Techno-economics of Industrial Scale β-D-fructofuranosidase and Short-chain Fructooligosaccharides Production

by

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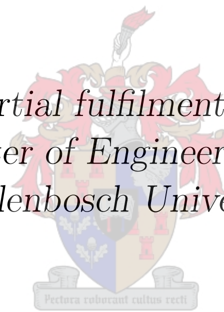

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Abstract

Techno-economics of Industrial Scale β-D-fructofuranosidase and Short-chain Fructooligosaccharides Production

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Enzymes are proteins which act as biological catalysts to enhance the rate of biochemical reactions. They function by reducing the activation energy of the reaction. There is currently no company that manufactures enzymes on an industrial scale in South Africa. A substance known as short-chain fructooligosaccharide (scFOS) is a product that has been growing in popularity on the prebiotics market. ScFOS is produced with an enzyme called β-D-fructofuranosidase (FFase) and sucrose. FFase is a speciality enzyme which is specifically manufactured for scFOS production. FFase can be produced with the choice of two different recombinant yeast-based production systems, where both were compared in this study. Regarding sucrose sugar supply, South Africa's sugar industry has export sugar that would be more beneficial to supply locally because of unattractive international sugar prices, where raw sugar is essentially sold at a loss. This makes for a good opportunity to produce scFOS.

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In this study, an economic model was developed as a tool to determine the economic feasibility of producing FFase and scFOS on a commercial scale locally. The model considered different production scales in order to meet differences in market demand. The facility focused on the production of FFase and scFOS. Scenarios were investigated where FFase is: (i) manufactured within the same plant as scFOS production, (ii) manufactured in a separate facility and bought in by the scFOS production facility, and (iii) manufactured in a toll manufacturing facility and supplied to a scFOS production facility.

When comparing the two production strains to produce FFase, alcohol oxidase (AOX) was found to be more effective than glyceraldehyde 3-phosphate dehydrogenase (GAP). The simulation results incorporating Monte Carlo analysis revealed that FFase should be sold as a product within a multi-product enzyme facility and not as a facility on its own because the demand for this enzyme alone is too low and will therefore not provide enough sales to sustain the business. To achieve the desired internal rate of return on investment (minimum of 30%) scFOS could be produced on an appreciable scale in South Africa at a minimum of 2 000 tonnes per annum irrespective of the scenario. For an scFOS production facility to be successful, it is recommended that FFase and scFOS are produced in the same facility.

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Uittreksel

Tegno-ekonomie van Industriële Skaal β-D-fruktofuranosidase en Kort-ketting Fruktooligosakkariede Produksie

("Techno-economics of Industrial Scale β-D-fructofuranosidase and Short-chain Fructooligosaccharides Production")

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Ensieme is proteïene wat as biologiese katalisators optree, wat die tempo van biochemiese reaksies bevorder. Die grondslag van die ensiem se funksie is om die aktiveringsenergie van die reaksies te verlaag. Tans is daar geen maatskappy in Suid Afrika wat ensieme op industriële skaal vervaardig nie. Kort-ketting frukto-oligosakkariede (kkFOS) is 'n verbinding waarin die prebiotiese mark toenemende belangstelling toon. Die kkFOS kan vanaf sukrose geproduseer word in 'n reaksie wat deur die ensiem β-D-fruktofuranosidase (FFase) gekataliseer word. FFase is 'n spesialiteit ensiem wat pertinent vir die produksie van kkFOS vervaardig word. Dié ensiem kan deur twee rekombinante gis-gebaseerde produksiesisteme geproduseer word, en beide sisteme is in hierdie studie ondersoek. Met verwysing na die sukrose suiker aanbod, Suid Afrika het groot volumes suiker wat vir die uitvoermark bestem is, maar weens hoë internasionale suikerpryse word die suiker effektief teen 'n verlies

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verkoop. Plaaslike produksie van kkFOS mag dus meer aantreklike geleenthede bied wat tot voordeel van die plaaslike ekonomie aanwend kan word. 'n Ekonomiese model is in hierdie studie ontwikkel wat as instrument kon dien om die ekonomiese haalbaarheid van plaaslike FFase en kkFOS produksie op kommersiële skaal vas te stel. Die gesimuleerde fasiliteit kom beide FFase en kkFOS produseer. Verder het die model verskillende skale van produksie in ag geneem, en sodoende kon die effek van verskille in die aanvraag om die produk gesimuleer word. Die volgende scenario's vir FFase produksie is ondersoek: (i) produksie in dieselfde aanleg as vir kkFOS produksie, (ii) ensiem word vanaf 'n onafhanklike vervaardiger aangekoop wat dan in 'n ander aanleg vir kkFOS vervaardiging gebruik word, en (iii) die ensiem word deur 'n tol-vervaardigingsaanleg aan die kkFOS aanleg verskaf. 'n Vergelyking tussen twee produksiesisteme het getoon dat die alkohol-oksidase (AO) sisteem meer doeltreffend vir FFase produksie aangewend kon word as 'n sisteem wat op gliseraldehied 3-fosfaat dehidrogenase (GAP) gebaseer was. Die resultaat van die simulasies, met Monte Carlo analises geïnkorporeer, het daarop gedui dat FFase in 'n meervoudige produk aanleg vervaardig moet word, en nie in 'n aanleg wat slegs op FFase produksie gebaseer is nie. In so 'n enkel-produk aanleg is die vraag na die ensiem nie groot genoeg om die besigheid volhoubaar te maak nie, aangesien die mark nie die nodige verkope sal kan ondersteun nie. Om die teiken interne opbrengskoers van minstens 30% te bereik, moet kkFOS op 'n aanvaarbare skaal van ten minste 2 000 ton per jaar vervaardig word, ongeag van die scenario waarin dit geproduseer word. Vir die suksesvolle produksie van kkFOS word dit aanbeveel dat FFase and kkFOS in dieselfde aanleg geproduseer word.

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Contents

Declaration i

Acknowledgements ii

Abstract iii

Uittreksel v

Contents vii

List of Figures xiii

List of Tables xix

Nomenclature xxiv

Glossary xxvii

1 Introduction and Theoretical Framework 1

1.1 Industrial Enzymes and ScFOS Background 1

1.1.1 Overview of Industrial Enzymes and ScFOS 1

1.1.2 Production History 2

1.1.3 Recent Developments 4

1.1.4 Industrial Importance 4

1.2 Research Proposal 5

1.2.1 Aim 5

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1.2.2 Objectives 5
1.2.3 Deliverables 6
1.2.4 Research Significance 7
1.2.5 Thesis Plan 7

2 Literature Review **9**

2.1 Overview 9
2.2 Previous Work 12
2.2.1 Enzymes 12
2.2.1.1 Enzyme Preparation 12
2.2.1.2 Advantages of Enzymes 12
2.2.1.3 General Enzyme Production 14
2.2.2 *Pichia Pastoris* 15
2.2.2.1 The Gene Expression Process 15
2.2.2.2 Promoters 17
2.2.3 FFase and scFOS Production 21
2.2.3.1 Factors Affecting scFOS Production 26
2.2.4 Literature Process Design 29
2.2.4.1 ScFOS Reactor Setup 33
2.2.4.2 scFOS Purification 35
2.2.4.3 scFOS Product Preparation 38
2.2.5 Process Hazards 40
2.2.6 Process Costing 41
2.2.6.1 Chemical Engineering Plant Cost Index 41
2.2.6.2 Costing Model 43
2.2.6.3 Engineering Economic Analysis 46
2.2.7 Economies of Scale 49
2.2.7.1 South African Sugar Production 49
2.2.7.2 Six tenths Rule 50
2.2.8 ScFOS Markets 53

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2.2.9 β-D-fructofuranosidase Market 57
2.2.9.1 FFase Markets and Freedom-to-Operate 57
2.2.9.2 China Market Research Center 58
2.2.10 Environmental Impact 63
2.3 Common Recommendations and Problems 64

3 Hypothesis and Key Questions 66

4 Design Approach 68
4.1 β-D-fructofuranosidase and ScFOS Process Outline 68
4.2 Process Description 71
4.2.1 β-D-fructofuranosidase Production Process 71
4.2.1.1 Process Overview 71
4.2.1.2 Equipment Selection 72
4.2.2 ScFOS Production Process 74
4.2.2.1 Process Overview 74
4.2.2.2 Equipment Selection 75
4.3 Process Designs 79
4.3.1 β-D-fructofuranosidase Production Process 79
4.3.2 ScFOS Production Process 82
4.4 Process Scheduling 84

5 Mass and Energy Balance 86
5.1 Mass Balance 86
5.1.1 FFase Production Process 86
5.1.1.1 Laboratory Operations 86
5.1.1.2 Reactor Setup (using GAP Production Strain) . 88
5.1.1.3 Reactor Setup (using AOX Production Strain) . 90
5.1.1.4 Downstream Operations 91
5.1.2 ScFOS Production Process Description 93
5.1.2.1 Reactor Feed 93

Stellenbosch University http://scholar.sun.ac.za

5.1.2.2 Reactor Set-up 93
5.1.2.3 scFOS Purification 94
5.1.2.4 scFOS Product Preparation 96
5.2 Energy Balance 99
5.2.1 Enzyme Production Process 99
5.2.1.1 Sterilisation 99
5.2.1.2 Aeration 100
5.2.1.3 Temperature Control 101
5.2.1.4 Centrifugation 102
5.2.1.5 Refrigeration 103
5.2.1.6 Total Energy Consumption 104
5.2.2 ScFOS Production Process 105

6 Process Economics 116
6.1 Economies of Scale 116
6.2 Process Costing 117
6.2.1 Assumptions 117
6.2.2 Scenarios 118
6.2.3 RiskSim 120
6.2.4 Costing Worksheet 122
6.2.5 FFase Production 126
6.2.5.1 Scenario 1 - FFase production using GAP and AOX production strain 126
6.2.6 scFOS Production 132
6.2.6.1 Scenario 2 - ScFOS production, self producing FFase, comparing GAP and AOX production strains 132
6.2.6.2 Scenario 3 - ScFOS production, buying locally produced FFase, comparing GAP and AOX production strains 134

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6.2.6.3 Scenario 4 - ScFOS production, buying FFase from a toll manufacturer, comparing GAP and AOX production strains 136
6.2.6.4 Scenario 5 - ScFOS production, buying locally produced FFase at an export price, comparing GAP and AOX production strains 137
6.2.6.5 Economic Summary 139

7 Results and Discussion 141
7.1 Scenario Comparisons 141
7.1.1 FFase Production 141
7.1.1.1 Scenario 1 - FFase production using GAP and AOX production strains 141
7.1.2 ScFOS Production 143
7.1.2.1 Scenario 2 - scFOS production at different production levels using FFase that is produced in the same facility as the scFOS, comparing GAP and AOX production strains 143
7.1.2.2 Scenario 3 - scFOS production at different production levels using FFase that is bought from a local producer comparing GAP and AOX production strains 146
7.1.2.3 Scenario 4 - scFOS production at different production levels using FFase that is bought from an external toll manufacturer, comparing GAP and AOX production strains 147
7.1.2.4 Scenario 5 - scFOS production at different production levels using FFase that is bought from a local producer at an export price, comparing GAP and AOX production strains 148

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7.1.2.5 Scenarios Summary 149

8 Conclusions 157

9 Recommendations 160

Appendices 162

A Physical Data 163

B Energy Data 164

C Steam Tables 165

D Literature Data 166

E Sample Calculations 170

E.1 Methanol Carry-over 170

E.2 Spray drying air 171

F Stream Tables 172

F.1 FFase Production Process Material Stream Tables 172

F.2 ScFOS Production Process Material Stream Tables 178

G Cash Flow Tables 184

H Fan Curves 190

List of References 192

Stellenbosch University http://scholar.sun.ac.za

List of Figures

1.1 Project plan for the research project showing deliverables and milestones to be completed for the project. 8

2.1 Enzyme production process illustration (Redrawn) (EuropeanProject(FP6), 2011). 14

2.2 Gene expression to produce a protein (Redrawn) (News-Medical, 2012). 16

2.3 Illustration of the polysaccharides that make up scFOS (Redrawn) (Shenzhen Victory Biology Engineering Co., 2011). 21

2.4 The effect of reaction temperature on the formation of scFOS (Muramatsu *et al.*, 1994). 27

2.5 The effect of initial sucrose concentration on the formation of scFOS (Muramatsu *et al.*, 1994). 28

2.6 Simplified FTase and FOS production process design adopted from the design of Vaňková *et al.* (2008). 30

2.7 Complete FTase production process design (Vaňková *et al.*, 2008). . 32

2.8 A schematic diagram of the reactor choice for the scFOS production process. The choice of the reactor depends on how the enzyme will be used in the reactor (immobilised or not). 33

2.9 A schematic diagram of a true moving bed chromatography column (redrawn) (Bailly and Nicoud, 1993). 36

2.10 A schematic diagram of a simulated moving bed chromatography column (redrawn) (Bailly and Nicoud, 1993). 37

Stellenbosch University http://scholar.sun.ac.za

2.11 The chemical engineering plant cost index. Figures from 1991-2006 obtained from Turton *et al.* (2008), figures from 2007-2010 obtained from Scribd (2012) and the figure from 2011 was obtained from Cheresources (2012). 41
2.12 Different shipping routes around the globe (Redrawn). South Africa has access to Europe along both the east and west coastlines of the continent of Africa (Kumar, 2012). 45
2.13 A cumulative cash flow diagram when evaluating a new project (Redrawn) (Turton *et al.*, 2008). 47
2.14 South Africa's Sugar Production (SASA, 2012) 50
2.15 The representation of the effect the six tenths rule has on the cost of equipment as it increases in size. 52
2.16 The cost per unit decreases as the output per time period increases illustrating economies of scale (Fourie *et al.*, 2008). 52
2.17 Japan's oligosaccharide market demand based on a study done in 2002 (Nakakuki, 2002). 54
2.18 China's β-D-fructofuranosidase Market Demand Statistics from 2008 to 2011 (CMRC, 2012). 60
2.19 Import volume of China's β-D-fructofuranosidase products from 2008 to 2011 (CMRC, 2012). 61
2.20 Export volume of China's β-D-fructofuranosidase products from 2008 to 2011 (CMRC, 2012). 61
2.21 China's β-D-fructofuranosidase demand forecasting from 2012 to 2015 (CMRC, 2012). 63
2.22 Enzyme production fitting into to an ecological cycle (Redrawn) (Falch, 1991). 64

4.1 Generic mind map of the process when designing and performing a costing analysis of the implementation of a production facility. 69

Stellenbosch University http://scholar.sun.ac.za

4.2 Enzyme block flow diagram illustrating the concept behind the design of the process. 72
4.3 ScFOS block flow diagram illustrating the concept behind the design of the process. 75
4.4 Schematic diagram of the setup and operation of a spray dryer (Redrawn) (Patel *et al.*, 2009). 78
4.5 Production process of β-D-fructofuranosidase. 81
4.6 ScFOS production process 83
4.7 Production schedule of the multi-enzyme production facility. FFase production schedule is to satisfy the needs of a continuous production process of scFOS. 85

5.1 Process flow diagram showing the heat flows within the scFOS production process. The light grey arrows indicate where heat/energy is put into the system and the dark grey arrows indicate where heat/energy is lost to the environment. 107
5.2 Heat capacity model to determine the heat capacity of complex organic compunds such as short chain fructooligosaccharides. The model fits well showing an R squared value of 0.9986. 108

6.1 FFase and scFOS project mind map showing the different scenarios that are proposed within the project. Two sets of results are used; the best from literature as well as the best results obtained from the experiments performed by the scientists within the scFOS project research group. 119
6.2 A cumulative chart and histogram of a simulation run to 3 000 iterations. The results of the simulation have the following results: mean = 0.998, st. dev. = 0.116, mean st. error = 0.002, minimum = 0.800, first quartile = 0.898, median = 0.998, third quartile = 1.097, maximum = 1.200, skewness = 0.0183. 120

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6.3 The expenses of an enzyme production facility producing FFase with GAP production strain (TPDC = total plant direct cost, TPIC = total plant indirect cost, DFC = direct fixed capital, OPEX = operating expenditure). 131

6.4 The expenses of an enzyme production facility producing FFase with AOX production strain. 132

6.5 The expenses of an FFase production facility comparing the GAP and AOX production strains, corresponding to the scFOS production scale that the enzyme production facility would supply to. 132

6.6 The expenses of an FFase production facility comparing the GAP and AOX production strains, corresponding to the scFOS production scale that the enzyme production facility would supply to. 133

6.7 The expenses of an FFase production facility comparing the GAP and AOX production strains, corresponding to the scFOS production scale that the enzyme production facility would supply to. 134

6.8 The expenses of an FFase production facility comparing the GAP and AOX production strains, corresponding to the scFOS production scale that the enzyme production facility would supply to. 135

6.9 The expenses of an FFase production facility comparing the GAP and AOX production strains, corresponding to the scFOS production scale that the enzyme production facility would supply to. 135

6.10 The expenses of an FFase production facility comparing the GAP and AOX production strains, corresponding to the scFOS production scale that the enzyme production facility would supply to. 136

6.11 The expenses of an FFase production facility comparing the GAP and AOX production strains, corresponding to the scFOS production scale that the enzyme production facility would supply to. 137

Stellenbosch University http://scholar.sun.ac.za

6.12 The expenses of an FFase production facility comparing the GAP and AOX production strains, corresponding to the scFOS production scale that the enzyme production facility would supply to. . . . 138

6.13 The expenses of an FFase production facility comparing the GAP and AOX production strains, corresponding to the scFOS production scale that the enzyme production facility would supply to. . . . 138

7.1 Enzyme selling price of pure protein in order for the facility to obtain a 30% IRR. 142

7.2 Enzyme selling price per fermentation broth in order for the facility to obtain a 30% IRR. 143

7.3 ScFOS production using FFase that has been produced in the same facility as the scFOS, comparing the GAP and AOX strains. 144

7.4 ScFOS production using FFase fermentation broth that has been bought from a local FFase producer, comparing the GAP and AOX strains. 146

7.5 ScFOS production using FFase that has been bought an enzyme toll manufacturer, comparing the GAP and AOX strains. 148

7.6 ScFOS production using FFase that has been bought from a local FFase producer offering the FFase at a competitive export price. The production strains of GAP and AOX are compared. The GAP is selling at R847/kg of fermentation broth and AOX is selling at R596/kg of fermentation broth. 149

7.7 ScFOS production that compares all the different options of obtaining the GAP produced FFase enzyme, namely: through self-production, buying from a local producer, buying from a toll supplier, and buying from a local producer that can supply at a low export price. 150

Stellenbosch University http://scholar.sun.ac.za

7.8 ScFOS production that compares all the different options of obtaining the GAP prodcued FFase enzyme, namely: through self-production, buying from a local producer, buying from a toll supplier, and buying from a local producer that can supply at a low export price. 151

7.9 ScFOS production that compares all the different options of obtaining the AOX prodcued FFase enzyme, namely: through self-production, buying from a local producer, buying from a toll supplier, and buying from a local producer that can supply at a low export price. 152

7.10 ScFOS production that compares all the different options of obtaining the AOX produced FFase enzyme, namely: through self-production, buying from a local producer, buying from a toll supplier, and buying from a local producer that can supply at a low export price. 153

7.11 IRR distribution from Monte Carlo analysis for scFOS production scale (tpa) for the scenario of self-producing FFase using the AOX production strain. 154

7.12 S-curves which represent the cumulative probabilities of IRR for scFOS production (tpa) for the scenario where FFase is self-produced in the same facility as the scFOS production using the AOX production strain. 155

7.13 The influence of scFOS selling price and scFOS production scale on IRR for and scFOS production plant that produces only enough FFase (using AOX production strain) for itself. 156

H.1 Industrial axial fan curves used to calculate the duty of a fan (Ciclo Software, 2013). 191

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List of Tables

2.1 Large scale enzyme applications (Leisola *et al.*, 2001) 13
2.2 Various promoters that can be used for the *P. pastoris* gene expression system 17
2.3 Components involved in the scFOS production 22
2.4 Costing method adopted from Petrides (2000) used to calculate the fixed capital cost estimation for the enzyme and scFOS production costs 43
2.5 Costing method adopted from Choi and Lee (1997) used to calculate capital and operating costs for the enzyme and scFOS production costs 44
2.6 Discounted Cash Flow Table (Turton *et al.*, 2008) 48
2.7 Oligosaccharides production in Japan (Taniguchi, 2004) 56

5.1 Pre-inoculum used for the production of FFase 87
5.2 Second stage inoculum used for the production of FFase 88
5.3 Industrial medium components used for the production of FFase using the GAP production strain 89
5.4 Industrial medium components used for the production of FFase using the AOX production strain 90
5.5 FFase Production stream table (to supply for 2 000 tpa of scFOS) . 92
5.6 Reactor product (FOS in syrup form before purification) 94
5.7 SMB column product (FOS in syrup form before product preparation) 95

Stellenbosch University http://scholar.sun.ac.za

5.8 SMB column raffinate (FOS in syrup form before product preparation) . . . 95
5.9 Dry scFOS product for selling . . . 96
5.10 Dry by-product for selling . . . 97
5.11 ScFOS Production stream table at 2 000 tpa of scFOS) . . . 98
5.12 Energy used for the high pressure steam sterilisation of the fermenter in the FFase production process, producing 14.43 tonnes per annum using the AOX production strain . . . 100
5.13 Energy used for aeration in the fermenter for the FFase production process producing 14.43 tonnes per annum using the AOX production strain . . . 101
5.14 Energy used for the agitation in the fermenter for the FFase production process producing 14.43 tonnes per annum using the AOX production strain . . . 101
5.15 Energy used for the temperature control in the fermenter for the FFase production process producing 14.43 tonnes per annum using the AOX production strain . . . 102
5.16 Energy used for the centrifugation of the fermentation broth for the FFase production process producing 14.43 tonnes per annum using the AOX production strain . . . 103
5.17 Energy used for the refrigeration of the fermentation broth for the FFase production process producing 14.43 tonnes per annum using the AOX production strain . . . 104
5.18 Total energy used for the FFase production process producing enough enzyme to supply for a scFOS production facility of 2 000 tpa (producing 14.43 tonnes per annum using the AOX production strain) . 105
5.19 Total energy needed for the FFase production process (using the AOX production strain), comparing all production scales . . . 106
5.20 Energy input to reactor R-102 in figure 4.6 . . . 109

Stellenbosch University http://scholar.sun.ac.za

5.21 Energy input to SMB chromatography column R-103 in figure 4.6 . 109
5.22 Energy input to SMB chromatography column R-103 in figure 4.6 . 110
5.23 Energy input for hot dry air to spray dryer F-104 in figure 4.6 . . . 110
5.24 Energy input for the extraction fan in the spray dryer F-104 in figure 4.6 111
5.25 Energy input for heating scFOS in spray dryer F-104 in figure 4.6 . 111
5.26 Energy input for the evaporator to treat the by-product, E-101 in figure 4.6 112
5.27 Energy input for the evaporator to treat the by-product, E-101 in figure 4.6 113
5.28 Total energy needed for the scFOS production process, focusing on 2 000 tpa (figure 4.6) 113
5.29 Summary of the total energy needed for the scFOS production process, comparing all production scales 115

6.1 Costing worksheet used to calculate capital and operating costs for the enzyme and scFOS production (2 000 tpa using the AOX production strain) 124
6.2 Costing worksheet continued... 125
6.3 Enzyme production direct fixed capital using the GAP production strain 127
6.4 Enzyme production direct fixed capital using the AOX production strain 128
6.5 Enzyme production operating costs for the various production levels using the GAP production strain 129
6.6 Enzyme production operating costs for the various production levels using the AOX production strain 130
6.7 Economic summary of scFOS production using the AOX gene at 2 000 tonnes per annum, comparing different scenarios 139

Stellenbosch University http://scholar.sun.ac.za

6.8 Economic summary of scFOS production using the AOX gene at 2 000 tonnes per annum, comparing different scenarios 140

A.1 Densities of Components in FFase Production 163

B.1 Heat capacity data 164

C.1 Steam Table (Spirax-Sarco, 2012) 165

D.1 Characteristics of equipment and operations used and compositions of outlet streams (Vaňková *et al.*, 2008) 167
D.2 List of equipment used for the production of immobilised FTase (Vaňková *et al.*, 2008) 167
D.3 List of raw materials for the production of immobilised FTase (Vaňková *et al.*, 2008) 168
D.4 List of equipment used for the production of FOS's (Vaňková *et al.*, 2008) 168
D.5 Main categories of operation costs for production of FOS's from food sucrose (Vaňková *et al.*, 2008) 169
D.6 Summary of costs for the production of FOS's (Vaňková *et al.*, 2008) 169

F.1 FFase Production (AOX) stream table for 3.60 tpa of fermentation broth (to supply for 500 tpa of scFOS) 173
F.2 FFase Production (AOX) stream table for 7.22 tpa of fermentation broth (to supply for 1 000 tpa of scFOS) 174
F.3 FFase Production stream table for 14.43 tpa of fermentation broth (to supply for 2 000 tpa of scFOS) 175
F.4 FFase Production (AOX) stream table for 36.09 tpa of fermentation broth (to supply for 5 000 tpa of scFOS) 176
F.5 FFase Production (AOX) stream table for 71.88 tpa of fermentation broth (to supply for 10 000 tpa of scFOS) 177
F.6 ScFOS Production stream table at 500 tpa of scFOS) 179

Stellenbosch University http://scholar.sun.ac.za

F.7 ScFOS Production stream table at 1 000 tpa of scFOS) 180
F.8 ScFOS Production stream table at 2 000 tpa of scFOS) 181
F.9 ScFOS Production stream table at 5 000 tpa of scFOS) 182
F.10 ScFOS Production stream table at 10 000 tpa of scFOS) 183

G.1 Discounted Cash Flow Table for scFOS Production of 500 tonnes per annum, self producing FFase using the AOX production strain(R in millions) 185
G.2 Discounted Cash Flow Table for scFOS Production of 1 000 tonnes per annum, self producing FFase using the AOX production strain(R in millions) 186
G.3 Discounted Cash Flow Table for scFOS Production of 2 000 tonnes per annum, self producing FFase using the AOX production strain(R in millions) 187
G.4 Discounted Cash Flow Table for scFOS Production of 5 000 tonnes per annum, self producing FFase using the AOX production strain(R in millions) 188
G.5 Discounted Cash Flow Table for scFOS Production of 10 000 tonnes per annum, self producing FFase using the AOX production strain(R in millions) 189

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Nomenclature

CAGR	Compound annual growth rate
CAPEX	Capital expenditure
CEPCI	Chemical engineering plant cost index
CSIR	The Council for Scientific and Industrial Research
CSTR	Continuously stirred tank reactor
DFC	Direct fixed capital
DNA	Deoxyribonucleic acid
FFase	β-D-fructofuranosidase
F	Fructose
FTase	Fructosyltransferase
FOS	Fructooligosaccharide
FOSHU	Foods for specified health uses
G	Glucose

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GF	Sucrose
GF_2	Kestose
GF_3	Nystose
GF_4	1-F-fructofuranosyl nystose
GOS	Galacto-oligosaccharide
GRAS	Generally recognised as safe
HTST	High temperature short time
IMO	Isomalto-oligosaccharide
IRR	Internal rate of return
MO	Malto-oligosaccharide
MOS	Mannan-oligosaccharide
NPV	Net present value
OC	Other costs
OPEX	Operating expenditure
PBR	Packed bed reactor
PDE	Potential daily exposure

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PPM Parts per million

RNA Ribonucleic acid

ScFOS Short-chain fructooligosaccharide

TEPC Total equipment purchase cost

TLPC Total land purchase cost

TPDC Total plant direct cost

TPIC Total plant indirect cost

XOS Xylo-oligosaccharide

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Glossary

Expression	Description
1-F-fructofuranosyl nystose	a carbohydrate sugar consisting of 4 parts fructose and 1 part glucose
β-D-fructofuranosidase	an enzyme with hydrolase and transferase activity
Antigenic	a substance that promotes the production of an antibody when introduced into the body (TheFreeDictionary, 2012)
Chromosome	a strand of DNA that contains hereditary information that is essential for the life of a cell (TheFreeDictionary, 2012)
Cytotoxic	substances that are toxic to cells (TrueKnowledge, 2012)
Eukaryote	a domain above the kingdom that includes organisms that have one or more cells containing visible nuclei and organelles (Merriam-Webster, 2012)
Exon	a sequence of DNA that codes information for the synthesis of protein that is transcribed to the messenger RNA (TheFreeDictionary, 2012)
Fructohydrolase	an alternative name for β-D-fructofuranosidase
Fructooligosaccharide	a polysaccharide comprised of one glucose molecule and and range between two and four fructose molecules
Fructose	a monosaccharide sugar
Fructosidase	an alternative name for β-D-fructofuranosidase
Fructosyltransferase	a variant enzyme of β-D-fructofuranosidase
Gene	a segment of DNA coding for an RNA molecule's sequence (Bailey and Ollis, 1986)
Glucose	a monosaccharide sugar
Heterologous	something that is immunologically related but not identical (TheFreeDictionary, 2012)
Hydrolysis	a chemical reaction where water reacts with a compound to produce other compounds; it involves the cleaving of a bond and the adding of the hydrogen cation and the hydroxide anion from the water (TheFreeDictionary, 2012)

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Expression	**Description**
Intron	a section of a gene that exists between exons that is removed before translation of messenger RNA and does not function in coding for protein synthesis (TheFreeDictionary, 2012)
Inulase	a β-D-fructofuranosidase variant (Grootwassink and Fleming, 1980)
Invertase	an alternative name for β-D-fructofuranosidase (Swiss Institute of Bioinformatics, 1998)
Kestose	a carbohydrate sugar consisting of 2 parts fructose and 1 part glucose
Methylotrophic	an organism that is able to use methanol as the sole carbon and energy source (Faber *et al.*, 1995)
Nucleoside	a glycoside formed by partial hydrolysis of a nucleic acid (WordNet, 2012)
Nucleotide	phosphoric ester of a nucleoside; the basic structural unit of nucleic acids (DNA or RNA) (WordNet, 2012)
Nystose	a carbohydrate sugar consisting of 3 parts fructose and 1 part glucose
Periplasm	the area in a gram-negative bacterium between the plasma membrane and a surrounding membrane that contains mostly enzymes and a thin layer of peptidoglycan (Merriam-Webster, 2012)
Peroxisome	a membrane-enclosed organelle that contains enzymes that are involved in a different variety of metabolic reactions, which include several aspects of energy metabolism (Cooper, 2000)
Plasmid	an additional small piece of DNA which is found in many bacteria and eukaryotes (Bailey and Ollis, 1986)
Promoter	a promoter is a region in DNA that facilitates the transcription of a particular gene under particular conditions
Proteolytic	An enzyme that stimulates proteolysis, which is the splitting of proteins by hydrolysis (Biology-Online, 2005)
Recombinant	a series of procedures that are used to recombine DNA segments. A recombinant DNA molecule is constructed from the segments of two or more different DNA molecules. Under certain conditions, a recombinant DNA molecule can enter a cell and replicate there, either on its own or after it has been integrated into a chromosome (MedicineNet, 1996-2012)
Ribosome	biochemical reaction sites for protein synthesis (Bailey and Ollis, 1986)
Splicing	the process of preparing recombinant DNA (Merriam-Webster, 2012)
Sucrase	an alternative name for β-D-fructofuranosidase
Sucrose	a di-saccharide sugar consisting of one fructose molecule and one glucose molecule
Transcription	making an RNA (ribonucleic acid) copy from a sequence of DNA (deoxyribonucleic acid) (a gene) (MedicineNet, 1996-2012)
Transfructolysation	the action that the enzyme of β-D-fructofuranosidase takes on sucrose
Translation	the step in gene expression where the information contained in the nucleotide sequence of a messenger RNA molecule is used to direct the synthesis of a peptide chain with a corresponding amino acid sequence (Bailey and Ollis, 1986)

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Chapter 1

Introduction and Theoretical Framework

1.1 Industrial Enzymes and ScFOS Background

1.1.1 Overview of Industrial Enzymes and ScFOS

Enzymes are important and useful components in biochemical processing. The challenge is to produce the enzymes to be able to meet the demands of large scale productions, which is why there is a desire to implement an industrial enzyme production facility. The present situation in South Africa is that enzymes that are required for industrial use are imported. The desire is to develop the local biotechnology industry, where the ideal situation would be to have an industrial enzyme production facility within the country so that the enzymes would be available locally and thus hopefully become a cheaper alternative to importing. The enzyme production facility would also help with the country's economy as the facility would create more jobs as labour would become necessary.

There has been a fair amount of work done in industrial enzyme production. This is no surprise as people have been working with industrial enzymes since the time of man. The main point is that man did not realise they were actually using the enzymes. There are many countries in the world that produce enzymes on an industrial scale for the various industries that demand these cer-

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tain enzymes. South Africa is a country that does not have an existing facility and therefore has an interest to see whether one should be implemented.

Enzymes are used in the manufacturing processes of many different products. These include the medical industry where a lot of vitamins and various other pharmaceuticals are produced, the food and beverage sector, where enzyme perform a pivotal role in the manufacturing of beer and wine. Enzymes are even used in the most unassuming applications such as the production of detergents and the stone washing of jeans, which shows the versatility of enzymes and shows a good reason why it would be beneficial to have a facility available.

There has been a growing interest in recent times over a product which is know as scFOS, which stands for short-chain fructooligosaccharide. This product has become popular in east Asia and there is also a keen interest in the United States of America and Europe (Charalampopoulos and Rastall, 2009). There is also an interest with scFOS in South Africa (Personal communication: Tongaat Hulett Ltd., Tongaat, KwaZulu-Natal, South Africa). This product is of attention because of its health benefits. The method to produce this product is by taking raw sucrose and putting it into contact with an enzyme by the name of β-D-fructofuranosidase. To be able to produce scFOS on a commercial scale, the enzyme would also have to be available on a large scale to meet the need of scFOS production.

1.1.2 Production History

ScFOS was first produced in 1984 by Meiji Seika Kaisha Ltd. using the organism of *Aspergillus niger* (Vaňková *et al.*, 2008). It was marketed in Japan using the brand name, 'Meioligo'. Meiji Seika Kaisha Ltd. later joined up with a French company, Beghin-Say. Together the FOS's were produced using the trademark name, 'Actilight' (Yun, 1996). This was followed by its successful production in an industrial scale by a South Korean company. Cheil Foods Chemical Co., using an immobilised of *Aureobasidium pullulans* (Mad-

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lová *et al.*, 2000).

Initially scFOS had a low amount of fibre in the product but the fibre content has gone up to a minimum of 95% (Cho and Terry Finocchiaro, 2009). Presently, scFOS is marketed under three different brand names: NutraFlora® in North America, South America and Australia; Actilight® in Europe; and Meioligo in Asia (Cho and Terry Finocchiaro, 2009). ScFOS has been approved in Japan as a food ingredient since 1980 and it also has FOSHU (Foods for Specified Health Uses) status. ScFOS has been recognised in Europe as a proper food ingredient since 1991 and was commercially available in the United States in 1988 and has the GRAS (generally recognised as safe) status (Cho and Terry Finocchiaro, 2009).

There have been further studies where scFOS is produced using fructosyl transferase (FTase), where the enzyme would perform the same function, but it would be manufactured in a different manner. There are many different types of organisms that are available which include different fungi and yeasts. The goal is to try and find an organism that will allow one to produce the most amount of enzyme at the lowest costs.

β-D-fructofuranosidase (FFase) is an enzyme that has many variants with similar functions. The difference is that they are produced in a different manner from each other. FFase is an enzyme that has been produced in countries where the scFOS market is already well developed such as countries in east Asia. Grootwassink and Fleming (1980) have shown to produce FFase from *Kluyveromyces fragilis*. There is a demand in South Africa for scFOS of approximately 200 tonnes (Personal communication: Tongaat Hulett Ltd., Tongaat, KwaZulu-Natal, South Africa) and through this interest, this has resulted in the desire for an industrial scale production of FFase and scFOS.

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1.1.3 Recent Developments

Since 2010, the University of Stellenbosch and Rhodes University began a joint project in the attempt to produce scFOS. Stellenbosch University performed research with the production of the enzyme, β-D-fructofuranosidase, which was used in the scFOS production process. Rhodes University took the enzyme that Stellenbosch produced and used it in their experiments to optimise the yield of scFOS. Later, more scFOS experiments were performed at Stellenbosch to find the best method and determine specific enzyme dosages for scFOS production.

When speaking about 'optimising' the yield of scFOS, the goal was to produce an scFOS that had the same composition as Actilight®, which is currently used as the benchmark by other scFOS producers.

1.1.4 Industrial Importance

If one wants to be successful in producing and selling a product such as scFOS; one needs to be able to market the product and be able to sell enough quantities so the project can be viable. The scale should be high enough to ensure that the investment is worthwhile.

The global and local markets were looked at to observe how large the production scale should be. Sugar, which is the raw ingredient for scFOS production in the country of South Africa, is a resource that is of abundance which is good news for scFOS production in South Africa. The other industrial issue is to be able to produce β-D-fructofuranosidase at a level that meets the demand for the scFOS production process.

Stellenbosch University has performed lab scale experiments but the challenge is to produce the enzyme commercially so they are available for sales in large quantities.

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1.2 Research Proposal

1.2.1 Aim

This project is aimed at the country of South Africa where enzymes are presently not produced but rather imported. The project can also be implemented in other countries in a similar situation. The project will aim initially to meet the local market demand of scFOS and hence the required FFase needed for the production of scFOS. The proposition of the implementation of a multi-enzyme production facility will be investigated to see if it will be viable. If this is viable, the process could be scaled up to supply on a national and international level, benchmarking against the product pioneers Béghin-Meiji who manufacture Actilight®.

1.2.2 Objectives

The project has certain objectives that it needs to achieve in order for it to be valuable research so that it may be used for further studies to come and also for a possibility for an implementation of the project in the country of South Africa. The research project has the following objectives that are set out to be achieved:

- The project will investigate if there is potential for the application of a feasible industrial enzyme production facility in the country of South Africa.

- The comparison of different scenarios were made. Scenarios were looked at where FFase was: i) produced in a facility to be sold to scFOS production process around the world, ii) manufactured within the same plant as, and used solely for that scFOS production process, iii) manufactured in a separate facility and bought in by the scFOS production facility, iv and manufactured in a toll manufacturing facility and supplied to a scFOS production facility.

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- An economic model will be built using the appropriate software, that will determine whether the concept of building an industrial enzyme production facility in South Africa will be a viable option or not. More specifically, the minimum scale of scFOS production required to achieve an IRR of at least 30%.

1.2.3 Deliverables

In order to ensure that the objectives of the project are met, deliverables are essential to monitor the progress of the research so that the project may stay on track and be completed within the intended time. The following deliverables are proposed for the project:

- Develop a standard design for the production of the enzymes as well as the production process of scFOS using Microsoft Office Visio®.
- Perform a mass and energy balance (using Microsoft Office Excel®) on the proposed design, to quantify the materials used and produced and to also determine the units operations that are to be used and the energy used within the process.
- Perform a costing analysis of the production plant to determine the viability of producing FFase and scFOS in South Africa.
- Use the software of RiskSim simulation program which is a Microsoft Office Excel® add-in to analyse the certain risks that are involved with the construction and running of an enzyme production plant. A full Monte Carlo simulation will be performed.
- Use Matlab®, mathematical computing software to plot surface plots from the RiskSim simulation results.

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1.2.4 Research Significance

With the new age of sustainable development, chemical process industries are looking for new and innovative ways to manufacture products, with a focus on 'greener' methods to produce products. The biotechnology world has now become a path that has received growing attention as it is an option where processes can substitute their previously inorganic chemical processes with organic ones using biological components in order to reduce any possible environmental harm.

Enzymes are a perfect candidate to replace potentially harmful inorganic chemicals. The challenge is to be able to produce the enzymes on an appreciable scale. Enzymes are used in many different industries and there is interest for enzymes to become a replacement in process where previously inorganic substances were used.

The growing interest of the product of scFOS has become significant in the sugar industry. If the sugar industry were to use the FFase enzyme, they could greatly increase the value of their sugar into scFOS just by using the FFase enzyme. The attractiveness about producing scFOS is that the main raw ingredient for the process is the raw sugar that is produced from a sugar mill. This means that no extra purification or refining needs to be done for the scFOS to be produced.

1.2.5 Thesis Plan

A Gantt chart is drawn up to show the proposed project plan (see figure 1.1). It is important to note that this plan is an estimation and the duration of some tasks may be shorter or longer in reality. The intended duration of the entire research project is as shown in the Gantt chart.

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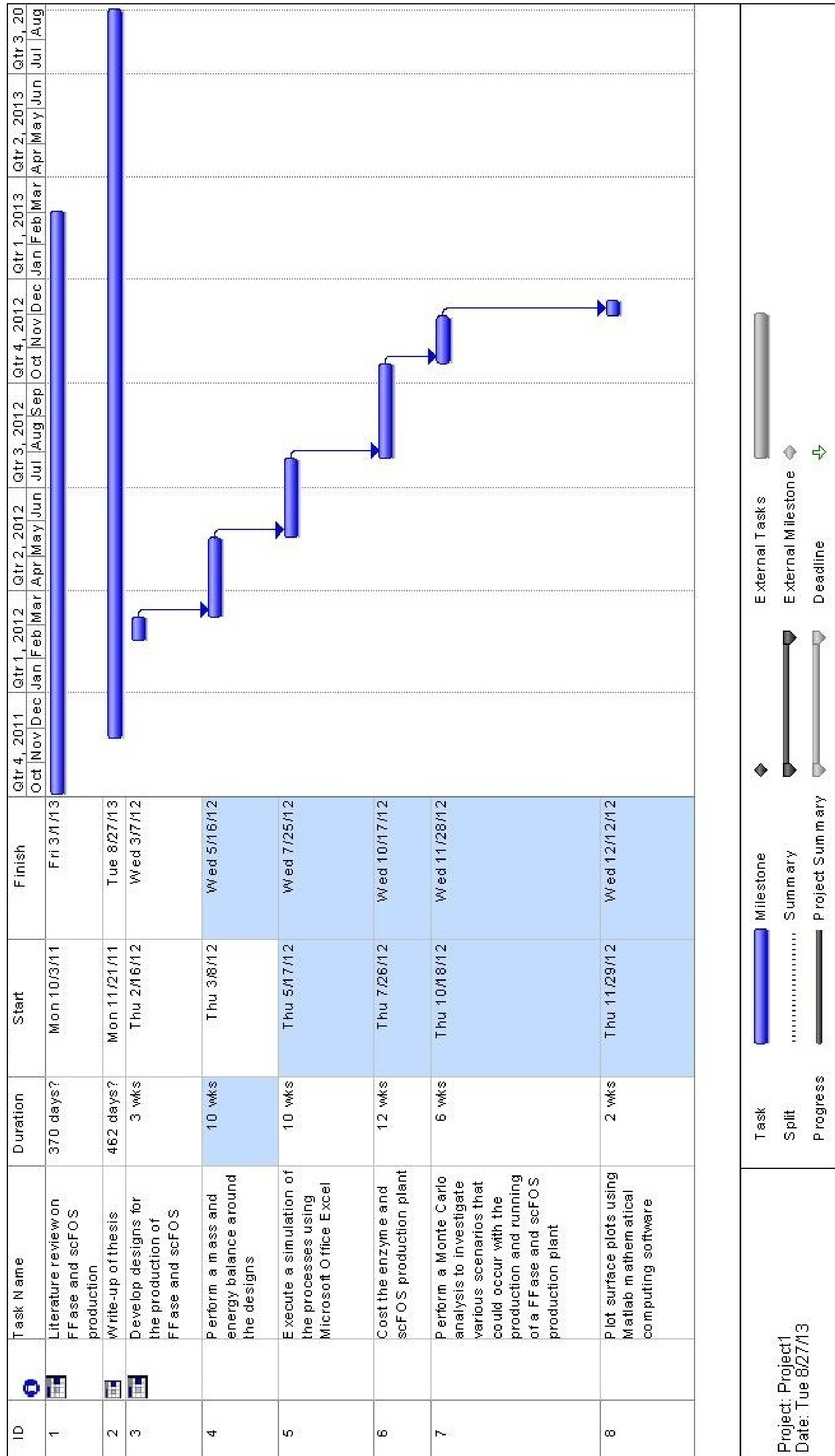

Figure 1.1: Project plan for the research project showing deliverables and milestones to be completed for the project.

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Chapter 2

Literature Review

2.1 Overview

Enzymes are natural catalysts and their function is to accelerate reactions that take place in a cell and its surroundings (Kirk *et al.*, 2004). A catalyst is something which is able to facilitate a chemical reaction, but it will not undergo any changes itself during the chemical reaction. Therefore an enzyme will be involved in a biochemical reaction where the enzyme will lower the activation energy needed to induce the chemical reaction. Enzymes are not stable and as a result lose their activity after a period of time. For this reason, there is a need for enzymes to be produced on an industrial level.

Enzymes have shown to be very useful in many different applications ranging from a lot of commercial foods that are sold in shops today which would include bakery products, alcoholic beverages, dairy products, fruit juice and textile industries to name a few (Vroemen, 1983). Enzymes are also used in other different applications such as dishwashing detergents, pulp and paper production and leather tanning (Enzyme Technical Association, 2001).

Enzymes are basically broken up into different categories according to their function and the compounds that they react with. They are named according to their biochemical reactions they catalyse. To name a few: *proteases* are enzymes that break down proteins, *cellulases* break down cellulose, *lipases* divide lipids (fats) into glycerol and fatty acids, and *amylases* are responsible

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for breaking down starch into simple sugars (Enzyme Technical Association, 2001).

It can be seen that enzymes form a very important part of the production process of many simple and complex products needed for everyday living. The fact that the enzymes are used for these everyday needs (e.g. dishwashing detergent) shows that there is a need to produce the enzymes on a large scale, otherwise the product would not be able the contend with the demands of the public. This gives support to the idea for the implementation of industrial scale production. One of the main reason driving large scale of production is economies of scale and the 'six tenths rule', which is explained in more detail further on in this document. A challenge is to be able to find or develop a big market to be able to sustain a facility of a large size.

Enzymes are usually acquired through the sources of animal tissue, microbes and plant material (Enzyme Technical Association, 2001). The only problem with the acquisition of enzymes in the manner of using plants and animal tissue is that it is difficult to produce enzymes in large quantities. If microbes are used; it is possible to produce the enzymes in larger quantities. The conditions for growing the organism need to be favourable so that there is prolific population growth.

This points again to large scale enzyme production. With industrial enzyme production; the desire is to be able to produce quantities of enzyme that are enough to meet the market demand. Industrial enzymes are pitched at different prices according to different demands. In this work, they will be divided into three categories.

1. Commodity enzymes: High demand, low price
2. Intermediate enzymes: Medium demand, average price
3. High value enzymes: Low demand, high price (i.e. speciality enzymes)

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The ideal situation would be if South Africa had an enzyme production facility that produced the enzymes locally, which in time would become a more economical option, for industries who need enzymes, than to import the enzymes from overseas countries. A question that would arise would be: does one focus on a production facility that produces enzymes from one category? For example, would it be more worthwhile to have a facility that produces only commodity enzymes? Or would it be better to have a facility that has multiple products, namely commodity, intermediate and high value enzymes all being produced in the same plant?

The initial thinking is that the original start-up cost of these facilities would be fairly similar. Equipment costs for the different types of enzymes may vary slightly, but their production method would use similar if not the same techniques. The next factor to think about is when will the facility be able to pay back the initial investment used to start up the plant? How will the three categories compare to each other in this respect as the three categories are different in their demands and prices? The controlling factor is obviously the price and the demand of the products as mentioned.

A big factor that plays a role in the construction of a plant is the idea of economies of scale which is linked with the 'six tenths' rule. The 'six tenths' rule shows that when the cost attribute of the piece of equipment increases, the cost of the the piece of equipment only increases by the power of six tenths as opposed to a linear increase. This essentially means that as the scale of production increases, the size of equipment would need to increase. When the size increases, the cost increases by a power of six tenths, which has the following effect: as the production output increases, the relative cost of equipment decreases for increasing production output. This means that the business will experience an economy of scale.

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2.2 Previous Work

2.2.1 Enzymes

Enzymes have been present since cheese manufacturing and indirectly via yeasts and bacteria in food production. In 1914, isolated enzymes were used in detergents. Enzyme's protein nature was proven in 1926 and large-scale microbial production began in the 1960s (Leisola *et al.*, 2001). Enzymes are types of proteins that have a unique function of catalysing certain biochemical reactions, depending on the type of enzyme. They are crucial for the maintenance and development of life (Kirk *et al.*, 2004). They are natural catalysts and are composed of up to 20 different amino acids (Kirk *et al.*, 2004).

The Enzymes are important for all metabolic processes, although they are not living (Enzyme Technical Association, 2001). The enzymes are made up of amino acids like all other proteins are, but the difference is shown in their catalytic ability, which makes them exclusive. Table 2.1 gives some examples of large scale enzyme applications.

2.2.1.1 Enzyme Preparation

The commercial types of enzymes are acquired from three primary sources: animal tissue, plants and microbes. These enzymes that occur naturally can typically cater for small amounts and are not enough for industrial applications, however, there is a way to select the best genetic strain and optimise the cultivation conditions with the preferred strain to get the desired output. Fermentation is the technique that is used to produce the enzyme and thereafter, the microorganisms are killed and the enzymes are separated, and can thus be used for industrial applications (Enzyme Technical Association, 2001).

2.2.1.2 Advantages of Enzymes

Using enzymes as opposed to normal chemical treatment can be very beneficial. Enzyme Technical Association (2001) says that the use of enzymes can

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Table 2.1: Large scale enzyme applications (Leisola *et al.*, 2001)

Industry	Enzyme	Effect
Detergent	proteinase lipase cellulase	protein degradation fat removal colour brightening
Textile	cellulase laccase	microfibril removal colour brightening
Animal feed	xylanase phytase	fibre solubility release of phosphate
Starch	amylases glucose isomerase	glucose formation fructose formation
Pulp and paper	xylanase	biobleaching
Fruit juice	pectinase cellulase xylanase	juice clarification juice extraction juice extraction
Baking	xylanase α-amylase glucose oxidase	dough conditioning loaf volume; shelf-life dough quality
Dairy	rennin lactase	protein coagulation lactose hydrolysis
Brewing	glucanase papain	filter aid haze control

improve the quality of products while lowering the manufacturing cost of the item. Less waste will be produced and also the energy use can be decreased. It is stated further by Enzyme Technical Association (2001) that traditional chemical treatments are sometimes harder to control, and have the possibility of creating some extreme conditions. Another shortcoming of traditional chemicals is that they tend to produce unwanted waste and by-products. Enzyme use can be controlled very well with the appropriate dosage, the correct operating temperature and the time. The fact that the enzymes act as catalysts means that only a small amount of the enzyme needs to be added in order for the reaction to proceed. The enzymes that were used in the food industry are usually terminated during the downstream process and thus do not exist in the food product (Enzyme Technical Association, 2001).

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2.2.1.3 General Enzyme Production

The generic enzyme production process is shown in Figure 2.1. The simple illustration shows the basic idea behind commercial enzyme production.

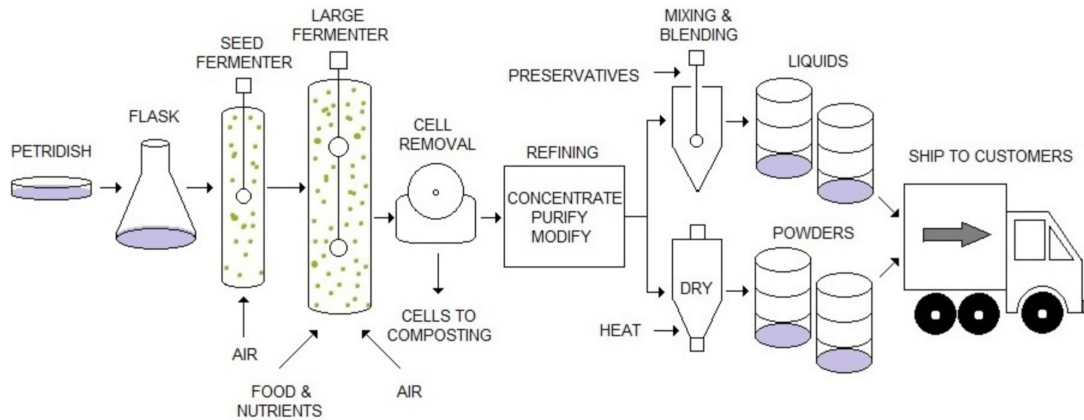

Figure 2.1: Enzyme production process illustration (Redrawn) (EuropeanProject(FP6), 2011).

The process simply begins with a cell growing in a small Petri dish. After the cell has increased its population to a stable amount, the cells are then transferred to shake flasks where they have a larger volume and cell mass to grow further. From the shake flask the medium is taken to a fermenter, where the cells are contacted with air and allowed to ferment. Once fermentation has been completed, the cells are removed, the enzymes that have been produced after fermentation are refined, where the procedures of concentration, modification and purification are done. It is important to know whether the enzymes will be transported and stored in the liquid form or powder form. In this way, from the refining step in the process the enzymes are either sent to a mixing and blending section, where the product is in the liquid phase, or the enzymes are sent to a drier, where the final product is in the form of a powder. From here the products are stabilised and are treated with preservatives before they are transported.

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2.2.2 *Pichia Pastoris*

Pichia pastoris is a yeast that has been chosen as the host strain for the production of β-D-fructofuranosidase in this project. Cereghino and Cregg (2000) say that *P. pastoris*, the methylotrophic yeast, has developed into a very successful scheme for producing a selection of different heterologous proteins. The reason, Cereghino and Cregg (2000) says that *P. pastoris* is becoming so popular is because of the following factors:

1. The methods necessary for molecular genetic modification of *P. pastoris* are very simple and also *P. pastoris* has shown to have very similar traits to *S. cerevisiae*, which is one of the best developed experimental systems in present day biology.

2. *P. pastoris* has a capability to produce foreign proteins in large amounts, where is it both intracellular and extracellular.

3. The *P. pastoris* also has the ability to undergo eukaryotic post-translational changes, which include glycosylation, disulphide bond formation and proteolytic processing.

4. The expression system of the *P. pastoris* is readily available as a commercially bought kit.

2.2.2.1 The Gene Expression Process

Gene expression is a complex process whereby the information supplied by a gene is utilised in the creation of a functional gene product. The products formed are proteins. Gene expression is used by all known life - eukaryotes (which includes organisms that are multicellular), prokaryotes (which include bacteria and archaea) and viruses (News-Medical, 2012).

The expression of a gene to form the sequence of amino acids and subsequent protein happens in a two-step process. The first step in the gene

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expression process is known as transcription (see figure 2.2) where an enzyme called RNA polymerase acts as the catalyst and synthesises the mRNA molecule using the gene as a template. Step two of the gene expression is known as translation. This step uses the information that exists in the nucleotide sequence of an mRNA molecule to direct the synthesis of a peptide chain with a corresponding amino acid sequence (Bailey and Ollis, 1986).

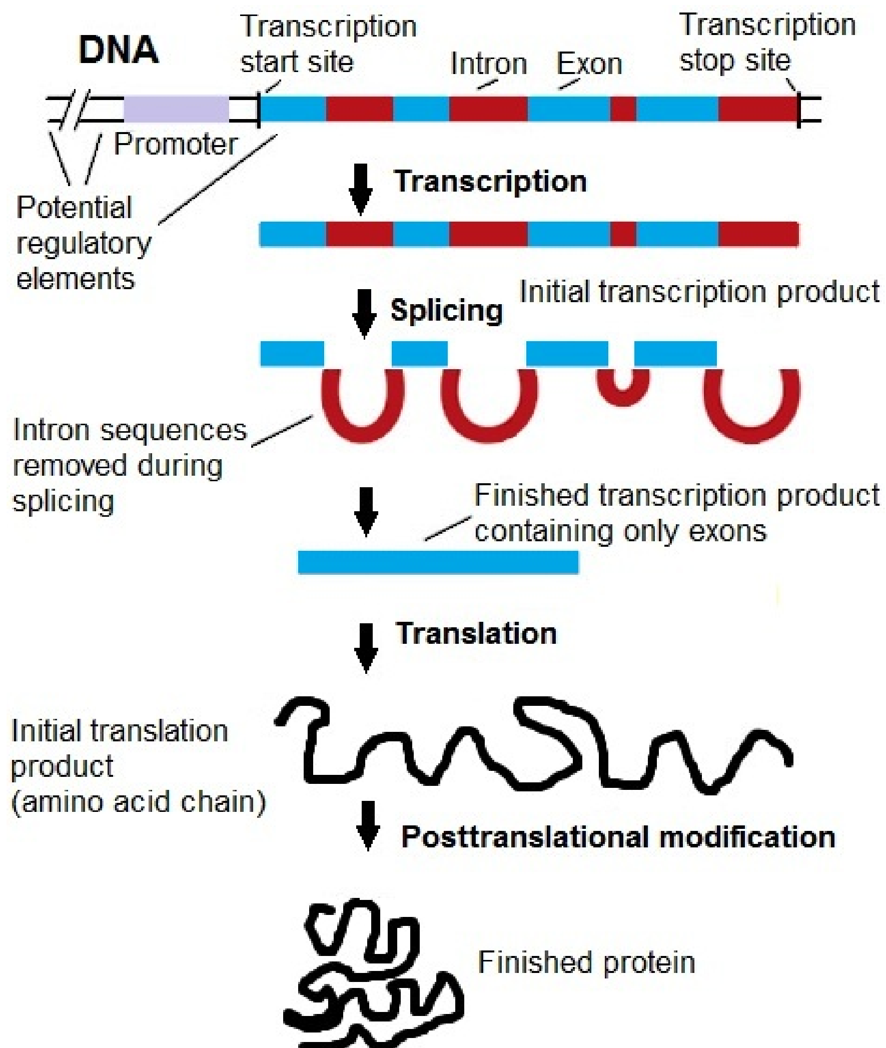

Figure 2.2: Gene expression to produce a protein (Redrawn) (News-Medical, 2012).

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2.2.2.2 Promoters

In order for the *P. pastoris* to be able to develop and express a certain gene within itself, the correct promoter needs to be chosen. A promoter is a region in DNA that facilitates the transcription of a particular gene under particular conditions (figure 2.2). From there the enzyme (in this case FFase), which is regarded as the protein (figure 2.2), starts to be produced.

There are a few promoters that can be chosen for the expression of a specific gene in *P. pastoris*, namely *AOX*, *GAP*, *FLD1*, *PEX8*, and *YPT1* (Cereghino and Cregg, 2000). Table 2.2 gives a more detailed explanation of the promoters.

Table 2.2: Various promoters that can be used for the *P. pastoris* gene expression system

Expression	Description
AOX	alcohol oxidase (Cereghino and Cregg, 2000)
GAP	glyceraldehyde 3-phosphate dehydrogenase (Cereghino and Cregg, 2000)
FLD1	formaldehyde dehydrogenase (Macauley-Patrick *et al.*, 2005)
ICL1	isocitrate lyase (Macauley-Patrick *et al.*, 2005)
PEX8	an intraperoxisomal peroxin of *Saccharomyces cerevisiae* required for protein transport into peroxisomes binds the PTS1 receptor Pex5p (Rehling *et al.*, 2000)
YPT1	a yeast gene encoding a protein homologous to the human c-has/bas proto-oncogene product (Gallwitz *et al.*, 1983)

According to Cereghino and Cregg (1999), *AOX* was a popular promoter from the options presented (see table 2.2) for the *P. pastoris* expression system where it was successfully used to produce over 300 foreign proteins. Lin Cereghino *et al.* (2001) further state that compared to other fungi, the benefit of the *P. pastoris* expression system is the use of an unassumingly effective and closely monitored promoter derived from AOX. Cereghino and Cregg (2000) explain that although the *AOX* has been a successful promoter in the expression sys-

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tem, there are some situations where *AOX* has not been the most appropriate promoter. The *AOX* needs to be induced by methanol, and for this reason, as scFOS is a product that is for human consumption, the level of methanol that could possibly carry over in the scFOS process could cause the scFOS to be potentially harmful to the people buying the product.

Another negative factor about using *AOX* is that methanol is a potential fire hazard as the substance is flammable and therefore if one were to use *AOX*, they would have to put safety measures in place, which would cost extra money and training, which is undesirable. The desire is to find a promoter that does not have to be induced so that no extra components have to be added to the system.

One promoter that serves as a very good alternative to *AOX*, is *GAP*. *GAP* has shown to be a very good constitutive promoter. What is meant by constitutive is that it does not need to be induced, or 'switched on', but instead from the start of inoculation, the *GAP* allows the production of the enzyme to happen, the gene expression happens from the start. *GAP* does not need methanol for induction. A downside to using *GAP*, however is that it does not cause the yeast of *P. pastoris* to produce as much protein (enzymes) as *AOX* does. Another disadvantage of *GAP* is that due to the fact that it expresses in a constitutive manner, it is not a advisable option when producing proteins that are toxic to the yeast (Cereghino and Cregg, 2000). This is backed up by Menendez *et al.* (2004), where it is stated that *GAP* has not been used extensively because it is believed that constitutive production of foreign proteins in *P. pastoris* could have cytotoxic effects.

FLD1, according to Veenhuis *et al.* (1983) is a very important enzyme that is used in the metabolic pathway in *P. pastoris*. *FLD1* promoter initiates the expression of a glutathione-dependent formaldehyde dehydrogenase, which is an important enzyme that is needed to metabolise certain methylated amines as nitrogen sources and methanol as a carbon source (Shen *et al.*, 1998).

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Cereghino and Cregg (2000) mentions that *FLD1* can be induced with methanol as its carbon source with ammonium sulphate as the nitrogen source, or methylamine as a sole nitrogen source and glucose as the carbon source. Once induction has occurred, with the methanol or methylamine, *FLD1* has the ability to express certain levels of a β-lactamase reporter gene, which shows similarity to those acquired with methanol induction from the *AOX1* promoter. The benefit of the *FLD1* promoter is that it offers the flexibility to be able to induce high levels of expression using either the methanol or methylamine, which shows to be an inexpensive nitrogen source that is also non-toxic. This is confirmed by Shen *et al.* (1998), where it is claimed that *FLD1* is involved in protecting the cell from toxicity, which is caused by the formaldehyde during the methylamine metabolism.

AOX1, *GAP* and *FLD1* are not always the most appropriate promoters for the given expression system. Sometimes, the aforementioned promoters are too strong, where they express the genes at a level that is too high. Brierley (1998) and Thill *et al.* (1990) have shown that for some specific foreign genes, the high level of expression from *AOX1* may damage the physical morphology of the cell after translation and will cause major part of the foreign protein to be misfolded, unprocessed, or mislocalised. For this reason, the promoter of *PEX8* is introduced, as it is not as 'harsh' as the previous three that have been mentioned. *PEX8* encodes a peroxisomal matrix protein that is vital for the biogenesis of the peroxisome (Liu *et al.*, 1995). The gene expresses itself at a low but substantial level and induction occurs when the cells are shifted to methanol. Segev *et al.* (1988) states that the *YPT1* gene encodes a GTPase involved in secretion, and its promoter offers a low but constitutive level of expression in media containing glucose or methanol or mannitol as the available carbon sources.

ICL1, which is a gene of *P. pastoris*, was investigated as another option as a promoter to the regularly used *AOX1* and *GAP* promoters (Menendez

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et al., 2003). Macauley-Patrick *et al.* (2005) explains further that a plasmid (pPICLDEX) that contains a single copy of the gene (which was controlled by *ICL1* that encodes dextranase from *Penicillium minioluteum* was converted to *P. pastoris*. High levels of dextranase yield was obtained when the culture contained a lower level of glucose or ethanol as the sole carbon source. On the contrary, a higher amount of dextranase was produced when there was no glucose available than after induction with ethanol. Macauley-Patrick *et al.* (2005) concluded that more work needed to be done on the promoter of *ICL1* to determine whether it is suitable and practical for the heterologous protein production in *P. pastoris*.

Many promoters are available for the expression of the yeast *P. pastoris* (Table 2.2). It is important that the most appropriate promoter for the job is chosen as each promoter has its advantages and disadvantages. For this project, the two promoters that were chosen were *AOX* and *GAP*. The *AOX* gene production system was chosen in this project because it has the ability to produce higher amounts of enzyme protein. A disadvantage of using *AOX* is that it needed to be induced by methanol, which is a component that is a potential fire hazard, and this is not ideal in large scale production as one would have to have safety precautions for it.

GAP on the other hand, is another useful promoter that is appealing as it is known as a constitutive promoter, and it therefore does not need to be induced (Waterham *et al.*, 1997). This means that a hazardous substance such as methanol would not need to be part of the process and therefore would not be a cause of concern in the facility. The negative of using *GAP* is that it does not promote as much protein formation compared to *AOX*. The choice of promoter depended on the economics of the process, i.e. which will be a more beneficial option with regard to cost.

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2.2.3 FFase and scFOS Production

Short chain fructooligosaccharide (scFOS) is a chemical that is regarded as a pre-biotic, and is used as a food supplement to improve dietary health in humans. As fructooligosaccharides are found in various fruit and vegetables, they have also shown to be consumed by animals, measured by an ion chromatographic method (Campbell *et al.*, 1997). Table 2.3 shows the chemical name, the abbreviated name as well as the molecular formula and the molecular weight of the different constituents that make up the chemical structure of scFOS (figure 2.3).

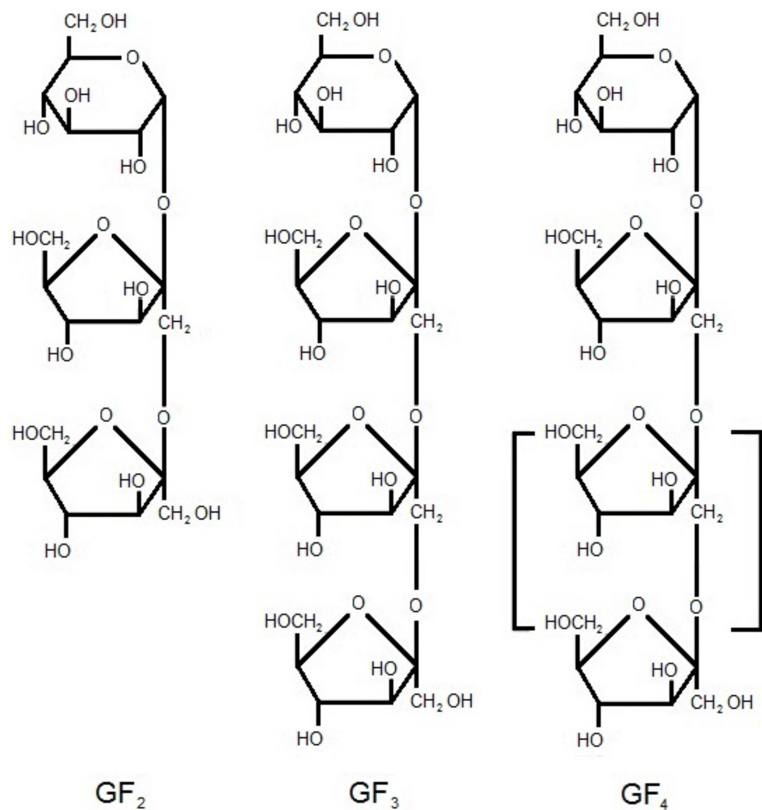

Figure 2.3: Illustration of the polysaccharides that make up scFOS (Redrawn) (Shenzhen Victory Biology Engineering Co., 2011).

Vaňková *et al.* (2008) did work on the design and economics of industrial production of fructooligosaccharides (FOS), where the study proposed two

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alternative processes for the production on 10 000 tonnes of FOS's every year. The different processes included the production of the FOS in the form of a powder as well as the FOS in the form of a syrup. The economic analysis by Vaňková *et al.* (2008) included the costs for all the pieces of the equipment in their design as well as prices of the raw materials in the process, which include the sugar needed (sucrose), the host strain that is used in the process, which is used to produce the enzyme (FTase) and all other utility costs. Vaňková *et al.* (2008)'s production method used the enzyme of FTase where the enzyme is produced intracellularly. To obtain the enzyme in this case, the cells needed to be broken open. In this study, the enzyme of FFase was used where in this case, the enzyme was produced extracellularly. The enzyme existed within the supernatant.

Table 2.3: Components involved in the scFOS production

Chemical name	Abbreviated name	Molecular formula	Molecular weight
Glucose	G	$C_6H_{12}O_6$	180 g/mol
Fructose	F	$C_6H_{12}O_6$	180 g/mol
Sucrose	GF	$C_{12}H_{22}O_{11}$	342 g/mol
Kestose	GF_2	$C_{18}H_{32}O_{16}$	504 g/mol
Nystose	GF_3	$C_{24}H_{42}O_{21}$	666 g/mol
1-F-fructofuranosyl nystose	GF_4	$C_{30}H_{52}O_{26}$	828 g/mol
A fructooligosaccharide	GF_5	$C_{36}H_{62}O_{31}$	990 g/mol
A fructooligosaccharide	GF_6	$C_{42}H_{72}O_{36}$	1152 g/mol
A fructooligosaccharide	GF_7	$C_{48}H_{82}O_{41}$	1314 g/mol

ScFOS has many benefits for health, and according to Shenzhen Victory Biology Engineering Co. (2011), the following benefits have been found:

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- **Intestinal flora is regulated** and the function of the gastrointestinal region in the body is greatly improved.
- **Blood lipid is reduced.** This means that the scFOS has proven to reduce cholesterol in the body as well as the free trans fatty acids and the blood sugar level balance.
- **Synthesis of vitamin intake is enhanced.** This implies that the scFOS can help improve the absorption of important vitamins such as vitamin B and folic acid that will promote the metabolism in the body. This can improve the immune system.
- **Blood endotoxins are eradicated.** This protects the liver and aids in the prevention of various chronic diseases and cancers.
- **The absorption of the minerals of Ca, Mg, Fe, Zn is enhanced** as well as other mineral matters in order to promote body growth development against the bone disease of osteoporosis.
- **Prevents obesity** because the scFOS is not decomposed easily by the gastric acid and enzymes inside alimentary tract. This makes the scFOS extremely difficult to be absorbed by the human body. Because of scFOS has a very low value in energy, it will not cause obesity, and can act as a very good low energy functional sweetening agent.
- **Preventing decayed teeth.** The scFOS is not used by the organism *Streptococcus mutans* to make the insoluble glucan and the secondary product, which is acidic. The scFOS will also not offer a base for the oral microbes' sediment or corrosion so that decaying teeth can be inhibited.
- **Removing of toxins for facial whitening and beautifying**. When the scFOS is taken, the human body can thoroughly clean out the endotoxins to ensure the prevention of facial sores, black spots, freckles, acnes and age pigments, and to prevent skin against ageing.

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It can be seen from the above list that scFOS would be a desirable product as it has many benefits to human health in maintaining the various parts of the body. In order to manufacture scFOS, the main constituents that are needed are the enzyme, which is β-D-fructofuranosidase (FFase) and the sugar of sucrose. The process that is of concern is:

a. the production of the enzyme FFase that is imperative for the scFOS manufacturing process.

b. the production of scFOS using FFase as the all important enzyme for the process as well as sucrose.

Vaňková *et al.* (2008) proposed a design of a fructosyltransferase (FTase) production facility as well as a FOS production design. The design synthesises the FTase production and FOS production, where it can be shown that the raw materials are the input parameters to the process. The design shows the units the materials pass through, all the way until the FOS production process is met. The final product comes out as the FOS, which is the desired product.

The goal of the study done by Vaňková *et al.* (2008), which is entitled 'Design and economics of industrial production of fructooligosaccharides' was to develop a process flow diagram and from this, investigate the viability of the industrial production of the FOS, for an annual production rate of 10 000 tonnes. This study was based on laboratory and semi-pilot scale experiments (Vandáková *et al.*, 2004). The FOS's production was performed in a packed bed reactor (PBR) and immobilised FTase was used, which was produced from *A. pullulans* (Onderková *et al.*, 2007). Gramblička and Polakovič (2007) determined the distribution coefficients and adsorption data that was needed for the design of the simulated moving bed (SMB) separation. From here Vaňková *et al.* (2008) stated that the material and energy balances as well as the equipment sizing and costing was done for a few different structures, where different sucrose sources and end products phases were considered.

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FOS's are present in various kind of vegetables and some plants. These include bananas, asparagus, onions, artichokes, shallots and wheat to name a few (Flamm *et al.*, 2001), (L'homme *et al.*, 2003). Vaňková *et al.* (2008) says that FOS's are produced industrially in two different manners, which results in two different products. The first process uses the degradation of inulin when the products are not fructo-oligomer chains any more (Franck *et al.*, 2002). The second process transforms sucrose by using the enzyme of FTase, which is obtained from bacterial and fungi sources (Bekers *et al.*, 2002), (Ghazi *et al.*, 2007), (L'Hocine *et al.*, 2000), (Sangeetha *et al.*, 2004). This is basically a case of hydrolysis versus synthesis.

Chien *et al.* (2001), Sangeetha *et al.* (2005a), Sangeetha *et al.* (2005b) and Tanriseven and Aslan (2005) have all made notable contributions with the research of FOS production. Many different organisms have been used when producing the enzymes needed for FOS production. Such examples of organisms that have been used are *Aureobasidium pullulans*, *Aspergillus japonicus*, *Aspergillus niger* and *Pichia pastoris* (Sánchez *et al.*, 2008). Lee *et al.* (1992) and Nguyen *et al.* (2005) say that during the production of FTase, the type of organism used during the production the enzyme had a strong influence in the outcome when cultivating, isolating and purifying the FTase.

Sangeetha *et al.* (2005c) found that when producing FOS, a yield of 53% (gram FOS per gram of sucrose) was obtained when using recycled pellets of *Aspergillus oryzae* CFR 202. The downside to this however was that the pellets lost their compactness and started to break up after the sixth cycle. FTase in the soluble form, in some applications, resulted in some disadvantages such as loss of enzyme activity during operation. After the conversion occurred, the enzyme needed to be destroyed as fructose began to form, which affected the activity (L'Hocine *et al.*, 2000).

Continuous processing is preferred with the enzyme being immobilised as the better option. The production of FOS's from sucrose in continuous re-

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actors was done by Won Yun *et al.* (1997) where the cells of the organism *Aureobasidium pullulans* was used as well as enzymes for two different experiment. The cells and enzymes were immobilised on a highly porous resin. These experiments resulted in a 57% and 58% yield respectively for each experiment.

Jung *et al.* (1989) says that the stoichiometry of the reaction of FTase and the sucrose, occurs with a two path parallel reaction mechanism. Furthermore, Aboudzadeh *et al.* (2006) mentions that a disproportionation reaction occurs and the FOS's and glucose (by-product) form from the activity of the FTase. The enzyme's hydrolytic activity causes the formation of the by-products of glucose and fructose. Sangeetha *et al.* (2004) found that chemical composition of the reaction mixture of FOS's, glucose, fructose and sucrose corresponded to mass percentages of 65%, 25%, 5% and 5% respectively. Nishizawa *et al.* (2001) and Yun *et al.* (1994) wanted to increase the amount of FOS's in the mixture by attempting to remove the glucose from the chemical mixture by employing an enzymatic reaction. Glucose has been found to inhibit FOS formation, which is the reason for the glucose removal (Yun, 1996).

After enzymatic reaction, FOS's are purified and it was found by Crittenden and Playne (1996), Charton and Nicoud (1995), Kim *et al.* (1992), Takahashi and Goto (1994) and Vente (2004) that a good way to concentrate the FOS's was to simulate them under moving bed chromatography. Aydoğan *et al.* (1998) and Goulas *et al.* (2002) used the technique of membrane filtration to concentrate the FOS's although it came at the expense of low selectivities and significantly higher costs.

2.2.3.1 Factors Affecting scFOS Production

Like all chemical reactions and chemical reactors, the conditions of the the inputs and the conditions of the unit operations will determine the state of the output or the product of the reaction and this is no different for scFOS production. The conditions of scFOS production have already been discussed

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so there is a good idea as to what are the most common conditions that are used in industry.

Temperature is a parameter that is very sensitive when chemical reactions occur. It can be seen, according to the results obtained in the patent by Muramatsu *et al.* (1994) that the most favourable conditions for high scFOS production are around 55°C and 60°C (Figure 2.4). The desire is to increase the concentration of the short-chain fructooligosaccharides while keeping the concentration of the other components low so the the scFOS percentage is highest.

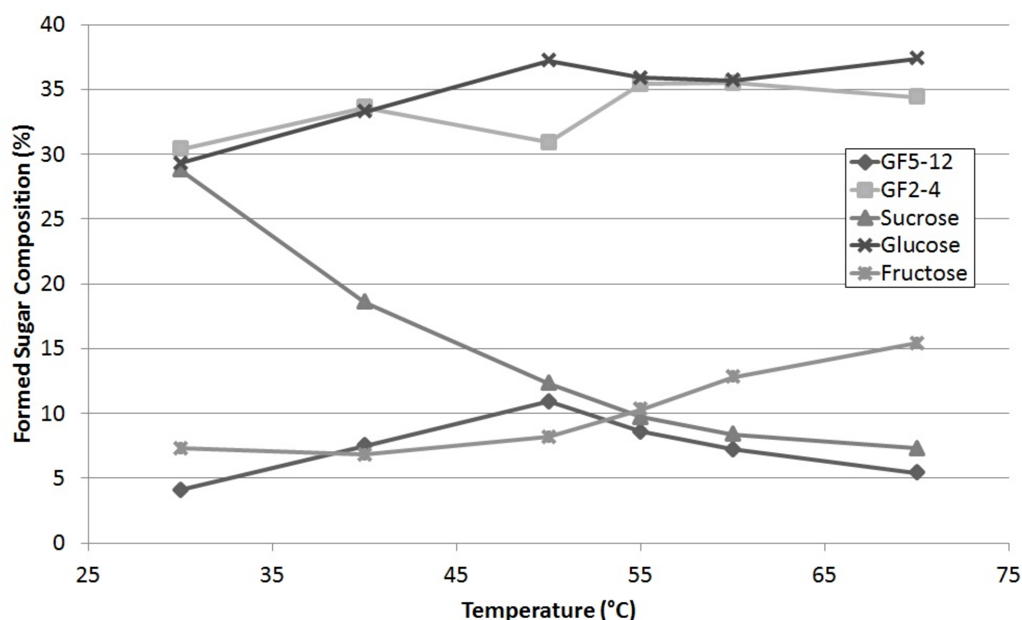

Figure 2.4: The effect of reaction temperature on the formation of scFOS (Muramatsu *et al.*, 1994).

Muramatsu *et al.* (1994) also published data with regard to the relationship between the incoming sucrose concentration and the composition of the sugars that were formed in the reactor (Figure 2.5). The GF2-4 showed to be highly dependent on the sucrose concentration as it increases substantially from 5% to 25% as the sucrose concentration increases from 1% to 10%. At a sucrose

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concentration of 30% and above, higher levels of scFOS (GF2-4) production are achieved (see figure 2.5).

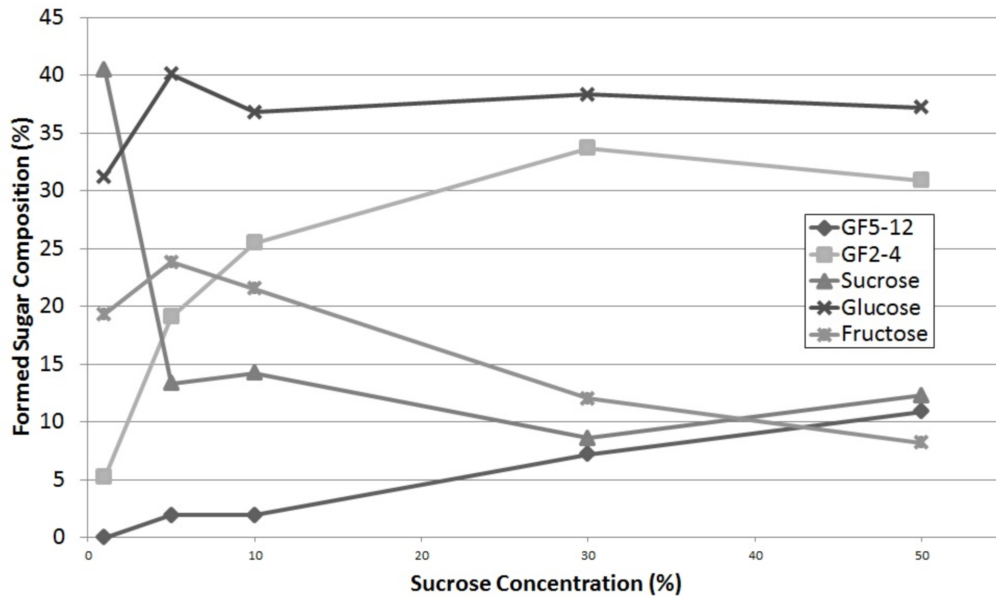

Figure 2.5: The effect of initial sucrose concentration on the formation of scFOS (Muramatsu *et al.*, 1994).

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2.2.4 Literature Process Design

Vaňková *et al.* (2008) proposed a design for the production of FOS (Figure 2.6). In Vaňková *et al.* (2008)'s design the enzyme of fructosyl transferase (FTase) is first produced. FTase is an alternative enzyme to FFase, where FTase is simply produced in a different manner to FFase, but performs the same function. The most important section is shown in Figure 2.6, where the FOS is produced. The FTase was produced by *A.pullulans* and it was the FTase that acted as the biocatalyst for the production of the FOS's from the sugar, sucrose. The FTase was present while the cells were cultivated in the periplasmic space of the cells and also in the cultivation medium (Vaňková *et al.*, 2005).

Onderková *et al.* (2007) described details of the processes of cultivation, separation and purification of the soluble FTase in their study, where it forms part of the initial section in Figure 2.6. The first section of the process flow diagram, is the storage tank of V-101, Vaňková *et al.* (2008) decided that the cultivation medium, was prepared in the tank V-101 and it consisted of sucrose, salts and 2% of inoculum. The holding times, temperatures, and compositions of the process streams involved are all shown in Table D.1. The cultivation medium that exits out the bottom of tank V-101 and is transferred via stream 4A to the 10m^3 fermentation bioreactor V-102. The purification and sterilisation of the air (at an average flow rate of 1m^3/min) needed for the cultivation of the medium in R-101 is performed in air compressor C-101 and the air filter F-101.

When the cultivation is complete in R-101, the medium is sent via stream 5A to the storage/blending tank V-102, where the medium is allowed to sit. From V-102, the material is sent along stream 6A to the microfiltration unit F-102, where the cells can be separated from the liquids. The liquid stream exiting the top of the microfiltration unit F-102, is sent to the ultrafiltration unit where the medium is separated further for any remaining cells in the liquid

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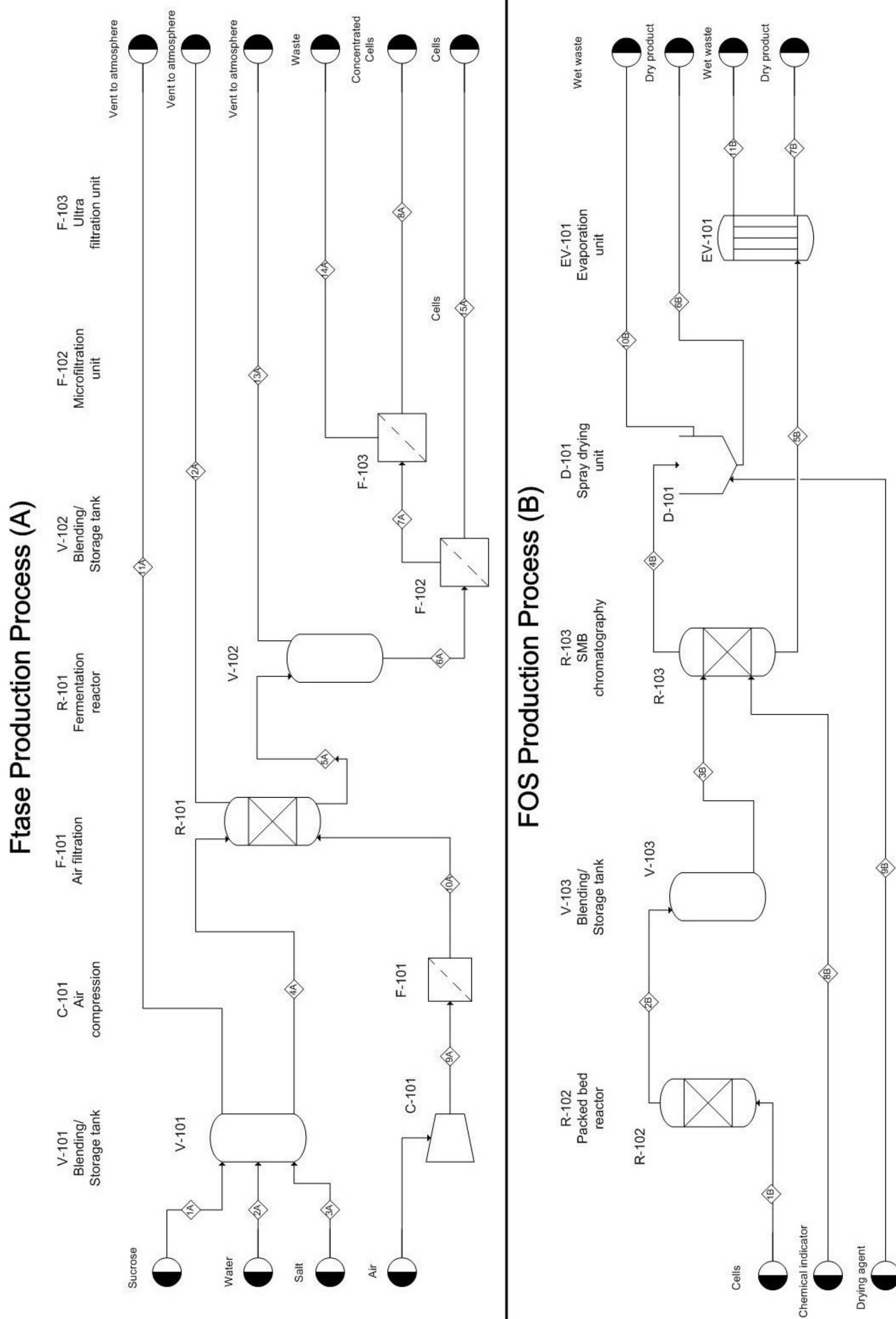

Figure 2.6: Simplified FTase and FOS production process design adopted from the design of Vaňková *et al.* (2008).

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medium. Note, the FTase is present in stream 7A, which flows out the top of F-102 (filtrate) as well as the stream 15A, which exits at the bottom of F-102 (retentate).

Vaňková *et al.* (2008) says that to increase the efficiency of the process to immobilise the cells, the solutes with low molecular mass that exist in the filtrate (7A), are removed by the ultrafiltration unit (F-103). The concentrated cells that exit the ultrafiltration unit F-103, exit via stream 8A and are processed further in the immobilisation process.

The design that is involved in the work of this thesis will not include an immobilisation process as the resources available at the University of Stellenbosch use a process that does not include the immobilisation process (compare Figure 2.6 with Figure 2.7). The FTase production design by Vaňková *et al.* (2005) also includes a bead milling process which effectively breaks open the cells so that the enzyme is released into the supernatant.

The design for this project uses the enzyme of FFase, which is produced differently to FTase and does not need cell breakage and immobilisation. This is due to the nature of the process where the enzyme is automatically secreted into the supernatant and the reactor set-up does not allow for cell immobilisation.

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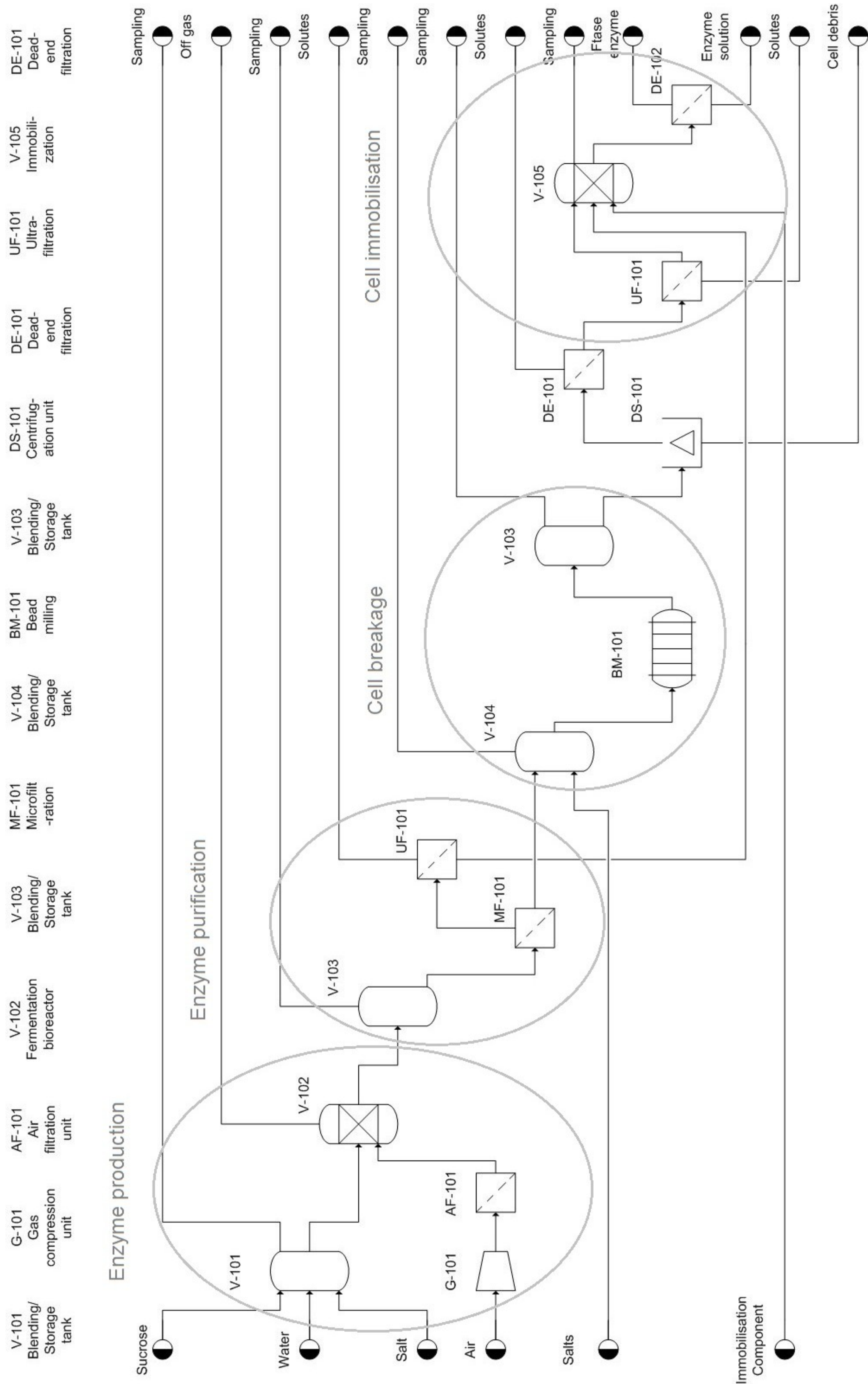

Figure 2.7: Complete FTase production process design (Vaňková *et al.*, 2008).

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2.2.4.1 ScFOS Reactor Setup

When producing scFOS, there is more than one method of production. One method of producing scFOS, is to immobilise the enzyme in the reactor in a packed bed reactor (PBR) setup and let the sucrose flow through the immobilised enzyme to produce the scFOS. Another way is to have the enzyme and the sucrose to flow into a continuously stirred tank reactor (CSTR) (see Figure 2.8). The reaction is catalysed by the enzyme and the scFOS is the product formed from the reaction.

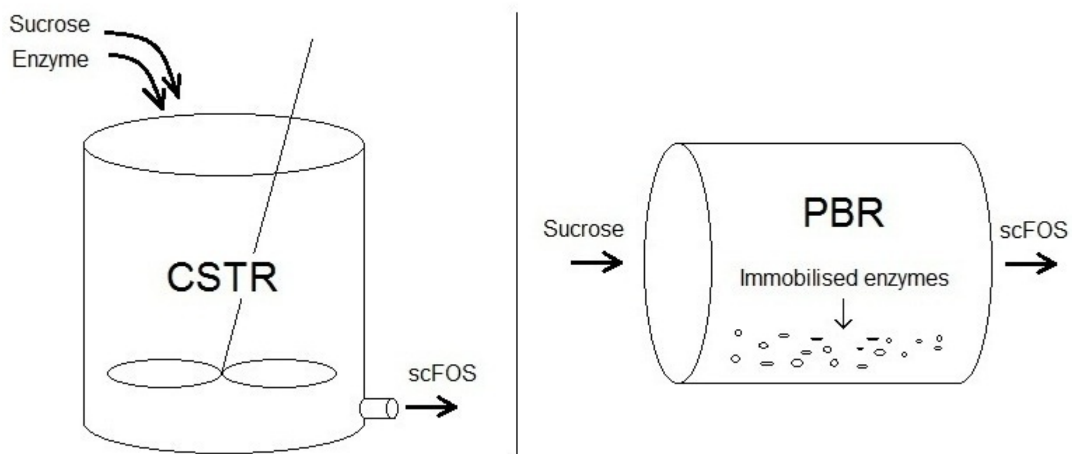

Figure 2.8: A schematic diagram of the reactor choice for the scFOS production process. The choice of the reactor depends on how the enzyme will be used in the reactor (immobilised or not).

Yun *et al.* (1992) have suggested that FOS production is more optimal when using a semi-batch operation compared to a continuously stirred tank reactor set-up. They mention further that the low yield of FOS's in the stirred tank reactor is because of the glucose inhibition that occurred in the reactor. Glucose inhibition occurs when there is an excess of glucose in the reactor and it inhibits the enzyme's function (invertase and tranferase activity). Santos and Maugeri (2007) conclude in their study that when comparing between a

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stirred tank reactor and a packed-bed reactor, they found to have no significant difference with regard to the yield.

With the specific production method of β-D-fructofuranosidase using *Pichia pastoris* as the host organism, the enzyme, when produced, is secreted into the supernatant. This means that there will have to be a more intense purification step to ensure that the enzyme is removed from the solution so that the scFOS may be more pure. Another point is that the enzyme will be wasted with the stirred tank reactor set-up because of the fact that it exits out with the scFOS in solution. The packed-bed reactor has the enzyme packed (which has been initially immobilised) and the sucrose flows through and the FOS's form without the enzymes leaving the reactor and thus being wasted. This method is preferred as the enzymes remain in the reactor and therefore a less intense purification step with the removal of the enzyme is not required and thus less costly.

In Vaňková *et al.* (2008)'s design, a PBR was chosen as they chose to immobilise their enzyme in the reactor and therefore have the enzyme in a packed bed setup. The reactor type chosen for this project is an agitated semi-batch reactor as the manner in which the enzyme was produced needed this type of setup. The enzyme and the sucrose will flow into the reactor simultaneously and reactor in the vessel to form the product.

The operating temperature is also another factor of importance as the conditions need to be right for the optimal production of the FOS's. The reactor is chosen to be operated at 50°C (Vaňková *et al.*, 2008). Yang *et al.* (2008), Yun and Song (1999) and Yun *et al.* (1994) suggested 55°C, which shows that the choice of operating temperature of 50°C is reasonable.

The product that forms in the reactor has a 89% conversion of sucrose (Vaňková *et al.*, 2008). Sheu *et al.* (2002) and Yun and Song (1993) found to have a sucrose conversion of 90%. The products that form are the following: G, F, GF_2, GF_3, GF_4, GF_5, GF_6 and GF_7 as well as unreacted sucrose (GF).

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This means that 11% of the sucrose is unreacted. From this, Sangeetha *et al.* (2004) says that of the 89% of the sucrose that has been converted, 58% forms into FOS's, 30% forms into glucose, 1% fructose and 11% sucrose. This split does not account for the other smaller yields of the components of GF_5, GF_6 and GF_7.

The assumption of the product splits for this design are: 65% FOS's, 23% glucose, 4% fructose, 4% sucrose, 2.5% GF_5, 1% GF_6 and 0.5% GF_7. The composition of the components that exit the reactor are shown in table 5.6. Nishizawa *et al.* (2001) and Yun (1996) claim to have found a 55-60% yield of FOS and Balken *et al.* (1991) also found to obtain a production yield of 60%.

2.2.4.2 scFOS Purification

There are a few different proposed methods for the purification of the fructooligosaccharides after they have been formed in the bio-reactor.

Vaňková *et al.* (2008) suggested the use of an SMB chromatography column where they achieved a 95% separation. Vaňková and Polakovič (2012) also proposed a method for FOS purification using SMB chromatography, where they obtained a 95% yield of FOS's. Vaňková and Polakovič (2010) investigated a method for the optimisation of a single column chromatographic separation of fructooligosaccharides and produced a yield of FOS's of 86%. The reason for this lower recovery is due to the fact that the single column has the one unit compared to the design of Vaňková *et al.* (2008), where four chromatography units in series were used. Nevertheless, 86% yield is still a good separation with the use of only one column.

Nobre *et al.* (2011) and Kuhn and Filho (2010) found an efficient method for fructooligosaccharide purification using a charcoal column adsorption technique where they obtained FOS recoveries of 97% and 97.8% respectively. Nobre *et al.* (2011) made the technique sound very attractive by stating that the method is a "simple and efficient way to purify FOS" and "it can be easily

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applied at a larger scale".

The downside however with the activated charcoal column is that ethanol, which is a hazardous material, is used as an eluent for the column. This means that extra process precautions would have to be put in place to ensure that all hazardous risks are taken care of. SMB chromatography uses water, which is a preferred option because of the non-hazard involved. Although the charcoal column shows to have slightly higher recoveries compared to the SMB chromatography column, the choice will still be to go with the chromatography column because it does not use the potentially hazardous ethanol.

The SMB chromatography column purifies the reactor product through size exclusion (Houwing *et al.*, 2003). The true moving bed was developed before the simulated moving bed and a schematic is shown in Figure 2.9.

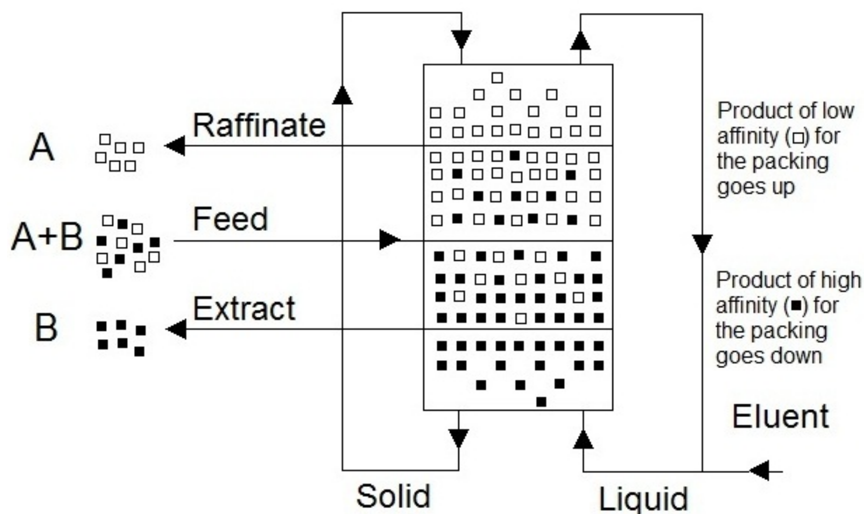

Figure 2.9: A schematic diagram of a true moving bed chromatography column (redrawn) (Bailly and Nicoud, 1993).

The contact between the solids and the eluent is counter-current. As shown (Figure 2.9), the feed which consists of components A and B, are input into the middle of the column. The assumption is that the attraction of A and B for the solid differs and therefore as a consequence, the two components of

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A and B will separate. Component A should travel up the column, while B drops down in the direction of the solid. B is the extract (desired) and A is the raffinate (undesired). The counter-current systems have said to be preferred because they have proven to be more effective than batch processes (Bailly and Nicoud, 1993).

The downfall of the true moving bed process is that it its function cannot be implemented correctly because the flow of the solids cannot be controlled correctly. From this, the SMB technique was developed. This difference with the SMB concept was that instead of moving the solid in a system that has inlet and outlet lines at fixed positions, rather move the inlet and outlet in the opposite direction of the desired solid flow (Bailly and Nicoud, 1993). Figure 2.10 shows the schematic setup of the simulated moving bed.

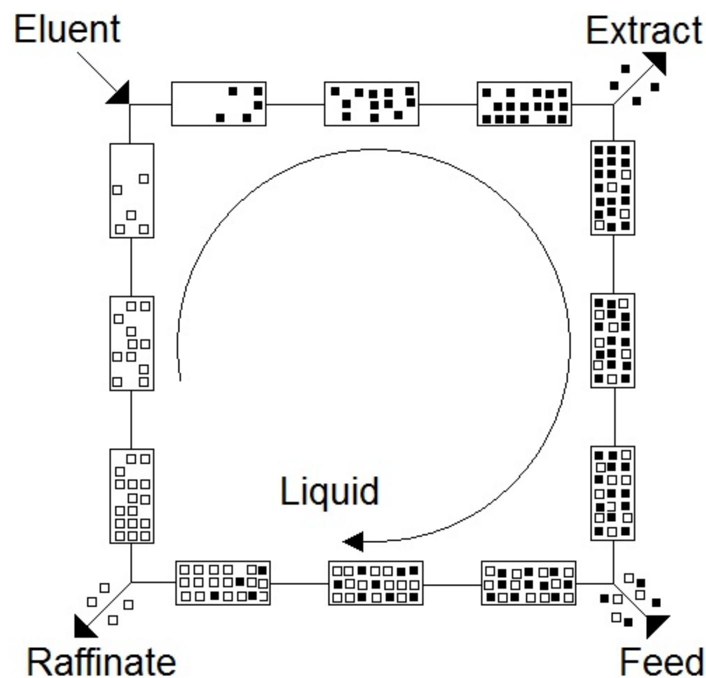

Figure 2.10: A schematic diagram of a simulated moving bed chromatography column (redrawn) (Bailly and Nicoud, 1993).

The temperature for the operation of the chromatography column is also

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a parameter that needs to be determined. Vaňková *et al.* (2008) operates their SMB column at 60°C, while Vaňková and Polakovič (2012) also used this operating temperature. Gramblička and Polakovič (2007) used a different technique to purify their FOS's, however they also used an operating temperature of 60°C to separate the sugars from the FOS's. For the design of this process the same temperature will be used.

2.2.4.3 scFOS Product Preparation

There has been work done on the applications of spray drying where processes have found to use dry air operating at temperatures above 100°C. A patent by Mysore *et al.* (2004) suggested that for preparation of FOS's, an inlet air temperature of 130 to 140°C is suitable and an outlet of 90 to 95°C is expected. Modler *et al.* (1993) used similar temperatures for the spray drying macerated Jerusalem artichoke tubers (JAT) for producing JAT flour. Air temperatures for the drying of FOS in this application were an inlet temperature of 200°C and an outlet air temperature of 95-102°C. In another application of the spray drying of *Lactobacillus paracasei* NFBC 338, Desmond *et al.* (2002) used a constant air inlet temperature of 170°C and the outlet temperature varied between 95 and 105°C. An industrial spray drying design application by Huang and Mujumdar (2007) used inlet and outlet temperatures of 250 and 110°C respectively, with a product flow rate of 1200 kg/h. The corresponding co-current air flow rate was 3.5-5.5 kg/s (12600-19800 kg/h).

From the experimental temperature values of the spray drying, the chosen temperature would be an inlet of the hot dry air of 180°C and an outlet temperature of the water vapour that has been 'caught' by the hot dry air of 90°C. The flow rate of the air would have to cater for an incoming product flow rate of 2 691 kg/h (for a scFOS production rate of 10 000 tonnes per annum), which suggests that the incoming air flow rate should be scaled up according to the ratio of 2 691/1200, which equates to 37 878 kg/h of hot air (appendix

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E.2 shows sample calculations for hot air required for an scFOS production scale of 2 000 tonnes per annum).

The evaporator was a unit operation that also needed to remove the moisture from the by-product stream. The by-product stream exited the bottom of the SMB chromatography column where most of the sugars of glucose, fructose and sucrose were found as well as a fair amount of water. The evaporator served its purpose to dry out the by product either for re-use or for sales. The evaporator was chosen to be operated at 105°C. This temperature choice was influenced by the work of Pridgeon and Badger (1924) and Radović *et al.* (1979).

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2.2.5 Process Hazards

In all process designs, the desire is to have a process that runs at optimal conditions, producing the most product with the least costs. This is an aspect that has good intentions but one must remember that the process must also run in a safe manner, i.e. contain any possible hazards and also comply with environmental regulations.

In the FFase production plant and subsequent scFOS production plant, it must be reiterated that scFOS is for human consumption and therefore one must ensure that the product is free of any components that could be harmful to humans.

During the FFase production, the FFase is produced using *P. pastoris* with either the GAPfopA gene or the AOXfopA gene. The AOXfopA gene uses methanol as an inducer during the enzyme production. This enzyme is used in scFOS production and thus poses a potential hazard in the process. It is both a process hazard in that it is a flammable substance and it is harmful to humans if consumed in quantities higher than 30 mg per day (USDHHS, 2012).

Environ© (2000) prepared a document for the GTC Nutrition Company to state that scFOS has been given the 'GRAS' status. The GRAS report is a large document that extensively researches scFOS to see whether it is safe for human consumption. It is positive knowledge to know that Environ© (2000) has given scFOS the status as generally recognised as safe.

According to the USDHHS (2012), in Table 2 of the document compiled by their department of health and human services, methanol's potential daily exposure is 30 mg per day and the concentration limit for methanol is 3 000 ppm, which is equivalent to 0.3%. In this process, it is mandatory to ensure that in the final scFOS product, the methanol content is below these figures stated above. The calculation shown in appendix E will show that the methanol composition in the scFOS is within the allowable limit.

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2.2.6 Process Costing

2.2.6.1 Chemical Engineering Plant Cost Index

The production of FTase and FOS's for annual production of 10 000 tonnes, where as shown in the design (Figure 2.6), the production is separated into two sections: a FTase production section, and a FOS production section (Vaňková *et al.*, 2008). Vaňková *et al.* (2008) has studied the costing of both the FTase and the FOS production section and costed them separately, to determine how much money is spent on the production of the separate processes.

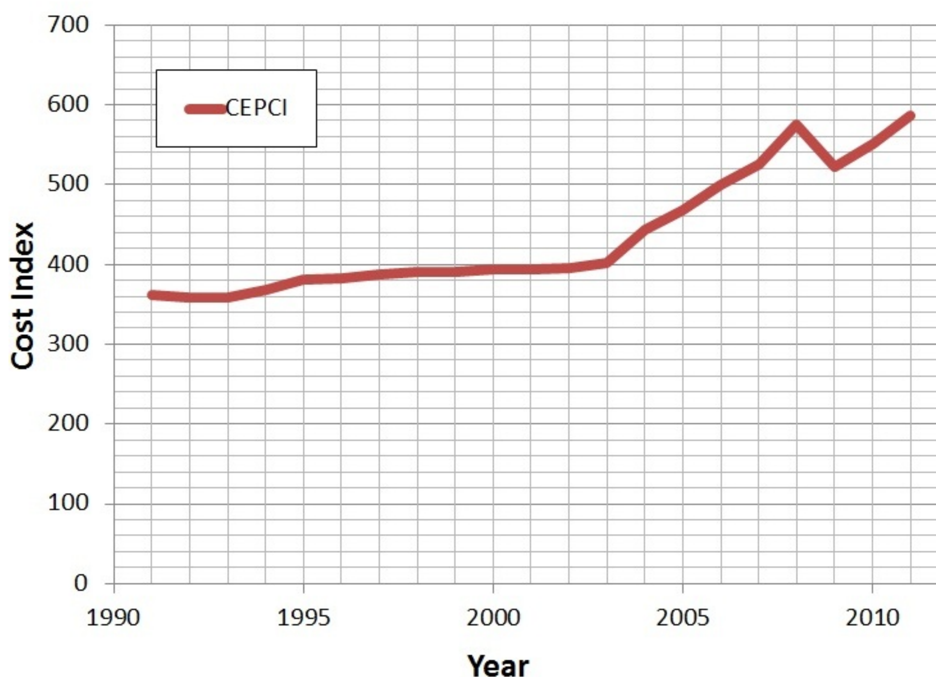

Figure 2.11: The chemical engineering plant cost index. Figures from 1991-2006 obtained from Turton *et al.* (2008), figures from 2007-2010 obtained from Scribd (2012) and the figure from 2011 was obtained from Cheresources (2012).

The list of equipment that was used for the FTase production process (Table D.2) showed the description of the unit used (that corresponds to figure 2.6) as well as the cost of the equipment given in Euros. This paper was written in 2008, so the figures would be adjusted according to the chemical

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engineering plant cost index (CEPCI). The CEPCI ensures that the historical prices of equipment units will be up to date with the present time (see Figure 2.11).

The CEPCI is an index that is based, like inflation, on the increase in cost with time. Turton *et al.* (2008) showed that the components of the CEPCI index were broken up into four main categories, where the equipment, machinery, and supports comprised of 61% of the total weighting. The remaining 39% was made up of erection and installation labour, buildings, materials and engineering of the plant.

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2.2.6.2 Costing Model

The costing of a production process is a task that has many components to take into account. Capital cost is one of the first factors to consider as this is the figure that possible investors would like to know when they have an interest in a project. The capital cost also includes many other costs which are based as a percentage of the equipment purchase cost. Potential investors would also like to have a good idea of the operating costs. This involves all the annual running costs, which includes raw materials, energy, utilities and labour that will accrue during each year of operation. Table 2.5 and table 2.4 gives a break-down of all the costs that are taken into account when performing a costing analysis of a production plant.

To begin with, the costing analysis must begin with the land that is available. This has to be the first factor to take into account so that the factory has a plot of land to be built on. The location of the land will be of much importance as it will have an effect on the transport costs of buying raw materials

Table 2.4: Costing method adopted from Petrides (2000) used to calculate the fixed capital cost estimation for the enzyme and scFOS production costs

COST ITEM	Average multiplier	Range of values
TOTAL PLANT DIRECT COST (TPDC)		
1. Equipment Purchase Cost (PC)		
2. Installation	(0.50 * PC)	0.2 - 1.5
3. Process Piping	(0.40 * PC)	0.3 - 0.6
4. Instrumentation	(0.35 * PC)	0.2 - 0.6
5. Insulation	(0.03 * PC)	0.01 - 0.05
6. Electrical	(0.15 * PC)	0.1 - 0.2
7. Buildings	(0.45 * PC)	0.1 - 2.0
8. Yard Improvement	(0.15 * PC)	0.05 - 0.2
9. Auxiliary Facilities	(0.50 * PC)	0.2 - 1.0
TOTAL PLANT INDIRECT COST (TPIC)		
10. Engineering	(0.25 * TPDC)	0.2 - 0.3
11. Construction	(0.35 * TPDC)	0.3 - 0.4
TOTAL PLANT COST (TPC)		
12. Contractor's fee	(0.05 * (TPDC + TPIC))	0.03 - 0.08
13. Contingency	(0.10 * (TPDC + TPIC))	0.07 - 0.15
DIRECT FIXED CAPITAL (DFC)	TPC = 12 + 13	

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Table 2.5: Costing method adopted from Choi and Lee (1997) used to calculate capital and operating costs for the enzyme and scFOS production costs

FIXED CAPITAL ESTIMATE SUMMARY	Method
A. TOTAL PLANT DIRECT COST (TPDC)	(physical cost)
a. Equipment Purchase Cost (PC)	(PC)
b. Installation	(summed over all units)
c. Process Piping	(0.35 * PC)
d. Instrumentation	(0.40 * PC)
e. Insulation	(0.03 * PC)
f. Electrical	(0.10 * PC)
g. Buildings	(0.45 * PC)
h. Yard Improvement	(0.15 * PC)
i. Auxiliary Facilities	(0.40 * PC)
TOTAL	
B. TOTAL PLANT INDIRECT COST (TPIC)	
a. Engineering	(0.25 * TPDC)
b. Construction	(0.35 * TPDC)
TOTAL	
C. OTHER COSTS (OTC)	
a. Contractor's fee	(0.05 * (TPDC + TPIC))
b. Contingency	(0.10 * (TPDC + TPIC))
TOTAL	
D. DIRECT FIXED CAPITAL (DFC)	(TPDC + TPIC + OTC)
ANNUAL OPERATING COST	
A. DFC-DEPENDENT ITEMS	
Depreciation	
Maintenance Material	(summed over all units)
Insurance	(0.01 * DFC)
Local Taxes	(0.02 * DFC)
Factory Expense	(0.05 * DFC)
TOTAL	
B. LABOUR-DEPENDENT ITEMS	
a. Operating Labour	(Working hours * rate * labourers)
b. Maintenance Labour	(summed over all units)
c. Fringe benefits	(0.40 * (a + b)
d. Supervision	(0.20 * (a + b))
e. Operating supplies	(0.10 * a)
f. Laboratory	(0.15 * a)
TOTAL	
C. ADMINISTRATION AND OVERHEAD EXPENSE	(0.6 * (a + b + c)
D. RAW MATERIALS	
E. OTHER CONSUMABLES	
F. UTILITIES	
G. WASTE TREATMENT/DISPOSAL	
TOTAL ANNUAL OPERATING COST	

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and sending out product. For the scFOS specifically, one would want to have a plant that is close to a sugar mill so that the sucrose is in close proximity to the scFOS plant.

Coincidently, in South Africa, sugar cane grows in the eastern parts of the country where the climate is conducive for good sugar cane production where in the summer months, there is a lot of heat and moisture. There also happens to be two large harbours on SA's east coast, namely Richards Bay and Durban harbour (see Figure 2.12). Richards bay and Durban harbours are areas that are close to large sugar mills and other industries. It would be a convenient location for a proposed FFase and scFOS production plant, as the scFOS would be produced close to the sugar mill where sucrose would be readily available and the close proximity to the harbour. Also in these sugar cane rich areas, there would be people that are experts in sugar production and similar sucrose derived products (such as fructose). It would be sensible to implement an scFOS production facility in an area such as this because there would be people with the knowledge in this field who could potentially provide expert knowledge in the field and make strong contributions to the success of the business.

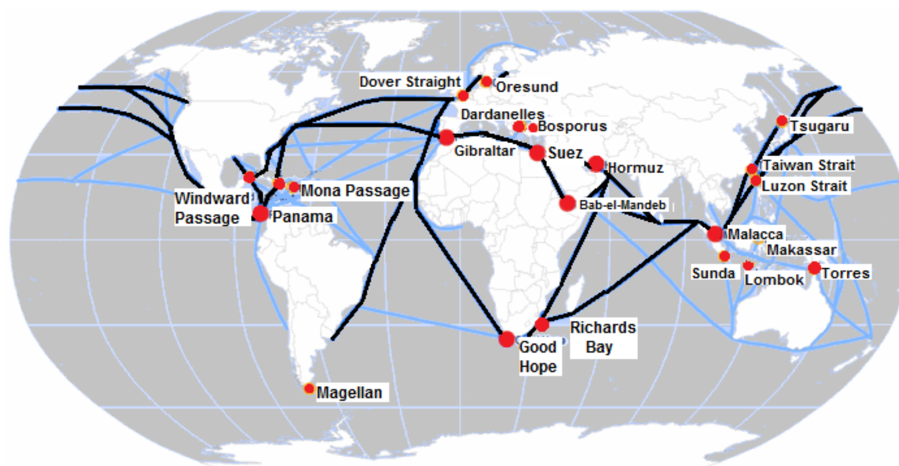

Figure 2.12: Different shipping routes around the globe (Redrawn). South Africa has access to Europe along both the east and west coastlines of the continent of Africa (Kumar, 2012).

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2.2.6.3 Engineering Economic Analysis

To decide whether a project will be viable or not, one needs to perform a costing of the proposed project. It is very important to ensure that all costs are taken into account so that the proposal is clear for possible investors to look at and determine whether it will be advantageous to them for investment.

To give a better idea of the economic analysis of the production plant, a cumulative cash flow diagram (see Figure 2.13) was set up to see the project lifespan, where this will give possible investors the opportunity to have a good idea if they would be interested in investing. The cash flow diagram shows the initial cost of the land and the fixed capital investment and the working capital costs. When the plant begins to operate, it will begin to pay back the initial amount of money that was invested into the facility.

The information that will be of interest to the investors is the time when the fixed capital investment is paid back otherwise known as the discounted payback period (DPBP). Also, the break-even period would be of interest and this is the time where the plant has paid back all its costs and starts to make profit from its production process. The next figure that is needed, is a projected value of the project at the end of its lifespan. The value must take into account that the money will devalue in the future due to inflation so that a true representation on the profits earned are shown. This figure is known as the net present value (NPV) and is a very important figure to present to investors.

The final figure that needs to be calculated for the cumulative cash flow analysis is the internal rate of return (IRR). The IRR is the discount rate that will cause the NPV to be equal to zero at the end of the project life. The IRR is a figure that possible investors will look at and their decision to invest will be strongly based on this figure.

The cash flow diagram corresponds to Table F.5, where the diagram is explained in tabular format. This method was based on a technique used by

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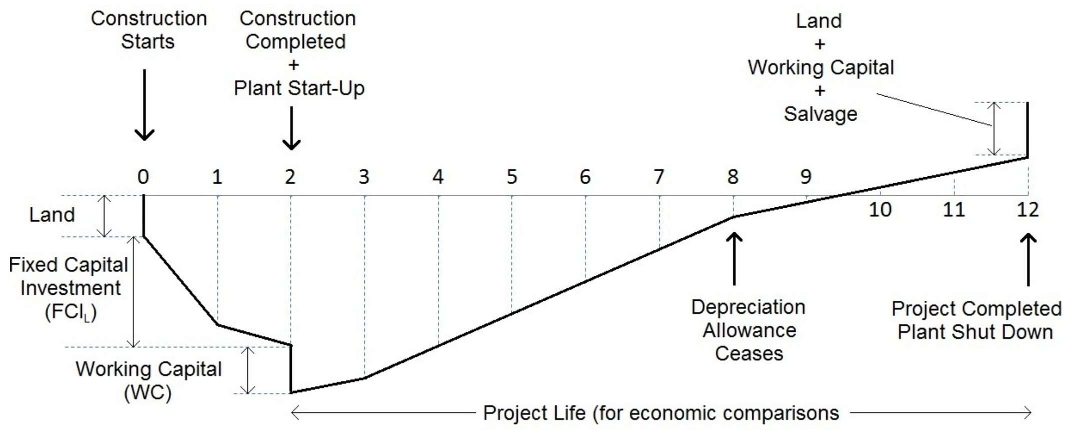

Figure 2.13: A cumulative cash flow diagram when evaluating a new project (Redrawn) (Turton *et al.*, 2008).

Turton *et al.* (2008) where the method of calculation is shown in Table F.5.

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Table 2.6: Discounted Cash Flow Table (Turton *et al.*, 2008)

Year End (k)	**Investment**	**d_k**	**FCI_L-Σd_k**	**R**	**COM_d**	**(R-COM_d-d_k)x(1-t)+d_k**	**Cash Flow**	**Cumulative Cash Flow**	**Discounted Cash Flow**	**Cumulative Discounted Cash Flow**
a	b	c	d	e	f	g	h	i	j	k
0	(Land)	-	d0	-	-	-	=b0	=h0	=h0/(1+r^{a0})	=j0
1	(FCI1)	-	d1	-	-	-	=b1	=i0+h1	=h1/(1+r^{a1})	=k0+j1
2	(FCI2)	-	d2	-	-	-	=b2	=i1+h2	=h2/(1+r^{a2})	=k1+j2
3	-	c3	=d2-c3	e3	f3	g3	=g3	=i2+h3	=h3/(1+r^{a3})	=k2+j3
4	-	c4	=d3-c4	e4	f4	g4	=g4	=i3+h4	=h4/(1+r^{a4})	=k3+j4
5	-	c5	=d4-c5	e5	f5	g5	=g5	=i4+h5	=h5/(1+r^{a5})	=k4+j5
6	-	c6	=d5-c6	e6	f6	g6	=g6	=i5+h6	=h6/(1+r^{a6})	=k5+j6
7	-	c7	=d6-c7	e7	f7	g7	=g7	=i6+h7	=h7/(1+r^{a7})	=k6+j7
8	-	c8	=d7-c8	e8	f8	g8	=g8	=i7+h8	=h8/(1+r^{a8})	=k7+j8
9	-	c9	=d8-c9	e9	f9	g9	=g9	=i8+h9	=h9/(1+r^{a9})	=k8+j9
10	-	c10	=d9-c10	e10	f10	g10	=g10	=i9+h10	=h10/(1+r^{a10})	=k9+j10
11	-	c11	=d10-c11	e11	f11	g11	=g11	=i10+h11	=h11/(1+r^{a11})	=k10+j11
12	-	c12	=d11-c12	e12+S	f12	g12	=g12+b12	=i11+h12	=h12/(1+r^{a12})	=k11+j12

Note: d_k = straight line depreciation; r = discount rate; S = Salvage value of the plant; t = tax rate (28%)

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2.2.7 Economies of Scale

In order for a production company to be successful, it needs to ensure that it is sustainable and makes a good profit. To make a good profit, of course one needs to produce a product that can be sold for a reasonable price while keeping the costs as low as possible.

With regards to industrial enzymes and scFOS production, there are different scales of production that can be considered. This would be based on the the amount of sugar that is available to be used in the scFOS production process and also the scale would be based on the viability of the production plant. There is no point designing a plant that is too large if it produces product that is too much for the demand of the market. Vaňková *et al.* (2008) proposed a design of a scFOS production rate of 10 000 tonnes per annum, which corresponds to a sucrose input of approximately 17 000 tonnes per annum. The range of production scales that were considered were: 1 000, 2 000, 5 000, 10 000, 20 000, 30 000, 40 000, and 50 000 tonnes per annum of scFOS production.

2.2.7.1 South African Sugar Production

An attractive aspect about the possibility of producing scFOS in South Africa is that there is sucrose that is readily available. This means that the transport costs will be much less when comparing with importing the raw material.

South Africa's export sugar for the past 5 years is approximately 850 000 tonnes per year and their total production rate of sugar has been above two million tonnes per year (except for 2011 because of bad droughts), see Figure 2.14 (SASA, 2012).

It is important to have the sugar information available for scFOS production so planning can be done about production levels because the trends in raw material production will show what to expect.

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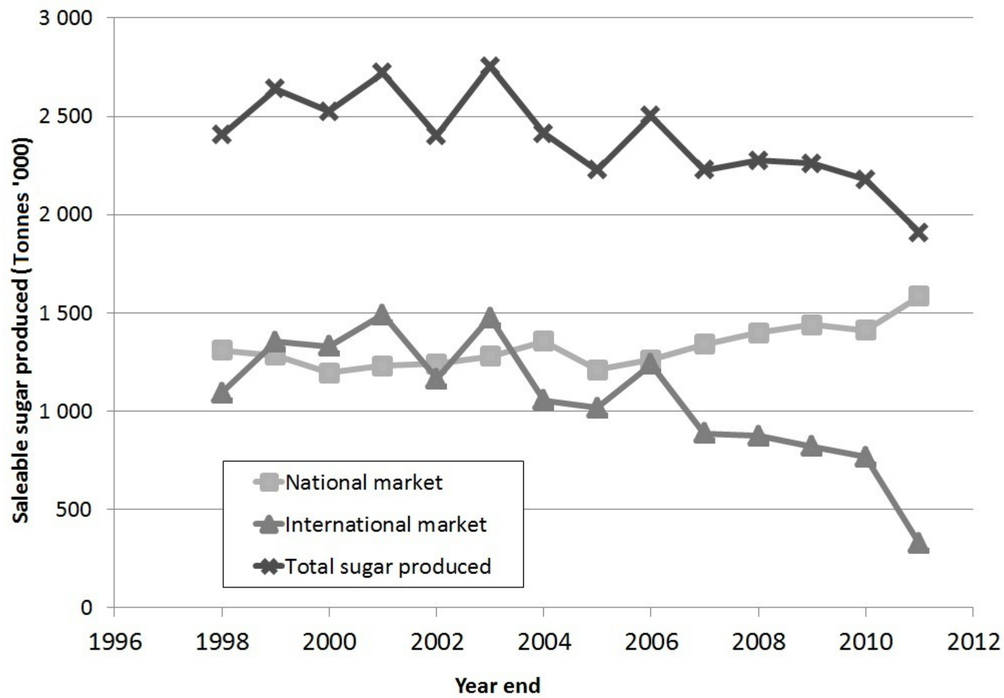

Figure 2.14: South Africa's Sugar Production (SASA, 2012)

2.2.7.2 Six tenths Rule

When costing a plant, one obviously needs to consider many costs that are involved with the start-up and running of the plant. Costs can be broken up into initial investment costs, fixed costs and running costs. This research involves the subject of the costing of a large/industrial scale enzyme and scFOS production facility. With regard to the initial investment or capital cost, the six tenths rule linked with economies of scale is a topic of high interest. The six tenths rule is given by equation 2.2.1, where the cost of equipment and the increase in capacity of the unit of operation is related (Tribe and Alpine, 1986), (Turton *et al.*, 2008).

$$C_B = C_A \left(\frac{A_B}{A_A} \right)^{\alpha} \tag{2.2.1}$$

where A = equipment cost attribute

C = purchased cost

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α = cost exponent

Subscripts: A refers to the equipment with the required attribute

B refers to the equipment with the base attribute

Equation 2.2.1, shows the relationship between the cost attribute of the equipment and the purchased cost, raised to the power of α. The six tenths rule, as the rule suggests, assumes that the value of α is equal to 0.6. This highlights an important concept of economies of scale and leads to the following generalisation according to equation 2.2.1:

> *The larger the equipment, the lower the cost of equipment per unit of capacity* (Turton *et al.*, 2008).

Fourie *et al.* (2008) backs this quote by saying that a firm will experience economies of scale if the production scale increases. This is the reason that there is an interest in the implementation of an industrial scale production facility.

The influence of the six tenths rule is represented graphically (Figure 2.15). It can be observed that as the cost attribute increases, the purchase cost will also increase as expected, but when the six tenths rule is applied, it will not increase as dramatically as the linear plot. Instead, the exponential line moves further away from the linear plot as the cost attribute increases. The same concept is illustrated in Figure 2.16.

This is why the scale up of a plant is attractive provided the product that is produced in the facility will exceed the demand of the market.

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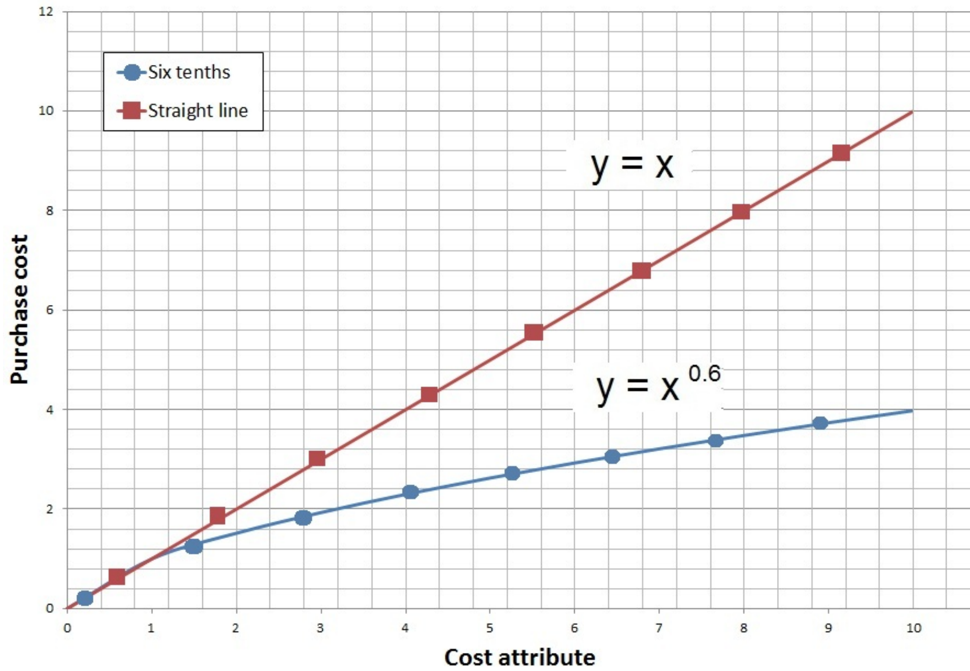

Figure 2.15: The representation of the effect the six tenths rule has on the cost of equipment as it increases in size.

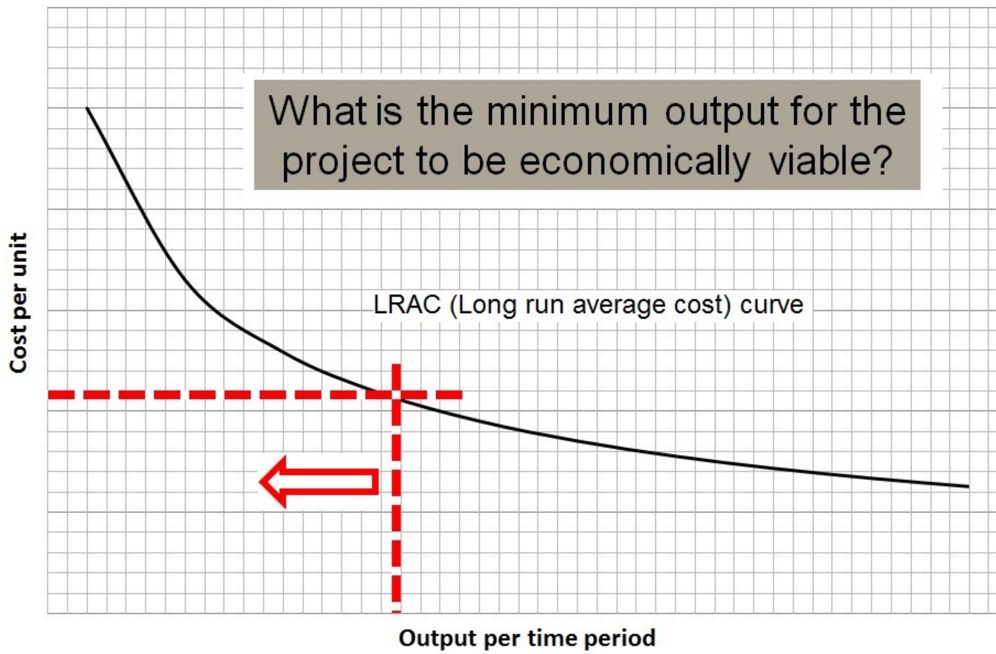

Figure 2.16: The cost per unit decreases as the output per time period increases illustrating economies of scale (Fourie *et al.*, 2008).

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2.2.8 ScFOS Markets

In order to be able to make money out of the proposed investment, the product that is produced in this facility needs to be sold. Information about the various markets is therefore very important and of very high interest.

ScFOS is a product that has recently become of interest in South Africa following a joint venture project with Stellenbosch University and Rhodes University. The product of scFOS is already well established in east Asia, which include countries such as Japan, South Korea, Taiwan and China. There is also other interest for the product in Europe and North America.

Charalampopoulos and Rastall (2009) said that in 1995, the global market for scFOS produced from sucrose was estimated to be 20 000 tonnes. They stated further that the growth rate for oligosaccharides was 15% per annum. If this was projected to present time the current scFOS global market demand would be around 240 000 tonnes. Nakakuki (2002) said that the fructooligosaccharide market was 6.5% of the global demand of prebiotics. The demand for prebiotic oligosaccharides in Japan was found to be 69 000 tonnes per year (Charalampopoulos and Rastall, 2009). It is important to remember that fructooligosaccharides are considered to exist in the field of prebiotics and furthermore, within the field of prebiotics, there is a group in oligosaccharides where there are many other types of oligosaccharides such as mannan-, xylo-, and galactooligosaccharides to name a few.

In a news article by 21tradenet (2010), it is stated that Japan's leading scFOS manufacturer, Meiji Seika Kaisha using their product 'Meioligo' found that its market share was 3 000 tons in 2007. An article by Sinosweeteners (2012) said that the development of functional oligosaccharides have opened up many new industrial applications and in 2002, the global production of oligosaccharides reached 150 000 tons. This created a US$40 billion functional food market, a US$10 billion functional (animal) feed market. If this figure of 150 000 tons was project with a CAGR of 15% and 6.5% of this value

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was assumed to be FOS's, then the current FOS market would be 45 000 tons. Oligosaccharides also showed opportunities listed in drugs, pesticides oligosaccharides and oligosaccharides fertilizer. After 1995, China's functional oligosaccharide began to produce oligosaccharides at an appreciable scale. The products listed were maltose oligosaccharides, fructooligosaccharides, galactooligosaccharides, soybean oligosaccharide, stachyose and some others (Sinosweeteners, 2012).

A study done by Nakakuki (2002) determined the oligosaccharide demand in Japan in 2002 (see Figure 2.17).

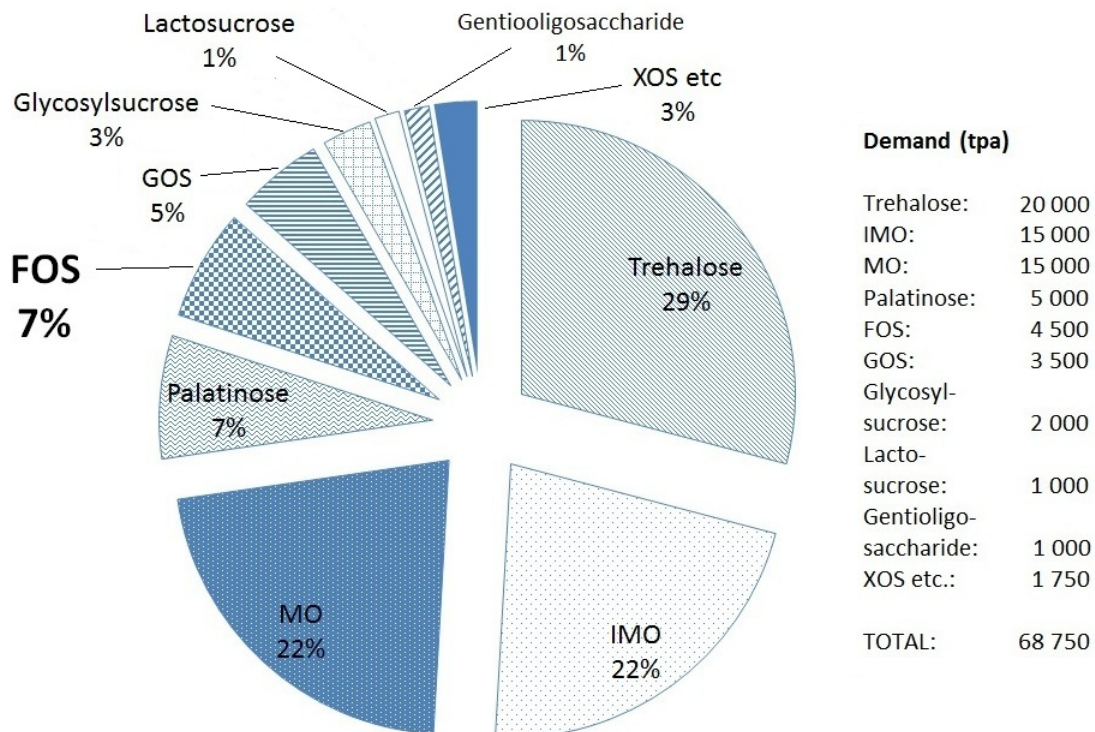

Figure 2.17: Japan's oligosaccharide market demand based on a study done in 2002 (Nakakuki, 2002).

As it can be seen in Figure 2.17, the FOS in Japan contributes to 7% of the total oligosaccharides which is 68 750 tonnes per annum (4 500 tonnes per year of FOS). If this figure of 4 500 tons was projected to 2013 with a 15% CAGR,

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then the present FOS market in Japan would be 21 000 tonnes. Taniguchi (2004) also did a study on the oligosaccharides production in Japan and found information that is shown in Table 2.7. It can be seen that this information correlates similarly to the figures given by Nakakuki (2002).

In an article by Watson (2011), they state that the United States prebiotic market is forecast to double in the next five years to a figure of greater than US$ 220 million. A research associate by the name of Tejaswini Prabhu said that the prebiotic market in the United States is estimated at US$ 110 million. Of this value, 35% is for inulin, 25% is for mannan oligosaccharides (MOS) and 10% is for fructan oligosaccharides (FOS). Therefore the FOS market would be US$ 11 million and if the price of scFOS was taken to be US$ 10/kg, then the FOS market would be 1.1 million kilograms or 1 100 tonnes.

GIA (2012) released a comprehensive report on the prebiotics market and they predicted also that the United States market would reach US$ 225.31 million by 2015, which is close to the previous figure given of US$ 220, and the European market would reach US$ 1.17 billion by 2015. If it was assumed that scFOS is 6.5% of the total prebiotics market (Nakakuki, 2002), then the US market would be US$ 14.3 million and the European market would be US$ 76.1 million. If the price of scFOS was taken to be US$ 10/kg, then the US market would be 1.43 millions kilograms or 1 430 tonnes and the European market would work out to be 7 610 tonnes. Having looked at all figures given the the respective sources, the global scFOS market could be assumed to be approximately 50 000 tonnes.

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Table 2.7: Oligosaccharides production in Japan (Taniguchi, 2004)

Oligosaccharides	**Production (tpa)**	**Biocatalyst**	**Price (Yen/kg)**	**Maker**
Trehalose	25 000	Transglucosidase	300	Hayashibara Biochemical Laboratories Inc.
Isomaltooligosaccharides	11 000	Transglucosidase	140	Showa Sangyo Co. Ltd
Galactooligosaccharides	6 000	β-galactosidase	500	Yakult Pharmaceutical Ind. Co. Ltd., Nissin Sugar
Fructooligosaccharides	4 000	β-fructofuranosidase	390	Meiji Seika Kaisha Ltd
Lactulose	2 800	Chemical isomerisation	1 000	Morinaga Milk Industry Co. Ltd
Lactosucrose	2 000	β-fructofuranosidase	500	Bioresearch Corporation of Yokohama
Cyclodextrins	1 800	Glucanotransferase	1 450	Bioresearch Corporation of Yokohama
Soy Oligosaccharides	1 000	Extraction	700	Calpis Co. Ltd
Gentiooligosaccharides	1 000	β-glucosidase	300	Nihon Shokuhin Kako Co. Ltd
Xylooligosaccharides	650	β-xylosidase	2 500	Suntory Ltd
Nigerooligosaccharides	300	α-glucosidase	300	Nihon Shokuhin Kako Co. Ltd
Raffinose	230	Extraction	2 000	Nippon Beet Sugar Mfg Co. Ltd
Palatinose	150	Glucosyltransferase	1 000	Shin Mitsui Sugar Co. Ltd

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2.2.9 β-D-fructofuranosidase Market

2.2.9.1 FFase Markets and Freedom-to-Operate

With the production of FFase and the subsequent production of scFOS, there is also an interest to know whether, in the dual production process of FFase and scFOS, there is a market for FFase alone? If this happened to be the case, FFase could be produced in excess so that the demand for scFOS production is met, while still having FFase left over for extra sales. This would be advantageous since it allows for FFase production at a larger scale thus realising lower production costs and improved returns on investment.

Before one can sell these products, one needs to know if it is allowed to produce these product first of all. There are patents that exist regarding the production of FFase as well as scFOS and one must be careful not to infringe on any of these patents as this is illegal and could cause potential trouble.

The first issue was to distinguish whether FFase (FopA enzyme) can be used for the production of scFOS and furthermore, can it be sold on the local and international markets. The patents US6337201 by Yanai *et al.* (2008) was examined and it was found that there are versions of this patent that match and have been filed, and are being implemented in Japan, Taiwan, and South Korea but no equivalent version has been granted in Europe and South Africa. The patent US6337201 implies that the FopA enzyme produced by the consortium cannot be sold in the USA, Japan, Taiwan or South Korea without infringing on the aforementioned patent. [1]

On the contrary, the consortium is allowed to commercialise the FopA technology in two ways: i) FopA enzyme may be produced in South Africa and exported to all countries in the world except for USA, Japan, Taiwan or South Korea. ii) FopA that is produced in South Africa may be used to

[1]The consortium is the association involved in the scFOS project which consists of TIA, University of Stellenbosch, and Rhodes University. TIA is a South African governmental organisation that stands for Technology Innovation Agency. TIA strives to enhance technological innovation to improve economic growth and quality of life for all South Africans.

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produce scFOS in South Africa and the scFOS product produced can be sold anywhere in the world.

From this information, it would be a good idea to know what the demand for FFase is on a local and global scheme as this FFase and scFOS production scheme could be able to sell scFOS and FFase together, which may increase the incentive for possible investors.

It is known that there is an increasing global demand for scFOS, and consequently, there is a need for FFase for scFOS production. Venkateshwar *et al.* (2009) backs this up by saying that the development of this line of research has become very significant and that the demand for FFase is increasing.

2.2.9.2 China Market Research Center

The China Market Research Center (CMRC), have compiled a market research report on China's β-D-fructofuranosidase only for scFOS Production. This report entailed a detailed analysis of the global β-D-fructofuranosidase market only for scFOS production which covered analyses such as operation and development, market environment, market competition, import and export, leading enterprises and development trend forecast (CMRC, 2012).

CMRC (2012) found that their country's market demand for β-D-fructofuranosidase only for scFOS production has been increasing steadily over the past three years. This would indicate that the market for scFOS is increasing also. Royal DSM is a company that is internationally known to produce β-D-fructofuranosidase as well as a large company that produces enzymes on an industrial scale by the name of Novozymes®. The important fact to note is that some countries manufacture β-D-fructofuranosidase, but it is not necessarily used for scFOS production. In some cases the β-D-fructofuranosidase is used to produce invert sugar and the β-D-fructofuranosidase is effectively an invertase. Royal DSM actually produces a β-D-fructofuranosidase that can only reduce sucrose to glucose and fructose (an invertase). Novozymes®

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produces a product called inulinase, which is an enzyme that breaks down inulin to form the fructooligosaccharides. This product of inulinase would not compete with β-D-fructofuranosidase for scFOS product as it performs a completely different function. Inulinase breaks down inulin to for scFOS, while β-D-fructofuranosidase (specifically for scFOS production) builds up sucrose to form scFOS. CMRC (2012) states that there is knowledge that there are other companies in the USA, France, Japan, and others that produce β-D-fructofuranosidase for scFOS production, but not on a large scale.

CMRC (2012) says that there is knowledge that other companies such as the USA, France, Japan, and others that produce β-D-fructofuranosidase for scFOS production, but not on a large scale. It was of recent knowledge that China are developing a technology for FFase and scFOS production, which will be implemented in a new manufacturing facility in China (Personal communication: Jorge Valdes Hernandez, Centro de Ingeniería Genética y Biotecnología (CIGB), Havana, Cuba). According to CMRC (2012), there are few countries that produce β-D-fructofuranosidase on an advanced level and the main areas are Holland, USA and Japan to name some important countries. It is concluded that the international producers of β-D-fructofuranosidase are relatively small, without strong market competition.

Everyone strives to improve their quality of life, and by doing so, this will mean that people will strive for better health, which mean that products like scFOS will have an increasing demand. If scFOS has an increasing demand; the enzyme of β-D-fructofuranosidase will have an increase in its demand, therefore the production and development of β-D-fructofuranosidase will definitely have a promising future. The present situation in China is that they are importing the β-D-fructofuranosidase but CMRC (2012) say that in the future, domestic research will take place and the enzyme will be developed locally creating an enzyme that should be available at a cheaper price and ultimately replace the imported enzymes. China's β-D-fructofuranosidase demand can be shown in

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Figure 2.18. As one can see, there is growth from 2008 and 2011, which shows to be a promising trend for the future of the β-D-fructofuranosidase market.

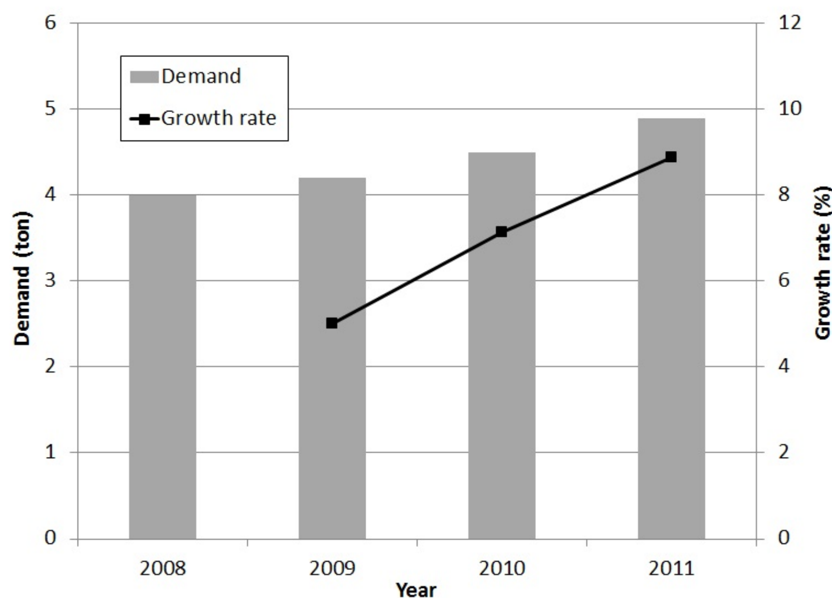

Figure 2.18: China's β-D-fructofuranosidase Market Demand Statistics from 2008 to 2011 (CMRC, 2012).

According to CMRC (2012), the import volume of β-D-fructofuranosidase was greater than 10 000 tons in 2008. The imports decreased in 2009 and stayed that way in 2010, but in 2011, the imports began to increase again (see figure 2.19). The decrease in imports in 2009 and 2010 could be due to increasing local β-D-fructofuranosidase manufacturing through domestic research as stated previously. The slight rise in imports thereafter could be as a result of increasing demand of scFOS as awareness of the product increases and therefore increasing β-D-fructofuranosidase demand.

Regarding the export situation, the exports between 2008 and 2011 grew every year, where there was good growth between 2009 and 2010, but from 2010 to 2011 the exports only grew by 141 tonnes (CMRC, 2012). The decrease in exports could be due to local demand increasing which meant that not as

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much β-D-fructofuranosidase could be exported but rather used to meet local demand. The export volumes reached 82 000 tons in 2011 (Figure 2.20).

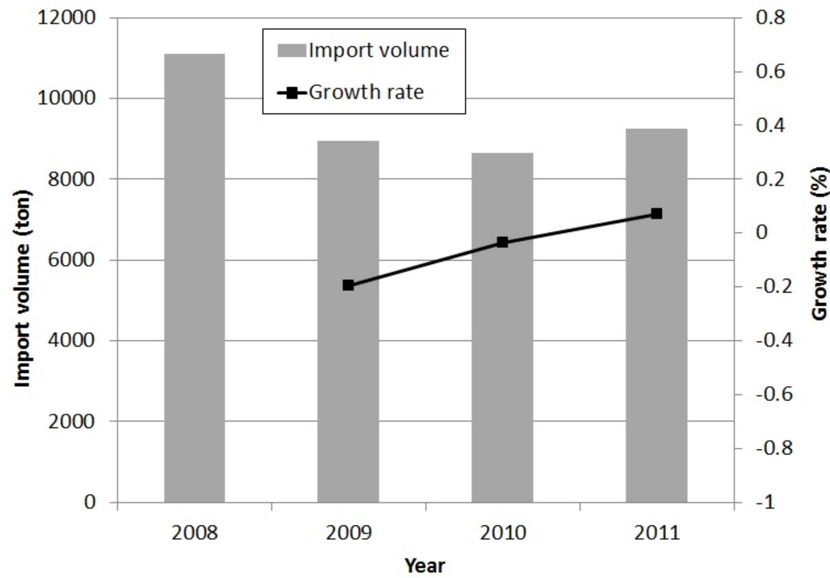

Figure 2.19: Import volume of China's β-D-fructofuranosidase products from 2008 to 2011 (CMRC, 2012).

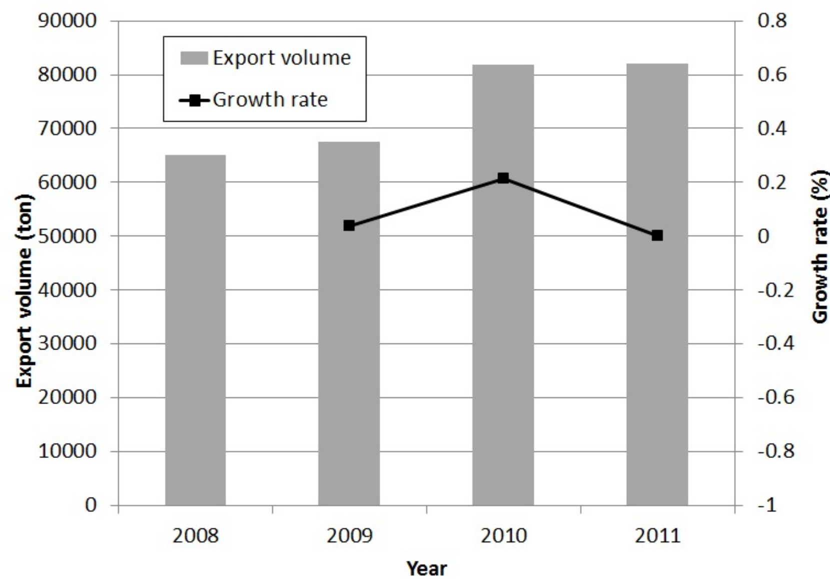

Figure 2.20: Export volume of China's β-D-fructofuranosidase products from 2008 to 2011 (CMRC, 2012).

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CMRC (2012) claim that at present, the total annual production of domestic oligosaccharides (all types of oligosaccharides) amounts to approximately 100 000 tons, and the annual demand of oligosaccharides in China has reached over 200 000 tons in 2011. As China is the most populated country in the world, it would be of useful knowledge to observe their trend with regard to their scFOS demand. East Asia would be a good indicator to follow to predict trends around the globe for further β-D-fructofuranosidase and scFOS development as countries in this part of globe have knowledge in scFOS production. Japan does sell one of the stronger brands of scFOS known as Actilight® and are therefore leaders in scFOS production (Vaňková *et al.*, 2008). China is also an influential East Asian country and the fact that they have a very large population would suggest that they would be a significant player in the scFOS market trends.

CMRC (2012) have forecasted their predicted market growth for the next three years (Figure 2.21) and it can be seen in the graph that the demand is predicted to grow at a steady rate every year until the year 2015. This will take the demand to around 6.1 tons of β-D-fructofuranosidase for the year 2015.

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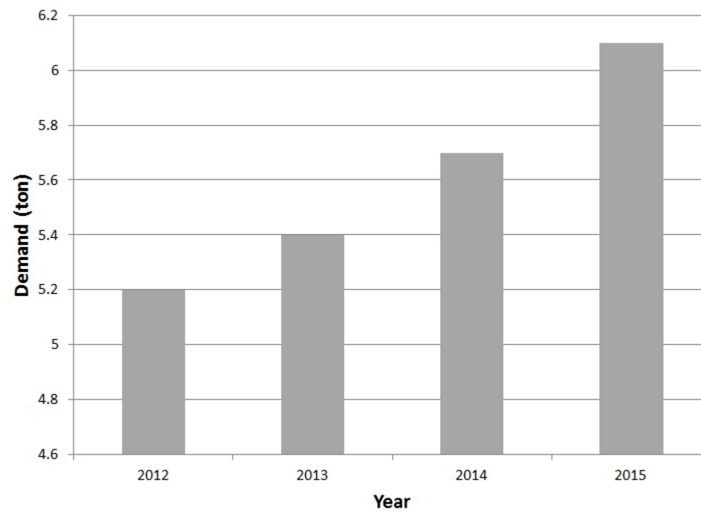

Figure 2.21: China's β-D-fructofuranosidase demand forecasting from 2012 to 2015 (CMRC, 2012).

2.2.10 Environmental Impact

Environmental awareness is always a factor of concern in process engineering to ensure that the implementation of the work does not disturb the surrounding system. Falch (1991) show this by illustrating how the enzyme production process, fits into an ecological loop.

Falch (1991) shows in Figure 2.22 that enzyme production can become part of a sustainable ecological loop. The unused matter that is of no use to the enzyme production plant, can be collected as a sludge and can then be spread on a farmland so it may be processed and decomposed (Larsen *et al.*, 1991). The compositions of the sludge includes: dead biomass, filter aid, nutrient surplus and insoluble debris (Falch, 1991).

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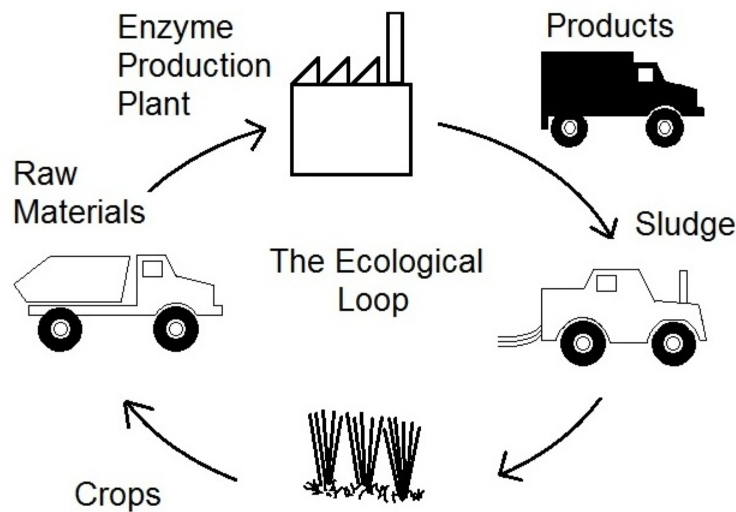

Figure 2.22: Enzyme production fitting into to an ecological cycle (Redrawn) (Falch, 1991).

2.3 Common Recommendations and Problems

Short-chain fructooligosaccharides production and β-D-fructofuranosidase production have been researched extensively for many years. The main challenge that is at hand is to ensure that one produces the scFOS of a high quality so that it is competitive in the international and national markets. In order for this to be a success, one will need to look at the product that is presently setting the standard. This product is Actilight® and it sets the standard of the quality what the GF_2, GF_3 and GF_4 ratios should be. To try and obtain the best quality scFOS while keeping costs down, a good enzyme needs to be produced.

The optimal conditions for the production of FFase are at a temperature of 30°C in the fermenter at a pH of 5.5 at atmospheric pressure. The agitation and the carbon food source must be linked so that the organism has the right sustenance and it can respire as needed so it can continue to grow and ferment properly to ultimately produce the highest concentration of enzyme possible.

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To produce scFOS, a variety of enzymes can be used. The most popular ones have shown to be fructosyltransferase and β-D-fructofuranosidase. This research focuses on scFOS production using β-D-fructofuranosidase. When considering the enzyme application for scFOS production, the enzyme must experience conditions, where its activity is at a favourable level. The pH in the bioreactor has shown to have an effect on the activity of the enzyme. Hydrolase/invertase activity, which separates the sucrose molecule into glucose and fructose, is favoured at pH 4 and transferase activity, which adds fructose molecules to form longer chain fructooligosaccharides, is favoured at pH 6 (Fernández *et al.*, 2004), (Katapodis *et al.*, 2003). It is important to know that glucose inhibits the enzyme, which decreases FOS production (Yun, 1996), (Duan *et al.*, 1994), (Jung *et al.*, 1989). This is why it is important to ensure a balance between the two enzyme activities. The best recommended conditions for scFOS production are a reactor temperature of 60°C and a pressure of 1 atmosphere and the pH must be around 5.5.

In terms of the equipment used for both the FFase and scFOS production processes. The recommended equipment for the FFase production process would be the standard procedure for enzyme production. It would consist of a starter culture lab, followed by a fermenter. A centrifuge removes the dead cells after fermentation and the broth is filtered further through nano-filtration. The enzyme would then be kept in cold storage rooms to preserve the enzyme.

Regarding recommended equipment for scFOS production; a CSTR would be the preferred choice of reactor because the FFase exists in the supernatant. Also, a CSTR can be run in the batch and semi-batch operation if need be. A buffer tank needs to be present after the reactor to control flow into the SMB chromatography column. The product from the column will then be prepared for packaging through a spray dryer to remove all moisture. The by-product is also treated by an evaporator to remove moisture for additional sales.

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Chapter 3

Hypothesis and Key Questions

Presently in South Africa, there is no commercial industrial enzyme production facility. The way companies have been getting around this problem is by importing their desired enzymes from overseas for their various industrial activities. Importing does not give any benefit to South Africa's economy and also to the companies that require enzymes. The companies would potentially pay higher prices for products attained overseas because of issues such as transport fees, import duties and exchange rates to name a few contributing factors to higher costs. Regarding South Africa's economy, producing enzymes locally would hopefully offer a lower price and jobs would be created through the construction, running and maintenance of the plant and business as a whole.

Another feature that is not desirable for companies that are obtaining their enzymes from overseas lands, are that the companies would have to manage their supply chain very carefully. If not, problems such as long arrival times or over ordering of raw materials could occur. With this issue, the companies will have to carefully plan their orders to ensure that they meet their local demand while not having too much stock on hand, but at the same time, have enough stock to be able to produce enough of their product for the local markets.

Another issue from the fact that the industrial enzymes are being bought from overseas lands is that enzymes tend to lose their reactive activity if they are left without being used for too long. The enzymes also need to be stored

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at low temperatures to preserve their activity level. To import enzymes would require a transport unit that would have to transport the enzyme in a timeous manner while maintaining the enzyme at a temperature of 4°C, which could turn out to be costly.

Some important questions in this research that need to be answered are:

- Is it feasible to create a facility that focuses and produces one enzyme or does one create a multiproduct facility, where many different enzymes are manufactured, be it commodity products, medium scale or high value products?

- Is it feasible to create a facility that produces both FFase and scFOS in the same facility or buy FFase from an external producer to produce scFOS? And at what scales?

- Can an economic model be developed as a tool to determine if a multi-product facility for industrial enzymes production in South Africa that is an economically viable investment?

- What is the demand for these enzymes and how much does it cost to manufacture the enzymes?

Hypothesis: FFase and scFOS production will be a viable economic venture provided the company can sell all of their product that they produce.

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Chapter 4

Design Approach

4.1 β-D-fructofuranosidase and ScFOS Process Outline

The core of the production facility is to produce enzymes on an industrial scale. The enzymes will be produced throughout the year based on their demand. FFase, as mentioned before is used in the production of the scFOS process, therefore the demand of FFase will be based on the availability of sugar as well as the market demand for scFOS.

When producing enzymes on an industrial scale, the culture first needs to be developed in the laboratory. Organisms must be grown from the Petri dish and developed further until they can become stable enough to be able to be used as inoculum for large scale fermenters. Fermenter sizes are scaled according to their demand for scFOS production. The organism grew in the first fermenter until it reached a population where it needed to be transferred into the final fermenter. The final fermenter was sterilised with high temperature steam to ensure a sterile environment for the culture. The final fermenter facilitates the actual fermentation process where the enzyme is produced.

The product from this fermenter was then sent to a large centrifuge where 98% of the cells were removed. The product was sent to storage where the temperature was maintained at 4 degrees Celsius.

FFase was then taken to the scFOS production process, where the enzyme

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came into contact with the sucrose. FFase and sucrose were the main raw materials for the scFOS production process. The sucrose and the FFase were sent into a continuously stirred tank reactor which operated at 60 degrees Celsius. The scFOS was formed in the reactor and was then sent to a simulated moving bed chromatography column, which purified the enzyme to a purity of approximately 90 percent. The purified scFOS was then dried using a spray drier so it can be in a powder form for sales. The by-product was also dried using an evaporator and was sent for sales.

When implementing the costing of a proposed design, a plan of the project needs to be set in place so that all aspects are covered and the project goes in the direction as planned. Figure 4.1 gives an idea of the how the project was planned.

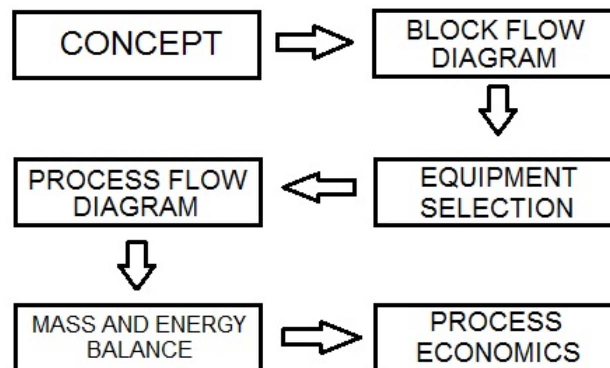

Figure 4.1: Generic mind map of the process when designing and performing a costing analysis of the implementation of a production facility.

As with all projects, one needs to come up with an initial idea or concept. The initial idea for this project, which has been stated before, is the question of the viability of the implementation of an enzyme production facility on a commercial scale. Furthermore, the construction of an enzyme production

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facility in conjunction with an scFOS production process and the question of viability of this process is also of interest.

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4.2 Process Description

In order to design the process of FFase and scFOS production, a detailed description of the process is needed, which will include details of exactly how the process will be run. Details will include quantities of incoming and outgoing streams of the process, as well as unit operations and an explanation of how they will be run according to their operating parameters.

The production process of FFase and scFOS are mutually exclusive as the scFOS production needs FFase as an enzyme and sucrose as the sugar to produce the FOS's. As a result, the processes needed to be operating in a sequential set-up.

4.2.1 β-D-fructofuranosidase Production Process

4.2.1.1 Process Overview

In order for scFOS to be produced, the enzyme of β-D-fructofuranosidase needed to be manufactured so that it can effectively perform its biochemical reaction with sucrose in the scFOS process.

The strain of *Pichia pastoris* was genetically engineered by biochemists to ensure that they employed an enzyme with the desired genes and that the organism was able to express these genes when the enzyme was being produced.

In the proposed production facility, the yeast of *P. pastoris* will be grown in Petri dishes in the preparation laboratory, where they will be grown to a size that is sufficient for them to be transferred into fermenters. The yeast will grow in the fermenters until it reaches a sufficient enough population for actual fermentation to start. The fermenters are sterilised before the culture enters the vessels. This is an imperative part of the process to ensure that the production medium will not become contaminated when entering a new vessel. The media is then sent to the final fermenter where the β-D-fructofuranosidase is produced.

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The enzyme is secreted into the supernatant which means that the remaining unwanted yeast cells will need to be removed from the enzyme 'broth'. The method to remove the unwanted cells is through centrifugation.

In any design project, one of the first steps in the design process is to develop a block flow diagram. The block flow diagram will determine the steps in the process that will convert the raw materials into the product. The block flow diagram will also give a good idea what process unit will be suitable for the respective steps in the production.

Before the process flow diagram can be drawn, an understanding of the enzyme production process needs to be attained where a detailed knowledge of the each unit operation must be known. Figure 4.2 shows a simple block flow diagram, where the process steps are shown between the incoming raw materials and the production of the enzyme.

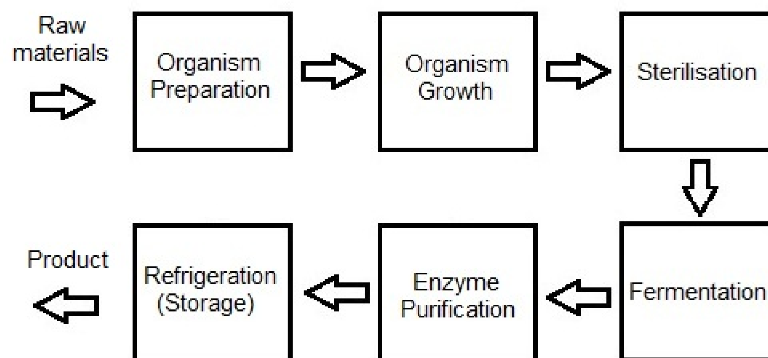

Figure 4.2: Enzyme block flow diagram illustrating the concept behind the design of the process.

4.2.1.2 Equipment Selection

When designing a production facility, the function of the process must be understood. There must be clarity in the goal of the process. A fair idea may be in mind for the process flow diagram of the plant, but before this can happen,

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one must break the process down into steps and ensure that the correct piece of equipment is chosen to satisfy the intended function of that step in the process.

Culture Preparation

To produce the enzyme, the culture needs to be prepared in a very careful manner to ensure that the organism grows in an optimal manner without contamination. The organism of *Pichia pastoris* was used as it is known that *P. pastoris* can grow to very large biomass quantities. The organism is placed initially in a Petri dish to be allowed to grow to a large enough population so that it can be transferred into a test tube.

The *P. pastoris*, once transferred into the test tube is then treated with a small amount of production medium. These are the nutrients needed for the organism to grow and survive inside the test tube and allow for the population to increase in size so that it can be moved to a larger vessel. Once the organism has grown to a sufficiently sized population, the culture is then transferred to a shake flask where it is treated with more production medium so that the culture can grow further.

The culture will then be sent to a seed train of fermentation tanks (increasing in size) where the culture will grow and will then be transferred to the larger tank when necessary.

Fermentation

When the population of the yeast *P. pastoris* has reached its maximum and the culture is in the final fermenter, the actual fermentation process begins. The enzyme of β-D-fructofuranosidase is secreted from inside the yeast cells out into the fermentation volume or into the supernatant. After fermentation, the tank is left with the enzyme that exists in the supernatant and the dead *P. pastoris* yeast cells at the bottom of the tank.

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Purification

Once the fermentation is complete, the dead yeast cells need to be removed from the supernatant so that the enzyme can be stored for further use in other processes. The dead yeast cells are removed through centrifugation and the supernatant is sent through ultrafiltration to remove any extra impurities. The protein now becomes more concentrated as the impurities are removed. The dead yeast cells are removed from the process, and can be used in the ecological loop for crop fertiliser.

Product Storage and Preparation

Once the enzyme has been produced; it is very important to store the enzyme at the correct conditions to ensure that the enzyme does not degrade. If the enzyme degrades then ultimately, it will ruin the quality of the short chain fructooligosaccharides in the scFOS process. The enzyme, after purification, needs to be stored at 4°C. This is imperative for the performance of the enzyme when it is used in downstream processes so that the enzymatic activity remains at its optimal value.

4.2.2 ScFOS Production Process

4.2.2.1 Process Overview

The scFOS production process follows a generic process pattern but the benefit is that the raw materials coming into the process do not need to be pre-handled, but only diluted. The block flow diagram is shown in Figure 4.3. The sucrose will arrive in bags where they will be mixed with hot water to bring the concentration down to the correct reaction specification. The FFase and the sucrose are sent straight to the bioreactor where the reaction can take place. There is a buffer tank that is placed after the reactor, to provide a flow control between the reactor and the SMB chromatography column.

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The purification step concentrates the product to a higher level, after which the product is then prepared for selling. The by-product, which also has its uses is also sold to a willing buyer in the sugar industry.

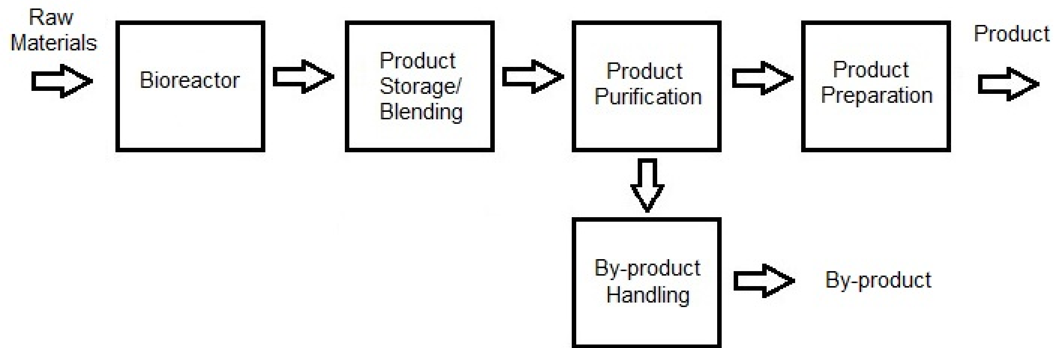

Figure 4.3: ScFOS block flow diagram illustrating the concept behind the design of the process.

4.2.2.2 Equipment Selection

Reactor

There were two different types of reactors that were considered for the operation of the scFOS process. The first reactor setup is a packed bed reactor (PBR). With this type of reactor, the enzyme needs to be fixed in a bed and the sucrose would flow through the fixed enzymes and react to form the scFOS. The challenge with this reactor setup is issue of immobilising the enzyme.

β-D-fructofuranosidase can be produced in numerous ways using different organisms and production techniques. Also, depending on the production method, the enzyme can be produced within the cells of the organism or the enzyme can be secreted in the supernatant. If the PBR reactor setup is chosen, the enzyme would have to be produced in a way that is conducive for the enzyme to be immobilised.

The other way to produce the scFOS is in a continuously stirred tank reactor (CSTR). In this method, the enzyme and the sucrose enter the reactor

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in separate streams, where they mix in the vessel and the reaction is allowed to take place. Using the CSTR, the enzyme can exist in the supernatant and flow into the reactor in a liquid form and mix with the sucrose to form the scFOS.

The CSTR reactor setup has been chosen because a very good method to produce β-D-fructofuranosidase has been found, where the organism of *Pichia pastoris* is used as the host for the enzyme production. Very good yields of enzyme are obtained by using *P. pastoris* and by using this method, the enzyme is secreted into the supernatant, which means that the CSTR reactor would be more suitable for the scFOS production.

Storage

Before the scFOS can be sent for purification, there needs to be a buffer tank between the two process steps so that there is control and time between the production of scFOS and the purification of the product. This is an important step of the process as it will allow time for the reaction mixture to settle and stabilise before it it sent to the SMB column.

Purification

The scFOS, before it is sold, needs to be sent to a purification unit to bring the purity up to a level of 95% or above. The impure scFOS leaves the buffer tank. There are two well known techniques that are used for the purification of the scFOS and they are simulated moving bed chromatography and activated charcoal column. They are both very effective techniques to remove the unwanted sugars (fructose, glucose and sucrose) from the process, both achieving purification rates of 95% or above. A factor that may deter the use of the charcoal column is the fact that it uses the potentially risky chemical of ethanol as an eluent in the process. SMB chromatography simply uses water as the eluent in the process.

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The choice would be to use the simulated moving bed chromatography technique as it is a well recognised method that is known to be used a lot in literature and it is also known to be used by local sugar producers in South Africa for fructose production. The technique has no hazardous materials and achieves a good separation, which are the influencing factors that will favour the choice of this technology.

Product Preparation

ScFOS is known to be sold in two different forms; in the syrup form and in the powder form. Depending on what the industry demands will determine in which form the product will be sold. The good news is that the process can cater for customers that request scFOS both in the powder and syrup form. To prepare the dry scFOS in powder form, the 'wet' scFOS that exits the SMB chromatography column is sent to a spray dryer. The spray dryer works in a way that is shown in Figure 4.4. The stream enters the hot drying chamber through a nozzle. The nozzle essentially sprays the entering 'wet' stream of scFOS into the hot chamber and the moisture is then absorbed by the hot dry air.

The dried product exits out the bottom of the chamber and is sent to packaging, while the cooled air exits out the side of the chamber and is sent to a cyclone where it is cleaned. The dry product is also filtered once more and the unwanted air is also sent to the cyclone where it is ultimately sent to the exhaust.

By-product Handling

From the SMB chromatography column, comes the by product which consists mainly of glucose, fructose, and sucrose. This stream can also be sold as there are still useful sugars in this stream. This stream, like the product stream from the SMB chromatography column also needs to be dried as it contains moisture that is undesirable. The piece of equipment that is used to treat

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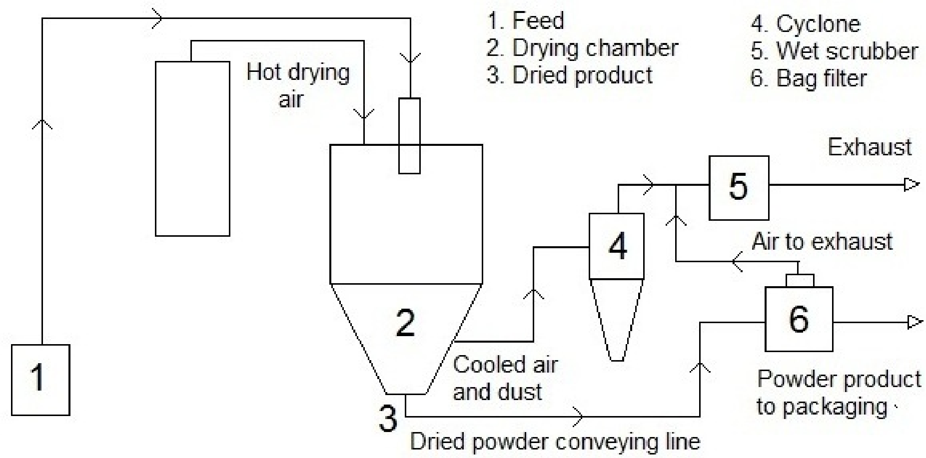

Figure 4.4: Schematic diagram of the setup and operation of a spray dryer (Redrawn) (Patel *et al.*, 2009).

the by-product is an evaporator. The stream will simply enter the evaporator and be subjected to high temperatures. As the temperature of the stream increases, the moisture will evaporate out and the by-product will become dry and be ready for selling.

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4.3 Process Designs

4.3.1 β-D-fructofuranosidase Production Process

The raw materials needed are the host organism (*P. pastoris*) with the impregnated gene (AOX). The organism is grown in Petri dishes, and then transferred into the shake flask. The production medium, which will allow for healthy growth in the shake flask is the next step of the enzyme production process.

Sterilisation of the fermentation vessels, is a very important part of the process. If the culture becomes contaminated, it would be a very big waste of time and money in the enzyme production process and it must be avoided at all costs. The fermentation vessels will be treated with cleaning in place systems using spray balls and caustic soda solutions. The final fermenter will also be treated with high pressure steam to ensure that there is absolute sterility in the fermenter.

The fermentation will begin once the culture has developed and begins to feed on the carbon source while receiving oxygen. The β-D-fructofuranosidase is produced within the cell and is then secreted into the supernatant. The supernatant is basically the entire liquid broth that includes all the ingredients needed for the fermentation to occur. The broth also includes other proteins that may have formed during the fermentation, but these are undesired. Only the pure β-D-fructofuranosidase is desirable, which means that the other constituents need to be removed.

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To purify the enzyme, the dead yeast cells need to be removed from the fermentation broth. This is done by centrifugation. The centrifuge will remove almost all of the cells but there will still be metabolites and other proteins that will form during the fermentation that need to be removed. These other components that form can be removed via ultrafiltration.

Once the block flow diagram was drawn, the actual process flow diagram could be constructed specifying the respective unit operations that need to be put in place to ensure that the process was designed correctly. The process flow diagram is shown in Figure 4.5 and can clearly show all the raw materials that are needed for the process, stream numbers and an explanation of the unit operations used in the process.

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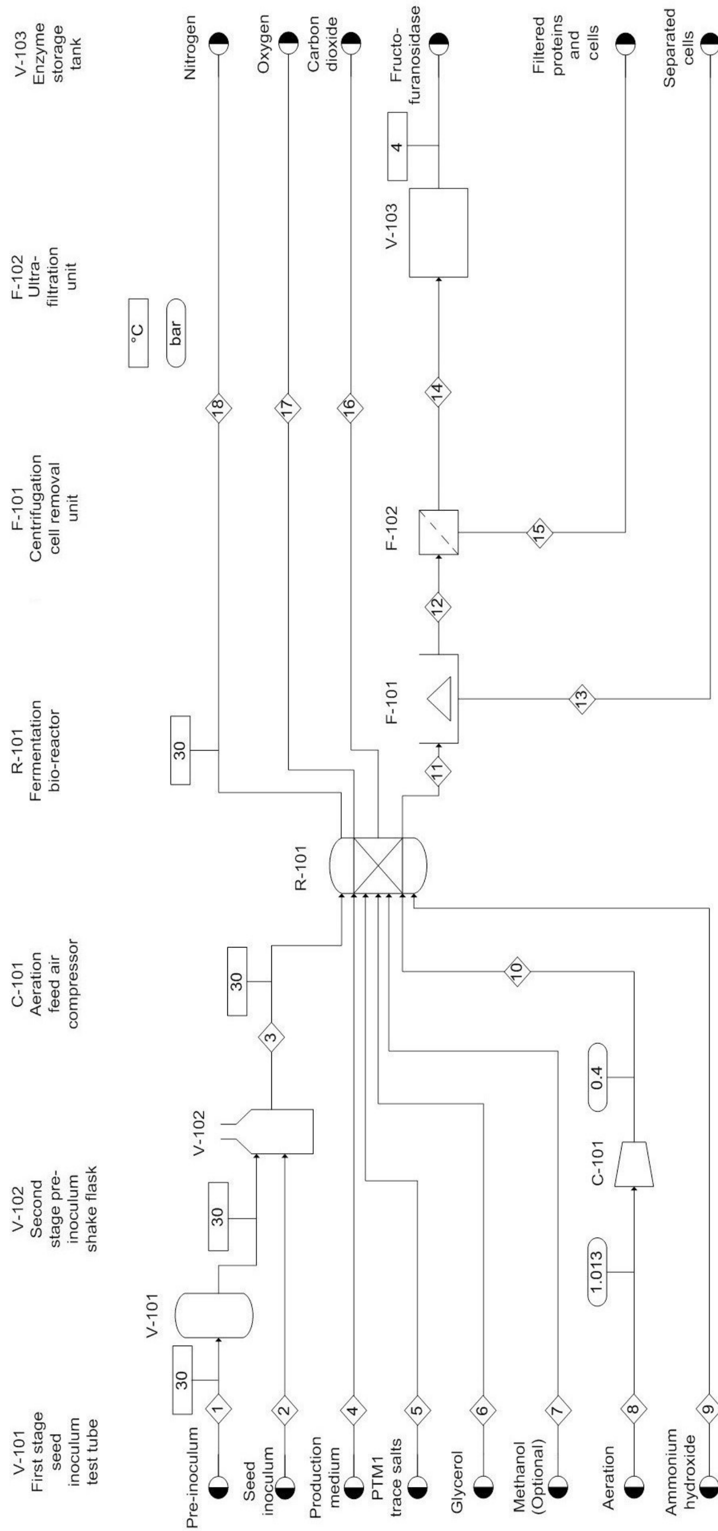

Figure 4.5: Production process of β-D-fructofuranosidase.

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4.3.2 ScFOS Production Process

The process flow diagram of the scFOS production process (Figure 4.6) is developed from the block diagram, where the unit operations and the choice of the equipment is combined to form a concise diagram with numbered flow streams and numbered equipment pieces.

The scFOS production process needs raw sucrose from a sugar production mill along with the enzyme β-D-fructofuranosidase. The enzyme which is produced in a separate enzyme production facility will be sent into the bioreactor along with the sucrose that would be shipped in from a mill. The pH and temperature are maintained at the desired level so that the biochemical reaction was allowed to take place. The typical conversion of the sucrose to scFOS is 60% so when the scFOS is produced in the reactor, there is still unreacted sucrose as well as remaining fructose and glucose, which needs to be removed to form a purer product.

The product from the bioreactor is then sent to a purification unit, which uses a simulated moving bed chromatography technique. This unit typically achieves a 95% purification rate and will remove the fructose, glucose and unreacted sucrose from the mixture, leaving a pure scFOS in syrup form. The raffinate from the SMB chromatography column is also treated further.

The scFOS is then sent to a spray drying unit where the moisture is removed from the scFOS that was in syrup form. The scFOS in powder form is then ready to be sold. The raffinate from the SMB column is also treated with an evaporator, where the moisture is also removed and the by product, which consisted mainly of fructose, glucose and sucrose is also sold. Heat exchangers are implemented to save any energy that would be lost in the process.

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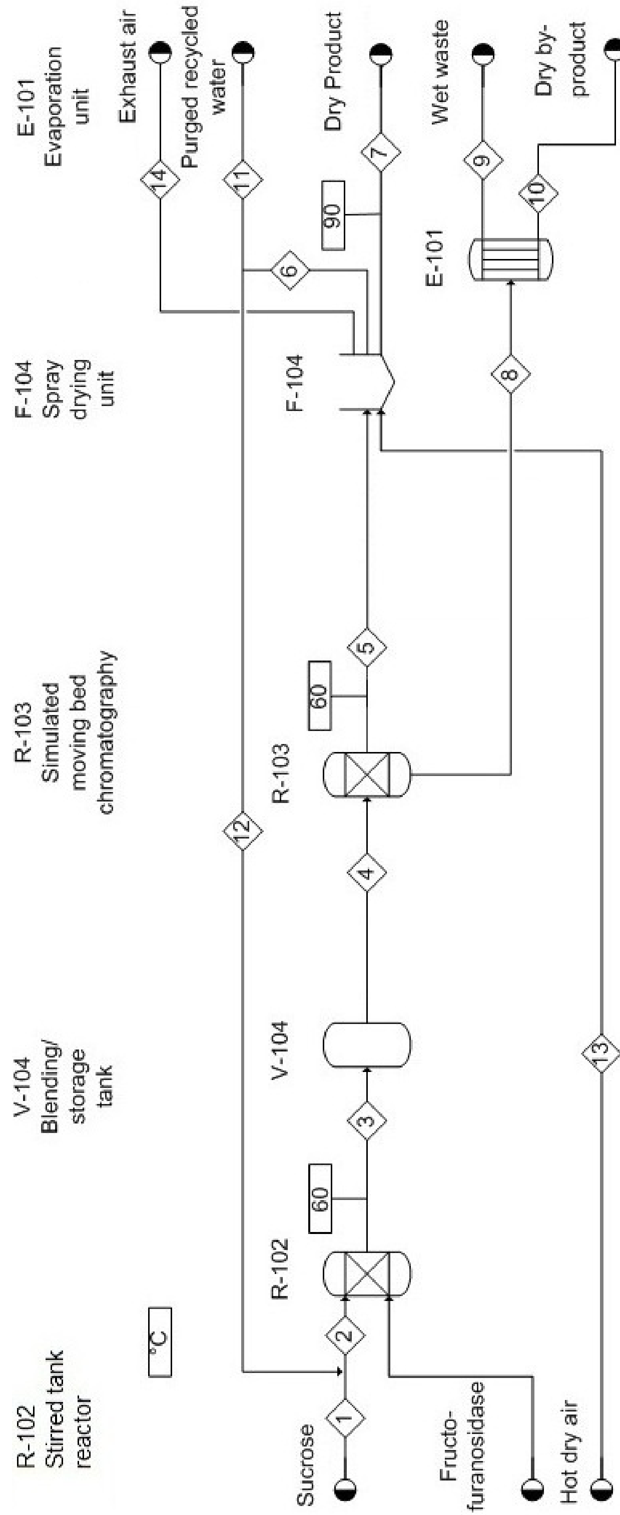

Figure 4.6: ScFOS production process

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4.4 Process Scheduling

In order for an enzyme production facility to be able to run properly, it needs to have a scheduled process to be able to operate in a orderly manner and optimise production. Not only is it important for production, but it is also important to have a schedule so that the equipment can be cleaned on a regular basis. This will ensure that the risk of contamination is reduced so that the culture will grow at an optimal rate.

During a run, the culture will be developed in labs and the culture would then be transferred into a seed train of fermentation vessels. These vessels before hand are washed with a cleaning-in-place system using spray balls and caustic soda solution. The final fermenter in the seed train is cleaned with high pressure steam to ensure absolute sterility.

The schedule is shown in Figure 4.7 where it can be seen that the facility can cater for a possible 4 strains/lines of enzyme production.

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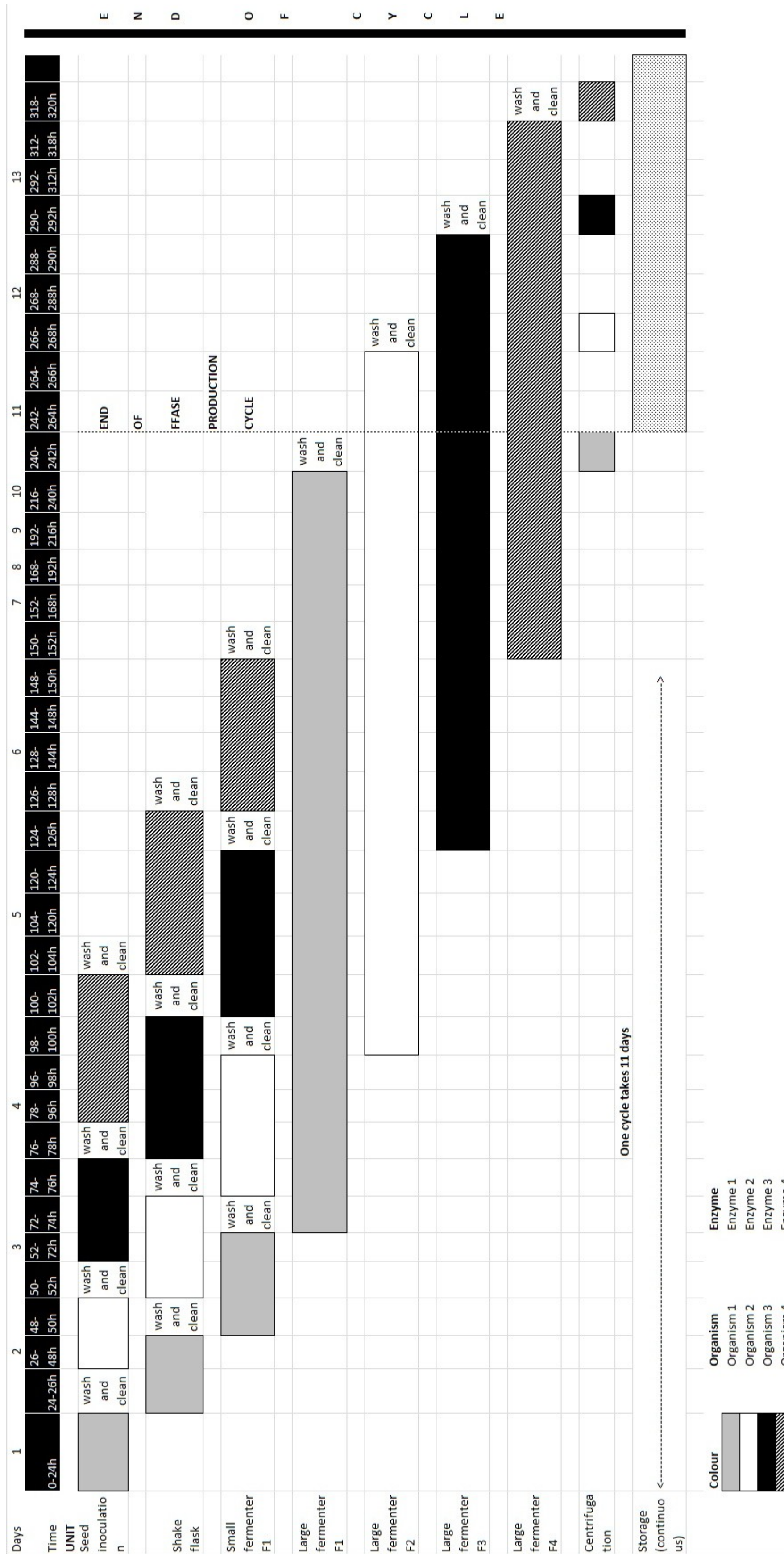

Figure 4.7: Production schedule of the multi-enzyme production facility. FFase production schedule is to satisfy the needs of a continuous production process of scFOS.

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Chapter 5

Mass and Energy Balance

The mass and energy balance forms the fundamental part of chemical engineering process design. It is essential to know exactly what is happening in the process with regards to the flow rates, pressures, and temperatures of the stream in the process. It is also important to know the specifications of the unit and to know all the information regarding the units. This will include sizing, operating conditions and stream composition entering and exiting the unit.

In this project, the enzyme production process and the scFOS production process are linked to one another in the way that the scFOS production levels will determine the amount of FFase that is needed for the scale and the FFase production process will be designed accordingly.

5.1 Mass Balance

5.1.1 FFase Production Process

5.1.1.1 Laboratory Operations

FFase production uses a fed batch fermentation process to produce the enzyme, but before one can put the ingredients into the reactor, preparation of the inoculum and production medium needs to be done.

The method that was used to produce the enzyme was practised by the University of Stellenbosch in small scale biological laboratories. However, this

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technique needed to be adjusted and adapted to industrial scale, where many of the more expensive ingredients where replaced with effective cheaper ingredients that are more feasible for industrial enzymes production. The benefit is that with industrial enzymes production, the product does not have to be entirely pure because the enzymes are produced on such a large scale. But at the same time, every effort must be made to ensure the product is as pure as possible.

The inoculation of the culture in the industrial enzyme production process will begin in a laboratory environment. The inoculation occurred in two stages. Initially, a 4 ml sample, which has the composition shown in Table 5.1, was prepared in a test tube (see V-101 in Figure 4.5). The ingredients were put into the test tube with one to three colonies of *P. pastoris* from the yeast peptone dextrose (YPD) agar plate. The organism was left to grow in the test tube for 16-24 hours at 30°C on a test tube spinning wheel.

Table 5.1: Pre-inoculum used for the production of FFase

Pre-inoculum	**Operation**
2.76 ml dH_2O	Autoclave
0.4 ml 1M Phosphate buffer, pH 6.0	
0.4 ml 10x Yeast nitrogen base (YNB)	Filter sterilise
8 μl 500x Biotin	
0.4 ml 10% Glycerol	

The second stage inoculum involved a volume of 40 ml, where the preparation amount was tenfold the amount inoculated in the seed inoculum (see Table 5.2). The medium was prepared in a 250 ml shake flask (V-102 in Figure 4.5), and included in the 40 ml solution was the seed inoculum prepared in

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the shake flask. The shake flask was incubated at 30°C and 200 rpm overnight (16-24 hours).

Table 5.2: Second stage inoculum used for the production of FFase

Pre-inoculum	Operation
27.6 ml dH_2O	Autoclave
4 ml 1M Phosphate buffer, pH 6.0	
4 ml 10x Yeast nitrogen base (YNB)	Filter sterilise
80 μl 500x Biotin	
4 ml 10% Glycerol	

5.1.1.2 Reactor Setup (using GAP Production Strain)

From the shake flask, the grown culture was sent to the fermenter (R-101 from Figure 4.5), where more additions were added to the reaction mixture to ensure that the enzyme production occurred as desired. The components that were put into the reactor was shown in Table 5.3, which is the industrial medium for FFase production using the GAP production strain.

In the reactor, the temperature is maintained at 30°C (Yun and Song, 1999) using a glycerol coolant, that does not form part of the mass balance. There is compressed air that is supplied to the system in order for the organism to respire, and it exits the compressor (C-101 from Figure 4.5) at a pressure of 0.4 bar and enters the bioreactor at a flow rate of 1 vvm (volume of gas per volume of medium per minute). The air is supplied when needed, according to how the organism is using its glycerol food source. The agitation is also linked to the the dissolved oxygen concentration to ensure the air that is supplied is dissolved in the medium (dissolved oxygen concentration is maintained above 30% air saturation). The agitation speed is cascaded from 200-1000 rpm. The

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pH of the medium was at pH 5, but the solution can deviate up to a pH of 5.5. A 25% solution of ammonium hydroxide was used to control the pH (Yun and Song, 1999).

The glycerol batch phase started when the 360 ml production medium (Table 5.3) was inoculated with the 40 ml inoculum that was prepared in the shake flask. The glycerol batch phase lasted for 24-27 hours and during this phase the aeration rate was increased with a decrease in agitation. A cell concentration of 90-150 grams of wet cells per working volume should be attained before the glycerol fed batch phase can proceed.

For the glycerol fed batch phase; 50% glycerol (stream 6 in Figure 4.5) was added by a constant feed at 18.15 ml/h/L of initial fermenter volume. The glycerol feed was controlled by virtue of the dissolved oxygen content, where if the dissolved oxygen rose above 30%, then the glycerol is fed, but when the dissolved oxygen drops below 30%, the glycerol feed is stopped.

Table 5.3: Industrial medium components used for the production of FFase using the GAP production strain

Industrial medium	Concentration (g/L)	1L reactor (g)	Mass (%)	Density (g/ml)	Volume (ml)
Glycerol (batch)	40.0	404.2	41.7%	1.26	400.0
Glycerol (fed batch)	700.0	473.1	48.9%	1.26	400.0
Yeast extract	10.0	8.0	1.0%	1.20	6.7
Phosphoric acid 85%	56.3	45.0	4.7%	1.69	26.7
Calcium sulphate	1.2	0.9	0.1%	2.32	0.4
Potassium sulphate	22.8	18.2	1.9%	2.66	6.8
Magnesium sulphate-7H_2O	18.6	14.9	1.5%	2.66	5.6
Potassium hydroxide	5.2	4.1	0.6%	2.04	2.0
TOTAL		968.4	100.0%		848.2

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5.1.1.3 Reactor Setup (using AOX Production Strain)

The experimental set-up when using alcohol oxidase (AOX) as a promoter is the same as when using GAP, except with AOX, the gene has to be induced with methanol. The reactor set-up procedure follows the same method as GAP until the glycerol fed batch phase. The glycerol fed-batch phase stops when the wet cell concentration reaches 180-220 grams per litre. The methanol fed batch phase is then introduced, where 100% methanol (stream 7 in Figure 4.5) is added. The methanol is fed initially at 1.8 ml/h/L of the initial fermenter volume for one hour. The feed rate is then increased incrementally to 3.6, 5.4, 7.3, 9.1 and 10.9 ml/h/L. The feed rate at 3.6 and 7.3 ml/h/L was fed for two hours and the feed rate at 5.4 and 9.1 ml/h/L lasted for one hour. At the end, 10.9 ml/h/L was kept for the remainder of the fermentation.

Table 5.4: Industrial medium components used for the production of FFase using the AOX production strain

Industrial medium	Concentration (g/L)	1L reactor (g)	Mass (%)	Density (g/ml)	Volume (ml)
Glycerol (batch)	40.0	404.2	47.9%	1.26	400.0
Glycerol (fed batch)	400.0	110.4	13.1%	1.26	100.0
Methanol (fed batch)	1000.0	237.5	28.2%	0.79	300.0
Yeast extract	10.0	8.0	1.0%	1.20	6.7
Phosphoric acid 85%	56.2	45.0	5.3%	1.69	26.7
Calcium sulphate	1.2	0.9	0.1%	2.32	0.4
Potassium sulphate	22.8	18.2	2.2%	2.66	6.8
Magnesium sulphate-7H_2O	18.6	14.9	1.8%	2.66	5.6
Potassium hydroxide	5.2	4.1	0.5%	2.04	2.0
TOTAL		843.3	100.0%		848.2

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5.1.1.4 Downstream Operations

The enzyme is slowly produced and secreted into the supernatant during fermentation. The FFase exists in the enzyme broth - it is an extracellular enzyme product. The components that exit the reactor are: carbon dioxide from the respiration of the organism, oxygen which has not been used by the organism, nitrogen which forms part of the air make up of the aeration during the fermentation. The main and most important component that exits the fermenter is the fermentation broth. This broth contains the β-D-fructofuranosidase as well as other proteins and metabolites and the dead *P. pastoris* cells.

The medium is sent to a centrifuge (F-101 in Figure 4.5), where approximately 98% of the dead *P. pastoris* cells are removed from the product medium. The medium is finally sent to a 22μm ultra-filtration unit (F-102 in Figure 4.5) to remove any unwanted proteins, metabolites and the rest of the *P. pastoris* cells. The enzymes are then sent to a refrigeration unit (V-103 in figure 4.5) to be stored.

Table 5.5 shows the material stream table of all the component flows and their mass percentages of all the important streams in the process. This stream table is based on the process flow diagram shown in Figure 4.5 and also the enzyme production levels that will be able to supply to a scFOS production facility that produces 2 000 tonnes per annum of scFOS.

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Table 5.5: FFase Production stream table (to supply for 2 000 tpa of scFOS)

Stream number	**4**	**6**	**8 (kg/hr)**	**10 (kg/hr)**	**11**	**12**	**13**	**14**	**15**
Temperature (°C)	30	30	30	30	30	30	30	30	30
Pressure (bar)	1.01325	1.01325	1.01325	0.4	1.01325	1.01325	1.01325	1.01325	1.01325
Total (g/h)	1125	790	6339587	6339587	2607	1823	245	1722	5
Mass (g/h)									
Glycerol	917.78	250.78	0	0	22.95	22.95	0	22.95	0
Yeast extract	18.17	0	0	0	0	0	0	0	0
H_3PO_4 85%	102.16	0	0	0	0	0	0	0	0
$CaSO_4$	2.11	0	0	0	0	0	0	0	0
K_2SO_4	41.33	0	0	0	0	0	0	0	0
$MgSO_4$-$7H_2O$	33.83	0	0	0	0	0	0	0	0
KOH	9.38	0	0	0	0	0	0	0	0
Air	0	0	6339.59	6339.59	0	0	0	0	0
NH_4OH	0	0	0	0	138.28	138.28	0	0	0
β-D-fructofuranosidase	0	0	0	0	15.99	15.99	0	15.99	0
Pichia pastoris cells	0	0	0	0	249.78	5.00	244.78	0	5.00
Supernatant	0	0	0	0	1567.49	1567.49	0	1567.49	0
Mass %									
Glycerol	81.60	31.74	0	0	1.11	1.26	0	1.26	0
Methanol	0	68.26	0	0	3.52	3.99	0	4.01	0
Yeast extract	1.62	0	0	0	0	0	0	0	0
H_3PO_4 85%	9.08	0	0	0	0	0	0	0	0
$CaSO_4$	0.19	0	0	0	0	0	0	0	0
K_2SO_4	3.67	0	0	0	0	0	0	0	0
$MgSO_4$-$7H_2O$	3.01	0	0	0	0	0	0	0	0
KOH	0.83	0	0	0	0	0	0	0	0
Air	0	0	100	100	0	0	0	0	0
NH_4OH	0	0	0	0	6.69	7.59	0	7.61	0
β-D-fructofuranosidase	0	0	0	0	0.77	0.88	0	0.88	0
Pichia pastoris cells	0	0	0	0	12.08	0.27	100	0	100
Supernatant	0	0	0	0	75.82	86.01	0	86.24	0

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5.1.2 ScFOS Production Process Description

5.1.2.1 Reactor Feed

The scFOS production process receives the enzyme β-D-fructofuranosidase along with sucrose which is fed at a flow rate of 421 kg/h into a semi-batch reactor with agitation (R-102 in Figure 4.6). The scFOS production rate of 2 000 tonnes per year, which equates to 253 kg/h. The sucrose enters at a concentration of 600 kg/m^3 and at a volumetric flow rate of 0.7 m^3/h. A recycled water stream from the from the FOS purification step joins the sucrose solution and this combined stream enters the semi-batch reactor.

5.1.2.2 Reactor Set-up

A semi-batch agitated reactor was chosen for the reactor setup of the scFOS production process. Both the enzyme and sucrose enter the semi-batch reactor at an enzyme flow rate of 0.0043 gram of average AOX fermentation broth per gram of sucrose. This effectively works out to be a incoming flow rate of 1.8 kg/h. The components will enter the reactor and be heated up to the designated temperature of 60°C and be held for the specified reaction time of 4 hours until the desired amount of kestose, nystose and 1^F-fructofuranosyl nystose is formed. The desired composition will be aimed at the composition of Actilight®, which is 35-40% GF_2, 50-55% GF_3 and 5-10% GF_4. The scFOS mixture will exit out of the reactor after the specified reaction time. The mixture will contain primarily the scFOS's as well as the unreacted sugars and some of the enzyme (see Table 5.6).

The calculated yield for the FOS's of this experiment is found to be 60%, which shows to be very similar to the yields found in literature. The FOS's that are produced from the reaction of the sucrose with the enzyme are sent to a blending/storage tank V-104 via stream 4 in the scFOS process flow diagram (Figure 4.6).

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Table 5.6: Reactor product (FOS in syrup form before purification)

Component	Flow rate (kg/h)	Mass %	Mass % (excluding water)
Kestose (GF_2)	94.10	12.74	23.10
Nystose (GF_3)	125.56	16.98	30.81
1^F-F-Nystose (GF_4)	23.54	3.18	5.78
GF_5	9.36	1.27	2.30
GF_6	3.74	0.51	0.92
GF_7	1.87	0.25	0.46
Fructose (F)	14.97	2.02	3.67
Glucose (G)	86.08	11.64	21.12
Sucrose (GF)	46.52	6.28	11.40
FFase	1.82	0.25	0.45

5.1.2.3 scFOS Purification

The scFOS that leaves the reactor needs to be purified to a high FOS percentage from 60% to 95%. The fructose, glucose and sucrose are extracted from the solution to give a FOS-rich product. The purification step is done via simulated moving bed (SMB) chromatography and is shown by the unit R-103 in Figure 4.6.

The design specified the SMB chromatography column to operate at 60°C to ensure a 95% FOS recovery. The extract (Table 5.7) from the column was then sent to a spray drying unit, F-104 (Figure 4.6), where the water was removed from stream 5. The raffinate (Table 5.8), which contained 95% of the mono and disaccharide sugars, exited out of the bottom of the column via stream 8 and entered an evaporation unit, E-101 (Figure 4.6).

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Table 5.7: SMB column product (FOS in syrup form before product preparation)

Component	Flow rate (kg/h)	Mass %
Kestose (GF_2)	89.40	15.75
Nystose (GF_3)	119.20	21.00
1-F-Nystose (GF_4)	22.35	3.94
GF_5	8.88	1.56
GF_6	3.55	0.63
GF_7	1.78	0.31
Fructose (F)	0.75	0.13
Glucose (G)	4.30	0.76
Sucrose (GF)	2.32	0.41
Water	315.06	55.51
Total	567.58	100

Table 5.8: SMB column raffinate (FOS in syrup form before product preparation)

Component	Flow rate (kg/h)	Mass %
Kestose (GF_2)	4.71	2.75
Nystose (GF_3)	6.27	3.66
1-F-Nystose (GF_4)	1.18	0.69
GF_5	0.47	0.27
GF_6	0.19	0.11
GF_7	0.09	0.05
Fructose (F)	14.21	8.29
Glucose (G)	81.72	47.69
Sucrose (GF)	44.10	25.74
Water	16.58	9.67
FFase	1.82	1.06
Total	171.34	100

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5.1.2.4 scFOS Product Preparation

The scFOS then entered the production preparation section of the scFOS production process. A spray dryer was used and the inlet temperature of the hot dry air was 180°C. The outlet temperature of the water vapour that has been 'caught' by the hot dry air was 90°C. The flow rate of the air would have to cater for an incoming product flow rate of 568 kg/h (see Table 5.7, which suggests that the incoming air flow rate should be 7 563 kg/h of hot air (spray dryer specification were influenced by the work of Huang and Mujumdar (2007), see Appendix E section E.2 for sample calculations).

Table 5.9: Dry scFOS product for selling

Component	Flow rate (kg/h)	Mass %
Kestose (GF_2)	89.46	35.40
Nystose (GF_3)	119.28	47.20
1-F-Nystose (GF_4)	22.36	8.85
GF_5	8.89	3.52
GF_6	3.56	1.41
GF_7	1.78	0.70
Fructose (F)	0.75	0.30
Glucose (G)	4.30	1.70
Sucrose (GF)	2.33	0.92
Total	252.71	100

The evaporator was a unit operation that also needed to remove the moisture from the by-product stream. The by-product stream exited the bottom of the SMB chromatography column where most of the sugars (glucose, fructose and sucrose) were found as well as a fair amount of water. The evaporator served its purpose to dry out the by product either for re-use or for sales. The evaporator operated at 105°C. Table 5.9 shows the dried scFOS that will be

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Table 5.10: Dry by-product for selling

Component	Flow rate (kg/h)	Mass %
Kestose (GF_2)	4.47	3.08
Nystose (GF_3)	5.96	4.10
1-F-Nystose (GF_4)	1.12	0.77
GF_5	0.44	0.31
GF_6	0.18	0.12
GF_7	0.09	0.06
Fructose (F)	13.51	9.30
Glucose (G)	77.69	53.41
Sucrose (GF)	41.99	28.87
Total	145.45	100

suitable for sales, where again the water was removed and it was assumed that 95% of the carbohydrates were separated and the dry by-products were sent for selling (see Table 5.10).

The entire stream table that corresponds to the process flow diagram in Figure 4.6 is shown in Table 5.11. The mass balance focuses on the production scale of 2 000 tonnes per annum, which is the scale of interest in this project.

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Table 5.11: ScFOS Production stream table at 2 000 tpa of scFOS)

Stream	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13 (t/h)**	**14 (t/h)**
T (°C)	25	25	60	25	60	90	90	60	25	25	25	25	180	25
P (bar)	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013
Total (kg/h)	420.23	737.10	738.93	738.93	567.59	315.06	252.53	171.35	24.23	145.29	0.01	315.06	7.56	7.56
Mass (kg/h)														
Kestose	0	0	94.11	94.11	89.40	0	89.40	4.71	0.24	4.47	0	0	0	0
Nystose	0	0	125.47	125.47	119.20	0	119.20	6.28	0.32	5.96	0	0	0	0
1^F-F-nystose	0	0	23.53	23.53	22.35	0	22.35	1.18	0.06	1.12	0	0	0	0
GF_5	0	0	9.35	9.35	8.89	0	8.89	0.47	0.03	0.45	0	0	0	0
GF_6	0	0	3.74	3.74	3.56	0	3.56	0.19	0.01	0.18	0	0	0	0
GF_7	0	0	1.87	1.87	1.78	0	1.78	0.10	0.01	0.09	0	0	0	0
Fructose	0	0	14.96	14.96	0.75	0	0.75	14.22	0.72	13.51	0	0	0	0
Glucose	0	0	86.02	86.02	4.31	0	4.31	81.72	4.09	77.64	0	0	0	0
Sucrose	420.23	422.05	46.43	46.43	2.33	0	2.33	44.11	2.21	41.90	0	0	0	0
Water	0	315.06	331.64	331.64	315.06	315.06	0	16.59	16.59	0	0.01	315.06	0	0
Air	0	0	0	0	0	0	0	0	0	0	0	0	7.56	7.56
FFase	0	0	1.82	1.82	0	0	0	1.82	1.82	1.82	0	0	0	0
Mass %														
Kestose	0	0	12.74	12.74	15.75	0	35.40	2.75	0.99	3.08	0	0	0	0
Nystose	0	0	16.98	16.98	21.00	0	47.20	3.67	1.32	4.10	0	0	0	0
1^F-F-nystose	0	0	3.18	3.18	3.94	0	8.85	0.69	0.25	0.77	0	0	0	0
GF_5	0	0	1.27	1.27	1.57	0	3.52	0.27	0.12	0.31	0	0	0	0
GF_6	0	0	0.51	0.51	0.63	0	1.41	0.11	0.04	0.12	0	0	0	0
GF_7	0	0	0.25	0.25	0.31	0	0.70	0.06	0.04	0.06	0	0	0	0
Fructose	0	0	2.02	2.02	0.13	0	0.30	8.30	2.97	9.30	0	0	0	0
Glucose	0	0	11.64	11.64	0.76	0	1.71	47.69	16.88	53.44	0	0	0	0
Sucrose	100	57.26	6.28	6.28	0.41	0	0.92	25.74	9.12	28.84	0	0	0	0
Water	0	42.74	44.88	44.88	55.51	100.00	0	9.68	68.47	0	100.00	100.00	0	0
Air	0	0	0	0	0	0	0	0	0	0	0	0	100.00	100.00
FFase	0	0	100.00	100.00	0	0	0	100.00	100.00	100.00	0	0	0	0

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5.2 Energy Balance

5.2.1 Enzyme Production Process

There were five main sections that were looked at for the energy balance around the FFase production process: i) the energy used for the high pressure steam sterilisation of the fermenters for each new batch, ii) the energy used for the continuous agitation in the vessel during fermentation, iii) the energy used to control the temperature of the fermenter at 30°C, iv) the energy used for the centrifugation step of the process when removing all the dead cells from the enzyme broth, and v) the energy used for the continuous cold room storage to ensure the enzymes maintain their level of activity.

5.2.1.1 Sterilisation

High pressure steam sterilisation is imperative for any biological process. Due to the nature of biology, there are so many organisms in the environment, it is important to keep the system sterile. The sterilisation conditions were high temperature short time (HTST), which were 141 °C and at a time in the fermenter for 10 seconds before the batch process. The energy calculation was calculated on the energy required to heat the inner walls of the fermenter to 141 °C. The necessary information was obtained and the calculation is shown in Table 5.12.

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Table 5.12: Energy used for the high pressure steam sterilisation of the fermenter in the FFase production process, producing 14.43 tonnes per annum using the AOX production strain

Item	Amount	Unit
Cp_{water}	4.186	kJ/kg.K
ρ_{water}	1 000	kg/m^3
Fermenter volume : boiler volume (Already Enterprises, 2013)	72 : 3	L
$V_{fermenter}$	0.620	m^3
V_{boiler}	0.026	m^3
ΔHv (Marsh, 1987)	2 233.901	kJ/kg
T_1	141	°C
T_2	25	°C
$E = \rho.V_{boiler}.Cp_{water}.dT_{(100-25)} + \rho.V_{boiler}.\Delta Hv + \rho.V_{boiler}.Cp_{water}.dT_{(141-100)}$	12 683.057	kJ
Steaming time	10	seconds
Power (P)	1 268.306	kW
Fermentations per year	26	times
Energy for a year	= 26*10s/3600*P	kW.h
Energy for a year	**91.600**	**kW.h**

Cp = specific heat, ρ = density, V = volume, T = temperature, dT = change in temperature, E = energy

5.2.1.2 Aeration

In order for the organism to be able to grow well, the organism needs the correct food source as well as to be able to respire. The fermenter is supplied with compressed air. Table 5.13 shows the energy required for the compressor to deliver the compressed air to the fermenter.

The agitation is important to allow the yeast to grow in the system so that when the enzyme is produced, its concentrations will be maximum. Regarding the energy consumed during the agitation of the process, Turton *et al.* (2008) proposed an energy correlation relating the energy consumptions and

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Table 5.13: Energy used for aeration in the fermenter for the FFase production process producing 14.43 tonnes per annum using the AOX production strain

Component	**Amount**	**(Unit)**
Air flow rate	0.012	kg/s
Enthalpy (0.400 bar)	301.370	kJ/kg
Enthalpy (1.013 bar)	600.712	kJ/kg
Energy	$E = m(h_2\text{-}h_1)$	kW
Energy	**3.661**	**kW**
Energy for a year	**28 995.112**	**kW.h**

the volume of the vessel. Table 5.14 shows the method of calculating the annual energy consumption for the agitation during fermentation when producing FFase.

Table 5.14: Energy used for the agitation in the fermenter for the FFase production process producing 14.43 tonnes per annum using the AOX production strain

Item	**Amount**	**Unit**
Energy (Turton *et al.*, 2008)	0.3	kW/m^3
Fermenter volume	0.561	m^3
Hours in a year	(330*24) = 7 920	h
Energy for a year	**1332.400**	**kW.h**

5.2.1.3 Temperature Control

Another important part of the fermentation is temperature control. The organism is very sensitive to the temperature it exits in and one can optimise its growth if it is subjected to a temperature that will be favourable to its development. According to Shay *et al.* (1987), the organism generates metabolic heat when it is growing and therefore the temperature in the fermenter will need to be controlled to ensure the best growing conditions. The correlation

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that Shay *et al.* (1987) used was 109 kcal/L/h of heat generated when the dissolved oxygen levels were between 20-25%. For this project the dissolved oxygen levels were specified to be at 30% and the correlation had to be adjusted accordingly. The rest of the information was known and the energy calculation method can be seen in Table 5.15.

Table 5.15: Energy used for the temperature control in the fermenter for the FFase production process producing 14.43 tonnes per annum using the AOX production strain

Item	**Amount**	**Unit**
Metabolic heat generation (22.5% dO_2)	109	kcal/L/h
From Shay *et al.* (1987)	= 456.36	kJ/L/h
dO_2 in Shay *et al.* (1987)	20 - 25	%
dO_2 in experiments	30	%
Metabolic heat generation (30% dO_2)	145.33	kcal/L/h
	= 608.48	kJ/L/h
E	V.E'.t	kW.h
V	560.77	L
E'	608.48	kJ/L/h
t	330*24	hours
P = V.E'	94.78	kW
E	P.t	kW.h
COP (New Buildings Institute, 1998)	4	
Energy for a year	= 24*330*P/COP	kW.h
Energy for a year	**187 671.52**	**kW.h**

5.2.1.4 Centrifugation

When fermentation is complete the dead cells need to be removed from the enzyme fermentation broth. This is done through centrifugation. Perry *et al.*

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(1984) has provided information for centrifugation power demand and the information is given in Table 5.16.

Table 5.16: Energy used for the centrifugation of the fermentation broth for the FFase production process producing 14.43 tonnes per annum using the AOX production strain

Item	Energy consumption	Unit
Automatic batch (constant speed) (E')	26	kJ/kg
Centrifuge time	30	mins
Number of runs	2	times
Total time	60	mins
Number of operating hours in a year (t)	26*2*30mins	26 hours
Incoming flowrate (F)	2.07	kg/hr
Energy for a year (E = F*E'*t)	**0.39**	**kW.h**

5.2.1.5 Refrigeration

Once the enzyme fermentation broth has been sent through the centrifuge, its will pass through an ultrafiltration plant where all the remaining contaminants are removed. The enzyme finally needs to be stored in a cool store room at 4°C. This cool room is essential for the preservation of the enzyme and to maintain its enzymatic activity. To calculate the energy needed for refrigeration, some assumptions had to be made. Firstly, then specific heat of the enzyme broth was taken to be that of water. This assumption was made because there is no information available for the enzymatic broth and it exists in a watery broth form, so the specific heat was taken to be 4.186 kJ/kg.K. The second assumption was that the coefficient of performance for the refrigeration was assumed to be 3.5 (Aneke *et al.*, 2012). This was made because there will be heat absorption through the walls of the cold room and the actual walls of the

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room will all be cooled during the cooling process. The following equation was proposed by Çengel and Boles (2007):

$$COP_R = \frac{desired\,output}{required\,input} = \frac{q_L}{W_{net,in}}$$

$$Therefore,$$

$$W_{net,in} = \frac{q_L}{COP_R}$$

The calculations are shown in Table 5.17.

Table 5.17: Energy used for the refrigeration of the fermentation broth for the FFase production process producing 14.43 tonnes per annum using the AOX production strain

Item	Energy consumption	Unit
Mass in (M)	1.823	kg/hr
$Cp_{stainlesssteel}$	4.186	kJ/kg.K
dT	21	K
Power (E') = M.Cp.dT	0.045	kW
Number of hours in a year (t)	24*330	hours
Energy required (E = E'*t)	352.460	kW.h
Coefficient of performance (COP) *	3.5	
Energy required for a year (= E / COP)	**100.703**	**kW.h**

* The coefficient of performance (COP) specification was influenced on work by Aneke *et al.* (2012) where it was mentioned that a formidable COP for vapour compression refrigeration was 3.5.

5.2.1.6 Total Energy Consumption

To calculate the total energy needed to run a FFase production facility that produces 14.43 tonnes per annum, the sum of all the energy requirements is taken and the total energy consumption is given in Table 5.18.

The comparison of the energy required for all scales of production is shown in Table 5.19. The energy consumption increases proportionally according to the production scale.

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Table 5.18: Total energy used for the FFase production process producing enough enzyme to supply for a scFOS production facility of 2 000 tpa (producing 14.43 tonnes per annum using the AOX production strain)

Item	Energy consumption	Unit
Energy consumption for sterilisation	91.60	kW.h
Energy consumption for compressed air	28 995.11	kW.h
Energy consumption for agitation	1 332.40	kW.h
Energy consumption for temperature control	187 671.52	kW.h
Energy consumption for centrifugation	0.39	kW.h
Energy consumption for refrigeration	100.70	kW.h
Total energy consumption	**218 191.72**	**kW.h**

5.2.2 ScFOS Production Process

The scFOS production energy balance was done using enthalpy flows from the incoming and outgoing components of each unit operation of the process. Figure 5.1 shows the process flow diagram indicating the the energy loss by the dark grey arrows and the energy input by the light grey arrows.

This chemical process had many complex carbohydrates involved in it. Physical properties of some of the compounds were obtained but not all of them because of the lack of knowledge of these complex carbohydrates. The physical properties are shown in Table B.1, where some of the components such as α-cyclodextrin and β-cyclodextrin were taken as reference compounds for the higher molecular weight fructooligosaccharides. The trend in Figure 5.2 was that the heat capacity increase linearly with molecular weight, and if this is correlated with Table B.1 and the trend-line in Figure 5.2, there is a good fit to the curve.

The heat capacity model development from Figure 5.2 allowed all the necessary thermodynamic data to be obtained so that the energy balance around the scFOS production process could be completed.

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Table 5.19: Total energy needed for the FFase production process (using the AOX production strain), comparing all production scales

Production	**500**	**1 000**	**2 000**	**5 000**	**10 000**	**20 000**	**30 000**	**40 000**	**50 000**
Sterilisation (kW.h)	25	46	91	229	451	902	1 356	1 807	2 255
Compressed air (kW.h)	7 249	14 498	28 995	72 488	144 400	305 806	458 709	611 612	764 515
Agitation (kW.h)	333	666	1 332	3 331	6 636	13 271	19 907	26 542	33 178
Temperature control (kW.h)	46 918	93 836	187 672	469 179	934 633	1 869 267	2 803 900	3 738 534	4 673 169
Centrifugation (W.h)	97	194	388	970	1 933	3 866	5 800	7 733	9 666
Refrigeration (kW.h)	25	50	101	251	502	1 003	1 504	2 006	2 508
Total (kW.h)	**54 550**	**109 096**	**218 912**	**545 799**	**1 086 624**	**2 190 253**	**3 285 382**	**4 380 509**	**5 475 633**

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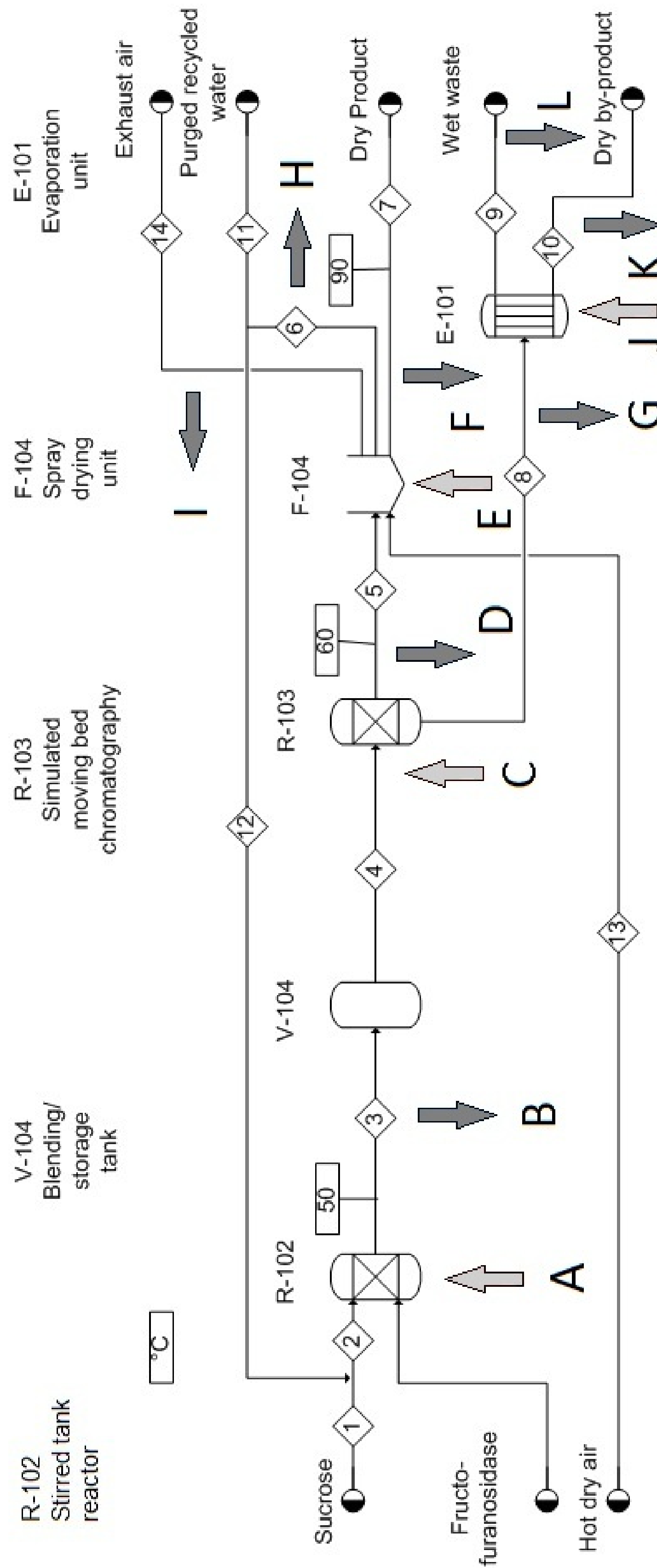

Figure 5.1: Process flow diagram showing the heat flows within the scFOS production process. The light grey arrows indicate where heat/energy is put into the system and the dark grey arrows indicate where heat/energy is lost to the environment.

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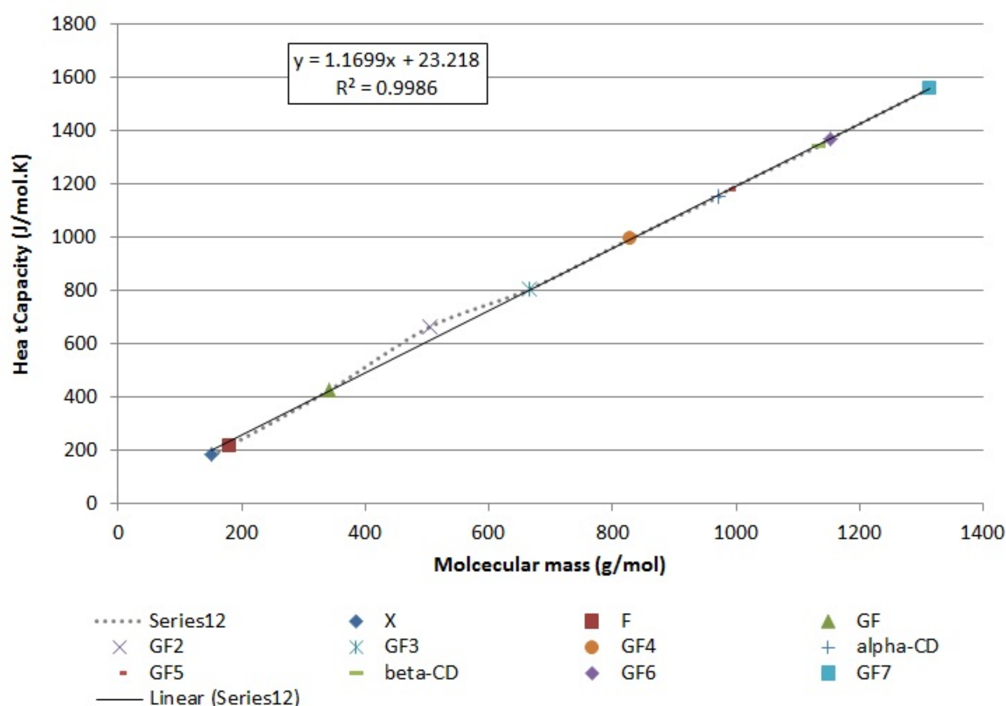

Figure 5.2: Heat capacity model to determine the heat capacity of complex organic compunds such as short chain fructooligosaccharides. The model fits well showing an R squared value of 0.9986.

The sucrose and the FFase entered the reactor, R-102 and were subjected to heating where the reaction temperature was specified to be 60°C. Table 5.20 shows the information about the energy required to heat all the relevant components in the reactor.

When the component in the reaction mixture leave the reactor, the energy is assumed to be lost from the time it moves from the reactor to the buffer tank, and then to the SMB chromatography column. The reaction mixture then enters the SMB column and is heated to 60°C, where the chromatographic separation takes place. The energy required to heat the components is given in Tables 5.21 and 5.22. The sum of these two energy figures should correspond to the energy required to heat the reaction mixture in the reactor.

The most energy intensive operation in the scFOS production process is the spray drying of the 'wet' scFOS to ensure that all the moisture is removed

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Table 5.20: Energy input to reactor R-102 in figure 4.6

Component	Flow rate (kg/h)	Mass %	Enthalpy (kW)
Kestose (GF_2)	94.17	12.49	1.20
Nystose (GF_3)	125.56	16.65	1.47
1^F-F-Nystose (GF_4)	23.54	3.12	0.27
GF_5	9.36	1.24	0.11
GF_6	3.74	0.50	0.04
GF_7	1.87	0.25	0.02
Fructose (F)	14.97	1.99	0.17
Glucose (G)	86.08	11.42	1.01
Sucrose (GF)	46.52	6.17	0.56
Water	345.81	45.52	13.92
FFase	2.41	0.32	0.10
Total	**754.03**	**100**	**18.90**

Table 5.21: Energy input to SMB chromatography column R-103 in figure 4.6

Component	Flow rate (kg/h)	Mass %	Enthalpy (kW)
Kestose (GF_2)	89.39	15.48	1.14
Nystose (GF_3)	119.19	20.65	1.40
1^F-F-Nystose (GF_4)	22.34	3.87	0.26
GF_5	8.88	1.54	0.10
GF_6	3.55	0.62	0.04
GF_7	1.78	0.31	0.02
Fructose (F)	0.75	0.13	0.01
Glucose (G)	4.30	0.74	0.05
Sucrose (GF)	2.32	0.40	0.03
Water	324.79	56.26	13.23
Total	**577.31**	**100**	**16.28**

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Table 5.22: Energy input to SMB chromatography column R-103 in figure 4.6

Component	**Flow rate (kg/h)**	**Mass %**	**Enthalpy (kW)**
Kestose (GF_2)	4.70	2.73	0.060
Nystose (GF_3)	6.27	3.64	0.073
1^F-F-Nystose (GF_4)	1.18	0.68	0.013
GF_5	0.47	0.27	0.005
GF_6	0.19	0.11	0.002
GF_7	0.09	0.05	0.001
Fructose (F)	14.21	8.24	0.17
Glucose (G)	81.72	47.37	0.96
Sucrose (GF)	44.16	25.60	0.53
Water	17.09	9.91	0.69
Total	**172.49**	**100**	**2.51**

and that it is ready for packaging and sales. The 'wet' scFOS enters the spray drying chamber where it is sprayed through a nozzle. The chamber is already heated with hot dry air at 180°C. The hot dry air absorbs almost all of the moisture in the scFOS leaving a dry powder and the hot 'wet' air leaves the spray dryer and is sent to a purge. A lot of hot dry air is needed to perform the moisture removal and Huang and Mujumdar (2007) provided valuable inlet air feed correlations with regard to the design parameters of the spray dryer. Table 5.23 shows the energy required to heat up the air and Table 5.25 shows the energy required to heat up the components that need to be dried.

Table 5.23: Energy input for hot dry air to spray dryer F-104 in figure 4.6

Component	**Flow rate (kg/h)**	**Mass %**	**Enthalpy (kW)**
Hot dry air	7 563.00	100	327.26
Total	**7 563.00**	**100**	**327.26**

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The spray dryer also needs the air to be extracted from the process. To determine the duty of the fan that needs to extract the air, the fan curves are used in appendix H. The volumetric air flow rate corresponds to the duty at a given propeller speed. For the given situation, Table 5.24 shows the energy needed to run the fan in the spray dryer.

Table 5.24: Energy input for the extraction fan in the spray dryer F-104 in figure 4.6

Component	**Flow rate (m^3/h)**	**Duty (kW)***
Hot dry air	6 290.20	1.60
Total	**6 290.20**	**1.60**

* Duty figure taken from Figure H.1 in appendix H.

Table 5.25: Energy input for heating scFOS in spray dryer F-104 in figure 4.6

Component	**Flow rate (kg/h)**	**Mass %**	**Enthalpy (kW)**
Kestose (GF_2)	89.39	15.48	4.57
Nystose (GF_3)	119.19	20.65	5.60
1^F-F-Nystose (GF_4)	22.34	3.87	1.04
GF_5	8.88	1.54	0.40
GF_6	3.55	0.62	0.14
GF_7	1.78	0.31	0.07
Fructose (F)	0.75	0.13	0.03
Glucose (G)	4.30	0.74	0.20
Sucrose (GF)	2.32	0.40	0.11
Water	324.79	56.26	52.91
Total	**577.31**	**100**	**65.09**

The raffinate from the SMB chromatography column is also treated in the scFOS production process as there are still valuable sugars that are present

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in this stream (stream 8 in Figure 4.6). To remove the unwanted moisture in the raffinate stream, an evaporator is used. The evaporator operates at 105°C (temperature choice influenced by work done by Pridgeon and Badger (1924)). The energy consumption from the evaporator is given in Tables 5.26 and 5.27.

Table 5.26: Energy input for the evaporator to treat the by-product, E-101 in figure 4.6

Component	Flow rate (kg/h)	Mass %	Enthalpy (kW)
Kestose (GF_2)	4.470	3.08	0.106
Nystose (GF_3)	5.960	4.10	0.130
1^F-F-Nystose (GF_4)	1.117	0.77	0.024
GF_5	0.444	0.31	0.010
GF_6	0.178	0.12	0.004
GF_7	0.089	0.06	0.002
Fructose (F)	13.501	9.29	0.292
Glucose (G)	77.630	53.41	1.698
Sucrose (GF)	41.955	28.87	0.942
Total	**145.343**	**100**	**3.208**

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Table 5.27: Energy input for the evaporator to treat the by-product, E-101 in figure 4.6

Component	Flow rate (kg/h)	Mass %	Enthalpy (kW)
Kestose (GF_2)	0.235	0.95	0.0056
Nystose (GF_3)	0.314	1.27	0.0068
1^F-F-Nystose (GF_4)	0.059	0.24	0.0013
GF_5	0.023	0.09	0.0005
GF_6	0.009	0.04	0.0002
GF_7	0.005	0.02	0.0001
Fructose (F)	0.711	2.87	0.0154
Glucose (G)	4.086	16.51	0.0893
Sucrose (GF)	2.208	8.92	0.0496
Water	17.094	69.09	1.2929
Total	**24.750**	**100**	**1.4621**

The sum of all the energy is given in Table 5.28, where the total annual power consumption is shown for an scFOS production rate of 2 000 tonnes per annum.

Table 5.28: Total energy needed for the scFOS production process, focusing on 2 000 tpa (figure 4.6)

Unit operation	Energy consumption	Unit
Bioreactor (R-102)	18.90	kW
SMB chromatography column (R-103)	18.80	kW
Spray dryer (F-104)	393.95	kW
Evaporator (E-101)	4.67	kW
Total	**436.22**	**kW**
Total energy for a year	**3 454 862.40**	**kW.h**

The summary of the total energy consumption is given in Table 5.29 where

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it shows that the spray dryer uses a lot of energy compared to the other unit operations. The total amount is given at the bottom of the table in kilowatts and kilowatt hours.

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Table 5.29: Summary of the total energy needed for the scFOS production process, comparing all production scales

Production scale	**500**	**1 000**	**2 000**	**5 000**	**10 000**	**20 000**	**30 000**	**40 000**	**50 000**
Reactor	5.65	8.57	18.45	29.26	84.09	169.80	255.13	340.60	425.89
SMB Column	5.57	8.49	18.45	29.19	84.02	169.73	255.06	340.53	425.82
Spray dryer	103.12	192.65	392.91	894.87	1 924.50	3 858.20	5 789.95	7 722.36	9 653.90
Evaporator	1.25	2.23	4.63	9.82	22.36	44.92	67.44	89.97	112.49
Total (kW)	**115.59**	**211.95**	**432.77**	**963.13**	**2 114.97**	**4 242.65**	**6 367.60**	**8 493.45**	**10 618.10**
Total (MW.h)	**915.49**	**1 678.61**	**3 427.57**	**7 628.03**	**16 750.53**	**33 601.75**	**50 431.32**	**67 268.15**	**84 095.38**

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Chapter 6

Process Economics

6.1 Economies of Scale

One of the main aims of this project was to discover the economic viability of the implementation of a β-D-fructofuranosidase and a scFOS plant to be built in South Africa. Various scales of production were looked at to see what would be the minimum scale of production that would allow the plant to be able to succeed. An important factor to consider in the scale-up of FFase and scFOS production was economies of scale. A company will experience an economy of scale if the cost per unit decreases as the output volume increases (Fourie *et al.*, 2008). In other words, as the levels of production increase, the equipment prices should become less per unit volume of the piece of equipment. This can also apply to some of the bulk raw material prices. Therefore, if one were to increase the scale of production, it would become more profitable for the company provided that the company reaches its sales target.

This is the biggest challenge of the company because it is good to know that the company will enjoy a higher IRR at larger production levels, but this is under the assumption that all the manufactured product is sold. This is an optimist view, but another way to approach this challenge is to find out information about the market, make a pessimistic assumption and try to determine what is the minimum level of production that one can produce at in order to achieve an IRR of a least 30%.

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6.2 Process Costing

6.2.1 Assumptions

The process costing model is based on a template suggested by Choi and Lee (1997) and in some aspects of the costing of the FFase and scFOS production plant, various assumptions needed to be made. The following assumptions were made:

- ScFOS is sold at a price of US$5-10/kg.
- The project life is 10 years.
- Tax rate is given as the South African governmental standard of 28%.
- Salvage value of plant is 10% of initial value.
- The fixed capital investment is implemented in the first two years of the project with 60% of the money being used in the first year and 40% of the money being used in the second year.
- The amount of labourers working in the facility was assumed to be 30 people.
- The wage for the labourers was R25 per hour. This was assumed from South Africa's national wage website (www.mywage.co.za).
- The working capital for the project was assumed to be 20% of the fixed capital investment (Turton *et al.*, 2008).
- The process costing method that was developed was partially based on work done by Choi and Lee (1997), Haas *et al.* (2006) and Petrides (2000).

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6.2.2 Scenarios

The project proposes some costing scenarios to choose the best conditions for the production of FFase and scFOS. Figure 6.1 gives an illustrative view of the different cases that are to be looked at. The first scenario is aimed at determining at what price the FFase needs to be sold at in order to achieve an IRR of at least 30% considering both production strains (AOX and GAP) as well as at different production levels.

Regarding the scFOS production; there are four scenarios that are looked at (scenario 2-5). Scenario 2 looks at the prospect of self producing the FFase using the GAP production strain to be able to supply for a local scFOS production plant (using results generated from the consortium's experiments) compared to using an external toll manufacturer to produce the enzyme. The external toll manufacturer would typically have a higher price compared to local production. The capital cost of equipment is looked at for both the FFase and scFOS production facilities as well as the operating costs. This scenario is based on the assumption that the FFase and the scFOS production plants run independently, but that the scFOS production plant is supplied with the amount of enzyme it needs from the FFase production plant.

Scenario 3 is the same as scenario 2 except it uses AOX instead of GAP. It looks at the prospect of self producing the FFase using the AOX production strain to be able to supply for a local scFOS production plant (using results generated from the consortium's experiments) compared to using an external toll manufacturer to produce the enzyme. Scenario 4 and 5 are also similar to scenario 2 and 3 except that the best results from literature are used instead of the best results obtained from the experiments performed by the consortium. All these scenarios are compared to each other in order to find the most feasible option, which would then be the best option to take if this project would come into fruition.

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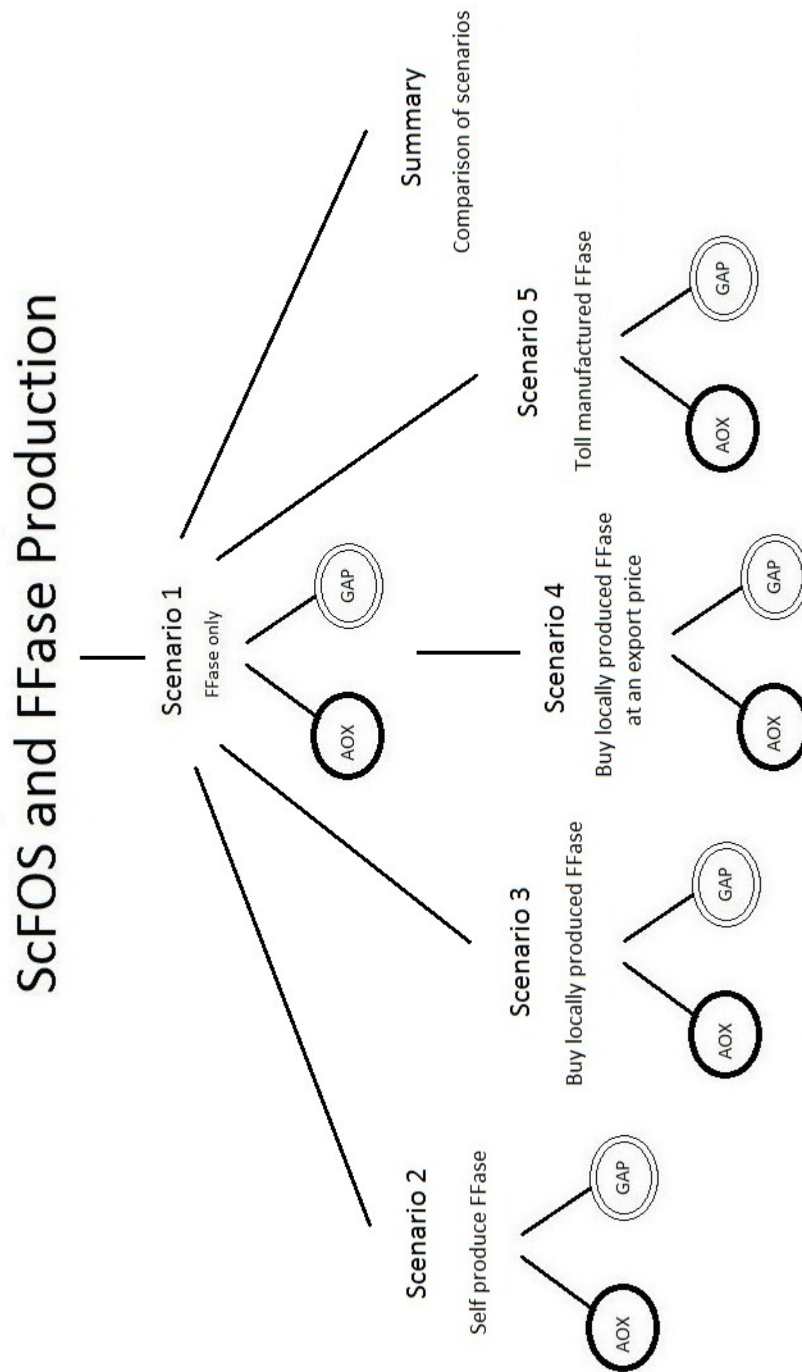

Figure 6.1: FFase and scFOS project mind map showing the different scenarios that are proposed within the project. Two sets of results are used; the best from literature as well as the best results obtained from the experiments performed by the scientists within the scFOS project research group.

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6.2.3 RiskSim

When performing a costing analysis on a large project, the requirement is to estimate the capital and operating expenditure as close as possible to what one would think would be an accurate portrayal of the cost estimation. Accurate quotes need to be obtained from potential suppliers for equipment as well as raw materials. Once all the prices are known the costing analysis can be done with the use of a costing worksheet.

In order for the cost estimation to be as realistic as possible, the Microsoft Excel® add-in, RiskSim was used to perform many Monte Carlo analyses on the different scenarios that are proposed in this project. The random number generator in Microsoft Excel®(2010) was used to generate random cost deviations so that a Monte Carlo simulation can be done on the costing of the project at the designated production scale.

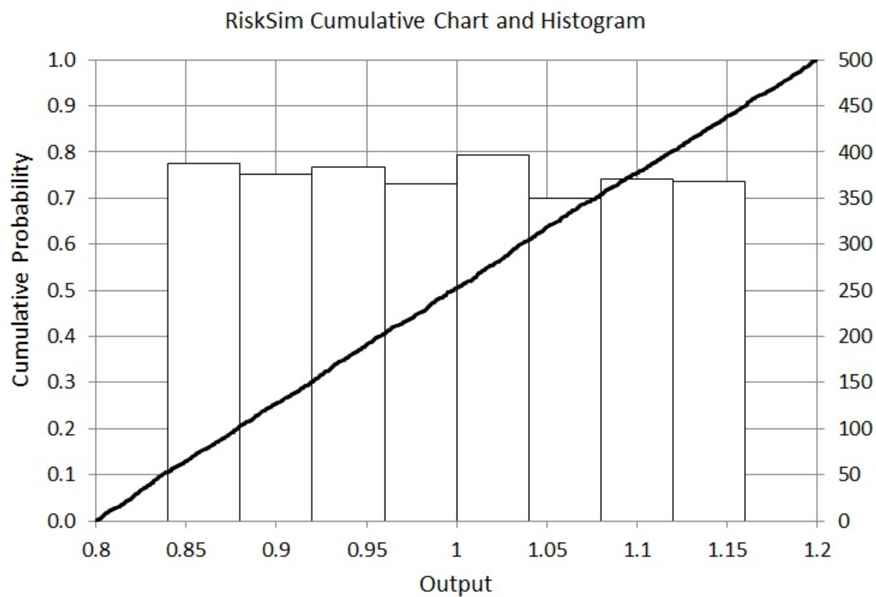

Figure 6.2: A cumulative chart and histogram of a simulation run to 3 000 iterations. The results of the simulation have the following results: mean = 0.998, st. dev. = 0.116, mean st. error = 0.002, minimum = 0.800, first quartile = 0.898, median = 0.998, third quartile = 1.097, maximum = 1.200, skewness = 0.0183.

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RiskSim, the Microsoft Excel (2010)® add-in, facilitates a Monte Carlo simulation by providing: nine random number generator functions, the ability to be able to set the seed for the random number generator, automatic repeated sampling for simulation, frequency distribution of simulation results, histogram and cumulative distribution charts. The random number generator in Microsoft Excel (2010)® is used to generate any number in the range that you specify. RiskSim then uses the random number generator to generate up to a maximum of three thousand iterations. Figure 6.2 shows an example of a RiskSim cumulative chart and histogram, showing three thousand iterations between 0.8 and 1.2. For the costing model in this project, the prices for the equipment are assumed to deviate 20% either way of its given value.

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6.2.4 Costing Worksheet

After the design and mass and energy balance of the process, a costing worksheet needed to be developed so that the important values such as capital expenditure (CAPEX) and operating expenditure (OPEX) can be calculated and ultimately work out the feasibility of the project.

The CAPEX is the figure that possible investors would like to know as this would be the money that they would have to put in, in order for the project to start. The investors also like to know the IRR of the project so that they will know what type of return they can expect back from their initial money input.

Table 6.1 shows the first section of the costing analysis where the capital expenditure is estimated. The construction of this worksheet was obtained from many different sources. The costing worksheet was based on two models proposed by Choi and Lee (1997) and Petrides (2000). It must be noted that the equipment prices for both the FFase and the scFOS production were obtained from the references shown and this was based on an scFOS production scale of 10 000 tonnes per annum and a corresponding FFase production scale (using the AOX production strain) of 72 tonnes per annum. The costs for the different production scales were adjusted according to the 'six tenths' rule.

Once a basic idea of a model was developed, the model had to be adjusted and changed according to the requirements of this project. Quotes for equipment needed to be obtained from reliable suppliers so that the model was accurate and had realistic estimations. In Table 6.1, it can be seen that the equipment quotes have been obtained from a few suppliers. SIRIUS Engineering (Pty) Ltd is an engineering consulting company in Paarl, South Africa that performed a costing analysis on the capital expenditure of industrial FFase and scFOS production related to this project. They have knowledge of local suppliers of equipment and their equipment reference has been shown in Table 6.1. SIRIUS Engineering (Pty) Ltd were also supplied with process design and the

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equipment sizing, where they performed their costing analysis on that basis. They performed their own capital cost estimation to be used as a verification of the estimate performed in this project. De Dietrich Process Systems Ltd is a global company that focuses on manufacturing process equipment. The South African branch exists in Boksburg, Gauteng.

Table 6.2 shows the operating costs of the costing model, where all the raw materials and operating costs of the company were looked at. Table 6.2 is also the second half of the entire costing worksheet and the total annual costs for the entire project is given as the sum of all the costs in both Tables 6.1 and 6.2. Sigma-Aldrich® is a company that, amongst others, manufactures and supplies chemicals. The prices for the necessary chemicals needed for this process were obtained from Sigma-Aldrich®. Alibaba.com® is a global trading site based in Asia where one can search for equipment and chemical product and be put into contact with suppliers. Some of the prices obtained in this costing worksheet were from suppliers that were contacted via email and were based in China.

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Table 6.1: Costing worksheet used to calculate capital and operating costs for the enzyme and scFOS production (2 000 tpa using the AOX production strain)

FIXED CAPITAL ESTIMATE			
Item	**Price**	**Unit**	**Reference**
Land	9 000 000	ZAR/15 000 m^2	SIRIUS Engineering (Pty) Ltd
TLPC		ZAR	
Enzyme Production			
Organism preparation laboratory	2 500 000	ZAR/system	SIRIUS Engineering (Pty) Ltd
Fermenters	500 000	EURO/30 m^3	De Dietrich Process Systems Ltd
Industrial chiller	9 000	USD/unit	Alibaba.com®
Spray balls	30 000	ZAR/system	SIRIUS Engineering (Pty) Ltd
Centrifuge	10 000	USD/unit	Alibaba.com®
Scrubber	10 000	USD/unit	Alibaba.com®
Ultrafiltration	10 000	USD/unit	Alibaba.com®
Storage tank	30 000	USD/30 000L	Alibaba.com®
Rotary yeast drier	3 000 000	ZAR/unit	SIRIUS Engineering (Pty) Ltd
Total		ZAR	
ScFOS Production			
Reactor	1 000 000	ZAR/unit	SIRIUS Engineering (Pty) Ltd
Storage Tanks	370 000	ZAR/4 units	SIRIUS Engineering (Pty) Ltd
SMB chromatography column	3 000 000	ZAR/unit	SIRIUS Engineering (Pty) Ltd
Spray dryer	20 000 000	ZAR/unit	SIRIUS Engineering (Pty) Ltd
Evaporator	185 000	ZAR/unit	SIRIUS Engineering (Pty) Ltd
Total		ZAR	
TEPC			
Installation	(0.7 * TEPC)	ZAR	Choi and Lee (1997), Petrides (2000)
Process piping	(0.35 * TEPC)	ZAR	Choi and Lee (1997), Petrides (2000)
Instrumentation	(0.4 * TEPC)	ZAR	Choi and Lee (1997), Petrides (2000)
Insulation	(0.03 * TEPC)	ZAR	Choi and Lee (1997), Petrides (2000)
Electrical	(0.1 * TEPC)	ZAR	Choi and Lee (1997), Petrides (2000)
Buildings	(0.45 * TEPC)	ZAR	Choi and Lee (1997), Petrides (2000)
Yard Improvement	(0.15 * TEPC)	ZAR	Choi and Lee (1997), Petrides (2000)
Auxiliary facilities	(0.4 * TEPC)	ZAR	Choi and Lee (1997), Petrides (2000)
TPDC		ZAR	
Engineering	(0.25 * TPDC)	ZAR	Choi and Lee (1997), Petrides (2000)
Construction	(0.35 * TPDC)	ZAR	Choi and Lee (1997), Petrides (2000)
TPIC		ZAR	
Contractor's fee	(0.05 * (TPDC + TPIC))	ZAR	Choi and Lee (1997), Petrides (2000)
Contingency	(0.1 * (TPDC + TPIC))	ZAR	Choi and Lee (1997), Petrides (2000)
OC		ZAR	
Working capital (WC)	(0.20 * (TPDC + TPIC + OC))	ZAR	Turton *et al.* (2008)
DFC	TPDC + TPIC + OC + WC	ZAR	

TLPC = total land purchase cost, TEPC = total equipment purchase cost, TPDC = total plant direct cost, TPIC = total plant indirect cost, OC = other costs, DFC = direct fixed capital

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Table 6.2: Costing worksheet continued...

Item Amount	Price	Unit	Reference
ANNUAL OPERATING COSTS		ZAR	
Salvage value (S)	(0.1 * DFC)	ZAR	Assumption
Depreciation (dk)	((DFC - S) / 5)	ZAR	Turton *et al.* (2008)
Maintenance Material	(0.1 * dk)	ZAR	Assumption
Insurance	(0.01 * DFC)	ZAR	Choi and Lee (1997)
Local Taxes	(0.02 * DFC)	ZAR	Choi and Lee (1997)
Factory Expense	(0.05 * DFC)	ZAR	Choi and Lee (1997)
DFC-DEPENDENT ITEMS		ZAR	
Operating Labour (OL)	25	ZAR/hour	mywage.co.za
Maintenance Labour (ML)	(0.35 * OL)	ZAR	Choi and Lee (1997)
Fringe benefits	(0.4 * (OL + ML))	ZAR	Choi and Lee (1997)
Supervision	(0.2 * (OL + ML))	ZAR	Choi and Lee (1997)
Operating supplies	(0.1 * OL)	ZAR	Choi and Lee (1997)
Laboratory	(0.15 * OL)	ZAR	Choi and Lee (1997)
LABOUR DEPENDENT ITEMS		ZAR	
ADMIN AND OVERHEADS		ZAR	
RAW MATERIALS			
Enzyme production ingredients			
Glycerol (batch)	28	ZAR/L	Alibaba.com®
Glycerol (fed batch)	28	ZAR/L	Alibaba.com®
Methanol (fed batch)	14.2	ZAR/L	Alibaba.com®
Yeast extract	858	ZAR/kg	Alibaba.com®
Phosphoric acid 85%	528	ZAR/L	Sigma-Aldrich®
Calcium sulphate	780	ZAR/kg	Sigma-Aldrich®
Magnesium sulphate-7H_2O	476	ZAR/kg	Sigma-Aldrich®
Potassium hydroxide	566	ZAR/kg	Sigma-Aldrich®
Caustic soda 1%	425	USD/ton	Alibaba.com®
TOTAL COST		ZAR	
ScFOS production ingredients			
Sucrose	0.2396	USD/lb	illovosugar.com
TOTAL COST		ZAR	
Utilities			
Electricity scFOS	0.65	ZAR/kW.h	durban.olx.co.za/
Electricity FFase	0.65	ZAR/kW.h	durban.olx.co.za/
TOTAL COST		ZAR	
GRAND TOTAL COST		ZAR	

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6.2.5 FFase Production

6.2.5.1 Scenario 1 - FFase production using GAP and AOX production strain

The first scenario compares the production of β-D-fructofuranosidase using the GAP and the AOX strains. From the experiments performed by the scientists it was found that when comparing GAP and AOX, AOX performed better where it produced a more concentrated enzyme. GAP also produced an appreciable amount of enzyme but the concentration level was not to the same degree as AOX.

Because AOX produced a more concentrated enzyme compared to GAP production process, the fermenter size for the AOX production process was smaller than GAP when producing the same amount of enzyme. As a result, the capital expenditure for the AOX production process was less than the GAP production process.

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Capital Expenditure

The capital expenditure for the FFase production system using GAP as the production strain was costed. Table 6.3 shows the capital expenditure (CAPEX) also known as the direct fixed capital (DFC) of an FFase production plant using GAP at different production levels corresponding to the respective scFOS production scale it would supply to.

Table 6.3: Enzyme production direct fixed capital using the GAP production strain

Item	**ScFOS production scale (tpa)**				
	2 000	**5 000**	**10 000**	**20 000**	**30 000**
	FFase production scale (tpa)				
	19	**48**	**95**	**191**	**286**
	Cost (Rand '000)				
Land	1 807	4 517	11 877	18 072	27 108
TOTAL	**1 807**	**4 517**	**11 877**	**18 072**	**27 108**
Organism preparation laboratory	1 127	1 953	2 953	4 486	5 722
Fermenters	790	1 369	2 070	3 145	4 012
Industrial chiller	101	175	264	401	512
Spray balls	14	23	35	54	69
Centrifuge	44	77	116	176	224
Scrubber	44	77	116	176	224
Ultrafiltration	44	77	116	176	224
Rotary yeast dryer	1 352	2 343	3 543	5 383	6 866
Storage tank	146	253	354	538	741
TOTAL	**3 662**	**6 345**	**9 595**	**14 578**	**18 593**
Installation	2 563	4 442	6 717	10 205	13 015
Process piping	1 282	2 221	3 358	5 102	6 508
Instrumentation	1 465	2 538	3 838	5 831	7 437
Insulation	110	190	288	437	558
Electrical	366	635	960	1 458	1 859
Buildings	1 648	2 855	4 318	6 560	8 367
Yard improvement	549	952	1 439	2 187	2 789
Auxiliary facilities	1 465	2 538	3 838	5 831	7 437
TOTAL	**9 448**	**16 371**	**24 755**	**37 611**	**47 971**
Engineering	3 277	5 679	8 588	13 047	16 641
Construction	4 588	7 951	12 023	18 266	23 297
TOTAL	**7 866**	**13 630**	**20 610**	**31 314**	**39 938**
Contractor's fee	1 049	1 817	2 748	4 175	5 325
Contingency	2 097	3 635	5 496	8 350	10 650
Working Capital	4 824	8 360	12 641	19 206	24 495
OTHER COSTS (OTC)	**7 970**	**13 812**	**20 885**	**31 731**	**40 470**
CAPEX	**30 753**	**54 677**	**87 722**	**133 306**	**174 080**

Table 6.4 shows the fixed capital expenditure of an FFase production plant

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using AOX as the production strain and it can be seen that less enzyme of the AOX production strain is needed compared to the GAP production strain for a respective production level of scFOS because AOX produces a more concentrated enzyme compared to GAP.

Table 6.4: Enzyme production direct fixed capital using the AOX production strain

Item	ScFOS production scale (tpa)				
	5 000	**10 000**	**20 000**	**30 000**	**40 000**
	FFase production scale (tpa)				
	14	**36**	**72**	**144**	**216**
	Cost (Rand '000)				
Land	1 807	4 518	9 000	18 000	27 000
TOTAL	**1 807**	**4 518**	**9 000**	**18 000**	**27 000**
Organism preparation laboratory	954	1 653	2 500	3 789	4 833
Fermenters	689	1 194	1 806	2 737	3 491
Industrial chiller	86	149	226	343	437
Spray balls	112	194	294	446	568
Centrifuge	37	65	98	149	189
Scrubber	37	65	98	149	189
Ultrafiltration	37	65	98	149	189
Rotary yeast dryer	1 145	1 984	3 000	4 547	5 800
Storage tank	124	214	324	491	626
TOTAL	**3 222**	**5 584**	**8 443**	**12 798**	**16 323**
Installation	2 256	3 908	5 910	8 958	11 426
Process piping	1 128	1 954	2 955	4 479	5 713
Instrumentation	1 289	2 234	3 377	5 119	6 529
Insulation	97	168	253	384	490
Electrical	322	558	844	1 280	1 632
Buildings	1 450	2 513	3 800	5 759	7 345
Yard improvement	470	815	1 233	1 868	2 383
Auxiliary facilities	1 289	2 233	3 377	5 119	6 529
TOTAL	**8 314**	**14 406**	**21 784**	**33 018**	**42 112**
Engineering	2 884	4 998	7 557	11 454	14 609
Construction	4 038	6 997	10 580	16 036	20 452
TOTAL	**6 922**	**11 994**	**18 136**	**27 490**	**35 061**
Contractor's fee	923	1 599	2 418	3 665	4 675
Contingency	1 846	3 198	4 836	7 331	9 350
Working Capital	4 245	7 356	11 124	16 860	21 504
OTHER COSTS (OTC)	**7 014**	**12 153**	**18 378**	**27 856**	**35 529**
CAPEX	**27 279**	**48 656**	**75 742**	**119 162**	**156 024**

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Raw material costs

Table 6.5 shows the operating capital expenditure of an FFase production plant using GAP as the production strain. Using the GAP production strain will have higher operating costs as the fermentation volume is larger when using GAP.

Table 6.5: Enzyme production operating costs for the various production levels using the GAP production strain

Item	**ScFOS production scale (tpa)**				
	2 000	**5 000**	**10 000**	**20 000**	**30 000**
	Production scale (tpa)				
	19	**48**	**95**	**191**	**286**
	Cost (Rand '000)				
Depreciation	5 535	9 842	15 790	23 995	31 334
Maintenance material	553	984	1 579	2 400	3 133
Insurance	308	547	877	1 333	1 741
Local taxes	615	1 094	1 754	2 666	3 482
Factory expenses	1 538	2 734	4 386	6 665	8 704
DFC DEPENDENTS	**8 459**	**15 200**	**24 386**	**37 059**	**48 394**
Operating labour	462	1 155	2 310	4 620	6 930
Maintenance labour	162	404	809	1 617	2 426
Fringe benefits	249	624	1 247	2 495	3 742
Supervision	125	312	624	1 247	1 871
Operating supplies	46	116	231	462	693
Laboratory	69	173	347	693	1 040
LABOUR DEPENDENTS	**1 113**	**2 784**	**5 567**	**11 134**	**16 701**
ADMIN & OVERHEADS	**524**	**1 310**	**2 620**	**5 239**	**7 859**
Raw materials					
Glycerol (batch)	304	761	1 516	3 044	4 565
Glycerol (fed batch)	356	891	1 774	3 562	5 344
Yeast extract	146	365	727	1 462	2 192
Phosphoric acid 85%	506	1 265	2 519	5 058	7 588
Calcium sulphate	8	19	38	77	116
Potassium sulphate	198	495	986	1 979	2 969
Magnesium sulphate-7H_2O	151	378	752	1 511	2 266
Potassium hydroxide	17	42	83	166	249
Caustic soda 1%	1	2	4	8	12
Sodium benzoate 0.2% w/w	13	33	66	132	197
Potassium sorbate 0.2% w/w	2	5	9	19	28
Sorbitol 30%	56	140	279	560	840
TOTAL	**1 758**	**4 394**	**8 754**	**17 578**	**26 366**
Utilities					
Electricity	183	457	910	1 826	2 739
TOTAL	**183**	**457**	**910**	**1 826**	**2 739**
OPEX	**12 127**	**24 144**	**42 237**	**72 835**	**102 059**

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Table 6.6: Enzyme production operating costs for the various production levels using the AOX production strain

Item	**ScFOS production scale (tpa)**				
	2 000	**5 000**	**10 000**	**20 000**	**30 000**
	Production scale (tpa)				
	14	**36**	**72**	**144**	**216**
	Cost (Rand '000)				
Depreciation	4 910	8 758	13 634	21 449	28 084
Maintenance material	491	876	1 363	2 144	2 808
Insurance	273	487	757	1 192	1 560
Local taxes	546	973	1 515	2 383	3 120
Factory expenses	1 364	2 433	3 787	5 958	7 801
DFC DEPENDENTS	**7 583**	**13 526**	**21 056**	**33 127**	**43 375**
Operating labour	396	990	1 980	3 960	5 940
Maintenance labour	139	347	693	1 386	2 079
Fringe benefits	214	535	1 069	2 138	3 208
Supervision	107	267	535	1 069	1 604
Operating supplies	40	99	198	396	594
Laboratory	59	149	297	594	891
LABOUR DEPENDENTS	**954**	**2 386**	**4 772**	**9 544**	**14 315**
ADMIN & OVERHEADS	**449**	**1 123**	**2 245**	**4 491**	**6 736**
Raw materials					
Glycerol (batch)	257	643	1 280	2 560	3 840
Glycerol (fed batch)	70	176	350	700	1 049
Methanol (fed batch)	77	192	382	763	1 145
Yeast extract	123	309	615	1 230	1 844
Phosphoric acid 85%	427	1 068	2 128	4 255	6 383
Calcium sulphate	7	16	33	65	98
Potassium sulphate	167	418	833	1 665	2 498
Magnesium sulphate-7H_2O	128	319	635	1 271	1 906
Potassium hydroxide	14	35	70	140	209
Caustic soda 1%	1	3	7	14	20
Sodium benzoate 0.2% w/w	10	25	50	99	149
Potassium sorbate 0.2% w/w	1	4	7	14	21
Sorbitol 30%	42	106	211	423	634
TOTAL	**1 325**	**3 313**	**6 599**	**13 198**	**19 796**
Utilities					
Electricity	142	355	707	1 425	2 138
TOTAL	**142**	**355**	**707**	**1 425**	**2 138**
OPEX	**10 454**	**20 704**	**35 381**	**61 788**	**86 367**

Table 6.6 shows the operating expenditure of an FFase production plant using AOX as the production strain.

Figure 6.3 shows the expenditure of an FFase production facility using the GAP production strain at different production levels. The comparison between TPDC, TPIC, DFC and OPEX is observed as the production levels increase.

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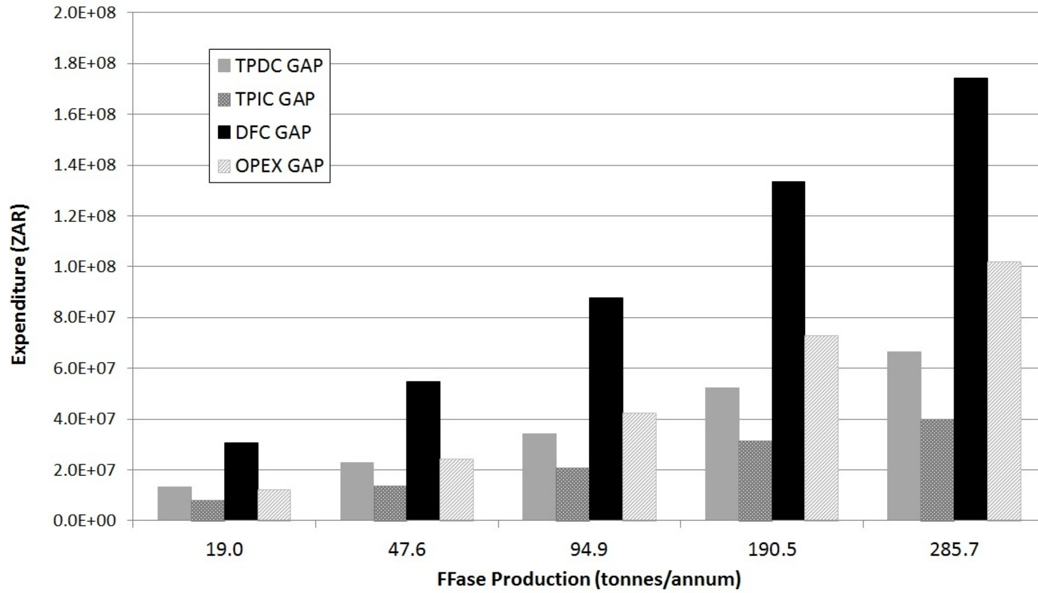

Figure 6.3: The expenses of an enzyme production facility producing FFase with GAP production strain (TPDC = total plant direct cost, TPIC = total plant indirect cost, DFC = direct fixed capital, OPEX = operating expenditure).

Figure 6.4 shows the expenditure of an FFase production facility using the AOX production strain at different production levels. The comparison between TPDC, TPIC, DFC and OPEX is again observed as the production levels increase.

Figure 6.5 shows a DFC/CAPEX and OPEX comparison between the GAP and AOX production strains when producing FFase. AOX is more attractive to use as it costs less money to buy and run the enzyme production facility.

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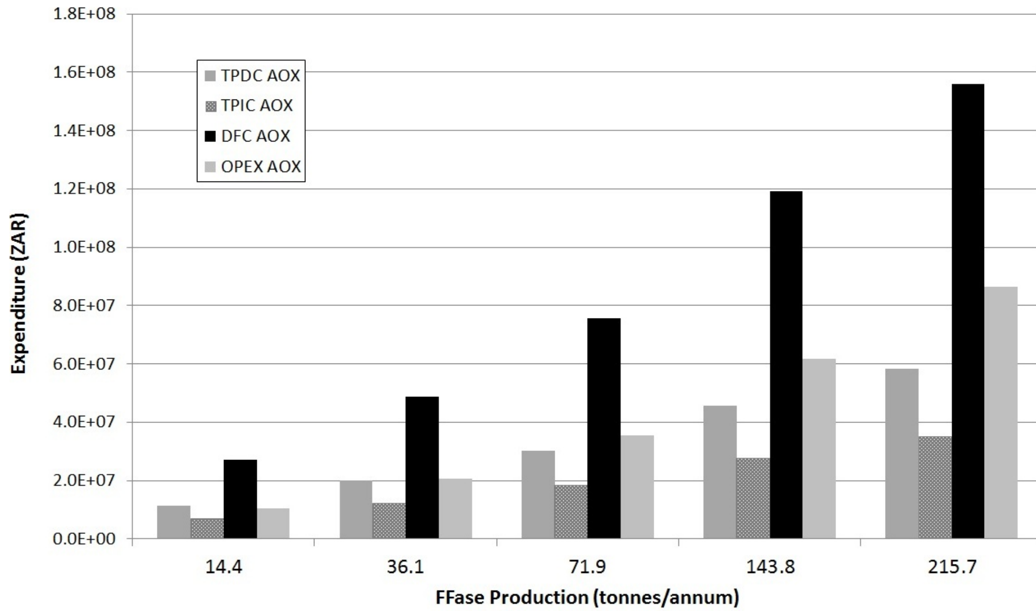

Figure 6.4: The expenses of an enzyme production facility producing FFase with AOX production strain.

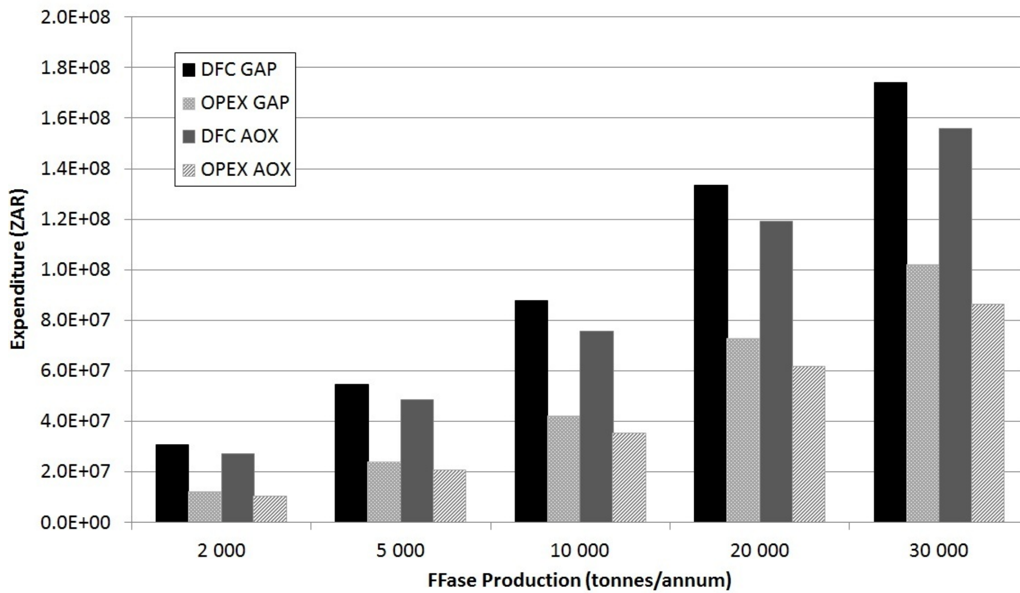

Figure 6.5: The expenses of an FFase production facility comparing the GAP and AOX production strains, corresponding to the scFOS production scale that the enzyme production facility would supply to.

6.2.6 scFOS Production

6.2.6.1 Scenario 2 - ScFOS production, self producing FFase, comparing GAP and AOX production strains

Producing FFase and scFOS in the same facility will have the highest capital costs and operating costs. However, even though this option shows to be the

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most expensive, it will still be a safe venture at production levels of 2 000 tonnes per annum and above. Figure 6.6 shows the expenditure using FFase that has been produced with GAP and Figure 6.7 shows the expenditure using FFase that has been produced with AOX.

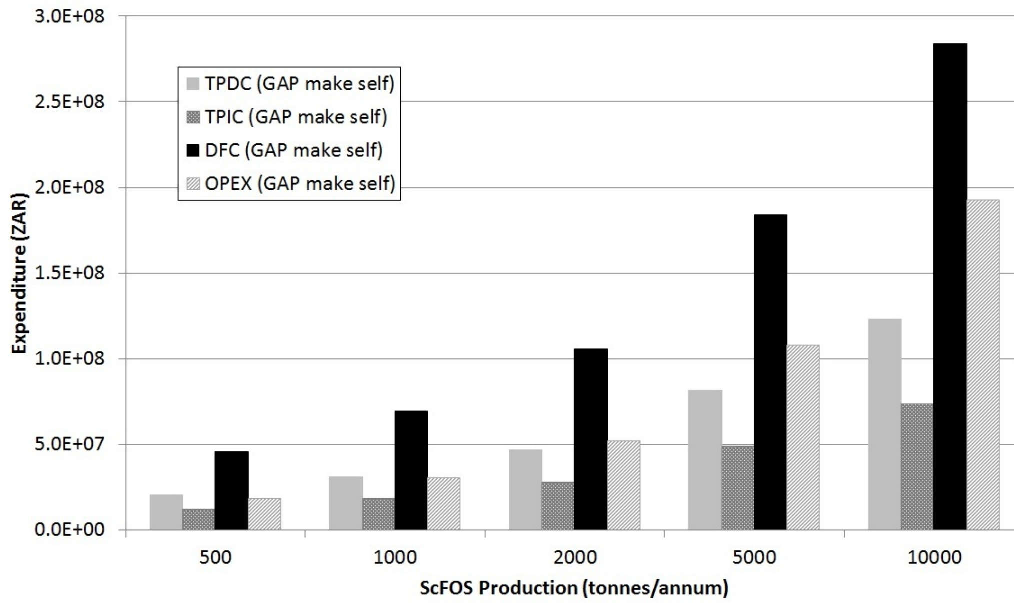

Figure 6.6: The expenses of an FFase production facility comparing the GAP and AOX production strains, corresponding to the scFOS production scale that the enzyme production facility would supply to.

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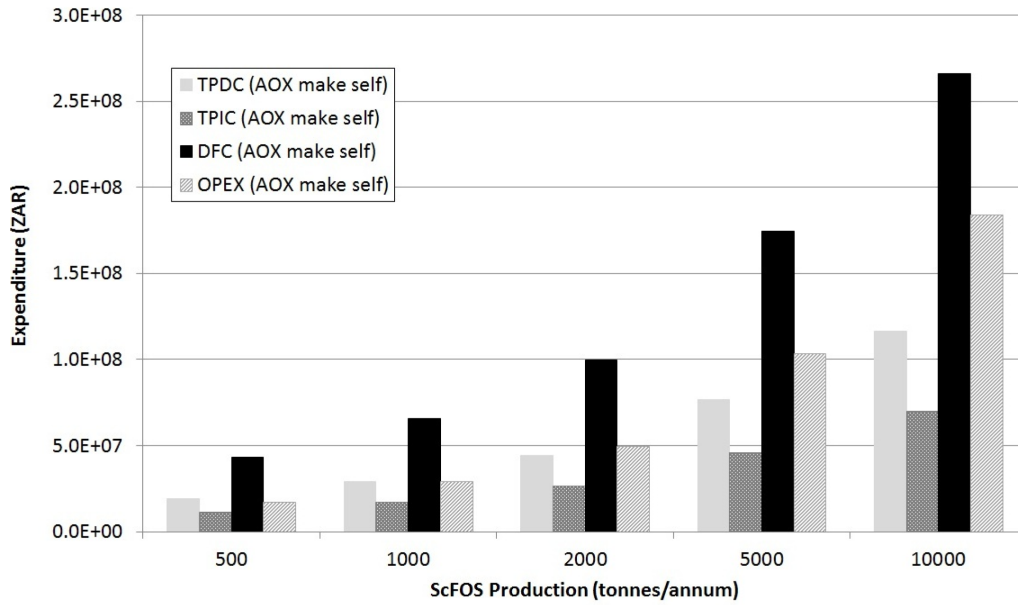

Figure 6.7: The expenses of an FFase production facility comparing the GAP and AOX production strains, corresponding to the scFOS production scale that the enzyme production facility would supply to.

6.2.6.2 Scenario 3 - ScFOS production, buying locally produced FFase, comparing GAP and AOX production strains

The second option to produce scFOS is to buy FFase from a local producer. This option will have less capital and operating expenditure compared to self producing the enzyme, but the price of the enzyme is based on the FFase production facility making a 30% IRR on sales. At low scFOS production levels and thus low FFase production levels, the price of the enzyme will be high, which is unfavourable for the scFOS producer. As the production level increases, the price of the enzyme will decrease. Figures 6.8 and 6.9 show the expenditure for scFOS production buying a GAP produced FFase and an AOX produced FFase respectively.

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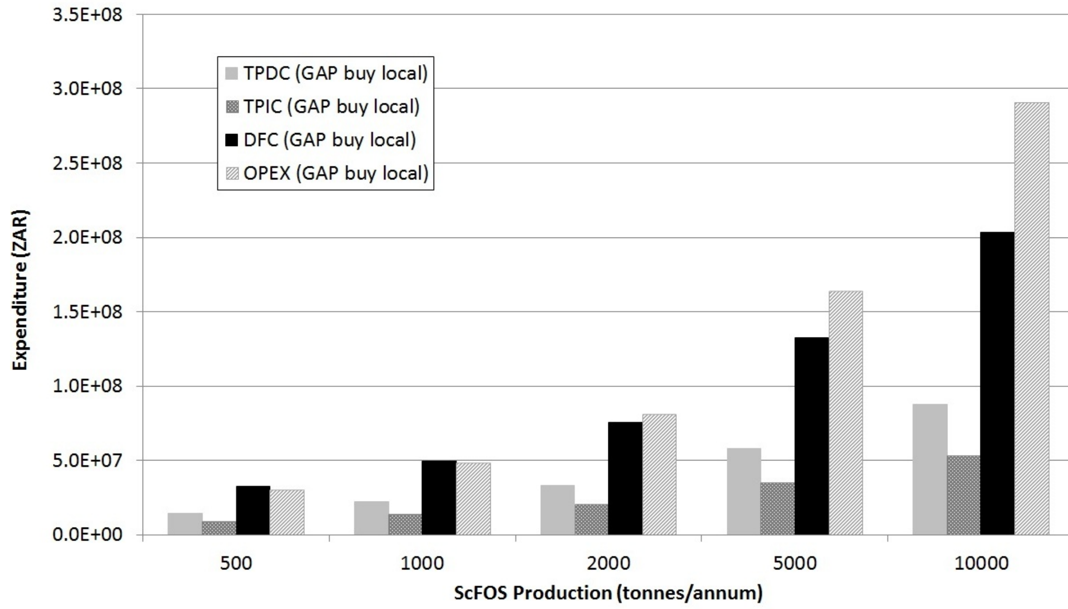

Figure 6.8: The expenses of an FFase production facility comparing the GAP and AOX production strains, corresponding to the scFOS production scale that the enzyme production facility would supply to.

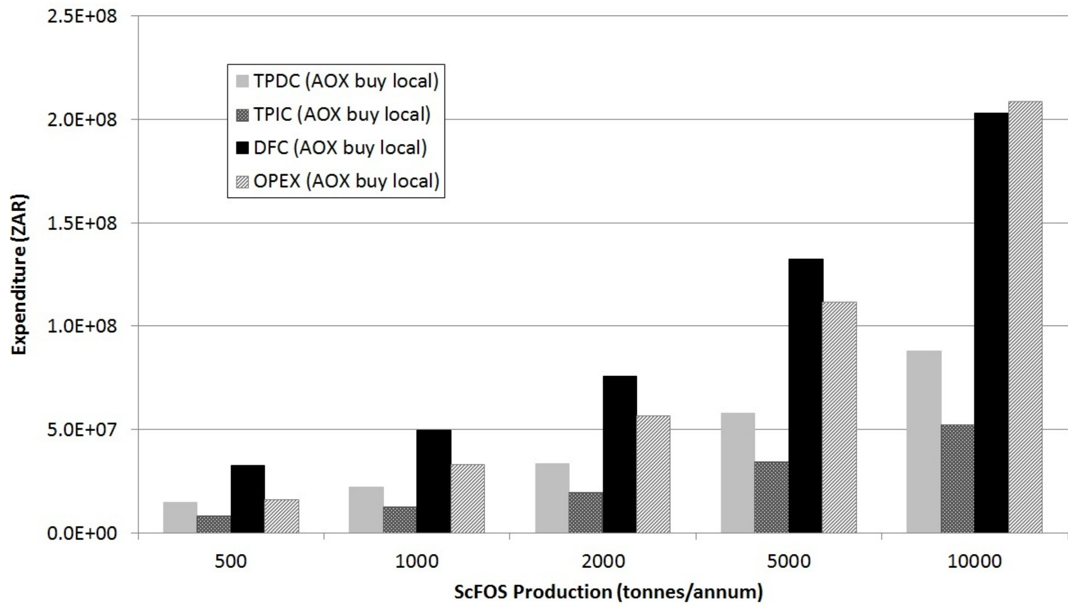

Figure 6.9: The expenses of an FFase production facility comparing the GAP and AOX production strains, corresponding to the scFOS production scale that the enzyme production facility would supply to.

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6.2.6.3 Scenario 4 - ScFOS production, buying FFase from a toll manufacturer, comparing GAP and AOX production strains

The toll manufacturer provides the best option for scFOS production. They can supply FFase at the best price. This is due to the nature of a toll manufacturing facility, where they have many application and supply to many industries, they have the freedom to lower prices to a very competitive level. Figures 6.10 and 6.11 compare the two production strains.

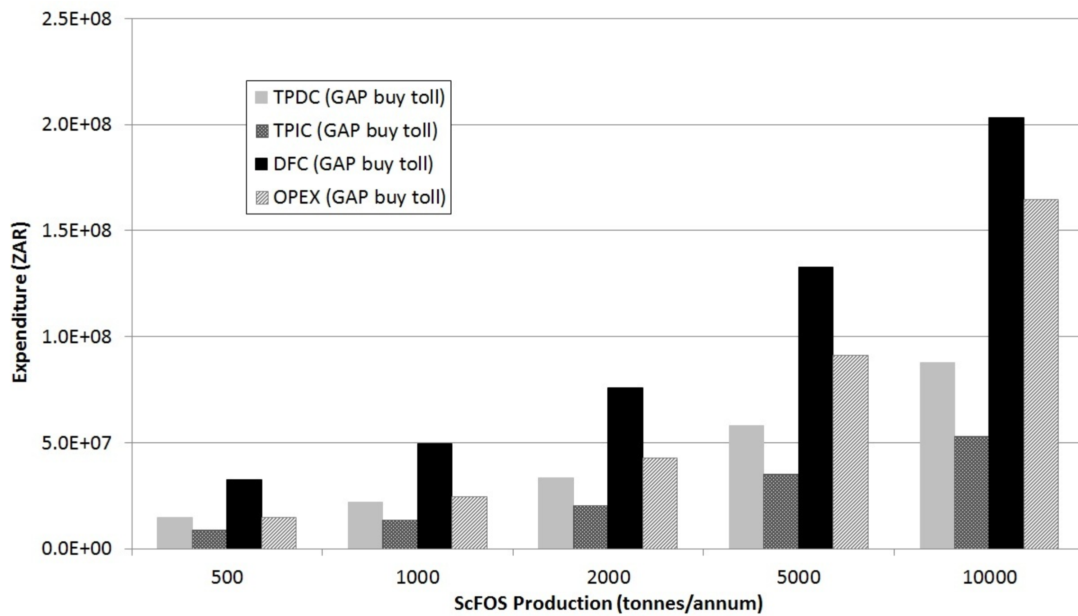

Figure 6.10: The expenses of an FFase production facility comparing the GAP and AOX production strains, corresponding to the scFOS production scale that the enzyme production facility would supply to.

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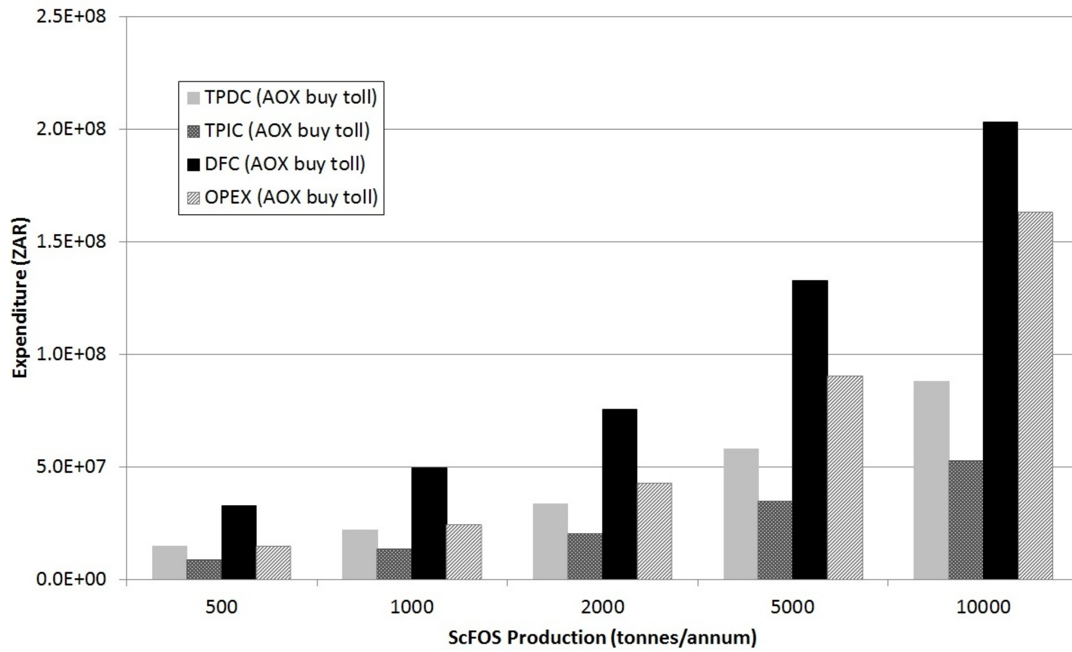

Figure 6.11: The expenses of an FFase production facility comparing the GAP and AOX production strains, corresponding to the scFOS production scale that the enzyme production facility would supply to.

6.2.6.4 Scenario 5 - ScFOS production, buying locally produced FFase at an export price, comparing GAP and AOX production strains

The final option that was looked at was the possibilty of buying FFase from a local supplier that offered the enzyme at an export price. This is under the assumption that the FFase production facility supplies to many different companies and can afford to drop their price to a low amount. This would be ideal for scFOS production as the enzyme would now be cheap and operating costs would be lower for scFOS production. Again, Figures 6.12 and 6.13 compare the GAP and AOX production strains respectively.

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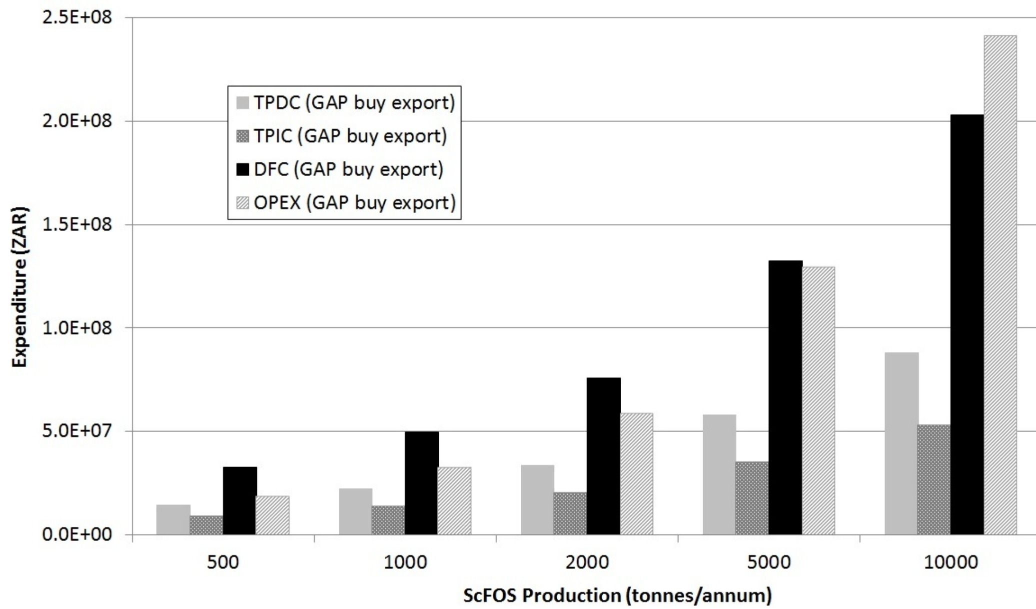

Figure 6.12: The expenses of an FFase production facility comparing the GAP and AOX production strains, corresponding to the scFOS production scale that the enzyme production facility would supply to.

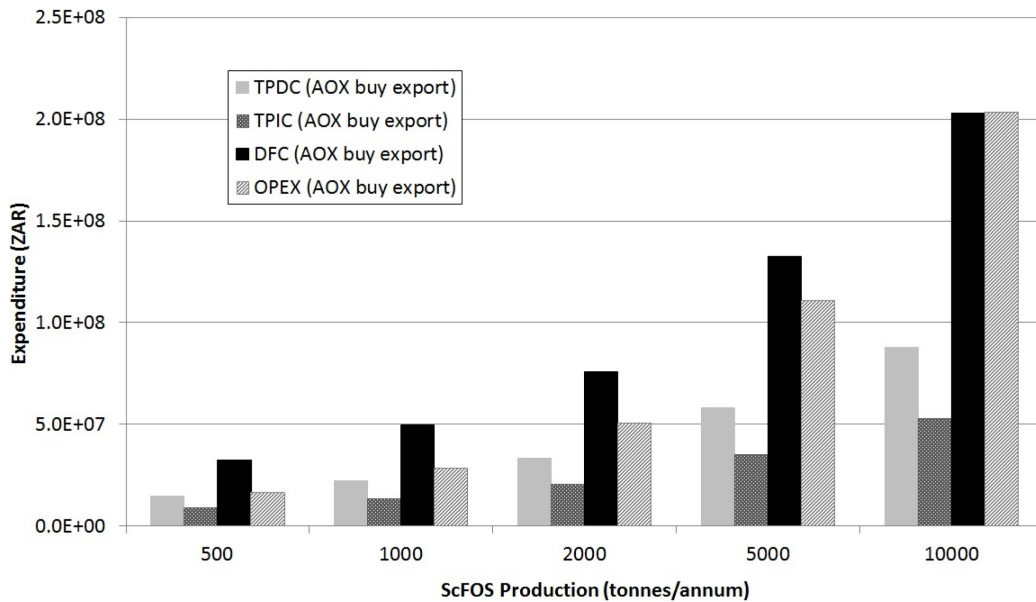

Figure 6.13: The expenses of an FFase production facility comparing the GAP and AOX production strains, corresponding to the scFOS production scale that the enzyme production facility would supply to.

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Table 6.7: Economic summary of scFOS production using the AOX gene at 2 000 tonnes per annum, comparing different scenarios

Production level 2 000 tpa	**Unit**	**Scenario 2 Make self**	**Scenario 3 Buy local**	**Scenario 4 Buy export**	**Scenario 5 Buy toll**
CAPEX	ZAR (millions)	99.8	75.7	75.7	75.7
NPV results					
Mean	ZAR (millions)	196.4	189.7	216.6	238.5
St. Dev.	ZAR (millions)	77.7	78.8	78.9	77.9
Mean St. Error	ZAR (millions)	2.5	2.5	2.5	2.5
Minimum	ZAR (millions)	38.6	31.5	66.1	89.4
First quartile	ZAR (millions)	127.9	123.9	147.6	175.3
Median	ZAR (millions)	201.0	184.6	216.1	233.2
Third quartile	ZAR (millions)	260.8	259.1	282.4	302.4
Maximum	ZAR (millions)	370.5	367.8	395.9	392.1
Skewness		-0.0004	0.1026	0.0339	0.0503
Prob NPV > 0		100%	100%	100%	100%
IRR Results					
Mean	%	38.0	42.0	45.0	46.6
St. Dev.	%	8.4	10.0	9.8	9.5
Mean St. Error	%	0.3	0.3	0.3	0.3
Minimum	%	17.9	18.8	22.9	25.9
First quartile	%	31.5	34.0	37.2	39.0
Median	%	38.8	42.8	45.5	46.7
Third quartile	%	44.8	49.5	53.0	53.9
Maximum	%	55.9	66.4	66.7	68.3
Skewness		-0.1774	-0.0844	-0.0265	-0.0161
Prob IRR > 25%	%	92%	96%	99%	100%

6.2.6.5 Economic Summary

The results in this chapter have shown that is it always more favourable to use AOX as the production strain when producing FFase and thus scFOS. Table 6.7 shows a comparison and statistical analysis of producing scFOS using an FFase produced with AOX, at a production rate of 2 000 tonnes per annum, comparing the scenario explained in this chapter.

After careful marketing and economic investigation it was decided that the most realistic manner to produce scFOS was to self produce the FFase in the same facility as scFOS production. This was due to the low demand of the enzyme and even though there is a higher capital cost, the economic analysis showed that the facility will still make good profit at production levels of 2 000

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tonnes per annum and above. Table 6.8 shows the base, worst and best case scenarios when producing scFOS using FFase that has been produced with AOX, comparing different production levels.

It can be seen in Table 6.8, that positive NPV's are attained for all cases expect for that of the worst case scenario producing 500 tonnes per annum of scFOS. Not surprisingly, the highest NPV is attained at the highest production level and the best case. The same pattern occurs with the IRR in Table 6.8. This is because the NPV and the IRR are mutually dependent.

Table 6.8: Economic summary of scFOS production using the AOX gene at 2 000 tonnes per annum, comparing different scenarios

	ScFOS production scale (tpa)				
	500	**1 000**	**2 000**	**5 000**	**10 000**
IRR					
Base Case	20.6	29.0	37.9	53.4	61.0
Worst Case	0.5	8.6	16.8	31.7	36.0
Best Case	37.3	47.0	57.5	74.5	84.8
NPV					
Base Case	24.2	77.3	199.3	666.9	1 324.8
Worst Case	-20.9	-7.2	39.4	301.0	591.3
Best Case	65.7	154.7	345.0	997.2	1987.3

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Chapter 7

Results and Discussion

7.1 Scenario Comparisons

7.1.1 FFase Production

7.1.1.1 Scenario 1 - FFase production using GAP and AOX production strains

The selling price of the enzyme of β-D-fructofuranosidase is determined by pitching a price that will enable the enzyme production facility to attain an IRR of 30%. Figure 7.1 shows the price of the pure protein against the pure protein production scale. Typically, the enzyme would be sold in a broth form as the enzyme dosage is very low for scFOS production. It also makes for easier handling when using the enzyme in broth form. Figure 7.1 illustrates the effect of economies of scale where the price drops significantly initially and flattens out as the production scale increases to much larger amounts. The FFase produced using the AOX gene can be sold for a lower price because it produces more enzyme in a smaller sized fermenter compared to the FFase produced with the GAP gene.

Figure 7.2 shows a comparison between the GAP and the AOX production strains with FFase in the broth form. The prices show to be very similar but the difference between the two production strains is that the AOX has a higher activity compared to the GAP. There is more protein concentrated in the AOX broth. A potential customer would have to buy less FFase produced with AOX

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compared to the GAP produced FFase. This could be a selling point in the manufacturing of this enzyme. An alternative option could be to dilute the enzyme produced with AOX to the same concentration as a GAP produced FFase or the industry standard. This would enable more enzyme to be sold and thus more profit in the enzyme production facility.

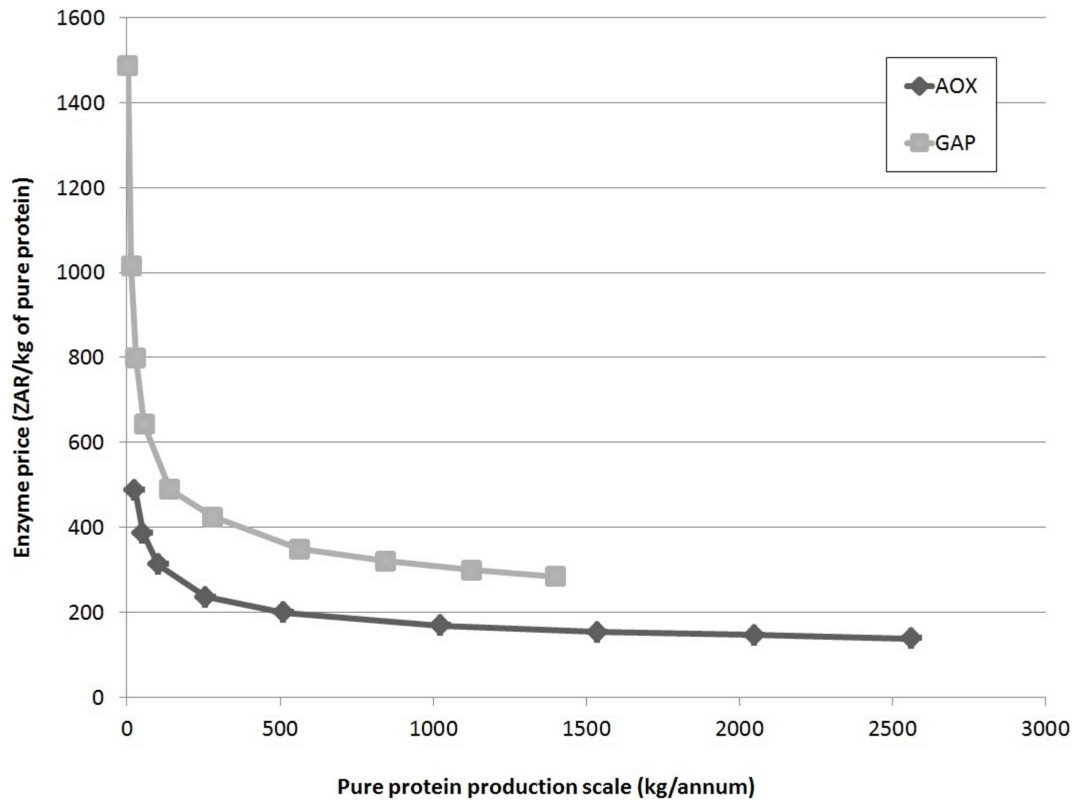

Figure 7.1: Enzyme selling price of pure protein in order for the facility to obtain a 30% IRR.

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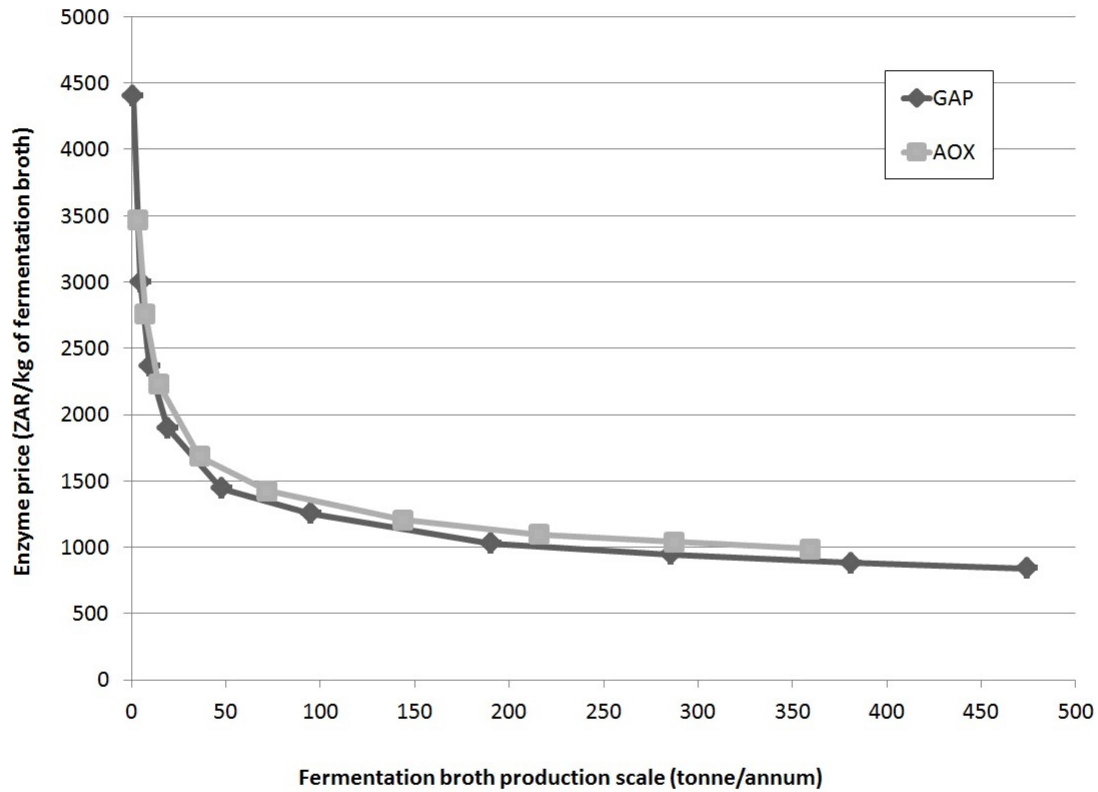

Figure 7.2: Enzyme selling price per fermentation broth in order for the facility to obtain a 30% IRR.

7.1.2 ScFOS Production

7.1.2.1 Scenario 2 - scFOS production at different production levels using FFase that is produced in the same facility as the scFOS, comparing GAP and AOX production strains

There are five scenarios that are considered in the project and four scenarios specifically to produce scFOS in this project (scenarios 2-5). 2) ScFOS can be produced by using an FFase that is produced in the same facility as scFOS. The enzyme production process feeds into the scFOS production process and all the manufacturing takes place in one facility. 3) ScFOS can be produced using an enzyme that is produced in a separate independent facility and that facility will sell FFase to the scFOS production plant. 4) ScFOS can be made using an enzyme that is bought from a toll manufacturer. These are companies which produce many different enzymes and have the capacity to manufacture

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an enzyme of choice on demand. 5) ScFOS can be produced using an enzyme that is produced in a separate independent facility and that facility will sell FFase to the scFOS production plant an an export price.

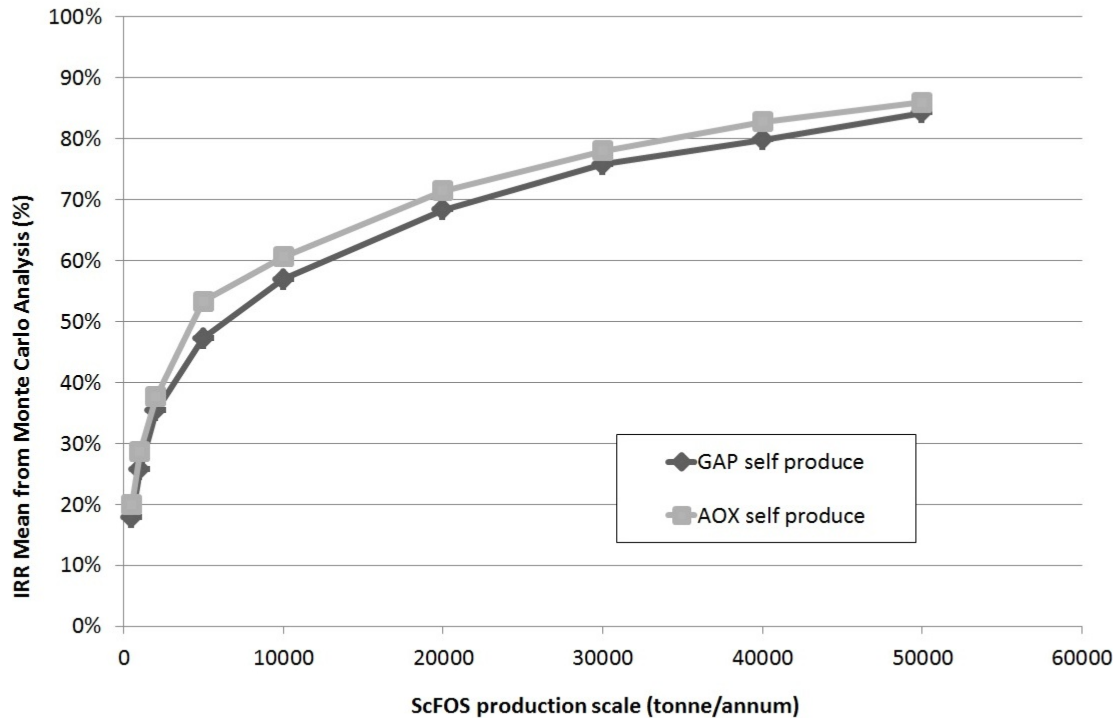

Figure 7.3: ScFOS production using FFase that has been produced in the same facility as the scFOS, comparing the GAP and AOX strains.

Figure 7.3 shows the scenario where scFOS is produced by self-producing FFase in the same facility and the production strain of AOX and GAP are compared. The FFase is produced in the first part of the process and the enzyme broth that is produced is fed straight to the scFOS production process. As the AOX gene produces a more concentrated enzyme, it would use less fermenter volume which would lessen the equipment cost.

Figure 7.3 shows that the IRR is higher using AOX compared to GAP and it is therefore better to produce scFOS while producing FFase with the AOX production strain. As the production levels increase, the difference between the two production strains increases at scFOS production levels between 2 000

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and 10 000 tonnes per annum. This difference then stays constant at scFOS production levels greater than 10 000 tonnes per annum.

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7.1.2.2 Scenario 3 - scFOS production at different production levels using FFase that is bought from a local producer comparing GAP and AOX production strains

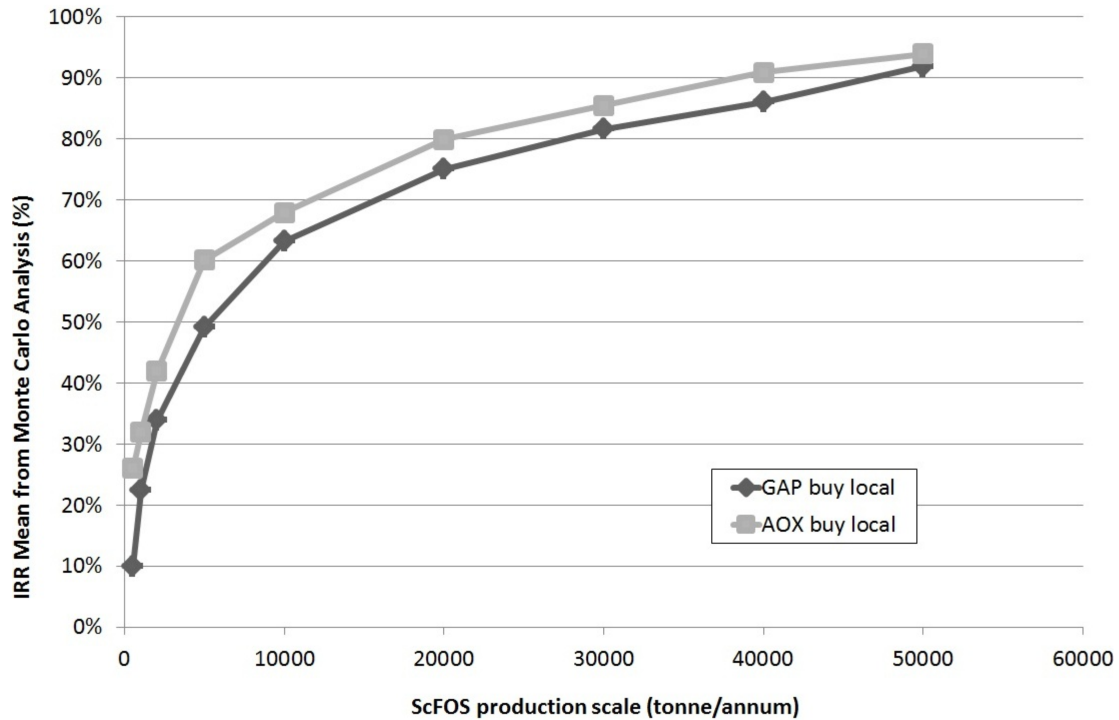

Figure 7.4: ScFOS production using FFase fermentation broth that has been bought from a local FFase producer, comparing the GAP and AOX strains.

Figure 7.4 shows that when producing scFOS, a local enzyme production facility can offer a better enzyme price when producing the FFase with the AOX production strain compared to GAP. It can also be seen that at production levels of 2 000 tonnes per annum, the scFOS production plant can achieve a healthy IRR above 30%.

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7.1.2.3 Scenario 4 - scFOS production at different production levels using FFase that is bought from an external toll manufacturer, comparing GAP and AOX production strains

The third point discussed in the different scenarios to produce scFOS considered the use of a toll supplier. A toll supplier is an enzyme production company that has the capacity to produce many different enzymes. They would produced the FFase as requested as well as many other enzymes to other companies according to their specified requirements. Figure 7.5 shows the comparison between the AOX and GAP production strains, where there is not much difference in the price. The reason for this is due to the fact that when producing scFOS, very little enzyme is needed to be added to the sucrose to produce scFOS. This means that there won't be a huge margin in difference when considering all the costs of the entire model.

This figure also corresponds with Figure 7.1 where one can see that there is not much difference at all with regards to the enzyme fermentation broth price. The toll manufacturing price is based on a quote of £60 000 per 20 000 litres and the required fermenter volume needed for each respective production strain. The costs for GAP is more expensive than AOX, but when considering the entire costs for the whole production plant, the difference is not large.

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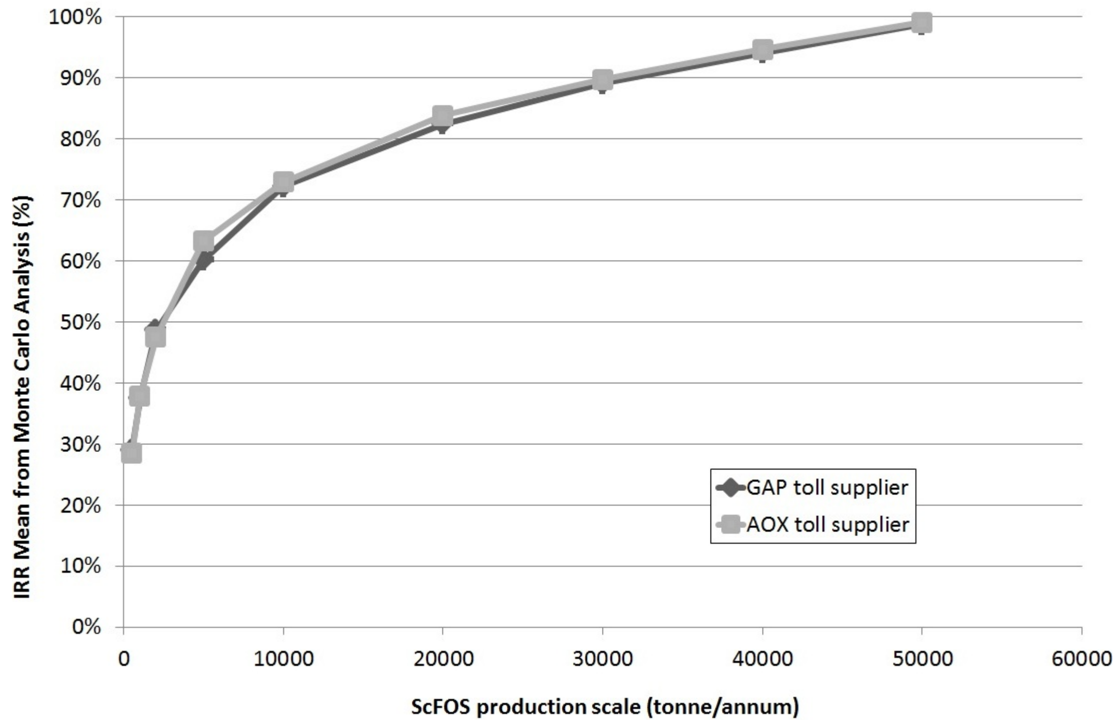

Figure 7.5: ScFOS production using FFase that has been bought an enzyme toll manufacturer, comparing the GAP and AOX strains.

7.1.2.4 Scenario 5 - scFOS production at different production levels using FFase that is bought from a local producer at an export price, comparing GAP and AOX production strains

An alternative buying option that was also investigated was the possibility of buying FFase at an export price. This is the best price the FFase production facility can offer, assuming that the enzyme production plant is producing and selling at high enough quantities to be able to afford a lower selling price. Figure 7.6 shows that when producing scFOS; an FFase that is bought with the export price offered by the AOX production strain is more attractive compared to FFase sold with the GAP production strain.

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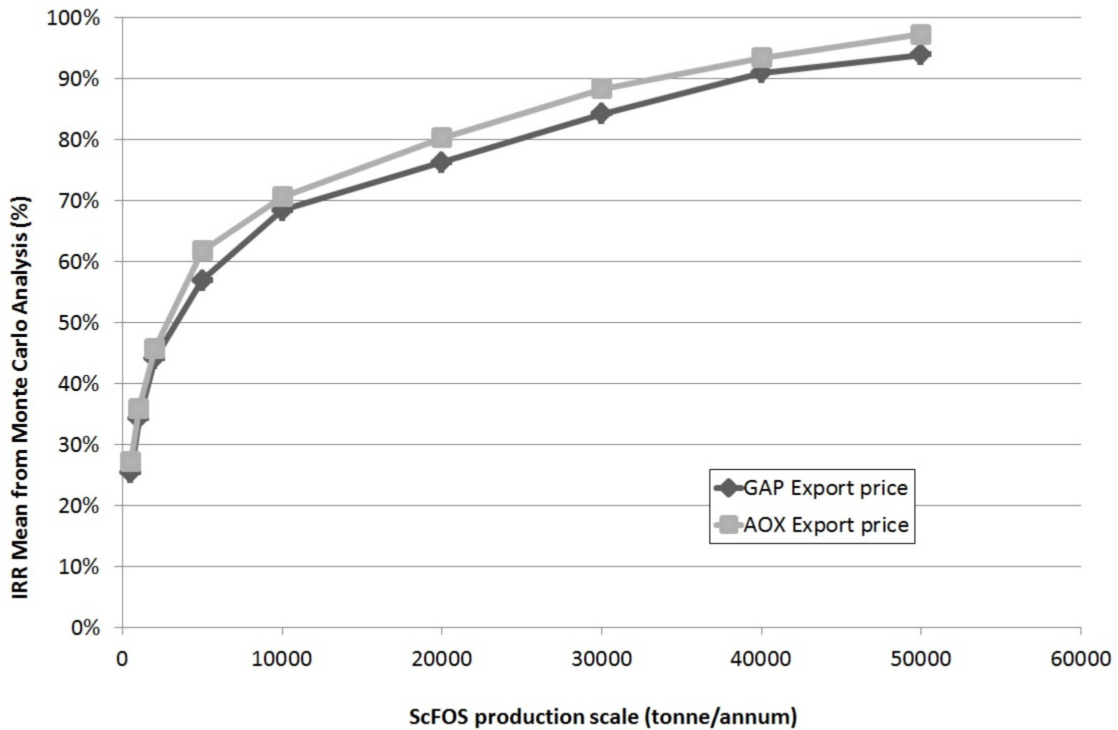

Figure 7.6: ScFOS production using FFase that has been bought from a local FFase producer offering the FFase at a competitive export price. The production strains of GAP and AOX are compared. The GAP is selling at R847/kg of fermentation broth and AOX is selling at R596/kg of fermentation broth.

7.1.2.5 Scenarios Summary

All the scenarios pose slightly different situations when regarding the IRR at different production levels but at the same time, the curves show similar trends with regards to their shape and the effect of economies of scale. Figure 7.7 shows scFOS production using the GAP production strain comparing four different scenarios, namely: i) self-producing FFase in the same facility, ii) buying FFase from a local producer at a price that would enable the supplier to make a 30% IRR while only selling to one scFOS production facility. iii) Using a toll manufacturer to supply the FFase for scFOS production, iv) buying FFase from a local producer who would supply FFase at an export price. The export price will be a price that is based on the FFase producer to make a

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30% on sale to many scFOS production companies.

Figure 7.7 shows that the most attractive option is to use a toll supplier. The reason the toll supplier would be the most favourable is that a toll supplier specialise in enzyme production so they would be able to provide an enzyme price that is very competitive. Buying from a local producer is also a good option but a potential problem with having an independent FFase producer is that the two facilities are co-dependent. If the local producer happens to receive better orders from elsewhere, the local scFOS production facility may run into some trouble. The least attractive, but the safest option to produce scFOS is to produce FFase and scFOS in one independent facility. This way there are no external influencing factors that can cause problems with regard to the scFOS production.

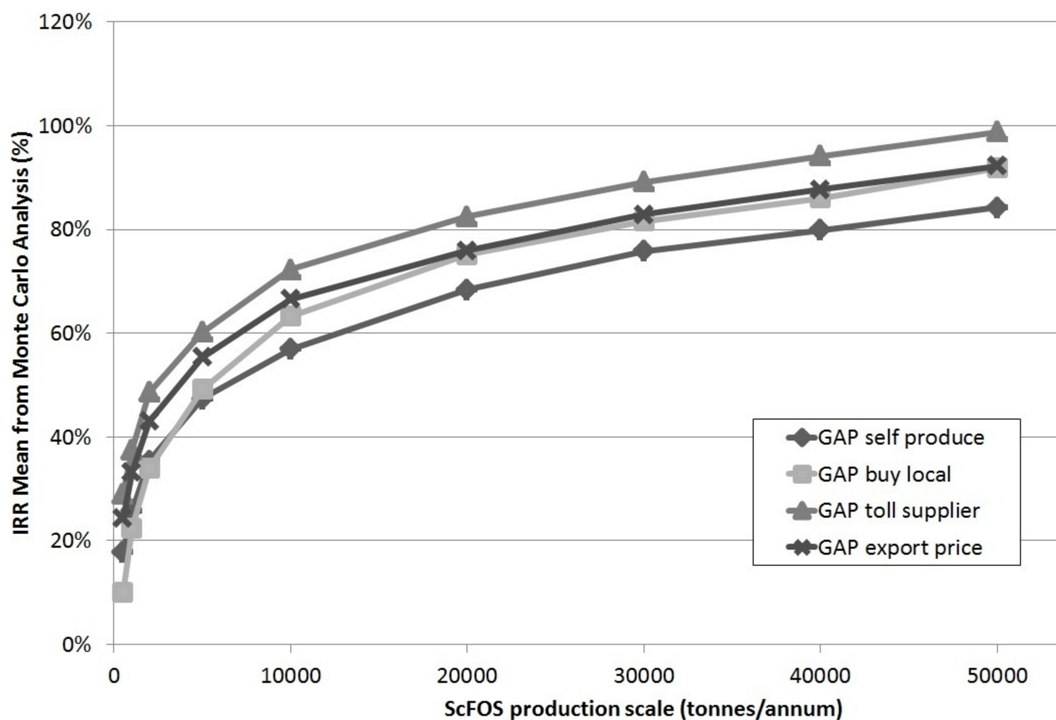

Figure 7.7: ScFOS production that compares all the different options of obtaining the GAP produced FFase enzyme, namely: through self-production, buying from a local producer, buying from a toll supplier, and buying from a local producer that can supply at a low export price.

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Figure 7.8 shows the same information as Figure 7.7 but it focuses on the lower scales of production. The lower areas of production are of interest because it is more realistic to enter the scFOS market at these production levels considering the estimated global market for scFOS. When looking at the 'buy local' option and the 'self-produce' option, the 'buy local' option becomes increasingly more favourable a larger scales of production. This is because the FFase supplier can afford to offer a better price as more enzyme is needed.

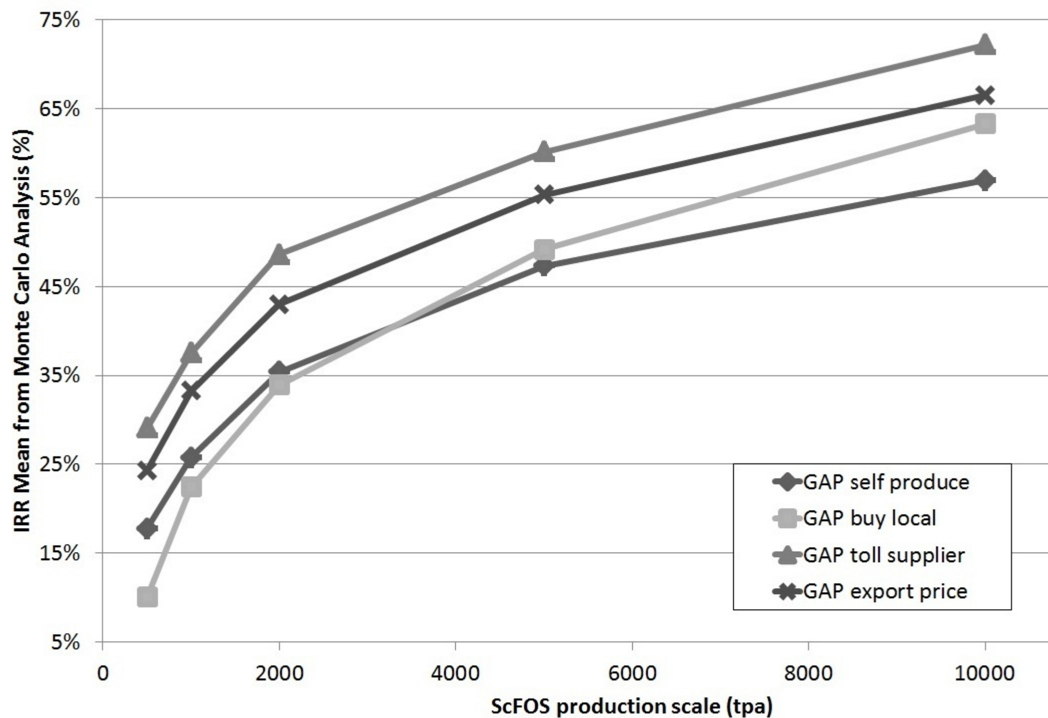

Figure 7.8: ScFOS production that compares all the different options of obtaining the GAP prodcued FFase enzyme, namely: through self-production, buying from a local producer, buying from a toll supplier, and buying from a local producer that can supply at a low export price.

Figure 7.9 is based on the same idea as Figure 7.7, but Figure 7.9 looks at the AOX production strain. The trends are very similar to that of Figure 7.7, where the most attractive option is to use a toll producer. Again, as the toll producer produces many enzymes and supplies to a number of industries, they have the freedom to put out competitive prices. This shows in Figure 7.9,

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where it is more favourable to use a toll supplier to obtain enzyme than it is to buy from a local producer who produces only FFase, or to produce FFase within an scFOS production facility.

The 'buy local' and 'self produce' option show to be close at low production levels but the curves part from each other more as the scFOS production levels increase. The curves part because at low production levels for 'buy local', the enzyme production facility will have to offer high enzyme prices to achieve a 30% IRR. At higher scFOS production levels, the enzyme production facility is now selling more enzyme, so they can afford to drop their enzyme prices and still achieve a 30% IRR.

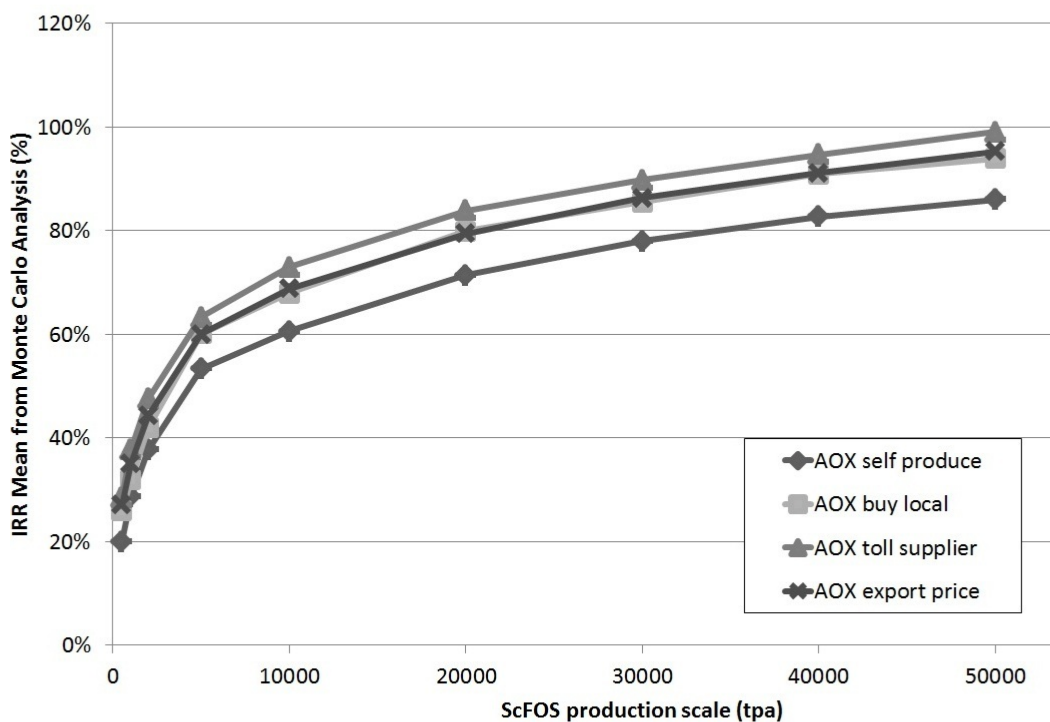

Figure 7.9: ScFOS production that compares all the different options of obtaining the AOX prodcued FFase enzyme, namely: through self-production, buying from a local producer, buying from a toll supplier, and buying from a local producer that can supply at a low export price.

Figure 7.10 shows the same information as Figure 7.9 but the lower scales of production are focused on. It is more apparent in Figure 7.10 when comparing

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'buy local' and 'self-produce', 'buy local' becomes more attractive than 'self-produce' at higher production levels for reasons already discussed. Looking at 'export price' and 'buy local', at low scFOS production levels, the export price shows to be more favourable and the 'buy local' option needs higher enzyme selling prices to be able to obatin a 30% IRR. When the scFOS production scale increases the local supplier can afford to drop their prices as they move towards the same price as the 'export price'. This is shown in Figure 7.10 where the 'buy local' curve moves closer to the 'export price' curve.

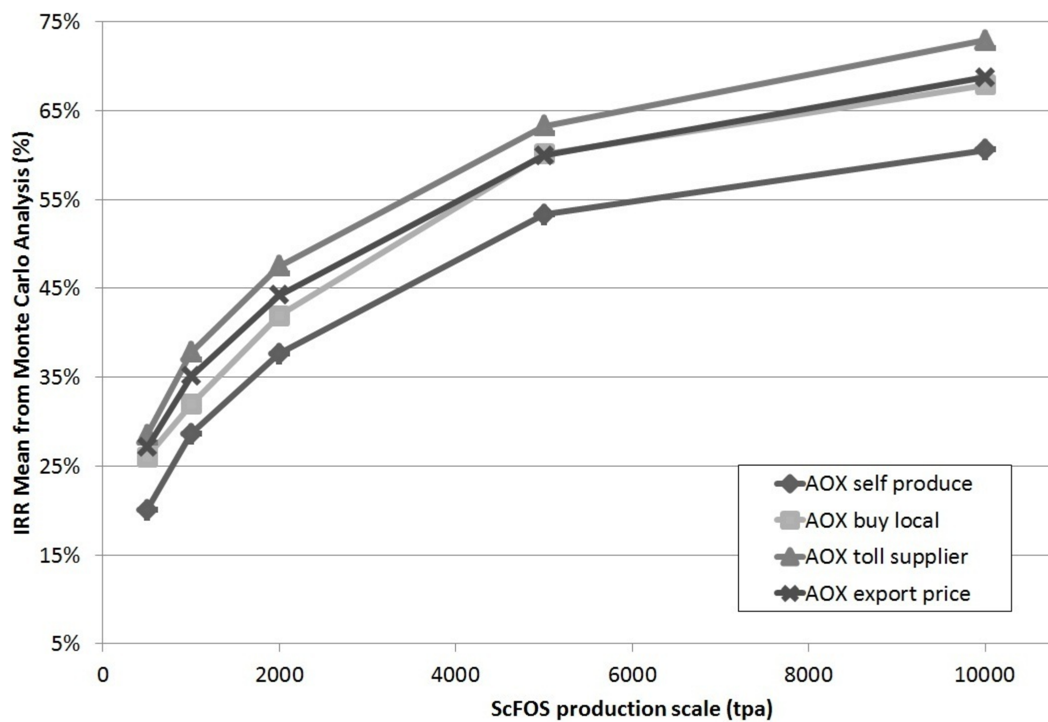

Figure 7.10: ScFOS production that compares all the different options of obtaining the AOX produced FFase enzyme, namely: through self-production, buying from a local producer, buying from a toll supplier, and buying from a local producer that can supply at a low export price.

All figures were plotted using Monte Carlo analyses and Figure 7.11 shows the distributions for scFOS production for the given production scales with the scenario of self-producing FFase using the AOX production strain for scFOS

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production. The mean from the curves from Figure 7.11 represents a point for the information shown in Figure 7.4 for the AOX production strain.

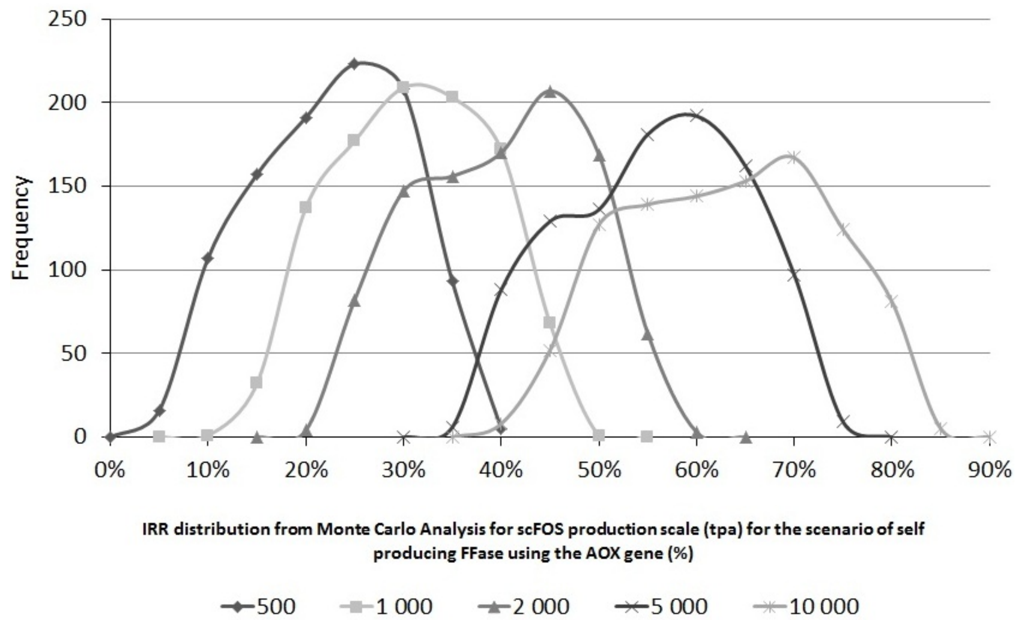

Figure 7.11: IRR distribution from Monte Carlo analysis for scFOS production scale (tpa) for the scenario of self-producing FFase using the AOX production strain.

Figure 7.12 shows the s-curves where cumulative probabilities are plotted and it can be seen that for an scFOS production plant producing 2 000 tonnes per annum, there is an 80% chance that the facility will achieve a 28.9% IRR and there is a 44% chance that the facility will achieve a 40% IRR. Hacura *et al.* (2001) say that in general, a project is relatively safe if the probability of a negative outcome of NPV is less than 0.2. This statement can be related to the scFOS production scenario of 2 000 tpa where there is 20% chance that the IRR will be less than 28.9%.

Figure 7.13 shows a scenario where the scFOS selling price is varied in conjunction with the scFOS production scale to determine what effect this would have on the IRR. For 2 000 tpa of scFOS production, the facility can afford to sell at USD6 to obtain 29.3% IRR or USD7 to obtain 35.5% IRR.

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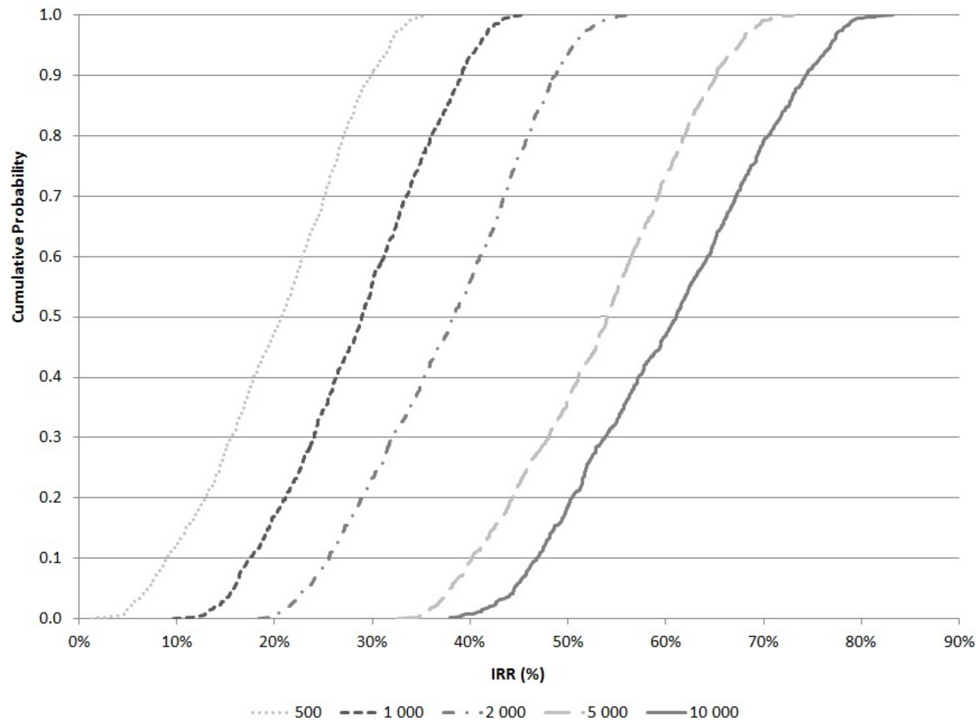

Figure 7.12: S-curves which represent the cumulative probabilities of IRR for scFOS production (tpa) for the scenario where FFase is self-produced in the same facility as the scFOS production using the AOX production strain.

These would be recommended selling prices for the 2 000 tonnes per annum production scale.

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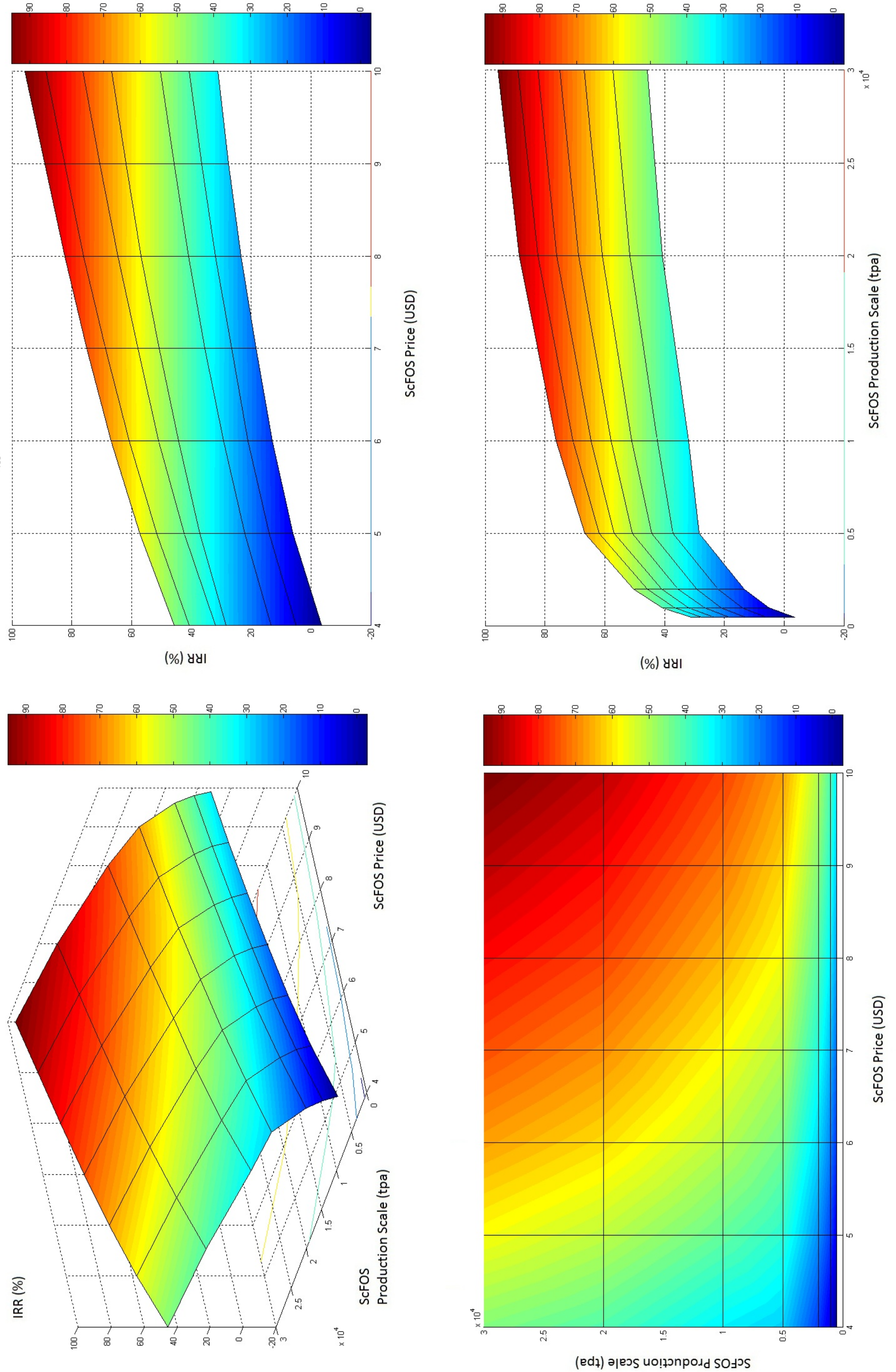

Figure 7.13: The influence of scFOS selling price and scFOS production scale on IRR for and scFOS production plant that produces only enough FFase (using AOX production strain) for itself.

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Chapter 8

Conclusions

The most significant findings from the objectives proposed in this project are as follows:

- ScFOS can be produced on an appreciable scale in South Africa.
- A facility that produces only β-D-fructofuranosidase specifically for scFOS production will have to sell its enzyme for R200/g of pure protein (using AOX production strain) provided they produce 512 kilograms per annum of β-D-fructofuranosidase (pure protein) in order for the production facility to succeed.
- Alternatively a facility that produces only β-D-fructofuranosidase specifically for scFOS production will have to sell its enzyme for R1 426/kg of fermentation broth (using AOX production strain) provided they produce 72 tonnes per annum of β-D-fructofuranosidase (fermentation broth) in order for the production facility to succeed.
- A facility that produces only β-D-fructofuranosidase specifically for scFOS production will have to sell its enzyme for R349/g of pure protein (using GAP production strain), provided they produce 562 kilograms per annum of β-D-fructofuranosidase (pure protein) in order for the production facility to succeed.

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- Alternatively a facility that produces only β-D-fructofuranosidase specifically for scFOS production will have to sell its enzyme for R1 254/kg of pure protein (using GAP production strain), provided they produce 95 tonnes per annum of β-D-fructofuranosidase (fermentation broth) in order for the production facility to succeed.

- For a 2 000 tonnes per annum scFOS production plant, 14.43 tonnes of enzyme fermentation broth (AOX production strain) is needed, which is 126 kg of pure enzyme.

- A single facility that produces β-D-fructofuranosidase (AOX production strain) and scFOS for a 2 000 tonnes per annum scFOS production will succeed having an IRR of 37.7%.

- The minimum production scale that scFOS can be produced while buying FFase (AOX production strain) from a local producer and achieving an IRR of 41.9% is 2 000 tonnes per annum.

- An scFOS production facility producing 2 000 tonnes per annum using β-D-fructofuranosidase (AOX production strain) bought from a contract toll enzyme manufacturer will show the best IRR rendering a value of 47.5%.

Other findings from this project that are also of useful knowledge are:

- When producing the enzyme β-D-fructofuranosidase, it is more favourable to use the AOX production strain compared to the GAP production strain.

- The AOX produces a more concentrated enzyme compared to GAP so effectively the equipment cost will be less with AOX because less fermenter space is needed.

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- Out of all the scenarios that were looked at, it is the most economical to use a toll manufacturer to buy β-D-fructofuranosidase when producing scFOS.

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Chapter 9

Recommendations

The most practical manner in which to produce scFOS in South Africa would be to manufacture β-D-fructofuranosidase and scFOS in the same facility. It is more sensible to produce scFOS and β-D-fructofuranosidase in the same facility as scFOS as there is a low FFase demand for the scFOS production and therefore the required enzyme production equipment wouldn't be excessively large. Also because there is a low FFase demand for scFOS production, the global market for the enzyme would not be high which means that there wouldn't be a lot of the enzyme available on the market. If there isn't a lot of enzyme available on the market, the likelihood is that the price of the FFase will be high, which is another reason to self-produce FFase.

Another good option would be to implement a multi enzyme production facility. A multi enzyme production facility should be the alternative where the facility would aim to produce at least four different types of enzymes (FFase to be one of them). This would work well because when regarding the capital costs involved with enzyme production, the enzyme production processes are similar for all the different enzymes. The main differences between the enzyme production processes would involve the raw materials which are the running costs where factors such as the production medium and cultivation conditions will change. One has to remember that some enzymes may only be required seasonally, which makes even more sense to employ a multi-product enzyme

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production facility.

Ultimately there are three options that can be taken from this project:

1. Implement an independent scFOS production plant in conjunction with β-D-fructofuranosidase production that forms one process, producing 2 000 tonnes per annum of scFOS and selling at USD7 per kilogram of scFOS.

2. Start up a multi enzyme production facility that will produce at least four different enzymes, and this enzyme production facility will supply its β-D-fructofuranosidase to an independent scFOS production facility. The enzyme production facility will essentially be a local toll manufacturer.

3. Start up a multi enzyme production facility that will produce at least four different enzymes (including FFase) and also include the scFOS production process within the facility.

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Appendices

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Appendix A

Physical Data

Table A.1: Densities of Components in FFase Production

Component	**Density (g/ml)**	**Reference**
Biotin	1.41	WolframAlpha (2011)
Boric acid	1.44	WolframAlpha (2011)
Calcium sulphate	2.96	Linstrom *et al.* (1997)
Casamino acids	1.41	Berlin and Pallansch (1968)
Cobalt chloride	3.35	ChemicalBook (2008)
Cupric sulphate-5H_2O	2.28	IowaStateUniversity (1995-2012)
Ferrous sulphate-7H_2O	1.90	ChemicalBook (2008)
Glycerol	1.26	WolframAlpha (2011)
Magnesium sulphate-7H_2O	2.66	IowaStateUniversity (1995-2012)
Manganese sulphate-H_2O	2.57	WolframAlpha (2011)
85% Phosphoric acid	1.63	Egan and Luff (1955)
Potassium hydroxide	2.04	WolframAlpha (2011)
Potassium sulphate	2.66	ChemicalBook (2008)
Sodium iodide	3.67	ChemicalBook (2008)
Sodium molybdate-2H_2O	3.28	ChemicalBook (2008)
Sulfuric acid	1.84	ChemicalLAND21 (2000-2010)
Water	1.00	Çengel and Cimbala (2006)
Zinc chloride	2.91	ChemicalBook (2008)

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Appendix B

Energy Data

Table B.1: Heat capacity data

Chemical name	Molecular formula	Molecular weight (g/mol)	Heat capacity (298K) (J/mol.K)	Reference
Xylose	$C_5H_{10}O_5$	150	182.0	Hernández-Segura *et al.* (2009), Goldberg *et al.* (1989)
Glucose	$C_6H_{12}O_6$	180	218.0	Douglas *et al.* (1951), Boerio-Goates (1991)
Fructose	$C_6H_{12}O_6$	180	215.8	Finegold *et al.* (1989), Kawaizumi *et al.* (1981)
Sucrose	$C_{12}H_{22}O_{11}$	342	425.4	Anderson Jr *et al.* (1950), Putnam and Boerio-Goates (1993)
Kestose	$C_{18}H_{32}O_{16}$	504	662.5	Briggner and Wadsö (1990), Hernández-Segura *et al.* (2009)
Nystose	$C_{24}H_{42}O_{21}$	666	805.1	Calculated from model
1^F-F nystose	$C_{30}H_{52}O_{26}$	828	993.3	Calculated from model
α-cyclodextrin	$C_{36}H_{60}O_{26}$	972	1151.5	Briggner and Wadsö (1990), Hernández-Segura *et al.* (2009)
GF_5	$C_{36}H_{62}O_{31}$	990	1181.5	Calculated from model
β-cyclodextrin	$C_{42}H_{70}O_{35}$	1134	1341.7	Briggner and Wadsö (1990), Hernández-Segura *et al.* (2009)
GF_6	$C_{42}H_{72}O_{36}$	1152	1369.7	Calculated from model
GF_7	$C_{48}H_{82}O_{41}$	1314	1557.9	Calculated from model
Water	H_2O	18	75.4	Çengel and Boles (2007), Linstrom *et al.* (1997)

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Appendix C

Steam Tables

Table C.1: Steam Table (Spirax-Sarco, 2012)

Pressure (barg)	Sat. temperature (°C)	Specific enthalpy (J/kg)	Density (water) (kg/m^3)
1	120.449	505725	942.793
2	133.705	562289	931.709
3	143.762	605453	922.801
4	151.966	640849	915.213
5	158.949	671117	908.527
6	165.059	697720	902.501
7	170.513	721561	896.984
8	175.451	743238	891.871
9	179.974	763168	887.091
10	184.154	781656	882.587
11	188.045	798931	878.320
12	191.691	815171	874.256
13	195.123	830515	870.369
14	198.368	845077	866.639
15	201.450	858947	863.048
16	204.384	872203	859.581
17	207.188	884907	856.226
18	209.873	897116	852.973
19	212.450	908873	849.812
20	214.930	920220	846.737
21	217.319	931192	843.739
22	219.626	941818	840.813
23	221.857	952125	837.953
24	224.017	962138	835.155
25	226.112	971876	832.415
26	228.145	981360	829.729
27	230.121	990605	827.092
28	232.044	999627	824.503
29	233.916	1010000	821.958
30	235.741	1020000	819.454
31	237.521	1030000	816.990
32	239.259	1030000	814.563
33	240.957	1040000	812.171
34	242.617	1050000	809.812
35	244.241	1060000	807.485
36	245.831	1070000	805.188
37	247.389	1070000	802.919
38	248.915	1080000	800.677
39	250.411	1090000	798.461
40	251.879	1090000	769.270

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Appendix D

Literature Data

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Table D.1: Characteristics of equipment and operations used and compositions of outlet streams (Vaňková *et al.*, 2008)

Equipment	Code	Operation	Duration time (h)	T (°C)	Stream	Composition (mass %)
Blending tank	V-101	P1	1.5	25	F1	Salts 2.51, sucrose 24.63, $NaNO_3$ 3.45, water 69.37
Bioreactor	V-102	P4	80	28	F6	Biomass 7.32, FTase 0.0035, proteins 0.46, salts 2.69, sucrose 12.76, water 76.78
Microfilter	F-102	P6	2.5	12	F9	FTase 0.0055, proteins 0.72, salts 4.24, sucrose 20.12, water 74.92
					F12	Biomass 19.87, proteins 0.01, salts 0.03, sucrose 0.13, water 79.97

Table D.2: List of equipment used for the production of immobilised FTase (Vaňková *et al.*, 2008)

Name	Description	Cost/10^3 EUR
V-101	Blending tank, vessel volume $10 m^3$ EUR	31
C-101	Compressor, power 400 kW	18.9
F-101	Air filter, rated throughput 0.06 m^3 s^{-1}	3.8
R-101	Fermenter (Bioreactor), vessel volume 10 m^3	151
V-102	Blending tank, vessel volume 10 m^3	31
F-102	Microfilter, membrane area 30 m^2	26.5
	Total	262.2

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Table D.3: List of raw materials for the production of immobilised FTase (Vaňková *et al.*, 2008)

Raw material	Cost ($EUR.kg^{-1}$)	Amount (kg)
Sucrose	0.9	2500
Yeast extract	2.6	100
$NaNO_3$	0.4	385
$MgSO_4.7H_2O$	0.5	25
K_2HPO_4	10.8	50
H_2O	0.1	63000
NaCl	2.1	298
Resin DOWEX MARATHON MSA	124.4	3600

Table D.4: List of equipment used for the production of FOS's (Vaňková *et al.*, 2008)

Name	Description	Cost/10^3 EUR
R-102	Packed-bed reactor, volume 1.6 m^3, 4 pieces	793
R-103	SMB column, volume 3.4 m^3, 4 pieces	454
D-101	Spray dryer, evaporation 100 $kg.m^3.h^-1$	90
V-103	Blending tank, vessel volume 5 m^3	27
	Unlisted equipment	303
	Total	1667

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Table D.5: Main categories of operation costs for production of FOS's from food sucrose (Vaňková *et al.*, 2008)

	Powdery FOS's/10^6 EUR	**Percentage (%)**	**FOS's syrup/10^6 EUR**	**Percentage (%)**
Raw materials	19.03	84.09	19.35	86.11
Consumables	1.40	6.19	1.37	6.10
Equipment-dependent	1.16	5.13	0.96	4.27
Utilities	0.72	3.18	0.48	2.14
Labour-dependent	0.16	0.71	0.16	0.71
Laboratory/QC/QA*	0.14	0.62	0.13	0.58
Waste treatment/disposal	0.02	0.09	0.02	0.09
Total	22.63	100	22.47	100

*QC - quality control, QA - quality assurance

Table D.6: Summary of costs for the production of FOS's (Vaňková *et al.*, 2008)

	Powdery FOS's		**FOS's syrup**	
Total investment costs/10^6 (EUR)	5.031		4.837	
	Food sucrose	**Industrial sucrose**	**Food sucrose**	**Industrial sucrose**
Operation costs/10^6 (EUR)	22.630	15.360	21.983	15.176
Production cost (EUR.kg^{-1})	1.47	0.74	1.42	0.73
Production cost/10^7 (EUR)*	1.47	0.74	1.42	0.73

* For an annual production of 10 000 tonne of FOS's (Vaňková *et al.*, 2008)

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Appendix E

Sample Calculations

E.1 Methanol Carry-over

Consider a scFOS production rate of 10 000 tonnes/year:

- The FFase production process, to meet a demand of 10 000 tonnes/year production, will need 400 kg/h of methanol for a FFase fermentation using the AOX production strain.
- If 90% of this methanol is consumed (which is a pessimistic assumption), 40 kg/h of methanol will go into scFOS process.
- The remaining 40 kg/h of methanol will be in the enzyme solution which will enter the reactor in the scFOS process.
- the reactor product produces 3 630 kg/h of non-purified scFOS. Adding the 40 kg/h of methanol 3 670 kg/h product flow rate.
- Enzyme percentage exiting the reactor would be 40/3 670, which will equal of 1.1% (mass) of methanol.
- If the purification step in the scFOS process achieves 90%; 0.11% (mass) of methanol will be present in final product.
- 0.11% (mass) equates to 1 100 ppm.
- This falls within acceptable range of 0.3% or 3 000 ppm.

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E.2 Spray drying air

The air flow rate for the industrial spray dryer was influenced by the work done by Huang and Mujumdar (2007). Their study specified operating and design parameter of a spray dryer.

- Feed rate: 1 200 kg/h (45% solids concentration) = 540 kg/h of solids
- Air inlet temperature: 250°C
- Air outlet temperature: 110°C
- Air mass flow rate: 3.5 to 5.5 kg/s

For an scFOS production rate of 2 000 tonnes per annum, the mass balance was simulated to determine the incoming flow rate of scFOS syrup entering the spray dryer. For 2 000 tonnes per annum of scFOS production, the incoming stream (stream 5 in Figure 4.6 leaving the SMB chromatography column that entered the spray dryer entered at a flow rate of 568 kg/h. The spray dryer specifications for this design were:

- Feed rate: 568 kg/h (44.5% solids concentration) = 252.42 kg/h
- Air inlet temperature: 180°C
- Air outlet temperature: 90°C
- Air mass flow rate: 3.5 to 5.5 kg/s (average is 4.5 kg/s)

Therefore the incoming air mass flow rate that will remove the moisture from the incoming scFOS 'syrup' stream will be:

$$568 * 44.5\% = 252.42\, kg/h$$

$$\begin{aligned} Air\, flow\, rate &= 4.5 * 252.42/540 \\ &= 2.104\, kg/s \\ &= 7\,563\, kg/h\ \ of\, air\, needed \end{aligned}$$

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Appendix F

Stream Tables

F.1 FFase Production Process Material Stream Tables

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Table F.1: FFase Production (AOX) stream table for 3.60 tpa of fermentation broth (to supply for 500 tpa of scFOS)

Stream number	**4**	**6**	**8**	**10**	**11**	**12**	**13**	**14**	**15**
Temperature (°C)	30	30	30	30	30	30	30	30	30
Pressure (bar)	1.01325	1.01325	1.01325	0.4	1.01325	1.01325	1.01325	1.01325	1.01325
Total (g/h)	281	135	1584897	1584897	517	456	61	454	61
Mass (g/h)									
Glycerol	229	63	0	0	6	6	0	6	0
Methanol	0	135	0	0	18	18	0	18	0
Yeast extract	5	0	0	0	0	0	0	0	0
H_3PO_4 85%	26	0	0	0	0	0	0	0	0
$CaSO_4$	1	0	0	0	0	0	0	0	0
K_2SO_4	10	0	0	0	0	0	0	0	0
$MgSO_4$-$7H_2O$	8	0	0	0	0	0	0	0	0
KOH	2	0	0	0	0	0	0	0	0
Air	0	0	1584897	1584897	0	0	0	0	0
NH_4OH	0	0	0	0	35	35	0	35	0
β-D-fructofuranosidase	0	0	0	0	4	4	0	4	0
Pichia pastoris cells	0	0	0	0	62	1	61	0	1
Supernatant	0	0	0	0	392	392	0	392	0

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Table F.2: FFase Production (AOX) stream table for 7.22 tpa of fermentation broth (to supply for 1 000 tpa of scFOS)

Stream number	**4**	**6**	**8**	**10**	**11**	**12**	**13**	**14**	**15**
Temperature (°C)	30	30	30	30	30	30	30	30	30
Pressure (bar)	1.01325	1.01325	1.01325	0.4	1.01325	1.01325	1.01325	1.01325	1.01325
Total (g/h)	562	395	3169794	3169794	1035	913	122	872	122
Mass (g/h)									
Glycerol	459	125	0	0	11	11	0	11	0
Methanol	0	270	0	0	36	36	0	36	0
Yeast extract	9	0	0	0	0	0	0	0	0
H_3PO_4 85%	51	0	0	0	0	0	0	0	0
$CaSO_4$	1	0	0	0	0	0	0	0	0
K_2SO_4	21	0	0	0	0	0	0	0	0
$MgSO_4$-$7H_2O$	17	0	0	0	0	0	0	0	0
KOH	5	0	0	0	0	0	0	0	0
Air	0	0	3169794	3169794	0	0	0	0	0
NH_4OH	0	0	0	0	69	69	0	0	0
β-D-fructofuranosidase	0	0	0	0	8	8	0	8	0
Pichia pastoris cells	0	0	0	0	127	4	122	0	4
Supernatant	0	0	0	0	784	784	0	784	0

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Table F.3: FFase Production stream table for 14.43 tpa of fermentation broth (to supply for 2 000 tpa of scFOS)

Stream number	**4**	**6**	**8**	**10**	**11**	**12**	**13**	**14**	**15**
Temperature (°C)	30	30	30	30	30	30	30	30	30
Pressure (bar)	1.01325	1.01325	1.01325	0.4	1.01325	1.01325	1.01325	1.01325	1.01325
Total (g/h)	1125	790	6339587	6339587	2607	1823	245	1722	5
Mass (g/h)									
Glycerol	918	251	0	0	23	23	0	23	0
Methanol	0	539	0	0	73	73	0	73	0
Yeast extract	18	0	0	0	0	0	0	0	0
H_3PO_4 85%	102	0	0	0	0	0	0	0	0
$CaSO_4$	2	0	0	0	0	0	0	0	0
K_2SO_4	41	0	0	0	0	0	0	0	0
$MgSO_4$-$7H_2O$	34	0	0	0	0	0	0	0	0
KOH	9	0	0	0	0	0	0	0	0
Air	0	0	6339587	6339587	0	0	0	0	0
NH_4OH	0	0	0	0	138	138	0	0	0
β-D-fructofuranosidase	0	0	0	0	16	16	0	16	0
Pichia pastoris cells	0	0	0	0	250	5	245	0	5
Supernatant	0	0	0	0	1567	1567	0	1567	0

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Table F.4: FFase Production (AOX) stream table for 36.09 tpa of fermentation broth (to supply for 5 000 tpa of scFOS)

Stream number	**4**	**6**	**8**	**10**	**11**	**12**	**13**	**14**	**15**
Temperature (°C)	30	30	30	30	30	30	30	30	30
Pressure (bar)	1.01325	1.01325	1.01325	0.4	1.01325	1.01325	1.01325	1.01325	1.01325
Total (g/h)	2812	1975	15848968	15848968	5168	4556	612	4362	12
Mass (g/h)									
Glycerol	2294	627	0	0	57	57	0	57	0
Methanol	0	1348	0	0	182	182	0	0	0
Yeast extract	45	0	0	0	0	0	0	0	0
H_3PO_4 85%	255	0	0	0	0	0	0	0	0
$CaSO_4$	5	0	0	0	0	0	0	0	0
K_2SO_4	103	0	0	0	0	0	0	0	0
$MgSO_4$-$7H_2O$	85	0	0	0	0	0	0	0	0
KOH	23	0	0	0	0	0	0	0	0
Air	0	0	15848968	15848968	0	0	0	0	0
NH_4OH	0	0	0	0	346	346	0	346	0
β-D-fructofuranosidase	0	0	0	0	40	40	0	40	0
Pichia pastoris cells	0	0	0	0	634	12	612	0	612
Supernatant	0	0	0	0	3919	3919	0	3919	0

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Table F.5: FFase Production (AOX) stream table for 71.88 tpa of fermentation broth (to supply for 10 000 tpa of scFOS)

Stream number	**4**	**6**	**8**	**10**	**11**	**12**	**13**	**14**	**15**
Temperature (°C)	30	30	30	30	30	30	30	30	30
Pressure (bar)	1.01325	1.01325	1.01325	0.4	1.01325	1.01325	1.01325	1.01325	1.01325
Total (g/h)	2812	1975	15848968	15848968	5168	4556	612	4362	12
Mass (g/h)									
Glycerol	4571	1249	0	0	114	114	0	114	0
Methanol	0	2686	0	0	363	363	0	0	0
Yeast extract	90	0	0	0	0	0	0	0	0
H_3PO_4 85%	509	0	0	0	0	0	0	0	0
$CaSO_4$	11	0	0	0	0	0	0	0	0
K_2SO_4	206	0	0	0	0	0	0	0	0
$MgSO_4$-$7H_2O$	168	0	0	0	0	0	0	0	0
KOH	47	0	0	0	0	0	0	0	0
Air	0	0	31572132	31572132	0	0	0	0	0
NH_4OH	0	0	0	0	689	689	0	689	0
β-D-fructofuranosidase	0	0	0	0	80	80	0	80	0
Pichia pastoris cells	0	0	0	0	1244	25	1219	0	1219
Supernatant	0	0	0	0	7806	7806	0	7806	0

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F.2 ScFOS Production Process Material Stream Tables

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Table F.6: ScFOS Production stream table at 500 tpa of scFOS)

Stream	1	2	3	4	5	6	7	8	9	10	11	12	13 (t/h)	14 (t/h)
T (°C)	25	25	50	25	60	90	90	60	25	25	25	25	180	25
P (bar)	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013
Total (kg/h)	105.04	183.96	184.42	185.79	141.61	78.48	63.14	44.18	6.05	36.32	0.003	78.47	1.90	1.90
Mass (kg/h)														
Kestose	0	0	23.53	23.53	22.35	0	22.35	1.18	0.06	1.12	0	0	0	0
Nystose	0	0	31.37	31.37	29.80	0	29.80	1.57	0.08	1.49	0	0	0	0
1^F-F-nystose	0	0	5.89	5.89	5.59	0	5.59	0.30	0.02	0.28	0	0	0	0
GF_5	0	0	2.34	2.34	2.23	0	2.23	0.12	0.01	0.12	0	0	0	0
GF_6	0	0	0.94	0.94	0.89	0	0.89	0.05	0.01	0.03	0	0	0	0
GF_7	0	0	0.47	0.47	0.45	0	0.45	0.03	0.01	0.03	0	0	0	0
Fructose	0	0	3.74	3.74	0.19	0	0.19	3.56	0.18	3.38	0	0	0	0
Glucose	0	0	21.51	21.51	1.08	0	1.08	20.43	1.03	19.41	0	0	0	0
Sucrose	105.04	105.49	11.61	11.61	0.59	0	0.59	11.03	0.56	10.48	0	0	0	0
Water	0	78.47	82.62	82.62	78.49	78.49	0	4.14	4.14	0	0.003	78.47	0	0
FFase	0	0	0.46	0.46	0	0	0	0.46	0	0.46	0	0	0	0
Air	0	0	0	0	0	0	0	0	0	0	0	0	1.90	1.90

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Table F.7: ScFOS Production stream table at 1 000 tpa of scFOS)

Stream	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13 (t/h)**	**14 (t/h)**
T (°C)	25	25	50	25	60	90	90	60	25	25	25	25	180	25
P (bar)	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013
Total (kg/h)	206.09	340.46	341.67	341.67	258.23	131.96	126.26	84.64	10.83	73.81	0.006	131.96	6.04	6.04
Mass (kg/h)														
Kestose	0	0	46.15	46.15	43.84	0	43.84	2.31	0.115	2.19	0	0	0	0
Nystose	0	0	61.54	61.54	58.46	0	58.46	3.08	0.154	2.92	0	0	0	0
1^F-F-nystose	0	0	11.54	11.54	10.96	0	10.96	0.58	0.029	0.55	0	0	0	0
GF_5	0	0	4.59	4.59	4.36	0	4.36	0.23	0.011	0.22	0	0	0	0
GF_6	0	0	1.83	1.83	1.74	0	1.74	0.09	0.005	0.09	0	0	0	0
GF_7	0	0	0.92	0.92	0.87	0	0.87	0.05	0.002	0.04	0	0	0	0
Fructose	0	0	7.34	7.34	0.37	0	0.37	6.97	0.349	6.62	0	0	0	0
Glucose	0	0	42.19	42.19	2.11	0	2.11	40.08	2.004	38.07	0	0	0	0
Sucrose	206.09	208.50	22.93	22.93	1.15	0	1.15	21.79	1.089	20.70	0	0	0	0
Water	0	131.96	141.44	141.44	134.37	134.37	0	7.07	7.072	0	0.006	131.96	0	0
FFase	0	0	1.20	1.20	0	0	0	1.20	0	1.20	0	0	0	0
Air	0	0	0	0	0	0	0	0	0	0	0	0	3.79	3.79

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Table F.8: ScFOS Production stream table at 2 000 tpa of scFOS)

Stream	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13 (t/h)**	**14 (t/h)**
T (°C)	25	25	50	25	60	90	90	60	25	25	25	25	180	25
P (bar)	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013
Total (kg/h)	422.61	747.40	749.81	749.81	577.32	324.79	252.53	172.50	24.75	145.35	0.01	324.79	12.24	12.24
Mass (kg/h)														
Kestose	0	0	94.10	94.10	89.40	0	89.40	4.71	0.24	4.47	0	0	0	0
Nystose	0	0	125.47	125.47	119.20	0	119.20	6.28	0.32	5.96	0	0	0	0
1^F-F-nystose	0	0	23.53	23.53	22.35	0	22.35	1.18	0.06	1.12	0	0	0	0
GF_5	0	0	9.35	9.35	8.89	0	8.89	0.47	0.03	0.45	0	0	0	0
GF_6	0	0	3.74	3.74	3.56	0	3.56	0.19	0.01	0.18	0	0	0	0
GF_7	0	0	1.87	1.87	1.78	0	1.78	0.1	0.01	0.09	0	0	0	0
Fructose	0	0	14.96	14.96	0.75	0	0.75	14.22	0.72	13.51	0	0	0	0
Glucose	0	0	86.02	86.02	4.31	0	4.31	81.72	4.09	77.63	0	0	0	0
Sucrose	422.61	422.61	46.49	46.49	2.33	0	2.33	44.17	2.21	41.96	0	0	0	0
Water	0	324.79	341.89	341.89	324.80	324.80	0	17.1	17.1	0	0.01	324.79	0	0
FFase	0	0	2.41	2.41	0	0	0	2.41	0	2.41	0	0	0	0
Air	0	0	0	0	0	0	0	0	0	0	0	0	7.56	7.56

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Table F.9: ScFOS Production stream table at 5 000 tpa of scFOS)

Stream	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13 (t/h)**	**14 (t/h)**
T (°C)	25	25	50	25	60	90	90	60	25	25	25	25	180	25
P (bar)	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013
Total (kg/h)	1050.57	1801.75	1807.76	1807.76	1382.56	751.19	631.37	425.20	58.69	369.08	0.01	751.18	18.94	18.94
Mass (kg/h)														
Kestose	0	0	235.26	235.26	223.50	0	223.50	11.77	0.59	11.18	0	0	0	0
Nystose	0	0	313.68	313.68	298.00	0	298.00	15.69	0.79	14.90	0	0	0	0
1^F-F-nystose	0	0	58.82	58.82	55.88	0	55.88	2.95	0.15	2.8	0	0	0	0
GF_5	0	0	23.38	23.38	22.21	0	22.21	1.17	0.06	1.12	0	0	0	0
GF_6	0	0	9.36	9.36	8.89	0	8.89	0.47	0.03	0.45	0	0	0	0
GF_7	0	0	4.68	4.68	4.45	0	4.45	0.24	0.02	0.23	0	0	0	0
Fructose	0	0	37.41	37.41	1.88	0	1.88	35.54	1.78	33.76	0	0	0	0
Glucose	0	0	215.06	215.06	10.76	0	10.76	204.30	10.22	194.09	0	0	0	0
Sucrose	1050.57	1050.57	115.83	115.83	5.80	0	5.80	110.04	5.51	104.54	0	0	0	0
Water	0	751.18	790.73	790.73	751.19	751.19	0	39.54	39.54	0	0.01	751.18	0	0
FFase	0	0	6.01	6.01	0	0	0	6.01	0	6.01	0	0	0	0
Air	0	0	0	0	0	0	0	0	0	0	0	0	18.94	18.94

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Table F.10: ScFOS Production stream table at 10 000 tpa of scFOS)

Stream	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13 (t/h)**	**14 (t/h)**
T (°C)	25	25	60	25	60	90	90	60	25	25	25	25	180	25
P (bar)	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013	1.013
Total (kg/h)	2101.14	3624.01	3635.99	3635.99	2785.50	1522.87	1262.63	850.54	118.37	726.16	0.01	1522.87	61.00	61.00
Mass (kg/h)														
Kestose	0	0	470.52	470.52	447.00	0	447.00	23.53	1.18	22.35	0	0	0	0
Nystose	0	0	627.36	627.36	596.00	0	596.00	31.37	1.57	29.80	0	0	0	0
1^F-F-GF_4	0	0	117.63	117.63	111.75	0	111.75	5.89	0.3	5.59	0	0	0	0
GF_5	0	0	46.76	46.76	44.42	0	44.42	2.34	0.12	2.23	0	0	0	0
GF_6	0	0	18.71	18.71	17.77	0	17.77	0.94	0.05	0.89	0	0	0	0
GF_7	0	0	9.36	9.36	8.89	0	8.89	0.47	0.03	0.45	0	0	0	0
Fructose (F)	0	0	74.81	74.81	3.75	0	3.75	71.07	3.56	67.51	0	0	0	0
Glucose (G)	0	0	430.11	430.11	21.51	0	21.51	408.60	20.43	388.17	0	0	0	0
Sucrose (GF)	2101.14	2101.14	231.79	231.79	11.59	0	11.59	220.20	11.01	209.19	0	0	0	0
Water	0	1522.87	1603.02	1603.02	1522.87	1522.87	0	80.16	80.16	0	0.01	1522.87	0	0
FFase	0	0	11.98	11.98	0	0	0	11.98	0	11.98	0	0	0	0
Air	0	0	0	0	0	0	0	0	0	0	0	0	37.89	37.89

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Appendix G

Cash Flow Tables

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Table G.1: Discounted Cash Flow Table for scFOS Production of 500 tonnes per annum, self producing FFase using the AOX production strain(R in millions)

Year End (k)	Invest-ment	d_k	FCI_L-Σd_k	R	COM_d	(R-COM_d-d_k)x(1-t)+d_k	Cash Flow	Cumu-lative Cash Flow	Discounted Cash Flow	Cumulative Discoun-ted Cash Flow
0	-0.45	0	42.35	0	0	0	-0.45	-0.45	-0.45	-0.45
1	-25.41	0	42.35	0	0	0	-25.41	-25.86	-22.09	-22.54
2	-16.94	0	42.35	0	0	0	-16.94	-42.80	-12.81	-35.35
2	-7.06	0	42.35	0	0	0	-7.06	-49.85	-53.37	-40.69
3	0	7.62	34.72	36.75	17.48	16.01	16.01	-33.85	10.52	-30.16
4	0	7.62	27.10	36.75	17.48	16.01	16.01	-17.84	9.15	-21.01
5	0	7.62	19.48	36.75	17.48	16.01	16.01	-1.84	7.96	-13.06
6	0	7.62	11.86	36.75	17.48	16.01	16.01	14.17	6.92	-6.14
7	0	7.62	4.23	36.75	17.48	16.01	16.01	30.18	6.02	-1.18
8	0	7.62	-3.39	36.75	17.48	16.01	16.01	46.18	5.23	5.11
9	0	7.62	-11.01	36.75	17.48	16.01	16.01	62.19	4.55	9.66
10	0	7.62	-18.63	36.75	17.48	16.01	16.01	78.19	3.96	13.62
11	0	7.62	-26.25	36.75	17.48	16.01	16.01	94.20	3.44	17.06
12	4.23	7.62	-33.88	40.98	17.48	19.05	19.05	113.25	3.56	20.62
12										24.86

d_k = straight line depreciation; r = discount rate; S = Salvage value of the plant; t = tax rate (28%); FCI = fixed capital investment

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Table G.2: Discounted Cash Flow Table for scFOS Production of 1 000 tonnes per annum, self producing FFase using the AOX production strain(R in millions)

Year End (k)	Invest-ment	d_k	FCI_L-Σd_k	R	COM_d	(R-COM_d-d_k)x(1-t)+d_k	Cash Flow	Cumu-lative Cash Flow	Discounted Cash Flow	Cumulative Discoun-ted Cash Flow
0	-0.90	0	64.18	0	0	0	-0.90	-0.90	-0.90	-0.9
1	-38.51	0	64.18	0	0	0	-38.51	-39.41	-33.49	-34.39
2	-25.67	0	64.18	0	0	0	-25.67	-65.08	-19.41	-53.80
2	-10.70	0	64.18	0	0	0	-10.70	-75.78	-8.09	-61.89
3	0	11.55	52.63	73.50	0	34.94	34.94	-40.84	22.98	-38.91
4	0	11.55	41.08	73.50	29.46	34.94	34.94	-5.90	19.98	-18.94
5	0	11.55	29.52	73.50	29.46	34.94	34.94	29.04	17.37	-15.64
6	0	11.55	17.97	73.50	29.46	34.94	34.94	63.99	15.11	13.54
7	0	11.55	6.42	73.50	29.46	34.94	34.94	98.93	13.14	26.68
8	0	11.55	-5.13	73.50	29.46	34.94	34.94	133.87	11.42	38.10
9	0	11.55	-16.69	73.50	29.46	34.94	34.94	168.81	9.93	48.03
10	0	11.55	-28.24	73.50	29.46	34.94	34.94	203.75	8.64	56.67
11	0	11.55	-39.79	73.50	29.46	34.94	34.94	238.70	7.51	64.18
12	0	11.55	-51.35	79.92	29.46	39.56	34.94	278.26	7.39	71.58
									77.99	

d_k = straight line depreciation; r = discount rate; S = Salvage value of the plant; t = tax rate (28%); FCI = fixed capital investment

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Table G.3: Discounted Cash Flow Table for scFOS Production of 2 000 tonnes per annum, self producing FFase using the AOX production strain(R in millions)

Year End (k)	Invest-ment	d_k	FCI_L-Σd_k	R	COM_d	(R-COM_d-d_k)x(1-t)+d_k	Cash Flow	Cumu-lative Cash Flow	Discounted Cash Flow	Cumulative Discoun-ted Cash Flow
0	-1.80	0	97.29	0	0	0	-1.80	-1.80	-1.80	-1.80
1	-58.37	0	97.29	0	0	0	-58.37	-60.17	-50.76	-52.56
2	-38.91	0	97.29	0	0	0	-38.91	-99.09	-29.42	-81.98
2	-16.21	0	97.29	0	0	0	-16.21	-115.30	-12.26	-94.24
3	0	17.51	79.77	14.70	50.26	74.56	74.56	-40.74	49.02	-45.22
4	0	17.51	62.26	14.70	50.26	74.56	74.56	33.82	42.63	-25.89
5	0	17.51	44.75	14.70	50.26	74.56	74.56	108.38	37.07	34.48
6	0	17.51	27.24	14.70	50.26	74.56	74.56	182.94	32.23	66.71
7	0	17.51	9.73	14.70	50.26	74.56	74.56	257.50	28.03	94.74
8	0	17.51	-7.78	14.70	50.26	74.56	74.56	332.06	24.37	119.12
9	0	17.51	-25.29	14.70	50.26	74.56	74.56	406.62	21.19	140.31
10	0	17.51	-42.81	14.70	50.26	74.56	74.56	481.18	18.43	158.74
11	0	17.51	-60.32	14.70	50.26	74.56	74.56	555.74	16.03	174.77
12	9.73	17.51	-77.83	15.67	50.26	81.56	81.56	637.30	15.24	190.01
12										199.74

d_k = straight line depreciation; r = discount rate; S = Salvage value of the plant; t = tax rate (28%); FCI = fixed capital investment

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Table G.4: Discounted Cash Flow Table for scFOS Production of 5 000 tonnes per annum, self producing FFase using the AOX production strain(R in millions)

Year End (k)	Invest-ment	d_k	FCI_L-Σd_k	R	COM_d	(R-COM_d-d_k)x(1-t)+d_k	Cash Flow	Cumu-lative Cash Flow	Discounted Cash Flow	Cumulative Discoun-ted Cash Flow
0	-4.50	0	168.58	0	0	0	-4.50	-4.50	-4.50	-4.50
1	-10.11	0	168.58	0	0	0	-10.11	-10.56	-87.96	-92.46
2	-67.43	0	168.58	0	0	0	-67.43	-17.31	-50.99	-143.45
2	-28.10	0	168.58	0	0	0	-28.10	-20.12	-21.25	-164.69
3	0	30.34	138.24	367.50	104.75	213.30	213.30	12.12	140.25	-24.45
4	0	30.34	107.89	367.50	104.75	213.30	213.30	225.41	121.95	97.51
5	0	30.34	77.55	367.50	104.75	213.30	213.30	438.71	106.05	203.55
6	0	30.34	47.20	367.50	104.75	213.30	213.30	652.00	92.21	295.77
7	0	30.34	16.86	367.50	104.75	213.30	213.30	865.30	80.19	375.95
8	0	30.34	-13.49	367.50	104.75	213.30	213.30	1 078.60	69.73	445.68
9	0	30.34	-43.83	367.50	104.75	213.30	213.30	1 291.89	60.63	506.31
10	0	30.34	-74.18	367.50	104.75	213.30	213.30	1 505.19	52.72	559.04
11	0	30.34	-104.52	367.50	104.75	213.30	213.30	1 718.49	45.85	604.88
12	16.86	30.34	-134.87	384.36	104.75	225.43	225.43	1 943.92	42.14	647.02
12										663.88

d_k = straight line depreciation; r = discount rate; S = Salvage value of the plant; t = tax rate (28%); FCI = fixed capital investment

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Table G.5: Discounted Cash Flow Table for scFOS Production of 10 000 tonnes per annum, self producing FFase using the AOX production strain(R in millions)

Year End (k)	Invest-ment	d_k	FCI_L-Σd_k	R	COM_d	(R-COM_d-d_k)x(1-t)+d_k	Cash Flow	Cumu-lative Cash Flow	Discounted Cash Flow	Cumulative Discoun-ted Cash Flow
0	-9.00	0	255.38	0	0	0	-9.00	-9.00	-9.00	-9.00
1	-153.23	0	255.38	0	0	0	-153.23	-162.23	-133.24	-142.24
2	-102.15	0	255.38	0	0	0	-102.15	-264.38	-77.24	-219.48
2	-42.56	0	255.38	0	0	0	-42.56	-306.94	-32.18	-251.66
3	0	45.97	209.41	735.00	186.39	407.87	407.87	100.93	268.18	16.52
4	0	45.97	163.44	735.00	186.39	407.87	407.87	508.80	233.20	249.72
5	0	45.97	117.47	735.00	186.39	407.87	407.87	916.67	202.78	452.50
6	0	45.97	71.51	735.00	186.39	407.87	407.87	1 324.54	176.33	628.84
7	0	45.97	25.54	735.00	186.39	407.87	407.87	1 732.41	153.33	782.17
8	0	45.97	-20.43	735.00	186.39	407.87	407.87	2 140.28	133.33	915.50
9	0	45.97	-66.40	735.00	186.39	407.87	407.87	2 548.15	115.94	1 031.44
10	0	45.97	-112.37	735.00	186.39	407.87	407.87	2 956.02	100.82	1 132.26
11	0	45.97	-158.33	735.00	186.39	407.87	407.87	3 363.89	87.67	1 219.93
12	25.54	45.97	-204.30	760.54	186.39	426.26	426.26	3 790.15	79.67	1 299.60
12										1 325.14

d_k = straight line depreciation; r = discount rate; S = Salvage value of the plant; t = tax rate (28%); FCI = fixed capital investment

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Appendix H

Fan Curves

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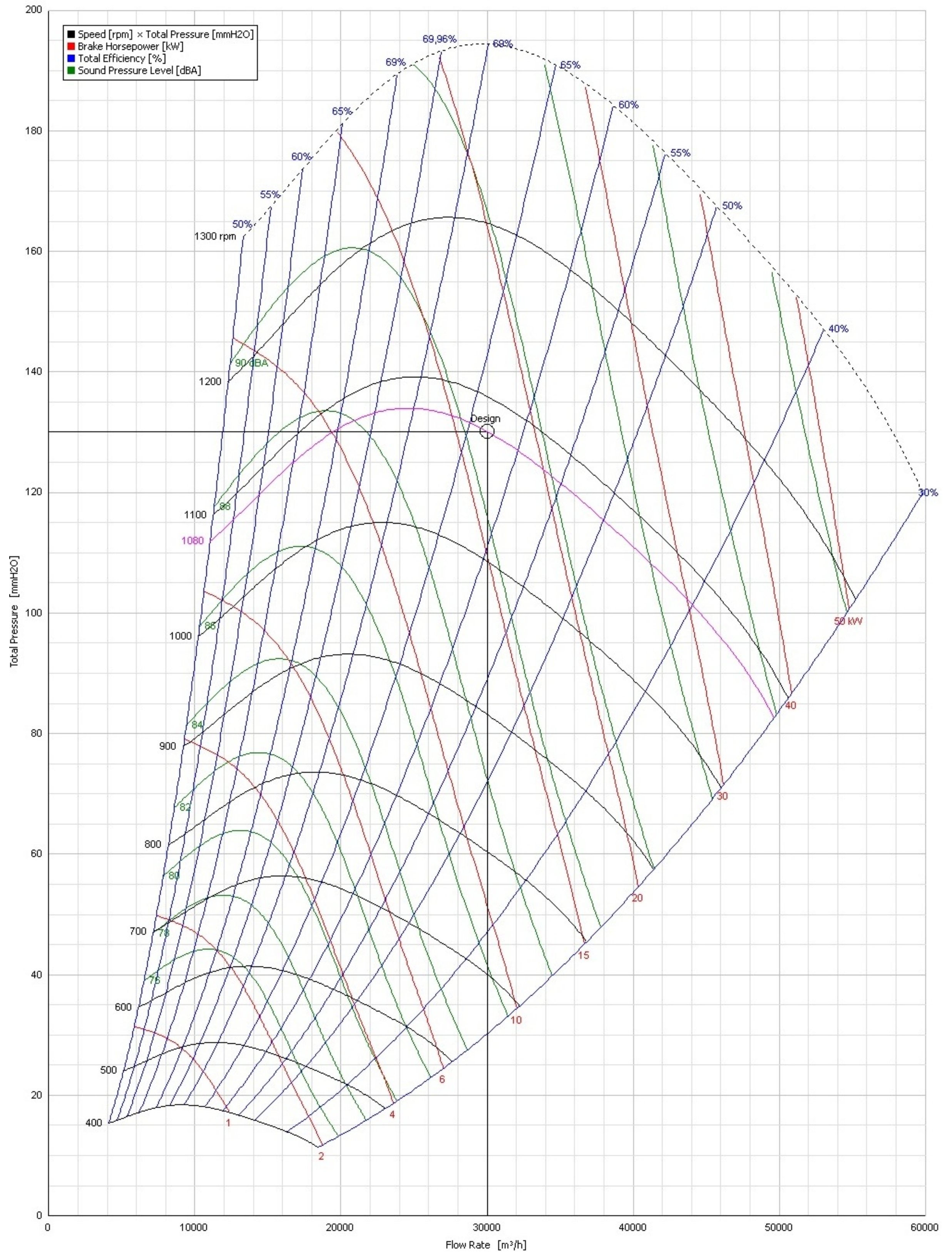

Figure H.1: Industrial axial fan curves used to calculate the duty of a fan (Ciclo Software, 2013).

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