Abstract:

Development and scale-up of a fed-batch process for heterologous hydrogenase production in *E. coli*

Co-supervisor 1: Prof. Dr. Peter Neubauer, Technische Universität Berlin, Institute of Biotechnology, Chair of Bioprocess Engineering, Straße des 17. Juni 135, D-10623 Berlin, Germany, Germany

Co-supervisor 2: Dr. Oliver Lenz, Technische Universität Berlin, Institute of Chemistry, Straße des 17. Juni 135, D-10623 Berlin, Germany, Germany

Biohydrogen produced by hydrogenases could be a green alternative to the use of fossil fuels. However, the availability of the required enzymes are complex, difficult to express and only available in small quantities from their native hosts. Thus the availability of hydrogenases is a bottleneck limiting basic research and biotechnological application. To overcome this bottleneck our project aims at the improvement of the existing bioprocess for the heterologous production of hydrogenases. Thus we aim at the development of a fed-batch process for the heterologous hydrogenase production in *E. coli*, its scale-up from shake flask to stirred-tank bioreactor scale (1L and 10L) using the *R. eutropha* RH as a model and the transfer of the obtained knowledge towards production of other more complex hydrogenases such as the soluble, NAD^+-reducing hydrogenase (SH) from *R. eutropha*..

Extended description version of the project:

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1. Overall goal of the project

The energy revolution, i.e. the transition from fossil fuels to alternative, CO_2-neutral energy sources, is currently one of the greatest and, at the same time, the most unavoidable social tasks in the fight against global warming. Thus, the search for and utilization of so called "green" alternative energy sources is an important task. A promising solution is the use of biohydrogen, which can be generated by specific bacterial biocatalysts called hydrogenases. However, in order to make the use of hydrogenases as tools for the production of hydrogen economically feasible and thereby enable the transition to a post-fossil age, it is necessary to improve existing bioprocesses for the production of hydrogenases and to develop more efficient ones. Besides developing a fed-batch process to increase the hydrogenase yield, the main focus of the process development will be on the scale-up of the production process from the shake flask to the bioreactor scale in order to meet the demand of enzyme required for scientific research and biotechnological application.

2. State of the art

Hydrogenases are abundant metalloenzymes that catalyze the reversible conversion of dihydrogen into protons and electrons. They can be found in many bacteria, archaea or unicellular eukaryotes. According to the metal composition of their active site, they are classified as [Fe]-, [FeFe]- or [NiFe]-hydrogenases. In addition to the metal ion containing active site hydrogenases contain different FeS clusters that serve as electron relay. A unique feature of some [NiFe]-hydrogenases is their O_2-tolerance that makes these enzymes particularly interesting as this facilitates their biotechnological application (e.g. for biohydrogen production, in fuel cells or for cofactor regeneration). Unfortunately, the low production yields in their native hosts result in high costs for cultivation and purification thus limiting their investigation and application. To circumvent this problem the heterologous production in a robust and genetically tractable host (e.g. *E. coli*) is a promising strategy. Moreover, the difficult maturation process required for the incorporation of the active site and the multisubunit architecture of the enzymes make them difficult-to-express proteins. However, until now different approaches have been made to improve the heterologous production of [NiFe]-hydrogenases[1]. Using the regulatory hydrogenase (RH) from *R. eutropha* as a model enzyme we already investigated relevant process parameters (e.g. strain, inducer concentration, temperature, production time) [2]. Moreover, implementing a fed-batch like production process further improved the RH yield in shake flask scales several 100-fold compared to RH yields obtained from *R. eutropha* [2]. Unfortunately, only an inactive, cofactor-free RH was produced in the initial attempts [2]. In a further step, an enzyme with similar activity to the native hydrogenase was produced by changing the culture conditions and additional expression of the specific maturation genes [3].

1. Specific aims and how they may be reached

So far, the heterologous hydrogenase production was only achieved in shake flasks with a scale up to 500 ml. Even though promising results could be achieved with fed-batch-like growth conditions using the EnPresso B medium, the scale for an economic biotechnological application of the hydrogenases is far too low. Furthermore, the heterologous production process has only been investigated for RH so far. However, compared to other [NiFe]-hydrogenases from *R. eutropha*, RH is significantly less processive, which limits its biotechnological applicability. Thus, the main objectives of the project are the development of a mineral salt-medium based high cell density fed-batch process in the bioreactor, its scale-up from 1L to 10L scale and the transfer of the process to other more complex hydrogenases such as the soluble, NAD^+-reducing hydrogenase (SH) from *R. eutropha*.

WP1: Development of a high cell density fed-batch

The switch from a standard complex medium-based batch cultivation to the EnPresso B-based fed-batch like cultivation already increased the biomass and RH production significantly. However, the obtained cell densities of about 30-40 are still very low compared to high cell density fed-batch processes typically reaching final ODs >300. In order to improve the hydrogenase production a mineral salt medium-based fed-batch process in a stirred tank bioreactor has to be implemented. To ensure high cell density as well as high specific hydrogenase yields and high activity, a fed-batch process with two separate feeding phases (1^{st} biomass production; 2^{nd} product formation) has to be developed. For both feeding phases it is necessary to evaluate the optimal phase length, the oxygenation level required for production of active hydrogenase and the need for supplementation with trace elements (mainly Ni and Fe). For these first experiments the Infors Multifors (Infors HT) bioreactor system will be used that allow cultivations in parallel bioreactors with a final volume of up to 1 L.

WP2: Upscaling of the hydrogenase production process

Scaling a bioprocess is an often underestimated problem. With increasing reactor size, the formation of zones in the bioreactor increases. In particular, the optimal distribution of O_2 and glucose is then no longer guaranteed. In order to enable the production of active hydrogenase on a larger scale, it is therefore necessary to investigate to what extent these conditions affect the formation of the product and its activity. To determine the robustness of the production process, the expected stress conditions due to zoning in the bioreactor can be simulated on a small scale using our automated high-throughput 2mag minibioreactor system. To confirm the results, the production scale can then be gradually scaled up from 1L to 10L bioreactor scale.

WP3: Production of the soluble hydrogenase (SH) from *R. eutropha*

In order to demonstrate the general usefulness of the developed production strategy with RH as a model protein for the production of hydrogenases in *E. coli*, the knowledge obtained shall be transferred to the production of SH from *R. eutropha*. To achieve this, first of all it is necessary to generate the required plasmids or strains respectively and then evaluate them according to the previous work [2–4].

References:

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