

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

*In Situ* Recovery of  
Secondary Metabolites Using  
Adsorption Resins

A thesis presented in partial fulfilment of the  
requirements for the degree of

Master of Philosophy

at Massey University, Palmerston North,  
New Zealand

Jason L J Ryan  
2006

## Abstract

Almost without exception a two to three fold increase in microbial secondary metabolite concentration was measured when adsorption resins were added *in-situ* during a submerged liquid fermentation. Anguidine was produced at a final concentration of 440 mg/L after five days in a shake flask that contained adsorption resin, compared to 300 mg/L without resin. Rapamycin was produced at a final concentration of 87 mg/L after six days in a shake flask that had resin present, compared to 28 mg/L without resin. Ansamitocin P3 was produced at a final concentration of 24 mg/L after six days in a shake flask with resin, compared to 9.75 mg/L without resin. The increase in secondary metabolite concentration confirmed that the resins used provided a positive influence on secondary metabolite production. Adsorption resins for shake flask studies were selected based on their ability to achieve maximum adsorption of specific secondary metabolites in various fermentation systems.

A library of adsorbed concentrations was collected for the three secondary metabolites studied. The lipophilicity of the metabolite, calculated by several software packages, was compared to the polarity of the adsorption resin to generate a relationship. By using the preceding set of data it is possible to select adsorption resins that improved the produced concentrations of the target organic secondary metabolites.

The fermentation media compositions tested appeared to have no effect on the final product concentration when adsorption resins were added *in situ* during the fermentations.

Based on the lipophilicity of the secondary metabolite and the polarity of the resins, it is possible to select a resin that achieves a high adsorption concentration of the target organic secondary metabolite.

## Acknowledgments

This thesis was undertaken with assistance from Industrial Research Limited.

It gives me great pleasure to acknowledge the many people who have assisted me throughout this work. I would like to thank my supervisors Professor Yusuf Chisti and Sarah Reader for their assistance and guidance over the course of this project.

I would like to thank Randy Greasham for providing the impetus upon which I started this work. Without his encouragement and support I would not have achieved this work.

For their support and insight over the length of this work and more I would like to thank my colleagues Max Kennedy, Tony Woolhouse, Sarah Underwood, Simon Hinkley and Yin Zhu.

1	Introduction.....	11
1.1	Motivation.....	11
1.2	Objectives .....	11
1.3	Approach.....	12
1.4	Overview.....	13
2	Literature Review.....	14
2.1	Fermentation technology .....	14
2.1.1	Aspects of submerged culture .....	14
2.1.1.1	Micro-organisms.....	14
2.1.1.2	Types of bioreactors.....	15
2.1.1.3	Operating modes for submerged bioreactors .....	17
2.1.2	Fermentation medium development .....	18
2.1.2.1	Complex medium.....	19
2.1.2.2	Defined medium .....	20
2.1.3	Overview of metabolite production.....	22
2.1.3.1	Primary metabolites or metabolism .....	22
2.1.3.2	Secondary metabolites .....	23
2.1.4	<i>Fusarium sambucinum</i> .....	25
2.1.4.1	Growth conditions .....	25
2.1.4.2	Trichothecene biosynthetic pathway .....	25
2.1.4.3	Anguidine .....	27
2.1.5	<i>Streptomyces hygroscopicus</i> .....	28
2.1.5.1	Growth conditions .....	28
2.1.5.2	Rapamycin biosynthetic pathway .....	29
2.1.6	<i>Actinosynnema pretiosum</i> .....	30
2.1.6.1	Growth strategies .....	30
2.1.6.2	Ansamitocin P-3 biosynthetic pathway .....	31
2.2	Adsorption resins .....	32
2.2.1	Resins: chemical and physical properties .....	32
2.2.1.1	Hydrophobic resins - XAD2/XAD16/XAD1180/SP207 .....	33
2.2.1.2	Hydrophilic resins – HP2MG/XAD761/L285 .....	34

2.2.2	Adsorption resin use in submerged culture fermentations.....	35
2.2.3	Mechanisms of action .....	38
2.2.4	Specific adsorption resin applications .....	39
2.2.4.1	Anguidine resin applications .....	39
2.2.4.2	Rapamycin resin applications.....	40
2.2.4.3	Ansamitocin P3 resin applications.....	40
2.3	Metabolite descriptions and interactions.....	40
2.3.1	Lipophilicity .....	40
2.3.2	Hydrophobic/hydrophilic interactions .....	41
3	Materials and Methods.....	42
3.1	Model system for cultivation studies .....	42
3.1.1	Organism .....	42
3.1.1.1	<i>Fusarium sambucinum</i> .....	42
3.1.1.2	<i>Streptomyces hygroscopicus</i> .....	42
3.1.1.3	<i>Actinosynnema pretiosum</i> .....	42
3.1.2	Cultivation medium .....	42
3.1.2.1	Cultivation medium for <i>F. sambucinum</i> .....	42
3.1.2.2	Cultivation medium for <i>S. hygroscopicus</i> .....	43
3.1.2.3	Cultivation medium for <i>A. pretiosum</i> .....	43
3.1.3	Adsorption resins.....	44
3.2	Protocol for cultivation studies .....	45
3.2.1	<i>F. sambucinum</i> cultivation .....	45
3.2.1	<i>S. hygroscopicus</i> cultivation.....	45
3.2.1	<i>A. pretiosum</i> cultivation .....	46
3.3	Measurements .....	47
3.3.1	Anguidine concentration .....	47
3.3.2	Rapamycin concentration.....	47
3.3.3	Ansamitocin P3 concentration.....	48
3.3.4	Cell mass and broth chemical composition.....	48
3.4	Experimental protocol.....	49
3.4.1	Adsorption of secondary metabolites using adsorption resins in aqueous phase .....	49

3.4.1.1	Experiment 1 .....	50
3.4.1.2	Experiment 2 .....	51
3.4.1.3	Experiment 3 .....	51
3.4.2	Addition of adsorption resin to fermentation system to enhance final concentration of metabolite .....	52
3.4.2.1	Experiment 4 .....	52
3.4.2.2	Experiment 5 .....	53
3.4.2.3	Experiment 6 .....	54
<b>4</b>	<b>Results and Discussion .....</b>	<b>56</b>
4.1	Adsorption of secondary metabolites using adsorption resins in aqueous phase .....	56
4.1.1	Experiment 1 .....	56
4.1.2	Experiment 2 .....	59
4.1.3	Experiment 3 .....	60
4.1.4	Discussion of adsorption data .....	62
4.1.5	Maximum adsorption analysis using dipole moment .....	63
4.2	Addition of adsorption resin to fermentation system to enhance final concentration of metabolite .....	71
4.2.1	Experiment 4 .....	71
4.2.2	Experiment 5 .....	73
4.2.3	Experiment 6 .....	74
4.3	Effect of resins on fermentations .....	76
<b>5</b>	<b>Conclusion .....</b>	<b>78</b>
5.1	Adsorption resin screening .....	78
5.2	Anguidine production .....	78
5.3	Rapamycin production .....	78
5.4	Ansamitocin P3 production .....	79
5.5	Selection of adsorption resin .....	79
<b>6</b>	<b>Appendix .....</b>	<b>80</b>
<b>7</b>	<b>References .....</b>	<b>82</b>

## **Figure**

Figure 1 Growth phases of micro-organisms in submerged culture (Pelczar and Reid, 1972).....	14
Figure 2 18L working volume stirred tank bioreactor.....	16
Figure 3 Proposed biosynthesis of trichothecenes (Blackwell <i>et al.</i> 1985) .	26
Figure 4 Trichothecene biosynthesis from tricodiene (Desjardins <i>et al.</i> 1993)	
.....	27
Figure 5 Conformation of anguidine (Uneo, 1980). ....	28
Figure 6 Structure of rapamycin (Ritacco <i>et al.</i> 2005) .....	29
Figure 7 Structure of ansamitocin P3 (Yu <i>et al.</i> 2002) .....	31
Figure 8 Ansamitocin biosynthesis deduced by Cassady <i>et al.</i> (2004) by addition of labelled precursors.....	31
Figure 9 XAD family chemical structure (Rohm and Haas 2003) .....	34
Figure 10 SP207 chemical structure (Mitsubishi Chemical Corporation, 2001).....	34
Figure 11 HP2MG chemical structure (Mitsubishi Chemical Corporation, 2001).....	35
Figure 12 Adsorption onto hydrophobic resin (Sigma 1996) .....	38
Figure 13 Adsorption of anguidine for various resins as maximum adsorption per mL of resin .....	56
Figure 14 Adsorption resin comparison for adsorbing of anguidine and total surface area per mL of resin .....	57
Figure 15 Adsorption of anguidine compared to pore size .....	58
Figure 16 Adsorption of rapamycin per resin type as maximum adsorption per mL of resin.....	59
Figure 17 Adsorption resin comparison of absorbed rapamycin and surface area .....	60
Figure 18 Adsorption of ansamitocin P3 (AP3) per resin type as maximum adsorption per mL of resin .....	61
Figure 19 Adsorption resin comparison of absorbed ansamitocin P3 and surface area .....	61
Figure 20 Maximum adsorption of three secondary metabolites on resins with differing surface areas .....	62
Figure 21 Maximum adsorption of anguidine based on resin polarity .....	64

Figure 22 Maximum adsorption of rapamycin based on resin polarity .....	65
Figure 23 Maximum adsorption of ansamitocin P3 based on resin polarity	66
Figure 24 Maximum adsorption per unit area for hydrophobic secondary metabolites based on the polarity of adsorption resins .....	67
Figure 25 Log <i>P</i> for anguidine evaluated using 6 computational programs	68
Figure 26 Log <i>P</i> for rapamycin evaluated using 6 computational programs .....	69
Figure 27 Log <i>P</i> for ansamitocin P3 evaluated using 6 computational programs .....	69
Figure 28 Average Log <i>P</i> resin gradient from Table 4.....	70
Figure 29 Average Log <i>P</i> versus lipophilicity intercept (Table 4).....	71
Figure 30 Anguidine production at day five with XAD1180 and SP207 resin addition on day 0, 1, 2, 3 and 4.....	72
Figure 31 Rapamycin production on day 7 based on day of resin added on day 0, 1, 2, 3, 4, 5 and 6 .....	73
Figure 32 Ansamitocin P3 production based on day of resin addition.....	75

## Tables

Table 1 Typical values of oxygen transfer coefficient ( $K_{La}$ ) value ( $\text{h}^{-1}$ ) in various systems .....	17
Table 2 Sample of resin types used in this work .....	33
Table 3 Adsorption resin surface areas and pore sizes .....	50
Table 4 Gradient and intercept for rapamycin, ansamitocin P3 and anguidine versus adsorption resin polarity .....	67
Table 5 Anguidine seed medium (GHY) .....	80
Table 6 Anguidine production medium DMP1 .....	80
Table 7 TJ seed medium .....	80
Table 8 SYLGG production medium.....	80
Table 9 Ansamitocin P3 seed medium.....	81
Table 10 Ansamitocin P3 production medium.....	81

## Equations

Equation 1 Oxygen transfer rate.....	16
Equation 2 LogP equation for the lipophilicity gradient .....	70

Equation 3 LogP equation for the lipophilicity intercept.....71

### **Nomenclature**

$\text{\AA}$	Angstroms
$k_{\text{LA}}$	Overall gas-liquid volumetric mass transfer coefficient ( $\text{h}^{-1}$ )
$\text{LogP}$	Logarithmic ratio of the concentrations of the solute in the solvent
$\pi$	$P_i$