ storage technologies. This group of microorganisms are ubiquitous in nature and they are characterised by a Wood-Ljungdahl pathway that combines CO₂ fixation with adenosine triphosphate (ATP) synthesis by using H₂ as electron donor. In this work the autotrophic production of acetate by a pure culture of *Acetobacterium woodii* has been tested under hydrogenophilic or bioelectrochemical conditions. More in details, the hydrogenophilic tests were conducted at two different pH values (5.5 and 7.5) with an H₂ partial pressure of 0.52 atm, while bioelectrochemical tests were performed at an applied cathodic potential of -0.90 V vs. SHE (Standard Hydrogen Electrode). The bioelectrochemical tests were set up in H-type reactors (250 mL), in which graphite rods were used as electrodic material and an anion exchange membrane served to separate the anodic and cathodic chambers while allowing anions migration for electroneutrality maintenance. The hydrogenophilic tests resulted in different kinetics depending on the applied pH value. The bioelectrochemical tests, performed at a pH value of 7.5, reached an acetate production rate 2 times higher than in the hydrogenophilic experiments at pH 7.5, as well as an increase in the efficiency of using the reducing power, suggesting an improvement in hydrogen uptake. At pH 5.5, on the other hand, production is improved by increasing the partial pressure of H₂



1. Introduction

One of the most important challenges is the mitigation of CO₂, to prevent damage to the ecosystem and the earth's climate due to its atmospheric accumulation (Wyns & Beagley, 2021). The transformation of industrial CO₂ into products with high added value represents a very attractive solution. The chemical transformation of CO₂, despite having the possibility of producing many substances, has many limits linked to the use of catalysts and energy requirement (Kamkeng et al., 2021). Biological transformation overcomes these limitations as it

operates under mild conditions. Biological transformation is possible through photosynthetic and non-photosynthetic fixation. Photosynthetic fixation is subject to photoinhibition and strictly depends on the day/night cycle and weather conditions, therefore it requires artificial lighting with increased production costs (Blanken et al., 2013). Non-photosynthetic fixation, on the other hand, has the advantages of operating in mild conditions (Salehizadeh et al., 2020). In fact, the production of ethanol, formate, butyrate, butanol and 2,3-butanediol has been reported (Lee et al., 2012). However, the most common products are methane and acetate, produced from methanogens and acetogens, respectively. Acetogens are interesting for the high acetate production from the reduction of CO₂ through the Wood-Ljungdahl pathway, using H₂ as an electron donor. A detailed review of acetogens and their metabolism is reported (Drake et al., 2008). A new perspective of using these organisms implement them in electrosynthesis cells (MES), in which a bioelectrode (cathode) is used to provide electrons for CO₂ reduction. This technology is based on the ability of some bacteria to interact directly with the electrode as an electron donor (Bajracharya et al., 2017). High level of hydrogenophilic acetate can be produced by *Acetobacterium woodii* starting from H₂ and CO₂ as substrates (Balch et al., 1977). *A. woodii* is a strictly anaerobic, non-spore forming bacterium, which grows well at moderate temperatures of about 30°C. *A. woodii* is the most studied and characterized bacterium, especially regarding the conservation of energy (Hess et al., 2013). But the possibility of *A. woodii* to produce acetate in a bioelectrochemical system has not been studied in depth. *A. woodii*'s inability to use direct current has been reported (Nevin et al., 2011), however, it could synthesize acetate using hydrogen produced directly from the electrode. This could overcome the limits of low H₂ transfer in the aqueous phase. Several studies have shown that *A. woodii* is often dominant in the microbial community in CO₂ reduction processes in bioelectrochemical systems for microbial electrosynthesis (Hou et al., 2006; Marshall et al., 2012). In this study, the hydrogenophilic productions of acetate through a pure culture of *A. woodii* under both hydrogenophilic and bioelectrochemical conditions were compared. Furthermore, hydrogenophilic production was tested at two pH levels, 7.5 and 5.5.

2. Material and methods

2.1 *Acetobacterium woodii* culture

The pure culture of *A. woodii* was obtained from the Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany) as active culture in Hungate-type tubes.

2.2 Hydrogenophilic tests

The hydrogenophilic tests were carried out in a 245 ml volume borosilicate glass serum bottle. The serum bottles were filled with 150 ml of mineral medium of composition: 1 g/L NH₄Cl, 0.33 g/L KH₂PO₄, 0.05 g/L K₂HPO₄, 0.12 g/L MgSO₄, 2 g/L yeast extract, 10 g/L NaHCO₃, 0.5 g/L Na₂S, 30 mg nitrilotriacetic acid; 10 mg MnSO₄ x H₂O; 20 mg/L NaCl; 2 mg/L FeSO₄ x 7 H₂O; 3.6 mg/L CoSO₄ x 7 H₂O; 2 mg/L CaCl₂ x 2 H₂O; 3.6 mg/L ZnSO₄ x 7 H₂O; 0.2 mg/L CuSO₄ x 5 H₂O; 0.4 mg/L KAl(SO₄)₂ x 12 H₂O; 0.2 mg/L H₃BO₃; 0.2 mg/L Na₂MoO₄ 2 H₂O; 0.5 mg/L NiCl₂ x 6 H₂O; 6 µg/L Na₂SeO₃ x 5 H₂O; 40 ng/L biotin; 40 ng/L folic acid; 0.2 mg/L pyridoxine-HCl; 0.1 mg/L thiamine-HCl x 2 H₂O; 0.1 mg/L riboflavin; 0.1 mg/L nicotinic acid; 0.1 mg/L D-Ca-pantothenate; 2 ng/L cyanocobalamin; 0.1 mg/L p-aminobenzoic acid; 0.1 mg/L lipoic acid. The pH of the medium was adjusted to 5.5 and 7.5 and was subsequently sterilized in a muffle at 120 ° C for 10 hours, and once cooled to room temperature, the serum bottles were inoculated with 2.5 ml of *A. woodii* strain 1030. The serum bottles were closed with rubber stoppers and flushed with a mixture of N₂/CO₂ (70-30% v/v) to ensure anaerobic conditions. For each test 2.04 mmol (50 ml) of H₂ (partial pressure equal to 0.52 atm) was initially added, and it was added whenever its concentration approached zero, to ensure that it was always present in the System. In the endogenous control tests, no hydrogen was added to determine the endogenous production of acetate from the residual organic carbon fermentation. Whenever gas was introduced into the bottles, it was filtered through cellulose acetate filters, diameter 25 mm, pore diameter 0.2 µm.

2.3 Bioelectrochemical test

A microbial electrosynthesis cell (MEC) was set up in an H-cell, consisting of two 450 ml borosilicate glass bottles fitted with a lateral flange for the junction between the two bottles. The two bottles, namely the anodic and cathodic compartments, were separated by an anion exchange membrane AEM (FUMASEP® FAS, Fumatech GmbH), which was pretreated in a solution of 5% by weight by volume of NaCl in distilled water for 24h. The cell set up was made to work in a configuration with three electrodes: the working electrode WE (cathode), the reference electrode Ag/AgCl and the counter electrode CE (anode). The WE and CE electrodes consisted of graphite rods, which were pretreated in a 34% HCl solution for 24h and washed with distilled water. The working electrode was kept constant at -0.9 V vs SHE through the IVIUM-N-STAT potentiostat. The anode and cathode compartments were filled up to 200 ml of the same sterilized mineral medium (see above), at pH 7, 5. The cathode was inoculated with 4 ml of pure culture of *A. woodii* strain 1030, while the anode was abiotic

and aimed to concentrate the acetate produced in the cathode compartment. Finally, the gas phase was flushed with a mixture of N₂/CO₂ (70-30% v/v).

2.4 Analytical methods and calculations

Analytical methods for CH₄, H₂, CO₂, and CH₃COO⁻ determination has been already described in (Zeppilli et al., 2020). Main calculations related to the anodic and cathodic bioelectrochemical reactions are summarized in Table

Table 1. Main parameters calculations

Parameter	Calculation
Conversion of concentration into meq	$meq = mM * ne^- * V$
CH₃COO⁻ production rate (rCH₃COO⁻)	
- rCH ₃ COO ⁻ (meq/Ld): daily milliequivalents in acetate per liter	$rCH_3COO^- = \frac{mmol_{CH_3COO^-}}{V * \Delta t} * 8 = \frac{meq_{CH_3COO^-}}{V * \Delta t}$
- 8 meq/mmol _{CH₃COO⁻} : conversion factor	
Cathode Capture Efficiency, CCE (%)	
- meq H ₂ : conversion of electricity into hydrogen (for the bioelectrochemical systems)	$CCE_{CH_3COO^-} = \frac{meq_{CH_3COO^-}}{meq_{H_2}}$ $meq_{H_2} = \int_{t=n}^{t=n+1} \frac{idt}{F}$
Reducing power consumption efficiency (η%)	
- meq _{H₂} : calculated with conversion factor of 2 meq/mmol (for the hydrogenophilic tests)	$\eta_{CH_3COO^-} = \frac{meq_{CH_3COO^-}}{meq_{H_2}}$

3. Results and discussion

3.1 Hydrogenophilic tests

The hydrogenophilic tests showed a different acetate production at pH 7.5 and 5.5 as reported in Figure 1-A and Figure 1-B, respectively. Hydrogenophilic and endogenous tests allowed the determination of the net acetate production considering the presence of residual organic matter in the cultivation media of the *A. woodii*. At pH 7.5 a net acetate production rate of 9.2 mg/Ld was observed up to day 8, in which a decrease of the production rate was observed according to the typical concentration profile of a batch hydrogenophilic test. The hydrogenophilic test at pH 7.5, in which a hydrogen partial pressure of 0.55 atm (corresponding to a H₂ concentration of 40 mg/L) was supplied, showed a higher hydrogen consumption rate during the first 25 days in according with the higher acetate production rate. Indeed, after hydrogen replacement at day 25, a low hydrogen consumption rate was observed in according to the lower acetate production rate (Figure 1-A). At pH 5.5, on the other hand, the net production was 4.5 mg/Ld measured up to the 12th day. Unlike the pH 7.5 test, the hydrogen was consumed very slowly (Figure 1-B), so more hydrogen was added to the system, reaching a hydrogen partial pressure of 0.90 atm. This partial pressure of hydrogen seems to have caused the increase in acetate production, with a rate of 9.7 mg/Ld. Comparing the net acetate production rates at the two different pH values, it is interesting to highlight similar performances of the *A. woodii* in terms of acetate production, indeed, the two operational pH allowed for acetate production with different kinetics (Figure 1-C). Moreover, the acetate production observed in both endogenous control tests at pH 7.5 and 5.5 can be attributed to the fermentation of the organic substance present in the yeast extract, and it is observed that *A. woodii* has a greater fermentative activity at pH 7.5 (Kremp et al., 2018). No methane production was observed in all the hydrogenophilic tests at pH 5.5 and 7.5, indicating no significative contamination of the reaction environments by methanogens.

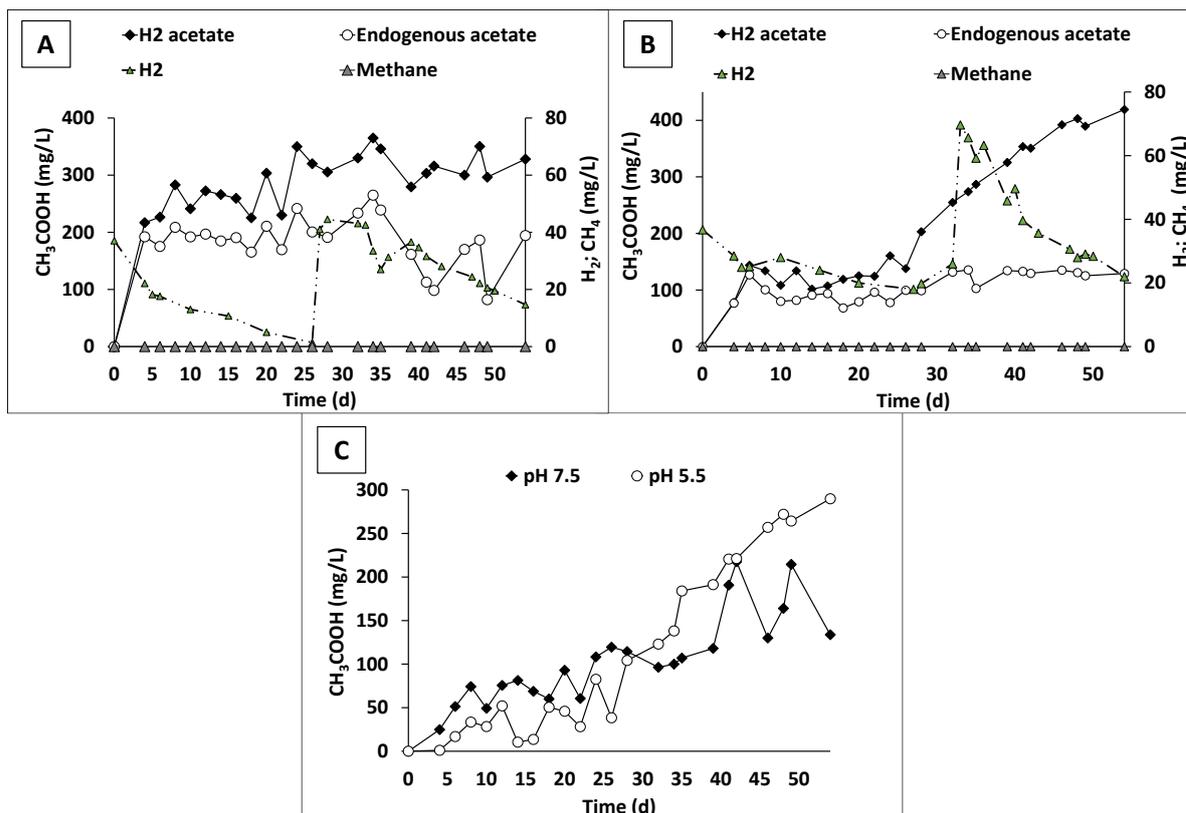


Figure 1. Concentration profile of acetate, methane and hydrogen in the hydrogenophilic and endogenous control, at pH 7.5 (A) and 5.5 (B); profile of the net acetate productions at pH 7.5 and 5.5 (C).

3.2 Bioelectrochemical test

In the microbial electrosynthesis cell was registered a current of 1.8 ± 0.3 mA which has led to an acetate production rate of 19 mg/Ld during the first days considering both cathodic and anodic acetate concentration (Figure 2-A) and considering the quantity of acetate recorded in the endogenous test performed in absence of hydrogen. Acetate concentration strongly decreased after day 10, moreover, according to Figure 2-B, acetate concentration in the anodic chamber of the bioelectrochemical cell followed the same trend, with a strong decrease of acetate concentration. A possible explanation of the acetate production drop can be explained by following the pH evolution in the cathodic chamber, indeed, a strong catholyte alkalization was observed: the catholyte pH had a value of 9.47 at day 10, whereas at day 22 pH reached a value of 11.5. Therefore, as recently reported in the literature (Zeppilli et al 2021), the proceed of the bioelectrochemical reactions, promotes the alkalization of the catholyte due the migration of different species from hydroxyls causing the cathode alkalization and anode acidification. Acetate depletion in the anodic chamber, reported in Figure 2-B suggests the possible acclimatation of a bioelectrochemical community capable to use the anode as electron acceptor for acetate consumption. Indeed, the oxidative conditions of the anodic chamber were suitable with a biological oxidation of the acetate migrated from the cathode to the anode. In addition, the stable pH around 7.5 in the anode compartment probably favored this degradation process (Nekliudov et al., 2008). The decrease of the acetate concentration in the bioelectrochemical system can be explained by a combination of effects in which alkalization of the cathodic chamber promoted the inactivation of *A. woodii* in the cathodic chamber whereas, the retro-diffused acetate in the anodic chamber was than consumed by biological activity, sustained by a pH around 7.5 (Figure 2-B) which would adopt an electroactive mechanism for acetate oxidation in which the graphite electrode was used as final electron acceptor.. The latter evidence is in according with recent literature (Gildemyn et al 2015) in which the anodic chamber of a microbial electrolysis cell reached an acid pH around 2 that allowed for the acetate preservation from the microbial consumption.

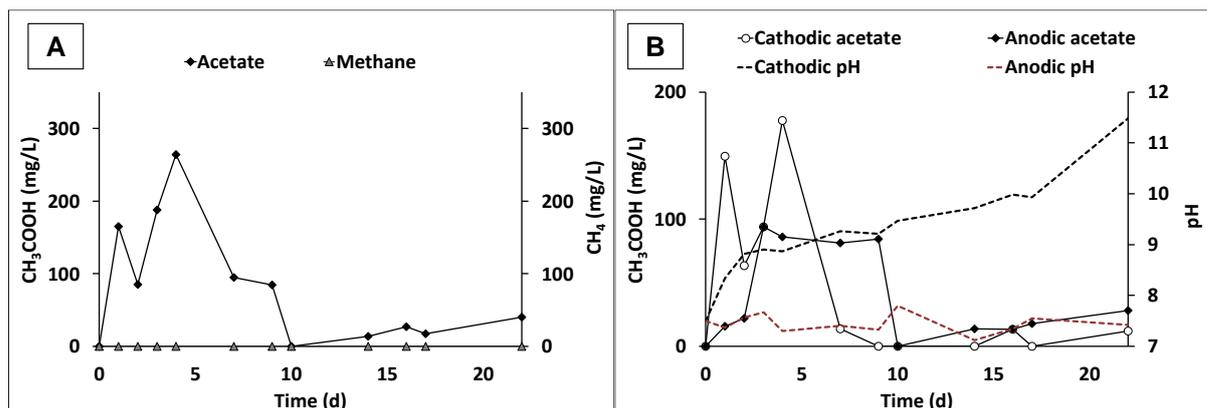


Figure 2. Cumulative acetate and methane production during the bioelectrochemical test (A); acetate concentration and pH profile in the anodic and cathodic chamber of the bioelectrochemical test (B).

3.3 Comparison of hydrogenophilic and bioelectrochemical production

In the hydrogenophilic tests, the efficiencies were measured on the 20th day considering the overall supplied hydrogen. On the contrary, in the bioelectrochemical test, the reducing power consumption efficiency (measured as cathode capture efficiency, CCE) was measured considering the cumulative charge supplied through the electrode polarization. The reducing power consumption efficiency was evaluated considering the net acetate production obtained in each condition taking into account the acetate produced by the endogenous control test. As reported in table 2, the highest acetate production rate under hydrogenophilic conditions was 9 mg/Ld at pH 7.5. The hydrogenophilic test performed at pH 5.5 resulted in an almost equal acetate production rate while a lower reducing power consumption (i.e. hydrogen utilization) efficiency even if its performance was significantly higher than the previous tests with a thermally treated mixed culture conducted at pH 5.5 (Zeppilli et al 2020). The best rate and efficiency were achieved by using the polarized bioelectrochemical system at -0.9 V vs SHE. In fact, the bioelectrochemical test, conducted at an initial pH of 7.5, showed an acetate production rate of 19 mg/Ld (calculated on day 4). It is fundamental to underline that during all the carried-out tests with *A. woodii* no methanogenic activity was detected, indicating a good preservation of the sterile condition.

Table 2. Reducing power consumption efficiency and acetate production rates in all the tests conducted with *A. woodii*

	Reducing power consumption efficiency (%)	Acetate production rate (mg/Ld)
Hydrogenophilic pH 7.5	36	9
Hydrogenophilic pH 5.5	17	10
Bioelectrochemical	58	19

4. Conclusions

The present experiment highlighted the possibility of using a pure culture of *Acetobacterium woodii* in a bioelectrochemical system for the autotrophic production of acetate. In hydrogenophilic conditions, a clear difference between pH 7.5 and pH 5.5 in terms of reducing power consumption efficiency, while an almost equal acetate production rate was observed at the two different level of pH. However, despite the lower stability of the acetate production, a higher production rate and efficiency was observed in the bioelectrochemical system, suggesting an improvement of H₂ uptake overcoming the limit of low mass transfer. However, it is necessary to investigate the aspect linked to microbiological stability for an optimization of the process.

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