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Biological Hydrogen Production from Sucrose and Sugar Beet by *Caldicellulosiruptor Saccharolyticus*

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Abstract

Hydrogen production needs to be based on renewable resources in order to be sustainable. Sugar beet is an ideal raw material for fermentative production of hydrogen in the EU and possibly in the USA due to its environmental profile and its potential availability in these areas. In this work, the fermentative production of hydrogen from sucrose of analytical grade and sugar beet extract by pure cultures of *Caldicellulosiruptor saccharolyticus* was investigated, under uncontrolled and controlled conditions. In the first case, growth of pure cultures of *C. saccharolyticus* on sucrose derived from sugar beet was compared to growth of the microorganism on sucrose of analytical grade. The production of hydrogen and organic acids (acetate and lactate) from sugar beet was largely equal to or slightly higher than the production of the control. In the second case, fermentation of sugar beet extract at sucrose concentration 10 g/l was comparable to the fermentation on pure sucrose except that the hydrogen yield was slightly higher on sugar beet extract. In particular, hydrogen yields of 2.9 and 3.0 mol/mol hexose were determined in fermentations of sucrose and sugar beet extract, respectively, corresponding to 73% and 75% of the theoretical value of 4 mol hydrogen/mol hexose. Acetic acid was the main product and very low production of lactic acid was observed.

1 Introduction

The looming energy challenges around the world will probably have to be tackled using a portfolio of different technologies. The production of hydrogen from biomass by biotechnological means is one of the most attractive technological options for efficiently producing clean energy [1,2]. Sugar-rich raw materials are advantageous in comparison to lignocellulosic ones, because they generally have a high content of readily fermentable sugars. Therefore, they are already well established in the literature [3,4,5,6] as substrates for fermentations.

Sugar beet (Table 1) is an ideal raw material for fermentative production of hydrogen in the EU [7] and possibly in the USA due to its environmental profile and its potential availability in these areas. The share of sugar beet in global sugar production is about 30%, with the rest coming mainly from sugar cane. Sugar beet (as well as sugar cane and sweet sorghum) is advantageous over the cereals (wheat, barley) in terms of yield of fresh biomass per hectare, yield of carbohydrates per hectare, and processing. Recently the comparison of the suitability

of several raw materials for fermentative hydrogen production was performed [2], and sugar beet was found one of the most technically attractive raw materials.

The objective of the present study is to assess (a) the fermentability of pure sucrose and sugar beet extract at sucrose concentration of 20 g/l, under fermentation conditions without control of pH and hydrogen pressure, and (b) the fermentative production of hydrogen from sucrose and sugar beet extract in batch fermentations under pH and hydrogen pressure controlled conditions, at an initial sucrose concentration of 10 g/l.

Table 1: Composition of sugar beet root expressed as % of dry matter.

Component	Amount %
Sucrose	67.3
Cellulose	4.2
Hemicellulose	5.2
Lignin	0.8
Protein	1.0
Ash	1.8

2 Materials and Methods

2.1 Raw material

Sugar beet was obtained from commercial farms, either in the south-west of The Netherlands or in Central-North Greece.

2.2 Experimental setup of the production of sugar beet extract

Sucrose extraction from sugar beet was designed to simulate the industrial sugar beet processing; the process included combined extraction of sugar and pressing, and has been previously described in details [2].

2.3 Fermentation

2.3.1 Microorganism, medium, cultivation

C. saccharolyticus DSM 8903 was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). The culture medium consisted of (per litre) KH_2PO_4 0.3 g, K_2HPO_4 0.3 g, $MgCl_2.6H_2O$ 0.4 g, NH_4Cl 0.9 g, yeast extract 1.0 g, cysteine-HCl 0.75 g, $FeCl_3.6H_2O$ 2.5 mg, SL-10 trace elements 1 ml, and resazurine 0.5 mg. 4-morpholine propanesulfonic acid (MOPS, 10.5 g/l) was used as a buffer under uncontrolled conditions. The pH was adjusted to 7.0 at room temperature. Inoculation was done by adding 5% or 10% (v/v) of a preculture that was grown overnight on sucrose. Medium was made anaerobic by flushing with nitrogen. Experiments were carried out under non-sterile conditions. The culture was grown at 72 °C.

Fermentability was tested under uncontrolled conditions with closed flasks of 118 ml with 20 ml culture medium under a nitrogen atmosphere. The sucrose concentration was 10 g/l coming from sucrose of analytical grade, the sugar beet extract or various combinations of both. The detailed procedure of the fermentability experiments is described by Panagiotopoulos et al. [2].

Batch fermentations under controlled conditions were performed in a jacketed 2 l bioreactor (Applikon, Delft, The Netherlands) at a working volume of 1 l. The pH was controlled at circa 6.8 (measured at room temperature) by automatic addition of 2 N NaOH. Cultures were continuously stirred at 350 rpm and sparged with nitrogen at 7 l/h. The concentration of sucrose was 10 g/l coming from sucrose of analytical grade (hereafter referred to as pure sucrose) or the sugar beet extract. Fermentation was considered to have ended when the hydrogen concentration was less than 0.1% in the off-gas. Samples of 7 ml were regularly taken from the culture medium for measurement of the cell density, and substrate and product analyses.

2.3.2 Hydrogen yield and productivity

Hydrogen production yield was calculated as the molar amount of hydrogen divided by the molar amount of consumed hexose equivalent (mmol hydrogen/mmol hexose). The consumed unknown organic compounds were considered as carbohydrates with the same product yield as for sucrose. COD in mmol O_2/l was converted to mmol hexose/l according to the equation $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$. Details of the calculations are described by de Vrije et al. [8].

2.4 Analytical methods

The determination of the chemical composition of sugar beet as well as the analysis of sucrose, organic acids, hydrogen and carbon dioxide were performed as described earlier [2,8].

3 Results

The present study investigated the fermentative production of hydrogen from pure sucrose and sugar beet extract in uncontrolled small-scale fermentations, and in controlled batch fermentations at an initial sucrose concentration of 20 and 10 g/l, respectively. Focus was given on hydrogen and organic acid production from the sugar beet extract-containing medium, compared to the control sample with pure sucrose, in order to evaluate the comparative suitability of sugar beet for hydrogen fermentation. Particularly interesting was the determination of the hydrogen yield (mol hydrogen/mol hexose) obtained when sugar beet is used as substrate.

3.1 Fermentability of sucrose and sugar beet extract on small scale

The fermentability of sugar beet extract was tested in small-scale experiments with closed flasks. The ratio of (pure sucrose)-(sucrose derived from sugar beet extract) ranged from 100-0 (control sample) to 0-100. The total sucrose concentration in all samples was 20 g/l. The production of hydrogen and the main organic acids (acetate and lactate) by *C. saccharolyticus* in fermentations with increasing amounts of sugar beet extract was determined. *C. saccharolyticus* was able to grow and produce hydrogen from sucrose in all experiments performed (Figure 1A). Hydrogen production roughly seemed to increase with increasing sugar beet extract. This is in accordance with the growth of *C. saccharolyticus* which was maximised when 100% sugar beet extract was used. Moreover, increased acetate production and decreased lactate production were observed with increasing sugar beet

contents (results not shown). In total, organic acid production after 16 and 40 h of fermentation was largely equal to the control (Figure 1B).

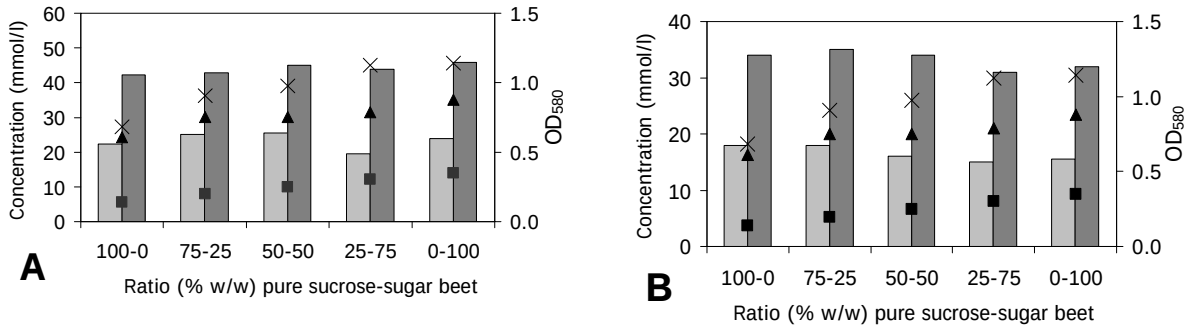

Figure 1: Hydrogen (A) and organic acid (sum of acetate and lactate) (B) production by culture of *C. saccharolyticus* grown on various ratios of pure sucrose and sugar beet extract. Measurements were done after 16 (light grey bars) and 40 (dark grey bars) h after the start of the fermentation. The growth of *C. saccharolyticus* (OD_{580}) after 0 (■), 16 (▲) and 40 (×) h of fermentation is also indicated.

3.2 Batch fermentations under controlled conditions in a bioreactor

Normal growth of *C. saccharolyticus* on pure sucrose and the sugar beet extract was achieved in batch fermentations under pH and hydrogen pressure controlled conditions, at an initial sucrose concentration of 10 g/l. Consumption of sucrose in both fermentations was almost complete after 26 h. The main products of the fermentations were hydrogen, acetate and carbon dioxide (Fig. 2). Production of lactate was minimal in both fermentations.

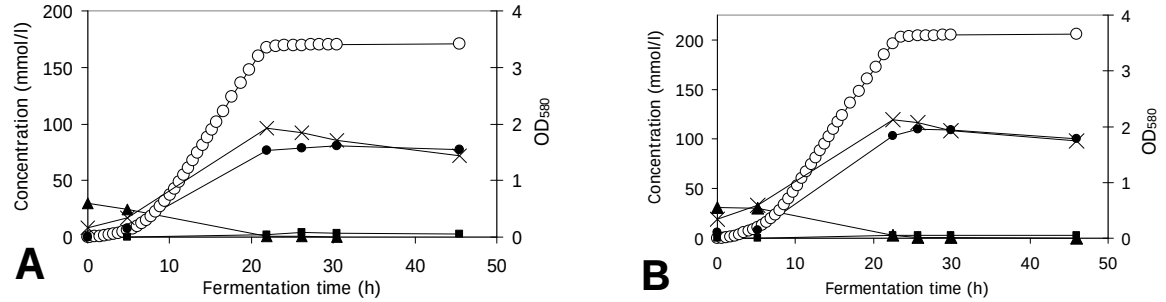

Figure 2: Fermentation profiles of batch cultivations of *C. saccharolyticus* in pH-controlled bioreactors on sucrose (A) and sugar beet extract (B) (10 g/l sucrose). The production of H_2 (○), acetate (●), lactate (■), the consumption of sucrose (▲) and the growth (×) of *C. saccharolyticus* are indicated. Amounts of sucrose and products are expressed in millimole per litre of culture.

For the pure sucrose-containing medium, a maximum hydrogen production rate of approximately 12.1 mmol/(l · h) was determined (Table 2), which was higher than the one of 8.4 mmol/(l · h) previously reported by van Niel et al. [9]. For the sugar beet extract-containing medium, maximum hydrogen production rate was approximately 12.8 mmol/(l · h).

The increased hydrogen production rate from the latter medium is possibly due to some compounds naturally present in sugar beet, such as phosphoric acid and amino acids, which may act as nutrients for cell growth. Hydrogen yields of 2.9 and 3.0 mol/mol hexose were observed in fermentations of sucrose and sugar beet extract, respectively, corresponding to 73% and 75% of the theoretical value of 4 mol/ hydrogen/mol hexose. The hydrogen to acetic acid molar ratio was 2.0-2.1 which is close to the theoretical value of 2.

Table 2: Molar yields and maximal volumetric hydrogen productivity (max. Q_{H2}) of *C. saccharolyticus* batch cultures grown on sucrose and sugar beet extract at 72 °C.

Substrate	Y_{H2}	Y_{HAc}	$Y_{HAc+HLac}$	max. Q_{H2}
	mol/mol C6	mol/mol C6	mol/mol C6	mmol/(l·h)
Sucrose	2.9	1.4	1.4	12.1
Sugar beet	3.0	1.5	1.6	12.8

Notable is the build-up of particulate matter, which took place during fermentation of the sugar beet extract, and did not cause any major disturbances in the process, but possibly led to a tendency to short periods of decreased hydrogen production after 4 and 12 h of fermentation (Fig. 3).

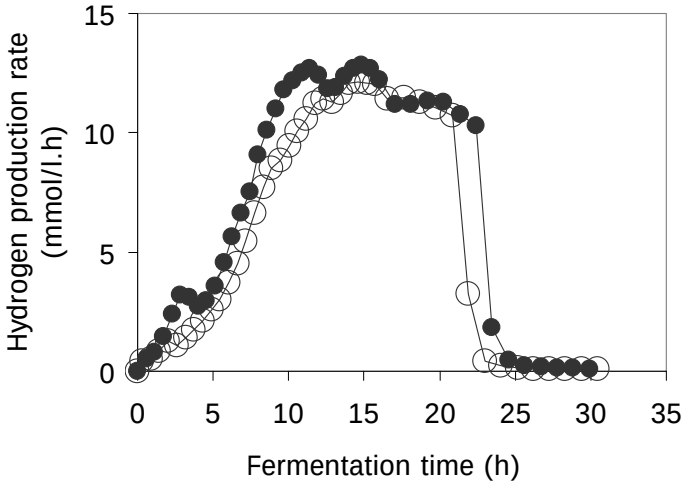

Figure 3: Hydrogen production rate by *C. saccharolyticus* grown on pure sucrose (open circles) and sugar beet extract (filled circles). The initial sucrose concentration was 10 g/l.

4 Conclusions

Sugar beet is a raw material well established in the literature as a substrate for fermentations, and can be instrumental in the middle-term in a sustainable hydrogen economy. The results of the present study suggest that *C. saccharolyticus* is suitable for efficient hydrogen production from sugar beet. In particular, *C. saccharolyticus* appears to

have a preference for sugar beet extract rather than for pure sucrose. Hydrogen yields of 2.9 and 3.0 mol/mol hexose were determined in controlled fermentations of sucrose and sugar beet extract, respectively, corresponding to 73% and 75% of the theoretical value of 4 mol hydrogen/mol hexose. Moreover, the results from the controlled fermentations are consistent with the notion that a fermentability experiment is a useful tool to rapidly determine the suitability of a raw material for hydrogen production.

Future research should address, from a biomass-oriented point of view, the utilization of sugar beet pulp for hydrogen production. The potential suitability of the pulp for hydrogen fermentation might contribute to the utilization of the whole plant as raw material for hydrogen production. From a biotechnological point of view, it might be interesting that future research focuses on the impact of several compounds naturally present in sugar beet on the fermentation of the sugar beet extract.

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