Volume 53(1):55-76, 2009
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

Biohydrogen production using the cellulose containing plant biomass

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There are many serious gobal problems caused by mankind in the last dacades. Among these, two have oustanding importance: a) the enviromentally friendly, biological degradation of the large amounts of organic waste poduced by the industrial sector; b) as well as the reinforcement of the energy supply by renewable energy source and the substitution of the current energy carriers by environmentally sound fuels. Hydrogen is considered as the best candidate for the future energy carriers, since just pure water is formed during its oxidation. There are biological tools for producing hydrogen and hydrogen evolving photosynthetic or fermentative microbes are primarily involved in these processes. In the dark fermantative processes, usually biopolymers of agricultural origin used to be the substrate, which have to be first converted to simpler monomers being fed to the hydrogen producing bacteria. Strains capable to convert and utilize complex biopolymers are of extrme importance. One of the best candidates is the Gram positive, hyperthermophilic, anaerobic *Caldicellulosiruptor saccharolyticus.* (Bagi et al. 2007) Its biotechnological importance is that it is capable to degrade cellulose-based biomasses, such as paper, or energy plants (which are found in the large quantities) and has hydrogenases for removal of excess electrons formed during the fermentative metabolism. The genome of this bacterium is available and the strain was shown to possess not only numerous glycosidases required for the hydrolysis of different kinds of polysaccharides, but also hydrogenase enzymes responsible for hydrogen production.

In our group an enviromentally friendly biological method have alredy been developed by wich we waste of animal origin could be transformed to hydrogen. (Bálint et al. 2005.)

In my work I aim at adapting this process to cellulose-based waste of plant origin. Morover,on the basis of the known genome of this organism, I intend to create a genetically modified strain which is capable of degrading the avaible biomass (currently filter paper) more efficiently and thereby producing significantly higher amounts of hydrogen.

According to our aims, I studied the hydrolysis of untreated filter paper in batch fermentation conditions in the presence of six various kinds of sugar using minimal media containing no other carbon sources. In paralel, I monitored the hydrogen evolution from sugars and filter paper alone and various combinations.

My results clearly showed, that *C. saccharolitycus* was able to degrade the untreated filter paper and to produce abundance hydrogen from this material in the presence of minimal amount of sugar. From the sugar specificity, it is likely that these sugars are basically necessary for the induction of cellulase enzymes., thus paper decomposition can be promoted by sugars.

For better understanding the molecular background of the events and for further improvement of the process genetically modified strains should be constructed. However, no protocol is available for gene transfer into *C. saccharolyticus*. In order to introduce foreign DNS into the cells, a functional genetic system has to be developed. As a first step, the efficient plating technique was ameliorated and numerous suitable vectors (replicative and non replicative) were collected and constructed to achieve sustainable vector replication or to modify the given part of the genome. Furthermore the development of an efficient method for introduction of foreign DNA is also requisite.

My future plans are the optimization of the paper decomposition conditions, getting deeper insight into the the molecular mechanism of the paper/sugar metabolism → hydrogen conversion.

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Construction and characterization of synthetic genetic oscillator in yeast

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The design of gene expression systems with both spatial and temporal regulation has been an area of intense scientific interest during the last ten years. Our aim is to create a synthetic genetic circuit in yeast based on transcriptional – translational feedback loops mimicking the structure (input, oscillator, output) and function (self-sustaining oscillation and resetting) of eukaryotic circadian clocks. The circuit will serve as an ideal test system for mathematical modeling describing oscillatory mechanisms (eg. circadian clocks), since all components are known, well characterized and can be easily modified/adjusted in order to test predictions from the model. The main requirements for such a system are: a genetic network with well defined components, which do not interfere with the physiology of yeast; option to set/modify the expression level/turn-over rates of components, proper input/resetting mechanism and easily measurable output (in vivo, real-time).

First we created and tested components for the input pathway of the oscillator. In the circadian systems of eukaryotes, external signals (e.g. light and/or temperature) reach the oscillating genetic network through the input pathway and cause an acute change in the expression