Physiological Engineering Aspects of *Penicillium chrysogenum*

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Aspects of
*Penicillium
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To Unni, Niklas and Mattias

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God did not create filamentous fungi
to grow in fermenters
A. P. J. Trinci

Preface

Penicillin production based on *Penicillium chrysogenum* is probably the best described large scale cultivation process in the open literature, and it is often considered as a mature science. However, there still remains a host of unanswered questions, which will only find their answer through fundamental research in the metabolism and morphology of *P. chrysogenum*. Furthermore, growth and product formation by *P. chrysogenum* is a good model system for the spectrum of industrially important products formed by filamentous fungi, e.g. other more valuable secondary metabolites and heterologous proteins. Finally, even 50 years after industrial penicillin production was initiated it is still by far the most important antibiotic with a total yearly market value close to 1.5 bio US\$.

This was the background for initiating in 1990 a research project on the penicillin production process at the Center for Process Biotechnology (which is hosted by the Technical University of Denmark in Lyngby). There was little previous experience with cultivations of filamentous fungi at the Center, and a close cooperation was therefore established with Novo Nordisk A/S, a producer of penicillin since 1944 and presently the world leader in production of industrial enzymes (of which many are produced by filamentous fungi). Novo Nordisk A/S supplied a high yielding strain (here designated NN-P8), which was necessary if the interplay between the primary and the secondary metabolism should be studied at nearly industrial conditions. Furthermore, the company kindly assisted in training the first ph. d. student working on the project, and thereby substantially supported the rapid implementation of a good experimental protocol at the Center. Gradually the fungal projects at the Center have matured in an open and always friendly cooperation with researchers at Novo Nordisk, thus serving as an excellent illustration of the mutual benefits of close cooperation between academia and industry.

This monograph is a state of the art review of the penicillin production by *P. chrysogenum*, with special emphasis on the activities at the Center for Process Biotechnology in the period between 1990 and 1995. The activities at the Center have focussed on bioreaction engineering aspects of the microbial physiology of *P. chrysogenum*, an approach which has been termed Physiological Engineering, and this text has therefore been given the title "Physiological Engineering Aspects of *Penicillium chrysogenum*". In 1995 the text was accepted by the Technical University of Denmark as a dissertation required to obtain the degree

dr. techn and it was defended in 1995. This is a modified version of the text especially prepared for publishing by World Scientific Publishing Co.

The findings presented in this text are the result of an extensive team work involving M. Sc. students, Ph. D. students, post docs, and laboratory technicians, and I acknowledge Lars H. Christensen, Annemarie Gade Pedersen, Claus Johansen, Henrik Jørgensen, Helmut Meier, Preben Krabben, Claus M. Henriksen, Rong Wei Min, Morten Carlsen, Mikael Bundgaard-Nielsen, Henrik Andersen, Bjarke Lorentzen, Michael Jacobsen, Henriette Ågård Jørgensen, Anders Spohr, Lars Bo Jørgensen, Tina Johansen and Lene Christensen for their contribution to this work. In writing this text I am very indebted to professor, dr. techn. John Villadsen, who strongly supported the build up of the group. He encouraged me to write the text and he has made a number of useful suggestions. Since I started as a student with John Villadsen we have worked closely together on many aspects of Bioreaction Engineering, and throughout I have enjoyed it and learned much. Finally I thank our librarian Jørgen Hedegaard, who has been very helpful in collecting the necessary literature, and all the people at Novo Nordisk, who have provided information on various aspects of penicillin production and other industrially important processes based on filamentous fungal cultivations especially Paul Hjortkjær, Henrik Møllgaard, Lars Guldbæk Carlsen, Henrik Kim Nielsen and Jens Gram.

Jens Nielsen

April 1996
Lyngby, Denmark

List of Symbols

a	Specific interfacial area [m^{-1}]
a_{cell}	Specific cell surface area [$\text{m}^2 (\text{g DW})^{-1}$]
$b(\mathbf{z}, t)$	Breakage function [h^{-1}]
c_i	Concentration of the i 'th chemical compound [M]
c_i^*	Saturation concentration of the i 'th chemical compound [M]
$c_{f,i}$	Concentration of the i 'th chemical compound in the feed [M]
c_{prim}	Concentration of the primary fluorophore [M]
c_s	Concentration of the limiting substrate (typically glucose) [M]
C	Number of pathway intermediates in a metabolic model
C_i^r	Flux control coefficient with respect to the i 'th enzyme activity
d_h	Hyphal diameter [m]
d_p	Pellet diameter [m]
$d_{p,\text{av}}$	Average pellet diameter [m]
$d_{p,\text{eq}}$	Equilibrium pellet diameter [m]
d_s	Stirrer diameter [m]
D	Dilution rate [h^{-1}]
$D_{i,\text{eff}}$	Effective diffusion coefficient [$\text{m}^2 \text{s}^{-1}$]
D_i	Diffusion coefficient of the i 'th chemical compound [$\text{m}^2 \text{s}^{-1}$]
e	Number of hyphal elements [m^{-3}]
e_p	Pellet concentration [m^{-3}]
e_{spore}	Spore concentration [m^{-3}]
$f(\mathbf{z}, t)$	Distribution function for cells with property y in the population
$f_m(l_i, n, t)$	Distribution function for hyphal elements with length l_i and number of tips n
$f_p(d_p, t)$	Distribution function for pellets with diameter d_p
F	Degrees of freedom
F_{rel}	Relative culture fluorescence [AFU]
$g(\mathbf{z}, t)$	Germination frequency
ΔG^0	Gibbs free energy [kcal mole^{-1}]
$h(\mathbf{z}, t)$	Net rate of formation of cells with property y [cells h^{-1}]
$h_m(l_i, n, \mathbf{z}, t)$	Net rate of formation of hyphal elements with property (l_i, n) [h^{-1}]

$h_f(l_i, n, z, t)$	Net rate of formation of hyphal elements with property (l_i, n) due to fragmentation $[h^{-1}]$
$h_g(l_i, n, z, t)$	Net rate of formation of hyphal elements with property (l_i, n) due to germination $[h^{-1}]$
H_A	Henry's constant $[atm\ m^3\ kmol^{-1}]$
J	Number of intracellular reactions
k_i	Rate constant $[h^{-1}]$
$k_t a$	Volumetric mass transfer coefficient $[s^{-1}]$
K_a	Acid dissociation constant $[M]$
K_m	Saturation constant $[M]$
K_i	Inhibition constant $[M]$
l_e	Effective length $[m]$
$l_{e,av}$	Average effective length in a population of hyphal elements $[n]$
$l_{e,eq}$	Equilibrium effective length $[m]$
l_{hgu}	Hyphal growth unit length $[m\ tip^{-1}]$
l_t	Total hyphal length $[m]$
$l_{t,av}$	Average total hyphal length in a population of hyphal elements $[m]$
L	Number of intracellular variables
m_{ATP}	Maintenance associated ATP consumption $[moles\ ATP\ (kg\ DW\ h)^{-1}]$
m_{hgu}	Hyphal growth unit mass given $[g\ DW\ tip^{-1}]$
m_s	Maintenance associated specific substrate consumption $[moles\ (kg\ DW\ h)^{-1}]$
m_p	Maintenance associated specific metabolic product formation $[moles\ (kg\ DW\ h)^{-1}]$
m_{pel}	Pellet mass $[g\ DW]$
M	Number of metabolic products
M_w	Molar mass $[g\ mole^{-1}]$
M	Matrix with the specific growth rates of morphological forms in the diagonal
n	Number of tips
n_{av}	Average number of tips in a population of hyphal elements
n_{tot}	Total number of tips in a population of hyphal elements
N	Number of substrates
N	Stirring speed $[s^{-1}]$
N_A	Aeration number

N_f	Flow number
N_p	Power number
p	Extracellular metabolic product
$p(\mathbf{z}', \mathbf{z})$	Partitioning function
$\mathbf{p}$	Extracellular metabolic product vector
P	Permeability coefficient [m s^{-1}]
P_g	Power input to a bioreactor at gassed conditions [W]
q_{bran}	Branching frequency [tip h^{-1}]
q_{frag}	Rate of fragmentation [h^{-1}]
q_i	Volumetric rate of formation of the i 'th compound [$\text{moles (m}^3 \text{ h)}^{-1}$]
$q_{p, \text{frag}}$	Rate of fragmentation of pellets [h^{-1}]
$q_{p, \text{growth}}$	Rate of growth in pellet diameter [m h^{-1}]
q_o^{mt}	Volumetric mass transfer rate of oxygen [$\text{moles (m}^3 \text{ h)}^{-1}$]
q_{tip}	Tip extension rate [m (tip h)^{-1}]
q_x	Volumetric rate of formation of biomass [$\text{g DW (m}^3 \text{ h)}^{-1}$]
Q	Number of morphological forms
r	Specific reaction rate [$\text{moles (kg DW h)}^{-1}$]
r_{ATP}	Specific ATP synthesis rate [$\text{moles ATP (kg DW h)}^{-1}$]
$r_{s, i}$	Specific uptake rate of the i 'th substrate [$\text{moles (kg DW h)}^{-1}$]
$r_{s, \text{max}}$	Maximum specific uptake rate of substrate [$\text{moles (kg DW h)}^{-1}$]
$\mathbf{r}$	Specific reaction rate vector [$\text{moles (kg DW h)}^{-1}$]
$\mathbf{r}_m$	Measured specific rate vector [$\text{moles (kg DW h)}^{-1}$]
$\mathbf{r}_p$	Specific metabolic product formation rate vector [$\text{moles (kg DW h)}^{-1}$]
$\mathbf{r}_s$	Specific substrate uptake rate vector [$\text{moles (kg DW h)}^{-1}$]
R	Pellet radius [m]
R_i	Net rates for the formation of the i 'th intracellular component [$\text{moles (kg DW h)}^{-1}$]
R_l	Thickness of active growth layer in pellets [m]
$R_i(\mathbf{z}, t)$	Net rate of increase in z_i for a cell in the state $\mathbf{z}$
$\mathbf{R}$	Vector of net rates for the formation of intracellular components [$\text{moles (kg DW h)}^{-1}$]
Re_s	Reynolds number for the stirrer
s	Extracellular substrate
$\mathbf{s}$	Extracellular substrate vector
t	Time [h]

t_c	Circulation time [s]
t_m	Mixing time [s]
$\mathbf{T}$	Total stoichiometric matrix
u_s	Superficial gas velocity [m s^{-1}]
$\mathbf{u}$	Vector containing the specific reaction rates of the metamorphosis reactions [kg (kg h)^{-1}]
v_f	Liquid feed flow to the bioreactor [$\text{m}^3 \text{ h}^{-1}$]
v_g	Gas flow [$\text{m}^3 \text{ h}^{-1}$]
$v_{\max}$	Maximum enzyme activity [moles h^{-1}]
v_{pump}	Impeller induced flow [$\text{m}^3 \text{ h}^{-1}$]
V	Medium volume [m^3]
V_{abio}	Volume of abiotic phase [m^3]
V_{cell}	Specific volume of cells [$\text{m}^3 \text{ (g DW)}^{-1}$]
V_{hgu}	Hyphal growth unit volume [$\text{m}^3 \text{ cell tip}^{-1}$]
V_z	Total property space
w	Water content of the biomass [$\text{kg water (kg biomass)}^{-1}$]
x	Biomass concentration [kg DW m^{-3}]
X_i	Concentration of the i 'th intracellular component [kg (kg DW)^{-1}]
$\mathbf{X}$	Vector of concentrations of intracellular biomass components [kg (kg DW)^{-1}]
$y_{\text{viab}}(z)$	Fraction of viable spores
Y_{ij}	Yield coefficient of j from i [$\text{kg } j \text{ (kg } i)^{-1}$]
Y_{xATP}	ATP consumption for biomass formation [moles ATP (kg DW) $^{-1}$]
$Y_{\text{xATP,growth}}$	ATP consumption for transport, biosynthesis and polymerization [moles ATP (kg DW) $^{-1}$]
$Y_{\text{xATP,lysis}}$	ATP consumption for repolymerization of degraded macromolecules [moles ATP (kg DW) $^{-1}$]
$Y_{\text{xATP,leak}}$	ATP consumption for leaks, futile cycles <i>etc</i> [moles ATP (kg DW) $^{-1}$]
Y_{xNADH}	NADH production in connection with biomass formation [moles NADH (kg DW) $^{-1}$]
Y_{xNADPH}	NADH consumption for biomass formation [moles NADH (kg DW) $^{-1}$]
z_i	The i 'th property of the cells
$\mathbf{z}$	State vector
Z_i	Concentration of the i 'th morphological form [kg (kg DW)^{-1}]
$\mathbf{Z}$	Vector of concentrations of morphological forms [kg (kg DW)^{-1}]

Greek letters

A	Matrix containing the stoichiometric coefficients for substrates
B	Matrix containing the stoichiometric coefficients for metabolic products
Γ	Matrix containing the stoichiometric coefficients for intracellular biomass components
$\delta(\gamma)$	Dirac delta function
Δ	Matrix of stoichiometric coefficients for morphological forms
ϵ	Porosity of a pellet
$\epsilon_{j,i}$	Elasticity coefficients
η	Viscosity [kg (m s) ⁻¹]
η_{eff}	Efficiency factor
μ	The specific growth rate of biomass [h ⁻¹]
μ_{max}	The maximum specific growth rate [h ⁻¹]
μ_q	The specific growth rate for the q'th morphological form [h ⁻¹]
ρ_m	Hyphal density [kg wet biomass (m ³ cell) ⁻¹]
ρ_l	Density of the liquid phase [kg m ⁻³]
ρ_{pel}	Density of pellet [kg DW m ⁻³]
τ_0	Yield stress [N m ⁻²]
τ_s	Shear stress [N m ⁻²]
τ_t	Tortuous factor
Φ	Thiele modulus
Φ_{gen}	Generalized Thiele modulus

Abbreviations

LLD-ACV	δ -(L- α -aminoadipyl)-L-cysteinyl-D-valine
ACVS	ACV Synthetase
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
6-APA	6-Aminopenicillanic acid
AT	Acetyl-CoA: Isopenicillin N Acyltransferase
ATP	Adenosine triphosphate
DNA	Deoxyribonucleic acid
DW	Dry Weight

EMP	Embden Meyerhof Parnas
FAD	Flavin adenine dinucleotide (oxidized form)
FADH ₂	Flavin adenine dinucleotide (reduced form)
GMP	Guanosine monophosphate
GTP	Guanosine triphosphate
8-HPA	8-Hydroxyphenillic acid
IPNS	Isopenicillin synthetase
NAD ⁺	Nicotinamide adenine dinucleotide (oxidized form)
NADH	Nicotinamide adenine dinucleotide (reduced form)
NADP ⁺	Nicotinamide adenine dinucleotide phosphate (oxidized form)
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
OPC	6-Oxopiperide-2-carboxylic acid
<i>pcbAB</i>	Gene for ACVS
<i>pcbC</i>	Gene for IPNS
<i>penDE</i>	Gene for AT
PP	Pentose phosphate
PSS	Protein synthesizing system
P/O-ratio	Number of ATP formed per atom of oxygen used in the oxidative phosphorylation
RNA	Ribonucleic acid
mRNA	Messenger RNA
rRNA	Ribosomal RNA
tRNA	Transfer RNA
RQ	Respiratory quotient
TCA	Tricarboxylic acid
UMP	uridine monophosphate
UTP	uridine triphosphate

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