3 INTRODUCTION

3.1 *Aspergillus niger* as Cell Factory and Model Organism

Industrial biotechnology as platform for sustainable growth uses microorganisms or enzymes, to make products in a wide range of industrial sectors including chemicals that can be used as fuels or building blocks for the production of solvents and materials. Industrial biotechnology offers opportunities for the production of highly specialised metabolites or enzymes for bioconversions and for sustainable and economic process alternatives.

The filamentous fungus *A. niger* (black mould) has been used for centuries as industrial versatile microbial cell factory for production of organic acids as well as various extracellular enzymes, native or heterologous proteins and antibiotics. *A. niger* shows an amazing nutritional flexibility and metabolic capacity, and produces high levels of secreted primary and secondary metabolites. Nowadays, it is difficult to think of filamentous fungus where the metabolic capabilities are of greater interest than *A. niger* (Andersen et al. 2008). As a eukaryotic organism, *A. niger* offers valuable advantages for enzyme secretion, such as facilitated proteolytic processing and protein folding as well as posttranslational modifications (Lubertozzi and Keasling 2009; Nevalainen et al. 2005). This has made this microorganism a potentially attractive host for the biotechnological industry. Especially the genus *Aspergillus*, frequently applied in enzyme production due to the GRAS status (generally regarded as save), has received particular attention. Due to enormous development of genetic engineering and efficient expression systems, *Aspergillus* species have also achieved increased attention as host for industrial production of homologues and heterologous proteins (Wang et al. 2005).

Industrial strains can secrete large quantities of many economically desired products, e.g. 25 g/L cephalosporin, 30 g/L glucoamylase, 40 g/L cellulase, 50 g/L penicillin, 140 g/L citric acid (Gordon et al. 2000a; Grimm et al. 2005; Papagianni 2004). It is estimated that there are many enzymes that have industrial relevance (see http://www.report2008.novozymes.com). A fraction of these is currently produced as mono-component enzymes (Novozymes A/S or see list of commercial enzymes of the Association of Manufacturers and Formulators of Enzyme Products: http://www.ampep.org/list/html or the Enzyme Technical Association:

http://enzymetechnicalassoc). Recently, according to an updated technical market research report, enzymes for industrial applications from BCC Research, the global market for industrial enzymes increased from $2.2 billion in 2005 to an estimated $3.3 billion by the end of 2010. The market is expected to increase to over $4.4 billion by 2015 based on three main application sectors including technical, food and animal feed enzymes (Figure 1).

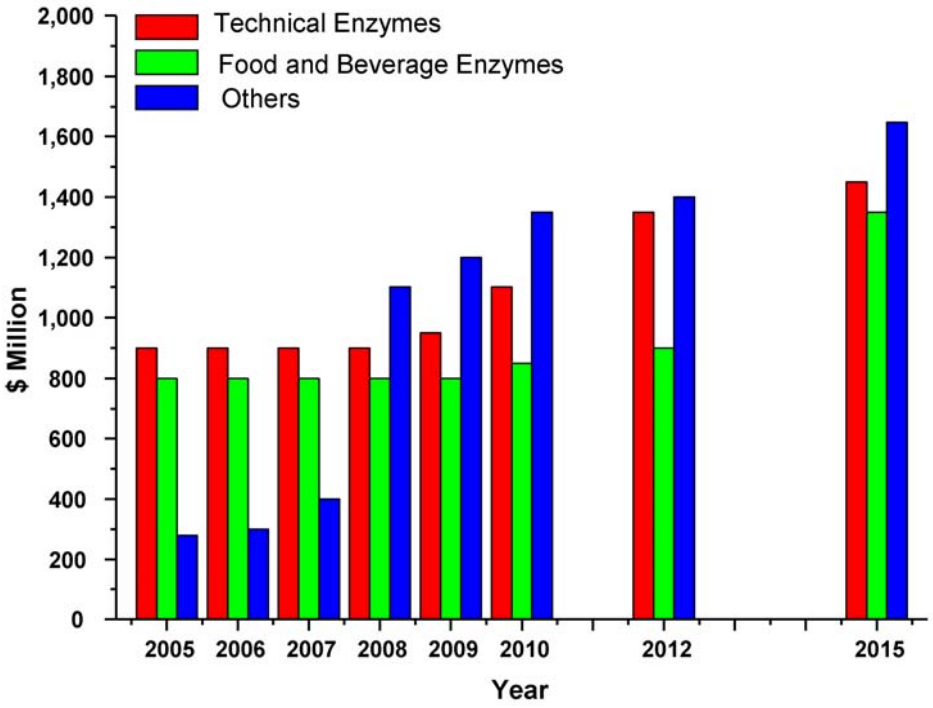

Figure 1. Global market of industrial enzymes based on three application sectors from 2005 to 2015 in $Millions - BCC Research.

Furthermore, *Aspergillus* species are capable to produce eukaryotic gene products such as tissue plasminogen activator (t-PA), Lactoferrin, and hen eggs lysozyme (HEWL) (Archer et al. 1990; Gheshlaghi et al. 2007; Ward et al. 1992; Wiebe et al. 2001).

Supplementary to the improvement of productivity by genetically modified strains (metabolic engineering), much research is focussed on the development of bioprocessing strategies resulting in an increased, controlled and tailored formation of a desired morphology and products while avoiding by-products. Still, the ability of *Aspergillus* to secrete the targeted metabolites is gaining ever increasing attention from the scientific community and industry. The bioprocessing strategies can be

applied to increase the production yield, titre and productivity in recombinant filamentous fungi in submerged culture, although is known that fungal cultivation system is recognized as a complicated multi-phase, multi-component process. For example the improvements in the cultivation and the productivity of the producer organisms have led to high recovery yields of penicillin (Nielsen et al. 1995a). However, *A. niger* has been widely applied as a model organism in studies of general cell physiology as a eukaryotic model or as a systems biotechnology workhorse. The idea of systems biotechnology is the understanding of the function of a cellular system as a whole towards improvement of desired product. As shown in various works there exists a close link between the bioprocess, the morphology and the underlying metabolism of the cell factory *A. niger* (Wucherpfennig et al. 2010). The scientific community is far from understanding of the underlying metabolic and regulatory mechanisms. Newly arising omic's (wet experimental) including genome, trancriptome, proteome, metabolome and fluxome and in silico design (dry experiments) in systems biotechnology, however, now provide a powerful toolbox to step towards understanding of this complex link between biological and engineering aspects of fungal cultures (Andersen and Nielsen 2009; Krull et al. 2010). Since the complexity of fungal systems is beyond intuitive comprehension, the core of systems biotechnology involves mathematical modelling of biological processes. Additionally, the significant similarity of the cell function among eukaryotic microorganisms offers promising prospects for *A. niger* models. In this thesis, different recombinant *A. niger* mutants as well as morphological forms such as pellet and free dispersed mycelia are investigated at various levels.