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ABSTRACT

Biohydrogen production by the hyperthermophilic and halophilic bacterium *T. maritima*, using fruit and vegetable wastes as the carbon and energy sources was studied. Batch fermentation cultures showed that the use of a culture medium containing natural seawater and fruit and vegetable wastes can replace certain components ($CaCl_2$, $MgCl_2$, Balch's oligo-elements, yeast extract, KH_2PO_4 and K_2HPO_4) present in basal medium. However, a source of nitrogen and sulfur remained necessary for biohydrogen production. When fruit and vegetable waste collected from a wholesale market landfill was used, no decreases in total H_2 production (139 mmol L^{-1}) or H_2 yield (3.46 mol mol^{-1}) was observed.

1. Introduction

The increasing world population and greater average per capita income have led to a rise in energy consumption, amounting to 553 $\times$ 10^{15} kJ in 2010. Currently 80% of most global energy demands are met by fossil fuels, such as oil, coal, and natural gas as main energy sources. Increasing energy demands will accelerate the depletion of fossil fuels, which in turn will raise energy costs and adversely affect national economies (Shafiee and Topal, 2009). Moreover, dependence on fossil fuels has created many environmental problems (e.g. emission of greenhouse gases and pollutants). This situation has prompted the development of renewable energy sources which are expected to provide a solution to the double challenge of environmental restoration and energy security (Turner, 2004). Renewable energy sources such as solar, wind, thermal, hydroelectric and biomass have thus recently attracted much interest internationally

Particular attention is being focused on research into hydrogen production and conservation. The use of hydrogen shows a 10% growth per year, leading to represent 8–10% of total energy in 2025. Today, hydrogen is almost exclusively used for industrial purposes in chemicals and refining. Hydrogen (H_2) is an attractive, clean future energy vector, and has the highest energy content per weight (143 kJ/g, against 54 kJ/g for methane, 29.7 kJ/g for ethanol and 47.3 kJ/g for gasoline). It can be easily and directly converted into water and electrical current (55–60%) in fuel cells. This electrical current can have a wide range of applications from transportation fuel to electricity generation (Mason and Zweibel, 2007). Hydrogen is currently generated by fossil resources, but it can also be produced from non-fossil fuel resources such as water by electrolysis, thermochemical processes, radiolytic processes, and biological processes (Chandrasekhar et al., 2015).

Biological processes such as photofermentation, dark fermentation and biophotolysis are environmentally friendly methods, and have low investment costs (Argun et al., 2017; Pathak et al., 2016). Anaerobic fermentation, also known as dark fermentation, seems a promising alternative for producing hydrogen in view of its high rates of hydrogen production, its low energy requirements, its feasibility (light-independent catabolic process), and its use of renewable feedstock sources (wastes, wastewaters or insoluble cellulosic materials) (Ramírez-Morales et al., 2015; Cardoso et al., 2014; Ruggeri and Tommasi, 2012; Das et al., 2014). Theoretically, the dark fermentation of 1 mol of glucose yields 4 mol of H_2 or 2 mol of H_2 through acetate or butyrate pathways (Kanchanasutaa

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et al., 2016). Several factors influence the fermentative hydrogen production process, such as type and pre-treatment of inoculum, substrate, type of reactor configuration, pH and temperature (De Gioannis et al., 2013).

The highest fermentative H_2 yields have been obtained with (hyper)thermophilic H_2 producers belonging to archaeal and bacterial domains (Guo et al., 2010; Cappelletti et al., 2012; Pradhan et al., 2015). They offer many advantages, such as lower viscosity of media, higher hydrogen production rates, less contamination level by H_2-consuming microorganisms and enhanced hydrolysis rates of complex substrates (Mohan, 2010; Pradhan et al., 2015). Some members of the order Thermotogales have been considered as ideal organisms for the industrial bioconversion of large quantities of waste materials into fuels. They allow high H_2 yields, ranging from 1.5 to 3.85 mol H_2 mol^{-1} hexoses from various carbohydrate-rich wastes (Cappelletti et al., 2012). Furthermore, de Vrije et al. (2009) showed that the rate of substrate consumption, biomass density and H_2 production of *T. neapolitana* were higher on the *Miscanthus* hydrolysate than on pure sugars (glucose/xylose). They have obtained a maximal volumetric hydrogen productivity of 12.6 mmol h^{-1} L^{-1} when *T. neapolitana* was fermenting 10 g L^{-1} of *Miscanthus* hydrolysates. These results could be attributed to the supplementation of the medium with some nutrients originating from the hydrolysate. The volumetric hydrogen productivity and the hydrogen yield of *Thermotoga neapolitana* with 10 g L^{-1} sugars from carrot pulp hydrolysate were 12.5 mmol h^{-1} L^{-1} and 2.8 mol H_2 mol^{-1} hexose, respectively (de Vrije et al., 2010).

In recent years, pure cultures of *Thermotoga maritima* have attracted considerable interest for their potential to produce hydrogen from many simple and complex carbohydrates (Huber et al., 1986; Chhabra, 2003; Nguyen et al., 2008; Boileau et al., 2016). This bacterium contains a wide range of thermostable hydrolytic enzymes (cellulases, invertase and xylanases), which are important for hydrolyzing the carbohydrate polymers into monomer sugars (Cappelletti et al., 2012).

Fruit and vegetable wastes (FVW) are produced in large quantities in wholesale markets; they raise serious environmental concerns, being rapidly contaminated during landfill disposal, especially after mechanical damage. In Tunisia, about 2.5 million tons per year of municipal solid wastes is generated, with an annual increase of about 2.5%. These wastes, characterized by a high moisture content (65%), consist mainly of a biodegradable organic fraction in the form of FVW (68%) (ANGED, 2016). The port of Tunis, with one quarter of the country's population, receives about 400 thousand tons of FVW per year (20% of the national wholesale production). Most wastes (25 tons per day) are transferred to landfills for burial or incineration without energy recovery, resulting in odor and toxic gas emissions, water pollution and costlier municipal landfills. Fermentative hydrogen production from FVW is widely recognized as an important strategy to reduce the escalating cost of landfill. Given their high organic content (75%) and ready biodegradability, FVW can be used as carbon and energy sources biofuel production (Bouallagui et al., 2009, 2005; Garcia-Peña et al., 2011; Mohan, 2010).

To our knowledge, no studies have been carried out with seawater as culture medium for biohydrogen production. One of the advantages of using seawater is to reduce fresh water losses knowing that less of 1% of the world's fresh water is accessible for human uses. Wu et al. (1993) have shown that the outdoor cultivation of *Spirulina* in seawater culture medium has potential for industrial production and has several advantages over its production in freshwater. It does not involve valuable farm land and employ less expensive culture. These results were confirmed by Leema et al. (2010) who have explained the advantage to use seawater media for the cultivation of *Arthrospira (Spirulina) platensis* at very low cost.

The main goal of this work was to study the feasibility of hyperthermophilic H_2 production from fruit and vegetable wastes by *Thermotoga maritima* in a simplified low-cost culture medium. The addition of natural seawater as an inorganic compound source was evaluated on the total H_2 production. The growth medium composition was simplified and optimized to achieve efficient H_2 production process from FVW harvested directly from landfill sites in Tunisia.

2. Material and methods

2.1. Strain and medium

The microorganism used in this study was the type strain of *Thermotoga maritima* DSM 3109 obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). Two mineral media were used to grow *T. maritima* that differed in their composition by the water used as solvent. A mineral basal medium (MBM) was made up with distilled water, while a natural seawater medium (NSM) was made with natural seawater taken directly from the bay of Gammarth located 15–20 km north of Tunis. This natural seawater was filtered under vacuum through a 0.45 μm cellulose nitrate filter (Sartorius, Germany).

The composition of the two media was (g L^{-1}): NH_4Cl (1), yeast extract (1), cysteine HCl (0.3), KH_2PO_4 (0.3), K_2HPO_4 (0.3), NaCl (25), $MgCl_2$ (0.25), KCl (0.5), $CaCl_2$ (0.1), and 10 mL Balch's oligo-elements solution. Balch's solution (pH 6.5) contained (g L^{-1}): nitrilotriacetic acid (1.5), $MgSO_4{\cdot}7H_2O$ (3.0), $MnSO_4{\cdot}H_2O$ (0.5), NaCl (1), $FeSO_4{\cdot}7H_2O$ (0.1), $CoSO_4{\cdot}7H_2O$ (0.18), $CaCl_2{\cdot}2H_2O$ (0.1), $ZnSO_4{\cdot}7H_2O$ (0.18), $CuSO_4{\cdot}7H_2O$ (0.01), $KAl(SO_4)_2{\cdot}12H_2O$ (0.02), H_3BO_3 (0.01), $Na_2MoO_4{\cdot}2H_2O$ (0.01), $NiCl_2{\cdot}6H_2O$ (0.025), $Na_2SeO_3{\cdot}5H_2O$ (0.0003), $Na_2WO_4{\cdot}2H_2O$ (0.0112) (Boileau et al., 2016).

2.2. Feedstocks: sampling, preparation and characterization

Two feedstocks were used in this study: (i) Model Fruit and Vegetable Wastes (MFVW), whose main constituents (g/kg) were: plums (207), peaches (207), apples (207), carrots (138), potatoes (130) and tomatoes (110), and (ii) Fruit and vegetable wastes (FVW) directly collected in a landfill near the Bir Kassa wholesale market of Tunis, in the winter season. The composition of FVW varied, reflecting the average production of these wastes in the wholesale market of Tunis during the winter season (apples, carrots, potatoes, tomatoes, pears, oranges, tangerines, onions, fennel, spinach and parsley, etc.). The two feedstocks were crushed with an electric blender into small pieces measuring less than about 2 mm in length and width, filtered, fully mixed and directly stored at −20 °C for later use.

2.3. Experimental system

The batch fermentation cultures of *T. maritima* for biohydrogen production were conducted in anaerobic conditions in a continuously stirred tank reactor (CSTR). A schematic diagram of the experimental process is shown in Fig. 1. The CSTR was composed of a 2.5 L glass vessel with a double envelope jacket for temperature regulation and a stainless steel lid with septum. The bioreactor was heated (80 ± 0.5 °C) by thermal recirculation of water in the jacket using a heat bath (Polystat 37, Fisher Scientific). The bioreactor was stirred at 150 rpm with an electric motor (IKA EUROSTAR 20 digital) and was equipped with pH and redox probes (Mettler Toledo InPro 3253, Switzerland), calibrated at 80 °C before

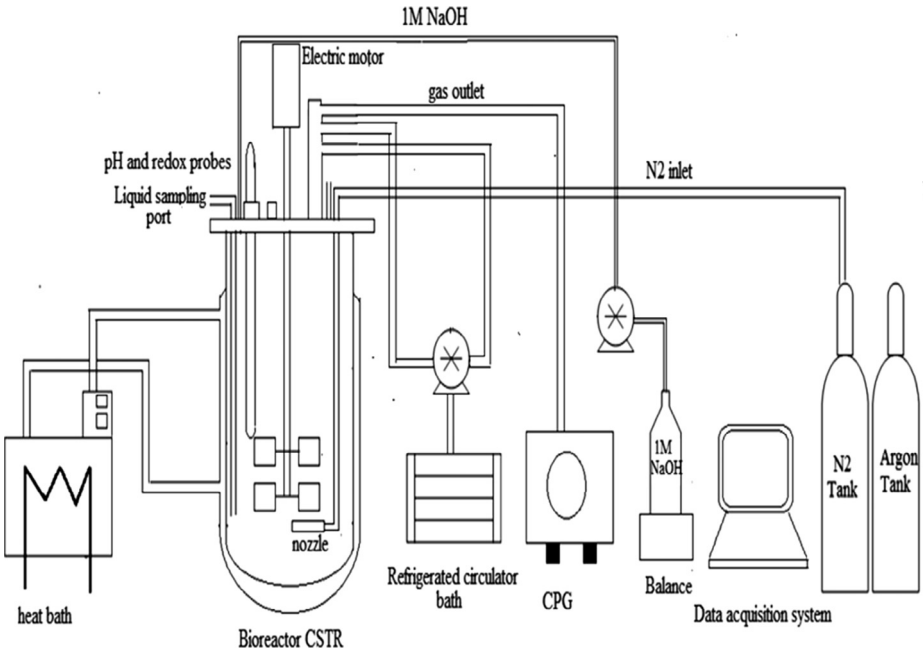

Fig. 1. Schematic representation of the H_2 experimental process composed of the bioreactor, heat bath, condenser, pH regulation and GC analyser.

fermentation as described by Lakhal et al. (2011). The regulation of pH at 7.0 ± 0.1 in the bioreactor was automatic, by adding 1 M NaOH whose consumption was tracked by a balance (AE Adam, France).

Anaerobic conditions were maintained by continuous injection of a stream of pure N_2 at 50 mL min^{-1} through a nozzle immersed in the bottom of the bioreactor. The N_2 gas inlet was equipped with a 0.20 μm PTFE membrane filter (Midisart® 2000, Sartorius Stedim) to sterilize the gas. To prevent loss of liquid caused by the high temperature, the outlet gas was condensed in a water cooler (PTC-2 Peltier Temperature Controller) whose temperature was controlled at around 7 °C with a refrigerated circulator bath equipped with a pump. The composition of outlet gas (H_2 and N_2) was measured at regular intervals (30 min) by automatically taking 1 mL from the headspace of CSTR and injecting it into the GC (Gas Chromatograph) via a valve. The CO_2 content in the outlet gas stream was measured in-line using a carbon dioxide probe (Vaisala Series GMT221, Finland) connected to a transmitter.

T. maritima was batch-cultured in a 2.2-L double-jacket glass bioreactor (FairMenTec, France) with a 1.1-L working volume. Before inoculation, the bioreactor was filled with the MBM, and 0.4 g L^{-1} of Na_2S was added. Experiments were set up by removing each of the nutrients one by one from the culture medium (Table 3) and evaluating impact on fermentative H_2 production. Each experimental condition was run in triplicate successive fermentation cycles. For the fermentation cycle, the bioreactor was emptied under a pure N_2 stream leaving a volume of 100 ml of fermentation liquid necessary to inoculate the next fermentation cycle. New culture medium supplemented with adequate nutrients was added to this volume of 100 mL, and a new fermentation cycle run.

2.4. Analytical methods

The total solids (TS), volatile solids (VS), total organic carbon, total nitrogen Kjeldahl (TNK), total chemical oxygen demand (tCOD), and the pH of the substrates were estimated according to APHA (2005). For total carbohydrate concentration, the anthrone-sulfuric acid method was used (Raunkjær et al., 1994) with modifications. A 0.2% solution of anthrone (w/v) was made up fresh in 75% (v/v) sulfuric acid on the day of measurement. The procedure consists in mixing a 1 mL sample with 2 mL 75% H_2S0_4 and 4 mL of anthrone reagent by vortex. Samples were placed on the heating block at 105 °C for 15 min and then cooled down to room temperature. Absorbance of each sample was determined at 625 nm using a UV–visible spectrophotometer. Measurement of biomass concentration could not be carried out due to the fact that fruits and vegetables (MFVW or FVW) interfered with the measurement of OD or proteins.

The reducing sugar concentrations were measured according to the method described by James (2013). The lignocellulose characterization of substrates including cellulose and hemicelluloses was carried out with reference to a gravimetric method described by Sun et al. (2003). The experiments related to the lignocellulose characterization of substrates were done in triplicate.

The concentration of H_2 in the bioreactor headspace was determined using a gas chromatograph (GC, Perichrom Company, France) equipped with a thermal conductivity detector (TCD) and a concentric CTR1 column (Alltech, USA). The operational temperatures of the detector, the injector and the oven were 100 °C, 100 °C and 40 °C, respectively. Argon was used as the carrier gas at a flow rate of 20 mL min^{-1}. This system was connected to a computer running WINILAB III software (Perichrom, France).

During fermentation, the concentrations of the main soluble metabolite products (acetate and lactate) and the residual sugars (sucrose, glucose and fructose) were measured. The liquid samples harvested from the CSTR were centrifuged at 14,000*g* for 5 min. The supernatants obtained were then filtered through a 0.45 μm cellulose acetate Minisart syringe filter (Sartorius Stedim). They were analyzed by HPLC (Agilent 1200 series, USA) equipped with a quaternary pump model coupled to a refractometer index (RI) detector and 300 × 7.8 mm Aminex HPX-87 H ion-exchange columns (Bio-Rad). This HPLC was connected to a computer running WINILAB III software (Perichrom, France). Sulfuric acid 5 mmol L^{-1} (in milliQ water) was used as mobile phase with a flow rate of 0.5 mL min^{-1}.

Table 1
Physical and chemical characterization of Fruit and Vegetable Wastes (FVW) and Model Fruit and Vegetable Wastes (MFVW).

Parameter	MFVW	FVW
Total solids (TS) (% wb)	10.1 ± 0.1	8.5 ± 0.3
Volatile solids (%/TS)	94.6 ± 1.7	92.7 ± 2.3
Total COD (g L^{-1})	148 ± 2.5	129 ± 6.3
Particulate COD (g L^{-1})	52 ± 4.1	38 ± 4.6
Soluble COD (g L^{-1})	96 ± 4	91 ± 4.3
Total organic carbon (g L^{-1})	103.6 ± 4.5	81.6 ± 1.5
Carbohydrates (g L^{-1})	106 ± 1.9	97.2 ± 1.8
Total nitrogen Kjeldahl (g L^{-1})	2.2 ± 0.04	2.5 ± 0.1
Reducing sugars (g L^{-1})	83.5 ± 5.6	79.5 ± 4.7
pH	4.07 ± 0.09	4.25 ± 0.05
Cellulose (g L^{-1})	4. 5 ± 0.7	7.5 ± 0.4
Hemicellulose (g L^{-1})	1.9 ± 0.2	2.5 ± 0.1
Reducing sugars (HPLC) (g L^{-1})	84.1 ± 0.3	76.1 ± 0.6

wb: wet basis.

2.5. Characteristics of the model fruit and vegetable wastes (MFVW) and fruit and vegetable wastes (FVW)

The characteristics of the MFVW and FVW are listed in Table 1. Total solid concentrations in MFVW and FVW were 10.1% and 8.5% with a total volatile solids content of about 94.6% and 92.7%, respectively; pH was around 4 for both substrates. The total chemical oxygen demand (tCOD) and the total organic carbon (TOC) concentrations for the MFVW were 148 and 103.6 g L^{-1}, respectively. These values were also high for the FVW (129 and 81.6 g L^{-1}, respectively). However, the nitrogen content, quantified as total nitrogen Kjeldahl (TNK), was low for both substrates (around 2.5 g L^{-1}). The corresponding C/N ratio of MFVW and FVW was balanced at around 47.1 and 33, respectively. The organic fraction for MFVW and FVW consisted of a large amount of carbohydrates (106 and 97.2 g L^{-1}, respectively), which have an important role during fermentative H_2 production. The low amount of cellulose and hemicellulose in MFVW (4.5 and 1.9 g L^{-1}) and in FVW (7.5, 2.5 g L^{-1}) was explained by the waste centrifugation step to eliminate the solid phase and facilitate sampling during the batch fermentations. However, fruit and vegetable wastes are known to be cellulose-poor, are easily biodegradable and release volatile fatty acids (Bouallagui et al., 2005; Mohan, 2010). The concentration of reducing sugars in MFVW and FVW was about 83.5 and 79.5 g L^{-1}, respectively.

3. Results and discussion

3.1. Physical and chemical characterization of feedstocks

For MFVW, the concentrations of reducing sugars obtained by HPLC were as follows: 163 mmol L^{-1} of glucose, 282 mmol L^{-1} of fructose and 22 mmol L^{-1} of sucrose for a total amount of about 467 mmol L^{-1} (84.1 g L^{-1}) (Table 1). Total carbohydrate content of MFVW was about 106 g L^{-1} (588 mmol L^{-1} equivalent glucose) (Table 1). The concentrations of reducing sugars and carbohydrates were also obtained from APRIFEL (2005) using the quantities of the different fruits and vegetables in the MFVW (Table 2). They were 86.7 g L^{-1} (481 mmol L^{-1}) and 114 g L^{-1} (633 mmol L^{-1}), respectively (Table 2).

Experimental concentrations of reducing sugars and total carbohydrate were comparable to those obtained from APRIFEL (2005). The differences between the concentrations of total carbohydrates and reducing sugars obtained from experiments (Table 1) and APRIFEL (2005) (Table 2) were about 22.5 g L^{-1} (125 mmol L^{-1}) and 27.3 g L^{-1} (151.7 mmol L^{-1}). We note that except for potatoes, reducing sugar concentrations were equivalent to carbohydrate concentrations of the remaining constituents of MFVW (Table 2). Potatoes contain mainly carbohydrates such as starch, and very low concentrations of reducing sugars. Concentration of carbohydrates in potatoes is about 20 g L^{-1} (111.3 mmol L^{-1}) taking into account their water content (78.9% wet basis) (Table 2). This concentration is comparable to those obtained experimentally (22.5 g L^{-1} or 125 mmol L^{-1}). It therefore seems that most of the carbohydrates came from the potatoes. Total solids (TS) (Table 1) of MFVW was about 117 g L^{-1} (considering a mean water content of MFVW of 85.2% (wet basis) (Table 2)). This value was lower than the average concentration of TS calculated from APRIFEL (2005), which was about 151.8 g L^{-1} (Table 2). This result seems correct because some fiber was removed after the centrifugation of MFVW.

FVW contained wastes obtained in the winter season, composed of citrus fruits (oranges and mandarins), apples and various vegetables (potatoes, carrots, tomatoes, onions, parsley, etc.). The heterogeneity of FVW from uncontrolled wastes precludes their characterization by APRIFEL (2005). The concentration of reducing sugars in FVW was about 79.5 g L^{-1}, confirmed by measurements by HPLC (76.1 g L^{-1}, 423 mmol L^{-1}: glucose 182 mmol L^{-1}, fructose 233 mmol L^{-1} and sucrose 8 mmol L^{-1}). Carbohydrate concentration was about 97.2 g L^{-1} (540 mmol L^{-1} equivalent glucose) (Table 1).

To evaluate the efficiency of fermentative H_2 production from organic wastes by *T. maritima*, the indigenous fermentative communities in MFVW and FVW were evaluated in CSTR under hyperthermophilic conditions (80 °C). Some studies have demonstrated the feasibility of H_2 production from self-fermentation of vegetables wastes without inoculum addition or pretreatments under mesophilic anaerobic conditions (28 °C and 37 °C) (Marone et al., 2014). In our culture conditions, experiments in batch reactors with only NSW, FVW and all components necessary for the medium culture (negative control) were conducted. During these experiments, we did not obtain any production of hydrogen nor of other compounds (acetate and lactate) (data not shown). These results may be explained by the absence of indigenous extremophilic and/or halotolerant microflora able to produce H2 by dark fermentation.

Table 2
Composition of different constituents of Model Fruit and Vegetable Wastes (MFVW). The composition of these different constituents (g/100 g) was taken from APRIFEL, (2005). The total concentrations of proteins, soluble sugars, carbohydrates, lipids and fiber (g L^{-1}) were calculated using the average of water content of the fruit and vegetable mixture, the concentration of each fruit or vegetable constituents (g/100 g) and the weight of each fruit or vegetable (g kg^{-1}).

Constituent	Weight (g kg^{-1})	Water content (% wb)	Proteins	Soluble sugars	Carbohydrates	Lipids	Fiber	Organic acids
Plums	207	81.9	0.8	9.6	9.6	0.3	2.3	–
Pears	207	85.1	0.4	10.4	10.8	0.2	3	0.1
Apples	207	85.3	0.3	11.3	11.3	0.2	1.9	0.5
Carrots	138	89.4	0.8	4.9	6.6	0.3	2.2	–
Potatoes	130	78.9	2	0.5	15.8	0.2	2.1	–
Tomatoes	110	94.5	0.8	1.7	1.7	0.3	1.4	0.4
Total	1000	85.2*	9 g L^{-1}	86.7 g L^{-1}	114 g L^{-1}	2.7 g L^{-1}	26.1 g L^{-1}	–

* Represents the average of water content of the fruit and vegetable mixture (MFVW).

Table 3
Experimental conditions for the CSTR batch fermentations E1 to E10 were performed using Model Fruit and Vegetable Wastes (MFVW) E11 and E12 were performed using a mixture of fruit and vegetable wastes (FVW) (+) with, (−) without.

Experiment	Natural sea water (NSW)	$CaCl_2$ & $MgCl_2$	Balch's oligo-elements	Yeast extract	KH_2PO_4 & K_2HPO_4	Na_2S	Cyst-HCl	NH_4Cl
E1	−	+	+	+	+	+	+	+
E2	+	+	+	+	+	+	+	+
E3	+	−	−	+	+	+	+	+
E4	+	−	−	−	+	+	+	+
E5	+	−	−	−	−	+	+	+
E6	+	−	−	−	−	−	+	+
E7	+	−	−	−	−	+	−	+
E8	+	−	−	−	−	−	−	+
E9	+	−	−	−	−	+	−	−
E10	+	−	−	−	−	−	+	+
E11	+	−	−	−	−	−	+	+
E12	+	−	−	−	−	−	+	+

3.2. Effects of natural seawater on growth and fermentative H_2 production of T. Maritima from MFVW

T. maritima was grown in a mineral basal medium (MBM) with a concentration of MFVW equivalent to 41.6 ± 2.2 mmol L^{-1} of total carbohydrates (experiment E1, Table 4). To reduce the production cost for *T. maritima* growth medium, natural seawater, a complex medium unlimitedly available and containing numerous minerals, was used for aqueous solution in a fermentation medium (NSM) (experiment E2, Table 3). The seawater used was directly harvested in the bay of Gammarth, Tunisia. Despite the advantages of seawater, the possible presence of heavy metals and/or hydrocarbons could inhibit microbial growth. There are few data available on the pollution of the coastal surface waters near Tunis. However, other studies have shown that samples collected from the Gulf of Gabès (south of the Gulf of Tunis) contain total dissolved aliphatic and polycyclic aromatic hydrocarbon concentrations ranging from 0.02 to 6.3 μg L^{-1} and from 8.9 to 197.8 ng L^{-1}, respectively (Fourati et al., 2017). These results confirm that this area is moderate-to-highly impacted by hydrocarbons and such compounds can be found in the bay of Gammarth, and so might impact the growth of *T. maritima*. However, *T. maritima* grew in an equivalent manner in both MBM and NSM media in the presence of MFVW as carbon and energy sources (Figs. 2a and 2b, Table 4).

In experiments E1 and E2, 33.6 mmol L^{-1} of total carbohydrates (42.4 ± 0.8 mmol L^{-1}) was consumed. Both cultures reached maximum H_2 productivity after approximately 6 h of fermentation. In these experiments, the maximum H_2 production rates were about 12.4 mmol h^{-1} L^{-1}, Table 4) showing the high potential of fermentative hydrogen production from fruit and vegetable wastes. Maximal hydrogen productivity of some *Thermotoga* strains has been reported to be between 2.7 and 14.5 mmol h^{-1} L^{-1} (Nguyen et al., 2008). Gomez-Romero et al. (2014) have obtained the highest overall productivity of biohydrogen (2.16 mm h^{-1} H_2 L^{-1}) and the maximum volumetric H_2 production rate (10.6 mmol h^{-1} L^{-1}) when they combined fruit and vegetable wastes (FVW) with crude cheese whey (CCW) (C/N ratio of 21). In the same way, Tenca et al. (2011) have obtained the highest production rate of 3.27 ± 0.51 L_{H2} L^{-1} d^{-1} after using a mixture of fruit and vegetable wastes with swine manure with ratio of 35/65.

The consumption of soluble sugars was almost complete within 24 h with fast utilization of about 85% of the glucose and 50% of the fructose during the first 10 h (Fig. 2b). The consumption of both sugars corresponded to 74% of the total consumed sugars. The remainder consumed (26%) corresponded to about 10 ± 2 mmol L^{-1} of complex carbohydrates, similar to the initial concentration of potato starch (11.8 mmol L^{-1}) in MFVW calculated from APRIFEL (2005) (Table 2). In our study, the growth rate of *T. maritima* could not be evaluated. However, recent studies showed that it depends on glucose, yeast extract, thiosulfate and dissolved hydrogen concentrations (Boileau et al., 2016; Auria et al., 2016).

T. maritima is known to metabolize both simple sugars and polysaccharides ranging from hexose and pentose monomers to starch and xylan polymers (Chhabra, 2003). The main fermentative end-products in both cases were essentially H_2, acetate and CO_2 with a little lactate production (1.75 ± 0.55 mmol L^{-1}, Table 4). The maximum H_2 production was about 127.6 ± 1.35 mmol L^{-1} equivalent to an average H_2 yield on total consumed sugars of 3.8 mol of H_2 per mole of total sugar, close to the theoretical H_2 yield of 4 mol of H_2 per mole of glucose in fermentation metabolism. Interestingly, total H_2 production and H_2 yield on consumed carbohydrates obtained in this study in 24 h were higher than those obtained with *T. maritima* cultured with optimal conditions (60 mmol L^{-1} glucose, 1 g L^{-1} yeast extract and 0.12 mmol L^{-1} thiosulfate) (Boileau et al., 2016). These authors obtained, at the end of the fermentation (23 h of culture), a maximum total H_2 production and H_2 yield of about 99.7 mmol L^{-1} and 2.2 mol mol^{-1}, respectively. However, compared with other hydrogen-producing microorganisms, *T. maritima* exhibited one of the highest H_2 yields, close to the theoretical maximum value (Thauer limit) of 4 mol of H_2 per mole of glucose (Cappelletti et al., 2012; Nguyen et al., 2008; Pradhan et al., 2015; Schröder et al., 1994). The hydrogen yield for *Thermotoga neapolitana,* with 10 g/L sugars from carrot pulp hydrolysate, was about 2.7 mol H_2 mol^{-1} hexose (de Vrije et al., 2010). These authors have also shown that *T. neapolitana* did not grow when using carrot pulp in the bioreactor.

During fermentation of *T. maritima* on MFVW in the presence of NS, the only noteworthy difference was that the redox potential (Eh) of the culture medium was significantly lowered to about −340 mV and −410 mV, respectively (Fig. 2b). Lakhal et al. (2011) showed that *T. maritima* was able to strongly reduce the redox potential of the culture medium, down to about −480 mV, so long as glucose was available. The higher Eh measured in the presence of NS (−340 mV) was probably due to oxidative compounds such as sulfate (2.8 g L^{-1}) contained in NS. However, this higher Eh value did not affect the total H_2 production (Fig. 2a).

3.3. Effects of the different nutrients in natural seawater medium (NSM) on fermentative H_2 production

T. maritima was grown on NSM using MFVW as a source of nutrients and energy. Consistent with the origin of this hyperthermophilic bacterium isolated from hot submarine regions, the culture medium enabling its optimal growth is usually complex (Childers et al., 1992; Huber et al., 1986; Nguyen et al., 2008). To simplify this medium by using seawater and fruit and vegetable wastes, many experiments have been carried out following the protocol defined in Table 3, which lists the main components used

Table 4
Results obtained for batch fermentations for experiments E1 to E12.

Experiments	E1 MBM	E2 NSM	E3 E2 w/o oligo & Ca/Mg	E4 E3 w/o YE	E5 E4 w/o KPO_4	E6 E5 w/o Na_2S	E7 E5 w/o Cys-HCl	E8 E5 w/o Na_2S & Cys-HCl	E9 E6 w/o NH_4Cl	E10 E6 + MFVW 2.6* [sugars]	E11 E6 + FVW	E12[a] E6 + FVW 3* [sugars]
Total carbohydrates (mmol L^{-1})	41.6 ± 2.2	43.2 ± 1.6	42.8 ± 2.1	45.9 ± 1.8	48.1 ± 1.9	47.7 ± 0.3	49.7 ± 3.2	48.4 ± 3.2	42.4 ± 1.8	124.1 ± 7.3	46.1 ± 3.4	143 ± 12.6
Glucose (mmol L^{-1})	11.3 ± 1.4	12.4 ± 0.9	13.9 ± 1.4	14.8 ± 2.1	15.7 ± 3.2	14.1 ± 2.6	16.5 ± 2.7	15.6 ± 1.2	12.6 ± 1.1	42.9 ± 4.6	16.3 ± 1.3	43.6 ± 7.6
Fructose (mmol L^{-1})	15.6 ± 1.7	17.6 ±1.8	17.3 ± 1.6	18.6 ± 2.2	20.3 ± 4.6	20.3 ±3.9	21.9 ± 5.1	18.9 ± 2.8	16.7 ± 2.3	52.9 ± 5.3	19.2 ± 2.6	50.6 ± 8.7
Others (Starch…) (mmol L^{-1})	14.7	13.2	11.6	12.5	12.1	13.3	11.3	13.9	13.1	28.3	10.6	48.8
Consumed carbohydrates (mmol L^{-1})	33.9 ± 0.6	33.2 ± 0.3	34.5 ± 1.3	33.9 ± 0.5	33.6 ± 0.5	34.1 ± 0.8	37.2 ± 0.9	2.3 ± 2	1.2 ± 0.8	62.2 ± 3.5	40.19 ± 1.6	124.3 ± 5.6
Glucose (mmol L^{-1})	10.3 ± 0.3	11.5 ± 0.5	13.2 ± 0.7	13.3 ± 1.2	14.5 ± 1	12.6 ± 1.6	15.6 ± 0.3	0.5 ± 0.1	0	20.4 ± 1.5	15.7 ± 0.6	37.2 ± 2.3
Fructose (mmol L^{-1})	11.5 ± 0.7	13.6 ± 0.6	12.7 ± 1.2	14.2 ± 0.8	15 ± 1.4	15.6 ± 0.8	15.7 ± 0.8	1.6 ± 0.2	0	28.3 ± 1.7	17.9 ± 1.2	40.4 ± 3.1
Others (Starch…) (mmol L^{-1})	12.1	8.1	8.6	6.4	4.1	5.9	5.9	0.2	1.2	13.5	6.6	46.7
H_2 total production (mmol L^{-1})	125 ± 2.7	129 ± 2.9	122.7 ± 3	120 ± 2.5	109 ± 1.9	98 ± 1.1	86 ± 1.6	1.3 ± 0.5	0.3 ± 0.1	165 ± 6.9	139 ± 2.7	446 ± 11.3
H_2 Yield (mol mol^{-1})	3.69 ± 0.2	3.89 ± 0.05	3.56 ± 0.1	3.54 ± 0.2	3.24 ± 0.1	2.87 ± 0.05	2.31 ± 0.1	0.57 ± 0.05	0.25 ± 0.2	2.65 ± 0.3	3.46 ± 0.1	3.59 ± 0.3
Maximal H_2 productivity (mmol h^{-1} L^{-1})	11.5 ± 1.1	12.4 ± 1.8	8.8 ± 0.8	8.1 ± 0.8	7.3 ± 1.3	5.8 ± 0.6	5.6 ± 0.8	0	0	11.8 ± 0.9	12.4 ± 0.1	18.1 ± 2.1
Acetate production (mmol L^{-1})	64 ± 1.2	65 ± 1.4	64 ± 1.5	61 ± 1.2	56 ± 0.9	50 ± 0.6	43.5 ± 0.8	1.6 ± 0.2	0.9 ± 0.1	96.8 ± 3.2	73 ± 1.4	226 ± 5.1
Lactate production (mmol L^{-1})	2.3 ± 0.3	1.2 ± 0.2	4.3 ± 1.2	6.1 ± 0.8	10.1 ± 1.1	17.3 ± 2.2	29.3 ± 3.6	0.6 ± 0.04	0.9 ± 0.05	24.36 ± 2.9	6.4 ± 1.2	19.6 ± 3.5
(lactate + acetate)/C_{tot} (mol mol^{-1})	1.96	1.99	1.98	1.98	1.97	1.97	1.96	0.96	1.5	1.95	1.98	1.98
acetate /C_{tot} (mol mol^{-1})	1.88	1.96	1.86	1.8	1.67	1.47	1.17	0.70	0.75	1.56	1.82	1.82
H_2/acetate (mol mol^{-1})	1.95	1.98	1.92	1.97	1.95	1.96	1.98	0.81	0.33	1.7	1.9	1.97
% of Consumed Sugars	81.49	76.85	80.61	73.86	69.85	71.37	74.85	4.75	2.83	50.12	87.18	86.92
CO_2 (mmol L^{-1})	52.3 ± 0.8	53.6 ± 1.1	51.1 ± 1.9	54.2 ± 2.1	49.3 ± 1.8	42 ± 2.6	39 ± 2.3	3.36 ± 0.6	2.23 ± 0.2	77.4 ± 4.3	68.8 ± 2.5	219 ± 9.6
H_2/CO_2 (mol mol^{-1})	2.39	2.41	2.4	2.21	2.21	2.33	2.21	0.39	0.13	2.13	2.02	2.04

[a] After 43 h of fermentation. w/o: without.

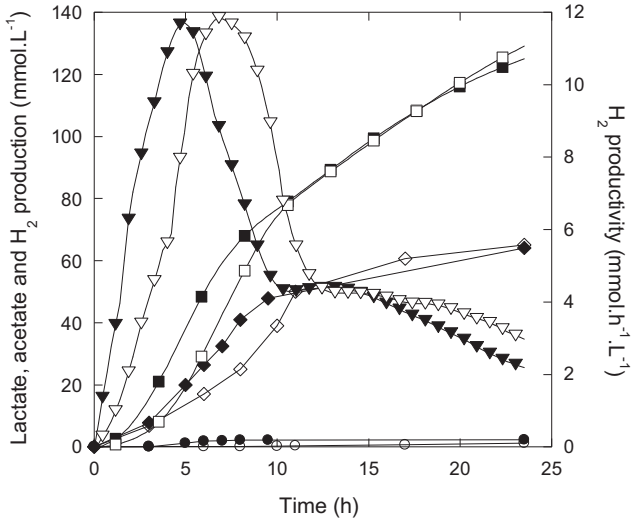

Fig. 2a. Lactate (E1: ●, E2: ○), acetate (E1: ◆, E2: ◇), H_2 production (E1: ■, E2: □) and H_2 productivity (E1: ▼, E2: ▽) versus time.

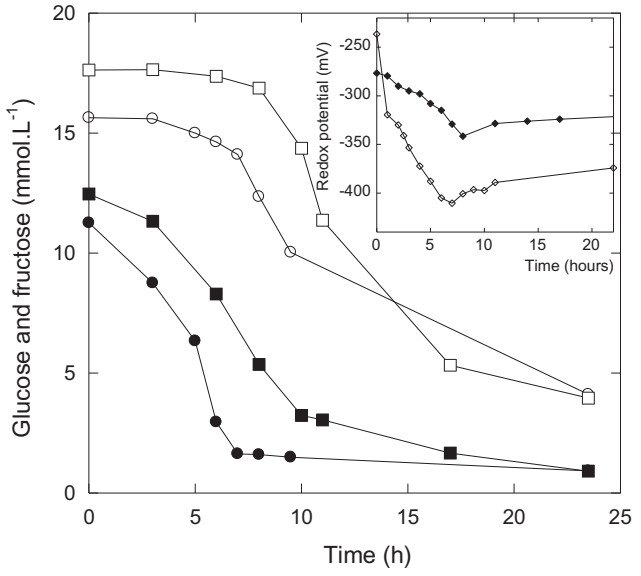

Fig. 2b. Glucose (E1: ●, E2: ■), fructose (E1: ○, E2: □), and redox potential (E1: ◇, E2: ◆) versus time.

in the preparation of classic culture media (MBM) of *T. maritima*. Seawater contains oligo elements (Culkin, 1965) which can partially replace those contained in the classical culture medium of. *T. maritima* Each of these components was removed one by one from the culture medium (NSM) in order to evaluate their effects on total H_2 volumetric production, consumed carbohydrates, H_2 yield and average H_2 productivity. The comparison of these experiments is summarized in Table 3.

In experiment E3, Balch's oligo-elements solution, $CaCl_2$ and $MgCl_2$ were removed from the culture medium. The results obtained (total H_2 volumetric production, carbohydrates consumption and H_2 yield) are listed in Table 4, and were similar to those of experiment E2. The highest H_2 yield obtained (3.56 mol H_2 mol^{-1} total sugars) confirms that a large part of the sugars in MFVW was consumed for H_2, CO_2 and acetate production. The ability of *T. maritima* to grow without addition of these elements could be explained by their presence in both seawater and MFVW. Seawater contains calcium and magnesium at concentrations of about 0.41

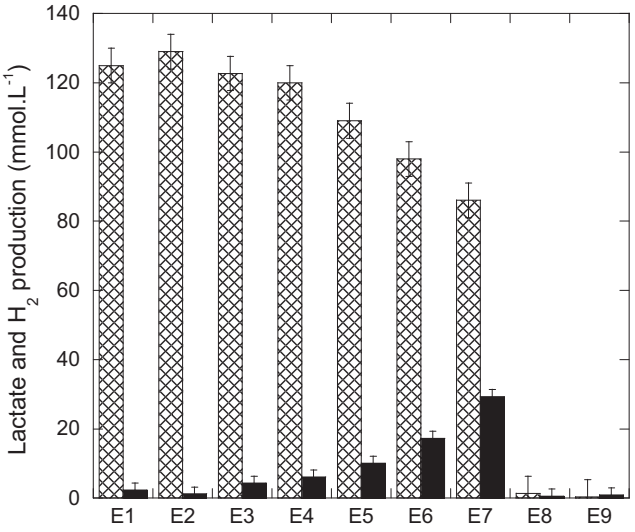

Fig. 3. Lactate (■) and H_2 production (⊠) at 24 h for the different experiments (E1 to E9).

and 1.29 g L^{-1}, respectively (Culkin, 1965), higher than those present in MBM ($CaCl_2$ 0.1 g L^{-1} and $MgCl_2$ 0.25 g L^{-1}).

In addition, MFVW contains many micro-elements (Mn, Fe, Zn, Co, etc.) normally provided by Balch's trace mineral elements added to MBM. Concentrations of Mn, Fe, Zn, Co are about 0.07, 0.2, 0.15 and 0.6 mg L^{-1}, respectively (APRIFEL, 2005), above the corresponding concentrations in Balch's trace mineral elements solution. Though at low concentrations, the presence of these elements is essential for growth. They are necessary for the cellular transport processes, and act as enzyme cofactors (Gomez-Romero et al., 2014). For example, iron is a major constituent of bifurcating Fe-Fe hydrogenase in *T. maritima*, the key enzyme involved in fermentative hydrogen production, and containing a bimetallic Fe-Fe active center (Schut et al., 2014). Its limitation can reduce biohydrogen production by deviating the fermentation pathways toward the production of more reduced end products such as lactate (Zhang and Shen, 2006). By contrast, it has been shown that supplementation of fermentative processes with Fe ion influences the system positively, and increases the fermentative hydrogen activity (Lee et al., 2001).

On the same basis as iron, the addition of tungsten to the growth medium of *T. maritima* increased both the cellular concentration of the Fe-Fe hydrogenase and it's in vitro activity. However, its function in the metabolism of this bacterium is still unknown: *T. maritma* can grow with or without added tungsten (Juszczak et al., 1991). However, tungsten is generally provided in the culture media via the oligo-element solution. In this experiment, Balch's oligo-element solution was removed from the medium, and its replacement by MFVW and seawater was not sufficient to obtain an optimal H_2 production. This result could explain the small increase in lactate production (from 1.2 mmol L^{-1} to 4.3 mmol L^{-1}, Table 4, and Fig. 3) and the decrease maximum H_2 productivity from 12.4 mmol h^{-1} L^{-1} to 8.8 mmol h^{-1} L^{-1} (Table 4).

In the next experiment (E4, Table 3), the yeast extract was removed from the culture medium (NSM). Compared with the previous experiment (E3), all the values of the fermentation process parameters remain unchanged (Fig. 3 and Table 4). Hence the absence of yeast extract does not affect the fermentation process of *T. maritima*, which produced a similar amount of hydrogen, CO_2 and fermentative end-products (acetate and lactate) as in experiment E3. However, Boileau et al. (2016) showed that in the absence of yeast extract in batch fermentation with glucose as sole carbon and energy source, *T. maritima* growth was very weak, and

biomass reached a limit of about 40 mg L^{-1}. Furthermore, Maru et al. (2012) found that reducing the level of yeast extract affected negatively H_2 production.

Other studies have obtained an improvement of biomass and H_2 production after increasing yeast extract concentration from 1 to 4 g L^{-1} in cultures on glycerol of *T. neapolitana*, a species very closely related to *T. maritima* (Ngo and Bui, 2013). The contribution of yeast extract to the growth of *T. maritima* is not yet clear, but it may play an important role in the fermentation process, providing nitrogen, mineral elements, amino acids and/or vitamins for bacterial growth. Rinker and Kelly (2000) showed that *T. maritima* did not significantly consume individual amino acids added to culture media. However, d'Ippolito et al. (2010) reported that consumption of protein sources (peptone, tryptone and yeast extract) accounts for 10–15% of the total H_2 production by *T. neapolitana*. Interestingly, most of the amino acids present in the yeast extract, such as lysine, phenylalanine, leucine, valine, methionine, cystine, tryptophan, threonine, isoleucine, aspartic acid and proline were also present in our model fruit and vegetable wastes (http://www.wh-foods.com/). Moreover, these concentrations were higher than or similar to those added to the medium by yeast extract (1 g L^{-1}). With regard to vitamins present in yeast extract, Childers et al. (1992) showed that only biotin was required for optimal growth of *Thermotoga maritima*. However, we underline that vitamins of yeast extract can be replaced by those contained in MFV, such as vitamin A, B, C and E, with average concentrations of around 0.25, 2, 4 and 0.37 mg L^{-1}, respectively (APRIFEL, 2005). Hence, in our study, it is clearly shown that seawater and fruit and vegetable wastes contain enough minerals and nutrient substances (peptides, amino acids and vitamins) to effectively replace the yeast extract of the classic medium (MBM). Ljunggren and Zacchi (2009) demonstrated that yeast extract was the main cost contributor during different hydrogen fermentation strategies (high H_2 productivity and high H_2 yield) which accounted for 49% and 93% of the nutrient cost, respectively.

Experiment E5 (Table 3) consisted in removing KH_2PO_4 and K_2HPO_4, considered as potassium and phosphate inorganic sources, from the culture medium (NSM). Compared with the previous experiment (E4), all the values of parameters describing the fermentation process (E5) such as total hydrogen production (Fig. 3), acetate and CO_2 production, H_2 productivities and yield H_2/C_{tot} (Table 4) were slightly decreased against an increase in lactate production (Fig. 3, Table 4). Potassium was supplied by the seawater (0.4 g L^{-1}), but phosphate was present at a low concentration. Phosphate is considered as an important inorganic nutrient for microbial growth and for optimal H_2 production (Liu et al., 2015). In our experiment, the decrease in the values of total hydrogen production, acetate and CO_2 production (Table 4) showed that the low phosphate supply was sufficient to enable the growth of *T. maritima*. However, we observed an increase in lactate production (Fig. 3) and a corresponding small decrease in H_2 productivity (Table 4), which demonstrates a slight orientation of the metabolism toward lactate production. Besides its function as a macro-element (phosphorus), phosphate can also increase H_2 production when it reacts with calcium (Liu et al., 2015). Chang and Lin (2006) have shown that an overly-high calcium concentration (0.3 g L^{-1}) with small phosphate concentrations can decrease H_2 productivity. In our experiment E5, the natural seawater provided 0.41 g L^{-1} of $CaCl_2$, which might explain the slight fall in H_2 productivity in the absence of phosphate. However, some other studies have found that excess amounts of phosphate can increase the production of volatile fatty acids, which is not desirable as this diverts the cellular reductants away from hydrogen production (Chandrasekhar et al., 2015).

Experiments E6 and E7 each eliminated one of the two sulfur compounds (Na_2S for E6 and cysteine-HCl for E7) from the culture medium specified for experiment E5 (Table 3). The absence of one of these sulfur compounds decreased total H_2 production (Fig. 3) concomitant with a decrease in H_2 productivity and yield H_2/C_{tot} for the two experiments (Table 4). Furthermore, lactate production was increased to 29.3 mmol L^{-1} for E7. Sulfur compounds under different forms (elemental sulfur, cysteine, Na_2S, thiosulfate, etc.) are essential for *T. maritima* growth and they can be used as an electron acceptor to remove the inhibition due to a high partial hydrogen pressure (Huber and Harning, 2006; Schröder et al., 1994; Ravot et al., 1995; Childers et al., 1992). Moreover, Boileau et al. (2016) have shown that adding thiosulfate at low concentrations (0.12 mmol L^{-1}) is sufficient to allow an optimum growth of this bacterium with a significant increase in hydrogen production. However, in our case, the significant input of sulfur compounds (Na_2S 5.12 mmol L^{-1} or cysteine-HCl 1.9 mmol L^{-1}) led to a significant reduction in growth parameter values of *T. maritima*. This negative effect may be explained by the oxidation of some Na_2S or cysteine-HCl by the oxygen introduced into the anoxic medium after adding MFVW, stored under aerobic conditions.

The oxidation of sulfur compounds could thus reduce the available sulfur concentration needed for *T. maritima* growth. Sulfur compounds are essential for protein, Fe-S clusters and ferredoxin synthesis. The low sulfur concentration with Na_2S addition in experiment E7 could result in priority being given to the protein synthesis rather than the Fe-S cluster formation (Ainala et al., 2016). This case could explain the strong increase in lactate production, since there is a limitation in *T. maritima*'s Fe-Fe hydrogenase synthesis. Besides, we note that seawater does not contain the sulfur compounds necessary for *T. maritima* growth. Experiment E8 (Table 3) showed that removing all sulfur compounds inhibits *T. maritima* growth, even in the presence of the sulfur-containing amino acids (methionine and oxidized cysteine) provided by MFVW (Table 4, Figs. 2a and 2b).

Experiment 9 (E9) involved removing the nitrogen source (NH_4Cl) from the culture medium specified for experiment E7 (Table 3). Rinker and Kelly (2000) showed that NH_4Cl and not amino acids serves as a nitrogen source for *T. maritima,* and no growth of this bacterium was found in the absence of this element. They also showed that increasing NH_4Cl concentrations up to 1.0 g L^{-1} in continuous culture stimulated biomass yields for *T. maritima.* In our study, MFVW and natural seawater did not contain inorganic nitrogen sources. Thus *T. maritima* did not grow without addition of this element.

From these different experiments, we specified a minimal culture medium containing natural seawater, fruit and vegetable wastes supplemented with cysteine-HCl and inorganic nitrogen source (NH_4Cl) (based on experiment E6). This simplified culture medium was then used as a base medium for testing different sugar concentrations, and the ability of *T. maritima* to produce H_2 from a mixture of fruit and vegetable waste (FVW) directly harvested from a landfill.

3.4. Effects of using FVW and higher sugar concentrations on fermentative H_2 production

After specifying a simplified culture medium (E6) and studying the growth of *T. maritima* in the presence of MFVW, the capacity of this bacterium to grow and produce H_2 from fruit and vegetable waste (FVW) was evaluated (E11). FVW was a mixture of fruit and vegetable waste collected from a wholesale market landfill (Bir Kassa, Tunis). The sampling was performed during the winter period, when the waste was composed of different quantities of apples, carrots, potatoes, tomatoes, pears, onions, fennel, spinach, parsley and citrus fruits (oranges and tangerines). Many studies have shown a decrease in H_2 production related to the presence of flavor compounds in citrus fruit (esters, alcohols, aldehydes, ketones, lactones and terpenoids), which inhibit the growth of

many microorganisms (Akinbomi and Taherzadeh, 2015). For instance, d-limonene (a citrus flavor belonging to a class of terpenoids) was found to have an antimicrobial effect at a very low concentration of 0.01% w/v (Mizuki et al., 1990). It can also cause the failure of the anaerobic digestion process, even at a very low concentration of 400 μL L^{-1} (Mizuki et al., 1990). However, in our study, the presence in FVW of citrus fruits (oranges and tangerines) did not affect H_2 production by *T. maritima*. On the contrary, H_2 production was promoted despite their presence (Fig. 4 and Table 4).

To compare with experiment E6, E11 was performed with an equivalent total carbohydrate concentration of about 46.1 mmol L^{-1}. Contrary to E6 and in 24 h of fermentation, FVW significantly improved the total hydrogen volumetric production (Table 4 and Fig. 4). Many factors related to the composition of FVW (rheological characterization, biodegradability of carbohydrates, mineral elements, etc.) could be responsible for increasing H_2 production. Nevertheless, the results obtained from E11 were similar to those obtained in experiments E1 and E2, which used the complete composition of MBM necessary for optimum growth. This increase is consistent with the consumption of total carbohydrates (essentially simple sugars) which was about 90%. This higher consumption could be explained by the microbial hydrolysis of complex carbohydrates during their landfilling. Furthermore, the fermentation with FVW allowed an increase in the maximum H_2 productivity up to 12.4 mmol h^{-1} L^{-1} (Fig. 4) similar to that obtained in experiment E2 with the complete medium (MBM) (12.4 mmol h^{-1} L^{-1}, Table 4). Hence the heterogeneous FVW composition is able to replace the different mineral compounds removed in NSM (E6). One example is the presence in FVW of onions rich in organosulfur compounds, which can replace the removed sulfur necessary for *T. maritima* growth (Ueda et al., 1994). In addition, onions are composed of many nutrients such as phosphorus, potassium and sulfur, with corresponding concentrations of about 193, 822 and 282 mg L^{-1}, respectively (Romano and Zhang, 2008). It was observed that adding 1% onion storage wastes to cattle dung in a bioreactor increased biogas production by 40–80% (Yadvika et al., 2004). The elements could therefore be easily assimilated by *T. maritima.*

The effect of MFVW and FVW concentrations on the production of H_2 was assessed using the simplified culture medium (NSM). The two initial equivalent C6 concentrations from MFVW and FVW were multiplied by about 2.8 ± 0.2 (124.1 mmol L^{-1} for E10

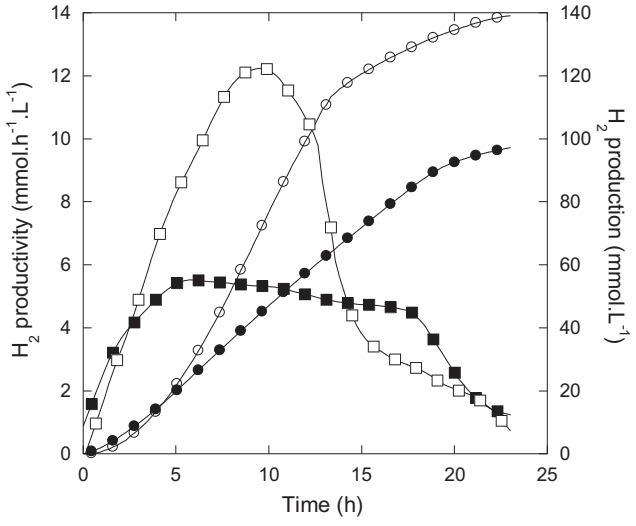

Fig. 4. H_2 productivity (E6:■, E11: □) and H_2 production (E6: ●, E11: ○) versus time.

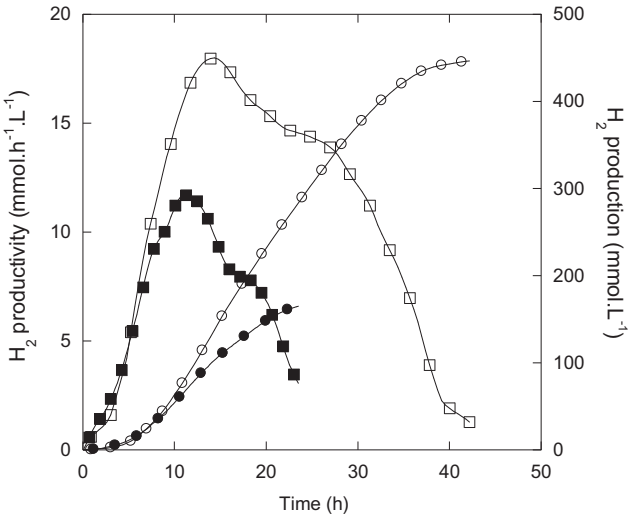

Fig. 5. H_2 productivity (E10:■, E12: □) and H_2 production (E10: ●, E12: ○) versus time.

and 143 mmol L^{-1} for E12) compared with experiments E6 and E11 (47.7 and 46.1 mmol L^{-1} respectively) (Table 4). The difference between the two experiments was noteworthy by the duration of fermentation, which was 24 h for E10 and 43.5 h for E12. In experiment E10, the fermentation was finished after 24 h with total carbohydrate consumption around 62.2 ± 3.5 mmol L^{-1} (Table 4). However, there was an increase in H_2 productivity (11.8 mmol h^{-1} L^{-1}) in comparison with experiment E6 (5.8 mmol h^{-1} L^{-1}) (Table 4). Thus, the addition of MFVW provided some elements that improved the consumption of total sugars and H_2 production, but still not enough to allow the consumption of total carbohydrates by *T. maritima*. However, unlike experiment E10 performed with MFVW, culture conditions in experiment E12 (with FVW), allowed the fermentation of a significant part of the carbohydrates (87%) in 43.5 h of culture (Table 4).

Furthermore, in our study, the maximum H_2 productivity obtained in E12 increased to 18.6 mmol h^{-1} L^{-1} (Fig. 5). This value was previously obtained only when *T. maritima* was cultivated on 4 g L^{-1} of yeast extract and 60 mmol L^{-1} of glucose (Boileau et al., 2016). Interestingly, the decrease in H_2 production rates seems to be explained by the consumption of most of the total carbohydrates, contrary to experiment E10. Hence FVW supplies all the compounds necessary for an optimal *T. maritima* growth such as organosulfur compounds and pre-hydrolysis complex carbohydrates. Moreover, in our culture conditions (high temperature and anaerobic conditions), FVW has other advantages. Indeed, the lysis of the microflora present within the waste provided usable compounds such as vitamins, amino acids, micro and macro-elements. In conclusion, *T. maritima* produced the equivalent of 446 mmol L^{-1} of H_2 (Fig. 5) in E12 in the presence of FVW, supplemented only with NH_4Cl as a nitrogen source, and cysteine HCl as a sulfur source.

4. Conclusion

This study demonstrates that the supply of seawater in fermentative H_2 production from fruit and vegetable wastes can replace certain nutrients necessary in mineral basal medium (MBM), and without modifying the total H_2 production. On the other hand, it is agreed from this work that the absence of a source of nitrogen and sulfur prevented H_2 production by *T. maritima*. Thus, the batch fermentation carried out in a culture medium containing organic market wastes and seawater appears as an alternative in the H_2

production. Such methods of H_2 production will encourage reducing the process-associated cost by simplifying the culture medium of hydrogen-forming bacteria.

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