BIOHYDROGEN PRODUCTION FROM CELLULOSIC BIOMASS

Ph.D Thesis

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INTRODUCTON

At present energy production takes place from fossil energy carriers most abundantly. The need for an alternative source to fossil fuels for powering an energy-hungry world is urgent for many political, social and environmental reasons: high oil prices, the dwindling supply of fossil fuels and the harmful effects of their use on the environment. Because of these the main task of the 21st century is to apply renewable energy resources instead of fossil fuels.

Hydrogen is a promising energy source because its use is entirely free of CO_2 emissions. It can be produced biologically: on one hand by photosynthetic organisms, directly using the energy of sunlight, and on the other hand, by heterotrophic hydrogen-producing creatures. For the propagation of this latter an external nutrient source is required, which is often glucose, starch or cellulose. Besides, the method can be combined with the decomposition of a variety of organic waste. The microbial hydrogen production is a more and more intensively studied route for the generation of renewable energy resources from biomass and other environmentally harmful waste. The thermophilic microorganisms are more suitable than their mesophilic counterparts for biohydrogen production from plant polysaccharides, because the anaerobic fermentation processes at higher temperature are thermodynamically more favorable and faster.

The model organism of my research was an anaerobic, extremely thermophilic bacterium *Caldicellulosiruptor saccharolyticus*. It can use a very broad spectrum of sugars, including mono-, oligo and polysaccharides. Under strict anaerobic conditions it ferments these sugars into hydrogen, acetic acid, carbon dioxide and few other by-products. Its genome sequence, which was described in 2007, revealed that the bacterium posesses an extensive polysaccharide degrading glycoside hydrolase enzyme system and it has a large number of ABC transporters which are necessary for transporting the synthesized sugars into the cells. In addition, it has two hydrogenase enzymes which are responsible for the formation of hydrogen. These characteristics make it an excellent candidate for hydrogen production from carbohydrate polymers implemented in the CBP (Consolidate Bioprocessing) process. However, the process requires in most cases the physical or chemical pre-treatment of the biomass.

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AIMS OF THE STUDY

The primary aim of my study was to study if hydrogen can be produced from the enzymatically non-pretreated biomass in a biological way, and moreover, to study if the efficiency of the conversion can be enhanced. In addition, I also wanted to characterize the background protein-associated processes using molecular methods.

Moreover, it was also important for me to ensure that the ensuing procedure is feasible in large-scale, because this is the prerequisite for long-term success in the industrial practice.

My exact tasks were as follows:

1. Utilization of enzymatically non-pretreated cellullosic biomass by *C. saccharolyticus* to produce hydrogen in a one-step process, and monitoring the measurable parameters during the degradation in small-volume batch fermentations.

2. Studying the effect of glucose on the degradation of cellulose.

3. Studying the effects of other sugars – xylose, mannose, fructose, rhamnose, lactose, sucrose – in order to optimalize the conversion of cellulose.

4. Increasing the yield of the bioconversion of cellulose,

5. Studying by molecular methods the way glucose exerts its positive action on cellulose degradation: studying the expression of genes responsible for cellulose hydrolysis and hydrogen production using whole cell transcriptome analysis and reverse transcription-coupled PCR.

6. Studying the biodegradation of a real cellulosic industrial waste.

MATERIALS AND METHODS

I studied the anaerobic degradation of two types of cellulosic waste in small-volume hypovial bottles and in 5.9-liter volume sectionally operated Biostat C fermentors. During experiments the *Caldicellulosiruptor saccharolyticus* was used as a model organism. The initial media was always salt-containign minimal medium and it was supplemented with cellulose biomass and/or the sugar. The hydrogen produced as a byproduct of the degradation was measured by gas chromatograph. The change in the cell number was counted by using a Bürker-chamber.

The whole-cell transcriptome analysis was carried out by a new generation sequencing (NGS)-based SOLiD4 equipment Validation of a part of obtained results was carried out by an Applied Biosystems 7500 Real-Time PCR equipment. Planning of the particular primers was made by Applied Biosystems Primer Express 3.0 software.

Propagation and inoculation work was carried out according to general practice.

RESULTS

1. My results clearly demonstrate that *Caldicellulosiruptor saccharolitycus* is able to break down the enzymatically untreated filter paper as the sole carbon source in batch flasks.

   I found that the number of cells, the pH change and the hydrogen production correlated with each other. The cell growth run paralel with the hydrogen production and the pH was shifted towards the acidic range as a result of the metabolic activity of the cells.

2. I studied the effect of several mono- and disaccharides (glucose, fructose, cellobiose, lactose, rhamnose) in various concentrations on cellulose driven hydrogen production. My conclusion is that almost all of the tested sugars promoted the degradation of paper to some extent, but glucose has the most

noticeable effect. In most cases, low concentration of sugar was enough for a faster and more efficient conversion.

3. I demonstrated, that the duration of biodegradation of cellulose supplemented with glucose not only significantly shortened but in the case of this medium produced twice the amount of hydrogen as compared to the culture containing cellulose only.

4. In order to decide whether the addition of glucose to the cellulosic substratesstimulated the cell growth and/or influenced the gene expression profile of the cells, total transcriptome analysis was performed. Genes from the large transcriptomic database were selected, which were assumed to be involved in the cellulose degradation and cellulose based hydrogen production and the results were validated by reverse transcription coupled real-time PCR. The study confirmed our hypothesis that glucose affects positively the production of cellulase enzymes and it has positive or negative influence on the expression of several genes which play role in the process of cellulose utilization. We have obtained a more precise view for the quantitative changes of the expression of selected genes during the various fermentations by using the qPCR techniqe.

   The results allowed us to concude that glucose primarily affects the biochemistry of the process, which is reflected by the transcriptional changes.

5. In addition to filter paper, bioconversion of industrial paper sludge from Dunapack Ltd., was investigated as well. The glucose stimulated cellulose-based biohydrogen production process could be adapted to industrial cellulosic waste albeit at lower efficacy. The reason for the weakerutilization efficiency of the complex substrate should be clarified in further experiments.

PUBLICATIONS RELATED TO THE THESIS

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