

Table of contents

Abstract	11
Index of abbreviations	17
I. Justification, objectives and contributions	19
II. Introduction	25
2.1 Mathematical modelling of microbial cultures	25
2.1.1 Bioreactors as dynamic systems	25
2.1.2 Constraint-based models of microbial metabolism	27
2.1.3 Representation of biomass in constraint-based models	32
2.1.4 Structural analysis of constraint-based models.....	33
2.1.5 Solving constraint-based models	33
2.2 Recombinant protein production in <i>Pichia pastoris</i>	37
2.2.1 General features	37
2.2.2 Fermentation guidelines for <i>P. pastoris</i> cultures.....	39
2.2.3 Process optimisation of <i>P. pastoris</i> cultures	41
2.2.4 Modelling and operating strategies in <i>P. pastoris</i> cultures.....	42
2.2.5 Modelling recombinant protein production	44
2.3 Glycosidases	47
2.3.1 Definition.....	47
2.3.2 Mechanism of action.....	48
2.3.3 Applications of glycosidases.....	50
2.3.4 α -L-arabinofuranosidase and β -N-glucosidase.....	52
2.4 Immobilisation of enzymes	53
2.4.1 Definition and advantages	53
2.4.2 Immobilisation methods	56
2.4.3 Molecular sieves as supports for enzyme immobilisation	57
III. Materials and Methods.....	61
3.1 Materials.....	61
3.1.1 Genetic materials.....	61
3.1.2 Microbial strains.....	63
3.1.3 Culture media	64
3.1.4 Mesoporous sieves.....	65
3.2 Microbial culture and transformation.....	67
3.2.1 General procedures	67
3.2.2 Microbial transformation	68
3.2.3 Transformant validation and characterisation	69
3.2.4 Activity plate screening.....	69
3.2.5 Expression assay in liquid flask cultures.....	70
3.2.6 Recombinant <i>S. cerevisiae</i> cultures.....	70
3.2.7 μ 24-microreactor cultures	71
3.3 Enzyme procedures and biochemical analysis	73
3.3.1 Enzyme recovery and purification.....	73

3.3.2 Protein gel electrophoresis	74
3.3.3 Deglycosylation.....	74
3.3.4 Enzymatic reactions.....	75
3.3.5 Subcellular location.....	75
3.3.6 Substrate specificity.....	76
3.3.7 Transglycosylation assays.....	76
3.3.8 Isoflavone release.....	77
3.3.9 HPLC analysis	78
3.4 Constraint-based model definition and analysis	78
3.4.1 Constraint-based model definition	78
3.4.2 Structural analysis	79
3.5 Flux calculations.....	80
3.5.1 Flux units	80
3.5.2 External flux calculation	80
3.5.3 Flux calculation methods: MFA, FS-MFA and PS-MFA.....	82
3.6 Consistency analysis based on PS-MFA.....	85
3.7 Ordinary least-squares regression and cross-validation.....	86
3.7.1 Ordinary Least Squares Regression.....	86
3.7.2 Cross validation	86
3.8 Immobilisation methods.....	87
3.8.1 Material and biocatalyst characterisation.....	87
3.8.2 Immobilisation procedures.....	87
IV. Expression of two glycosidases in <i>Pichia pastoris</i>	89
4.1 Background	89
4.2 Objectives.....	92
4.3 Results	93
4.3.1 Obtention of recombinant <i>P. pastoris</i> strains expressing <i>A. niger abfB</i> and <i>C. molischiana bglN</i> genes.....	93
4.3.2 Characterisation of the selected transformants.....	99
4.3.3 Characterisation of the recombinant enzymes.....	109
4.4 Discussion	122
V. Validation of a constraint-based model of <i>Pichia pastoris</i> metabolism	125
5.1 Background	125
5.2 Objectives.....	128
5.3 Results	129
5.3.1 Constraint-based metabolic model	129
5.3.2 Structural analysis	135
5.3.3 Validation against literature datasets.....	135
5.3.4 Structural analysis	140
5.3.5 Using the model to predict growth	143
5.3.6 Using the model to predict the intracellular flux distribution.....	147
5.4 Discussion	152

VI. Constraint-model based estimation of recombinant protein production in <i>Pichia pastoris</i> cultures.....	155
6.1 Background	155
6.2 Objectives.....	160
6.3 Results	160
6.3.1 Constraint-based metabolic model	160
6.3.2. Literature datasets	162
6.3.3 Stoichiometric estimation of protein	164
6.3.5 Protein estimator: definition	168
6.3.6 Protein estimator: parameter fitting.....	170
6.3.7 Applications of protein model.....	173
6.3.8 Application of the model in X33-Abf, X33-Bgl cultures.....	183
6.4 Discussion	193
VII. Glycosidase immobilisation on bimodal organosilicas	197
7.1 Background	197
7.2 Objectives.....	201
7.3 Results	202
7.3.1 Support and immobilisation method selection.....	202
7.3.2 Set-up of immobilisation procedure – adsorption assays.....	202
7.3.3 Biocatalyst obtention.....	206
7.3.4 Physicochemical characterisation.....	208
7.3.5 Biochemical characterisation of immobilised enzymes	210
7.3.6 Technological applications.....	221
7.4 Discussion	227
VIII. Future Work	233
8.1 Glycosidase expression and use in biocatalysis.....	233
8.2 Modelling extensions	234
8.3 Monitoring, optimisation and control	236
IX. Conclusions	241
X. References	245