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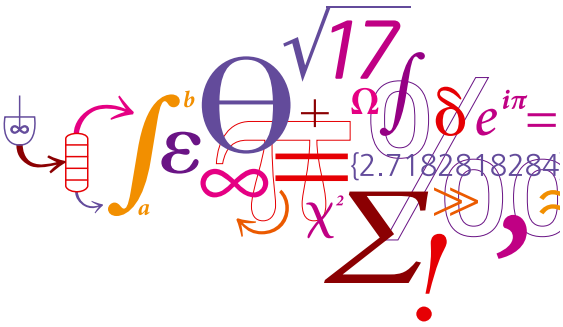

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Ph.D. Thesis
July 2011

DTU Chemical Engineering
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PhD Thesis

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Preface

This thesis is submitted as partial fulfillment of the requirements for the degree of Doctor of Philosophy (PhD) at the Technical University of Denmark (Danmarks Tekniske Universitet, DTU). The work has been carried out at the Computer Aided Process-Product Engineering Center (CAPEC) at Department of Chemical and Biochemical Engineering (Institut for Kemiteknik) from August 2008 to July 2011 under the main supervision of Professor Rafiqul Gani and co-supervision of Professor John M. Woodley. The project has been financed by PROMEP (Programa del Mejoramiento del Profesorado), Mexico.

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I am most grateful to my beloved family, my mother Alicia, my father Vitelio, and my sister Lorena for all their love. Thanks to my cherished brother, Vitelio, whose love and joy have remained in me.

Last, but for sure not least, thank you very much to my soul mate and great teacher of life, Ivan, for being the light unto my path.

Alicia Román Martínez

Nørrebro, København, July 2011

Abstract

This thesis presents a generic and systematic model-based framework to design intensified enzyme-based processes. The development of the presented methodology was motivated by the needs of the bio-based industry for a more systematic approach to achieve intensification in its production plants without an excessive investment in experimental resources. Process intensification has recently gained a lot of attention since it is a holistic approach to design safer, cleaner, smaller, cheaper and more efficient processes. This dissertation proposes a methodological approach to achieve intensification in enzyme-based processes which have found significant application in the pharmaceutical, food, and renewable fuels sector. The framework uses model-based strategies for (bio)-chemical process design and optimization, including the use of a superstructure to generate all potential reaction(s)-separation(s) options according to a desired performance criterion and a generic mathematical model represented by the superstructure to derive the specific models corresponding to a specific process option. In principle, three methods of intensification of a bioprocess are considered in this thesis: 1. enzymatic one-pot synthesis, where, for example, the combination of two enzymatic reactions in one single reactor is examined; 2. chemo-enzymatic one pot synthesis, where, for example, one enzymatic reaction and one alkaline catalytic reaction occur simultaneously in a single reactor; and 3. *in-situ* product recovery/removal (ISPR), where, for example, a separation step is integrated with the reaction step.

Often, enzyme-based processes have limited productivity and yield, which may be due to the unfavorable reaction equilibrium, product inhibition to the enzyme and/or product degradation. Additionally, downstream processing for enzyme-based processes is difficult and a way to simplify it is by reducing the reaction and separation steps by for example, combining the reaction and separation in a single processing step. The implementation of intensification methods usually involves experiment-based investigation which causes limitations in the search space of process options leading to

a high risk of implementing sub-optimal processes. Therefore, applying the framework presented in this thesis, all possible process options can be considered, and using a hierarchical decomposition approach for optimization, the search space is reduced to locate the candidate process options, giving an optimal design where further experimental efforts can be focused on.

The application of a generic and systematic model-based framework is illustrated through a case study involving the production of an important intermediate pharmaceutical: *N*-acetyl-D-neuraminic acid (Neu5Ac). A second case study is added and deals with the enzymatic production of biodiesel.

Resume på dansk

Denne afhandling omhandler et generelt og modelbaseret framework til design af intensiverede enzymbaserede processer. Udviklingen af den præsenterede metodik var motiveret af biotekindustriens behov for en mere systematisk metode til intensivering i sin produktion, uden at behøve at investere unødvendige midler i eksperimentelle undersøgelser. Procesintensivering har på det seneste fået meget opmærksomhed, fordi det er en holistisk metode til at designe sikrere, renere, billigere og mere effektive processer. Denne afhandling foreslår en metodisk fremgangsmåde til at opnå intensivering af enzymbaserede processer, som har fundet vigtige anvendelsesmuligheder inden for lægemiddel-, fødevare- og biobrændselsindustrien. Dette framework bruger modelbaserede strategier til (bio)kemisk procesdesign og –optimering, herunder brugen af en superstruktur til dannelse af alle muligheder for reaktion(er) – separation(er) i forhold til ønskede ydelseskriterier, og en generel matematisk model, repræsenteret af superstrukturen, til at udlede de specifikke modeller, som hører til en specifik procesmulighed. Der er tre metoder at finde i denne afhandling: 1. enzymatisk "one-pot" syntese, hvor for eksempel kombinationen af to enzymatiske processer i én reaktor er undersøgt; 2. kemo-enzymatisk "one-pot" syntese, hvor for eksempel en enzymatisk reaktion og en basisk katalytisk reaktion finder sted samtidig i én reaktor; og 3. *in-situ* produkt genindvinding/fjernelse (ISPR), hvor for eksempel et krystalliseringstrin er integreret i reaktionstrinnet.

Ofte har enzymbaserede processer begrænset produktivitet og udbytte, hvilket kan skyldes en ufavorabel reaktionsligevægt, enzymets produktinhibering og/eller nedbrydning af produktet. Herudover kan deres senere behandlingstrin være svære, og en måde at simplificere dem på er ved at fjerne separationstrinnet, ved for eksempel at kombinere reaktions- og separationstrinnet i ét procestrin. Implementeringen af intensiveringsmetoderne omfatter som regel eksperimentelt arbejde, hvilket er årsag til begrænsninger i områder der bliver undersøgt som mulige processer, hvilket igen fører

til implementering af suboptimale processer med høj risiko. Ved anvendelse af dette framework bliver alle muligheder for proceskombinationer genereret, og ved at bruge en hierarkisk nedbrydningsmetode til optimering, vil undersøgelsesområdet blive reduceret, mulige kandidater til den bedste proceskombination kan lokaliseres, og et optimalt procesdesign, som kan undersøges yderligere eksperimentelt, kan findes.

Anvendelsen af det generelle og modelbaserede framework er illustreret gennem to cases. Den første case omhandler produktionen af et vigtigt mellemprodukt i lægemiddelproduktionen: N-Acetylneuraminsyre (Neu5Ac). Den anden case handler om enzymatisk produktion af biodiesel.

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Abbreviations

BSTR	Batch stirred tank reactor
CEOPS	Chemo-enzymatic one-pot synthesis
CHRO	Chromatography
CRYST	Cristallization
CSTR	Continuous stirred tank reactor
DF	Driving force
DSP	Downstream processing
EOPS	Enzymatic one-pot synthesis
FAAE	Fatty acid alkyl ester
FAME	Fatty acid methyl esther
FBSTR	Fed-batch stirred tank reactor
FFA	Free fatty acid
ISPR	*In situ* product removal
LB	Lower boundaries
MINLP	Mixed integer nonlinear programming
MR	Membrane reactor
NIU	Number of identified operation/equipment in each process unit
NPO	Number of possible options
OPS	One-pot synthesis

PFR	Plug-flow reactor
PI	Process intensification
PSE	Process systems engineering
TRIZ	Teoriya Resheniya Izobretatelskikh Zadatch (Theory of inventing problem solving)
UP	Upper boundaries

Nomenclature

F_{OBJ}	Objective function
$\bar{X}$	Vector of design variables
$\bar{Y}$	Vector of decision variables
$\bar{d}$	Vector of known parameters
w_k	Weight for a specific criterion k
$\bar{g}$	Vector of constraints functions
$\bar{h}_p$	Vector of process models
u	Processing unit
r	Number of streams
t	Time
f	Number of phases
P	Pressure
T	Temperature
x	Composition
n	Number of moles
F	Flowrates
H	Enthalpy

Q	Heat
r_i	Reaction rate of compound *i*
m	mass

Subscripts or Superscripts

in	Inlet
out	Outlet
i	Number of compounds
j	Number of constraints
j_L	Number of logical constraints
j_s	Number of structural constraints
j_o	Number of operational constraints
k	Number of criterion
u	Number of unit

Greek letters

$\bar{\theta}$	Vector of product parameters
α	Phase alpha
β	Phase beta
σ	Separation factor
ξ	Connectors in superstructure
λ	Conversion rates

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CHAPTER ONE
Introduction

1.1 Research Motivation

Enzyme-based production processes are processes that use enzymes, in one or more of their processing steps, to obtain desired products. They are an essential part, at different development stages, of many chemical, pharmaceutical and food production processes (Table 1.1). Due to sustainability and environmental concerns, substitution of chemical routes by enzymatic routes has been recently the subject of investigation and the replacement of petrochemicals with renewable products is a desired trend. Hence, there is an interest in seeking more environmentally benign alternatives. Enzymes are a promising option since they offer mild reaction conditions (physiological pH and temperature), a biodegradable catalyst and environmentally acceptable solvent (usually water), as well as chemo-, regio- and stereo-selectivities. Furthermore, the use of enzymes generally obviates the need for functional group protection and/or activation, affording synthetic routes which are shorter, generating less waste and hence, are both environmentally and economically more attractive than conventional organic synthesis. One important factor that has allowed new enzyme-based processes to be implemented is the recent advance in enzyme production for industrial applications in chemical synthesis. Industrial enzyme sector is growing rapidly due to improved production technologies, engineered enzyme properties and new application fields. Over the next few years, an increasing number of chemicals and materials will be produced using enzymes in one or more of the processing steps.

Table 1.1

The application of enzyme technology in the chemical industry (modified from Schmid *et al.*, 2002)

Industry sector	Impact (estimate)*		
	Today	Near Future	Distant future
Organics			
Food and feed additives	+++	+++	+++
Fine chemicals	+	++	+++
Drugs (antibiotics, intermediates)	++	++	+++
Plastic materials and synthetics	+	++	++
Soaps, cleaners, personal care products (lipases, proteases)	+	++	+++
Inorganics	-	+	++
Miscellaneous chemical products (adhesives, pulp, textile and oil processing, waste water treatment)	+	++	+++
Agricultural chemicals (herbicides, intermediates)	+	+	++
Renewable sources of energy (biodiesel, bioethanol)	+	++	+++

*+++, very high; ++, high; +, moderate; -, low.

In general, enzyme-based processes are cleaner and greener compared to chemical alternatives. They offer novel, high-selective, shorter processing routes and lower temperature and pressure conditions. In many cases, the enzymes and the raw materials used are renewable and the generation of mass and energy waste is considerable reduced. It has been reported, as outlined in Table 1.2, that some of these processes have contributed to the sustainability of the chemical industry. Process improvements such as increase in yield and reductions in raw material demand, emissions (e.g. carbon dioxide emissions) energy consumption, water use and waste result in process cost savings and can give enzyme-based processes advantages over traditional chemical routes (Schmid *et al*., 2002).

Nevertheless, there are some limitations that make enzyme-based process implementation difficult and not straightforward (Table 1.3). Usually, they have limited reaction productivity and yield due to the unfavorable reaction equilibrium and product inhibition to the enzyme. Because of their resultant low product concentrations and product specifications of high purity, especially in the pharmaceutical industry, the downstream processing (DSP), e.g., the separation and purification stages of a process,

is difficult and thus, expensive. For many of these processes, the major cost in manufacture lies in the downstream process operations where product separation and purification is carried out (Schügerl and Hubbuch, 2005). Another limitation is the high cost of the enzyme compared to chemical catalysts which limit a replacement to the catalytic route. In addition, these types of processes have been designed and partially developed in laboratories, and are designed on a case-by-case basis, leading to a high risk of implementing sub-optimal processes, and using considerable experimental resources and time for development.

Different solutions have been proposed and applied to tackle the above mentioned difficulties. Concerning the enzyme development, one strategy to overcome the loss of enzyme activity and the optimal conditions of pH and temperature is the alteration of the enzyme (e.g, via recombinant DNA and directed evolution technologies). Another strategy is the engineering design of novel enzymes and the characterization and application of new enzymes to catalyze reactions with commercial potential and industrial applications (Kirk *et al*., 2002).

Table 1.2

Enzyme-based processes increasing the sustainability of the chemical industry (Griffiths, 2001).

Product	Enzyme*	Comparison with conventional process	Company
Ammonium acrylate	Nitrilase	◦ High yield ◦ Easy quality control of product ◦ No emission of toxic vapour	Ciba
Polyester	Lipase	◦ High-quality of product ◦ No alternative conventional process	Baxenden
(*S*)-Chloropropionic acid	(*R*)-Specific dehalogenase	◦ Simple one-step process ◦ High-quality of product ◦ No involvement of toxic raw materials	Avecia
7-ACA	D-Amino acid oxidase, glutaryl amidase	◦ No involvement of toxic raw materials ◦ Mild reaction conditions ◦ Tenfold reduction of waste	Biochemie
SO_4^{2-} removal	Sulfate-reducing microbe	◦ SO_4^{2-} and F^- load in waste water is very low ◦ Reduction of gypsum in waste water from 18 tons/day to essentially 0 tons/day	Budel Zink
Removal of hydrogen peroxide from textiles	Catalase	◦ High-quality of product ◦ Simple process ◦ Decrease of waste water	Windel
Removal of fatty acid esters from oil	Phospholipase	◦ Simple process ◦ Tenfold reduction of waste water ◦ Eightfold reduction of sludge ◦ Reduction of raw materials	Cereol

*Origin of enzymes: nitrilase, *Rhodococcus* sp.; lipase, *Candida Antarctica*; (*R*)-specific dehalogenase, *Pseudomonas* sp.; D-amino acid oxidase, not mentioned; glutaryl amidase, *E. coli*; catalase, not mentioned; phospholipase, *Hyphozyma* sp.
Abbreviation: 7-ACA, 7-aminocephalosporanic acid.

Table 1.3

Main limitations in enzyme-based processes

Process element	*Limitation*
Substrate	◦ Non-availability ◦ Variable composition and source ◦ Inhibition to the enzyme ◦ Limited solubility in water ◦ Limited dissolution rate
Enzyme	◦ Non-availability in bulk quantities ◦ High cost ◦ Substrate/product inhibition ◦ Deactivation ◦ Different optimal conditions than ones of the reaction medium
Bioconversion	◦ Unfavorable equilibrium ◦ Low conversions ◦ Slow reaction rates ◦ Low yields
Product	◦ Limited solubility ◦ Inhibition to the enzyme ◦ Diluted concentrations
Downstream processing	◦ Difficult, many steps ◦ Loss of product yield ◦ High cost

Concerning the structure and operating mode of the processing steps of an enzyme-based process, the combination of operations (reaction(s) and/or separation(s)) in a single-pot operation using new processing techniques have been proposed: (1) The direct removal of product while the reaction is progressing, named *in situ* product removal (ISPR), which has two main purposes, to avoid the inhibition of the enzyme activity due to high product concentrations and to overcome the limitation of thermodynamically unfavorable reactions to achieve a substantial product concentrations (Woodley *et al*., 2008); and (2) The complete or partial combination of the reactions (enzymatic and/or chemical) occurring in the process in a single reactor, named one-pot synthesis, (enzymatic one-pot synthesis EOPS, and chemo-enzymatic

one-pot synthesis CEOPS) with the purpose of reducing the total number of steps to avoid the isolation of intermediate products after the initial conversion (Dalby *et al*., 2005). These methods of reaction/reaction and reaction/separation integration (methods for process intensification) in enzyme-based processes have as a consequence the reduction of the total number of processing steps and therefore the overall process yields can be increased by the omission of associated handling losses in each piece of a plant process.

Process System Engineering (PSE) approaches, methods and tools, which have been widely applied in chemical process systems, are now becoming of particular interest in industrial biotechnology to design and operate processes effectively and efficiently. PSE is concerned with understanding and development of systematic procedures for design and operation of (bio)-chemical process systems, ranging from micro systems to industrial-scale continuous, fed-batch and batch processes (Grossman and Westerberg, 2000). PSE can contribute to the design, development and improvement of enzyme-based processes providing process modeling and analysis, process simulation and optimization, and process integration and intensification, applied in a systematic manner with supporting methods and tools.

To overcome the difficulties presented using the approaches mentioned above, with less time and effort, systematic and generic model-based design methodologies are needed for a fast and reliable identification and selection of new high-performance enzyme-based process configurations, which involve process intensification (Lutze *et al.*, 2010) and, consequently, integration approaches (Mitkowski *et al*., 2008). Therefore, in this work the intention is to attempt to integrate the mentioned methods of combination of operations (one-pot synthesis and ISPR) together with a model-based systematic methodology to intensify enzyme-based processes developed and presented in this thesis. This allows exploitation of the synergistic relationship between process intensification and process systems engineering. Approaches concerning the modification of the enzyme by, directed evolution, for example, are not considered in the methodology presented here.

1.2 Objectives

1.2.1 General Objective

The general objective of this thesis is to propose and apply a systematic model-based generic framework for the conceptual synthesis and design of intensified enzyme-based processes, to identify and select improved, efficient and novel high performance reaction/separation process configurations.

1.2.2 Specific Objectives

The specific objectives of this thesis consist on the characteristics and requirements that the framework must meet. The framework developed should fulfill the following:

- It should use a hybrid approach of process synthesis, since it combines knowledge-based with optimization-based methods for process synthesis and design (d'Anterroches, 2005). This allows the use of physical insights of the knowledge based methods to narrow the search space and decompose the general mathematical formulation of the process optimization problem into a collection of related but smaller mathematical problems.

- It should contain an objective function, which is the performance criterion or criteria (multi-objective function) to be used for selection of the best intensified process option.

- It should generate all possible process options, by the implementation of a mathematical combinatorial expression and a superstructure with all available operational units, including the integrated ones of one-pot reactors and for the ISPR procedures.

- It should include a mathematical generic model representing the implemented superstructure, from which specific process sub-models for the options generated are derived and subject to subsequent simulation.

- It should propose a decomposition approach to solve the whole complex optimization formulation, where a hierarchical use of constraints, including logical, structural and operational, are used to screen out unfeasible process options.

1.3 Thesis organization

This PhD thesis is organized in eight chapters, including this current introduction chapter, where the motivation and the objectives of the thesis are presented. Chapter 2 presents the concepts and research aspects related to process intensification and methods for enzyme-based process intensification. Chapter 3 presents a review of solution approaches in process synthesis and design problem, which, together with chapter 2, leads to a discussion about the issues and needs to be addressed and included in the framework. Chapter 4 presents a description of the proposed framework. First the problem formulation is stated and the second part describes the stages of the framework, their methods, algorithms and tools. In chapter 5, the methodology is highlighted through two case studies: the *N*-acetyl-D-neuraminic Acid (Neu5Ac) synthesis and the enzymatic production of biodiesel. Chapter 6 presents a discussion of the results.

Chapter 7 presents the conclusions. Finally, chapter 8 presents directions for future work.

CHAPTER TWO

Enzyme-based Process Intensification

2.1 Introduction

The research motivation and the objectives of this thesis were presented in the previous chapter. Basically, it was pointed out the necessity of developing a systematic and generic model-based methodology for synthesis and design of intensified enzyme-based processes, which are becoming increasingly important with many applications in the industrial sector. In addition, there is an urgent requirement for intensification of these kinds of processes, among others. In this chapter, the concepts and research aspects related to process intensification and methods for enzyme-based process intensification are reviewed, to identify important aspects that the framework must address.

2.2 Process Intensification

More than ever, biochemical and chemical industries are facing important challenges due to economic, environmental and societal concerns. Energy consumption, safety, non-renewable feedstock depletion as well as environmental impact (e.g. global warming) are nowadays receiving increased attention by the society. At the same time, those industries are required to be more responsive to market needs and develop processes in a fast, reliable and efficient way, without consuming excessive effort,

investment and time. Process Intensification (PI) is a fashionable and promising development path which helps to overcome these concerns.

2.2.1 Historical background

PI, as a chemical engineering discipline, appeared first in Colin Ramshaw´s work (Ramshaw, 1983) concerning the application of centrifugal fields ("HiGee") in distillation processes. In his work, Ramshaw discussed the concept of process intensification, i.e. devising exceedingly compact plant which reduces both the 'main plant item' and the installation costs. He also presented a description of 'HiGee', which substantially increases mass transfer rates compared with a conventional distillation plant.

From its beginning until the early 1990´s, the British mainly worked on PI focusing primarily their research on four areas: the use of centrifugal forces, compact heat transfer, intensive mixing, and combined technologies. They organized the first international conference on PI (1995). From this time onwards, PI started to be an international discipline and many research centers in different countries began to work in the area. For example, in the Netherlands, at Delft University of Technology together with DSM, research was done on structured reactors (Smits et. al., 1997) and centrifugal adsorption (Bisschops et. al., 1997). In France, research was aimed to the development of a design method for heat transfer devices (Thonon, 1995) and the introduction of very compact heat exchangers for the process industry (Thonon and Mercier, 1997). In Germany, research in micro-systems for the chemical industry, mainly micro-reactors, prospered at the end of the past century (Jäkel, 1995; Ehrfeld et. al., 2000). China and United States also increased their research activities in the PI area mainly in high-gravity processing (Zheng et. al., 1997), micro-channel heat exchanger and micro-reactors (Tonkovich et. al., 1996; Quiram et. al., 2000). Practical applications also were

outlined such as the intensification of a hydrogen peroxide system (Meili, 1997) and the hypochlorous acid process intensified reaction step (Trent et. al., 1999).

The beginning of this century has witnessed a rapid evolution in the chemical and biochemical engineering PI-related activities both in academia and industry, mainly in the development of process-intensifying equipment and process-intensifying methods (Stankiewicz and Moulijn, 2000). A considerable amount of novel equipment has been developed to intensify chemical and biochemical processes, just to mention a few: static mixers (Taylor et. al., 2005), structured catalysts and reactors (Cybulski and Moulijn, 2006), microreactors (Ehrfeld et. al., 2000). Rotating devices such as the spinning disk reactor (Oxley et. al., 2000) and rotating packed beds (Woyuan et. al., 2009), have also been developed.

Developed methods for PI have been classified according to Stankiewicz and Drinkeburg (2004) into a) novel processing methods, such as integration of reaction and one or more unit operations (e.g. multifunctional reactors and hybrid separations); b) use of alternative forms and sources of energy, such as solar energy, ultrasound waves and microwave dielectric heating; and c) novel methods of process/plant development and operation such as process synthesis and dynamic reactor operation. Figure 2.1 shows a classification of process intensification viewed as a toolbox.

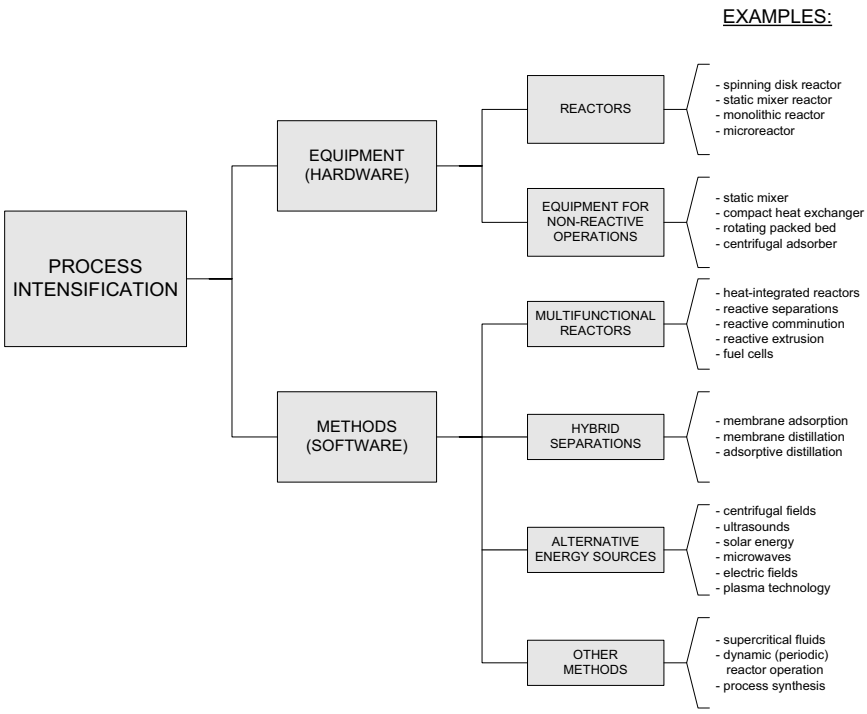

Figure 2.1 PI viewed as a toolbox (Taken from Stankiewicz & Drinkenburg, 2004).

2.2.2 Definition

As presented in the previous section (2.2.1) PI has only been considered as a kind of "toolbox" for a little more than two decades (Van Gerven and Stankiewicz, 2009). Indeed, different definitions of PI have been reported, as showed in Table 2.1, with diverse interpretations.

Table 2.1 PI Definitions

Defintion	*Reference*
”PI is devising an exceedingly compact plant which reduces both the ’main plant item’ and the installation costs”.	Ramshaw (1983)
“PI is the strategy of reducing the size of a chemical plant needed to achieve a given production objective”.	Cross and Ramshaw (1986)
“PI consists of the development of novel apparatuses and techniques that, compared to those commonly used today, are expected to bring *dramatic* improvements in manufacturing and processing, substantially decreasing equipment-size/production-capacity ratio, energy consumption, or waste production, and ultimately resulting in cheaper, sustainable technologies”.	Stankiewicz and Moulijn (2000)
“PI comprises novel equipment, processing techniques, and process development methods that, compared to conventional ones, offer substantial improvements in (bio)chemical manufacturing and processing”	Stankiewicz (2001)
“PI refers to technologies that replace large, expensive, energy intensive equipment or processes with ones that are smaller, less costly and more efficient or that combine multiple operations into fewer devices (or a single apparatus)”.	Tsouris and Porcelli (2003)
“PI defines a holistic approach starting with an analysis of economic constraints followed by the selection or development of a production process. Process intensification aims at drastic improvements of performance of a process as a whole. In particular it can lead to the manufacture of new products which could not be produced by conventional process technology. The process-intensification process itself is ‘constantly financially evaluated’”.	Degussa (now Evonik) (2005)
“Any chemical engineering development that leads to a substantially smaller, cleaner, safer and more efficient technology is process intensification”.	Reay, Ramshaw and Harvey (2008)
“PI presents a set of often radically innovative principles (‘paradigm shift’) in process and equipment design which can benefit (often with more than a factor of two) process and chain efficiency, capital and operating expenses, quality, wastes, process safety and more”.	ERPI (2008)
“PI is a revolutionary design philosophy that delivers highly efficient processes involving several combined advantages”	Arizmendi-Sánchez and Sharratt (2008)
“PI is a proven approach to process and plant design which concentrates the reaction in a chemical process in a small space with the precise environment it needs to flourish. This results in better product quality and processes which are safer, cleaner, smaller and cheaper”.	BHR (2008)
“PI stands for an integrated approach for process and product innovation in chemical research and development, and chemical engineering in order to sustain profitability even in the presence of increasing uncertainties”.	Betch, Franke, Geißelmann and Hahn (2009)
“PI is the improvement of a process by adding/enhancing phenomena in a process through the integration of operations, integration of functions, integration of phenomena or alternatively through the sole enhance of phenomena in a given operation”.	Lutze, Gani and Woodley (2010)

Although there is not a general agreement on the meaning of PI, all reported definitions have many features in common, especially with regard to the goals pursued by PI application. This leads to the establishment of principles that motivate its development, application and research.

2.2.3 PI goals

By analyzing the definitions shown above, the purpose fulfilled in PI is the development of novel apparatus, equipment, techniques (e.g. processing techniques) and methods (e.g. process development methods) for the chemical and biological processes to achieve the following goals:

- Reduction of process steps
- Use of novel and more eco-efficient synthesis routes
- Enabling greater production
- Miniaturization
- Drastic improvement of equipment and process efficiency
- Overall capital cost reduction
- Reduction of processing time (e.g. switch from batch to continuous)
- Decreasing of costs (with reduced equipment size, increased energy efficiency, less waste and pollution, improved safety).
- Development of greener routes
- Recycling
- Energy efficiency
- Substantially cheaper processes, particularly in terms of: land costs (higher production capacity and/or number of products per unit of manufacturing area), investment costs (cheaper, compact equipment, reduced piping, reduced civic works and installation costs, integrated

processing units, etc.), raw material costs (due to higher yields/selectivities), costs of utilities (in particular costs of energy, due to higher energy efficiency) and cost of waste processing (less waste generated in process-intensive plants).

- Shorter time to the market
- Smaller equipment/plant
- Safer processes
- Less waste/by products
- Smaller quantities (or even absence of) solvents
- Better possibilities for keeping processes under control.
- Elimination of one or more of the process components
- Producing much more with much less
- Increase efficiency
- Reduction of residence time
- Increase the flexibility
- Reduction of the volume/equipment size
- Reduction of the complexity of the flowsheet

These PI pursued goals are complementary, i.e. the achievement of one goal may lead to the achievement of other(s). These goals can be measured by the use of metrics that are classified by Lutze and co-workers (2010) in economic, environmental, safety and intrinsic intensified.

2.2.4 PI Principles

All the PI definitions and goals share in their rationale the same principles. Generic principles, on which process intensification is based, have been reported. Arizmendi-Sánchez and Sharrat (2005) identified two main design principles for PI: synergistic integration of process tasks and coupling of phenomena and targeted intensification of transport processes.

Van Gerven and Stankiewicz (2009) distinguished four principles: Principle 1: Maximize the effectiveness of intra- and intermolecular events; which is mainly concerned with changing the kinetics inherent in a process to improve the effectiveness (better conversions and selectivities) of a reaction. Principle 2: Give each molecule the same processing experience; in order to approximate to the ideality of delivering of uniform products with minimum waste. Principle 3: Optimize the driving forces at every scale and maximize the specific surface area to which these forces apply; which is concerned with maximization of the interfacial area, to which the driving forces (e.g. concentration difference) apply. Principle 4: Maximize the synergistic effects from partial processes; e.g. utilization of the multi-functionality on the macro-scale, such as that in the reactive separation units.

Lutze and co-workers (2010) classified four principles associated with PI as enhancements achieved through (1) integration of operations, (2) integration of functions, (3) integration of phenomena and/or (4) targeted enhancement of a phenomenon of a given operation. This phenomena-based rationale has been previously reported in the general principles for process phenomena manipulation (Rong et. al., 2008), dividing them into (1) Enhance a favorable phenomenon, e.g. enhance an oxidation reaction by using oxygen instead of air; (2) Attenuate an unfavorable phenomenon, e.g. decrease side-reactions by shortening residence time; (3) Eliminate a phenomenon, e.g. eliminate an azeotropic behavior by adding a solvent in a distillation system; (4) Combine several process phenomena, e.g. combine reaction and distillation

into a reactive distillation; (5) Separate phenomena, e.g. external catalyst packages in reactive distillation; (6) Mitigate the effect of a phenomenon by combining it with another, e.g. transfer reaction equilibrium limit by removing desired product immediately; and (7) Create a new phenomenon, e.g. create new phase interface for mass transfer.

Integration, synergy, modification (enhacement) of phenomena and optimization, are the common concepts required to develop PI. Although not new to the chemical engineering field, they are here seen as important targets that an intensified process aims to accomplish.

2.2.5 PI methods and approaches

As outlined previously in Figure 2.1, PI methods have been grouped into four defined areas: integration of reaction together with one or more unit operations, integration of more than one separation method (hybrid separations), use of alternative forms and sources of energy for processing and other methods, in which here process synthesis methods for intensification are emphasized.

A preferred principle here is process integration, especially integration of reaction and separation operations, which is one of the most important methods of process intensification (Schmidt-Traub and Górak, 2006). Table 2.2 has been created in this work and outlines the most common integrated operations used up till now to intensify different processes.

Table 2.2 Examples of integrated operations

Type	Example	Reference
Multifunctional reactors		
Integration of reaction and heat transfer		
Reverse-flow reactors	Catalytic partial oxidation of methane	Neumann and Veser, 2005.
Reactive separations		
Reactive distillation	1,1 diethoxy production	Agirre, et. al., 2011
Reactive condensation	Methanol synthesis	Ben Amor and Halloin, 1999
Reactive extraction	Hexanoic acid synthesis	Wasewar and Shende, 2011
Reactive crystallization or precipitation	Synthesis of $CaCO_3$ nanocrystals	Varma et. al., 2011
Reactive absorption	Synthesis of fatty esters	Kiss and Bildea, 2011
Reactive gas adsorption	Upgrading synthetic natural gas	Gassner et. al., 2009
Membrane reactors	Lactic acid production	Pal et. al., 2009
Reactive distillation with membrane separation	Production of tert-amyl ethyl ether	Arpornwichayop et. al., 2008
Combination of reaction and phase transition		
Reactive extrusion	Polyurethane synthesis	Puaux et. al., 2006
Chemical reaction with generation of electric power	Waste water treatment	Aoudj et. al., 2010
Hybrid Separations		
Integration of membranes with another separation technique		
Membrane adsorption and stripping	Hydrogen production	Harale et. al, 2010
Membrane distillation	Tetrahydrofuran (THF) recovery	Koczka et. al., 2007.
Membrane chromatography	Albumin downstream process	Bengtson et. al., 2004.
Adsorptive distillation	Distillation of isopropanol-water	Mujiburohman et. al., 2006

In order to extend the potential for the application of the essential ideas and concepts, a deep understanding and systematic approaches to the principles involved in PI should be a first step. To address this, process synthesis methodologies which generate intensified options of chemical and biochemical processes have been developed. Arizmendi-Sánchez and Sharratt (2008) developed a phenomena-based methodology to approach intensive options based on four levels: The structural level, consisting of region of elements and connection elements; the behavioral level, defined by physicochemical phenomena and represents the accumulation, generation and transport of material and energy; the teleological level, related to the design goal assigned to a certain component (e.g. device); and the functional level, which is used to represent the function that the component should perform to achieve the goal.

Rong and co-workers (2008) presented a methodology of conceptual process synthesis for process intensification. In their methodology, first, an analysis of relevant physical and chemical phenomena to investigate the various concepts and principles of the processing tasks is done. Then, the various partial solutions for process and equipment intensification are generated through phenomena-based reasoning. Next, the feasible conceptual process alternatives are synthesized by combining the generated partial solutions.

Van Gerven and Stankiewicz (2009) classified four approaches to realize the PI principles in the following domains: Spatial (structure) introduced to avoid spatial randomness; thermodynamic (energy) where not only heat but also pressure and movement are considered; functional (synergy), related to bring multiple functions together in one component; and temporal (time), which has to do with manipulations of the time scales at which different process steps proceed or the introduction of dynamic states into a process, usually in the form of periodicity.

Finally, Lutze and co-workers (2010) proposed a general systematic framework for synthesis and design of PI options consisting in six steps: (1) Problem definition where

the process/operation scenario, the process boundaries, an objective function and the selection of metrics for evaluation are investigated. (2) Collect data and identify bottlenecks/limitations to collect feasible PI equipment/strategies. (3) Select and develop models, to provide the process/operational mathematical models needed for the subsequent calculation/evaluation steps. (4) Generate feasible flowsheet options, to obtain all PI options based on the equipment obtained in the previous steps and afterwards reducing the search space by screening for feasibility using structural and operational constraints. (5) Fast screen for process constraints based on shortcut models, where the remaining options are screened by performance metrics using simulation results based on shortcut/simple models to identify process constraints and to further reduce the search space of PI options. And (6) Minimize the objective function and validate the most promising options via experimentation, to identify the optimal feasible PI option through optimization and benchmarking of the options with respect to the objective function.

To achieve step two in the methodology proposed by Lutze and co-workers, PI strategies according to the type of system have to be developed. This thesis provides a PI strategy for enzyme-based processes, where specific methods for intensification are identified: one-pot synthesis (OPS) and *in situ* product removal (ISPR).

2.3 Methods for Enzyme-based Process Intensification

The industrial success of many enzyme-based processes is often limited by inherent low productivity. In the last decades the enhancement of the enzyme-based process productivity has been pursued by manipulating some aspects of the process. There are listed below those considered the most relevant for intensification purposes.

2.3.1 Enzyme immobilization

An immobilized enzyme is an enzyme that is attached to an inert, insoluble material. This can provide increased resistance to changes in conditions such as pH or temperature. It also allows enzymes to be held in a fixed place throughout the reaction, following which they are easily separated from the products and may be used again. There are a number of advantages of attaching enzymes to a solid support and several major reasons are, in addition to more convenient handling of the enzyme, the facilitation of efficient recovery and reuse of costly enzymes, which enables their use in continuous, fixed-bed operation. In addition, immobilization provides a facile separation from the product. A further benefit is often enhanced stability, under both storage and operational conditions. Improved enzyme performance via enhanced stability and repeated re-use is reflected in higher biocatalyst productivities (kg product/kg enzyme), which, in turn, determine the enzyme cost per kg product (Sheldon, 2007; Tufvesson *et al*., 2011).

2.3.2 Substrate supply and delivery

According to Kim and co-workers (2007), the supply and method of delivery of a given substrate are key determinants in the effectiveness of an enzymatic reaction, but so too is the need for controlled addition such that known concentrations are available to the enzyme. They pointed out two reasons for control of substrate concentration in the enzymatic medium. First, if the enzymatic reaction is negatively affected by the presence of a toxic or inhibitory substrate above a certain concentration, the control beneath this critical concentration will be essential. Second, there is an inverse correlation between substrate concentration and enantiomeric purity of products. The

methods for substrate supply in enzymatic reactions vary depending mainly on the natural, pre-existing phase of the substrate and reactor operation (batch, fed-batch or continuous). For example, most substrates are delivered in the same phase (aqueous liquid) as the reaction media containing the biocatalyst: either in its original phase or concentrated in a suitable, inert co-solvent. When the substrate exists naturally as either a gas or a solid (at given reaction conditions), dissolution into the liquid phase is usually necessary before the reactions can take place. Also, there are different auxiliaries, such as organic solvents, ionic liquids and resins, which help to the transport of the substrates to the reaction medium.

2.3.3 One pot synthesis

It has been reported (Hailes et al., 2007) that reactions, whether enzymatic or chemical can be run in a truly integrated one-pot operation. This results in potential improvements for capital expenditure, improved equilibrium, higher reaction rates, higher product-to-enzyme ratio and reduction of the total number of processing steps.

One pot synthesis operations can be divided, according to the systems under study in this thesis, into (a) multiple enzymatic reactions in one reactor, since new enzymes that can substitute chemical reaction steps are being added into the market (Enzymatic one-pot synthesis, EOPS) and (b) chemical and enzymatic reactions together in one-pot (chemo-enzymatic one pot synthesis, CEOPS), due to the broad field of systems where the enzymatic reactions are preceded by and/or followed by chemical conversions.

Since the different reactions proceed alone under different conditions, the reactions in one-pot cannot usually be carried out under optimal conditions but under compromised conditions (Dalby et al., 2005). In many cases the solution choice may be to modify the enzyme to operate most effectively under conditions that favor the chemical

transformation (i.e. in an organic solvent or an aqueous-organic solvent biphasic mixture) or by directed evolution to modify enzyme properties.

2.3.4 *In situ* product removal (ISPR)

As mentioned at the beginning of this section (2.3), enzyme-based processes often suffer from a limited productivity. One of the reasons for this is the presence of reaction products which cause inhibitory or toxic effects to the enzyme, product degradation and/or the existence of unfavorable reaction equilibrium, giving low substrate conversions. To address each case, the product can be removed from the reaction medium as soon as it is generated (*in situ* product removal, ISPR), causing a productivity enhancement of the process (Lye and Woodley, 1999).

ISPR methods can increase the productivity or yield of a given enzymatic reaction by any of the following means: (1) overcoming inhibitory or toxic effects; (2) shifting unfavorable reaction equilibrium; (3) minimizing product losses owing to degradation or uncontrolled release; and (4) reducing the total number of downstream processing (DSP) steps. This leads to several benefits and their corresponding impacts are summarized in Table 2.3.

Table 2.3 Benefits of ISPR (Lye & Woodley, 1999).

Benefit	Impact	Basis
Increased product concentration	Reduced reactor volume, easier DSP	$g_p l^{-1}$
Increased yield on biocatalyst	Reduced enzyme cost	$g_p g_e^{-1}$
Increased yield on substrate	Reduced substrate cost	$g_p g_s^{-1}$
Increased volumetric productivity	Reduced reactor volume and or processing time, easier DSP	$g_p l^{-1} h^{-1}$

Abbreviations: DSP, Downstream processing; g_e, grams enzyme; g_P, grams product; g_s, grams substrate; l, liter; h, hour.

2.3.4.1 Selection of the appropriate ISPR technique

The selection of an ISPR method depends on many factors, ranging from the type of reaction being formed, whether there is an immobilized or free enzyme, the design and operation of the reactor, the physical and chemical properties of the compounds involved, the mode of operation (whether they are batch, fed-batch or continuous; internal or external with direct and indirect enzyme contact), and the degree of technological advancement of the techniques (e.g. adsorption resins with increased capacity). Novel separation techniques to meet the requirements for ISPR have been emerging via different science and process engineering disciplines. Systematic methods for selection and development of novel and economic ISPR methods need to be developed (Woodley et al., 2008)

ISPR is designed and affected via exploitation of molecular properties by which the product differs from the background medium. Chauhan and Woodley (1997) proposed five principal product properties to help choose the most suitable ISPR techniques:

- Volatility (boiling point $<80^{o}C$)
- Hydrophobicity ($logP_{oct} > 0.8$)
- Size (molecular weight < 1000 dalton(uma))
- Charge (positive, negative, neutral)
- Specific binding properties of a compound:
  - Hydrophobic – volatile
  - Hydrophobic-non-volatile
  - Hydrophilic-neutral-volatile
  - Hydrophilic-neutral-non-volatile

- Hydrophilic-charged

According to Stark and von Stockar (2003) a product may be removed from its producing enzyme by five possible main techniques:

- Evaporation via
  - Stripping
  - (Vacuum-) distillation
  - Membrane supported techniques
    - Pervaporation
    - Transmembrane distillation

- Extraction into another phase
  - Use of water-immiscible organic solvents
  - Techniques including an organic phase can be supported by a membrane (perstraction)
  - Supercritical fluids
  - Aqueous two-phase system
  - Reactive extraction (incl. perstraction)

- Size selective permeation techniques that take advantage of membranes
  - Dialysis
  - Electrodialysis
  - Reverse osmosis
  - Nanoflitration

- Immobilization procedures
  - Adsorption on hydrophobic carriers
  - Affinity adsorption techniques on the basis of molecular recognition
  - Ion-exchange resins

- Precipitation or crystallization

The success of an ISPR process does not depend only on the chosen separation technique but also on the configuration of the bioreactor/separation, mode of operation (batch, fed batch or continuous), additional process and economic constraints. Figure 2.2 was created for this thesis and shows a classification scheme for ISPR process according to its mode of operation, internal or external removal of the product (within or outside the reactor) and the way of contact between the enzyme and the separation phase that removes the product from the vicinity of the enzyme.

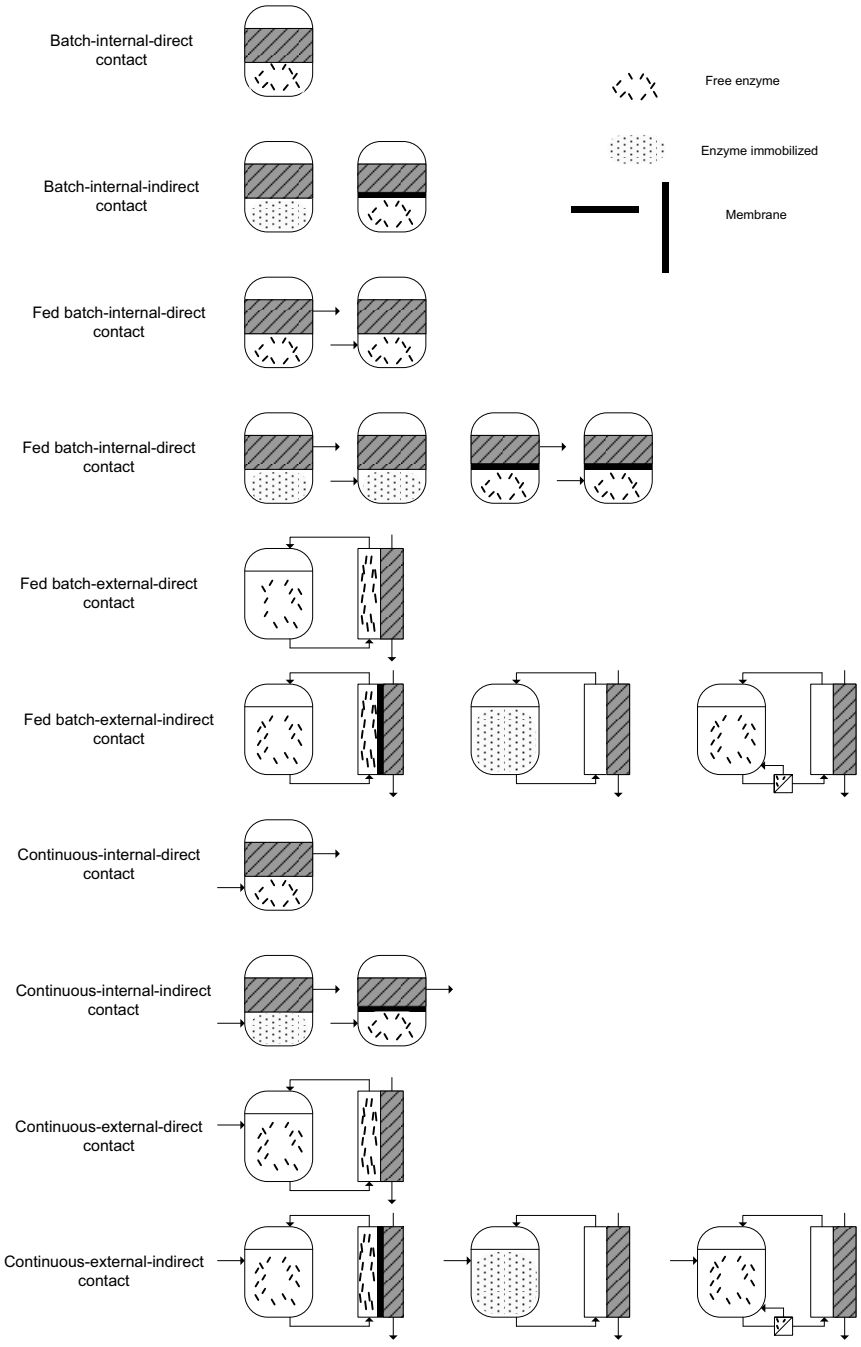

Figure 2.2 ISPR configurations

CHAPTER THREE

Solution Approaches in Process Synthesis and Design: Review & Challenges

3.1 Introduction

In the previous chapter, an overview of the concepts and research aspects related to process intensification and some methods for enzyme-based process intensification were reviewed. These identified elements for enzyme-based process intensification are implemented in a model-based systematic methodology for design of intensified enzyme-based processes. In this chapter, a brief review of the features of the different solution approaches for synthesis and design of chemical processes and bioprocesses is presented to lead to a discussion, at the end of this chapter, concerning the issues and needs to be addressed in the framework proposed.

3.2 Solution Approaches in Process Synthesis and Design

Essentially, a synthesis and design problem is solved in the framework proposed for intensification of enzyme-based processes. The synthesis and design problem for enzyme-based processes can be stated as an adaptation from Hostrup (2002), defined as:

Given the substrate(s) and product(s) specifications in the process, determine a flowsheet with the required tasks of reaction and separation, appropriate equipments, solvents, catalysts and enzymes needed (See Figure 2.2). The flowsheet must be capable of converting input (substrate streams) to output (product streams). Furthermore,

determine the design of the equipments in the flowsheet and the appropriate conditions of operation.

According also to Hostrup (2002), two flowsheet synthesis problems exist: grass-root design (where the process is designed from scratch) and retrofit design, where an existing process flowsheet has to be modified or changed in order to match new objectives, such as the case in this thesis when an enzyme-based process is intensified.

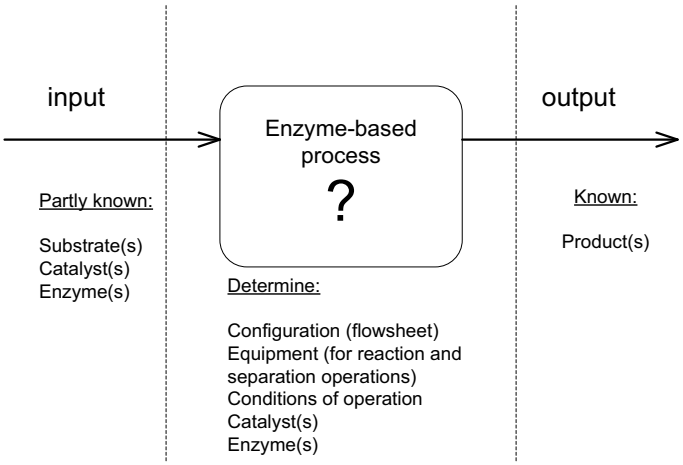

Figure 3.1 Definition of problem synthesis and desing problem for enzyme-based processes (adapted from Hostrup, 2002)

To solve the synthesis design problem, different methods have been reported. Conventionally, the design methods for conceptual process synthesis can be classified into three groups: knowledge-based methods, mathematical optimization-based methods and hybrid methods.

3.2.1 Knowledge-based approaches

Knowledge-based methods employ rules based on experience and available data and information. Different knowledge-based methods are discussed in this section.

3.2.1.1 Heuristic approach

Heuristic methods are founded in experience, using 'rules of thumb'. The first attempt to develop a systematic heuristic approach was made by Siirola and Rudd (1971). They proposed twelve alternating synthesis and analysis steps to take the process from the reaction path stage to the isolation of material separation problems, to the discovery of the useful physical principles for the solution of the separation problems, and on to the synthesis of the process task network. From there, significant research has been carried out based on this approach. A number of heuristic methods have been reported in the open literature, e.g. Powers (1972), Seader and Westerberg (1977), Nath and Motard (1978), Douglas (1985), Douglas (1985), Barnicky and Fair (1990, 1992), Douglas (1992), Chen and Fan (1993), Rapoport *et al*. (1994), Smith (1995), Pahl *et al*. (1996), Schembecker and Simmrock (1997), Pennington (1997), Butner (1999), Martin *et al.* (2006), Vanderfeesten *et al*. (2008), Adams and Seider (2009).

Heuristic methods have the main advantage of allowing the quick location of solutions, sometimes "near" the optimal, therefore, they are good to be applied to make fast estimates and preliminary process designs.

One main disadvantage of the heuristic method is the impossibility to manage the interactions between the different design levels. This causes problems in the systematic handling of multi-objective issues within hierarchical designs. This method offers no guarantee of finding the optimal design (Li and Kraslawski, 2004). Also, the heuristics

may fail since they are based on the analysis of a set of simplified rules, avoiding the complexity of more realistic systems.

3.2.1.2 Means-end analysis

In Means-End Analysis (Simon, 1969) in (bio)-chemical process design, the raw materials are taken as the initial state and the desired products the final state (the goal state). Raw materials and products are described and characterized by a number of physical and chemical properties. If the value of a particular property of a raw material is different from the value of the corresponding property in the desired product, a property difference is detected. The objective of a (bio)-chemical process is to apply technologies in a way that the property differences are eliminated so that the raw materials become the desired products. Means-end analysis consists of the systematic detection of these differences and the identification of the technology to eliminate such differences (e.g. to change the molecular identity a reactor is used, and a separation like distillation to change concentration and purity). This method is generally applied in a forward direction, beginning with the initial state and systematically applying transformation operators to produce at each step fewer differences until the final state is achieved (Siirola, 1971).

The means-end analysis approach is convenient for an early and fast systematic process synthesis method for overall process flowsheet synthesis, if the specifications of the initial state of the starting materials and the final state of the desired products are well known.

The main limitations of this method are the impossibility to consider all the properties. Therefore many of them are ignored, leading to a risk of discarding a big number of feasible and better options.

3.2.1.3 Driving force methods

These methods use thermodynamic insights (Linnhoff and Hindmarsh, 1983, El-Halwagi and Manousiouthakis, 1989, Jaksland *et al*, 1994) and fundamentals of separation theory, utilizing property data to predict feasible configurations of reaction and separation flowsheets. They are related to analysis of the driving forces of physical and chemical changes. Sauar *et al*. (1996) proposed design principle based on the equipartition of forces. Based on the definition of driving force (*DF*), as the difference in chemical/physical properties between two coexisting phases that may or may not be in equilibrium, Bek-Pedersen and Gani (2004) developed a framework for synthesis and design of separation schemes. The framework includes methods for sequencing of distillation columns and the generation of hybrid separation schemes. The *DF* approach makes use of thermodynamic insights and fundamentals of separation theory, utilizing property data to predict optimum or near optimum configurations of separation flowsheets. This approach allows identifying feasible distillation sequences as well as other separation techniques (different than distillation).

3.2.1.4 Conflict-based approach

The conflict-based approach is based on the TRIZ approach for the identification of the conflicts that limit the development of a technical system (Altshuller, 1998). The design problem is represented by the conflicts among the multiple design objectives and the characteristics of the process flowsheets (Li et al, 2003).

It is an efficient method for the preliminary design in terms of the screening of unfeasible options at an early stage for example, of reactor/separator systems and waste management (Li *et al.*, 2001; Li *et al.*, 2003(b)).

3.2.2 Mathematical optimization-based approaches

The optimization of a process synthesis problem can be stated as follows: for a given process superstructure representing different process options described mathematically, find the best solution (the best process included in the superstructure) within constraints. The best solution is quantified by means of an objective function. A superstructure includes all possible interconnected unit operations in a potential flow sheet. Decision variables (describing presence of the unit operation) and structural parameters (like size of reactor, number of plates in distillation column, membrane area) are included in the mathematical formulation of the problem. This kind of problem formulation leads to mixed-integer linear programming (MILP) and mixed-integer nonlinear programming (MINLP) problems (Biegler and Grossmann, 2004).

Optimization-based methods are advantageous since they provide systematic mathematical frameworks to manage a variety of process synthesis problems and a more rigorous analysis of features like structure interactions and economical aspects.

The optimization methods present some disadvantages, such as the heavy mathematical programming and with this, the requirement of huge computational efforts and consequently excessive amount of time to obtain results. Other drawbacks are the lack of ability to automatically generate a flowsheet superstructure, the dependency on the availability of reliable process models, the difficulty of involving all possible alternatives in the mathematical model and of extending the models in order to make them generic enough to be applicable to any (bio)-chemical process. This approach faces difficulties for the optimization of poorly defined design problems and uncertainties coming from the multi-objective functions of the design problem.

3.2.3 Hybrid approaches

Hybrid methods combine the advantages of the knowledge-based methods and the optimization method. For example, a hybrid method could use the heuristics of knowledge-based methods to narrow the search space, and decomposes an optimization problem into a collection of related but smaller mathematical problems. It is usually applied in a step-by-step procedure in which solution of one problem provides input information to the subsequent steps where other smaller mathematical problems are solved. Finally, such a procedure leads to an estimate of one or more feasible process flowsheets. The final step of hybrid methods is a rigorous simulation or experimental validation for verification of a proposed process flowsheet.

In this thesis, the techniques for enzyme-based process intensification, mentioned in Section 2.3, are included in a generic and systematic model-based framework for synthesis and design. The framework is proposed from the perspective of a hybrid approach of process synthesis and design (knowledge-based and optimization-based methods are combined). The framework is developed in such a way that, starting from a performance criterion or criteria, given by an objective function in an optimization problem, all possible intensified options for a specific system to make a product are generated, and then, through an hierarchical screening through logical, structural, operational constraints, and the process model; the best intensified option and the optimal operational conditions for the reaction and separation steps in an enzyme-based process, is obtained.

3.3 Issues and needs

To overcome the challenge that is confronted to achieve the objectives of this thesis, many and diverse research issues and needs arise, which can be organized under the following generic points.

3.3.1 Problem definition

The reliability of a solution to a process synthesis/design problem largely depends on the problem definition. In the framework developed here for intensification of the reaction and separation steps of enzyme-based processes, this step consists in identifying the limitations/bottlenecks of the existent process in its reaction and separation step (observed from a base-case design), the needs that the new process sought must address; the boundaries, constraints and metrics for comparing performance also are identified in this step to define the goals and create an objective function that the method of problem solution has to solve.

3.3.2 Metrics for process intensification

Part of the problem definition consists in the identification of metrics for performance evaluation among the process options generated for selection of the best intensified process. While in the past economic criteria mainly drove the decision for choosing and implementing a particular chemical process; now the trend is the use for sustainability metrics to select between process options (Carvalho et al, 2008). Sustainability metrics (economic, environmental) and intrinsic intensified can be used for the decision making of the best intensified enzyme-based process. They are outlined in Table 3.1.

For assessing/ranking the performance in biocatalytic processes, thus, enzyme-based processes, the most common metrics are related to the productivity and product purity (Law et al, 2008):

- *Space-time-yield* (g product/L reactor/h), as an indicator of reactor cost.
- *Biocatalyst yield* (g product/g catalyst), as an indicator of biocatalyst cost.
- *Product concentration leaving the reactor* (g product/L reactor) and *purity of the product*, as an indicator of downstream processing cost.

Table 3.1 Direction of improvement through PI for each metrics (Lutze et al, 2010)		
Environmental	Waste	⇩
	Efficiency	⇧
	Energy	⇩
Safety	Safety	⇧
	Health risks	⇩
	Operability	⇧
Economic	Capital costs	⇩
	Operational costs	⇩
	Productivity	⇧
	Product purity	⇧
Intrinsic intensified	Residence time	⇩
	Controllability	⇧
	Flexibility	⇧
	Modularity	⇧
	Maintainability	⇧
	Ease of construction	⇧
	Volume/equipment size	⇩
	Complexity of the flowsheet	⇩
Social Factors	Sustainability, life cycle impact, climatic impact, labor utilization, risk minimization, security	

3.3.3 Objective function definition

The definition of the objective function is also part of the problem definition. It consists of determining the criterion or criteria for optimization, and specifying the objective function in terms of the variables of the system. In this way the performance model is provided with a mathematical expression. The definition of the objective function strongly depends on the analysis of bottlenecks and limitations of the base-case that should be done previously to set the goal necessary to achieve the designed, identified and selected process. This analysis generally leads to an objective function based on multiple criteria (multi-objective optimization), where there can be conflicts between objectives; for example, to increase the yield of an enzyme-based process one must use the maximum possible substrate concentration and the maximum possible amount of enzyme, which leads to an increase in the processing costs, that is, the improvement of one will result in the worsening of other. This will require that the synthesis system generates not only one optimum design, but rather whole families of designs. Each may need to be evaluated from distinct points of view. Due to the complexity of the problem addressed in this work, there will be many variables and many constraints involved, the problem formulation will be too large, then the mathematical statement of the problem should be simplified as much as possible without losing the essence of the problem. Sometimes a simplification of the objective function will be necessary. This can be done by ignoring those variables that have an insignificant effect on the objective function, either based on engineering judgment or by performing a mathematical sensitivity analysis and determining the weights that should be assigned to each variable. In general, the determination of weights for each criterion depends on experience and knowledge of the specific case.

3.3.4 Bottlenecks identification

Bottlenecks/limitations analysis will also be useful for the identification of process techniques to overcome them, as shown in Table 3.2, and with this, the generation of new/intensified process options. Since the intensification of a process has as a starting point an established process that needs to be improved, then a retrofit design problem is addressed. The principal objectives of process retrofits are to identify the bottlenecks in the process, recognize the bottlenecks that when removed will lead to improvements and suggest new design alternatives that match the identified bottlenecks (Carvalho, 2009). Typically, these objectives are related to process intensification aims, like reducing the environmental impact, increasing the capacity in a plant without increasing the size of it, utilizing new process technologies to improve the energy-use efficiency, increasing the safety of the process and/or reducing the operating costs.

3.3.5 Knowledge management.

Collection and management of the data and information necessary to solve our synthesis/design problem for intensified enzyme-based process are fundamental tasks. The amount and complexity of information of different types, ranging from experimental data to complex mathematical models that need to be managed is immense. Questions arise when decisions about what to collect and what should have to be made to discern among the vast amount of information which will be important for the specific problem and to help us for rational decision making.

Table 3.2 Some techniques to solve a given bottleneck in enzyme-based processes

Bottleneck	Process Techniques					
	Two-liquid phase	ISPR	Immobilization	Excess of reactant	Substrate feeding	One-pot synthesis
Reactant/product pH/T lability		●			●	
Thermodinamically unfavourable		●		●	●	●
Reactant/product low water-solubility	●				●	
Reactant toxic/inhibitory to biocatalyst	●		●			
Product toxic/inhibitory to biocatalyst	●	●	●		●	
Difficult/expensive downstream processing		●				
Many processing steps		●	●			●

3.3.6 Identification and classification of constraints

Constraints are conditions that a solution to an optimization problem must satisfy. In general they are classified in equality constraints and inequality constraints. For the type of problems that are solved by the framework proposed in this thesis, different kinds of constraints (equality and inequality constraints) are involved: logical, structural, operational and the process model. The set of process options that satisfy all the constraints is called the feasible set. Logical constraints represent the selection of a processing unit/equipment and the logical sequence of allowed operations in the processing steps. Structural constraints represent the allowed inlet, outlet and recycle

streams in the flowsheets. Operational constraints are related to process design specifications, such as reflux ratio, operation pressure and temperature, etc. There is also a set of equality constraints representing the process model equations (i.e., mass and energy-balance equations). The application of these design constraints must be systematic, i.e. they are to be applied inside a hierarchical screening procedure of all the options generated in the framework.

Table 3.3 Type of data/information necessary for enzyme-based process intensification

Compounds involved in the process and their properties	
	Substrates
	Enzymes
	Catalyst
	Solvents
	Water
Mixture properties	
Maximum number of processing units	
Type of processing units:	Reactors
	Separators
	Reactive-Separators
Number of phases per processing unit	
Number of streams in the flow sheets	
Class of equipment for each processing unit	e.g. membrane reactor, packed bed column, evaporator, crystallizer, etc.
Process models	
Equipment models	
Kinetic models	Kinetic parameters
Experimental data	Process
	Properties

The identification of constraints depends largely on the analysis of the reactions, equipment, processes, data and information collected about the system under consideration. In general, the knowledge of the constraints can be obtained for information reported in literature, experimental data and experience. Windows of operation (Woodley and Titchener-Hooker, 1996) are tools which help in the

identification of key constraints. Windows of operation are graphical representations of the design constraints that define the feasible operational region of a process.

Constraints have to be used for screening of the generated process options in a hierarchical manner to structure and speed up the process of the location of the best option which performs superior to all other possible processes.

3.3.7 Generation of options

Intensified process design involves making a search of improved process options and matching these against specific objectives. For that, systematic generation methods are the most effective and broadly applicable in the future. These methods build up one or more designs given the goals and constraints of the processing problem. Even more, PI involves invented new unit operations of combined reactions and separations that come from the phenomena exploited to perform desired tasks. For example, reactive distillation has found a broader application, while more novel concepts such as membrane reactors are beginning to be exploited. A problem arises when there is an inability to guarantee that, among all the possibilities, the best option is selected. For that, the framework proposed here should be able to generate systematically all possible options in so that they can be used to create the superstructure for simultaneous discrete and continuous variable optimization. The generation of options has to be represented, like the process performance, by a mathematical expression which will allow us to generate all the possible options. This mathematical representation is highly combinatorial and must be considered as a constraint in the whole problem representation.

3.3.8 Superstructure development

A superstructure is used to determine optimally and simultaneously the structure of a process design, i.e., equipment identity and connectivity, as well as all the design and operating parameters for each piece of equipment. The superstructure includes all paths and equipment options for achieving the design objectives. To find the feasible options, the redundant paths and equipment alternatives are stripped away by the use of logical, structural and operational constraints. The mathematical flow through each interconnection, as well as scale, operating conditions, and other design parameters for each piece of equipment are then determined in a whole simultaneous mathematical program by optimization of a desired performance criterion.

Two separate and distinct problems still limit the use of superstructure optimization techniques:

(1) How to generate the initial superstructure while guaranteeing it contains the "best" solution. There is a lack of methods for generating good and complete superstructures.

(2) How to solve the large optimization problem inherent in practical system problems. In our case, a MINLP (Mixed integer non linear problem) has to be solved, and due to its size and complexity (indifferentiable, discontinuous and nonconvex nature, etc), it is impossible to solve the whole problem simultaneously in a mathematical program.

In any event, neither computer software nor hardware has been able to perform the task except for very simplified sub-problems. These more complex problems can be addressed by the framework proposed in this work.

3.3.9 Models, model development and model analysis

The framework proposed in this thesis is model based since we are making use of different types of mathematical models to represent the system to study its behavior under specific conditions, since it is difficult to observe it in reality. The whole mathematical problem formulation contains different types of mathematical expressions which are used as a means to solve the problem we are solving:

- The objective function
- The expression for option generation
- Equality and/or inequality logical constraint expressions
- Equality and/or inequality structural constraint expressions
- Equality and/or inequality operational constraint expressions
- Generic superstructure model from which all specific flow sheet process models are derived
- Constitutive relations of the process models

It is necessary that the framework provides the methods and tools to define, setup, analyze, test, validate and verify these models in order to have systematic procedures to solve the problem stated in this project, which is to find the values of the variables that describe the best intensified enzyme-based process option (optimization variables). They must satisfy all the constraints and the objective function.

3.3.10 Sensitivity Analysis

Sensitivity analysis is necessary to be implemented in the framework to identify the inputs (some process variables) that have most influence on the results (outputs) of the

system studied. Given the immense number of variables involved, sensitivity analysis allows the identification of the critical variables, and/or building different possible scenarios which allow the analysis of the behavior of a result (e.g. on chosen optimization criteria) under different assumptions.

3.3.11 Solution technique of the optimization problem

As stated before in section 3.3.9, the solution of the problem is to find the values of the optimization variables that satisfy all the multiple constraints and the objective function. This optimization problem consists of non-linear equations, continuous and discrete variables that result in a Mixed Integer Non-Linear Programming (MINLP) problem, whose complexity is increased by the introduction of the superstructure that implies a large number of processing options, and the process sub-model for each option, that, including the unit operations models and constitutive equations like the kinetic expressions for the enzymatic reactions, are highly nonlinear. The solution technique in a simultaneous and efficient manner is impossible, therefore, the framework aims to contain an efficient solution approach based on decomposing the whole complex problem into a set of sub-problems.

3.3.12 Methods and tools

Development of generic and systematic methods for intensified enzyme-based process design has not been addressed yet in much extension because of the lack of information on how these problems can be formulated in a general manner and the tools that would be needed to solve them. Design algorithms that do not focus primarily on the process cost but take into consideration the various aspects and implications of process intensification need to be developed. Flexible solution strategies are necessary. Flexible, simple and accurate computer aided methods and tools are also needed. Design of enzyme-based intensified processes can be viewed as a reverse problem for synthesis and process design (d'Anterroches & Gani, 2005). Flexible solution approaches should be able to solve the problem tackled here with the reverse approach.

3.3.13 Methodologies

Biocatalytic processes, including enzyme-based processes, have been developed through costly and time consuming trial and error design procedures. The development of systematic methodologies, with the related work-flows and data-flows for the inter-related activities involved in the design of new processes has been recognized as one of the main research challenges in the context of chemical and biochemical engineering. The solution of the problem of intensification of enzyme-based processes should be obtained via a systematic methodology.

3.3.14 Systematic frameworks

In order to increase the efficiency of the different tasks involved in enzyme-based process intensification, a general framework with a user friendly environment should be developed. The framework should allow the use of the methods and tools in an integrated manner, allowing inter-changes of information, data and results.

3.4 Addressing issues and needs

This PhD project will address the issues and needs in a way that, given the reactions – the compounds (substrate(s), product(s)), the catalyst(s) (chemical, enzyme(s)) - , and the base-case design, a user should be able to identify the optimal intensified enzyme-based process, that is, its configuration (reaction and separation and or integrated reaction/separation stages, type of equipment and their interconnection) and its operational conditions (e.g. concentrations, catalyst and enzyme amount, flow rates, temperature, pH, etc.). The optimal intensified enzyme-based process will be obtained by means of a combined (hybrid) knowledge-based and model-based framework. The framework will be a systematic methodology with the data, information, methods, algorithms and tools that can be used to the analysis of the base-case design to know the bottlenecks/limitations, to define the objective function, to generate all possible configurations, to screen hierarchically not feasible options by use of logical, structural, operational and process model constraints. At the end, an evaluation of the objective function will give the user the best option.

Figure 3.2 shows a graphical representation of the problem addressed in this thesis.

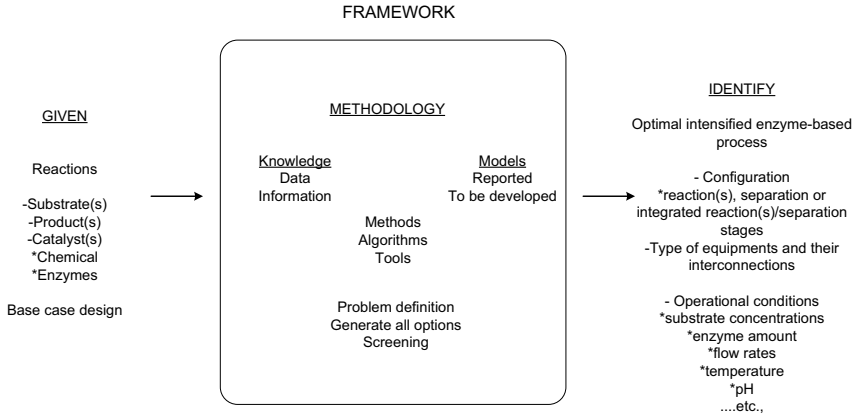

Figure 3.2 Representation of the issues and needs addressed in this thesis

CHAPTER FOUR

Framework for Design and Analysis of Intensified Enzyme-based Processes

4.1 Introduction

The first three chapters of this thesis a general justification of this project has been described. The intention was to introduce the motivation, objectives, needs and challenges that thesis will address. In chapter one, the growing importance of enzyme-based processes is highlighted. The benefits and drawbacks that enzyme-based processes offer were explained, and based from these, the objectives were formulated. Chapter two describes the historical and conceptual review of Process Intensification (PI) and important methods for enzyme-based process intensification are emphasized. In the third chapter there is a review of the methods in the Process Systems Engineering (PSE) area that have been used to design chemical and biochemical processes and thus, enzyme-based processes. It also showed the issues and needs to be addressed in order to achieve the objectives of the thesis, as well as how they are going to be addressed. Here, in chapter four, the developed framework needed for design and analysis of intensified enzyme-based processes is presented. First, the problem formulation is explained, prepared for the description of the solution technique, incorporated in a systematic methodology for design of intensified enzyme-based processes.

4.2 Problem formulation

The synthesis/design problem presented in this thesis can be stated in a mathematical form as the following: taking into account different generated process options – from a previous base-case design analysis and data collection - (in their reaction(s) and separation(s) steps) including in a superstructure, where NIU_u is the number of identified equipment in each process unit u, optimize a determined performance criterion (or several performance criteria) defined by an objective function F_{OBJ} subject to a set of optimization variables, which include design variables $\bar{X}$, decision variables $\bar{Y}$, known parameters $\bar{d}$, product parameters $\bar{\theta}$, a set of weights for each criterion w_k ,a set of constraint functions $\bar{g}$, and the process models $\bar{h}_p$. The decision variables $\bar{Y}$ describe the existence of processing units, streams, operations and equipment in a processing unit u. Each processing unit has r streams. Subscripts k and j represent a certain criterion and constraint, respectively. The process variables $\bar{X}$ can be, for example, flow rates, substrate concentrations, temperature, etc. There is a subset in $\bar{X}$ representing spatial process variables $\bar{X}_{espatial}$, referred to the spatial coordinates, for example, in a rectangular system, $\bar{X}_{espatial} = [x, y, z]$. The known parameters $\bar{d}$ can be, for example, equipment parameters (e.g. reactor geometry, heat transfer coefficients) and known kinetic constants (e.g. Michaelis-Menten constants, inhibition constants). The constraint functions include logical, structural and operational constraints bounded by lower boundaries LB and upper boundaries UB. Therefore, the mathematical formulation of the problem is given by the overall optimization model:

Maximize or minimize the objective function,

$$\max/\min \; F_{\mathrm{OBJ}} = \sum_{k=1}^{k} f_k\left(\bar{X},\bar{Y},\bar{d},\bar{\theta}\right) w_k \tag{4.1}$$

s.t. $\bar{X},\bar{Y},\bar{d},\bar{\theta},w_k$

the number of options (combinatorial constraint)

$$NPO = \sum_{u=1}^{u}\left(\prod_{u=1}^{u} NIU_u\right)\left(\frac{(r\cdot u)!}{u!(r\cdot u-u)!}\right) \tag{4.2}$$

the logical constraints

$$g_{j_L,Logical,LB} \le g_{j_L,Logical}\left(\bar{Y}\right) \le g_{j_L,Logical,UB} \tag{4.3}$$

$$g_{j_L,Logical} = g_{j_L,Logical}(\bar{Y}) \tag{4.4}$$

the structural constraints

$$g_{j_S,Structural,LB} \le g_{j_S,Structural}\left(\bar{Y}\right) \le g_{j_S,Structural,UB} \tag{4.5}$$

$$g_{j_S,Structural} = g_{j_S,Structural}\left(\bar{Y}\right) \tag{4.6}$$

the operational constraints

$$g_{j_O,Operational,LB} \le g_{j_O,Operational}\left(\bar{Y},\bar{X},\bar{d},\bar{\theta}\right) \le g_{j_O,Operational,UB} \tag{4.7}$$

$$g_{j_O,Operational} = g_{j_O,Operational}\left(\bar{X},\bar{Y},\bar{d},\bar{\theta}\right) \tag{4.8}$$

and the process models constraints

$$h_{p,j}\left(\bar{Y},\bar{X},\frac{\partial\bar{X}}{\partial\bar{X}_{espatial}},\bar{d},\bar{\theta}\right)=\frac{\partial\bar{X}}{\partial t} \tag{4.9}$$

Equations (4.1)-(4.9) represent the mathematical formulation of the synthesis/design of the optimal intensified enzyme-based process. The solution of the problem is to find the values of the optimization variables that satisfy all the constraints and the objective function. It is an optimization problem that consists of nonlinear equations (e.g. constitutive kinetic equations of the process model) continuous and discrete variables that result in a Mixed Integer Nonlinear Programing (MINLP) problem, whose complexity is increased by the introduction of the superstructure (detailed description in Section 4.4) that implies a large number of processing options, and the process sub-model for each option. To clarify the difficulty of the solution procedure of this process synthesis problem, the incidence matrix for Equations (1) to (9) is derived (Table A.1 in appendix A), in which the variables are arranged horizontally and the equations vertically. In this, a cross indicates the occurrence of a variable in a corresponding equation.

4.3 Solution technique: Systematic methodology for design and analysis of intensified enzyme-based processes.

To make the problem described in Section 4.2 solvable, a systematic generic model-based framework for design of enzyme-based processes is introduced which will guide the user by a step by step procedure for analysis of the design problem and data/knowledge about it, generation of all process configurations and screening of them to finally give an optimal processing route (see Figure 4.1). The solution strategy is reflected in a methodology schematically shown in Figure 4.2, which uses different types of methods, knowledge and tools to find the solution. The stages outlined in this figure (stages of the methodology) are explained in the subsequent sections of this chapter. Hence, instead of solving the whole synthesis problem together, this will be difficult to solve and may not lead to the optimal process, the mathematical problem is divided into manageable sub-problems (blocks), presented in the incidence matrix (Table A.2 of Appendix A). Each block needs therefore information from a block before. In each block all equations can be solved (either simultaneously or sequentially).

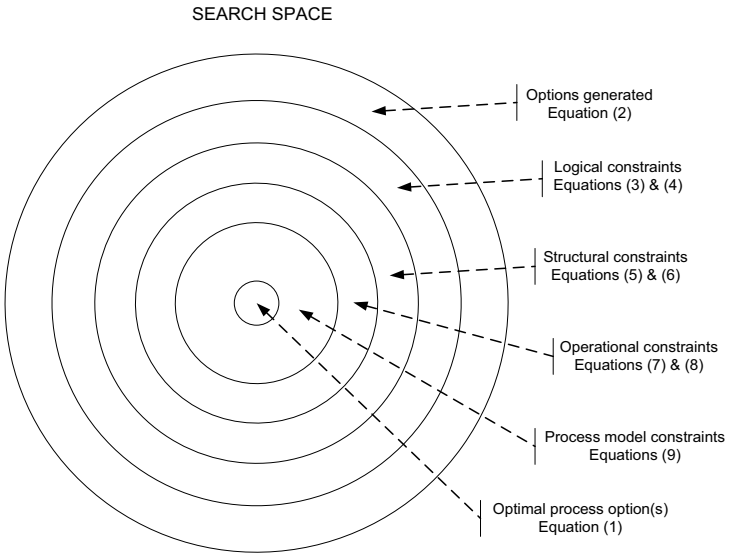

Figure 4.1 Search-space narrowing for location of the optimal process option by hierarchical screening

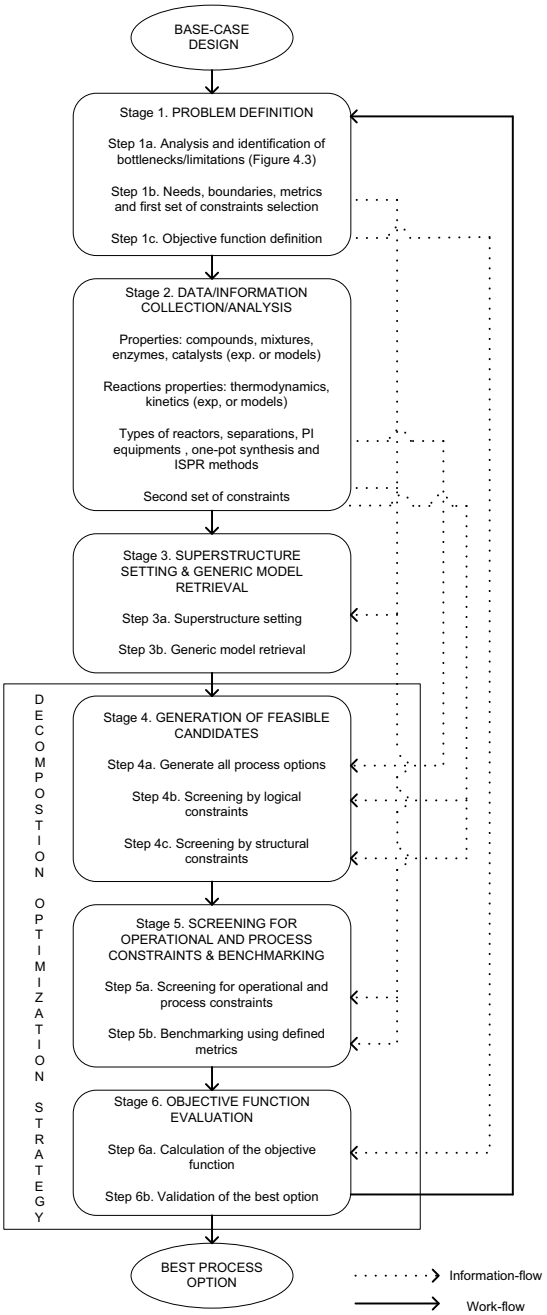

Figure 4.2 Work- and data-flow of the methodology

4.3.1 Stage 1: Problem definition

The first stage of the methodology consists in the problem definition. For this, the starting point is a base-case design of the system to be intensified. The base-case design can be an existing or a conceptual process. This base-case design is subject to analysis and identification of the bottlenecks/limitations in its reaction and separation steps (Step 1a); followed by the identification of the needs, preliminary definition of boundaries, an initial set of constraints and decided metrics for performance ranking (Step 1b) and the definition of the objective function (Step 2c).

4.3.1.1 Step 1a. Analysis and identification of bottlenecks /limitations

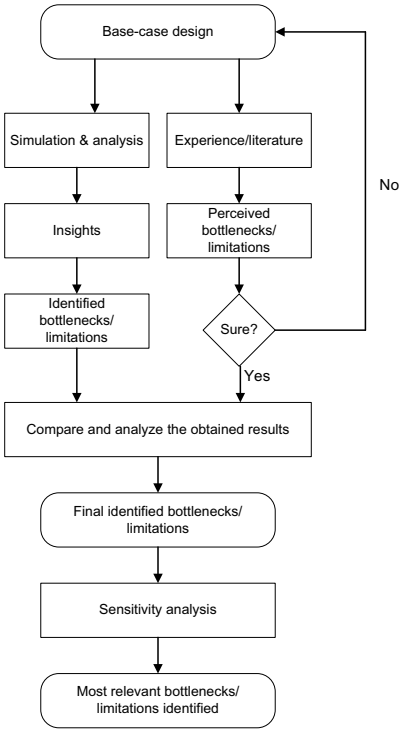

Figure 4.3 Algorithm for identification of bottlenecks/limitations (Modified from Beng-Guang et al., 2000)

Bottlenecks/limitations (some examples are outlined in Table 4.1, which is an extended version of table 1.3) identification can be done by different methods (Beng-Guang et al, 2000): by experience, where the engineers can indicate the part of the process which is the source of bottleneck problems; by experimental tests, where bottlenecks can be testing from the real equipment in the process; or by computer simulation. In this methodology, a combined experience (or knowledge reported in literature) and simulation is employed. Figure 4.3 shows the detailed procedure for bottlenecks/limitations identification, where a sensitivity analysis should be done to identify the most relevant bottlenecks that can limit the achievement of the design needs. Process simulation of the base-case design can be done using process simulators such as PRO-II and ProSim.

4.3.1.2 Step 1b. Needs, boundaries, metrics and first set of constraints selection

After analysis of the base-case design and identification of the bottlenecks/limitations, we are able to identify the needs, related to finding a better alternative. Also a preliminary selection of boundaries can be done is this part of the methodology. Some boundaries can be translated to constraints. Also, selected PI metrics can be translated into heuristic rules integrated in logical and structural constraints, such as connection rules for synthesis. Examples of needs, questions to define the boundaries and examples of constraints are presented in Table 4.2. Additional PI metrics for benchmarking are also selected in this step (Table 3.1).

4.3.1.3 Step 1c. Objective function definition

Also, once the most important bottlenecks/limitations have been identified, the objective function (Eq. 1 in the problem formulation) can be defined. In this step, in the case of a multi criteria function, the weights of each term have to be assigned. They can be assigned if there is knowledge about them. In case there is not knowledge about the weights, a sensitivity analysis of the objective function can be done to have a full definition of the multi-criteria objective function. Table 4.3 shows possible objective functions that are defined for a specific bottleneck/limitation.

Table 4.1 Examples of bottlenecks/limitations

Process component	***Bottleneck/limitation***
Substrate(s)	-Non-availability -Need to process new substrates -Excessive consumption of substrates -Low water solubility -pH/T lability -Inhibition to the enzyme -Limited dissolution rate
Catalyst(s)/Enzyme(s)	-Need to process new catalyst(s)/enzyme(s) -Excessive consumption of catalyst(s)/enzyme(s) -Substrate inhibition/toxicity -Product inhibition/toxicity -Non-availability in bulk quantities -High cost -Deactivation -Different optimal conditions than those of the reaction medium
Product(s)	-Low water solubility -pH/T lability -Inhibition to the enzyme -Very diluted concentrations
Reaction(s)	-Generation of toxic products and/or pollutants (Bottlenecks of environmental impact) -Thermodynamically unfavorable -Low conversion -Slow reaction rates -Low yields
Separation(s)	-Difficult downstream processing -Expensive downstream processing -Many steps -Loss of product yield
Units and Interconnections (Streams)	-Bottlenecks of process structures -Many processing steps
Equipment	-Size specifications (Bottlenecks of scale)
Operational	-Stirring velocity (Bottleneck of scale) -Retention time of reactors (Bottleneck of scale) -Equipment operating temperature and pressure (Bottleneck of scale and energy consumption)

Table 4.2 Examples of needs, boundaries and constraints that can be identified in Stage 1

Needs
Increase the production capacity
Efficiently processing new raw material feed stocks
Utilizing a certain type of catalyst and/or enzyme
Utilizing new process technologies
Reducing environmental impact
Reducing operating costs
Reduction of the processing steps
Boundaries
How many and what substrates?
How many and what products?
How many and what reactions?
How many and what possible reactors?
How many and what possible separations?
How many phases in the reaction and separation steps?
How many and possible interconnections (inlet, outlet, recycle streams)?
Constraints
Logical constraints: Logical sequence of reaction(s)/separation(s)
Structural constraints: Number of processing units
Operational constraints: Ranges of pH Ranges of temperatures

Table 4.3 Identified bottleneck/limitation and example of corresponding objective function

Bottleneck/limitation	*Example of objective function*
Size specifications	Maximization of the space-time-yield (g product/L/h)
Unfavourable reaction equilibrium (Low K_{eq} value)	Maximization of yield (g product end of reaction/g substrate)
Excessive catalyst consumption due to Inhibition (High k_I value)	Maximization of catalyst yield (g product/g catalyst)
Many processing steps	Maximization of product purity and space-time-yield (g product/g other compounds) & (g product/L/h)
Difficult downstream processing	Maximization of overall yield (g product/g substrate) Maximization of product concentration leaving the reactor + purity (g product/L reactor) + (g product/g all compounds)

4.3.2 Stage 2: Data/information collection/analysis

In stage 2, necessary and reported data/information for the subsequent stages in the methodology are collected. In principle, there are some previous data that were obtained from the first stage, in the analysis of bottlenecks/limitations and definition of needs, boundaries and some constraints. At first, there are specific kind of data/information that is known to be collected, e.g. properties of the compounds and mixtures (e.g. solubilities), property models in case a property is not reported, kinetic expressions, types of reactors, types of separation methods (including possible ISPR techniques) models of equipments and operations (e.g. distillation column, crystallization), a second set of constraints (e.g. allowed unit operations, redundant

operations) etc. Even some information collected can join into the second set of constraints. Figure 4.3 shows the different types of data/information that can be collected.

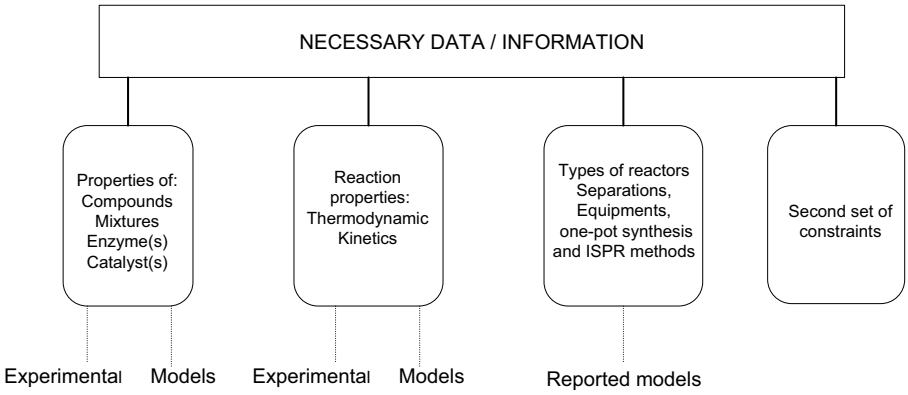

Figure 4.4 Different types of data/information collected in Stage 2.

4.3.3 Stage 3: Superstructure setting & generic model retrieval

4.3.3.1 Step 3a. Superstructure setting

A superstructure is a graphical representation which features a number of different processing units and their interconnections. The superstructure contains all possible options of a potential process, including the optimal solution that is hidden, and it is

located through screening by logical, structural, operational constraints, the process model and evaluation of the objective function. The constituent elements of the superstructure developed here are the processing units, represented by square blocks (▯) – with a certain number of phases $f(\alpha,\beta,...)$, pressure P_u, temperature T_u, pH pH_u, composition(s), $x_i^{u,f}$, numbers of moles of a certain compound $n_i^{u,f}$ and separation factors for each compound $\sigma_i^{u,f}$ -, the streams, that contain different sub-streams for each compound i (⟶) – inlet $F_{i,in}^{u,f}$, outlet $F_{i,out}^{u,f}$, product $F_{i,p}^{u,f}$ recycle and by-pass streams $F_i^{u_o u_e,f}$, where the flow rates are indicated – junction connectors (▷) and split connectors (◁) namely $\zeta_{in}^{u,f}$ and $\zeta_{out}^{u,f}$, respectively (They can take values between 0 and 1). The processing units are limited to perform the operations of reaction and/or separation or integrated reaction/separation. The integration can be realized externally or internally, meaning that two tasks are realized in one unit, such as reactive extraction, or a reactor consisting of two reactions.

In order to build the superstructure required for solving the types of problems postulated in this thesis, specific information is needed, basically: maximum number of processing units (any can have the function of reactor, separator, or integrated reaction/separation equipment) and maximum number of phases per processing unit. This information can be retrieved from Step 1b. and Stage 2.

First, with the maximum possible number of phases, the maximum inlet streams to the processing unit, the maximum outlet streams from the processing unit, the number of junction connectors (connections that bring together several streams into one) and splitter connectors (connections that split one stream into several streams), can be set by using Table 4.4. Then, with the maximum number of processing units, the maximum number of recycling streams and maximum number of by-pass streams are also set, and then the total number of streams can be set (see Table A.3 in Appendix A); and the superstructure can be constructed. The workflow of the superstructure building is detailed in Figure 4.5.

Table 4.4 Maximum inlet streams outlet streams and connectors per processing unit				
Max. Number of phases	1 *(monophasic)*	*2 (biphasic)*	*3(triphasic)*	…
Max. inlet streams	1	2	3	…
Max. outlet streams	1	2	3	…
Total	2	4	6	…
Junction connectors	1	2	3	…
Split connectors	1	2	3	…

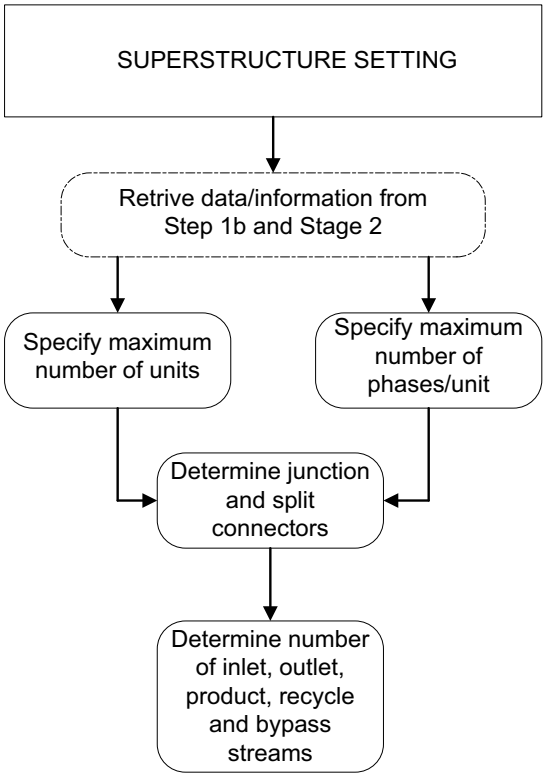

Figure 4.5 Superstructure setting workflow

The following figures are examples of superstructures. Figures 4.6, 4.7 and 4.8 are the superstructures of one, two and three processing units with maximum two phases, respectively.

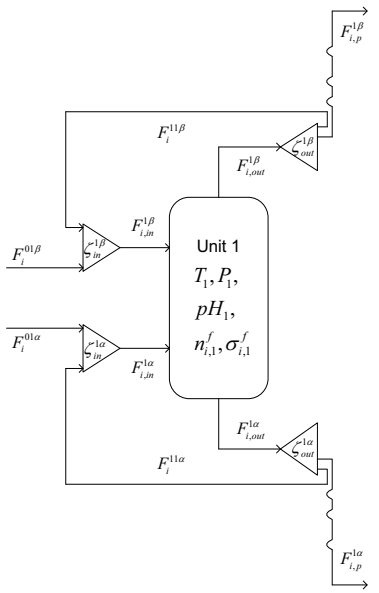

Figure 4.6 Superstructure with maximum one processing unit with maximum two phases

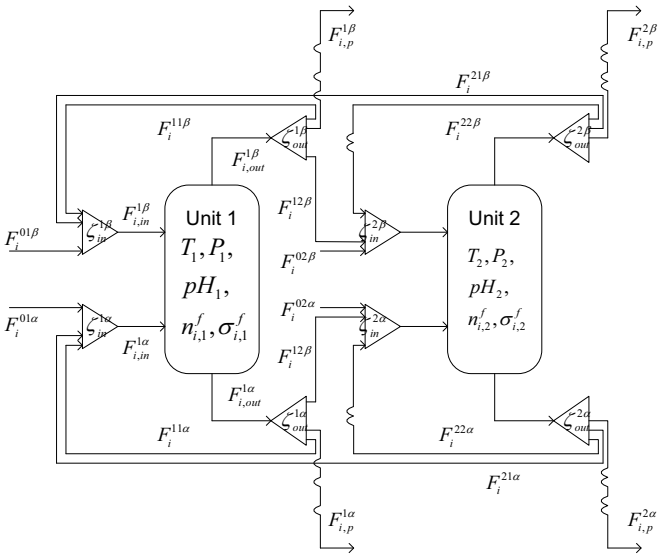

Figure 4.7 Superstructure with maximum two processing units with maximum two phases

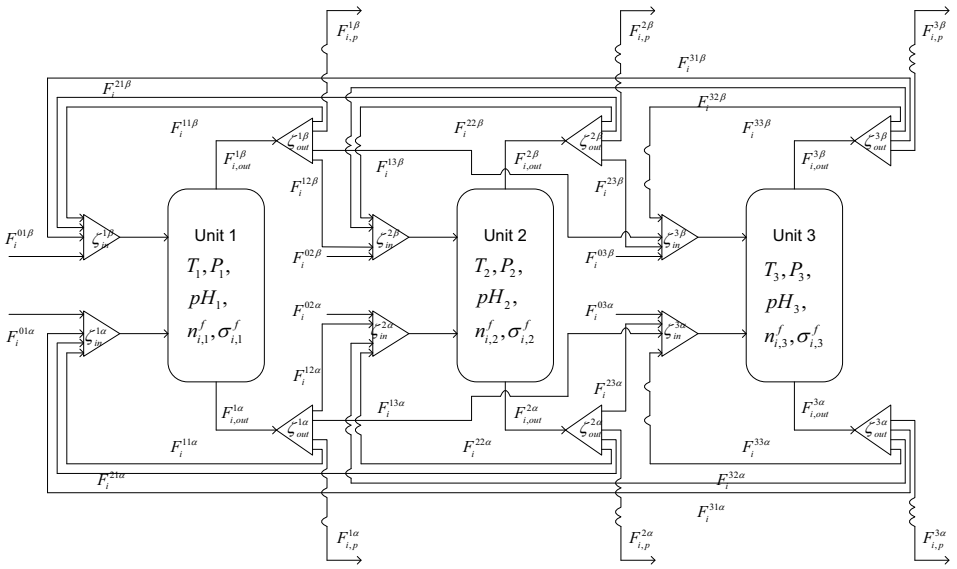

Figure 4.8 Superstructure with maximum three processing units with maximum two phases

4.3.3.2 Step 3b. Generic model retrieval

After the superstructure is set, a generic process model which represents the superstructure, and therefore, all potential process options, can be applied. The generic model represents the physical and chemical changes that happen in the superstructure and consists of mass and energy balance equations, connection equations, as well as constitutive equations.

The generic model consists of the following equations:

Mass balance of compound i around the superstructure:

$$\frac{\partial n_i}{\partial t} = \sum_{u=1}^{u} Y^u \left(Y_{in}^{u,\alpha} x_{i,in}^{u,\alpha} F_{in}^{u,\alpha} + Y_{in}^{u,\beta} x_{i,in}^{u,\beta} F_{in}^{u,\beta} \right) - \sum_{u=1}^{u} Y^u \left(Y_{out}^{u,\alpha} x_{i,out}^{u,\alpha} F_{out}^{u,\alpha} + Y_{out}^{u,\beta} x_{i,out}^{u,\beta} F_{out}^{u,\beta} \right) + \sum_{u=1}^{u} Y^u \left(\lambda_i^{u,\alpha} + \lambda_i^{u,\beta} \right) \tag{4.10}$$

Where, Y^u can take the values of 0 or 1 and represents the existence of a unit u; $Y_{in}^{u,\alpha}, Y_{in}^{u,\beta}, Y_{out}^{u,\alpha}, Y_{out}^{u,\beta}$ represent the existence of inlet and outlet streams in phases α and β, respectively; and $\lambda_i^{u,\alpha}$ and $\lambda_i^{u,\beta}$ are the conversion rates of the compound i in phases α and β, respectively.

Energy balance around the superstructure:

$$\frac{\partial H}{\partial t} = \sum_{u=1}^{u} Y^u \left(Y_{in}^{u,\alpha} \dot{H}_{in}^{u\alpha} + Y_{in}^{u,\beta} \dot{H}_{in}^{u\beta} \right) - \sum_{u=1}^{u} Y^u \left(Y_{in}^{u,\alpha} \dot{H}_{out}^{u\alpha} + Y_{in}^{u,\beta} \dot{H}_{out}^{u\beta} \right) + \sum_{u=1}^{u} Y^u \left(\dot{Q}_{u,HX} \right) \tag{4.11}$$

Where H is the enthalpy and $\dot{Q}_{u,HX}$ is the heat flow exchanged in a unit u.

The constitutive equations:

$$\dot{H}^{u,f} = f\left(\overline{F}, x_i^{u,f}, n_i^{u,f}, T^u, P^u \right) \tag{4.12}$$

$$\lambda_i^{u,f} = f(r_i^{u,f}, n_{catalyst}^{u,f}, x_i^{u,f}, n_i^{u,f}, T^u, P^u) \tag{4.13}$$

Where r_i is the reaction rate of compound i, and $n_{catalyst}$ is the moles of catalyst (in these systems the catalyst can be chemical or an enzyme). The constitutive models can be retrieved from Stage 2 of the methodology (See Figure 4.3).

For each unit u involved, the connection equations for the inlet streams for each phase, the conversion in each unit, the outlet streams to the environment for each phase and the connection streams to each unit u_e have to be solved.

$$Y_{in}^{u\alpha} \cdot F_{in}^{u\alpha} = \sum_{u_e=0}^{u} Y^{u_o u_e \alpha} \cdot F^{u_o u_e \alpha} \tag{4.14}$$

$$Y_{in}^{u\beta} \cdot F_{in}^{u\beta} = \sum_{u_e=0}^{u} Y^{u_o u_e \beta} \cdot F^{u_o u_e \beta} \tag{4.15}$$

$$Y_{out}^{u\alpha} \cdot F_{i,out}^{u\alpha} = f\left(F_{in}^{u\alpha}, F_{in}^{u\beta}, x_i^{u\alpha}, x_i^{u\beta}, \sigma_i^{u\alpha}\right) \tag{4.16}$$

$$Y_{out}^{u\beta} \cdot F_{i,out}^{u\beta} = f\left(F_{in}^{u\alpha}, F_{in}^{u\beta}, x_i^{u\alpha}, x_i^{u\beta}, \sigma_i^{u\beta}\right) \tag{4.17}$$

$$Y_P^{u\alpha} \cdot F_P^{u\alpha} = Y_{out}^{u\alpha} \cdot F_{out}^{u\alpha} \cdot \zeta_{out}^{u\alpha} \tag{4.18}$$

$$Y_P^{u\beta} \cdot F_P^{u\beta} = Y_{out}^{u\beta} \cdot F_{out}^{u\beta} \cdot \zeta_{out}^{u\beta} \tag{4.19}$$

Where Y_{in}, Y_{out}, Y_P $Y^{u_o u_e \alpha}$ and $Y^{u_o u_e \beta}$ are decision binary variables (0 or 1 values) that refer to the existence of a specific stream. The superstructure and the process model are

generic for all enzyme-based processes and hence, only have to be developed once and be retrieved for subsequent applications. From this generic model, different specific process/operation sub-models are derived for the subsequent screening steps. Process options based on unreliable constitutive models are removed.

4.3.4 Stage 4: Generation of feasible candidates

The core of the methodology lies in Stage 4, where all possible options are generated using the combinatorial equation (4.2), and the generated options are screened by logical and structural constraints. The intention of generating all possible options is to not have any doubt that all the potential options are considered and do not let any outside of the optimization problem.

4.3.4.1 Step 4a. Generation of all process options

All possible options are generated using the combinatorial mathematical expression (equation 4.2) explained in the problem formulation:

$$NPO = \sum_{u=1}^{u} \left(\prod_{u=1}^{u} NIU_u \right) \left(\frac{(r \cdot u)!}{u!(r \cdot u - u)!} \right) \tag{4.2}$$

Where, as stated previously, NIU_u is the number of identified operations/equipment per processing unit u (retrieved from Stage 2), u is the number of processing units and r is the number of streams per processing unit u (retrieved from the superstructure, Stage 3, see Table 4.5). For example, if the set superstructure consists of two processing units of

maximum two phases, and in the first unit, two operations/equipments are identified and in the second unit, three operations/equipments are identified, therefore $u = 2$, $r = 12$ (see Table A.3), $NIU_1 = 2$, $NIU_2 = 3$, and the total number of options generated is:

$$NPO = 2\left(\frac{(12\cdot 1)!}{1!(12\cdot 1-1)!}\right) + 2\cdot 3\left(\frac{(12\cdot 2)!}{2!(12\cdot 2-2)!}\right) \tag{4.20}$$

$NPO = 1680$ possible options generated from the superstructure.

The optimal option is among all the generated options.

4.3.4.2 Step 4b. Screening by logical constraints

Logical constraints, indicated by equations (4.3) and (4.4) in the problem formulation (Section 4.2) are functions of decision variables $\bar{Y}$ and represent the logical sequence of the processing units and the allowed unit operations in each step, matches not allowed are also taken. The logical constraints are formulated by retrieving information collected in Stage 2 of the methodology.

$$g_{i,Logical,LB} \le g_{i,Logical}\left(\bar{Y}\right) \le g_{i,Logical,UB} \tag{4.3}$$

$$g_{i,Logical} = g_{i,Logical}(\bar{Y}) \tag{4.4}$$

For example, to avoid the overlapping in operations/equipment for, e.g. a superstructure made of four processing units, the following logical constraints are set:

$$Y^u = 0,1 \tag{4.21}$$

$$1 \le \sum_{u=1}^{u} Y^u \le 4 \tag{4.22}$$

4.3.4.3 Step 4c. Screening by structural constraints

Structural constraints, indicated by equations (4.5) and (4.6) in the problem formulation (Section 4.2) are functions of decision variables $\overline{Y}$ and represent the allowed inlet, outlet and recycle streams in the flowsheets. They are also retrieved from the information collected in Stage 2 of the methodology.

$$g_{i,Structural,LB} \le g_{i,Structural}\left(\overline{Y}\right) \le g_{i,Structural,UB} \tag{4.5}$$

$$g_{i,Structural} = g_{i,Structural}\left(\overline{Y}\right) \tag{4.6}$$

For example, in a superstructure with maximum two phases per processing unit,

$$Y_{in}^{u\alpha}, Y_{in}^{u\beta}, Y_{out}^{u\alpha}, Y_{out}^{u\beta}, Y_{P}^{u\alpha}, Y_{P}^{u\beta}, Y^{u_o u_e} = 1,0 \tag{4.23}$$

The binary variables representing the existence of streams can take the values of 0 or 1 depending on the specific option. Also, for example, for a superstructure of four processing units, a structural constraint is set to allow certain streams for processing unit, according to table 4.4, maximum streams for processing units are 16,

$$0 \leq \bar{Y}_{(streams)} \leq 16 \tag{4.24}$$

4.3.5 Stage 5: Screening for operational and process constraints, benchmarking criteria.

In Stage 4, the two first levels of screening are done, in order to reduce the search space of process options, leading to a reduced set of options that can be screened through evaluation through their specific process sub-models and operational constraints. The specific process sub-models for each option are derived from the generic model explained in section 4.3.3.2 and the operational constraints are retrieved from Stage 2 of the methodology.

4.3.5.1 Step 5a: Screening for operational and process constraints.

The derivation of the specific process sub-models from the generic model is based on a generic systematic modeling procedure, (Sales-Cruz, 2006) but adapted to a specific advantage of the framework. This specific advantage consists in saving model development efforts since the specific process sub-models are derived from the generic model (presented in section 4.3.3.2). The constitutive equations (e.g. kinetic models), corresponding to each option are retrieved from stage 2 of the methodology. The systematic process sub-model derivation procedure is outlined in Figure 4.9.

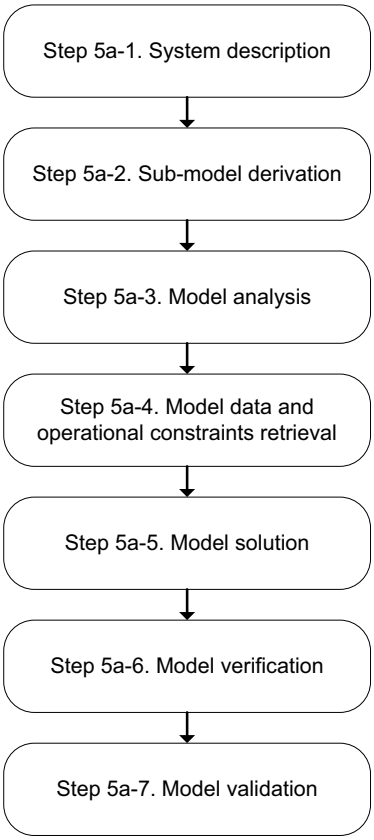

Figure 4.9 Systematic process sub-model building steps.

Step 5a-1. System description.

This step consists in describing the process option (its main characteristics such as the variables, constants, inputs and outputs and the time characteristics -static versus dynamic-), identifying the controlling factors or mechanisms (the physico-chemical phenomena that take place in the system) and making assumptions to possible the mathematical representation and reduce the complexity of the problem (by reducing the number of factors under consideration, by neglecting some of the independent variables,

for instance those variables whose effect may be relatively small compared to other factors involved in the behavior, and by assuming relatively simple relationships).

The controlling factors or mechanisms that are common in the possible options generated inside the superstructure can be:

- Chemical and enzymatic reaction
- Diffusion of mass
- Forced and free convection heat transfer
- Evaporation and other phase-change mechanisms (e.g. crystallization)
- Turbulent mixing
- Fluid flow

The assumptions common in the generated options can be:

- Perfect mixing in each phase
- Constant physic-chemical properties
- Equal inflow and outflow (implying constant liquid volume)
- Constant pressure and temperature in each unit
- Adiabatic operation

Step 5a-2. Sub-model derivation.

The specific process sub-models are derived from the generic model from Step 3b, and the collected constitutive models retrieved from Stage 2.

Step 5a-2.1. Identify existent process units, equipments and streams. Specify values of Y´s.

Step 5a-1.2. Obtain the specific process model from the generic model represented by Equations 4.10 to 4.19. Retrieve constitutive models from Stage 2 of the methodology.

Step 5a-3. Model analysis.

In this step, the following procedure is done:

Step 5a-3.1. Classify variables according to Table 4.7.

Step 5a-3.2. Specify total number of equations and total number of variables

Step 5a-3.3. Determine the degrees of freedom (DOF)

$$N_{DOF} = N_u - N_e \tag{4.25}$$

Where, N_{DOF} is the number of degrees of freedom, N_u is the number of unknown variables and N_e is the number of independent equations in the model. There are three possible values for N_{DOF} to take:

(a) $N_{DOF} = 0$. This implies that the number of independent variables (unknowns) and independent equations is the same. Therefore a unique solution exists.

(b) $N_{DOF} > 0$. This implies that the number of independent variables is greater than the number of independent equations. Therefore the problem is underspecified and a solution is possible only if some of the independent variables are fixed by some external considerations in order to reduce N_{DOF} to zero. In the case of optimization (Stage 6 of the Methodology) these N_{DOF} variables will be adjusted to give a "best" solution to the problem.

(c) $N_{DOF} < 0$. This implies that the number of independent variables is less than the number of independent equations. Therefore the problem is overspecified. The solution to this problem is one which best "fits" all the equations.

Step 5a-4. Model data and operational constraints retrieval

In this step, model data are retrieved from Stage 2 of the Methodology. Those data retrieved are operational constraints, parameters values, known variables, design variables, constants, initial conditions for dynamic models, etc.

Step 5a-5. Model solution

In this step, the evaluation for operational constraints and process sub-models is done by simulation, leading DAE systems of equations representing each remaining option. The process sub-models are evaluated and solved with the computational tool ICAS MoT® (Sales-Cruz, 2006).

Step 5a-6. Model verification.

In this step the behavior of the model is checked in order to see if the model is behaving correctly.

Step 5a-7. Model validation. The behavior of the model against the reality must be checked. There are several questions to answer before designing validation tests (Sales-Cruz, 2006):

- Does the model answer the problem identified in step 5a-1?
- Can one really gather the data necessary to operate the model?
- Does the predicted curve fit experimental data?
- Does the model make common sense?

- Are the results obtained from the model logic?

Once the common sense test is passed, the model should be tested many times using experimental observations. The same modelling procedure is done for all the remaining options.

Table 4.5 Variable Classification (Sales-Cruz, 2006)

Variable type	*Definition*
Parameter	Variables with known values
Explicit	Variables that are function only of parameters and/or dependent-prime variables
Implicit-Unknown	Variables related to AEs (algebraic equations) where there is more than one unknown variable per equation
Dependent	Variables appearing with the differential operators on the LHSs (Left hand side of equations) of ODEs (Ordinary differential equations) and /or PDEs (partial differential equations)
Dependent prime	The derivative operator related to the dependent variable

4.3.5.2 Step 5b: Benchmarking using defined metrics

Once the options have been subject to process simulation, they are benchmarked using criteria derived from the definition of the metrics in Stage 1, Step 1b. For example, in a pharmaceutical process to be economically viable, certain process metrics must be achieved. In the case of a biocatalytic process, two are particularly important. The usual requirement is to achieve product concentrations of at least 50-100 g/l. The other metric is dependent on the cost of the enzyme and is best expressed as the gram

product/gram or activity unit of biocatalyst (defined here as enzyme yield). For commercial processes, this metric needs to be at least 1000 for an enzyme and 15 for a whole-cell system (Pollard and Woodley, 2006).

4.3.6 Stage 6: Objective function evaluation

4.3.6.1 Step 6a: Calculation of the objective function

The best option(s), obtained from the benchmarking, is (are) subjected to the evaluation of the objective function, defined in Stage 1, Step 1c, Equation 4.1 in the problem formulation in section 4.2. That can be done by relaxing the operational constraints related and adjusting the design variables (a sensitivity analysis can be done to identify the most influencing to the process performance) in order to optimize the process.

$$\max/\min \; F_{\mathrm{OBJ}} = \sum_{k=1}^{k} f_k\left(\bar{X},\bar{Y},\bar{d},\bar{\theta}\right) w_k \tag{4.1}$$

This evaluation can be done by using the software ICAS-MoT®.

4.3.6.2 Step 6b: Validation of the best option

Once Step 6a is done, the best option can be identified, and validated through rigorous simulation using process simulators like PRO-II, or otherwise experimentally. In case the validation is unsatisfactory, then, the procedure in the methodology is done again until a satisfactory process option is identified.

CHAPTER FIVE

Application of the framework for intensification of enzyme-based processes

5.1 Introduction

In this chapter, the application of the framework for intensification of enzyme-based processes proposed in this thesis is highlighted through two case studies. One case study is related to the pharma-sector: the synthesis of *N*-acetyl-D-neuraminic Acid (Neu5Ac), an important pharmaceutical intermediate. The second case study deals with the enzymatic production of biodiesel.

5.2 N-Acetyl-D-Neuraminic Acid (Neu5Ac) synthesis

5.2.1 Introduction

Neu5Ac, (molecular formula $C_{11}H_{19}NO_9$) is the most prevalent type of sialic acid in nature and has numerous important physiological functions (Figure 5.1 presents the current and potential applications). Neu5Ac is a high-priced raw material for many pharmaceuticals. It is a precursor for producing several anti-viral, anti-cancer and anti-inflammatory drugs, especially zanamivir, the active ingredient in Relenza, marketed by

GlaxoSmithKline. During the last years, Relenza´s sales have been substantial (Figure 5.2), since it is being used in the treatment and prophylaxis of influenza caused by influenza A virus and influenza B virus (such as the avian influenza virus H5N1 and the 2009 H1N1, the virus of swine flu) (Zhang et al., 2010).

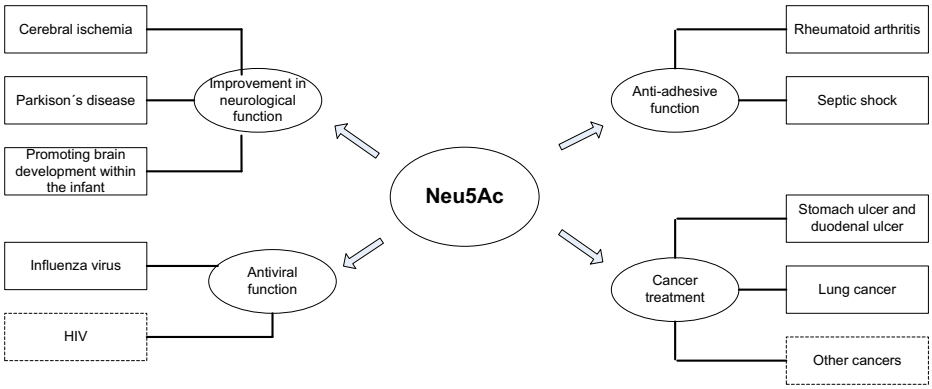

Figure 5.1 Applications of Neu5Ac. The solid line indicates the current uses of this compound, and the dashed line represents potential uses (Tao et al., 2010).

The current methods for producing Neu5Ac range from the natural product extraction, chemical synthesis, biotransformation (whole-cell) and biocatalytic (enzymatic) processes. The traditional methods for Neu5Ac production are the extraction from natural sources, such as edible bird´s nest (Martin et al., 1977) and chalaza and egg yolk membrane (Juneja et al, 1991); and the hydrolysis of colominic acid using microbial neuramidase (Uchida et al., 1973). Often, the processes from natural product extraction are inefficient, since the Neu5Ac contents are too low to be isolated with sufficient recovery and purity (Maru et al., 2002). Whole-cell biocatalytic processes have significant drawbacks such as the mass transfer resistance, lower reaction rates than enzymatic processes, presence of byproducts and cellular components and the

occurrence of side reactions (Tao et al., 2010). The chemical synthesis of Neu5Ac from a non-carbohydrate source (Banwell et al., 1998) requires a fifteen step reaction sequence, making them unsuitable for large scale production. Other chemical methods (Cornforth et al, 1958; Danishefsky et al., 1988; DeNinno, 1991) also require laborious steps, leading to the formation of many intermediates, making a very complex and difficult separation process (Tao et al., 2010). The chemo-enzymatic (Mahmoudian et al, 1997; Dawson et al., 2000) and the enzymatic production of Neu5Ac (Kragl et al., 1991) can be achieved in large scale due to the renewable sources of carbohydrates and the high stereoselectivity of the enzymes involved (Hsu et al., 2005; Hu et al., 2010). Due to some of the discussed reasons in Chapter 1 of this thesis, e.g. environmentally friendly operations under mild conditions, enzyme-based methods have high potential for Neu5Ac production (Schmid et al., 2001; Schoemaker et al., 2003). Two enzymes have been utilized for Neu5Ac production, Neu5Ac synthase (EC 4.1.3.19) and Neu5Ac aldolase (NAL, previously named Neu5Ac lyase, EC 4.1.3.3). NAL is preferred to Neu5Ac synthase because its substrate, pyruvate, is cheaper and more available in bigger amounts than phosphoenolpyruvate, the substrate for Neu5Ac synthase (Rodriguez-Aparicio et al., 1995).

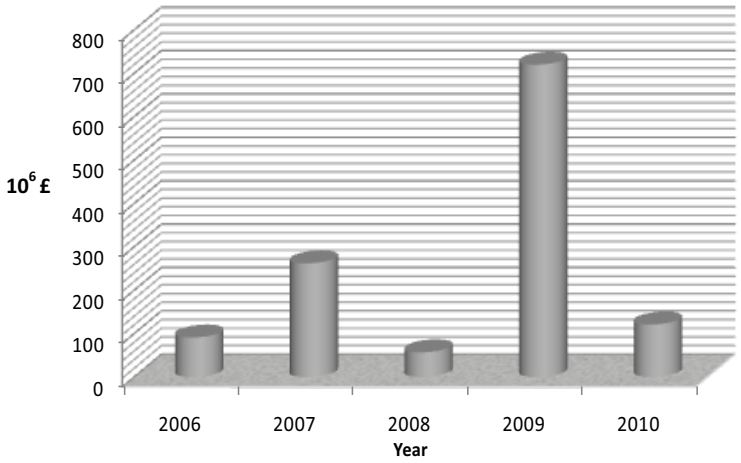

Figure 5.2 Sales of Relenza (GlaxoSmithKline, 2006-2010)

Usually, on industrial scale, Neu5Ac is produced enzymatically in two reaction steps from *N*-acetyl-D-glucosamine (GlcNAc, molecular formula $C_8H_{15}NO_6$), which is the monomer of chitin $(C_8H_{13}O_5N)_n$, found in many places throughout the natural world (e.g. shrimp shells). The first reaction step is the epimerization of GlcNAc to *N*-acetyl-D-manosamine (ManNAc). This step can be achieved either by chemical alkaline epimerization or enzymatic epimerization with N-acylglucosamine-2-epimerase (AGE, EC 5.1.3.8). The second reaction step is an aldol condensation of ManNAc with pyruvate (Pyr, molecular formula $C_3H_4O_3$) catalyzed by NAL. The reactions taking place during the synthesis can be distinguished in Figure 5.3. In this Figure, compound B, ManNAc, is not recommended as starting point because B is very expensive as a raw material, therefore, compound A, GlcNAc, obtained by acid hydrolysis of shrimp shells, is preferred as the starting substrate.

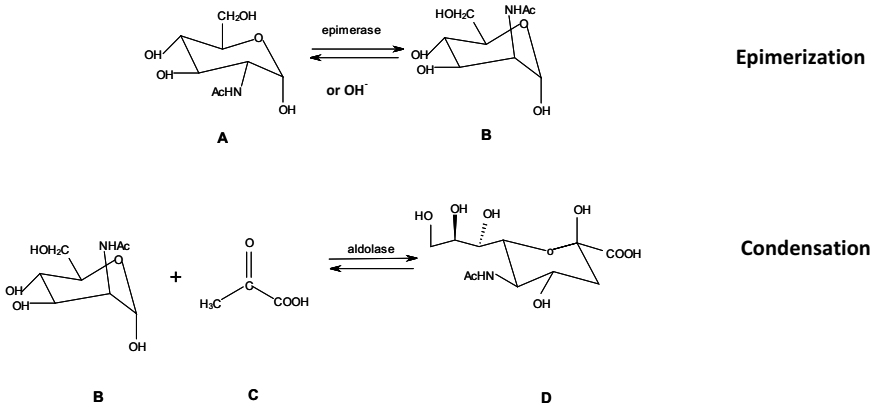

Figure 5.3 Synthesis of Neu5Ac acid from GlcNAc in two reaction steps (In the reactions, **A**: *N*-acetyl-D-glucosamine (GlcNAc); **B**: *N*-acetyl-D-manosamine (ManNAc); **C**: pyruvate (Pyr); **D**: *N*-acetyl-D-neuraminic acid (NeuAc); Epimerase: N-acylglucosamine-2-epimerase (AGE, E.C. 5.1.3.8); Aldolase: neuraminic acid aldolase (NAL, E.C. 4.1.3.3))

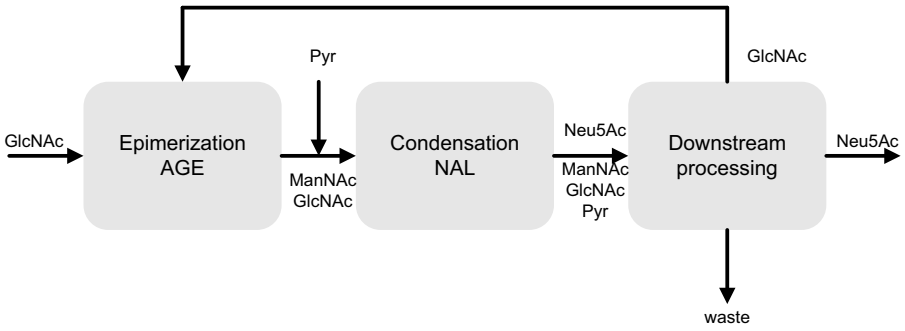

Figure 5.4 Conventional sequence of steps for Neu5Ac production

A conventional sequence of steps for Neu5Ac production is shown in Figure 5.4, where the reactions steps are made first, followed by the downstream processing steps (separation and purification steps). In this simplified scheme, Neu5Ac is produced in a batch process with considerable pyruvate excess and precipitation of the product with glacial acetic acid (Zimmermann et al., 2007). This process is characterized by significant waste, and difficult downstream processing, drawbacks which may potentially be overcome with intensification techniques explained in section 2.3 of this thesis. This explains why this system is an interesting case study in this thesis. Here the main goal is to propose an intensified enzyme-based option for production of Neu5Ac. To reach this objective, the systematic framework of design and development of intensified enzyme-based processes proposed in this thesis, and described in chapter four, is applied.

5.2.2 Stage 1. Problem Definition

For this system, the problem formulation statement (adapted from Section 3.4 of this thesis) is: given GlcNAc (A) and Pyr (C) as substrates to make the desired product, Neu5Ac (D), through enzyme-based intensification methods, find the best way to make it, that is, find the optimal route, according to a criteria given by the objective function, from A and C to D, specifying the separations and reactions in the process. All the possible combinations are considered and analyzed, including the integration of reaction/reaction, (e.g. one-pot synthesis), reaction/separation (e.g. in situ product removal) and separation/separation (hybrid separations). In order to avoid any doubt, all the possibilities are considered to ensure that the best option will be selected. First of all, a base-case design is needed which is used to evaluate the performance of intensified process solutions against it. A simplified process scheme of the base-case design (Mahmoudian et al, 1997) is outlined in Figure 5.5.

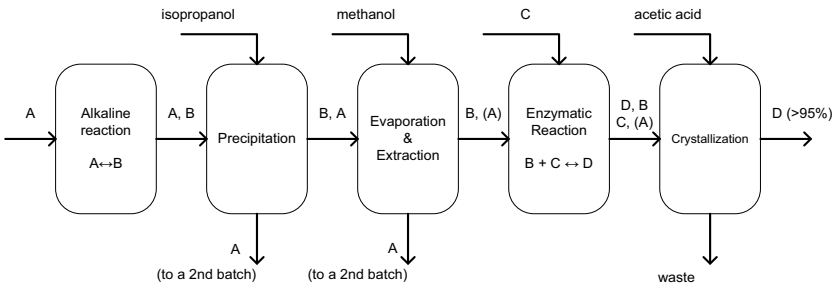

Figure 5.5 Simplified process scheme for a conventional two-step chemo-enzymatic synthesis in batch mode (Mahmoudian et al., 1997).

In Figure 5.5, the first reaction is homogeneously alkaline catalyzed with sodium hydroxide reaching a molar ratio of 20% of compound B. The enrichment of B is proposed in order to enhance the reaction rate in the subsequent enzymatic reaction.

Hence, the enrichment of B to a molar ratio 1:4 (compound A: compound B) is realized through precipitation of A by adding isopropanol followed by evaporation and extraction of the residues by methanol. The second reaction achieving the final product D is using an immobilized enzyme (NAL) and a low excess of C (molar ratio 1:1.3 for B: C) to improve the reaction rate. Purification of D to a purity exceeding 95% (molar basis) is done by crystallization with glacial acetic acid. The whole process is run in batch mode.

5.2.2.1 Step 1a. Analysis and identification of bottlenecks /limitations

An important identified bottleneck/limitation from simulation of the base-case design is the low volumetric productivity (Equation 5.1), defined as the grams of product produced per day per volume (around 0.25 g l^{-1} day^{-1}). Other process bottlenecks/limitations are identified as low overall product yield (0.17) with respect to GlcNAc (Equation 5.2), defined as final grams of product per initial grams of substrate. The product yield with respect to ManNAc is calculated with Equation 5.3 and is 0.88.

$$productivity_{Vol} = \frac{m_D}{V_0} \cdot \frac{1}{t} \tag{5.1}$$

$$yield_{Neu5Ac,GlcNAc} = \frac{m_D}{m_{A_0}} \tag{5.2}$$

$$yield_{Neu5Ac,ManNAc} = \frac{m_D}{m_{B_0}} \tag{5.3}$$

The process bottlenecks/limitations identified in the literature are:

- *Diluted concentrations.* Since the equilibrium conversion is proportional to the initial reactant concentrations, for both reactions there should be a compromise in operating with the highest possible substrate molarities but with the minimum product inhibition possible (Blayer et al, 1999).

- *Substrate and product inhibition.* The aldolase enzyme activity is inhibited by both Neu5Ac and GlcNAc while epimerase enzyme activity is inhibited by both pyruvate and Neu5Ac (Kim et al., 1988; Kragl et al., 1992).

- *Unfavorable equilibrium.* Both the equilibrium for the epimerization ($K_{eq,epi} = 0.24$) and to a lesser extent the aldolase condensation ($K_{eq,epi} = 28.71$ L/mol) are unfavorable for Neu5Ac synthesis. That means low conversion of the substrates into products. (Ghosh and Roseman, 1965; Kragl et al., 1992).

- *Difficult downstream separation.* The main problem in the separation process is the separation of Neu5Ac from Pyr since they are both negatively charged and have similar pKa. The pKa values for Neu5Ac (2.5) (Kragl, 1992) and pyruvate (2.6) (Dawson et al., 1993) are similar, which may lead to a potential difficulty in downstream separation unless pyruvate concentration is kept low (Blayer et al., 1999). In some current processes a large amount of pyruvate is used (up to ten-fold to shift the equilibrium towards the product formation, which increases the complexity of downstream processing) (Dawson et al, 2000).

By doing a sensitivity analysis of the bottlenecks/limitations with respect to the final amount (moles) of product obtained n_{NeuAc}, the most sensitive parameters are, the equilibrium constant (K_{eq}) for the first reaction and the inhibition constant of Neu5Ac (K_I) of the second reaction which affects the resulting amount of moles of Neu5Ac.

5.2.2.2 Step 1b. Needs, boundaries, metrics and first set of constraints selection

The process needs are selected as producing D with purity over 95% (requirements according to Kragl et al, 1991; Mahmoudian et al, 1997), with less processing steps than the base-case design (Figure 5.5), meaning that only options with maximum four processing steps will be generated, this with the purpose of simplification, one of the main goals of PI (Section 2.2.3 of this thesis). Boundaries are selected as maximum four processing steps of maximum two phases per processing unit, either reaction or separation steps.

Some metrics have been selected, namely waste generation, energy consumption and simplification of the process. These will be translated into heuristic rules integrated in logical constraints such as connection rules for synthesis, e.g. "Only generate options with less than five processing steps" due to simplification of the process; and in structural constraints, e.g. "Not use two different solvents for base catalyzed epimerization" due to waste generation. As an additional metric for benchmarking in Stage 5 of the Methodology, the productivity (defined in Equation 5.1) is selected, since it will influence the cost-effectiveness of the process (see Table 3.1, economic metrics). Processes with overall productivities over 150 g/day are selected. By analyzing the base-case design, a first set of constraints is identified. Some of these identified constraints are some types and sequences of the operations (logical constraints), some of allowed inlet, outlet and recycle streams (structural constraints), and some process specifications (operational constraints). This first set of identified constraints is presented in Appendix B.1 of this Thesis.

5.2.2.3 Step 1c. Objective function definition

Since the most sensitive bottlenecks/limitations, identified in Step 1a, are the unfavorable equilibrium of the first reaction and the product inhibition of the second reaction. The criteria for optimal selection (see Table 4.3) are the maximization of the product yield and the maximization of the Neu5Ac aldolase enzyme yield, therefore, the objective function is given by the summation of those two criteria, with their respective weights:

$$\max F_{OBJ} = \sum_{k=1}^{k=2} f_1 w_1 + f_2 w_2 \tag{5.4}$$

where

$$f_1 = yield_{Neu5Ac,GlcNAc} = \frac{m_D}{m_{A_0}} \tag{5.5}$$

$$f_2 = yield_{Neu5Ac,NAL} = \frac{m_D}{m_{NAL}} \tag{5.6}$$

The weights w_1 and w_2 for each function f_1 and f_2, respectively, are determined by the sensitivity analysis and the values are 0.73 for w_1 and 0.27 for w_2.

5.2.3 Stage 2. Data/Information Collection/Analysis

Process data (components properties, reactions) have been collected (Auge et al, 1984; Juneja et al, 1991; Kragl, 1991; Ohta, 1995; Mahmoudian et al, 1997; Maru et al, 1998; Blayer et al, 1999; Dawson, 2000; Tabata et al, 2002; Lee et al, 2004; Xu et al, 2007; Lee et al, 2007; Zimmermann et al, 2007 & 2008;Wang et al, 2009; Tao et al, 2010). The data is listed in Appendix B.2, where compounds and mixtures solubilities are needed to know the limits of the initial substrate concentrations. Other properties like enzyme activities and reaction thermodynamics and kinetics reported in the literature are listed. Information about different types of reactors, separations, equipments, the options of enzymatic and chemo-enzymatic one-pot synthesis and ISPR options, is also collected. With this information, a second set of constraints is defined, e.g, different decision variables representing the reaction and separation options, logical sequences of the operations, reflected in logical constraints. Also, with this information, the allowed inlet, outlet and recycle streams of the reaction and separation options are identified and reflected in structural constraints. The operational constraints, such as allowed temperatures, pH´s, feed concentrations, etc., are also collected in this stage and reflected in operational constraints.

5.2.4 Stage 3. Superstructure setting and generic model retrieval

5.2.4.1 Step 3a. Superstructure setting

The maximum number of processing units and the maximum number of phases per processing units are information that is retrieved from Step 1b to build the superstructure (4 processing steps and two phases). Therefore, by looking at table 4.4,

the maximum number of inlet, outlet streams, the maximum number of junction and split connectors per processing unit can be set. Subsequently, by looking at Table A.3, the remaining number of streams (recycle streams, inlet streams before and after connections) can be known. With this, the superstructure is created and is shown in Figure 5.6. It consists of four processing units, two phases per processing unit, named α and β, and 64 streams in the whole superstructure (16 streams per processing unit). After collecting the data in Stage 2 concerning the reactions and the methods of separation, a diagram (Figure 5.7), which includes the different collected methods of reaction, separation, one-pot synthesis and ISPR, was created. With this, now the information for generation of options (Equation 4.2) is complete. Each process configuration derived from Figure 5.7 and the superstructure will match the generic model and the decomposition strategy explained in section 4.3.

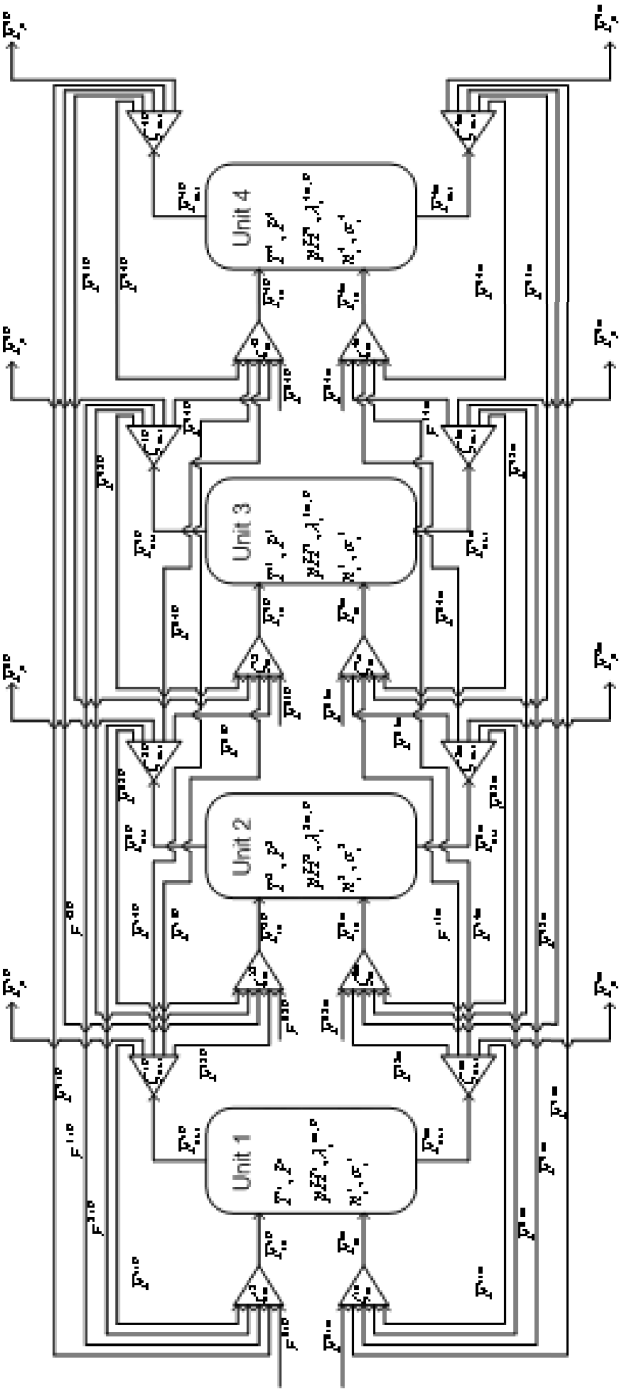

Figure 5.6 Superstructure for reaction-separation configuration of enzymatic processes *(symbols: streams F [mol], temperature T [K], pressure P [bar], total number o moles n [mol], separation factors σ [-], binary existence variable ζ [0,1]; Subscripts: Bottom flow α, top flow β, product p)*

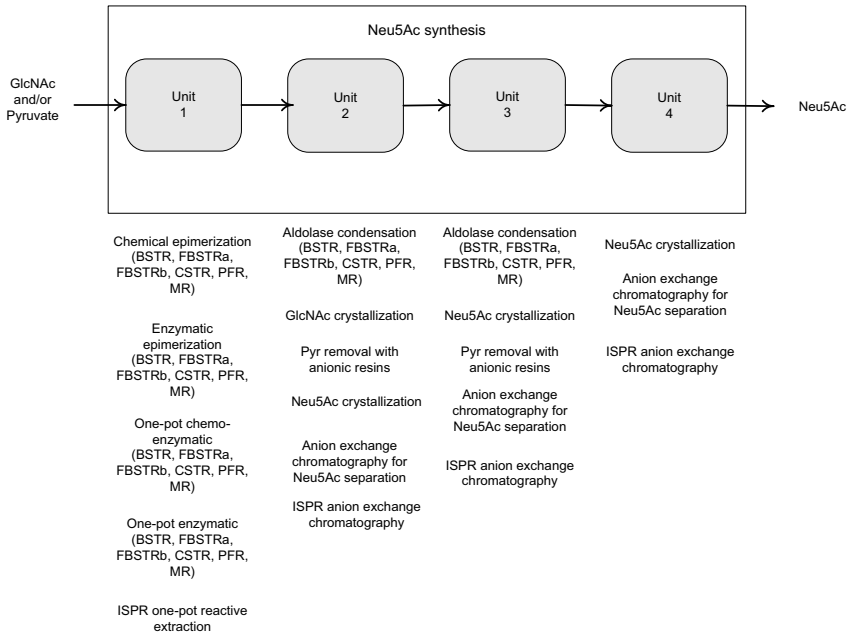

Figure 5.7 Reaction/Separation options for the chemo-enzymatic and enzymatic synthesis of Neu5Ac (BSTR: Batch stirred tank reactor; FBSTRa: Fed-batch stirred tank reactor with intermittent feeding, FBSTRb: Fed-batch stirred tank reactor with continuous feeding; CSTR: Continuous stirred tank reactor; PFR: Plug flow reactor; MR: Membrane reactor).

5.2.4.2 Step 3b. Generic model retrieval

The generic process model derived in Section 4.3.3.2 of this thesis is retrieved. For the superstructure developed in this case study, the mass balance (Equation 4.10), is for four compounds ($i = 1,2,3,4$), $i = 1$ is for GlcNAc, $i = 2$ for ManNAc, $i = 3$ for Pyr and $i = 4$ for Neu5Ac and the $u = 4$, the maximum number of processing units. The different values for each variable in the model, including the decision variables $\overline{Y}$,

flowrates $\bar{F}$, the constitutive equations $\bar{\lambda}$, the separation factors $\bar{\sigma}$, etc, retrieved from Stage 2 of the methodology, are listed in Appendix B.4.

$$\frac{\partial n_i}{\partial t} = \sum_{u=1}^{4} Y^u \left(Y_{in}^{u,\alpha} x_{i,in}^{u,\alpha} F_{in}^{u,\alpha} + Y_{in}^{u,\beta} x_{i,in}^{u,\beta} F_{in}^{u,\beta} \right) - \sum_{u=1}^{4} Y^u \left(Y_{out}^{u,\alpha} x_{i,out}^{u,\alpha} F_{out}^{u,\alpha} + Y_{out}^{u,\beta} x_{i,out}^{u,\beta} F_{out}^{u,\beta} \right) + \sum_{u=1}^{4} Y^u \left(\lambda_i^{u,\alpha} + \lambda_i^{u,\beta} \right) \tag{5.7}$$

5.2.5 Stage 4. Generation of feasible candidates

5.2.5.1 Step 4a. Generation of all process options

The maximum number of options was generated from the superstructure (Figure 5.6) and from the Figure 5.7 that represents the different possible operations for each processing unit (For unit 1: 25 options; for unit 2: 11 options; for unit 3: 10 options and for unit 4: 3 options). The number of total combinations of flowsheets with u=4 processing units interconnected by r=16 streams is given by the combinatorial expression represented by Equation (4.2).

$$NPO = \sum_{u=1}^{u} \left(\prod_{u=1}^{u} NIU_u \right) \left(\frac{(r \cdot u)!}{u!(r \cdot u - u)!} \right) \tag{4.2}$$

$$NPO = 25\left(\frac{(16 \cdot 1)!}{1!(16 \cdot 1 - 1)!} \right) + 11 \cdot 25\left(\frac{(16 \cdot 2)!}{2!(16 \cdot 2 - 2)!} \right) + 10 \cdot 11 \cdot 25\left(\frac{(16 \cdot 3)!}{3!(16 \cdot 3 - 3)!} \right) + 3 \cdot 10 \cdot 11 \cdot 25\left(\frac{(16 \cdot 4)!}{4!(16 \cdot 4 - 4)!} \right) \tag{5.8}$$

Therefore, the total number of options is ***5,289,552,800***

5.2.5.2 Step 4b. Screening by logical constraints

According to the superstructure in Figure 5.6 and the Figure 5.7, integer variables and equations for the selection of the logical sequence of processing step and option j in each processing unit are introduced. The screening by logical constraints for this is summarized in Table 5.1.

Table 5.1 Screening by logical constraints

Constraint statement	*Reaction-separation sequence*	*Screened options*	*Remaining options*
A.Minimun 2 & maximum 4 processing units	1.All possible sequences	400	5,289,552,400
B.Constraints related to the logical sequence of reaction and separation of options four, three and two processing units	2.R-R-S-S	548,964,864	4,740,587,536
	3.R-S-R-S	928,735,360	3,811,852,176
	4.R-S-S-S	1,777,700,224	2,034,151,952
	5.R-R-S	37,486,853	1,996,665,099
	6.R-S	128,951	1,996,536,058
C.Anion exchange chromatography cannot exist after enzymatic AGE reactions and enzymatic one.pot synthesis since the cofactors Mg^{++} and ATP interfere with the performance of the resins	7.R-S-R-S	789,909,826	1,206,626,232
	8.R-S-S-S	362,164,320	844,461,912
	9.R-S	16368	844,445,544
D.Logical feeding and/or removal to maintain high yields and low subtrate concentrations for separations: BSTR discarded	10.For 4 units	5,718,385	838,727,159
	11.For 3 units	51,888	838,675,271
	12.For 2 units	1,488	838,673,783
E. MR not considered since the enzymes are strongly inhibited by products	13.For 4 units	7,624,512	831,049,271
	14.For 3 units	69,184	830,980,087
	15.For 2 units	1,984	830,978,680
F. CSTR is not considered since enzymes are inhibited by the highest concentration of products	16.For 4 units	7,624,512	823,353,591
	17.For 3 units	69,184	823,284,407
	18.For 2 units	1,984	823,282,423
G. PFR is only effective in NAL reaction separation with excess of ManNAc	19.R-S-R-S	205,861,824	617,420,599
	20.R-S-S-S	4,384,512	613,036,087
H.Chemo-enzymatic one-pot synthesis discarded since itcauses compromise in conditions of pH, yield, becuase enzyme inhibition and substrate and product degradation	21.R-S-S-S	228,735,360	384,300,727
	22.R-S-S	2,075,520	382,225,207
	23.R-S	14,880	382,210,327
I. Other redundancies and matches not allowed (e.g. discard chemical reaction to avoid neutralization steps, etc.)	24.For 4, 3 and 2 units	374,585,815	7,834,048

5.2.5.3 Step 4c. Screening by structural constraints

The screening by structural constraints consists of the allowed streams for each of the remaining options after logical constraints. Table 5.2 summarizes the screening by structural constraints.

Table 5.2 Screening by structural constraints

Constraint statement	*Screened options*	*Remaining options*
J. Maximun allowed streams for options of two processing units	25. 1,359,200	6,474,848
K. Maximun allowed streams for options of three processing units	26. 4,756,400	1,718,448
L. Maximun allowed streams for options of four processing units	27. 1,718,434	14

After the screening by all logical and structural constraints, the total number of remaining options is 14. The remaining options are indicated in Table 5.3.

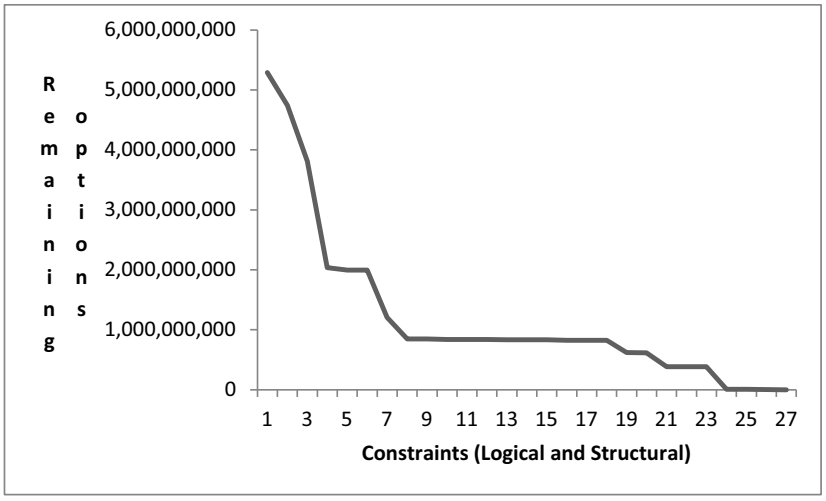

Figure 5.8 Screening of the options by logical and structural constraints

Table 5.3
Options after screening by structural constraints.
OPR: One-pot reactions, OPRS: One-pot reactive extraction, R_{AGE}: Epimerization Reaction, R_{NAL}: Aldolase Reaction, CRYST: Crystallization, CHRO: Chromatography, EVAP: Evaporation.

Remaining Option No.	*Process*
1	$OPRS\text{-}CRYST_{Neu5Ac}$
2	$OPRS\text{-}CHRO_{ISPR}$
3	$OPR_{BSTR}\text{-}CRYST_{Neu5Ac}$
4	$OPR_{FBSTR}\text{-}CHRO_{Neu5Ac}$
5	$OPR_{FBSTR}\text{-}CRYST_{GlcNAc}\text{-}CRYST_{Neu5Ac}$
6	$OPR_{FBSTR}\text{-}CHRO_{PYR}\text{-}CRYST_{Neu5Ac}$
7	$OPR_{FBSTR}\text{-}CHRO_{Pyr}\text{-}CHRO_{Neu5Ac}$
8	$R_{AGE}\text{-}R_{NAL}\text{-}CRYST_{Neu5Ac}$
9	$R_{AGE}\text{-}R_{NAL}\text{-}CHRO_{Neu5Ac}$
10	$R_{AGE}\text{-}CRYST_{GlcNAc}\text{-}R_{NAL}\text{-}CRYST_{Neu5Ac}$
11	$R_{AGE}\text{-}CRYST_{GlcNAc}\text{-}R_{NAL}\text{-}CHROM_{Neu5Ac}$
12	$R_{AGE}\text{-}R_{NAL}\text{-}CRYST_{GlcNAc}\text{-}CRYST_{Neu5Ac}$
13	$R_{AGE}\text{-}R_{NAL}\text{-}CHRO_{Pyr}\text{-}CRYST_{Neu5Ac}$
14	$R_{AGE}\text{-}R_{NAL}\text{-}CHRO_{Pyr}\text{-}CHRO_{Neu5Ac}$

5.2.6 Stage 5: Screening for operational and process constraints, benchmarking criteria.

5.2.6.1 Step 5a. Screening for operational and process constraints

The 14 remaining options after screening by logical and structural constraints are subjected to the screening by operational and process constraints. The process model for each specific option is derived from the generic model. An example is presented here.

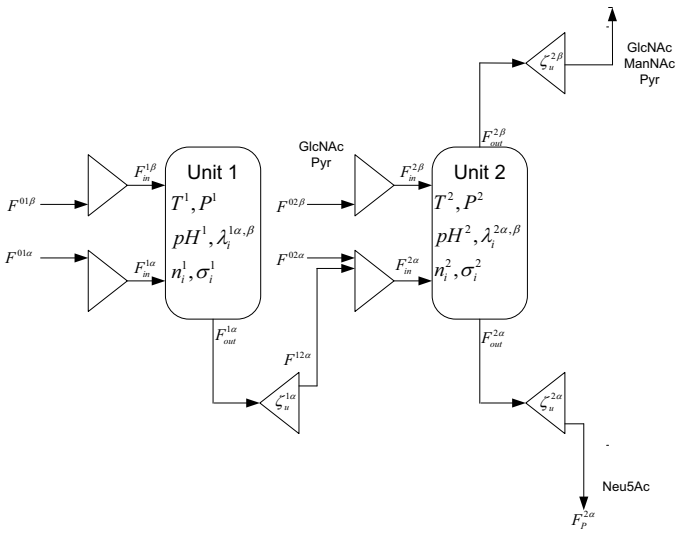

Figure 5.9 Process configuration OPR_{BSTR}-$CRYST_{Neu5Ac}$

For example, taking the option No. 3, which is OPR-CRYST (One-pot reaction followed by crystallization, represented in Figure 5.9), the specific process model for this process configuration is derived from the generic model developed in Stage 3. Therefore, the equations of the specific process model are taken from the generic molar

balance equation 9, and its corresponding constitutive equations together with other process constraints.

$$\frac{\partial n_i}{\partial t} = \sum_{u=1}^{u} Y^u \left(Y_{in}^{u,\alpha} x_{i,in}^{u,\alpha} F_{in}^{u,\alpha} + Y_{in}^{u,\beta} x_{i,in}^{u,\beta} F_{in}^{u,\beta} \right) - \sum_{u=1}^{u} Y^u \left(Y_{out}^{u,\beta} x_{i,out}^{u,\alpha} F_{out}^{u,\alpha} + Y_{out}^{u,\beta} x_{i,out}^{u,\beta} F_{out}^{u,\beta} \right) + \sum_{u=1}^{u} \left(\lambda_i^{u,\alpha} + \lambda_i^{u,\beta} \right) \tag{5.14}$$

For this case, the following binary variables exist, while all other binary variables in the superstructure are zero:

$$Y^{01\alpha} = Y_{in}^{1\alpha} = Y_{out}^{1\alpha} = Y^{12\alpha} = 1 \tag{5.15}$$

$$Y^{02\alpha} = Y_{in}^{2\alpha} = Y_{out}^{2\alpha} = Y_{out}^{2\beta} = Y_P^{2\alpha} = Y_P^{2\beta} = 1 \tag{5.16}$$

As seen in Figure 5.9, there is no splitting of streams, therefore, the connection scheme of the existing flow streams F is given by:

$$\zeta_u^{1\alpha} = \zeta_u^{2\alpha} = \zeta_u^{2\beta} = 1 \tag{5.17}$$

$$F^{01\alpha} = F_{in}^{1\alpha} \tag{5.18}$$

$$F_{out}^{1\alpha} = F^{12\alpha} \tag{5.19}$$

$$F_{in}^{2\alpha} = F^{12\alpha} + F^{02\alpha} \tag{5.20}$$

$$F_{out}^{2\beta} = F_{P}^{2\beta} \tag{5.21}$$

$$F_{out}^{2\alpha} = F_{P}^{2\alpha} \tag{5.22}$$

Since the defined scenario is run in batch, the generic mass balance (Equation 5.7), is split into two time domains, one for the reaction and one for the separation afterwards:

$$\frac{dn_i}{dt} = \left[\frac{dn_i}{dt}\right]_{t=0}^{t=t_{reaction}} + \left[\frac{dn_i}{dt}\right]_{t=t_{reaction}}^{t=t_{end}} \tag{5.23}$$

During the reaction time, the initial concentration (Equation 5.24) is changing due to the two reactions. The conversion rates are functions of component and enzyme concentrations determined from literature:

$$\left[\frac{dn_i}{dt}\right]_{t=0} = x_{i,in}^{1\alpha} F_{in}^{1\alpha} \tag{5.24}$$

$$\frac{dn_i}{dt_{reaction}} = \lambda_i^{reaction1} + \lambda_i^{reaction2} \tag{5.25}$$

The conversion rates are replaced by kinetic reaction expressions derived by Zimmermann and co-workers (2007, 2008a, 2008b). Validation of the kinetic model has been done and results are shown in Figure 5.10.

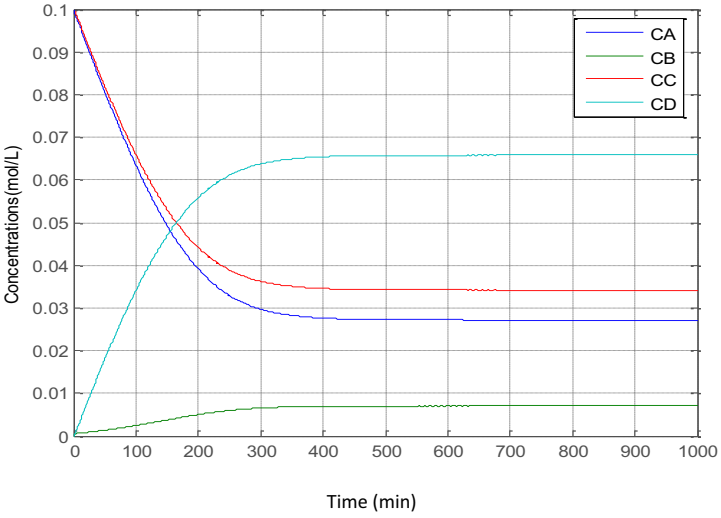

Figure 5.10 Simulation of the kinetic model of the one-pot enzymatic synthesis of Neu5Ac (D) from GlcNAc (A) and Pyr (C). ManNAc (D) is the intermediate compound (Equations outlined in Appendix B.3). Model was taken as reliable since the conversion to product obtained by the model is 0.66 whilst experimental reported is 0.63 (Zimmermann *et al*., 2007).

When the reaction is in equilibrium or complete, the contents of the reactor are emptied and sent to the crystallization step where the feed is initially mixed with glacial acetic acid.

$$x_{i,out}^{1\alpha} F_{out}^{1\alpha} = \left[\frac{dn_i}{dt} \right]^{t=t_{reaction}} \tag{5.26}$$

$$\left[\frac{dn_i}{dt}\right]_{t=t_{reaction}} = x_i F_{in}^{2\alpha} \tag{5.27}$$

The product stream $F_P^{2\beta}$ can be calculated with equation (5.20) in which the crystallization time t_{cryst} is a function of the volume of the solution and the concentrations:

$$x_D F_P^{2\beta} = \sigma^{\beta} \cdot \frac{t}{t_{cryst}} \cdot x_D F_{in}^{2\alpha} \tag{5.28}$$

All the other components are found in the product stream $F_P^{2\alpha}$.

The following operational constraints have been identified, that is the ratio of A over D (Mahmoudian et al, 1997) and C over D (Yamaguchi et al, 2006) on a molar basis:

$$\frac{n_A}{n_D} \leq 0.3 \tag{5.29}$$

$$\frac{n_C}{n_D} \leq 2.2 \tag{5.30}$$

The systematic modelling steps, described in Section 4.3.3.2 and Section 4.3.5.1 of this thesis, have been applied for each option. The results of the process simulations (calculation of the criteria for benchmarking, the productivity) for all the 16 remaining options, using the tool MoT of ICAS12® are listed in Table 5.4.

Table 5.4
Results of process simulation at same initial substrates amounts ($n_{GlcNAc,0}$ =1.3 mol, $n_{Pyr,0}$ = 2.6 mol) and same enzyme concentrations (C_{epi} =1500 U/L, C_{ald} = 24000 U/L).

Option No.	*Process*	*Reaction Neu5Ac productivity (gNeu5Ac/L.day)*	*Overall Neu5Ac Productivity (gNeu5Ac/L.day)*
1	OPRS-$CRYST_{Neu5Ac}$	269	201.02
2	OPRS-$CHRO_{ISPR}$	269	216.49
3	OPR_{FBSTR}-$CRYST_{Neu5Ac}$	213.4	160.82
4	OPR_{FBSTR}-$CHRO_{Neu5Ac}$	213.4	120.61
5	OPR_{FBSTR}-$CRYST_{GlcNAc}$-$CRYST_{Neu5Ac}$	217	151.54
6	OPR_{FBSTR}-$CHRO_{PYR}$-$CRYST_{Neu5Ac}$	217	160.82
7	OPR_{FBSTR}-$CHRO_{Pyr}$-$CHRO_{Neu5Ac}$	217	173.19
8	R_{AGE}-R_{NAL}-$CRYST_{Neu5Ac}$	72.37	54.28
9	R_{AGE}-R_{NAL}-$CHRO_{Neu5Ac}$	72.37	57.90
10	R_{AGE}-$CRYST_{GlcNAc}$-R_{NAL}-$CRYST_{Neu5Ac}$	180.92	135.69
11	R_{AGE}-$CRYST_{GlcNAc}$-R_{NAL}-$CHROM_{Neu5Ac}$	180.92	144.73
12	R_{AGE}-R_{NAL}-$CRYST_{GlcNAc}$-$CRYST_{Neu5Ac}$	72.37	40.71
13	R_{AGE}-R_{NAL}-$CHRO_{Pyr}$-$CRYST_{Neu5Ac}$	72.37	43.42
14	R_{AGE}-R_{NAL}-$CHRO_{Pyr}$-$CHRO_{Neu5Ac}$	72.37	43.42

5.2.6.2 Benchmarking using defined metrics

The options are benchmarked using the defined metric of overall productivity, defined in Step 1b (Section 5.2.2.2). Processes with overall productivities over 150 g/day are selected. Now, there are 6 remaining options, number 1,2,3,5,6 and 7 from Table 5.4.

5.2.7 Stage 6. Objective function evaluation

5.2.7.1 Step 6a. Calculation of the objective function

In this Stage the most promising options (the six remainig options after benchmarking) are subjected to calculation of the objective function (Eq. 5.4). The most promising candidates are listed in Table 5.5, and are those that satisfy all the constraints including the benchmarking criteria. These 6 options are subjected to the calculation of the objective function (results presented in Table 5.5).

Table 5.5
Results of the objective function of the feasible alternatives

Option No.	*Process*	*Product yield*	*NAL Enzyme yield*	F_{Obj} *(Equation5.4)*
1	OPRS-$CRYST_{Neu5Ac}$	0.50	10699	2889
2	OPRS-$CHRO_{ISPR}$	0.54	11522	3111
3	OPR_{FBSTR}-$CRYST_{Neu5Ac}$	0.39	8560	2311
4	OPR_{FBSTR}-$CRYST_{GlcNAc}$-$CRYST_{Neu5Ac}$	0.38	8062	2177
5	OPR_{FBSTR}-$CHRO_{PYR}$-$CRYST_{Neu5Ac}$	0.40	8558	2311
6	OPR_{FBSTR}-$CHRO_{Pyr}$-$CHRO_{Neu5Ac}$	0.43	9217	2489

The option with the maximum yield is OPRS-CHRO (One-pot reactive extraction followed by ISPR by chromatography), achieving a percentage product yield of 53.85%. This option (see Figure 5.5) consists of an integrated reaction and extraction in a batch reactor with continuous renewal of the organic phase, followed by a chromatographic step.

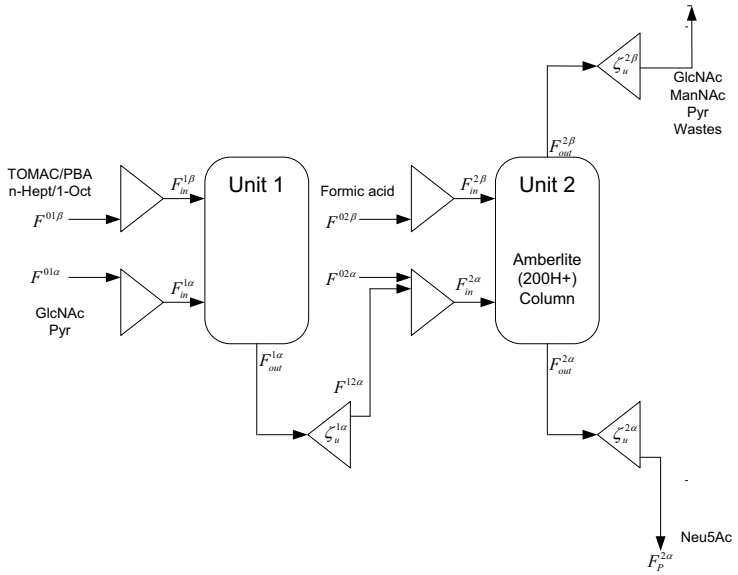

Figure 5.5 Option OPRS-CHRO

5.2.7.2 Step 6b. Validation of the best option

The final validation of the proposed design will be possible by comparing the model-based results with experimental data if available. Since the option presented is an improvement achieved by the PI framework proposed here, it has not been implemented yet. On the other side, a validation by rigorous simulation will require further work for model development including experimentation. The validation can be done by implementing the equipments of the proposed option and performing experiments to check if the results between the model-based simulations with the experimental simulations are consistent.

5.3 Enzymatic production of biodiesel

5.3.1 Introduction

Biodiesel is an important vegetable oil- or animal fat-based diesel fuel consisting of long-chain alkyl esters (methyl, ethyl, propil, butyl or isobutyl esters, etc.). These molecules are called fatty acid alkyl esters (FAAE) obtained by vegetable oils or animal fats by transesterification. Due to the worldwide confrontation with depletion of energy fossil resources and increased environmental problems, biodiesel has been attracting increasing attention during the last decades since it is a sustainable and environmentally friendly alternative fuel (Barnwal and Sharma, 2005). Biodiesel is renewable, biodegradable and nontoxic. It does not contain sulfur or aromatics. Its oxygen contents enhance its ability towards combustion. With biodiesel content up to 20%, conventional diesel engines can run without requiring any modification.

Biodiesel is a mixture of fatty acid alkyl esters (FAAE), the most common nowadays being fatty acid methyl esters (FAME) that can be obtained by several methods (Ma and Hanna, 1999; Ranganathan *et al*., 2008). The options vary from the type of feedstock (raw materials) and the methods of biodiesel production. Figure 5.12 outlines different raw materials/pretreatment/reaction/separation options for the production of biodiesel.

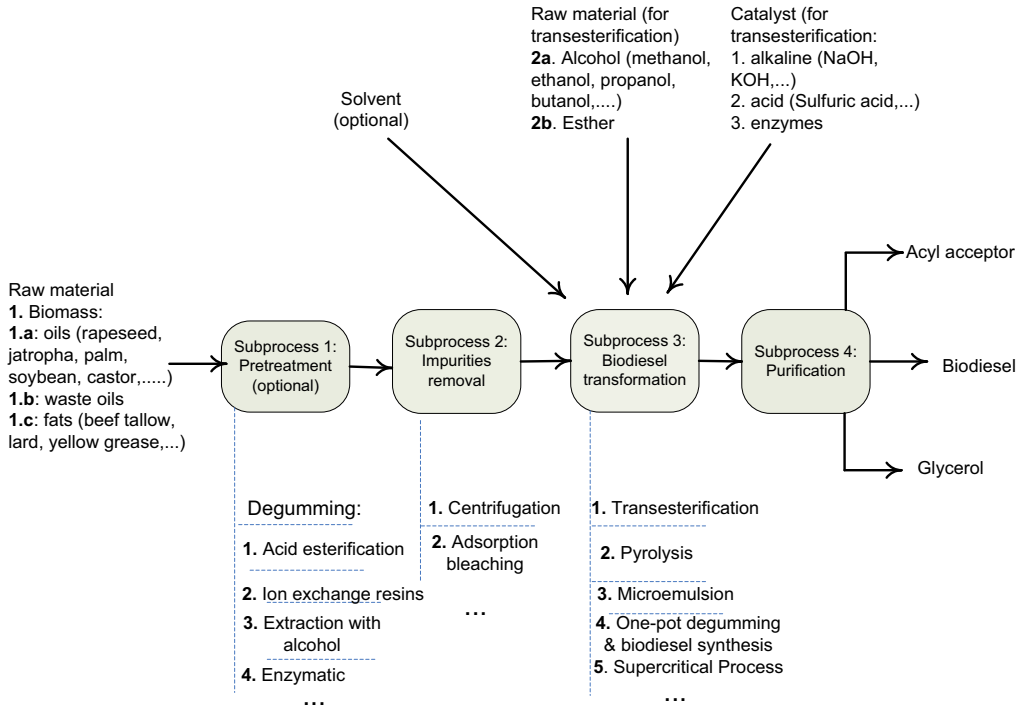

Figure 5.12 Options for biodiesel production

The most common method of biodiesel production is the alkali transesterification. Biodiesel produced by transesterification consists of reacting the oil with an acyl acceptor, preferably an alcohol in the presence of a catalyst to form FAAE as biodiesel and glycerol as by product. The transesterification reaction is outlined in figure 5.13.

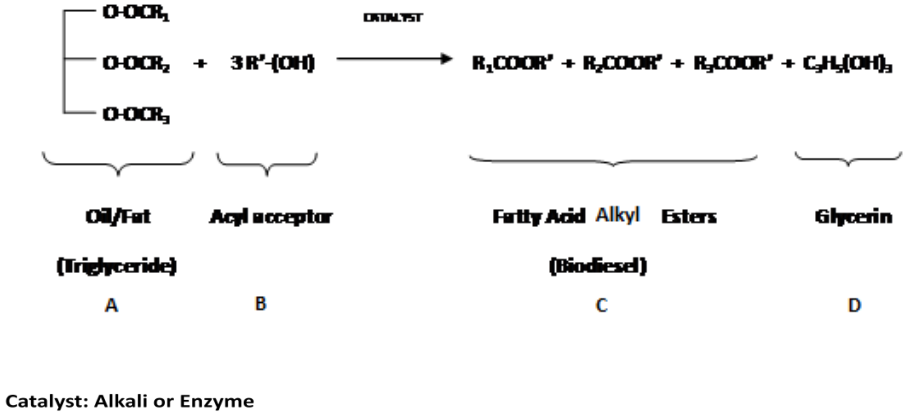

Figure 5.13 Transesterification reaction for biodiesel production

Alkaline transesterification of oil, with methanol, is most often used industrially because of its high reaction yield and efficient time, high conversion of triglycerides and the cheap catalyst.

A conventional alkali process for biodiesel production is shown in Figure 5.14, where a pretreatment applies only to fats and oils containing high levels of free fatty acids (FFA) leading to soap formation. This pretreatment step consists of esterification adding an acid catalyst (e.g. H_2SO_4) and methanol to the oil to reduce the amount of FFA to less than 1%. This processing step is especially important in the case of biodiesel from animal fats or waste cooking oil, where the level of FFA is generally high and varies from batch to batch of the raw material. Once the base oil/fat is cleaned, it is subjected to the main process, known as tranesterification. Through this reaction, the oil is transformed into biodiesel (FAME) and glycerin by heating and mixing alcohol and a base catalyst such as NaOH. The reaction product, consisting of methyl ester, glycerin, excess methanol and catalyst must be neutralized. Later there is a methanol recovery step usually employing flash distillation. Methanol vapors are condensed and sent to a storage tank, from where it is recycled to this process. In the following step is the

separation of the two phases by settling is employed, one rich in FAME, and the other rich in glycerol. This is followed by the neutralization of the biodiesel-glycerol phase and settling is employed to separate both phases. A salt removal step is done to separate salts of the catalyst. A FAME recovery is done by washing with water to remove any substance from the biodiesel. A drying step is done to remove the remaining water from the washing process. The by-product glycerol (about 10% of biodiesel produced), must be refined to obtain a product with added value.

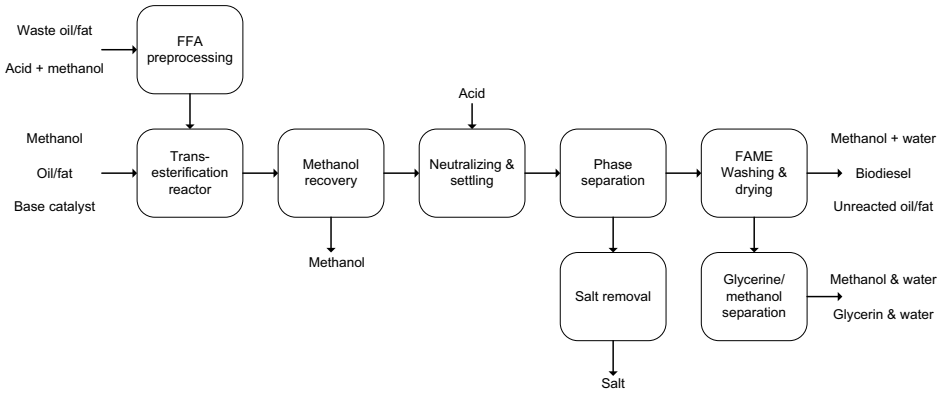

Figure 5.14 Simplified flowsheet of a conventional alkali biodiesel production process

There are some limitations in this process such as negative environmental impact mainly because of the waste water treatment and high energy consumption due to its extensive downstream processing.

Finally, enzymatic biodiesel production has been investigated as a promising option for its benefits to offer a simpler, less energy consuming process and more compatible with variation of the raw material. However, its production by enzymatic routes has not been completely implemented at industrial scale mainly due to the very high costs of the enzymes (lipases) and their easy loss of activity and low reaction rates.

Process intensification techniques, like the integration of reaction and separation, can help to solve these difficulties and lead to obtaining a feasible enzymatic process to make biodiesel. Therefore, the proposed framework for intensified enzyme-based processes in this thesis is applied to find an improved alternative for biodiesel production.

5.3.2 Stage 1. Problem definition

For this system, the problem formulation statement (adapted from Section 3.4 of this thesis) is: given tryglicerides (A) and acyl acceptor (B) as substrates to make the desired product, Biodiesel (C), through enzyme-based intensification methods, find the best way to make it, that is, find the optimal process route, according to the criteria given by the objective function, from A and B to C, specifying the separations and reactions in the process. All the possible combinations are considered and analyzed, including the integration of reaction/reaction, (e.g. one-pot synthesis), reaction/separation (e.g. in situ product removal) and separation/separation (hybrid separations). In order to avoid any doubt, all the possibilities are considered to ensure that the best option will be selected.

Following the methodology proposed and as in the first case study, a base-case design is needed which is going to be used to evaluate the performance of intensified process solutions against it. A simplified process scheme of the base-case design (Ranganathan *et al*., 2008) is outlined in Figure 5.15.

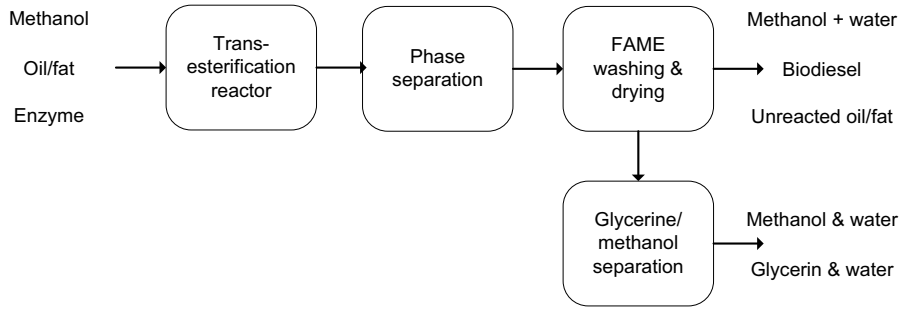

Figure 5.15 Simplified process scheme for a conventional enzymatic process for biodiesel production (Ranganathan *et al*., 2008).

5.3.2.1 Step 1a. Analysis and identification of bottlenecks /limitations

In the base-case design the following limitations were found:

- Low reaction rates
- Costs of lipase
- Enzyme inhibition (by alcohol and glycerol)

5.3.2.2 Step 1b. Needs, boundaries, metrics and first set of constraints selection

Boundaries are selected as maximum four processing steps of maximum two phases per processing unit, either reaction or separation steps.

Some metrics have been selected, namely waste generation, energy consumption and simplification of the process. These will be translated into heuristic rules integrated in logical constraints such as connection rules for synthesis, e.g. “Only generate options with less than five processing steps” due to simplification of the process; and in structural constraints, e.g. “Not use solvents for lipase transesterification” due to waste

generation an environmental concerns. As an additional metric for benchmarking in Stage 5 of the Methodology, the productivity (defined in Equation 5.1) is selected again, since it will influence the cost-effectiveness of the process (see Table 3.1, economic metrics). By analyzing the base-case design, a first set of constraints is identified. Some of these identified constraints are some types and sequences of the operations (logical constraints), some of allowed inlet, outlet and recycle streams (structural constraints), and some process specifications (operational constraints).

Immobilized enzyme and not free is selected for industrial purposes because its handling is easier and allows the reuse of the enzyme without the need to separation of the reaction mixture before downstream processing

5.3.2.3 Step 1c. Objective function definition

Since the main problem for implementation here is the cost of enzyme, the objective function is defined to maximize the productivity, that is the amount of biodiesel generated per amount of enzyme, which may lead to a reduction of the overall process cost.

$$OF = \max \left(productivity = {}^{kg\ biodiesel}\!/_{kg\ lipase} \right) \tag{5.31}$$

5.3.3 Stage 2. Data/Information Collection/Analysis

In the second stage, different data were collected such as types of raw materials, acyl acceptors, catalyst, and possible solvents to use, since solvents can solve the problem of miscibility, mass transfer limitations, and enzyme inhibition (Table 5.6).

Table 5.6 Different options of raw material, catalyst and solvent for enzymatic biodiesel production

Oil/fat	*Acyl acceptor*	*Catalyst*	*Solvent*
Rapeseed oil	Methanol	Novozym 435	Solvent free
Soybean oil	Ethanol	Lypozyme RMIM	n-hexane
Jatropha oil	Propanol	Lypozyme TLIM	tert-butanol
Palm oil	Butanol	Combined enzymes	...
Waste oil	Isobutanol	...	
Beef tallow	Isopropanol		
...	...		
25 options	11 options	12 options	7 options

Process and property data, like enzyme activity and reaction thermodynamics and kinetics reported in the literature, were collected. Information about different types of reactors, separation methods and equipment were also collected. With this information, a second set of constraints is defined, e.g, different decision variables representing the reaction and separation options, logical sequences of the operations, reflected in logical constraints. Also, with this information, the allowed inlet, outlet and recycle streams of the reaction and separation options are identified and reflected in structural constraints. The operational constraints, such as allowed temperatures, pH´s, feed concentrations, etc., are also collected in this stage and reflected in operational constraints.

Figure 5.16 shows the superstructure that is created by including the different collected methods/equipment of reaction and separation.

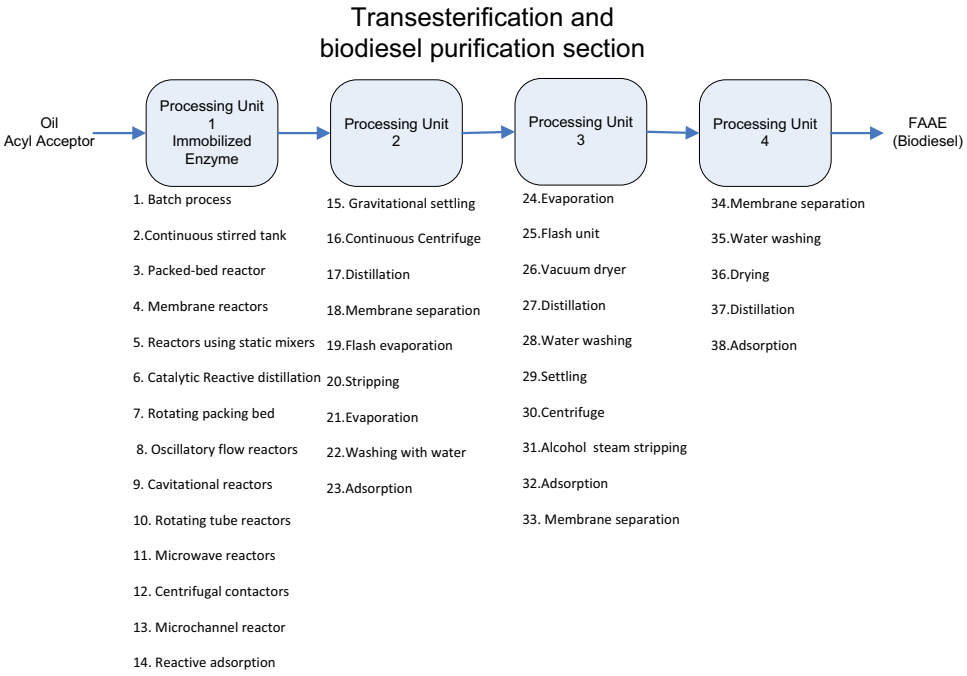

Figure 5.16 Operations/equipment options for enzymatic biodiesel production

5.3.4 Stage 3. Superstructure setting and generic model retrieval

5.3.4.1 Step 3a. Superstructure setting

The maximum number of processing units and the maximum number of phases per processing units are information that is retrieved from Step 1b to build the superstructure (4 processing steps and two phases). Therefore, by looking at table 4.4,

the maximum number of inlet and outlet streams, the maximum number of junction and split connectors per processing unit can be set. Subsequently, by looking at Table A.3, the remaining number of streams (recycle streams, inlet streams before and after connections) can be identified. With this, the superstructure is created and is shown in Figure 5.17.

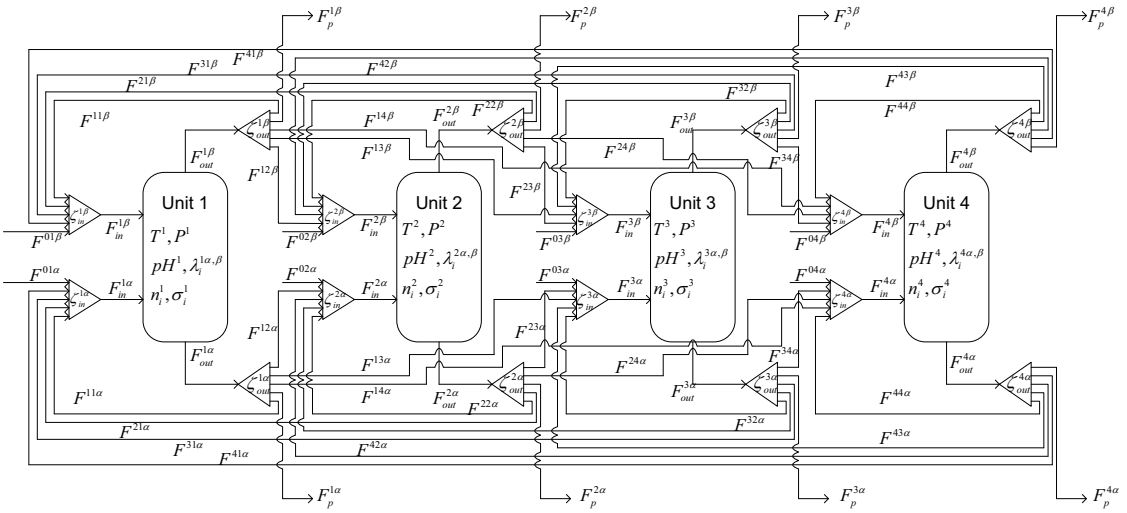

Figure 5.17 Superstructure for generation and evaluation for intensified options for biodiesel production

5.3.4.2 Step 3b. Generic model retrieval

The generic process model derived in Section 4.3.3.2 of this thesis is retrieved. For the superstructure developed in this case study, the mass balance (Equation 4.10), is for four compounds ($i = 1,2,3,4$), $i = 1$ is for tryglicerides, $i = 2$ for metahnol, $i = 3$ for FAME and $i = 4$ for glycerol and the $u = 4$, the maximum number of processing units.

$$\frac{\partial n_i}{\partial t} = \sum_{u=1}^{4} Y^u \left(Y_{in}^{u,\alpha} x_{i,in}^{u,\alpha} F_{in}^{u,\alpha} + Y_{in}^{u,\beta} x_{i,in}^{u,\beta} F_{in}^{u,\beta} \right) - \sum_{u=1}^{4} Y^u \left(Y_{out}^{u,\alpha} x_{i,out}^{u,\alpha} F_{out}^{u,\alpha} + Y_{out}^{u,\beta} x_{i,out}^{u,\beta} F_{out}^{u,\beta} \right) + \sum_{u=1}^{4} Y^u \left(\lambda_i^{u,\alpha} + \lambda_i^{u,\beta} \right) \tag{5.32}$$

5.3.5 Stage 4. Generation of feasible candidates

5.3.5.1 Step 4a. Generation of all process options

The number of total combinations of flow sheets with four processing units interconnected by 16 streams each is given by the combinatorial expression represented by equation 4.2 in the problem formulation explained previously with the added options for raw material N_{OR}, acyl acceptor N_{OA}, enzyme N_{OE} and solvent N_{OS}.

$$NPO = N_{OR} \cdot N_{OA} \cdot N_{OE} \cdot N_{OS} \sum_{u=1}^{u} \underbrace{\left(\prod_{u=1}^{u} NIU_u \right)}_{\text{options trough units}} \underbrace{\left(\frac{(r \cdot u)!}{u!(r \cdot u - u)!} \right)}_{\text{options through recycle}} \tag{5.33}$$

$NPO = 9.3E13$ before constraints

5.2.5.2 Step 4b y 4c. Screening by logical and structural constraints

In the screening for logical and structural constraints different information and knowledge were translated into decision variables. For the decision on the raw material used in Europe, rapeseed oil is considered. The alcohol selected is the cheapest, in this case, methanol. The commercial available enzymes are considered, and the reaction is solvent free. Considering the maturity of PI technology, etc. and the existence of specific streams in the superstructure, at the end of the screening step, 32 options are found.

5.3.6 Stage 5: Screening for operational and process constraints, benchmarking criteria.

5.3.6.1 Step 5a. Screening for operational and process constraints

Simulations of batch reactors were done for the selected enzymes (see Figure 5.18)and it was found that Novozym 435 gave the highest activity compared to Lipozymes, but the combination 50:50 of Novozym 435 and Lipozyme TL gave better performance achieving the highest yield to methyl esters and showing positive synergistic effects. Together they can eliminate the rate limiting step in the transesterification. With this a reduction of the cost of biodiesel can be achieved since Novozym 435 is partially replaced by the less expensive Lipozyme TL IM. So, 24 options are now discarded and there are eight remaining options.

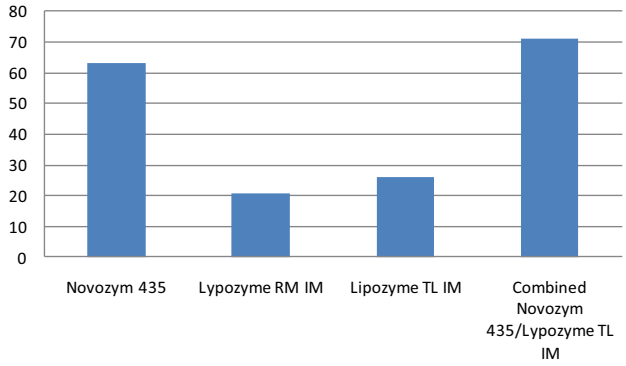

Figure 5.18 Screening of lipase for biodiesel production in a solvent-free medium. Calculated methyl ester yield for different types of enzymes. Reaction conditions: 45◦C, 150 RPM, methanol/rapeseed oil molar ratio 6:1, 5% enzyme Based on oil weight, reaction time 20 h.

Validation of the kinetic models was performed by simulation and comparison of the numerical results with reported data. Model validation was done for the kinetic of the enzymes Lipozyme RM IM, Lipozyme TL IM, and Novozym 435, which are commercially available enzymes. Figure 5.19 shows the validation results for the enzyme lipozyme RM IM.

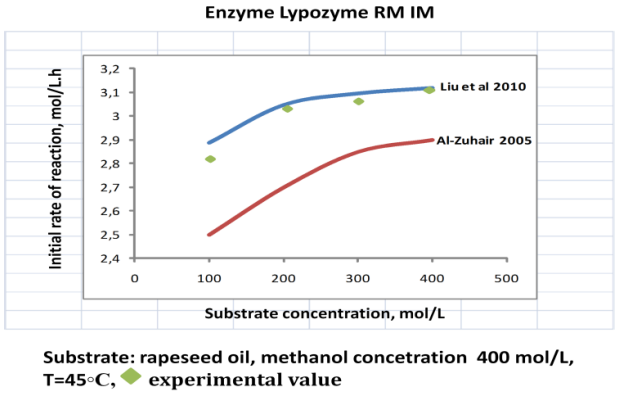

Figure 5.19 Validation of de enzymatic models for the enzyme Lipozyme RM IM

5.3.6.2 Benchmarking using defined metrics

The results of productivity of process model calculations are summarized in Table 5.7. The highest productivity is given by the option that performs the transesterification option of a packed bed reactor integrated with glycerol removal and then removal of glycerol from biodiesel by adsorption. In table 5.7 it can be observed that the highest productivities are obtained by integrated options like membrane reactors and the lowest productivities are obtained by the batch reactors.

Table 5.7 Screening for process constraints and benchmarking. MR: Membrane Reactor; BR: Batch Reactor; CSR: Continuous Stirred Reactor; PBR: Packed Bed Reactor; PBR/S: Packed Bed Reactor Integrated w/Glycerol Removal; D: Distillation; C: Centrifuge; M: Membrane Separation; A: Adsorption Column. 1:1 enzyme Novozym 435/Lypozyme TL IM weight ratio

Option number	*Process path*	*Productivity (kg biodiesel/kg enzyme)*
1	MR-D-C	6700
2	MR-C-D	6520
3	BR-C-D-M	5260
4	BR-C-D-A	4870
5	CSR-D-C-M	5920
6	CSR-D-C-A	5450
7	PBR-D-M-A	4040
8	PBR/S-A	6970

5.3.7 Stage 6. Objective function evaluation

5.3.7.1 Step 6a. Calculation of the objective function

The best option is the ones indicated in Table 5.7 as number 8. It consists of a packed bed reactor integrated with a container at the bottom for intermittent removal of glycerol, and the effluent is passed through an adsorption column to remove the residual glycerol in the biodiesel from 0.053 wt% to 0.003 wt%. (Figure 5.20).

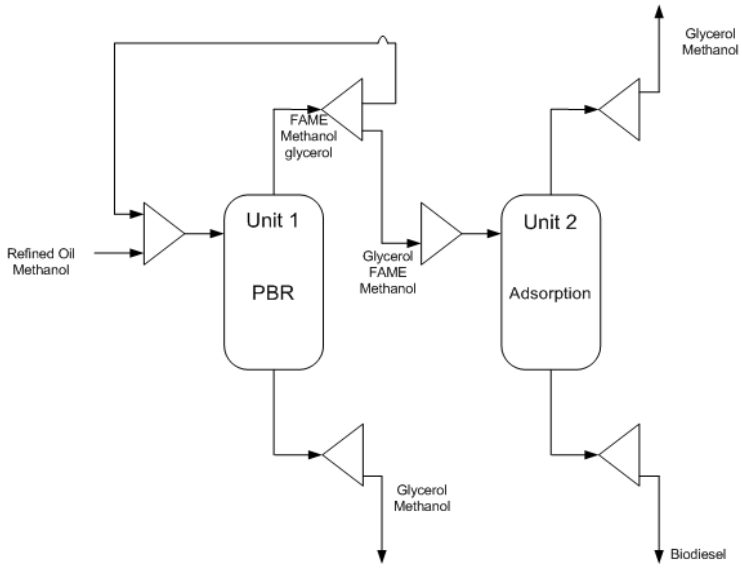

Figure 5.20 Best identified reaction/separation configuration for enzymatic biodiesel production

The maximization of the productivity was done evaluating a range of enzyme ratios, methanol to oil ratio and pass number giving a maximization of the productivity of 9040 kg/biodiesel/kg enzyme.

Table 5.8 Maximization of the objective function for the best process alternative

Novozym435/Lypozyme TLIM weight	*Methanol/Oil ratio*	*Pass number*	*Oil conversion %*	*Productivity(Kg biodiesel/Kg enzyme*
2:4	0.5	12	99.7	9040

5.3.7.2 Step 6b. Validation of the best option

The final validation of the proposed design is possible by comparing the model-based results with experimental data, if available. Since the option presented is an improvement achieved by the PI framework proposed here, it has not been implemented yet. On the other side, a validation by rigorous simulation requires further work, such as model development including experimentation. The validation can be done by implementing the equipment of the proposed option and performing experiments to check if the results between the model-based simulations with the experimental simulations are consistent.

CHAPTER SIX
General Discussion

In this Thesis, a combined knowledge- and model-based generic framework for intensification of enzyme-based processes is proposed. The framework provides a methodology that systematically finds an intensified process configuration to make a product with enzyme-based reactions.

The type of systems for the framework to be applied are named here as enzyme-based production process. An enzyme-based production process is defined in this work as a process that uses commercially produced enzymes, in one or more of its processing steps, to obtain desired products. During the last years, there has been an increasing interest of this type of processes due to the benefits they may offer in the industrial chemical, pharmaceutical, food and renewable fuels industry. Examples of these benefits are mild reaction conditions, easy processing of renewable raw materials and the growing development and production of commercial enzymes for the process industry. Enzyme-based production processes are cleaner and greener compared to the chemical processes.

Due to the process limitations that enzyme-based processes present, e.g., limited reaction productivity and difficult downstream processing (DSP), process intensification (PI) is exposed here as a promising approach to design improved enzyme-based process options. The most important process intensification methods for enzyme-based processes are related to the integration of processing steps and identified here as one-pot synthesis (OPS) and *in situ* product removal (ISPR).

In order to design an intensified enzyme-based production process, in a systematic and efficient manner, different issues and needs have to be addressed. These issues and

needs can be addressed using process systems engineering (PSE) approaches, methods and tools. The main issues and needs identified and partially addressed are: problem definition, metrics for PI, objective function definition, bottlenecks identification, knowledge management, identification and classification of constraints, generation of options, superstructure development, model development and analysis and sensitivity analysis. With this, the identification of an optimal intensified enzyme-based process configuration with its corresponding equipment and operation conditions, using a methodology, and specific methods and tools, included in a framework, is achieved.

The synthesis/design problem addressed in this work could be stated as identifying the optimal enzyme-based path to reach a given product in the desired quality and quantity with respect to defined constraints in the process. Since the framework uses model based techniques it was necessary to give the formulation of the process synthesis problem presented in a mathematical form. The problem formulation is presented in the form of an optimization problem, where an objective function (the criterion or criteria for final selection), the expression of generation of options, the logical, structural, and operational constraints; and the process models, are given. This kind of problem is very complex and impossible to be solved by optimization programming techniques (MINLP). Therefore, a solution strategy is proposed here and reflected in the methodology of the framework.

The methodology consists of six stages. In the first stage, named problem definition, an analysis of the bottlenecks/limitations of a base case design is done. Identification of needs, boundaries, first constraints and decided metrics for performance ranking is also done in this stage. The definition of the objective function is the last action of this stage. In the second step, necessary and reported data/information for the subsequent stages in the methodology are collected, (e.g. compounds and mixture properties, reaction properties, types of reactors, different methods of separation, OPS and ISPR methods). In the third stage, a superstructure is generated. The superstructure represents all options. A generic process model which represents the superstructure is used in the subsequent process model evaluation stage. In the fourth stage, a generation of feasible

candidates is achieved by using logical constraints and structural (identified in the stage two of the methodology) to be used for screening of options in the superstructure. In stage five, the feasible options are screened by using the operational constraints and the process model (derived from the generic model representing the superstructure) for each remaining option. A benchmarking of the options using PI selected criteria in stage one, is done. The last stage consists of the evaluation of the objective function, defined in stage 1, and the option that remains after all the stages is selected as the best intensified process option. In this manner, the methodology has the ability to generate all reaction and separation options, and, through the systematic use of many types of constraints, logical, structural and operational and process models, it is capable to find the optimal option, according to an objective function, to make a desired product. To perform all the stages, different knowledge and tools like heuristics, databases, literature, operational windows, a superstructure, model libraries and model developers and solvers like ICAS-MoT are used to perform the stages in the methodology.

The framework was highlighted and applied through two case studies. One case study deals with Neu5Ac synthesis since it is an example of equilibrium controlled enzyme-based reactions and this case presented the challenge of observing the benefits and drawbacks of intensification: mainly consisting of integrating the enzymatic step with either the epimerization (chemical or enzymatic) step upstream and the product separation downstream of the reaction (ISPR). The second case study is related to the enzymatic production of biodiesel due to the importance that this renewable biofuel is adquiring and diverse enzymatic alternatives have been investigated.

CHAPTER SEVEN
Conclusions

In this work a combined knowledge- and model-based (hybrid) generic methodology for design of intensified enzyme-based processes has been developed along with the methods and tools which assist in the systematic investigation of intensified/integrated enzyme-based production process systems. The main advantages of this framework are the following:

- It uses a combined knowledge- and mathematical optimization-based approach for process synthesis and design (Hybrid approach), using the advantages of both: e.g. early and fast screening of unfeasible options (advantage of a knowledge-based method) and the possibility to manage the interactions between the different design levels (advantage of optimization-based methods).

- The objective function can be defined easily by the identification of the bottlenecks/limitations of the base case design.

- The framework facilitates the generation of all possible options, by the implementation of a mathematical combinatorial expression and a superstructure (also easily to be generated) which includes all available operational units, including the integrated ones of one-pot reactors and the ISPR methods. With this, any doubt of avoiding any potential option is discarded.

- With the use of this framework it is possible to rule out unfeasible process options at early stages of process research, this is convenient because it accelerates the design procedure and process development time is saved.

- It proposes a decomposition approach to solve the whole complex optimization formulation. In this approach, the whole problem is divided into sub-problems (easier to handle) and the solution is obtained in a step-by-step well defined procedure.

- The framework is generic. This means that it is applicable for many different systems where different products are made by enzyme-based reactions.

- Using the framework, process improvements by intensification methods, without the excessive use of resources, e.g. experimental, can be achieved.

However, this framework has certain limitations. The framework is limited by the availability of data and information. Also the lack of reliable models, especially concerning to the enzymatic kinetics. Further work needs to be done to address these limitations.

CHAPTER EIGHT
Future work

The main drawback of using the developed framework is the availability of data and models describing the separation methods and the reactions. Therefore, the quality of the design is highly dependent on the quality of the data and models. An other disadvantage is the need of manual generation of the specific process configurations from the superstructure. Based on these drawbacks and other features including in the framework, the recommendations for future work are the following:

- The framework has been used for one case study from the pharmaceutical sector, the synthesis of Neu5Ac and for the enzymatic production of biodiesel. The framework can be applied to other case studies where enzyme-based reactions are taking part, such as other biofuels and products related with the food industry and/or fine and bulk chemicals. The generic nature of the framework can be proved by using the framework to intensify these systems.

- The framework provides at the end an intensified enzyme-based process option, whose validation is needed to prove that the designs behave in reality as predicted by the models. Therefore, the experimental validation of the resulted intensified process configuration has to be done in the future.

- Databases for the models and properties need to be extended for other enzyme-based systems.

- Due to the lack of availability of models describing enzyme-based reactions and operations, specific and detailed methodologies for creating these models should be developed and included in the framework.

- The framework includes the enzyme-based intensification methods of OPS and ISPR. It can be extended to PI equipments, such as, spin disk reactors, microbioreactors, etc. Data , models and information for these type of intensified equipment need to be investigated.

- Additional constraints dictated by process economics and safety may be incorporated. Better yet, the defined objective function can include an economic function, for this, accurate models describing the process economics should be developed.

- Data/Information management should also be systematized. For this, it is needed to prioritize and classify the type of data required for any enzyme-based process. These can be achieved by applying the framework to more case studies and inquire into a common trend of type of data required at different levels of the methodology.

- Detailed methods for weights definition in multi-criteria objective functions and sensitivity analysis techniques have to be further developed.

- The framework developed may be integrated into a generic methodology for PI, such as the ones described in chapter two of this thesis.

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APPENDIX A.1

Incidence matrices of the problem formulation and decomposition approach

Table A.I Incidence matrix for process design/synthesis (incorporating PI)

Equation/Method	Product parameters	Equipment parameters	Integer variables			Binary variables			Process variables	Objective function	
	$\bar{\theta}$	$\bar{d}$	u	r	NIU_{1_u}	$\bar{Y}^{u}_{1\_u}$	$\bar{Y}^{u,u_{out}}_{1\_r_{out}}$	$\bar{Y}^{u}_{1\_NIU}$	$\bar{X}$	w_{1_k}	F_{OBJ}
Define product	x										
Identify operations		x	x		x						
Generate options			x	x	x						
Logical constraints						x	x	x			
Structural constraints						x	x	x			
Operational constraints	x	x				x	x	x	x		
Process models	x	x				x	x	x	x		
Optimization residuals	x	x				x	x	x	x		
Objective function	x	x				x	x	x	x	x	x

Table A.2 General decomposition strategy for process design/synthesis (incorporating PI)

Equation/ Method	Product parameters	Equipment parameters	Integer variables			Binary variables			Process variables 1..v				Benchmark criteria	Process variables u-1..n		Objective function		Block
	$\bar{\theta}$	$\bar{d}$	u	r	$NIU_{1..u}$	$\bar{Y}^{u}_{1..u}$	$\bar{Y}^{u,k_c}_{1..n_{out}}$	$\bar{Y}^{u}_{1..NIU}$	$\bar{X}_{1..r}$	$\bar{X}_{r+1..t}$	$\bar{X}_{t+1..v}$		$\bar{X}_{u+1..p}$	$\bar{X}_{p+1..n}$	$w_{1..k}$	F_{OBJ}	
Define product	x																	**1**
Identify operations		x																**2**
Generate options (superstructure)			x	x	x													**3**
Logical constraints						x	x	x										**4**
Structural constraints						x	x	x										
For each subset of feasible flowsheet/set of fixed Y's																		
Operational constraints	x		x	x	x	x	x	x	x	x	x							**5**
Operational constraints		x																
Short cut process models	x	x	x	x	x	x	x	x	x	x	x							
Benchmark criteria	x	x	x	x	x	x	x	x	x	x	x		x					
For most promising options																		
Detailed process model	x	x	x	x	x	x	x	x	x	x	x			x	x			**6**
Optimization residuals	x	x	x	x	x	x	x	x	x	x	x			x	x			
Objective function	x	x	x	x	x	x	x	x	x	x	x			x	x	x	x	

APPENDIX A.2

Determination of the streams in the superstructure

Table A.3 Maximum number of streams for processing units (U) with maximum two phases (named phase α and β)

Number of units	1	2		3			4				5					6						…
	U1	U1	U2	U1	U2	U3	U1	U2	U3	U4	U1	U2	U3	U4	U5	U1	U2	U3	U4	U5	U6	
Max. inlet streams	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	…
Max. outlet streams	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	…
Max. recyc. & bypass streams phase α	1	2	2	3	3	3	4	4	4	4	5	5	5	5	5	6	6	6	6	6	6	…
Max. recyc. & bypass streams phase β	1	2	2	3	3	3	4	4	4	4	5	5	5	5	5	6	6	6	6	6	6	…
Initial inlet streams before junction connectors	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	…
Final outlet streams after split connectors	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	…
Total streams per unit	10	12	12	14	14	14	16	16	16	16	18	18	18	18	18	20	20	20	20	20	20	…
Total streams in superstructure	10	24		42			64				90					120						…

APPENDIX B.1

First set of identified constraints

Table B.2.1 First set of identified constraints

No.	Constraint	Type	Mathematical form
1	D purity over 95%	Operational	$\frac{[D]}{[A]+[B]+[C]+[D]} > 0.95$
2	Options with maximum four processing steps will be generated	Structural	$\sum_{u=1}^{u} Y^u \leq 4$
3	Options with minimum two processing steps and maximum processing steps	Logical	$2 \leq \sum_{u=1}^{u} Y^u \leq 4$
3	Maximum two phases per processing unit	Structural	$\sum_{f=1}^{f} Y^f \leq 2 \qquad f = \alpha, \beta$
4	Four reaction components	Structural	$i = 1,2,3,4$ Where 1 refers to A(GlcNAc), 2 to B(MAnNAc), 3 to C(Pyr) and 4 to D(GlcNAc)

APPENDIX B.2

Data/Information Collected

Table B.2.1 Neu5Ac synthesis components properties (Blayer, 1997)

	Pyr	**GlcNAc**	**ManNAc**	**Neu5Ac**
Charge	Negative pKa=2.39	/	/	Negative pKa=2.0
Hydrophobicity	No	No	No	No
Volatility	No	No	No	No
Specific group	1 carboxyl 1 acetyl	4 hydroxyls 1 N-acetyl	4 hydroxyls 1 N-acetyl	1 carboxyl 1 N-acetyl
Solubility	Water (3.6 M) EtOH	Water (1.3 M) EtOH low solubility in propanol	Water (1.6 M) EtOH	Water (0.95 M) EtOH low solubility in acetic acid
Others	Alkali very labile	Alkali labile	Alkali labile Hydrate form	Heat labile Alkali and acid labile

Table B.2.2 Enzymes properties*

Enzyme	**Specific Activity**	**Optimum pH**	**Optimum T**	**Inhibitors**
Immobilized NAL	10 U/mg	7.0 – 7.5	25^{o}C	Pyr ManNAc GlcNAc
Immobilized AGE	32 U/mg (in the presence of 1mM ATP)	7.0	30^{o}C	Pyr (50% reduced activity at 0.2 M)

*Data taken from BRENDA enzyme database (http://www.brenda-enzymes.org)

Table B.2.3 Possible reactor configurations for Neu5Ac synthesis

Reactor type and configuration	Notes
Batch stirred tank reactor (BSTR)	Initial concentrations once of Pyr and ManNAC (GlcNAc for upstream and in case of one-pot synthesis)
Fed-batch stirred tank reactor (FBSTR)	Intermintent feeding of Pyr Intermitent feeding of Pyr and ManNAc (or GlcNAc in case of upstream and one-pot synthesis)
Fed-batch stirred tank reactor (FBSTR)	Continous feeding of Pyr
Continuous stirred tank reactor (CSTR)	Continuous feeding of ManNAc (or GlcNAc) and Pyr and Continuous removal of outlet stream
Plug flow reactor (PFR)	Continuous feeding and removal
Membrane reactor (MR)	Continuous feeding and removal Feasibility of using free enzyme

Table B.2.4 Separation methods for Neu5Ac process

Separation method	Notes
Crystallization of GlcNAc with isopropanol	
Crystallization of Neu5Ac with glacial acetic acid	Crystallization occurs at low pH≈2.0
Removal of Pyr by anionic resins after enzymatic reactions	
Anion exchanger chromatography for Neu5Ac separation	

Table B.2.5 Potential ISPR methods for Neu5Ac synthesis

Separation	Basis	Notes
Anion exchange	Negative charge	Pyr binding due to charge, feeding strategy required. Counter anions leakage into the system
ISPR by reactive Extraction (Zimmermann et al, 2008a,b)		

Table B.2.6 Other information collected about the reactions

Information from reaction characterization	Reference
1. In enzymatic one-pot synthesis both enzymes are under inhibition. The product stream is diluted. Mg^{++} and ATP interfere with anion exchange chromatography.	Blayer, 1997
2. In enzymatic one-pot synthesis both enzymes are under inhibition. The product stream is diluted. Mg^{++} and ATP interfere with anion exchange chromatography.	
3. In chemo-enzymatic one-pot synthesis the epimerization is not feasible below pH 8 and the enzyme stability decreased vigorously at pH 10.5. There is degradation of Neu5Ac and Pyr.	Blayer, 1997
4. For batch reactions, Pyr is used in excess to obtain a high yield on ManNAc, which is expensive.	
5. Pyr has a strong inhibitory effect on initial rates of reaction. The aldolase activity decreases above 0.5 M Pyr up to 3.6 M (Saturation concentration)	Blayer, 1997
6. At high ManNAc concentrations the activity falls. A maximum activity was found around 750 mM on this substrate	Blayer, 1997
7. Non-specific inhibition at high molarities of all components of the medium, on account of viscosity increases	Kragl et al., 1992
8. Suggestion of logic substrate feeding in order to maintain low concentrations of pyruvate and resultant high reaction rates. Also could be beneficial for DSP demands, reducing enzyme inhibition and further enhancing productivity	Blayer, 1997
9. In chemo-enzymatic one-pot synthesis the alkaline conditions deactivate the aldolase enzyme which optimum pH is 7.0-7.5. There is also significant Pyr degradation at alkaline conditions	Blayer, 1997

Table B.2.7 Other information collected about the reactors	
1. In a one-pot enzymatic or chemo-enzymatic synthesis run in a MR, the enzymes are strongly inhibited by Neu5Ac	Kragl et al., 1991
2. In a one-pot enzymatic or chemo-enzymatic synthesis run in a CSTR, the enzymes are strongly inhibited by Neu5Ac	Blayer, 1997
3. BSTR and PFR are more beneficial considering Neu5Ac inhibition (exposing the catalyst to high product concentration only at the end of the reaction)	Blayer, 1997
4. CSTR and PFR are advantageous to avoid Pyr inhibition	Blayer, 1997
5. Feeding strategies are likely to overcome kinetic limitation (advantageous for enzyme limiting processes) and provide conditions beneficial to ion exchange separation	Blayer, 1997
6. PFR has the advantages of both continuous operation and batch kinetics. By operating substrate feeding with excess of ManNAc, the PFR can achieve high conversion rates and maintain low pyruvate concentration leaving the reactor, achieveing high yields on this limiting substrate	Blayer, 1997

Table B.2.8 Other information collected about the separation methods	
1. Crystallization of Neu5Ac with acetic acid occurs at very low pH. The enzymes lose activity below pH 3.5. Therefore, the integration of the reaction with crystallization is unfeasible.	Uchida et al, 1984 Blayer, 1997
2. ISPR by using ion exchange chromatography may improve the conversion yield and the reaction yield	Freeman et al., 1993

APPENDIX B.3

Constitutive models, variables and parameters

B.4.1 Aldolase Condensation Kinetic Model (Kragl et al., 1992)

$$\frac{d[D]}{dt} = \frac{[NAL]\cdot\left(\frac{A\max h\cdot[C]\cdot[B]}{k_{I,C}\cdot k_{I,B}} - \frac{A\max r\cdot[D]}{k_{m,D}}\right)}{1+\frac{[C]}{k_{I,C}}+\frac{[B]\cdot k_{m,C}}{k_{I,C}\cdot k_{m,B}}+\frac{[C]\cdot[B]}{k_{I,C}\cdot k_{m,B}}+\frac{[D]}{k_{I,B}\cdot k_{m,D}}+\frac{[D]}{k_{m,D}}}$$

Where

$[B]$	mol/L	ManNAc concentration	
$[C]$	mol/L	Pyr concentration	
$[D]$	mol/L	Neu5Ac concentration	
$[NAL]$	g/mol	NAL concentration	
$A\max h$	U/mg	Maximal specific activity, synthesis	(13.8 U/mg)
$A\max r$	U/mg	Maximal specific activity, cleavage	(8.5 U/mg)
$k_{m,B}$	mol/L	Michaelis-Menten constant, ManNAc	(402.2 mmol/L)
$k_{m,C}$	mol/L	Michaelis-Menten constant, Pyr	(0.136 mmol/L)
$k_{m,D}$	mol/L	Michaelis-Menten constant, Neu5Ac	(9.44 mmol/L)

$k_{I,B}$	mol/L	Inhibition constant for ManNAc	(23.76 mmol/L)
$k_{I,C}$	mol/L	Inhibition constant for Pyruvate	(1.301 mmol/L)
t	min	Time	
U	μmol/min	Enzyme unit	

B.4.2 Alkaline epimerisation kinetic model (Salo et al., 1976)

$$\frac{d[B]}{dt} = k_a[A] - k_b[B]$$

Where

$[A]$	mol/L	GlcNAc concentration
$[B]$	mol/L	ManNAc concentration
k_a	h^{-1}	Kinetic constant for GlcNAc epimerization ($12x10^{-3}$ h^{-1})
k_b	h^{-1}	Kinetic constant for ManNAc epimerization ($4.82x10^{-2}$ h^{-1}) at pH 10.5, T=25°C

B.4.3 Integrated chemo-enzymatic (alkaline epimerization-aldolase condensation) (Blayer, 1997)

Combined equilibrium constant:

$$k_{eq} = \frac{[D]}{[A][C]} = 6.728 \text{ M}^{-1}$$

B.4.4 Double enzymatic synthesis (one-pot synthesis)

$$k_{eq} = \frac{[D]}{[A][C]} = 6.76 \text{ M}^{-1}$$ (Kragl et al., 1991)

Epimerisation (Zimmermann et al., 2007)

$$r_{AGE} = \frac{\left(\frac{A^A_{V,AGE} \cdot [D]}{K^A_M} - \frac{A^B_{V,AGE} \cdot [B]}{K^{B,AGE}_M} \right)}{1 + \frac{[A]}{K^A_M} + \frac{[B]}{K^{B,AGE}_M} + \frac{[C]}{K^{C,AGE}_i} + \frac{[D]}{K^{D,AGE}_i}}$$

Aldolase condensation (Zimmermann et al., 2007)

$$r_{NAL} = \frac{\left(\frac{A^f_{V,NAL} \cdot [B] \cdot [C]}{K^C_i \cdot K^B_M} - \frac{A^r_{V,NAL} \cdot [D]}{K^D_M} \right) \cdot \frac{1}{1 + \frac{[C]_0 + [B]_0 + [A]_0 + [D]_0}{K_V}}}{1 + \frac{[C]}{K^C_i} + \frac{K^C_M \cdot [B]}{K^C_i \cdot K^B_M} + \frac{[B] \cdot [C]}{K^C_i \cdot K^B_M} + \frac{[D]}{K^D_M} + \frac{[D] \cdot [B]}{K^B_i \cdot K^D_M}}$$

Where

$$A^A_{v,AGE} = k^A_{AGE}[AGE]$$

$$A^{B}_{v,AGE} = k^{B}_{AGE}\left[AGE\right]$$

$$A^{f}_{v,NAL} = k^{f}_{NAL}\left[NAL\right]$$

$$A^{r}_{v,NAL} = k^{r}_{NAL}\left[NAL\right]$$

$$\left[AGE\right] = \frac{C_{AGE}}{\Omega_{w,AGE} \cdot M_{AGE}}$$

$$\left[NAL\right] = \frac{C_{NAL}}{\Omega_{w,NAL} \cdot M_{NAL}}$$

And

r_{AGE}	epimerization reaction velocity, mol/L.min
r_{NAL}	aldolase condensation reaction velocity, mol/L.min
k^{A}_{AGE}	Kinetic constant for the enzyme epimerase, forward reaction, mol(s)/mol(e).min
k^{B}_{AGE}	Kinetic constant for the enzyme epimerase, reverse reaction, mol(s)/mol(e).min
k^{f}_{NAL}	Kinetic constant for the aldolase enzyme, forward reaction, mol(s)/mol(e).min
k^{r}_{NAL}	Kinetic constant for the aldolase enzyme, reverse reaction, mol(s)/mol(e).min
K^{A}_{M}	Michaelis-Menten constant for GlcNAc (epimerization), mol/l
$K^{B,AGE}_{M}$	Michaelis-Menten constant for ManNAc (epimerization), mol/l
K^{B}_{M}	Michaelis-Menten constant for ManNAc (aldolase condensation), mol/l
K^{C}_{M}	Michaelis-Menten constant for Pyr (aldolase condensation), mol/l
K^{D}_{M}	Michaelis-Menten constant for Neu5Ac (aldolase condensation), mol/l
$K^{C,AGE}_{i}$	Pyr inhibition constant (epimerization), mol/l
$K^{D,AGE}_{i}$	Neu5Ac inhibition constant (epimerization), mol/l

K_i^B	ManNAc inhibition constant (aldolase condensation), mol/l
K_i^C	Pyr inhibition constant (aldolase), mol/l
$[AGE]$	Enzyme epimerase concentration, mol/l
$[NAL]$	Enzyme aldolase concentration, mol/l
$[A]_0$	GlcNAc initial concentration,mol/l
$[B]_0$	ManNAc initial concentration,mol/l
$[C]_0$	Pyr initial concentration,mol/l
$[D]_0$	Neu5Ac initial concentration,mol/l
$[A]$	GlcNAc concentration,mol/l
$[B]$	ManNAc concentration,mol/l
$[C]$	Pyr concentration,mol/l
$[D]$	Neu5Ac concentration,mol/l
K_V	Inhibition constant describing the viscosity of the medium,mol/l
C_{epi}	Enzyme epimerase concentration,U/l
C_{ald}	Enzyme aldolase concentration,U/l
$\Omega_{w,epi}$	Epimerase specific activity,U/g
$\Omega_{w,ald}$	Aldolase specific activity,U/g
$\gamma_{v,epi}^A$	Epimerase volume-specific activity for the forward reaction,U/l
$\gamma_{v,epi}^B$	Epimerase volume-specific activity for the reverse reaction,U/l

$\gamma_{v,ald}^{f}$	Aldolase volume-specific activity for the forward reaction,U/l
$\gamma_{v,ald}^{r}$	Aldolase volume-specific activity for the reverse reaction,U/l
M_{epi}	Epimerase molecular weight,g/mol
M_{ald}	Aldolase molecular weight,g/mol

Parameters values:

k_{AGE}^{A}	9.77 x 10^{-5}	mol substrate/mol enzyme.min
k_{AGE}^{B}	2.13 x 10^{-3}	mol substrate/mol enzyme.min
k_{NAL}^{f}	4.80 x 10^{-6}	mol substrate/mol enzyme.min
k_{NAL}^{r}	6.71 x 10^{-6}	mol substrate/mol enzyme.min
K_{M}^{A}	1.76 x 10^{-2}	mol/l
$K_{M}^{B,AGE}$	9.93 x 10^{-2}	mol/l
K_{M}^{B}	1.31 x 10^{-2}	mol/l
K_{M}^{C}	9.41 x 10^{-2}	mol/l
K_{M}^{D}	4.26 x 10^{-2}	mol/l
$K_{i}^{C,AGE}$	0.146	mol/l
$K_{i}^{D,AGE}$	0.719	mol/l
K_{i}^{B}	1.19 x 10^{-2}	mol/l
K_{i}^{C}	8.49 x 10^{-3}	mol/l
K_{V}	0.035	mol/l

APPENDIX B.4

Operational Constraints

For aldolase condensation:

Experimental limits (Kragl et al., 1992) :

305 mM ManNAc	0.55 M ManNAc	750 mM ManNAc
562 mM Pyr	1.05 M Pyr	
	Mass balance complete	
	94.4 conversion ManNAc	

Solubility limits (Blayer, 1997):

1.6 M ManNAc

3.6 M Pyr

pH 7.5

Optimum pH between 7.0-7.5

Final obtainable concentration arbitrarily fixed to 0.2 M Neu5Ac at equilibrium

Demands of subsequent DSP: arbitrarily set to a ratio of 10-fold Neu5Ac to Pyruvate, important in ion exchange chromatography (Auge et al., 1984)

T = 25^{o}C is suitable operation condition

For Batch Stirred Tank Reactor Aldolase Condensation

Neu5Ac aldolase immobilized on Eupergit beads. 33 g/L

990 mM Pyr

456 mM ManNAc

2.17 fold Pyr molar excess used to drive the equilibrium towards a higher yield on ManNAc, which is more expensive (Sigma, 2010)

Immobilised aldolase concentration of 43% w/v chosen to test the higher limits of operation.

For Fed-Batch Stirred Tank Reactor Aldolase Condensation (intermittent feeding)

Pyruvate feeding minimizes the detrimental effects of enzyme kinetics and evaluates reactor options for the implementation of continuous ion exchange chromatography integrated with the biotransformation step

Initial conditions:

200 mM Pyr

500 mM ManNAc

An aldolase condensation with Pyr performed with the same amount of substrates as those used in the batch reaction. A 34% increase in the initial rate of reaction observed. The reaction rate decreased when the reaction approached equilibrium.

Both ManNAc and Pyr were pulse fed in the reactor in order to maintain the advantageous initial rate, to minimize both the effect of ManNAc consumption on rate and Pyr concentration at the end of reaction and to observe due to build up to the Neu5Ac. This effect is more dominant towards the equilibrium.

Comparison of batch with double substrate fed aldolase condensation indicated the fed-batch could produce the same amount of Neu5Ac with a decrease of 90% of the residula Pyr at the end of the reaction. This decrease in residual pyruvate concentration is clearly beneficial to ion-exchange chromatography.

For Fed-Batch Stirred Tank Reactor Aldolase Condensation (continuous feeding)

Initially 550 mM ManNAc

3M Pyr solution fed at 2.8 μL/min

The reaction rate is limited by substrate feeding. Therefore, in order to achieve higher conversion rates, Pyr feeding was increased two fold, however the reaction became enzyme limited and Pyr accumulation took place. Benefical effects on reaction rate were achieved only when Pyr was fed at a constant concentration of 130 mM. In this case, a 59% increase over the batch initial reaction rate was achieved together with a Neu5Ac/Pyr ratio of 2.7 at the end of the reaction.

For Plug Flow Reactor Aldolase Condensation (continuous feeding)

19.5 mL PFR

459 mM ManNAc

182 mM Pyr

91% equilibrium conversion at 0.3 bv/h (bv, bed volumes)

Under the same conditions, only 35% conversion took place when 550 mM ManNAc with 1.6 Pyr molar excess was used.

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