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Biohydrogen production from hyperthermophilic anaerobic digestion of fruit and vegetable wastes in seawater: Simplification of the culture medium of *Thermotoga maritima*

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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.

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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), $MgSO_4·7H_2O$ (3.0), $MnSO_4·H_2O$ (0.5), NaCl (1), $FeSO_4·7H_2O$ (0.1), $CoSO_4·7H_2O$ (0.18), $CaCl_2·2H_2O$ (0.1), $ZnSO_4·7H_2O$ (0.18), $CuSO_4·7H_2O$ (0.01), KAl $(SO_4)_2·12H_2O$ (0.02), H_3BO_3 (0.01), $Na_2MoO_4·2H_2O$ (0.01), $NiCl_2·6H_2O$ (0.025), $Na_2SeO_3·5H_2O$ (0.0003), $Na_2WO_4·2H_2O$ (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

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