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Assessment of the adequacy of different Mediterranean waste biomass types for fermentative hydrogen production and the particular advantage of carob (*Ceratonia siliqua* L.) pulp

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ABSTRACT

The conversion of agro-industrial byproducts, residues and microalgae, which are representative or adapted to the Mediterranean climate, to hydrogen (H_2) by *C. butyricum* was compared. Five biomass types were selected: brewery's spent grain (BSG), corn cobs (CC), carob pulp (CP), *Spirogyra* sp. (SP) and wheat straw (WS). The biomasses were delignified and/or saccharified, except for CP which was simply submitted to aqueous extraction, to obtain fermentable solutions with 56.2–168.4 g total sugars L^{-1}. In small-scale comparative assays, the H_2 production from SP, WS, CC, BSG and CP reached 37.3, 82.6, 126.5, 175.7 and 215.8 mL (g biomass)$^{-1}$, respectively. The best fermentable substrate (CP) was tested in a pH-controlled batch fermentation. The H_2 production rate was 204 mL (L h)$^{-1}$ and a cumulative value of 3.9 L H_2 L^{-1} was achieved, corresponding to a H_2 production yield of 70.0 mL (g biomass)$^{-1}$ or 1.6 mol (mol of glucose equivalents)$^{-1}$. The experimental data were used to foresight a potential energy generation of 2.4 GWh per year in Portugal, from the use of CP as substrate for H_2 production.

Introduction

Global warming and issues of national security due to dependence on oil and gas imports have increased the renewable energy research at an unprecedented rate during the last decade [44]. Regarding biomass use for biofuels, efforts based on the rational use of waste, crop leftovers and agro-industrial byproducts must be undertaken, to avoid any competition between food and energy production [17]. Any analysis concerning the production and conversion of biofuels must take into consideration which renewable resources are available at a local and regional level, therefore depending on geographic location, climate specifications and biomass availability [55], while ensuring their possible exploration preserves the natural biodiversity, and soil, fodder and water

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supply [15]. For example, the biomass attractiveness of some Mediterranean crops may depend on their drought resistance, typical of a coast associated dry-land agriculture [31]. In addition, the biomass composition should be a factor of selection, as well as the concentration and ease of depolymerisation of the constituent carbohydrates in the case of further bioconversion, whereas extractable compounds can confer added value to the biomass [15,45].

Previous studies considering the flora characteristic of, or adaptable to, the Mediterranean region identified several cultures as potentially adequate energy crops, such as *Ceratonia siliqua* L. or carob tree, brewery's spent grain (BSG) and microalgae. The carob tree is a highly drought resistant species, requires little maintenance and is recommended for afforestation in coastal areas threatened by soil erosion and desertification [28]. The carob pulp (CP) is the byproduct of galactomannan extraction from the carob seeds and represents about 90% (w w^{-1}) of the total dry weight of the fruit, containing a high percentage of readily soluble sugars (up to 54% w w^{-1}). Brewery's spent grain (BSG) is a byproduct of the beverage industry, being composed by the residue of malt and grain after the production of beer, and shares a comparable content in polysaccharides (40–45% (w w^{-1}) [37]. Unlike carob, barley is not a culture of Mediterranean origin but this cereal crop is well adapted to dry regions [56] and the beer industry is representative in countries like Spain, France and Portugal [12]. Microalgae are considered a third-generation feedstock for biofuels production, as they are photosynthetic organisms with exceedingly fast grow and high CO_2 sequestration rates, which do not need arable land or potable water [7], do not compete with the food-market either in resources or final destination of the product, and have already been successfully used for wastewater depuration and conversion into several types of biofuels [1,39]. The proximity to the sea and the climate characteristics, such as the high number of sunny days and hours of sunlight per day, the mild temperatures and thermal amplitudes are excellent for microalgae cultivation in the Mediterranean basin [16,22]. Corn cobs (CC) and wheat straw (WS) were included in this study for comparison purposes, as the two cultures accounted for the second highest share (21.0%) of the cereals produced in the EU-28 (Eurostat, 2017). In their majority, both materials are composed by cellulose and hemicellulose at, approximately, 45% and 35% (w w^{-1}) in CC, and 30% and 50% (w w^{-1}) in WS, respectively [51].

The suggested feedstocks – CP, BSG, CC, WS and microalgae - are considerably rich in carbohydrates, a characteristic that makes them suitable as substrate for dark fermentation (DF) by microbial consortia or microorganisms like *Enterobacter*, *Clostridium* or *Bacillus* [19] for hydrogen (H_2) production. Hydrogen possesses a high energy content (120 MJ kg^{-1}), is easily convertible into energy by combustion or into electricity through the use of fuel cells, and generates no greenhouse gases in its conversion [10]. The fermentative H_2 production is accompanied by the production of a vast array of organic acids which are considered high-value products and can be further valorised *e.g.* for photoproduction of H_2 [27] or for the production of polyhydroxyalkanoates for bioplastics [34]. Furthermore, this H_2 production process requires neither light or O_2, can convert a vast array of carbon sources, including waste streams, and achieves higher H_2 production rates when compared to other biological H_2 production systems [48]. Naturally, the fermentative H_2 production also has limitations, which are mostly associated with the low production yields and the cost of the fermentable raw materials [4]. The strategies used to overcome these problems include, for example, the integration of dark and photofermentation into a single production system [18], the application of different reactor configurations, flow dynamics [47] or cell immobilisation [49] and the removal of dissolved H_2 [29]. Additionally, metabolic engineering and gene regulation strategies can be employed to increase conversion efficiency, and mesophilic pure cultures can be replaced by thermophilic strains or microbial consortia as biocatalysts as the latter can achieve higher production yields and rates [19]. The use of carbohydrate-rich byproducts or waste biomass as fermentation substrate is generally accepted as the best option to reduce the overall process costs [42].

Current sustainability concerns about resources scarcity and the preservation of natural biodiversity make it important to benchmark the locally best-positioned waste feedstocks for the production of bioenergy and bioproducts. As such, this study focused on the comparison of the H_2 fermentative performance of waste lignocellulosic biomasses adapted to the characteristics of the Mediterranean climate, and the selection of the best feedstock for which the saccharification and conversion yields may boost a future process scale-up. *C. butyricum* was chosen as a model microorganism by its robustness, its capability to attain high H_2 production yields and rates, the possibility of using a vast range of substrates, and the fact that it can be cultured efficiently at mild mesophilic conditions [6]. By the first time, the potential of CP for fermentative hydrogen production was clearly evidenced by a thorough comparison with other Mediterranean waste biomass counterparts.

Material and methods

Raw biomass

Corn cobs, BSG and WS were obtained from Companhia das Lezírias (Samora Correia, Portugal), Central Society of Beers and Beverages (SCC, Vialonga, Portugal) and National Plant Breeding Station (ENMP, Elvas, Portugal), respectively. The materials were dried to reduce moisture below 10% (w w^{-1}) and ground to a particle size of less than 3 mm (CC) or approximately 0.5 mm (BSG and WS). The CP kibbles were obtained from Chorondo & Filhos Lda. (Loulé, Portugal). The microalga used in this work was *Spirogyra* sp. (SP) (division Chlorophyta, family *Zygnemaceae*), from Sammlung von Algenkulturen Göttingen (SAG) of the University of Göttingen (reference: SAG 170.80) and cultured as described elsewhere [39].

Biomass pretreatment and saccharification

The methods for the pretreatment and hydrolysis of each feedstock were selected from the literature (Table 1) [8,11,39]. Wheat straw and CC required a previous delignification stage. Carob pulp was submitted to an aqueous extraction to obtain a