Hydrogen production from biomaterials by the extreme thermophile *Caldicellulosiruptor saccharolyticus*

Ph.D. Thesis

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Introduction

The popularity of hydrogen as an energy carrier increased after the energy crisis during the 1970s. The worldwide energy crisis originates from the excessive use of non-renewable fossil fuels. In the 1990s concerns about the approaching environmental disaster due to the phenomenon called global warming due to an increased greenhouse effect caused also by the fossil fuels triggered renewed interest in hydrogen as a fuel. Nowadays it is widely acknowledged that hydrogen is an attractive energy carrier to replace conventional fossil fuels, both from environmental and economic points of view. When hydrogen is used as a fuel it generates no pollutants since upon burning hydrogen produces water which can be recycled to generate more hydrogen. A substantial amount of hydrogen is produced in industry today, but nearly 90% of this hydrogen is obtained by steam reformation of petroleum, coal tar or natural gas.

Molecular hydrogen, produced from renewable sources (biomass, water, organic wastes) biologically is called “biohydrogen”. Biohydrogen production can be achieved by the use of two main approaches, photosynthetic (photoautotrophic and photoheterotrophic) and fermentative. The production of H_2 is one of the specific mechanisms to dispose excess electrons through the activity of hydrogenase enzymes present in H_2 producing microorganisms. Hydrogenases have various physiological roles. They may also have different localization in the cell and different subunit composition. Three main classes have been recognized: iron-only ([FeFe] hydrogenases), nickel-iron ([NiFe] hydrogenases), "iron-sulphur-cluster-free" hydrogenases, the latter contain no redox active metal ion in their active centre.

Biohydrogen production may involve direct photolysis, indirect photolysis, photofermentation or dark fermentation. Our work focuses on the dark fermentative approach. The hydrogen-producing microbes in dark fermentation processes can be classified into two categories: facultative anaerobes (enteric bacteria, e.g. *Escherichia coli*, *Enterobacter*, and *Citrobacter*) and strict anaerobes (clostridia, methylotrophic mathanogens, and rumen bacteria). The extreme thermophile *Caldicellulosiruptor saccharolyticus* is related to clostridia. The genus Clostridium has been widely studied for H_2 production. Clostridia species are capable of using various organic substrates such

as proteins, starch, animal manure and sewage sludge. Some clostridia are both proteolytic and saccharolytic. Despite the relatively low yields of hydrogen, the fermentative route is a promising method for biohydrogen production due to its high rate of the H_2 evolving [FeFe] hydrogenase and the versatility of the substrates used.

Biomass, a product of photosynthetic conversion of solar energy, is a versatile renewable source that can be utilized for sustainable production of hydrogen. Biomass of low moisture content, such as wood residue, wood scrap and solid household garbage can be incinerated directly to produce heat. Energy from biomass having high water content, such as sewage sludge, agricultural and livestock effluents as well as animal excreta is recovered mainly by microbial fermentation. Wood biomass is mostly composed of cellulose, hemicellulose, lignin, ash and soluble substances called extractives. Hydrolysis is the step that breaks down the cellulose and hemicellulose polymers into their basic sugars. There are different hydrolysis technologies using energy demanding physico-chemical methods. Enzymatic hydrolysis is viewed as an environmentally friendly technology for decomposing biomass. It involves microorganisms that produce enzymes degrading cellulose into sugars. Using properly selected microorganisms, many agricultural feedstocks and their residues can be exploited for renewable biofuel production. Some agricultural plants and their residues like wheat straw, maize leaves, sweet sorghum and sugarcane bagasse have been utilized for biogas, bioethanol and biohydrogen production. The first two approaches have reached the level of elaboration into industrial technologies.

The main aim of this study was to examine different biomaterials, including biopolymers and plant biomass, which can be used as a substrate for H_2 production by *Caldicellulosiruptor saccharolyticus*. *C. saccharolyticus* was selected for fermentative biohidrogen production because it is an extreme thermophile that has benefits in industrial applicaitons, it has been reported to possess various hydrolitic enzymes and a very active hydrogenase enzyme. I aimed at developing an immobilization system to stabilize the hydrogen production system by selecting suitable and cheap immobilization support matrices and the determination of the optimal range of substrates and conditions for maximum hydrogen production.

Methods

Batch cultures of *Caldicellulosiruptor saccharolyticus* were grown on DSMZ medium 640 and the viability was determined by plating, using gelrite solidifying agent at high temperature. Cell biomass was determined as cell dry weight. Hydrogen was measured in a gas chromatograph using TCD detector and molecular sieve column. Organic carbon was determined on TOC analyzer. An immobilization procedure was developed to establish the cell attachment capacity to the support matrices. One-dimensional SDS PAGE analysis was employed for the analysis of proteins. Glucose concentration was analysed by the DNSA method.

Results

1. I demonstrated that *C. saccharolyticus* possesses an agarolytic activity in the presence of cellobiose, with a possible practical application in biohydrogen production, involving a simple way of utilizing agarose as a discarded waste.

2. I performed preliminary studies on the protein profile of agarose-induced *C. saccharolyticus* cells and detected a number of newly synthesized proteins due to the use of agarose as an additional carbon and energy source.

3. I proved that *C. saccharolyticus* has the ability to catabolise alginic acid and alginates in the presence of cellobiose, and produces hydrogen from this substrate.

4. I confirmed the cellulolytic activity of *C. saccharolyticus* using pine tree wood shavings, demonstrating the metabolic versatility of that extremophile.

5. I have selected cheap, widely available and non-toxic support matrices for the stabilization of the hydrogen-producing system by immobilization.

6. I selected for a support matrix, which has a dual function for the cells – on one hand, providing a solid surface for the cells, and on the other, playing the role of a substrate, which maintains the viability and preserves the physiology status during storage.

7. The optimal storage conditions of *C. saccharolyticus* cells have been established and their storability has been improved by immobilization. The hydrogen productivity of immobilized cells has been preserved for a period of 30 days compared to 8 days of their freely suspended counterparts.

8. I demonstrated that energy plants and agricultural waste resources were suitable for use by *C. saccharolyticus* for hydrogen production.

The experimental results confirm that *C. saccharolyticus* is particularly useful microorganism for fermentative biohydrogen production. For practical applications it has a remarkable diversity of potential substrates, several of them are components of the waste stream in agricultural activities and food processing. Equally important is the stabilization of the cells by a cost effective and environmentally friendly immobilization technique and the preservation of their biological activity for an extended period of time.

Publications

Ivanova, G., Rakhely, G. and Kovacs, K. L. Hydrogen production from biopolymers by *Caldicellulosiruptor saccharolyticus* and stabilization of the system by immobilization. Int J Hydrogen Energy 2008; doi:10.1016/j.ijhydene.2008.08.058.

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Co-author’s disclaimer declaration

I (undersigned) declare, that I am familiar with the candidate’s thesis points, I have not used the scientific achievements declared in her thesis points to acquire any scientific degree, and I will not use those for such reasons in the future.

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