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Thermophilic biohydrogen production from energy plants by *Caldicellulosiruptor saccharolyticus* and comparison with related studies

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ABSTRACT

Air-dried samples of sweet sorghum, sugarcane bagasse, wheat straw, maize leaves and silphium were utilized without chemical pretreatment as sole energy and carbon sources for H_2 production by the extreme thermophilic bacterium *Caldicellulosiruptor saccharolyticus*. The specific H_2 production rates and yields were determined in the batch fermentation process. The best substrate was wheat straw, with H_2 production capacity of 44.7 L H_2 (kg dry biomass)$^{-1}$ and H_2 yield of 3.8 mol H_2 (mol glucose)$^{-1}$. Enzymatically pretreated maize leaves exhibited H_2 production of 38 L H_2 (kg dry biomass)$^{-1}$. Slightly less H_2 was obtained from homogenized whole plants of sweet sorghum. Sweet sorghum juice was an excellent H_2 source. *Silphium trifoliatum* was also fermented though with a moderate production. The results showed that drying is a good storage method and raw plant biomass can be utilized efficiently for thermophilic H_2 production. The data were critically compared with recently published observations.

1. Introduction

Sugarcane, sweet sorghum, wheat, maize and their byproducts, for instance, are currently subjects of studies concerning their ability to serve as substrates for biofuels' production. One of the advantages using such material as biomass for biofuel production is that these plants are short-rotation crops. Some relatively new and less well-studied energy crops, e.g. *Silphium trifoliatum*, have considerable potential as they re-grow from their rhizome after each harvest, which allows multiple harvests without the need to re-plant. Maize and sorghum silage can serve both as energy plants and as fodder, while sugarcane, sweet sorghum syrup, corn and wheat starch are used in the food industry. The leftover biomass, however, is mostly unexploited and poses a disposal problem.

Abbreviations: BGS, sugarcane bagasse; CFU, colony forming unit; E, extract; PML, pretreated maize leaves; PWS, pine wood shavings; RB, residual biomass; SSC, sweet sorghum concentrate 65° Bx; STR, *Silphium trifoliatum* leaves; SSJ, sweet sorghum juice; SSP, sweet sorghum plant (*Sorghum bicolor*); TC, total carbon; TOC, total organic carbon; UML, untreated maize leaves; WST, wheat straw.

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By virtue of its unrivaled environmental benefits, H_2 is widely considered to be the energy carrier of the future [1–3]. H_2 can be generated via a number of established technologies, including renewable biological routes [4,5]. Strategies for H_2 production from plant sources essentially follow two major routes: the photochemical conversion of sunlight or dark fermentative processes. Although many scientific issues still remain to be understood, the fermentative path currently appears to be closer to practical utilization. The benefits of this approach include the low cost of biomass, and the fact that byproducts of agricultural food production can be used as feedstocks in the processes [6–8].

Biogas and bioethanol production from wheat straw have been studied [9–11], but results on the fermentative production of H_2 from wheat and maize residues appeared in the literature, recently. Hussy et al. [12] produced H_2 from wheat starch via dark fermentation by using mixed microflora. The efficient conversion of wheat straw into H_2 by cow dung compost [6], through the degradation of wheat bran [13] or wheat powder [14] was demonstrated using poorly defined, pretreated anaerobic sludge inocula. The compositions of the wheat bran and the solubilized wheat hemicellulose are well characterized [13,15,16].

Sweet sorghum juice is used for bioethanol production, and was recently tested for biogas and H_2 production [17,18]. The feasibility of bioH_2 production from sweet sorghum has been established [19,20]. These studies have demonstrated that sweet sorghum extract can be used for H_2 and biogas production in a two-stage process where the effluent from the hydrogenogenic reactor can be used for biogas production without any additional treatment. *Ruminococcus albus* is one of the outstanding candidates for H_2 production [20], and in chemostat cultures at 37 °C, significant amount of H_2 has been generated from sorghum stalks, an aqueous extract of sorghum containing the free sugars, and the sorghum residues after the sugar extraction process. The H_2 yield from the aqueous extract of sorghum was the same as that from glucose batch experiments, i.e. approximately 2.5 mol H_2 (mol glucose)$^{-1}$. Sweet sorghum juice supported the growth and H_2 production of the extreme thermophile *Caldicellulosiruptor saccharolyticus* for about 60 h with an average production rate of 10 mmol H_2 L^{-1} h^{-1} (0.24 mol H_2 L^{-1} day^{-1}) during the first 16 h and a maximal production rate of 21 mmol H_2 L^{-1} h^{-1} (0.5 mol H_2 L^{-1} day^{-1}) at 10 h after the start of fermentation [18].

Bagasse has been recognized as an excellent biomass for biogas production [21,22]. The economic advantages and disadvantages of using bagasse as an energy source in Latin America have been analyzed [21]. Bagasse utilization for energy production can reduce the emission of CH_4 and CO_2 into the atmosphere [22]. It was pretreated with acid or base in order to increase the fermentability of the biomass. The best results were obtained after alkaline pretreatment, where 37.1 g of glucose, corresponding to 60% cellulose conversion, was obtained from 100 g of sweet sorghum bagasse [18].

C. saccharolyticus is an extremely thermophilic, Gram-positive, fermentative anaerobe, which can utilize a wide range of substrates including cellulose, hemicellulose, starch, pectin, pentoses and hexoses [23–26]. The capability of simultaneous utilization of a range of complex biomass feedstocks represents a highly attractive feature of this bacterium for H_2 [5,24,25] and biogas production [27].

A burst of reports on fermentative H_2 production has been published in the past 2 years. Many of them (Tables 3 and 4) employed pretreated natural biogas-producing consortia in which the methanogenic population had been eliminated by heat treatment, leaving the spore-forming Clostridia alive for H_2 generation experiments [28–32]. Moreover, in many cases the experimental conditions were simplified by adding pure glucose or sucrose as substrate. Because of the undefined and irreproducible composition of the H_2-producing bacterial mixture these studies were of limited scientific value, and the use of the relatively expensive feedstock suggests that the systems are unlikely to be of practical value for large-scale applications. In studies using well-defined microbes or consortia thereof as biocatalysts, with energy plants, biomass residues or waste streams as substrates [4,5,28,31–33], H_2 productivity data may facilitate scientific understanding of the process and lead to practical applications.

In the present study, we set out to determine the bioH_2 production potential of a number of plant biomass sources that are widely available for renewable energy production and are relatively cheap for potential practical applications. A single extreme thermophilic strain in pure culture was used in order to acquire reproducible data on H_2 productivity under anaerobic batch fermentation conditions. Our results are critically compared with those on recent H_2 production systems and some general recommendations for the planning of future R&D are given.

2. Materials and methods

2.1. Microorganism, medium and culture conditions

C. saccharolyticus (DSM8903) was purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) and propagated at 70 °C on DSMZ medium 640, 1 L of which contained 0.90 g NH_4Cl, 0.90 g NaCl, 0.40 g $MgCl_2 \times 6H_2O$, 0.75 g KH_2PO_4, 1.50 g K_2HPO_4, 1.00 g yeast extract, 1.00 g cellobiose, 0.75 g cysteine-HCl × H_2O, 0.50 mg resazurin and 1 mL trace element solution SL-10 (10.00 mL HCl (25%, 7.7 M), 1.50 g $FeCl_2 \times 4H_2O$, 70.00 mg $ZnCl_2$, 100.00 mg $MnCl_2 \times 4H_2O$, 6.00 mg H_3BO_3, 190.00 mg $CoCl_2 \times 6H_2O$, 2.00 mg $CuCl_2 \times 2H_2O$, 24.00 mg $NiCl_2 \times 6H_2O$, 36.00 mg $Na_2MoO_4 \times 2H_2O$, 990.00 mL distilled water). pH was adjusted to 7.2. The culture was grown in anaerobic 50 mL hypovials (Supelco) until OD600 = 0.5 cm^{-1}, (corresponding to 3×10^7 CFU mL^{-1}) was attained. Routine manipulations were performed in an anaerobic chamber (Bactron IV, Sheldon Manufacturing, Inc., Canada). The inoculum size was 3% (v v^{-1}).

2.2. Viable cell counts

Viable cell counts were determined as CFU by plating on DSMZ medium 640 solidified with 2.5% (w v^{-1}) Gelrite Gellan Gum (Sigma–Aldrich, Germany) [34]. In order to develop the colonies, the plates were incubated at 70 °C for 3–4 days in the