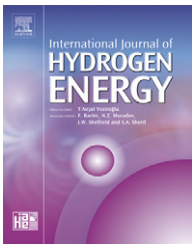
INTERNATIONAL JOURNAL OF HYDROGEN ENERGY 33 (2008) 1153–1163

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/ijhydene



Hydrogen production from sugars and sweet sorghum biomass using *Ruminococcus albus*

I. Ntaikou[a,b], *H.N. Gavala*[a,b,*], *M. Kornaros*[a], *G. Lyberatos*[a,b]

[a]*Department of Chemical Engineering, University of Patras, Karatheodori 1 st., 26500 Patras, Greece*
[b]*Institute of Chemical Engineering and High Temperature Chemical Processes, 26504 Patras, Greece*

ARTICLE INFO

Article history:
Received 12 July 2006
Received in revised form
3 September 2007
Accepted 19 October 2007
Available online 25 January 2008

Keywords:
Hydrogen
Sugars
Sweet sorghum
Ruminococcus albus

ABSTRACT

In the present work the production of hydrogen from sorghum biomass by pure cultures of the bacterium *Ruminococcus albus* was investigated. *R. albus*, an important fibrolytic bacterium of the rumen, can ferment hexoses and pentoses as well as cellulose and hemicelluloses. Therefore, *R. albus* seems to be very promising for the production of hydrogen from energy crops such as sweet sorghum, with the potential of utilizing not only the free sugars but also the cellulosic/hemicellulosic biomass as well. Batch and continuous stirred tank reactor (CSTR) experiments were carried out using glucose as carbon source in order to investigate the metabolism and calculate the growth kinetics of *R. albus*. Besides hydrogen, the main metabolic products detected were acetic and formic acids and ethanol. Hydrogen yield ranged from 0.47 to 2.52 mol of hydrogen per mole of glucose in continuous and batch experiments, respectively. Moreover, sorghum water extract containing soluble sugars and the lignocellulosic sorghum biomass before and after water extraction were also tested as potential substrates for hydrogen production using *R. albus*. The hydrogen productivity of sorghum extract plus that of sorghum residues equaled the hydrogen productivity obtained from the sorghum stalks suggesting that the process could be designed as a single-step process, thus avoiding the separate fermentation of soluble and insoluble carbohydrates as well as the extraction process. Hydrogen productivity has been estimated to be approximately 60 l of hydrogen per kg of wet sorghum biomass, thus suggesting that *R. albus* is suitable for efficient hydrogen production from sweet sorghum biomass.

1. Introduction

The world population and consequently energy demands seem to grow following exponential rate [1]. The impending shortage of energy resources together with the environmental fall off due to unreasonable use of fossil fuels lead many scientists to the search for alternative energy sources. Among others, research has been focused on the hydrogen production field, either by physicochemical or biological methods. Biological hydrogen production can be achieved by the use of microorganisms of two main types, photosynthetic (photoautotrophic and photoheterotrophic) [2] and fermentative. The main source of hydrogen during a biological, fermentative process is carbohydrates, which are very common in plant tissues, either in the form of oligosaccharides or as their polymers, cellulose, hemicellulose and starch. Thus, the

*Corresponding author at: Department of Chemical Engineering, University of Patras, Karatheodori 1 st., 26500 Patras, Greece. Tel.: +30 2610997858; fax: +30 2610993070.
E-mail addresses: gavala@chemeng.upatras.gr, hng@biocentrum.dtu.dk (H.N. Gavala).
0360-3199/$ - see front matter © 2007 International Association for Hydrogen Energy. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.ijhydene.2007.10.053

biomass of certain plants with high content in carbohydrates could be considered as a very promising substrate for biohydrogen production. Such plants are misckanthus, sugarcane and sorghum, the so-called energy crops. In addition, using properly selected microorganisms, many rural residues can be exploited for hydrogen production as well. The maximum theoretical hydrogen yield is 4 mol per mole of utilized carbohydrates, expressed as glucose equivalents when carbohydrates are used as substrate [3].

The microorganism used in the present study was the bacterium *Ruminococcus albus*. *R. albus* is a non spore-forming, obligatory anaerobic, coccoid bacterium [4,5], the natural habitat of which is the first stomach (rumen) of the ruminants. It produces extracellular hydrolytic enzymes (exoglucanases and endoglucanases), which break down cellulose [6–8] and hemicellulose [9], whereas it cannot break down pectin and starch [10]. The oligosaccharides produced from cellulose and hemicellulose degradation—cellobiose and the respective pentoses xylose and arabinose—are further metabolized [11,12]. *R. albus* can grow on glucose as well, but in cultures containing both glucose and cellobiose, it seems to prefer the cellobiose utilization [13]. In pure cultures of *R. albus* the metabolic products are acetate, ethanol, formate, hydrogen and carbon dioxide in different stoichiometric ratios depending on the environmental and operating conditions [14–16], whereas some strains produce lactate as well [17,18].

Sweet sorghum (*Sorghum bicolor* (L.) Moench) is an annual C4 plant of tropical origin, well adapted to sub-tropical and temperate regions and highly productive in biomass. Sweet sorghum stalks are rich in sugars, mainly in sucrose that amounts up to 55% of dry matter and in glucose (3.2% of dry matter). They also contain cellulose (12.4%) and hemicelluloses (10.2%) [19]. Sweet sorghum has been used for dietary reasons for many years in many parts of Africa, Asia and semi-arid tropics worldwide. More recently it has been considered as feedstock for ethanol production, since it is very rich in sugars, has low nutrition and water demands [20,21], and it consequently can be cultivated over a large geographic region.

The most common process for ethanol production from sorghum is the direct fermentation of the soluble sugars of the juice using the yeast *Saccharomyces cerevisiae*, though other species of the genus *Saccharomyces* have also been used successfully [22]. The remaining lignocellulosic material can also be converted to ethanol either after the enzymatic hydrolysis of the biopolymer [23] or directly using mixed cultures of cellulolytic and fermentative microorganisms [24,25]. The conversion of the grain sorghum starch to ethanol has also been investigated through a co-culture of amylolytic yeasts together with *S. cerevisiae* [26].

Although sorghum has been thoroughly investigated as an energy crop for bioethanol production, it has not been used so far as a potential source for hydrogen production. Sorghum biomass could be fully exploited for hydrogen production since both soluble and complex carbohydrates can be utilized, either in a single step or separately after extraction. Extraction of free sugars from the stalks is easily achieved using water at 30 °C. After the extraction process, a liquid fraction, rich in sucrose, and a solid fraction, containing the cellulose and hemicelluloses, are obtained.

The aim of the present study was to investigate the hydrogen production potential from sweet sorghum biomass using pure cultures of *R. albus*. Since sorghum biomass has almost equal amounts of soluble and complex carbohydrates which can easily be separated with the extraction method described above, the exploitation of each fraction separately and of the total biomass as a whole was investigated for hydrogen production. Experiments were carried out with different carbohydrate substrates as carbon sources in order to understand the metabolism of the microorganism. Glucose was mainly used in experiments aiming at the determination of growth kinetics on this substrate and for studying the distribution of fermentation products under different conditions. Cellobiose, xylose and arabinose, which are the main products of cellulose and hemicellulose hydrolysis, were also used as substrates for *R. albus*. Finally, hydrolysis of sweet sorghum biomass was investigated and hydrolysis kinetics were determined.

2. Materials and methods

2.1. Organism, medium and growth conditions

R. albus, strain DSMZ 20455 [17,27], was obtained from the Deutsche Sammlung von Microorganismen und Zellkulturen (DSMZ) culture collection and was maintained in a modified DSMZ 453 medium of the following composition: 5 g/l glucose, 5 g/l tryptone, 2 g/l yeast extract, 8.24 g/l KH_2PO_4, 1.84 g/l K_2HPO_4, 0.48 g/l NaCl, 0.8 g/l $(NH_4)_2SO_4$, 0.1 g/l $MgSO_4 \cdot 7H_2O$, 0.03695 g/l $CaCl_2 \cdot 2H_2O$, 4 g/l Na_2CO_3, 0.5 g/l cystein-HCl, 0.1 ml/l isovaleric acid, 0.1 ml/l isobutyric acid and 0.1 ml/l methylbutyric acid. The medium was prepared by mixing anaerobically and under sterile conditions separate solutions of the organic compounds (sugars), the salts, the acids mixture and the cystein-HCl. The different solutions had been separately sterilized at 121 °C for 20 min and cooled down before mixing. Stock cultures were stored at −22 °C in 20% glycerol and inoculation cultures were transferred twice before use. The basal medium was used in all cultures while the carbon source, which also was the limiting nutrient, was varied depending on the substrate tested, i.e. glucose, whole sorghum stalks, sorghum extract and sorghum cellulosic–hemicellulosic residues. All cultures were grown in a CO_2/N_2 (71:29, v/v) atmosphere, at 37 ± 1 °C and continuous stirring of 200 ± 10 rpm. Batch experiments were performed under the same conditions.

2.2. Sweet sorghum biomass

The sweet sorghum biomass (*S. bicolor* L. Moench) used in the present study was produced in field experiments through biological farming techniques according to European Regulation EC 2092/91. The experiments were conducted at the University of Patras experimental station. Sweet sorghum var. Keller seeds were sown at mid of May and the stalks were harvested at mid of October. After the harvesting of sorghum stalks, the fresh stems were stripped from the leaves, were chopped to a size of 20 cm and were stored in the freezer at −20 °C. Subsequently, the stalks were milled by a laboratory