



**LIFE CYCLE ASSESSMENT COMPARISON
BETWEEN PEFACTANT[®] AND CHEMICAL
SURFACTANT PRODUCTION**

MASTER THESIS

By

Huai HUANG

B.E. (Beijing University of Chemical Technology, China)

**School of Chemical Engineering
Faculty of Engineering, Computer & Mathematical Sciences**

April 2008

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

Huai HUANG

04/2008

Acknowledgements

During this 18-month research study period, I have obtained the support and assistance from many people. Firstly, I would like to express the sincerest appreciation to my supervisors, Dr. David Lewis, Prof. Anton Middelberg and Dr. Peter Ashman, for offering me academic supervision, technical assistance, financial support and enthusiastic encouragement. My principal supervisor Dr. David Lewis has spent much time on reviewing my thesis and helping me correcting my English writing as English is not my first language. My external co-supervisor Prof. Anton Middelberg has provided me with collaborative support within his research team, at the Australian Institute for Bioengineering and Nanotechnology, University of Queensland.

The other people I also would like to thank are the research team of Prof. Anton Middelberg, in particular Dr. Robert Falconer. He has supplied me the most important technical information for this project and assisted me in solving the technical problems for this study. Without his help, this project wouldn't be completed as what it is like today. Additionally, Dr. Annett Dexter, Mr. Andrew Malcolm, Ms. Belinda Hartmann and Miss Mirjana Dimitrijevic have given me valuable suggestions relating to the research methods and have facilitated the completion of experimental activities.

Last but not least, I will forever be grateful to my parents who support me both financially and mentally to complete this study and pursue a Masters Degree from overseas.

Contents

CONTENTS	I
LIST OF FIGURES	IV
LIST OF TABLES	VI
ABSTRACT	A
1. INTRODUCTION	1
1.1 SUMMARY	1
1.2 PEPTIDE SURFACTANTS	2
1.2.1 <i>Novel Surface Theory</i>	2
1.2.2 <i>Novel Peptide Surfactants</i>	4
1.2.3 <i>Pepfactants[®] Manufacture</i>	5
1.3 CHEMICAL SURFACTANTS	8
1.3.1 <i>Definition and Classification of Surfactants</i>	9
1.3.2 <i>Chemical Surfactants Production</i>	10
1.3.3 <i>Applications of Chemical Surfactants</i>	11
1.4 RESEARCH GAP	11
1.5 OBJECTIVES OF THIS RESEARCH	12
2 METHODOLOGY- LCA	13
2.1 STEPS AND PHASES	13
2.2 GOAL AND SCOPE DEFINITION	15
2.3 LIFE CYCLE INVENTORY (LCI)	16
2.4 LIFE CYCLE IMPACT ASSESSMENT (LCIA)	18
2.5 LIFE CYCLE INTERPRETATION	23
2.6 LCA IMPLEMENTATION	23
3 LCA ON PEFACTANT[®] AND CHEMICAL SURFACTANT MANUFACTURE	25
3.1 BACKGROUND	25
3.2 SCOPE OF THE LCA	26

3.2.1	<i>Function of Surfactants</i>	26
3.2.2	<i>Functional Unit, Assumptions and Limitations</i>	27
3.2.3	<i>System Boundaries</i>	29
3.3	LIFE CYCLE INVENTORY	31
3.3.1	<i>Procedures for Data Collection</i>	31
3.3.2	<i>AM1 Manufacture Model and Process Assumptions</i>	32
3.3.3	<i>LAS Production Process and Assumptions</i>	41
3.3.4	<i>Life Cycle Inventory</i>	42
3.4	IMPACTS ASSESSMENT	50
3.4.1	<i>Impact Categories</i>	50
3.4.2	<i>Classification and Characterisation</i>	50
3.4.3	<i>Processing the Inventory and Assessment</i>	53
3.5	EXTENDED LCA FOR PEPFACTANT [®] AM1	63
3.6	CONCLUSIONS	69
4	FOAMING TESTS ON SURFACTANTS	70
4.1	BACKGROUND	70
4.2	METHODOLOGY	71
4.2.1	<i>Foaming Experiment Overview</i>	71
4.2.2	<i>Determination of Foaming Experiment Method</i>	72
4.3	MATERIALS AND TESTS	73
4.3.1	<i>Materials</i>	73
4.3.2	<i>Tests</i>	74
4.4	RESULTS	76
4.5	CONCLUSIONS	77
5	RESULTS AND DISCUSSION	79
5.1	COMPARISON RESULTS TOWARDS LCA PROFILES	79
5.1.1	<i>Raw Materials</i>	79
5.1.2	<i>Energy Requirements</i>	82
5.1.3	<i>Atmospheric and Aqueous Emissions</i>	83
5.1.4	<i>CO₂ Emissions (for energy requirements)</i>	86
5.1.5	<i>LCA Comparison</i>	87
5.2	FOAMING ABILITY COMPARISON	89

5.3 LCA IMPROVEMENT	91
6 CONCLUSIONS	93
6.1 CONCLUSIONS	93
6.2 FUTURE WORK	95
REFERENCES	96
APPENDIX A CHEMICAL SURFACTANTS PRODUCTION	107
APPENDIX B APPLICATIONS OF CHEMICAL SURFACTANTS	110
APPENDIX C SHORT DESCRIPTION OF LCIA METHODS	114

LIST OF FIGURES

Figure 1.1 Flow sheet of purification process of AM1	8
Figure 2.1 Phases and applications of an LCA (AS/NZS ISO 14040, 1998)	15
Figure 2.2 A “from cradle to grave” LCA system boundary	16
Figure 3.1 Assessment Boundary of LCA on Pepfactant® AM1 Manufacture	30
Figure 3.2 Assessment Boundary of LCA on LAS production	30
Figure 3.5 Percentage contributions of AM1 production across all LCA impact categories	56
Figure 3.6 Percentage contributions of LAS production across all LCA impact categories	61
Figure 3.7 Direct carbon dioxide emission trace with raw materials	64
Figure 3.8 Indirect carbon dioxide emission trace with energy depletion	65
Figure 3.9 Relative public power generation by sources in 2003 (IEA, 2005)	66
Figure 3.10 Energy reduction potential with highest efficiencies included countries (Graus <i>et al</i> , 2007)	67
Figure 3.11 CO ₂ savings potential with highest efficiencies included countries (Graus <i>et al</i> , 2007)	67
Figure 3.12 Scenario of Pepfactant® AM1 manufacture model with recycling water	69
Figure 4.1 Schematic of apparatus used for foaming tests	74
Figure 4.2 Experimental set up of foaming tests	75
Figure 4.3 Concentration dependence of Pepfactant® AM1 foams (25mM HEPES pH 7.4 200µM Zn)	76
Figure 4.4 Concentration dependence of LAS foams (25mM HEPES pH 7.4)	77
Figure 5.1 Raw materials depletion for Pepfactant® AM1 production	80

Figure 5.2 Raw materials depletion for LAS production	81
Figure 5.3 Energy requirement comparison between Pepfactant® AM1 and LAS production	82
Figure 5.4 Atmospheric and aqueous emissions for Pepfactant® AM1 production	83
Figure 5.5 Atmospheric and aqueous emissions for LAS production	84
Figure 5.6 CO ₂ emissions for energy requirements	86
Figure 5.7 LCA comparison between Pepfactant® AM1 and LAS production	87
Figure 5.8 Extended LCA comparison between Pepfactant® AM1 and LAS taking account of usage	90

LIST OF TABLES

Table 1.1 Classification of chemical surfactants	10
Table 2.1 LCIA methods and software	19
Table 3.1 Process Conditions for the production of 1000 kg AM1	39
Table 3.2 Raw material, energy and products including emissions data for AM1 manufacture on industrial scale and functional unit	43
Table 3.3 Raw materials, energy and emissions process data for discrete processes in AM1 manufacture	45
Table 3.4 Breakdown of Electricity Requirements for AM1 Manufacture	46
Table 3.5 Raw materials, Energy and Emissions Process Data for Discrete Operations Involved in LAS Production (Pittinger <i>et al</i> , 1993)	48
Table 3.6 LCA Characterisation factors for AM1 manufacture (Heijungs, 1992)	51
Table 3.7 LCA Characterisation factors for LAS production (Heijungs, 1992)	52
Table 3.8 LCA impact indicators for 1000 kg of AM1 manufacturing process (CML 1992)	54
Table 3.9 LCA impact indicators for 1000 kg of LAS manufacturing process (CML, 1992)	59

Abstract

Recently designed Pepfactants[®] are an innovative type of nano-technological products, which could potentially replace conventional surfactants in broad-ranging applications. Currently, Pepfactants[®] technology is still in an initial design period at the laboratory scale. In order to develop the industrial-scale production of Pepfactants[®], the design group has proposed simulated strategies for industrial-scale Pepfactants[®] manufacture and a desire to improve these strategies with regards to sustainability.

This project aimed to assist Pepfactants[®] designers to understand the environmental footprint of simulated Pepfactant[®] AM1 manufacturing process, using the methodology of Life Cycle Assessment (LCA) – a comprehensive tool to quantify the environmental impacts from products and processes. To find the environmental shortcomings of the proposed manufacturing process for Pepfactant[®] AM1, the LCA outcomes were compared with published life cycle information of traditional chemical surfactant Lineal Alkylbenzene Sulphonate (LAS) production. Following LCA methodology, a life cycle inventory was compiled based on the simulated AM1 manufacture, which determined the environmental impact assessment for both AM1 and LAS production. In the LCA boundaries disregarding the usage of both surfactants, the quantitative LCA comparison results indicated that raw material and energy requirements of AM1 manufacture were much higher than LAS production, estimated to be 3,186 t/t AM1 against 31.1t/t LAS and 1,564,000MJ/t AM1 against 69,870MJ/t LAS respectively. Additionally, compared with LAS production, enormous water consumption (2,651 t/t AM1) and CO₂ emission (522 t/t AM1) were also shown to be severe environmental problems for AM1 manufacture. Furthermore, the AM1 manufacture presents apparent problems with environmental impacts of nitrification, human toxicity, photochemical oxidant formation and acidification in comparison with LAS production.

Other than providing the optimisation point in the view of environmental impacts for Pepfactant[®] AM1 manufacture, the results of experimental work in this project

showed that as the surfactant concentration increases a greater foam height of Pepfactant[®] AM1 was achieved than when (from 7mm to 52mm between 15 μ M and 100 μ M) compared with LAS (from 8mm to 53mm between 31.3 μ M and 2,000 μ M) in the same aeration duration. This result demonstrated the great potential of AM1 to replace LAS based on the LCA functional unit – 1 tonne of products. The experiments results implied that 1 tonne of AM1 is able to have the same foaming ability as approximate 25 tonnes of LAS. Consequently, the environmental impacts from Pepfactant[®] AM1 manufacture are reduced by 25 times in the extended LCA boundaries linked to the quantitative usage comparison of these two surfactants.

1. INTRODUCTION

1.1 Summary

Biotechnology and nanotechnology have been applied to synthesising an increasing range of chemical products in many areas, such as engineering, manufacturing, medicine, food and water treatment. Recently, the research group led by Prof. Anton Middelberg from the University of Queensland (UQ) has designed synthetic peptides using nano-technological approaches to produce novel self-assembled materials (Dexter *et al*, 2006). This group of researchers has found that these peptide materials with self-assembled configurations and structures present novel stimuli-responsive properties.

Molecular self-assembly is a material synthesis technique which can design and construct molecules at nano-scale so that shape-complementarity causes them to aggregate into desired structures (Whitesides, 1995). The new peptide materials designed at UQ are based on the self-assembly in bulk aqueous solution or at solid-fluid interfaces (Dexter *et al*, 2006). They have been designed to be stimuli-responsive surfactants and patented as Pepfactants[®] (International Patent Application PCT/AU2006/000236). They are able to switch molecular structures at solution interfaces by responding to the adjustments in the composition of the aqueous environment. Pepfactants[®] are believed to be the first surfactants that can reversibly control the formation, stabilisation and destabilisation of emulsions and foams.

Since the importance of environmental awareness and protection has been recognised, it has become necessary to pay attention to reducing environmental impacts associated with the manufacture and utilisation of products. Life Cycle Assessment (LCA) is a tool used for the purpose of developing methods to better comprehend and quantify environmental impacts (Azapagic, 1999).

Currently, Pepfactants[®] are still at the design stage and the manufacturing process needs optimisation before industrial scale production can be established. Of the

Pepfactants[®] family, industrial manufacturing process has been proposed for AM1 – a 21 residue self-assembled peptide, functionalised as a stimuli-responsive surfactant. To improve this Pepfactant[®] manufacture, there are some supportive and strategic considerations contributing to decision-making for optimising the proposed process at this early stage. One of these is from the sustainability point of view, in particular approaching Life Cycle Assessment, with respect of evaluating the sustainability and environmental compatibility of this new production.

The main objective of this research was to perform a LCA to evaluate the environmental sustainability of a proposed large scale process for the manufacture of Pepfactant[®] – AM1. To achieve this, the Pepfactant[®] AM1 was compared with the chemical surfactant LAS (linear alkylbenzene sulphonate), which is one of the most widely used surfactants, primarily in laundry detergents and cleaning products (P&G Scientists, 2002). The LCA on LAS used the existing life cycle information produced by Pittinger *et al* (1993). Additionally, to extend the LCA comparison boundaries, which covered the functionality of different surfactants, this research also included a foaming ability comparison based on test result analysis.

1.2 Peptide Surfactants

The recently deigned self-assembling functional peptides not only have the fundamental properties of surfactants but also have superior foaming attributes that are important properties of stimuli-responsive surfactants, i.e. solid foaming ability as well as the switchibility and reversibility between different states.

1.2.1 Novel Surface Theory

As a result of self-assembly strategy applied to synthesise novel nano-materials, the macroscopic properties of a surface can be intelligently controlled by altering the states of the modified molecules (Liu *et al*, 2005).

Switchability and reversibility of the designed surfaces enables the properties of a surface to be changed reversibly by responding to the external stimulatives. Recently, increasing attention has been paid to the development of controlled switchable surfaces, also known as “smart surfaces” (Milner, 1991). Reversible control of the surface properties have been achieved with various methods, including photo illumination, thermal driving, electrical potential effects and surrounding media treatment. Various surface properties, such as wettability, adhesive features, roughness and biocompatibility have been demonstrated to switch reversibly on different kinds of specially designed surfaces of nano-materials based on polymers, self-assembly monolayers (SAM) and metal oxides (Liu *et al*, 2005).

Other studies have implied the control of properties of fluid-fluid interfaces are important for industrial applications using surfactants (Bos and Vliet, 2001; Wilde, 2000). Particularly, the interfacial effects between fluids that result in various application of foams, emulsions, and detergents. Preparation of a stable foam or emulsion requires the formation of an interfacial architecture that can inhibit coalescence of the dispersed phases (air and water, or oil and water) (Dexter *et al*, 2006).

The theory behind solid foam ability is complex. Generally, there are two forms of surfactants capable of forming stabilising interfacial architectures: low-molecular weight detergents with high lateral mobility in the interface, and polymers, including proteins, which form a cohesive interfacial film (Dexter *et al*, 2006). Although the Gibbs-Marangoni mechanism is understood, by which interfacial architectures with high mobility contribute to foam and emulsion stability, the mechanisms by which cohesive films contribute to the stability of foams and emulsions are still unclear. Wilde *et al* (2004) postulated that the Gibbs-Marangoni mechanism requires fast diffusion or movement of emulsifiers at the interface to reduce surface concentration gradients that may occur. However, more detailed understanding of other unknown mechanisms could be attained with the benefit of improved molecular approaches in future work.

1.2.2 Novel Peptide Surfactants

On the basis of the effects of hydrogen-bonding, hydrophobic, charge-charge and metal-ion binding related to the structures of dissolved peptides, it has been possible to create peptide sequences that self-assemble in liquid or at solid interfaces with a predictable manner (Dexter *et al*, 2006). Previous researches demonstrated that self-assembly is able to produce stimuli-responsive peptides that gel by responding to the variations in pH, temperature or salt concentrations (Petka *et al*, 1998; Schneider *et al*, 2002; Hong *et al*, 2004).

Despite extensive work on the behaviour of switchable bulk structures, there has been only one focused on switchable modulation at a soft interface (i.e. air, oil, water), reported by Dexter *et al* (2006). In this work, the researchers introduced the recently designed 21-residue peptide - AM1, based on the amphipathic peptide Lac21 (peptide sequence Ac-MKOLADSLHQLARQVSRLEHA-CONH₂) (Fairman *et al*, 1995) and its reversibility and switchability of converting between a cohesive 'film state' and a mobile 'detergent state' at a fluid-fluid interface. The importance of AM1 is that self-assembly can lead to active and reversible inter-conversion between film and detergent states. This mesoscopic change causes a previously unreported and remarkable macroscopic change – the rapid coalescence of a previously stable foam or emulsion, in a reversible manner (Wilde, 2000). Possessing new foaming properties, this designed peptide enables the emergence of a novel class of surfactant that diminishes the use of harsh chemicals functionalised as surfactants and thus offers environmentally friendly approaches to control the stability of interfaces in foams, emulsions and fluid-fluid interfaces.

In regard of chemical surfactants, it is widely known that pH and the concentration of mono- and divalent counter-ions in bulk solutions contribute to the surface potential and pressure area isotherms of ionisable surfactants. Recently, switching of interfacial tension has been reported for designed chemical surfactants using triggers such as pH (Sengupta and Papadopoulos, 1994; Kanidky *et al*, 2000), metal binding (Sengupta and Papadopoulos, 1999), chemo degradation (Jong and Abbott, 2000; Chen *et al*, 2000), electrochemical oxidation (Gallardo and Abbott, 1997) and ultraviolet irradiation (Shin and Abbott, 1999). However, none of these triggers cause rapid and

complete coalescence on a timescale of seconds, as achieved for Pepfactants[®] and many display restricted functionality compared with Pepfactants[®] (e.g. chemodegradable surfactants are not reversible, oil-soluble surfactants are applicable for emulsions but not foams.)

So far, the Pepfactants[®] design group has been the first to realise that peptide structures are able to be controlled at a fluid-fluid interface. The designed amphipathic peptide creates interfacial assembly reversibly and active switching between movable and cohesive states in aqueous conditions (Dexter *et al*, 2006). Pepfactant[®] AM1 operates by a unique mechanism – foams and emulsions are stabilised by a cohesive film with no change in surface tension. The outcome offers significant potential for the development of processes for current products and novel film- or detergent-based products (Bos and Vilet, 2001; McClements, 2004; Martin *et al*, 2002; Abbott, 2001; Whidhab *et al*, 2005; Middlelburg and Jones, 2003).

1.2.3 Pepfactants[®] Manufacture

Currently, the industrial scale manufacture of Pepfactants[®] has not been established. However, the designers have proposed some options for scale-up process strategies for Pepfactant[®] AM1 manufacture based on an economics study of inclusion body processing by Lee *et al* (2006). To support the proposed strategy, it was necessary to make a series of process assumptions, regarding both mass and energy balance. There are three primary options for the strategy, summarised by Yadav (2006), the common principle for which is that soluble peptide AM1 is attached to a soluble fusion vector – Maltose Binding Protein (MBP) with a tobacco etch virus protease (TEVP) consensus sequence in between as well as *E. coli* is used as the host for the fermentation process.

The first simulation strategy employs enzymatic cleavage and ethanol precipitation. In this process, the recombinant *E. coli* is stimulated to produce soluble fusion peptide in the cytoplasm, cells were homogenised and the rest of the cell proteins were precipitated out by the Cohn's ethanol precipitation method. For the first step of

precipitation 30% ethanol solution is used. After the precipitation reaction, the solution is filtered and the fusion peptide is cleaved using TEVP. The cleaved peptides are dissolved in 70% EtOH solution that precipitates out MBP and TEVP, and the pure AM1 can be derived. The precipitated peptides are filtered out while the solution is vacuum distilled to concentrate AM1 and recover ethanol, which is then recycled.

The second option is chemical cleavage and ethanol precipitation, which is similar to the precipitation method except in place of the enzymatic cleavage, the process involves chemical cleavage using 1-Cyano-4-dimethylaminopyridinium tetrafluoroborate (CDAP) and tris (2-carboxyethyl) phosphine (TCEP). A different fusion peptide is used to accommodate the chemical cleavage step. Instead of a TEVP consensus sequence a long cysteine sequence was used to create a fusion peptide of MBP and AM1. CDAP is used for cyclizing free cysteines, which form the link between MBP and AM1 in the fusion peptide. It is important the cysteine remains independent and does not form a sulphide link or pair up with other cysteines, for this purpose, 8M urea and TCEP are added. These are established denaturant and reducing agents respectively. A pH of 7.0 is maintained throughout the reaction. After chemical cleavage, MBP is precipitated with 70% EtOH, the solution is filtered, vacuum distilled and pure AM1 is recovered.

The third strategy is to utilise the affinity tag property of Maltose Binding Protein (MBP). After the fermentation and homogenisation, the broth is diluted with water and passed through a cellulose based affinity column. This cellulose resin is assumed very cheap, but with affinity similar to the commercial amylose resin, which has an affinity of 3 g/l. After the fusion peptide is purified, it is cleaved with help of TEVP, therefore this process utilises a fusion peptide similar to the one used in the enzymatic cleavage process. After the enzymatic cleavage the same affinity column is utilised to remove MBP and purify AM1. TEVP is precipitated out with help of ethanol.

To determine an economical strategy for AM1 manufacture process, Engelbrecht (2007) has investigated the production and purification of the peptide AM1 using the

previous three strategies. For estimating the gross expense of industrial scale AM1 processing, each scale-up strategy was simulated under appropriate process assumptions by using the Super Pro Designer software Version 6 (Intelligen Inc., Scotch Plains, NJ, USA). The reason for the designers selecting this simulation tool is its advantage in estimating and analysing the cost for the entire manufacture process. Also, the Super Pro Designer software is powerful enough for estimating the component data of operations, the amount of material and waste disposal, based on the mass balance in the life cycle of the manufacture of AM1.

According to Engelbrecht's work (2007) with simulation results, the first strategy, employing enzymatic cleavage and ethanol precipitation was identified as the most economic process for AM1 production. The flow-sheet of the scaled up process based on the first strategy is shown in Fig.1.1. This industrial scale process was assumed to produce purified functional peptide AM1 from an annual operating time of 330 days (Yadav, 2006).

Briefly, the process can be described using the following steps. The prepared media was fermented with the inoculated *E.coli*. Biomass would be obtained after diafiltration and homogenisation. Then, peptide MBP-AM1 was cleaved and MBP was precipitated with the addition of ethanol. Finally, peptide AM1 was purified by spray drying. Importantly, to reduce the use of ethanol, the designers have proposed the operation of vacuum distillation to recycle the excess ethanol.

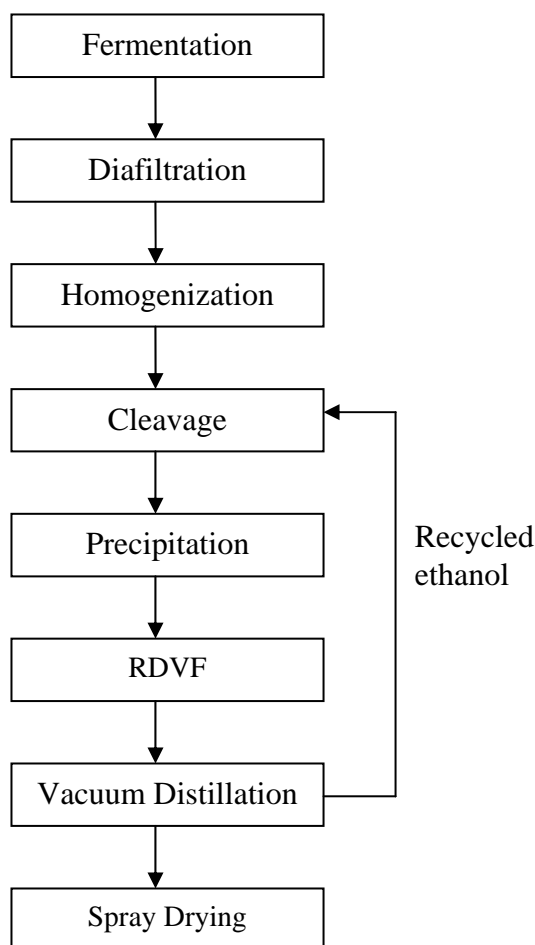


Figure 1.1 Flow sheet of purification process of AM1

1.3 Chemical Surfactants

Surfactants are a distinctive category of chemical compounds, versatile and adaptable in many applications, including mineral and petroleum processing, biological systems, health and personal care products, foods, and crop protection. Generally, they are chemically synthesised. As a class of vital products, surfactants should be studied in terms of sustainability, particularly when the novel competent Pepfactants® emerge. In this way, it is feasible to identify the environmental advantages or disadvantages for different surfactant products.

1.3.1 Definition and Classification of Surfactants

Surfactants are amphiphilic compounds with both hydrophobic tails – lacking affinity with water and hydrophilic heads – easily contactable with water. The common property of these compounds is they can reduce surface and interfacial tensions by accumulating at the interface of immiscible fluids and increase the solubility, mobility, bioavailability and subsequent biodegradation of hydrophobic or insoluble organic compounds (Ajay *et al*, 2006).

Surfactants reduce the surface tension of water by adsorbing at the liquid-gas interface and reduce the interfacial tension between oil and water by adsorbing at the liquid-liquid interface. Many surfactants are able to form micelles in the bulk solution due to the presence of both hydrophobic and hydrophilic groups in one molecule. The concentration at which this occurs is known as the Critical Micelle Concentration or CMC (Haigh, 1996). When dissolved in water at low concentration, surfactant molecules exist as monomers. At higher concentrations, the system's free energy can be reduced by the aggregation of the surfactant molecules into micelles with the hydrophobic groups located at the centre and hydrophilic head groups towards the solvent (Ying, 2005).

Surfactants have a very broad range of applications, including detergents, fabric softeners, emulsifiers/demulsifiers, paints, adhesives, inks, alveoli, wetting agents, wax, foaming/defoaming, laxatives, agrochemical formulations, quantum dot coating, biocides, hair conditioners and spermicide.

The compounds are classified according to the charge present in the hydrophilic groups: anionic surfactants, cationic surfactants, nonionic surfactants and ampholytic surfactants. The basic classification of chemical surfactants in accordance with the different hydrophilic groups is shown in Table 1.1.

Table 1.1 Classification of chemical surfactants

<i>Classifications</i>	<i>Surfactants</i>
Anionic (based on sulfate, sulfonate or carboxylate anions)	Sodium dodecyl sulfate (SDS), ammonium lauryl sulfate, and other alkyl sulfate salts; sodium laureth sulfate (SLES); alkyl benzene sulfonate; soaps or fatty acid salts
Cationic (based on quaternary ammonium cations)	Cetyl trimethylammonium bromide (CTAB), cetypyridinium chloride (CPC), polyethoxylated tallow amine (POEA), benzalkonium chloride (BAC), benzethonium chloride (BZT)
Nonionic	Alkyl poly, alkyl polyglucosides, fatty alcohols
Ampholytic	Dodecyl betaine, dodecyl dimethylamine oxide, cocamidopropyl betaine, coco ampo glyciante

1.3.2 Chemical Surfactants Production

The work of Davidson *et al.* (1987), Painter (1992) and Porter (1991) describes the synthesis approach for various surfactants, classified as anionic, cationic, non-ionic and ampholytic. The detail is referred to Appendix A.

1.3.3 Applications of Chemical Surfactants

Attributed to the unique properties and multitalented functions, surfactants play a critical role in widespread fields with practical applications. They are mainly used for ore flotation, detergency, foams and froths, defoaming, emulsions and demulsification (referred to Appendix B).

1.4 Research Gap

As an innovative nano-technological product, synthetic peptide surfactants have the potential to replace the harsh chemical surfactants that have a negative environmental impact, e.g. release toxic pollutants (Venhuis *et al*, 2004). However, it is at the initial design period for this product and tremendous work is required to be undertaken in order to develop this new type of surfactant.

At present, Pepfactants® designers have started to consider exploiting the industrialisation and commercialisation for their recently patented surfactants. They have proposed the industrial manufacture process for specific Pepfactant® and performed the economic evaluation for the proposed case. In the mean time, Pepfactants® designers have realised that sustainability study is also of significance for optimising the process in the long term. Hence, the aim of this research is to assist Pepfactants® designers to determine the environmental footprint of the proposed manufacturing process in terms of sustainability. In particular, this sustainability study will primarily implement Life Cycle Assessment (LCA) on Pepfactant® AM1 and compare it with a chemical surfactant manufacturing process. The LCA comparison results will provide supportive feedback for Pepfactants® designers before they make the decisions on the production strategy with the respective of sustainability. The knowledge gained from this study will help researchers with determining the marketability of nano-technological products.

1.5 Objectives of this Research

The primary objective of this project was to determine the sustainability of simulated industrial Pepfactant manufacturing process, by comparing the environmental impacts from Pepfactant production and conventional chemical surfactants production. This comparison was undertaken by carrying out a partial Life Cycle Assessment (LCA) on Pepfactant[®] manufacture and environmental impact assessment on chemical surfactant manufacture with available life cycle inventory from the literature.

Additionally, in order to extend the LCA boundaries for the comparison on Pepfactant[®] and chemical surfactant, this research aimed to investigate the basic functionality difference between them, i.e. to compare the directly measurable functional attributes. This comparison result was also linked to the LCA. The integrated comparison outcomes were expected to outline an initial LCA sketch and suggest Pepfactants[®] designers the directions leading to improving the sustainability design of the novel peptide nano-products.

2 METHODOLOGY- LCA

LCA is a powerful tool, often used as an aid to decision making in industry and for public policy (Gauthier, 2005). On the basis of the heightened awareness of sustainability and the possible environmental impacts associated with the products manufactured and consumed, LCA is applied as a methodology to comparing the possible shortcomings of Pefactants® and chemical surfactant manufacture in respect of environmental protection.

AS/NZS ISO 14040 (1998) has defined that “*LCA is a technique for assessing the environmental aspects and potential impacts associated with a product, by*

- *Compiling an inventory of relevant inputs and outputs of a product system;*
- *Evaluating the potential environmental impacts associated with those inputs and outputs;*
- *Interpreting the results of the inventory analysis and impact assessment phases in relation to the objectives of the study.”*

Therefore, conducting LCA in this research is beneficial for Pefactants® designers to determine the environmental footprint of the proposed industrial-scale process and make informed decisions on process optimisation.

2.1 Steps and Phases

Generally, life cycle refers to the whole process in a product’s life, “from the cradle to grave” (Todd and Curran, 1999) and takes account of the following steps (Ciambrone, 1997):

- Raw materials acquisition – the removal of raw materials and energy sources from the earth.
- Bulk material processing – the conversion of raw materials into a form that can be used to create a finished product.
- Product fabrication –the processing of manufactured material into a product ready to be packaged.

- Packaging/Transportation/Distribution – finished products are packaged and distributed to retail outlets or the consumer. This step also includes the transportation of the product between stages and the resulting environmental impact.
- Use, reuse and maintenance – involves all of the activities involved with the product's useful life, including energy requirements and wastes from storage and consumption.
- Recycling – includes the energy requirements and environmental wastes associated with recycling of the product.
- Disposal – includes the energy requirements and environmental wastes associated with the final disposal of the product.

For the purpose of improving product or process design, LCA should be carried out as early as possible. AS/NZS ISO 14040 (1998) has identified the LCA framework, including the following phases as shown in Fig 2.1.

- Goal and scope definition
- Life cycle inventory (LCI)
- Life cycle impact assessment (LCIA)
- Interpretation

The Goal and scope definition is the planning phase of LCA study, to identify the chosen system boundary and functional unit for comparison as well as the required level of the study detail and strength. Inventory analysis aims to quantify the inputs and outputs that cross the system boundary. In this phase, the data are obtained by calculating mass and energy balances of the system. Impact assessment is the phase to categorise, characterise and evaluate the environmental impacts of the system based on the list of inputs and outputs in the inventory. The final phase is interpretation, to report the LCA results and evaluate the possible ways to reduce the impacts of the process on the environment.

NOTE: This figure is included on page 15 of the print copy of the thesis held in the University of Adelaide Library.

Figure 2.1 Phases and applications of an LCA (AS/NZS ISO 14040, 1998)

2.2 Goal and Scope Definition

Before undertaking an LCA, it is important to identify design scenarios that will likely be involved in the life cycle stages of the process or product to be assessed. It is also important to decide the strengths of the investigation and how the methods are to be executed in regard to the objectives as well as how the results are to be reflected and interpreted from the detailed information.

The scope definition of an LCA provides a description of the product or process system boundary in terms of the life-cycle stages. The system boundary can separate the evaluated system from the environment, or separate the subsystem being studied from other subsystems or between processes from the evaluated system. For example, Fig 2.2 (SAIC, 2006) presents a typical “from the cradle to grave” system boundary for a full LCA. Furthermore, following AS/NZS ISO 14040 (1998), goal and scope definition needs to define a functional unit, preliminary assumptions and limitations of the study as well as data quality requirements for comparisons between systems.

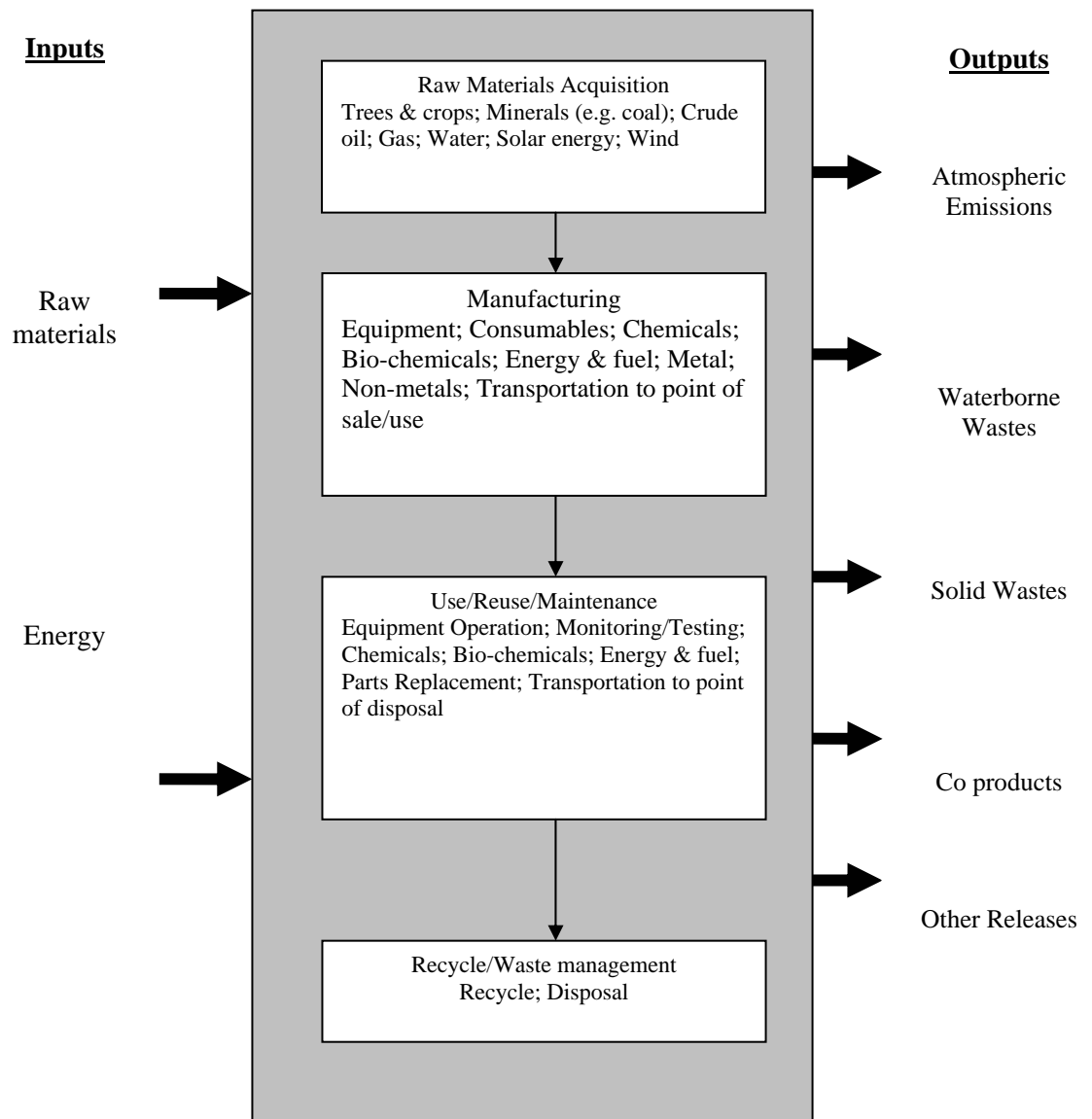


Figure 2.2 A “from cradle to grave” LCA system boundary

2.3 Life Cycle Inventory (LCI)

AS/NZS ISO 14041 (1999) has introduced that “*Life cycle inventory (LCI) involves the collection of the data necessary to meet the goals of the defined study. It is essentially an inventory of input/output data with respect to the system being studied.*” Using this explanation, a life cycle inventory refers to a series of procedures of quantifying energy and raw material requirements, atmospheric

emissions, waterborne emissions, solid wastes, and other releases or co-products for the entire life cycle of a product, process, or activity. In the life cycle inventory phase of an LCA, all relevant data is collected and organised. It provides a basis to evaluate comparative environmental impacts or potential improvement of different process scenarios or products. The components of LCI include the following steps:

- Develop the process flow being evaluated – within of the specified system boundaries, a process flow is constructed to combine all of unit processes and form a life cycle picture of the required inputs and outputs to the system. Flow diagrams are used to model all alternatives under consideration. For a comparative study, it is important that both the evaluated systems use the same system boundary and are modelled to the same level of detail (SAIC, 2006).
- Collect the process data – the data that are required are quantified, including values for the inputs to and outputs from each individual process. These data can be obtained via modelling calculations, scientific literature, published data files used by LCA practitioners, industry and government records. Sometimes collecting the data for an LCA may be the most time consuming and perhaps the most difficult of all LCA activities.
- Create the environmental data – after collecting the process data, a calculation will have to be made in order to generate the inventory list or table of the environmental data, which is derived from the data of energy and raw material consumption, emissions or releases from the system. This calculation calls for applying scaling factors to convert the process data to the values that relate to single functional unit. Aggregation of all data through compiling, namely results are listed in the inventory table.
- Evaluate and document the LCI results – the evaluation and report of the inventory should explicitly define the systems analysed and the boundaries that were set. All assumptions made in performing the inventory should be clearly explained. Life cycle inventory studies generate a great deal of information, often of a disparate nature (SAIC, 2006). The analyst needs to select a presentation format and content that are consistent with the purpose of the study.

2.4 Life Cycle Impact Assessment (LCIA)

Life cycle impact assessment (LCIA) is the phase where the results of the inventory analysis are interpreted in terms of the impacts they have on the environment. LCIA provides the basis for analysing the potential contributions of the resource extractions and waste/emissions in an inventory to a number of potential impacts. The result of the LCIA is an evaluation of a product life cycle, on a functional unit basis, quantified within several impacts categories and in some cases, in an aggregated way.

ISO 14044 (2006) illustrated both objective (mandatory) and subjective (optional) steps for conducting an LCIA, which are described below:

- Selection of the impact categories of interest – identifying relevant environmental impact categories (e.g., global warming, energy depletion, human health). For a LCA, impacts are defined as the consequences that could be caused by the input and output streams of a system on human health, plants, and animals, or the long term availability of natural resources. Usually, LCIA focuses on the potential impacts to three main categories: human health, ecological health and resource depletion.
- Classification – assigning LCI results to the impact categories (e.g., classifying carbon dioxide emissions to global warming). In some cases, emissions can contribute to several types of problem: NH₃ emissions, for example, have an effect on acidification and human health.
- Characterisation – modelling LCI impacts within impact categories using empirical conversion factors (e.g., modelling the potential impact of CO₂ emissions on global warming). This is the major step to quantify the environmental impacts and provides a way to directly compare the LCI results within each impact category. So, characterisation enables direct comparisons on impact indicators translated by inventory inputs. Impact indicators are typically characterised using Eq 2.1:

$$\text{Inventory Data} \times \text{Characterisation Factor} = \text{Impact Indicators} \quad \text{Eq 2.1}$$

- Normalisation (optional) – As impact categories have different units,

normalization is used to assist in comparing categories. Using Eq 2.2, this step normalise the indicator results by dividing a selected reference value i.e. normalisation factor. The subjectivity of normalisation lies in the dependence of normalisation factor. Different normalisation factors can lead to different normalisation results.

$$N = S / R \quad \text{Eq 2.2}$$

where N is the normalised result, S is the impact indicator from the characterisation phase and R is the reference value. For example, the normalised results can use the total emissions or resource use for a given area on a per capita basis.

- Grouping and Weighting (optional) – sorting or ranking indicators and assigning weights or relative values based on practitioners’ emphasise and concerns on the issue of environmental protection.

Currently, for performance of LCIA, there are a number of method and model options introduced in various literature and LCA software packages, which are summarised in Table 2.1. The further details of these methods are referred to Appendix C.:

Table 2.1 LCIA methods and software

<i>Method / Software Package</i>	<i>Links</i>
CML Guide	http://www.leidenuniv.nl/interfac/cml/ssp/
Eco-indicator 99	http://www.pre.nl/eco-indicator99/
EDIP 97	http://ipt.dtu.dk/~mic/EDIP97
EDIP 2003	http://ipt.dtu.dk/~mic/EDIP2003
EPS 2000d	http://eps.esa.chalmers.se/
Impact (2002)+	http://www.epfl.ch/impact
JEPIX	www.jepix.org
LIME	http://www.jemai.or.jp/lcaforum/index.cfm
Swiss Eco-Scarcity	http://www.e2mc.com/BUWAL297%20english.pdf
TRACI	http://epa.gov/ORD/NRMRL/std/sab/iam_traci.htm

It should be realised that due to the subjectivity in selection of LCIA methods, the choice of different methods could have an effect on the overall LCA results. In this research, CML (1992) was selected as the LCIA method template, as CML is an original systematic LCIA method covering different environmental impacts. With this method, the major environmental impacts could be classified as Depletion and Pollution Potentials (Heijungs, 1992).

Specifically, Depletion Potentials contain:

- a) Raw material requirement is the sum of the net quantities (kg or tonne) of each raw material used within the process – subtraction between material flowed in and out of the system:

$$\text{Raw material requirement} = \sum_i \text{raw material used}_i \text{ (tonne)} \quad \text{Eq 2.3}$$

- b) Energy requirement is the sum of electricity consumption (the primary energy source based on the simulated process), which is converted to the energy requirement:

$$\text{Energy requirement} = \sum_i \text{factor}(MJ / kwh) \times \text{electricity}(kwh) \quad \text{Eq 2.4}$$

Pollution Potentials include:

- a) Global warming potential is a relative parameter which uses CO₂ as a reference. The global warming potential of a given substance is defined as the extent to which a mass unit of the substance can absorb infrared radiation compared with a mass unit of CO₂. In this way atmospheric emissions can be converted to CO₂ emissions with an equivalent greenhouse effect:

$$\text{Greenhouse effect (GWP)} = \sum_i \text{GWPI} \times \text{emissions}_i \text{ to the air (tonnes)} \quad \text{Eq 2.5}$$

For example, a process has 2.4 tonnes CO₂ emissions and 1.5 tonnes CH₄ emissions. The GWP factor for CO₂ and CH₄ is 1 tonne/tonne and 4 tonne/tonne, respectively. Thus, the GWP for this process = 2.4 × 1 + 1.5 × 4 = 8.4 tonnes.

- b) Nutrication made by various forms of intervention in the environment can be determined by weighting with NP factors which are a measure of the capacity to form biomass, compared with phosphate (PO_4^{3-}). Emissions to the atmosphere, water or soil (tonnes) are converted, using the NP_i to an equivalent phosphate emissions (tonnes) in terms of eutrophication:

$$\text{Nutrication (NP)} = \sum_i \text{NP}_i \times \text{emissions}_i \text{ (tonnes)} \quad \text{Eq 2.6}$$

For example, a process has 1.5 tonnes NH_3 emissions to the air. The NP factor for NH_3 (air or water) is 0.33 tonne/tonne. Thus, the NP for this process = $1.5 \times 0.33 = 0.495$ tonnes.

- c) Human toxicity is assessed by a conversion made so that emissions exposed through air, water or the soil are potential to harm human health. This result in a definition of human toxicological classification factors which depend on the substance and the environmental medium concerned: for the atmosphere (HCA), for water (HCW) and for soil (HCS). The unit of the effect score is tonne and it is calculated as follows:

$$\begin{aligned} \text{Human toxicity (HT)} = & \sum_i \text{HCA}_i \times \text{emissions}_i \text{ to the air} + \sum_i \text{HCW}_i \times \\ & \text{emissions}_i \text{ to water} + \sum_i \text{HCS}_i \times \text{emissions}_i \text{ to the soil} \end{aligned} \quad \text{Eq 2.7}$$

For example, a process has 0.5 tonnes NH_3 emissions and 1 tonnes NH_4^+ emissions. The characterisation factor for NH_3 (air) and NH_4^+ (water) is 0.02 tonne/tonne and 0.0017 tonne/tonne respectively. Thus, the HT for the process = $0.5 \times 0.02 + 1 \times 0.0017 = 0.0117$ tonnes.

- d) Photochemical oxidant formation potential (POCP) is a relative measure which uses carbohydrates as a reference and is defined as extent to which a mass unit of a substance forms oxidants compared with a mass unit of carbohydrates. In this way atmospheric emissions can be converted to carbohydrates emissions (reference to effect of 1 kg C_2H_4) with equivalent oxidant formation:

$$\text{POCP} = \sum_i \text{POCP}_i \times \text{emissions}_i \text{ to the air} \quad \text{Eq 2.8}$$

For example, a process has 2.1 tonnes ethanol emissions. The POCP factor for ethanol (air) is 0.268 tonne/tonne. Thus, the POCP for this process = $2.1 \times 0.268 = 0.5628$ tonnes.

- e) Solid wastes are the overall net quantity (kg or tonne) of the non-biodegradable wastes in solid.
- f) Acidification potential (AP) is the result of the release of nitrogen and sulphur oxides into the atmosphere, on soil and water that can vary the acidity in soil and water, with influences on both plants and fauna. It is measured with a reference to the effect of 1 kg SO₂.

$$\text{Acidification potential (AP)} = \sum_i AP_i \times \text{emissions}_i \text{ to the air} + \sum_i AP_i \times \text{emissions}_i \text{ to water} + \sum_i AP_i \times \text{emissions}_i \text{ to the soil} \quad \text{Eq 2.9}$$

For example, a process has 1.5 tonnes NH₃ emissions to the air and 0.8 tonnes NH₃ emissions to the water. The AP factor for NH₃ (air) and NH₃ (water) is both 1.88 tonne/tonne. Thus, the AP for this process = $(1.5+0.8) \times 1.88 = 4.324$ tonnes.

- g) Ecotoxicity potential (EP) is the effect of toxic substances that can damage plants and fauna. Ecotoxicity is defined for both water (aquatic ecotoxicity, i.e. ECA) and soil (terrestrial ecotoxicity, i.e. ECT). ECA and ECT are measured as the volume of water and weight of soil that would be polluted to a critical level by 1 kg of substance respectively.

$$\text{Ecotoxicity potential (EP)} = \sum_i EP_i \times \text{emissions}_i \text{ to water} + \sum_i EP_i \times \text{emissions}_i \text{ to the soil} \quad \text{Eq 2.10}$$

For example, a process has 1.1 tonnes phenol emissions to the water. The EP factor for phenol (air) is 5.9 tonne/tonne and phenol (water) is 5.3 tonne/tonne. Thus, the EP for this process = $1.1 \times 5.3 = 5.83$ tonnes.

- h) Odour potential (OP) is the effect of the substances that release the smelly odour, defined by the substances on dependence of odour potential factors.

$$\text{Odour potential (OP)} = \sum_i \frac{\text{emissions}_i}{\text{OdourThresholdValue}} \quad \text{Eq 2.11}$$

For example, a process has 0.01 tonnes phenol emissions to the air. The odour factor for phenol (air) is 0.039 mg/m³. Thus, the OP for this process = 0.01 × 10⁹ / 0.039 = 2.6 × 10⁸ m³.

2.5 Life Cycle Interpretation

Life cycle interpretation occurs at every stage in an LCA. If two product alternatives are compared and one alternative shows higher consumption of each material and of energy and other impact indicators, an interpretation purely based on the LCIA can be conclusive. In interpretation, a practitioner needs to analyse results, reach conclusions, explain the limitations and provide recommendations in accordance with the findings of previous phases of LCA, and to report the LCA results.

2.6 LCA Implementation

In this research, the LCA comparison of Pepfactant® and chemical surfactant LAS manufacture is achieved by conducting the following LCA activities:

- Goal and scope definition - the system boundary and functional units will be identified for both of the surfactants manufacture process. Additionally, the assumptions and limitations will be analysed for the LCA.
- Life Cycle Inventory (LCI) analysis – the fundamental phase where the mass and energy balance will be calculated so that the resources, energy inputs as well as emissions, releases and wastes as outputs will be summarised at the specific system boundary. This particularly aims to launch a series of lists for the environmental data.
- Life Cycle Impact Assessment (LCIA) - to quantify the environmental impacts based on the inventory with the steps of classification, characterisation (the core step), and evaluation. And, in the discussion, the LCA results of

Pepfactant and chemical surfactant manufacture will be compared and interpreted in combination with the basic functional attributes of both surfactants.

It must be pointed out that if not all of the Life Cycle Assessment (LCA) can be carried out on the full life cycle (from cradle to grave), in which case the analysis is possible when terminating at an intermediate stage (from cradle to gate) or starting and finishing at certain intermediate stages (from gate to gate) (Josa *et al*, 2007). For this LCA study, the complete life cycle inventory of large-scale Pepfactant® production is unavailable at the early design stage, which makes the partial LCA (from gate to gate) appropriate and practical for evaluating possible environmental impacts from the Pepfactant® manufacture process as well as for planning more complete LCA for optimisation. In the gate-to-gate LCA, the upstream and downstream of the Pepfactant® production will not be considered while the manufacture phase will be a fundamental part in the assessment boundaries.

3 LCA on PEPFACTANT® and CHEMICAL SURFACTANT MANUFACTURE

In most situations, product design decisions and options made at an early stage are likely to result in adverse environmental impacts when the products are manufactured and used (Peregrina *et al*, 2006). As a type of innovative product, Pepfactants® are designed with biodegradable materials – self assembled peptides - in order to render novel switchability and to possess stable capability when functionalising as surfactants.

Pepfactant® designers are exploring a wide-ranging family of functional peptides and using them to synthesise more industry-applicable and environmentally compatible surfactant products (Dexter and Middelberg, 2007). It is necessary to pay attention to sustainability at the design stage to minimise the environmental impacts of Pepfactant® production, which was investigated with an LCA study. The LCA is a critical part of sustainability assessment for decision support in bio-nano-technological produced peptides at a design stage.

Furthermore, it is helpful to undertake a LCA comparison with the literature work on life cycle inventory of a widely-used chemical surfactant. The comparison performed between the LCA on both surfactants will provide instructive information for revising and optimising the Pepfactant® manufacture process.

3.1 Background

The aim of this LCA is to investigate the possible environmental impacts of Pepfactant® AM1 manufacture using an industrial-scale computer simulation model. This will assist Pepfactant designers in understanding the environmental footprint of AM1 manufacture. To display the differences on the environmental impacts from Pepfactant® and chemical surfactant production, an assessment is also conducted on

the traditional surfactant LAS (Linear Alkylbenzene Sulphonate) using an existing life cycle inventory from the literature that relates to detergent-grade surfactant production (Pittinger *et al*, 1993 and Patel *et al*, 1998). The LCA study offers base information to compare the sustainability of the proposed Pepfactant® manufacture process with traditional chemical surfactant LAS production.

3.2 Scope of the LCA

3.2.1 Function of Surfactants

➤ *Pepfactants®*

Pepfactant® technology employs the reversible switching of a collection of weak bonds, implying that unlike cleavable surfactants, the Pepfactants® are not covalently altered by the switching process, which can be repeated multiple times and is complete within seconds. Switching is achieved by a change in the bulk solution composition, such as a change in pH, or the addition or sequestering of metal ions. It enables peptides to reversibly and precisely control the stability of foams. Foam stabilisation occurs when the peptide forms a cohesive interfacial film that is bound by metal ions, while foam destabilisation occurs when peptide-metal binding fails (Dexter and Middelberg, 2007). Pepfactants® are designed to be a novel class of surfactant with many properties superior to those of traditional emulsifying and foaming agents and have the potential to yield a new class of industrially important, stimuli-responsive foams and emulsions. Importantly, the superior properties of Pepfactants® could lead to reductions in usage rates when compared with chemical surfactants.

➤ *Chemical Surfactants*

Conventionally, surfactants are synthesised chemically and applied as detergents in people's daily life. They are able to eliminate dirt or fats and make them dissolvable in the water phase. Chemical surfactants are the main components in many cleaning

and cleansing products such as laundry detergents, shampoos and hair conditioners, etc. Also, in industrial manufacturing processes, surfactants are used as antistatic agents, lubricants and levelling agents (e.g. in mining, oil production and wastewater treatment, textile production), flotation agents and emulsifiers in the food industry and for the production of colorants, coatings and plastics. One of these – LAS, Linear Alkylbenzene Sulphonate is widely used as a detergent, playing a significant role in current surfactant field (Penteado *et al*, 2006).

3.2.2 Functional Unit, Assumptions and Limitations

- Functional Unit

The purpose of a functional unit is to provide a reference to which the inputs and outputs are related (AS/NZS ISO, 1998). This reference is necessary to ensure comparability of LCA results assessed on a common basis. Functional unit describes the main function performed by a product and indicates how much of this function is considered (Heijungs, 1992). To be consistent with the surfactant manufacture processes, the functional unit of both surfactants manufacture was defined as the environmental performance per 1000 kg or per tonne of final surfactant product. For example, the functional unit of CO₂ emission for Pepfactants® production is kg CO₂/1000 kg Pepfactant® or tonne CO₂/tonne Pepfactant® (t CO₂/t Pepfactant®).

- Assumptions and Limitations

- *Pepfactants®*

Since the full-scale manufacturing of Pepfactants® is still under development, a scaled-up production and application process has been proposed with a series of assumptions. To collect data for life cycle inventory, these assumptions provide important prerequisites to carry out calculations and estimations of the process data. However, due to the limited availability of data for the large-scale process, the LCA focuses on the simulation model of Pepfactant AM1 manufacture phase. This model

(Yadav, 2006) has not included the upstream or downstream phases of Pepfactant AM1 production e.g. application, disposal and recycle. Therefore, the LCA undertaken on the AM1 manufacture process model is “from gate to gate” or partial rather than “from cradle to grave”.

➤ ***Chemical Surfactants***

The appropriate assumptions for chemical surfactant production were considered on energy, emissions and process data, which were detailed in the study of Pittinger *et al* (1993) and used with the estimation methods of FAL (Franklin Associates, Ltd.). However, the life-cycle inventory of surfactants in this study was limited to the detergent-grade surfactant technology of 1990's. Thus, the LCA may not be able to accurately reflect the environmental problems of current chemical surfactant production processes. Broadly speaking, there are an extensive series of LCA literature on surfactants for different purposes of study with various assessment methods. In this LCA, the available life cycle inventory of LAS summarised by Pittinger *et al* (1993) and Patel *et al* (1998) was applied as the source for comparison with the Pepfactant® LCA.

3.2.3 System Boundaries

Generally, system boundaries of LCA include the processes of raw material acquisition, manufacturing, use/reuse/maintenance, and recycle/disposal combined with the inputs and outputs related to the individual process (i.e. raw materials, energy, emissions, wastes and releases).

➤ *Pepfactant®AM1*

Disregarding the raw materials acquisition, energy extraction, product delivery or application, the system boundary was restricted on the currently available simulated large-scale manufacturing process model. It focused on the primary unit processes of manufacture and the proposed recycle utility of ethanol, as shown in Fig 3.1. Technically, this simulation model (Yadav, 2006) was developed with the concern of the economics of the Pepfactant AM1 processing, i.e. to reduce the ethanol use. In this system boundary, the gate-to-gate LCA analysis resources were inputs (raw materials and consumed energy) and outputs (emissions in air, water or solid wastes) associated with each life cycle stage of the manufacture process.

➤ *Chemical Surfactant LAS*

Similar with the LCA on Pepfactant AM1, the life cycle boundaries of chemical surfactant LAS were focused on the manufacturing phase of production, taking into account the environmental impact contributors on the basis of inputs and outputs through the selected production phase, including raw materials and energy requirements, aquatic, atmospheric and solid pollutions, shown in Fig 3.2.

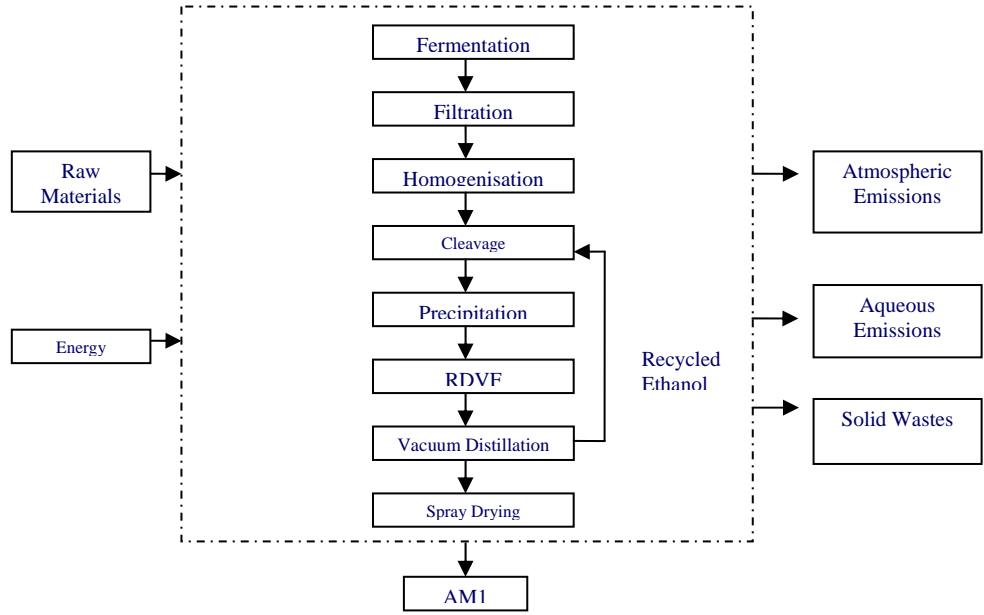


Figure 3.1 Assessment Boundary of LCA on Peppactant® AM1 Manufacture

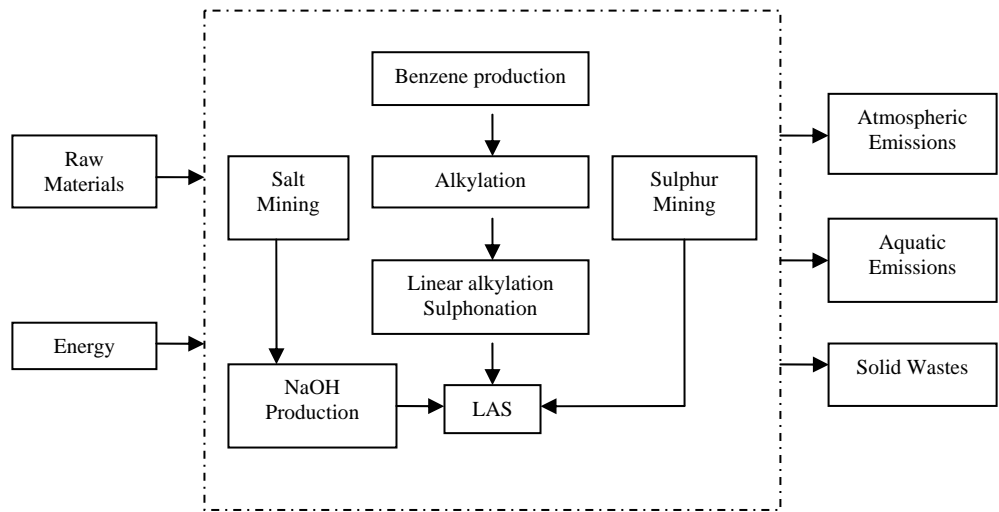


Figure 3.2 Assessment Boundary of LCA on LAS production

3.3 Life Cycle Inventory

Upon completion of identifying the scope of LCA, the next step is to conduct a life cycle inventory analysis (LCI), covering the data collection and calculation procedures (AS/NZS ISO, 1998). Inventory analysis aims to quantify the inputs and outputs that cross the system boundary. The result of an inventory is a long list of material and energy requirements, products and co-products as well as waste and releases into air, soil and water. This list is referred to as the mass and energy balance or the inventory table (Miettinen *et al*, 1997).

3.3.1 Procedures for Data Collection

To establish a life cycle inventory, the first phase is to survey and collect the life cycle data related to the product system, from inputs to outputs. In this case, the data collection was conducted on the entire Pepfactant® manufacture process, as follows:

1. Identify the inputs and outputs for each individual unit process for the industrial scale simulation of Pepfactant manufacture;
2. Undertake mass and energy balance for the entire process;
3. Quantify the amount of product, waste, material source and energy consumption;
4. Convert the industrial scale data to the baseline of selected functional unit (mass per 1000kg or tonne Pepfactant®).

3.3.2 AM1 Manufacture Model and Process Assumptions

Originally, there were three broad strategies for the production of peptides: chemical synthesis (Merrifield, 1963), transgenic production and recombinant production (Latham, 1999). Chemical synthesis is a rapid and effective method for the production of custom-made peptides in small quantities. However, this method has major drawbacks on expenses under scaled-up processes. In addition, the use of transgenic animals may be a cost-effective method for large peptide quantities but the long lead times to develop productive animal clones, and the potential to generate negative public opinion, diminish the attractiveness of this route. Recombinant peptide production in *E.coli* has the potential to avoid some of the above problems and replace other harmful chemicals in fast moving consumer goods such as detergents and emulsifiers (Morreale *et al*, 2004).

The simulation of the large scale manufacture of Pepfactant® AM1 was achieved with the use of SuperPro Designer. The software is capable of handling material and energy balances, equipment sizing and costing, economic evaluation, environmental impact assessment, process scheduling and debottlenecking of batch and continuous processes (Harrison *et al*, 2002).

- **AM1 Manufacture Model**

The simulated batch process flowsheet of Pepfactant® AM1 manufacture is displayed in Fig 3.3 and described in following sections:

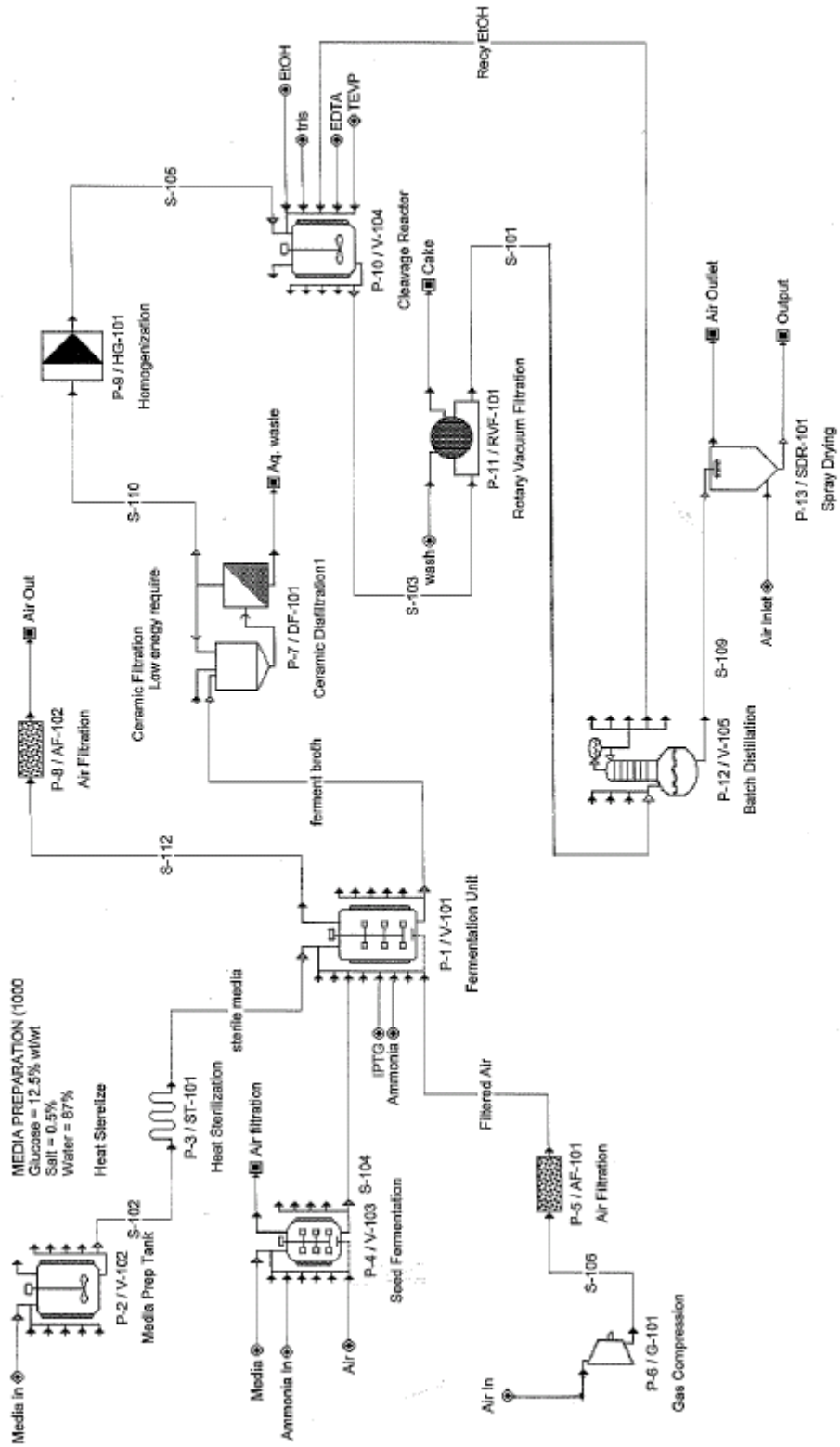


Figure 3.3 SuperPro Designer Flowsheet of Peppactant® AM1 Batch Production

- **Process Descriptions**

The inventory data for the LCA of AM1 production was based on the economic study by Yadav (2006), which was linked to an industrial scale process. This work used a variation of the SuperPro Designer flowsheet, developed from high efficiency extraction of cytoplasmic viral coat inclusion body protein processes (Lee *et al*, 2006).

From this study, the manufacturing process was scaled up using the simulation data to give an annual AM1 production of 19,000 kg, which required 422 batches, where the final output of one batch comprised 45 kg main product, 2.3 kg H₂O and 4.5 kg salt.

The industrial scale production of Pepfactant AM1 was achieved using a fusion protein containing Maltose Binding Protein (MBP) as the fusion partner and a consensus sequence enabling it to be cleaved by Tobacco Etch Virus Protease (TEVP). Recombinant *E. coli* was used as the host for the fermentation process. Downstream processing was used to liberate, cleave and purify the peptide product.

The unit operations (Fig 3.3) are described as follows and the process conditions for AM1 production are summarised in Table 3.1.

Media Preparation

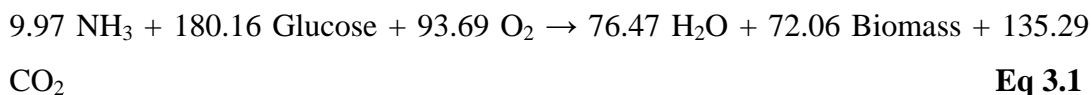
The media, containing Glucose (9.26% wt/wt), IPTG (isopropylthiogalactopyranoside, 0.01% wt/wt), Salts (0.37% wt/wt, K₂HPO₄, Na₂SO₄, NaH₂PO₄, (NH₄)₂SO₄, NH₄Cl, MgSO₄.7H₂O, citrate, Thiamine, trace salts), and Water (90.36% wt/wt) was mixed in a stirred mixing tank and passed to the fermenter through a plug flow steriliser. The sterilisation temperature was set at 140 °C, and the final exit temperature was maintained at 35 °C.

Seed Fermentation

In seed fermenter (1,390 L), media (5 L) was transferred and inoculated with *E.coli* containing the plasmid for AM1 production. Fermentation occurred in batch mode for 8 h, at 37 °C and at a pressure of 1.013 bar. A mixture of air and ammonia was provided throughout the fermentation process at 0.5 vvm (volume per volume per minute). The product obtained from the seed reactor was used for seeding the large fermenter.

Fermentation

E.coli was the host cell, capable of producing the soluble fusion peptide MBP-TEVP consensus sequence-AM1. Based on the default chemical formula for biomass ($\text{CH}_{1.8}\text{O}_{0.5}\text{N}_{0.2}$) employed in the software of SuperPro Designer package (Intelligen Inc., Scotch Plains, NJ, USA), with an assumed yield of 0.4 g biomass/g glucose, the following mass based stoichiometric equation was used to describe the fermentation reaction.



Mass-based stoichiometric coefficients were used to describe this reaction and each stoichiometric coefficient represents the mass of particular reactant used or product formed. Fermentation was carried out using a 60,000L fermenter at 37 °C, for 16 h, yielding a final cell concentration of 50 g/L dry cell weight. Glucose and ammonia were used as the carbon and nitrogen source, respectively. Compressed air was used to supply the required oxygen at 0.5vvm (volume per volume per minute).

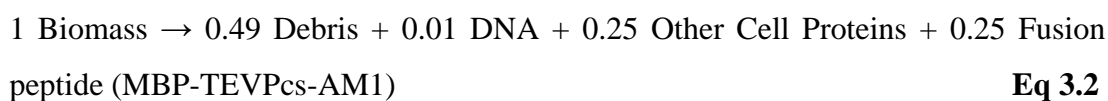
Diafiltration

A diafiltration unit was used to concentrate the fermentation broth to one third of the initial volume. The operation time for the filtration step was set as 4 h. The rejection coefficient (Smith *et al*, 2004) for the filter with respect to biomass and salts was set at 100% and 1.5% respectively, namely biomass was completely retained (retentate)

whereas 1.5% wt/wt of salts was retained on the membrane of the filter. The filtered broth was transferred to the homogeniser where the cells were ruptured.

Homogenisation

The fermentation broth was homogenised for 3 h at 25°C. The pressure drop during homogenisation was 800 bar, and exit temperature was 25 °C. The mass based stoichiometry for homogenisation is as follows:



DNA was assumed to be 1% (w/w) of the biomass (Lodish *et al.*, 1995). It was assumed that all of the biomass was broken up in a single pass through the homogeniser (Yadav, 2006).

Enzymatic Cleavage

The filtered fusion peptide solution was then transferred to a reactor and mixed with activated TEVP enzyme, where enzymatic cleavage of MBP-TEVP consensus-AM1 occurred. The reaction was carried out with the utility of 2.9 kg of TEVP. The reaction was performed at 30 °C for 2 h. The Reaction stoichiometry is as follows:



After the reaction was completed, ethanol was added to achieve a final concentration of 70% ethanol, which precipitated the cleaved MBP and TEVP.

Precipitation

With addition of 70% ethanol, all the protein was precipitated out of the solution except the AM1 peptide. This precipitation reaction was carried out for 8 hour at 25 °C. The following reaction was assumed:

1MBP (Maltose Biding Protein) → 1 Cell Debris **Eq 3.4**

The cell debris precipitated out from the protein as a precipitated globulin that was easily filtered out of the solution.

Rotary Drum Vacuum Filtration (RDVF)

After the precipitation reaction AM1 still remains soluble in 70% ethanol solution, so the solution was filtered to recover pure AM1. As AM1 is soluble, the rejection coefficient of AM1 is set at 0% whereas the precipitated cell debris, remainder of MBP, DNA and other cell proteins were set at 100%. All of these biomass residues co-produced from this operation were assumed to be non-wastes and able to be used for animal feed. The purity of AM1 filtered was assumed to be acceptable as “industrial-grade” and it would contain some salts and trace levels of bacterial cell components. Overall, the procedure can be described as below:

MBP + Cell Debris + DNA + Other Cell Proteins + Salts → Filtered Solids

Eq 3.5

Vacuum Distillation

After RDVF of AM1, the 70% ethanol solution remained and was distilled in vacuum at 0.7 bar pressure. The reboiler and condenser temperature were maintained at 50°C and 25°C respectively. Distillation was performed for 6 hours with AM1 as the only non-volatile component in the mixture. The reflux ratio for the distillation simulation was maintained at 0.5 and the mole percentage for distillation was set at 50%. In this operation, it was assumed that 98% wt/wt of the ethanol solution could be recycled while 2% wt/wt of the ethanol solution would be processed with the AM1 stream to the next unit operation.

Spray Drying

The unit process of spray drying which is used to separate and purify the peptide AM1 product from the remaining water and ethanol, from a moisture content of 91.8% wt/wt to 5% wt/wt. The final product per batch was composed of 45kg AM1 peptide, 2.43 kg water and 0.6 kg salts.

Cleaning in Place (CIP)

The other operation in the simulation model was Cleaning in Place (CIP), which was for cleaning the reactors and accessories for each unit operation. Water was the primary source for rinsing the unit operations and the NaOH was used for cleaning the salts and biomass residues. To neutralise NaOH, HCl at the same concentration was added to the processes. Therefore, the production of NaCl would be the outlet from CIP. It was assumed that 45.1 tonnes of water was required for CIP of 1 batch AM1 production, which was drained after processing. Also, 675 kg HCl with 774 kg NaOH was consumed for one CIP which subsequently neutralised to NaCl in the aqueous outlet.

Table 3.1 Process Conditions for the production of 1000 kg AM1

	Unit	Specifications
Media preparation	P-2/V-102	Glucose (9.26% wt/wt), IPTG (isopropylthiogalactopyranoside, 0.01% wt/wt), Salts (0.37% wt/wt, K ₂ HPO ₄ , Na ₂ SO ₄ , NaH ₂ PO ₄ , (NH ₄) ₂ SO ₄ , NH ₄ Cl, MgSO ₄ .7H ₂ O, citrate, Thiamine, trace salts), and Water (90.36% wt/wt) CIP (Cleaning in Place)
Seed fermentation	P-4/V-103	<i>E.coli</i> (inoculated), Media Reactor Volume: 1,390L Temperature: 37°C Pressure: 1.013 Bar Ammonia Feed: 0.5 vvm (volume/volume/minute) Reaction time: 8 h CIP (Cleaning in Place)
Heat Sterilization	P-3/ST-101	Temperature: 139°C Continuous sterilisation – including heat integration Time: 4 h CIP (Cleaning in Place)
Gas Compression	P-6/G-101	Air Feed Temperature: 40°C Pressure: 5.01 Bar
Air Filtration	P-5/AF-101	Air Feed: N ₂ 3,794.4 tonnes; O ₂ 1,151.9 tonnes Temperature: 40°C Pressure: 6.01 Bar
Fermentation	P-1/V-101	<i>E.coli</i> (inoculated), Media Reactor Volume: 60,000 L Temperature: 37°C Pressure: 1.013 Bar Ammonia Feed: 0.5 vvm (volume/volume/minute) IPTG Feed: 0.2 kg Reaction time: 16 h CIP (Cleaning in Place)
Ceramic Diafiltration	P-7/DF-101	Biomass Retentate: 100% Salts Retentate: 1.5% Temperature: 37.5°C Pressure: 1.013 Bar Residence Time: 4 h CIP (Cleaning in Place)

Homogenisation	P-9/HG- 101	No. of passes: 1 Pressure Drop: 800 Bar Temperature: 30 °C Time: 3 h CIP (Cleaning in Place)
Cleavage & Precipitation	P-10/V- 104	Cleavage: Reaction Time: 2 h Temperature: 30 °C Pressure: 1.01 Bar TEVP Feed: 2.9 tonnes Precipitation: Reaction Time: 8h Temperature: 25 °C Ethanol Feed: 225.3 tonnes Reactor Volume: 99,540 L Pressure: 1.01 Bar CIP (Cleaning in Place)
RDVF	P- 11/RVF- 101	Biomass Retentate: 0% Salts Retentate: 2% Temperature: 25 °C Pressure: 1.013 Bar Residence Time: 4 h CIP (Cleaning in Place)
Distillation	P-12/V- 105	Pressure: 0.7 Bar Residence Time: 6 h Temperature : 50 °C (Reboiler) 25 °C (Condenser) CIP (Cleaning in Place)
Spray Drying	P- 13/SDR- 101	Initial moisture content: 91.8% Final moisture content: 5% Drying Rate: 4.8 GJ/t Temperature: 120 °C Residence time: 3 h CIP (Cleaning in Place)

3.3.3 LAS Production Process and Assumptions

Pittinger *et al* (1993) investigated the major operations of LAS production including Benzene production, Alkylation, Salt mining, NaOH production, Sulphur mining and linear alkylbenzene (LAB) sulphonation. To specify and launch the inventory of the LAS production, proper assumptions were made for materials and energy estimation of the production process as well as the process data and emissions estimation.

Energy estimation and assumptions

To be consistent with FAL methodology (Hunt *et al*, 1992), Pittinger *et al* (1993) considered assumptions on three types of energy: transportation energy, process energy and material resource energy. Transportation energy was calculated as the energy required per metric ton-kilometre to transport raw materials, process intermediates and finished surfactants. Process energy required for non-transport related industrial operations included fuels combusted on-site for utility heating and the consumption of electrical power generated off-site. Material resource energy was estimated as the energy equivalence of fossil fuel-derived feedstocks conventionally used as fuel sources.

Emissions estimation and assumptions

Pittinger *et al* (1993) also applied the procedures of FAL to calculate the process and fuel-related emissions released to air, water and land from each operation. The composition of process emissions from each operation were characterised to the level of detail commensurate with the data received. These process emissions were those resulting directly from agricultural or industrial operations excluding energy usage.

Process data and assumptions

As for process data, Pittinger *et al* (1993) started with LAB (linear alkylbenzene) production from benzene and paraffin. Data were weighted for the two commercial processes according to typical U.S. production practices as determined by FAL. Use of 75% hydrogen fluoride and 25% aluminium chloride as catalysts was assumed. Feedstock volumes were assumed to be the same for both processes. Production of 1,000 kg benzene was estimated to require 1,105 kg naphtha. Process data for LAB sulphonation were averaged as a composite of conventional processes using sulphur trioxide gas, sulphur trioxide liquid or oleum as the sulphonating agent. Production of 1,000 kg LAS was estimated to require 710 kg LAB; other feedstock volumes can be calculated proportionally from Fig 3.4. Caustic soda (NaOH) and chlorine were assumed to be produced electrolytically from salt deposits mined by Fransch process solution methods. Sulphuric acid production data were based on the sulphur-combustion contact process and included sulphur-mining wastes.

3.3.4 Life Cycle Inventory

The life cycle inventory was based on the material and energy requirements and environmental emissions associated with AM1 manufacture. The LCI the quantification of raw materials, energy, final products and emissions based on the functional unit for the entire process and discrete process respectively.

➤ *Pepfactant®AM1*

The integrated quantification of raw materials and energy requirements as well as amount of final product and emission for the AM1 large-scale production (19 tonnes/year) and functional unit (1 tonne) is presented in Table 3.2. Also, the distribution of these inventory data for discrete processes was estimated, as listed in Table 3.3. The estimate of energy consumption was based on the assumption of total power requirements used in Pepfactant® AM1 manufacturing process. The power consumption is listed in Table 3.4, including the electricity consumed by main unit operations plus 20% used for transport of the streams in processing.

Table 3.2 Raw material, energy and products including emissions data for AM1 manufacture on industrial scale and functional unit

<i>Items</i>		<i>tonnes or MJ /year</i>	<i>tonnes or MJ</i>
		<i>(19t scale)</i>	<i>/tonne AM1</i>
Raw Materials	Water	31,320	1,648
	Water (CIP)	19,044	1,002
	Glucose	3,209	169
	Total Salts	129	6.8
	_ Na ₂ SO ₄	12.4	0.6
	_ K ₂ PO ₄	85.5	4.5
	_ NaH ₂ PO ₄	22.8	1.2
	_ MgSO ₄	1.9	0.1
	_ Citrate	5.7	0.3
	N ₂	72,094	3,794
	O ₂	20,243	1,065
	Ethanol	4,281	225
	TEVP	55.1	2.9
	IPTG	3.8	0.2
	Ammonia	198	10.4
	Ammonium Formate	13.3	0.7
	HCl	285	15
	NaOH	327	17.2
Electrical Power		29,719,876	1,564,204
Products	AM1	19	1
	Total Salts	1.9	0.1
	_ Na ₂ SO ₄	0.17	0.009
	_ K ₂ HPO ₄	1.27	0.067
	_ NaH ₂ PO ₄	0.34	0.018
	_ MgSO ₄	0.06	0.003
	_ Citrate	0.08	0.004
	H ₂ O	23.8	0.054

Emissions & Wastes	Water	32,661	1,719
	Water (CIP)	19,044	1,002
	Glucose	49.4	2.6
	CO ₂	2,373	125
	Ammonium Formate	13.3	0.7
	O ₂	20,243	1,065
	N ₂	72,094	3,794
	Total Salts	129	6.7
	_ Na ₂ SO ₄	11.4	0.6
	_ K ₂ HPO ₄	83.6	4.4
	_ NaH ₂ PO ₄	22.8	1.2
	_ MgSO ₄	3.8	0.2
	_ Citrate	5.7	0.3
	Ammonia	22.5	1.2
	Reusable Co-products & residues	Ethanol	85.6
DNA		13.3	0.7
Cell Debris		1,290	67.9

Table 3.3 Raw materials, energy and emissions process data for discrete processes in AM1 manufacture

<i>AM1 production</i>	<i>Raw materials^a (t/t)</i>	<i>Energy^b (MJ/t)</i>	<i>Atmospheric emissions^c (t/t)</i>	<i>Aqueous emissions (t/t)</i>	<i>Reusable co-products & residues (t/t)</i>
Fermentation	Glucose: 169, Salts: 6.8, PW: 1,648, WW: 99, O ₂ : 1,152, N ₂ : 3,794, NaOH: 17.2, HCl: 15 Ammonia: 10.4, IPTG: 0.2, Ammonium Formate: 0.7, WW: 41.2	P: 640,022 T: 128,004	NOx (Indirect): 0.6 SOx (Indirect): 0.8 CO ₂ (Direct): 125, CO ₂ (Indirect): 195; O ₂ : 1,065, N ₂ : 3,794 Ammonia: 1.2	(Na ⁺ : 0.98 - CIP)	
Filtration		P: 12,793 T: 2,559	CO ₂ (Indirect): 3.9 NOx (Indirect): 0.01 SOx (Indirect): 0.017	Glucose: 2.6, Na ⁺ : 0.425, NH ₄ ⁺ : 0.7, HPO ₄ ²⁻ : 2.43, H ₂ PO ₄ ⁻ : 0.97, SO ₄ ²⁻ : 0.57, K ⁺ : 1.97 Mg ²⁺ : 0.04 Citrate: 0.3, (Na ⁺ : 0.41 - CIP) (Na ⁺ : 0.41 - CIP)	
Homogenization	WW: 41.2	P: 4,441 T: 888	CO ₂ (Indirect): 1.4 NOx (Indirect): 0.004 SOx (Indirect): 0.005		
Cleavage	TEVP: 2.9, WW: 164.	P: 5,967 T: 1,193	CO ₂ (Indirect): 1.8 NOx (Indirect): 0.006 SOx (Indirect): 0.009	(Na ⁺ : 1.62 - CIP)	
Precipitation RDVF	Ethanol: 225, WW: 164 WW: 164	P: 2,640 T: 528	CO ₂ (Indirect): 0.8 SOx (Indirect): 0.0034 NOx (Indirect): 0.0024	(Na ⁺ : 1.62 - CIP)	Cell Debris: 67.9, DNA: 0.7
Vacuum Distillation	WW: 164	T: 528	CO ₂ (Indirect): 0.07 NOx (Indirect): 0.0004 SOx (Indirect): 0.0005	(Na ⁺ : 1.62 - CIP)	
Spray Drying	WW: 164	P: 637,200 T: 127,440	CO ₂ (Indirect): 194; PW: 61.8 Ethanol: 4.5 SOx (Indirect): 0.8 NOx (Indirect): 0.6	(Na ⁺ : 1.62 - CIP)	

a. PW: process water, WW: wash water

b. P: process energy; T: transfer energy

c. Indirect emissions are associated with the coal-fuelled power generation

Table 3.4 Breakdown of Electricity Requirements for AM1 Manufacture

<i>Unit Procedure</i>	<i>Operation</i>	<i>422 runs kWh/year</i>	<i>kWh/t AM1</i>
Media Preparation Tank (Type: Blending Tank)	Agitation	633	33
Seed Fermentor	Batch Heating	1,013	53
Fermentation Unit	Agitation		
	Fermentation	30,384	1,599
	Inline Sterilizer	1,043,184	54,904
	Agitation	635	33
Centrifugal Compressor	Batch Stoichiometric Fermentation	1,218,360	64,124
	Centrifugal gas compression	1,083,696	57,037
Diafilter	Batch concentration	67,520	3,554
Homogenizer	High pressure homogenization	23,436	1,234
Blending Tank	Agitation	31,492	1,658
RDVF		13,933	733
Spray Drier		3,363,000	177,000
Total (plus assumed 20% on transport)		8,249,214	434,169

➤ ***Chemical Surfactant LAS***

Pinttinger *et al* (1993) compiled the environmental life cycle inventory of detergent-grade surfactant production, one of which - LAS production was selected here to proceed with impact assessment. Process data related to the raw materials, energy and emissions for LAS production is listed in Table 3.5 and primary mass requirements for the pre-production of LAB (linear alkylbenzene) are estimated and shown in Fig 3.4.

Table 3.5 Raw materials, Energy and Emissions Process Data for Discrete Operations Involved in LAS Production (Pittinger *et al*, 1993)

NOTE: This table is included on page 48 of the print copy of the thesis held in the University of Adelaide Library.

a: Energy key: P = process energy, T = transportation energy, MR = material resource energy.
b. Emissions key: Hyd = hydrocarbons, Part = particulates, SOX = sulfur oxide, Alde = aldehydes, NH₃ = ammonia, NOX = nitrogen oxides, CO = carbon monoxide, HF = hydrogen fluorides, Orgs = organics, Cl = chlorine, VOC= volatile organic carbons, EO = ethylene oxides, DSol = dissolved solids, BOD = biological oxygen demand, COD = chemical oxygen demand, TSS = total suspended solids, O&G = oil and grease, Phen = phenols and phenolics, Cr = chromium, SSol = suspended solids, Sulf = sulfides/sulfuric acid, F = fluorides, Met = metals, CN = cyanide, TDS = total dissolved solids, TOC = total organic carbon, Acet = acetaldehyde. c. CO₂ emissions for LAS production process was totally estimated as 4,200kg/1,000kg products

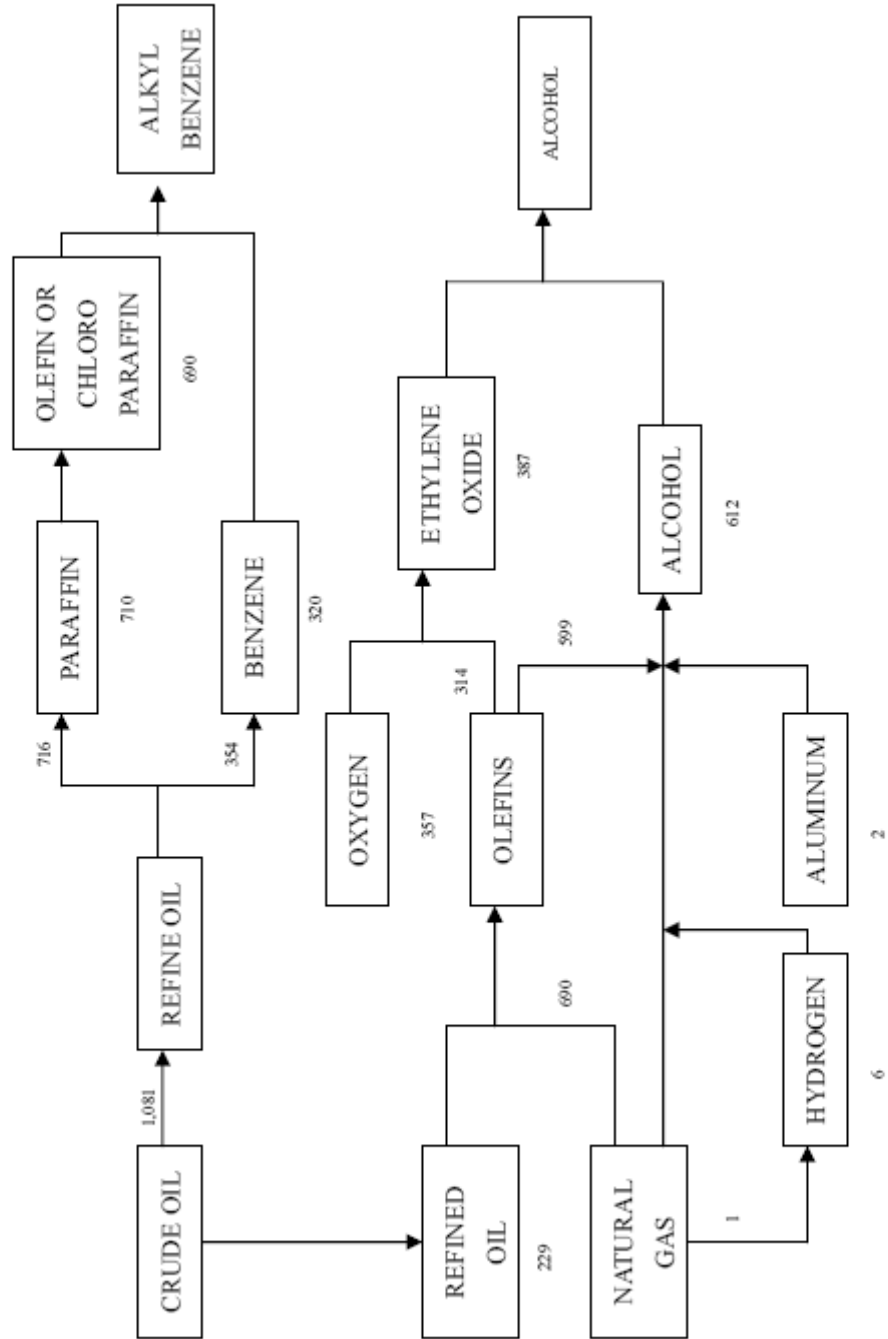


Figure 3.4 Production of linear dodecyl alkylbenzene (LAB) and alcohol ethoxylate from petroleum and natural gas (Mass requirement (kg) for each stage are expressed on the basis of 1000 kg LAB produced)

3.4 Impacts Assessment

3.4.1 Impact Categories

To investigate the environmental impacts of Pepfactant® production, it was essential to identify the relevant impact categories to proceed with calculation of impact effect scores, namely quantification of impacts. According to the life cycle inventory, the impact categories involved in the Pepfactant® AM1 manufacture basically take account of raw material depletion, energy depletion and other environmental problems associated with the emissions.

3.4.2 Classification and Characterisation

Referring to the life cycle assessment guide (Heijungs, 1992), based on the life cycle inventory data of the processes, the environmental problems of the Pepfactant® AM1 and chemical surfactant LAS manufacture can be classified as: raw material requirement, energy requirement, global warming potential (GWP), nutrification potential (NP), human toxicity potential (HP), photochemical oxidant formation potential (POCP), solid wastes, acidification potential (AP), ecotoxicity potential (EP) and odour potential (OP). The characterisation method has been mentioned individually for these impacts in Chapter 2.

In principle, the characterisation of the inventory was to calculate the indicators of the classified environmental impacts. Specifically, in this study, the characterisation was undertaken with approaches of the CML method (Heijungs, 1992) for both surfactants manufacture processes i.e. Eq 1.4-1.12. The characterisation factors used in calculations for Pepfactant® AM1 and chemical surfactant LAS production are listed in Table 3.6 and Table 3.7 respectively.

Table 3.6 LCA Characterisation factors for AM1 manufacture (Heijungs, 1992)

NOTE: This table is included on page 51 of the print copy of the thesis held in the University of Adelaide Library.

Table 3.7 LCA Characterisation factors for LAS production (Heijungs, 1992)

NOTE: This table is included on page 52 of the print copy of the thesis held in the University of Adelaide Library.

3.4.3 Processing the Inventory and Assessment

After identifying the classification and characterisation of impacts, the next step is to quantify the environmental impacts by processing the life cycle inventory. The approach involves calculating the different categorised impact score for each unit process in the life cycle of Pepfactant® AM1 manufacturing as mentioned before.

➤ ***Peppactant® AM1***

**Table 3.8 LCA impact indicators for 1000 kg of AM1 manufacturing process
(CML 1992)**

NOTE: This table is included on page 54 of the print copy of the thesis held in the University of Adelaide Library.

The results listed in Table 3.8 are the quantified characterisation scores for Pepfactant AM1 manufacture, covering the unit processes: fermentation, filtration, homogenisation, cleavage, precipitation, RDVF, vacuum distillation and spray drying. Using the CML 1992 assessment method, for 1000 kg of Pepfactant AM1 produced, seven environmental impact categories were quantified, which were associated with the manufacture process, including raw material depletion, energy depletion, global warming potential (GWP), nutrification potential (NP), human toxicity potential (HT), photochemical oxidant formation potential (POCP) and acidification potential (AP). To obtain the quantification results for impacts, characterisation scores were calculated for each impact category by using Eq 2.1. The amounts of material and energy requirements as well as the emissions from AM1 manufacture process were referred to life cycle inventory (summarised in Table 3.3) and characterisation factors (Table 3.6) were applied to the emissions when they were allocated to the different impact categories. All of these impacts were estimated for the baseline of 1000 kg AM1 products.

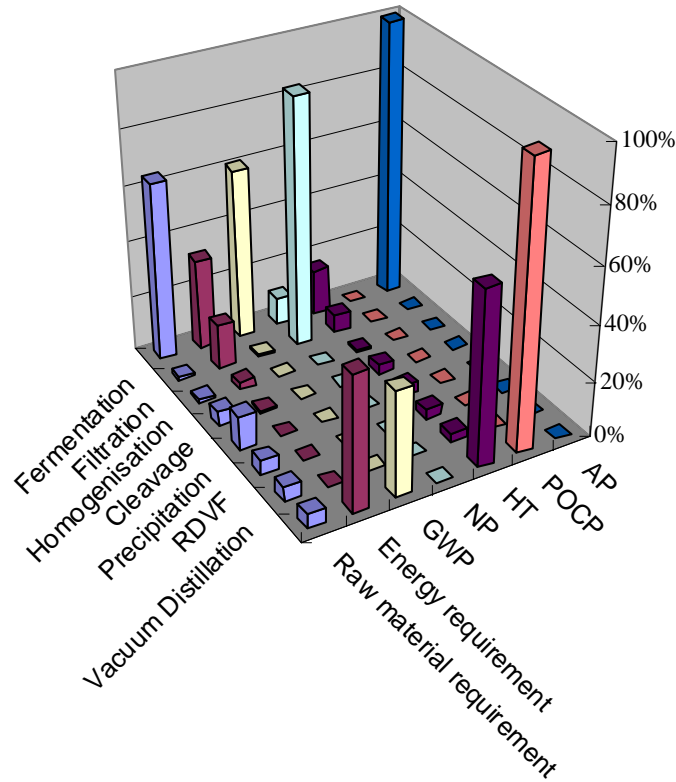


Figure 3.5 Percentage contributions of AM1 production across all LCA impact categories

Figure 3.5 displays the fractional contribution of each unit operation (Fermentation, Diafiltration, Homogenisation, Cleavage, Precipitation, RDVF and Vacuum Distillation) to the total LCA impact within each impact category (e.g. GWP) on the vertical axis and reveals the major processes contributing to each LCA impact in the manufacture of AM1.

As seen in Fig 3.5, for this AM1 manufacture model, fermentation is the most significant contributor to the raw material requirement. Of estimated raw material consumption, the water requirement is considerable in this process model with 1,648 t/t AM1 consumed by processing and 1,002 t/t AM1 consumed by cleaning in place operations according to designers’ simulation data of this model.

Energy requirement is also a significant environmental burden for producing Pepfactant® AM1. The simulated process was estimated to require electrical energy 1,564,204 MJ/t AM1, almost half of which (48.9%) was estimated to be attributed to spray drying.

The problems of human toxicity and eutrophication are primarily contributed by the releases from fermentation (1.2 t NH₃/t AM1), diafiltration (0.7 t NH₄⁺/t AM1, 2.85 t metallic ions/t AM1) and spray drying (4.5 t ethanol/t AM1) as well as the accounted indirect emissions NO_x and SO_x (Delta Electricity, 2006) from coal-fuelled electricity generation. The potential impacts of photochemical oxidant formation and acidification are respectively caused by emissions from spray drying (4.5 t ethanol/t AM1) and fermentation (1.2 t NH₃/t AM1) plus harsh indirect emissions from power generation. As for the co-products i.e. the cell debris (67.9 t/t AM1) associated with minor amount of DNA (0.7 t/t AM1) precipitated out from the process were regarded as the ideally reusable bio-mass to be used for creating animal feeding stocks.

Similar with the above impacts, the global warming potential, primarily resulting from the enormous CO₂ emissions from two sources: the direct CO₂ released from fermentation (125 t/t AM1) and the indirect CO₂ emission (397 t/t AM1) linked to the sources of electricity production in Australia, where coal is the main resource which has high CO₂ emissions. This quantification result indicates that global warming potential is a heavy environmental burden for Pepfactant® AM1 production, particularly processed in Australia in terms of the consideration of the types of processing energy and relevant source.

However, when it comes to the ecotoxicity and odour potential, it is essential to notify that the current LCI data is unable to identify and quantify the related emissions causing these two environmental impacts due to the difficulty in obtaining the actual releases from the process which does not exist at present. Hence, at this very early design stage, this LCA of Pepfactant® production excludes the investigation of ecotoxicity and odour potential, which does not imply that Pepfactant® production has no impacts on ecotoxicity and odorous air.

Overall, as Fig 3.5 presents, the process unit of fermentation, diafiltration and spray drying contribute an obviously higher LCA impact as compared with other environmental impacts. This finding is supported by analysing the individual impact categories with the CML Guide assessment method, which could be beneficial for Pepfactant® designers to optimise the large scale production when they adopt these unit operations.

➤ *Chemical Surfactant LAS*

**Table 3.9 LCA impact indicators for 1000 kg of LAS manufacturing process
(CML 1992)**

NOTE: This table is included on page 59 of the print copy of the thesis held in the University of Adelaide Library.

Likewise, the results listed in Table 3.9 are the quantified characterisation scores for chemical surfactant LAS manufacture, covering the operations: Benzene production, Alkylation, Salt mining, NaOH production, Sulphur mining and LAB production. Using the CML Guide assessment method, for 1000 kg of LAS produced, ten environmental impact categories were analysed associated with the manufacture process, including raw material depletion, energy depletion, global warming potential (GWP), nitrification potential (NP), human toxicity potential (HT), photochemical oxidant formation potential (POCP), Solid waste, acidification potential (AP), ecotoxicity potential (EP) and odour potential (OP). To obtain the quantification results for impacts, characterisation scores were calculated for each impact category by using Eq 2.1. The amounts of material and energy requirements as well as the emissions from LAS manufacture process were referred to life cycle inventory (summarised in Table 3.5) and characterisation factors (Table 3.7) were applied to the emissions when they were allocated to the different impact categories. All of these impacts were estimated for the baseline of 1000 kg LAS products.

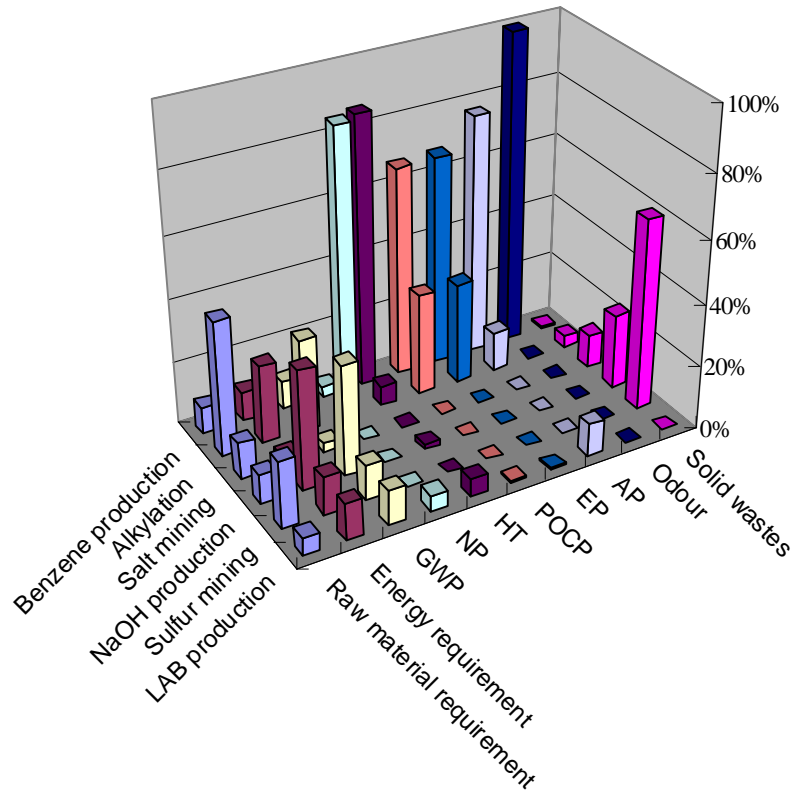


Figure 3.6 Percentage contributions of LAS production across all LCA impact categories

Furthermore, to disclose the major processes contributing to the LCA impacts in the manufacture of LAS, Fig 3.6 displays the fractional contribution of each process (Benzene production, Alkylation, etc) to the total LCA impact with each impact category (e.g. GWP) on the vertical axis.

From the results shown in Fig 3.6, each unit operation of LAS production has contributions to at least 4 categories of impact. In other words, the complexity of the environmental burden is the result of the diverse emissions and releases from the LAS production.

The major processes contributing to the LCA impacts in the production of LAS are benzene production and alkylation. Within the impacts, compared with other unit operations, benzene production has a dominant potential on human toxicity (86.5%),

photochemical oxidant formation (67.1%), ecotoxicity (67.5%), acidification (77.2%) and complete odours contribution (100%) and as for alkylation it plays a main role on raw material requirement (43.4%), nitrification (91.6%), energy requirement (25.2%), photochemical oxidant formation (32.5%) and ecotoxicity (32.3%).

It is necessary to point out that global warming potential resulting from the CO₂ emission was estimated proportionally to the energy requirements as there is no direct CO₂ emission during LAS production. Therefore, the unit operation of NaOH production has the highest contributions (38%) to the 2 categories followed by alkylation (25.2%), sulphur mining (12.3%) and LAB production (11.5%).

In this section, the quantitative environmental impact results were obtained for Pepfactant® AM1 and LAS manufacture process with LCA profiles. Hence, it is feasible to compare the environmental impacts from both processes. The LCA comparison between them will be presented in detail in Chapter 5.

3.5 Extended LCA for Pepfactant® AM1

In order to make the assessment and comparison as transparent as possible, this LCA study does not carry out the optional phases - normalisation and weighting. As stated earlier, the system boundaries of this LCA were restricted on the manufacture phase of Pepfactant® AM1 production. However, it is important to extend the system boundaries to provide a comprehensive LCA in view of more general environmental considerations and feedbacks. In this section, the major LCA outcomes of high CO₂ emissions and water requirement will be discussed, taking account into the inclusion of feedstock resources and energy generation as well as proposed recycling approach for water utility.

- ***Carbon Dioxide Emission***

From the LCA on Pepfactant® AM1 manufacture, it is clear that the global warming potential is a dominant environmental burden associated with the assumed large-scale process. Essentially, this impact results from the large quantities of CO₂ emissions – 125 t/t AM1 directly and 397 t/t AM1 indirectly. However, these figures are improved if the LCA boundaries are extended, namely the raw material acquisitions and energy sources involved in the LCA.

Direct Carbon Dioxide Emission

Reviewing the raw materials for AM1 manufacture, glucose, used for an important nutrient in fermentation media, is the exclusive source of influent carbon and responsible for the direct CO₂ emission as the co-product from the fermentation. It was estimated that 169 tonne of glucose would be needed to produce 1 tonne AM1.

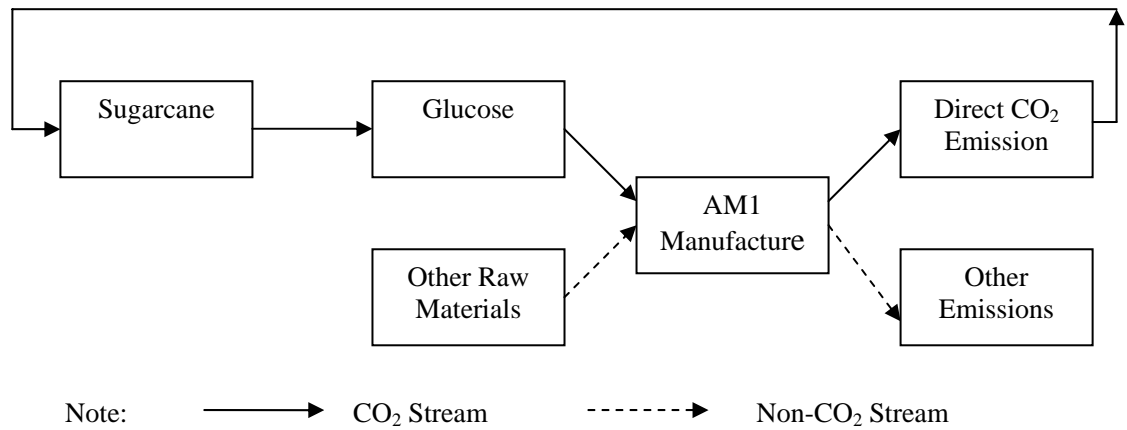


Figure 3.7 Direct carbon dioxide emission trace with raw materials

If the LCA boundaries was extended as the CO₂ trace shown in Fig 3.7, it can be seen that glucose is sourced from sugary plants, typically sugarcane in Australia. In fact, these crops play significant roles as greenhouse gases sinks for carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O) (Weier, 1998). Afterwards, the traditional industrial sugar or syrup production is used to attain glucose or fructose products, including the major the unit operations – enzymatic hydrolysis and saccharification.

Weier (1998) estimated that in 1994 Australia 35 million tonnes of sugarcane was produced, emitting 7.6 million tonnes CO₂ while absorbing 13.4 million tonnes CO₂. Thus, the net CO₂ intake ability of sugarcane is approximately estimated to be 0.2 tonne CO₂/tonne sugarcane. Additionally, scientists from CSIRO's Davies Laboratory in Townsville affirmed that 18% higher sugar yield could be achieved with cane (CSIRO, 1998).

Based on the information above, in the extended LCA covering direct CO₂ emission from the raw material acquisition, the recovery rate of glucose or sugar is assumed as 25% from sugarcane. In other words, to produce 1 tonne AM1, 169 tonnes of glucose would consume 676 tonnes sugarcane, which is likely to decrease direct CO₂

emission by 112.2 tonnes. So, the direct CO₂ emissions can drop from 124.9 tonnes to 12.7 tonnes.

Furthermore, the same relevancy between ethanol extracted from sugarcane and CO₂ emission could be looped in the LCA boundaries, namely 100 g of glucose (sugar) able to produce 51.4 g of ethanol (Badger 2002). In this case, 1 tonne AM1 production demands 4.5 tonne ethanol, equivalent to 8.8 tonne glucose, which is produced from 35.2 tonne sugarcane. The capability of this amount of sugarcane as the sink of CO₂ is approximately 6 tonne. Therefore, plus the CO₂ emission reduction estimated from last paragraph, the direct CO₂ emission from Pepfactant® AM1 production can be decreased to 6.7 tonne/tonne AM1.

Indirect Carbon Dioxide Emission

The indirect carbon dioxide emission for manufacture AM1 was regarded as the emission generated in the activities of energy depletion, i.e. the consumption of electricity - the major energy employed for the manufacture processing. In the previous LCA chapter, the estimate of indirect CO₂ emission of AM1 manufacture was on the base of Australia power generation sources and relevant greenhouse gas emission factors. On this point, the LCA boundaries are extended as presented in Fig 3.8, to demonstrate the reducible indirect CO₂ emission in terms of the power generation source.

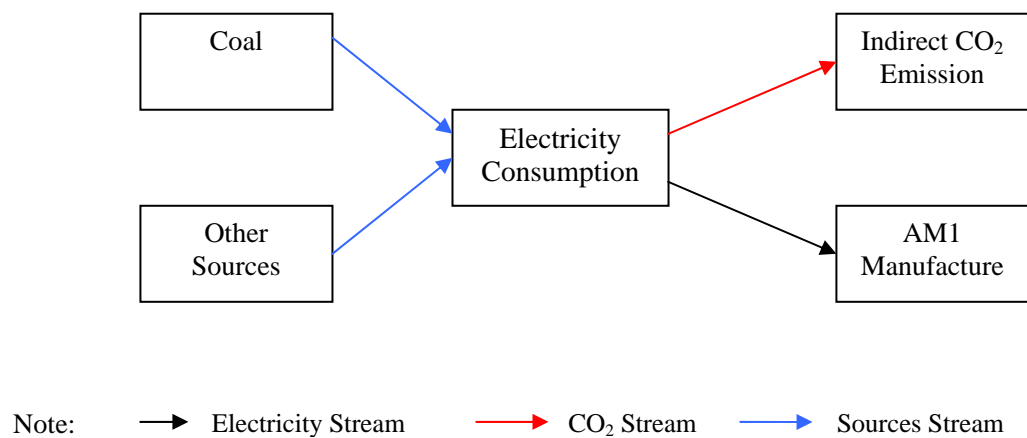


Figure 3.8 Indirect carbon dioxide emission trace with energy depletion

NOTE: This figure is included on page 66 of the print copy of the thesis held in the University of Adelaide Library.

Figure 3.9 Relative public power generation by sources in 2003 (IEA, 2005)

According to the International Energy Agency's (2005) statistics over power generation of 10 typical countries (Fig 3.9), the sources to produce electricity in Australia substantially derive from fossil fuels, coal-fired power (higher than 80%) and gas-fired power (about 10%). Compared with other countries, electricity production in Australia is the major producer of CO₂ emissions. In this LCA on AM1 manufacture, it is crucial to emphasise that the AM1 production in Australia has a great potential of global warming impact (roughly 397 t CO₂/t AM1) because of the enormous dependence on electricity as the processing energy.

Furthermore, Graus *et al* (2007) found the highest efficiencies of electricity production in the above countries (Fig 3.9), 42% for coal, 52% for gas and 45% for oil-fired power generation, estimating the energy savings potential and corresponding CO₂ emission reduction potential providing all of these countries produce electricity at the highest efficiencies, as shown in Fig 3.10 and Fig 3.11. For AM1 production, hypothetically, the production of AM1 being carried out in Nordic countries, substituting hydro-power or nuclear power for the fossil-fired power, is an optional scenario to foresee the possibility to reduce the severe indirect CO₂ emissions.

NOTE: This figure is included on page 67 of the print copy of the thesis held in the University of Adelaide Library.

Figure 3.10 Energy reduction potential with highest efficiencies included countries (Graus *et al*, 2007)

NOTE: This figure is included on page 67 of the print copy of the thesis held in the University of Adelaide Library.

Figure 3.11 CO₂ savings potential with highest efficiencies included countries (Graus *et al*, 2007)

- ***Water Requirement***

The simulated AM1 manufacture process uses considerable amounts of water – 1,648 t/t AM1 for processing and 1,002 t/t AM1 for CIP. From the perspective of sustainability, the enormous water requirement of this AM1 production model is hardly an acceptable scenario.

Broadly speaking, water resource and consumption has been encountered challenges in Australia, with annual use of approximately 1.3 million litres per person, ranking at the third highest consumption rate in the world (ATSE, 2004). The National Land and Water Resources Audit (NLWRA, 2002) asserted that 26% of Australia's surface water management areas are either close to or overused compared with their sustainable flow regimes. Nowadays, less than 10% of the wastewater resource is treated and utilised with the remainder being discharged at various points in the environment. However, the increase of the use of recycled water for a variety of purposes, providing benefits for the community and the environment by increasing available water resources and decreasing nutrient and contaminant loads to surface and coastal waters (ATSE, 2004).

In this context, for the industrial and manufacture use, there is also a necessity to highlight that the use of the recycled water is able to offset the substantial water demand. Specifically, in AM1 processing, even though water was unavoidably consumed as the main materials for biomass fermentation and cleaning, the recycle of effluent water would reduce the burden of the current water requirement. Technically, for processing water, a large quantity of waste water was emitted after the filtration in the AM1 manufacture model – 95% of the media water. To reduce the processing water use, it is feasible to add a water treatment process to the outlet of filtration as shown in Fig 3.12. Assuming a recycle rate of 95%, the processing water use would be reduced to 161 t/t AM1. Likewise, it is critical to manage the disposal of the CIP water, which could be reduced to 50 t/t AM1, assuming a recycle rate of 95%.

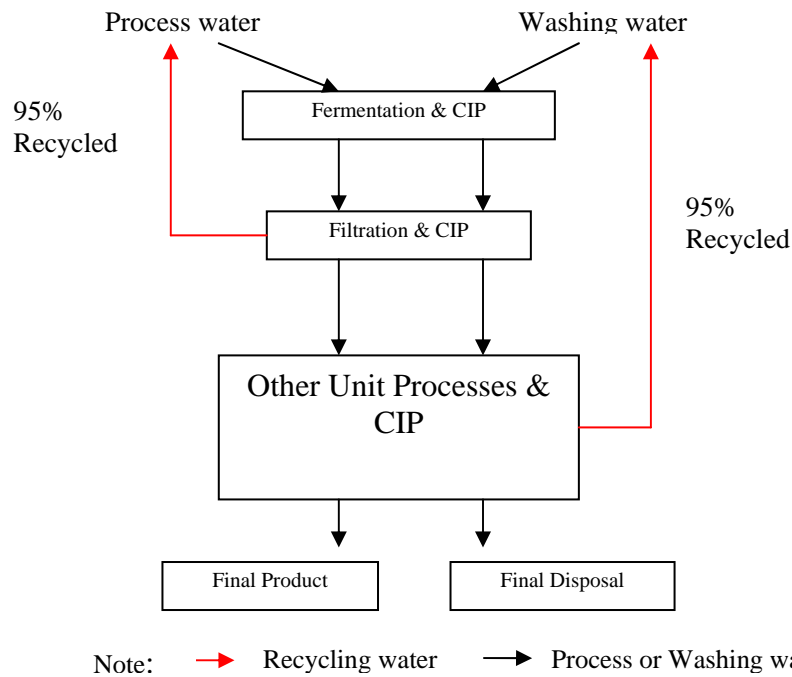


Figure 3.12 Scenario of Pepfactant® AM1 manufacture model with recycling water

3.6 Conclusions

At the first stage, it can be concluded from the characterisation results of this partial LCA study that the Pepfactant® AM1 manufacture presents more environmental burdens compared with chemical surfactant LAS manufacture. Some further considerations were also involved in the extended LCA according to the results, such as the great global warming potential and water consumption. More detailed comparison on LCA between both surfactants manufacture will be discussed in Chapter 5. However, it needs to notice that differences of manufacture approaches between AM1 and LAS cause the LCA lack comparability. Since Pepfactants® were designed as new surfactants with superior functional attributes, it is meaningful to extend the LCA boundaries to applicable functions of the two surfactants, so that the LCA can be incorporated with comparable functional attributes between AM1 and LAS.

4 FOAMING TESTS on SURFACTANTS

In the previous chapter, a LCA study was undertaken on Pepfactant[®] AM1 manufacture and an environmental impact assessment was analysed on the chemical surfactant LAS production with an available life cycle inventory. This enabled quantification of the possible environmental impacts associated with AM1 and LAS manufacturing processes.

4.1 Background

On the basis of the simulated Pepfactant[®] manufacturing process, the LCA boundaries have not incorporated upstream and downstream processes such as application and disposal of AM1 in possible industrial situation. However, the expanded boundaries of LCA on AM1 compared with LAS, incorporating applicable functions of surfactants will be more informative for the comparison between both products. The goal of the experimentation work reported in this chapter was to perform tests to measure one key parameter of surfactant function, namely foaming height (a measurable form of foaming ability), on Pepfactant[®] AM1 and chemical surfactant LAS solution respectively. The test results demonstrating the quantified foaming ability between AM1 and LAS could be linked to the LCA comparison of the two surfactants. Particularly, in accordance with the functional unit of LCA studies - 1 tonne of surfactant, foaming height measurement results were able to demonstrate the superior foaming ability of 1 tonne of AM1 product compared with 1 tonne of LAS product.

4.2 Methodology

The measurement of a surfactant's function is a complex activity and covers a wide range of physical and chemical parameters and factors. As foaming is an important functional attribute of surfactants, which results from the property of lowering surface tension of substances, foaming ability can be an indicative parameter to measure a surfactant's function. Theoretically, the measurement of foaming ability of surfactant solutions is a difficult problem due to the volume and quality of the foam produced by any method on a given solution as well as the complexity of affecting factors on foaming persistence (Ross *et al*, 1941).

4.2.1 Foaming Experiment Overview

In various published works, with regard to studying foaming mechanisms or theories, there were several experiments performed on foam comparison. The methods used are summarised below.

- 1) Method with Manual Shaking (Mikitenko *et al*, 1982): In a graduated cylinder (250 ml), a 100-ml sample of the emulsion is shaken for 1 min at constant amplitude. The volume of foam that is formed (in ml) is measured and then taken as an index of foaming ability. After 2 min of settling, the foam volume is again measured and is taken as a measure of the foam stability.
- 2) Method of Ross and Miles (Ross *et al*, 1941): A 50-ml sample of surfactant is poured carefully into a graduated cylinder. A 150-ml quantity of surfactant is placed in a burette, which is then mounted in a strictly vertical position in the upper part of the cylinder so that the distance from the surfactant surface in the cylinder to the tip of the burette is 90 cm. The stopcock of the pipette is opened, and the surfactant is allowed to flow out. The volume of the foam that has been formed is measured at the instant when the emulsion flow from the burette has been completed, then repeated after 5 min.

- 3) Method Using Porous Filter (Christmann, 1928): A 190-ml sample of surfactant is placed in a 1-Litre graduated cylinder and a porous filter is lowered to the bottom of the cylinder. Air is passed through the porous filter for 5 min at a constant rate of 94 ± 5 ml/min. At the end of this time, the air flow is shut off, and the foam volume is measured. The volume of remaining foam is measured after 10 min.
- 4) American Standard Method ASTM D 892 (Mikitenko *et al*, 1982): This test method is similar to the method just described with respect to the test conditions and the equipment set up. The only difference is with the use of a porous diffuser with a known pore diameter.
- 5) Pulsating Jet Method (Mikitenko *et al*, 1977): This method is based on the circulation of the test surfactant in a closed system consisting of a pump and a cylinder.
- 6) Method Using a Perforated Disk. (Griffith *et al*, 2002): A perforated disk mounted on a stainless steel rod is placed in a 1-liter graduated glass cylinder with 200 ml of the test emulsion. The rod with the disk is put into reciprocating motion at a rate of 100 strokes per minute by means of an eccentric mounted on a motor. The perforated disk is thus moved up and down in the layer of test surfactant without coming any closer than 0.5 cm to the bottom of the cylinder or the surfactant surface. After 5 min of mixing, the foam volume is measured, and the remaining foam volume is measured after 10 min settling time.
- 7) Method Using a Paddle Stirrer (Preston *et al*, 1929): A paddle stirrer on a stainless steel rod is placed in 1-Litre graduated cylinder with 200 ml of the test surfactant; the stirrer is rotated at a speed of 3000 rpm. After 5 min of stirring, the foam volume is measured, and the remaining foam volume is measured after 10 min settling time.

4.2.2 Determination of Foaming Experiment Method

Of the mentioned methods, Mikitenko *et al* (1982) justified that the paddle stirrer or the perforated disk methods present the smallest deviations between values obtained for the foaming ability on different batches of a given product. They also pointed out

that pulsating jet and porous filter methods give the highest values of the foaming ability with subtle deviations between the results obtained in parallel tests; Ross and Miles (1951) method can not be used to rate the foaming behaviour of tested fluids as the error of the determination is much greater at low levels of foaming; the manual shaking method and the ASTM D 892 methods give the greatest differences between foaming ability for different batches of the same foaming fluids.

Despite the recommendation of Mikitenko *et al* (1982) that the paddle stirrer and perforated disk methods are superior, the method using a Porous Filter was used for Pepfactant[®] foaming measurement by Malcolm *et al* (2006). In this work, this method was selected to determine the comparable dependence of the foam height on concentrations of surfactant AM1 and LAS not only for securing the measurement performed with identical methods but also considering bench-scale simplifications and apparatus availability for the tests. The relation between foam height and surfactant concentration will reflect foaming ability difference between Pepfactant[®] AM1 and surfactant LAS solutions in the same situation. The results will be converted to 1 tonne base comparison consistent with LCA comparison.

4.3 Materials and Tests

4.3.1 Materials

The 21-residue peptide AM1 (Dexter *et al*, 2006) was produced by Genscript Corporation (Piscataway, New Jersey, USA). The purity was >95% by RP-HPLC. All other reagents and chemicals used were of analytical grade. Water was obtained from a Milli-Q system (Millipore, North Ryde, NSW, Australia) with a 0.22 µm filter and had a resistivity of >18.2 MΩ cm. The commercial LAS powder was obtained from Albright & Wilson Ltd. (Wetherill Park, NSW, Australia). The purity of (C-10-16) alkylbenzene sulphonic sodium salt was > 80% for this LAS sample.

4.3.2 Tests

A custom built glass foaming apparatus (Fig 4.1 and Fig 4.2) consisted of a glass tube (10 cm × 1 cm diameter), open at the top and fitted with a porous glass frit at the bottom. Below the glass frit an air inlet connection and a valve for draining liquid from the tube were fitted. The air inlet was connected by plastic tubing to an air filled 60 ml syringe, mounted on a syringe pump (Pump 11 Plus, Harvard Apparatus, Holliston, MA, USA).

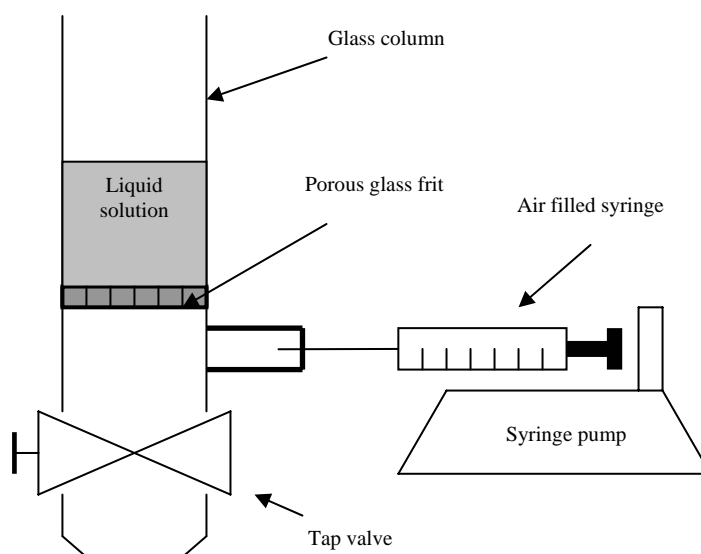


Figure 4.1 Schematic of apparatus used for foaming tests

For foaming ability tests, 0.5 ml AM1 solution with concentration of 15 μM was prepared in 25 mM sodium 4-(2-hydroxyethyl)-1-piperazine ethanesulfonate (HEPES) to ensure pH 7.4. HEPES was selected as the buffer as it provides buffering capacity in pH ranges ($\text{pK}_{\text{a}1}$ of ~ 3 to $\text{pK}_{\text{a}2}$ of 7.55) where AM1 is able to present film state when binding Zn ions (Malcolm *et al*, 2006). The test sample was pipetted onto the glass frit through the top opening in the tube. Air was bubbled through the solution at a rate of 0.2 ml/min and timing started when the first bubble appeared in the solution. The test time was set as 25 min, assuming the foaming efficiency of solution was 100%. Hypothetically, when the air was ran out at the end of 25 min, foam of 5.5 ml or 55 mm would be obtained. In practise, at the end of 25 min, air bubbling was stopped and foam height of the solution was measured. The same tests were

conducted on 30 μM , 40 μM , 50 μM , 75 μM and 100 μM AM1 solutions respectively. All of the measures were undertaken at room temperatures.

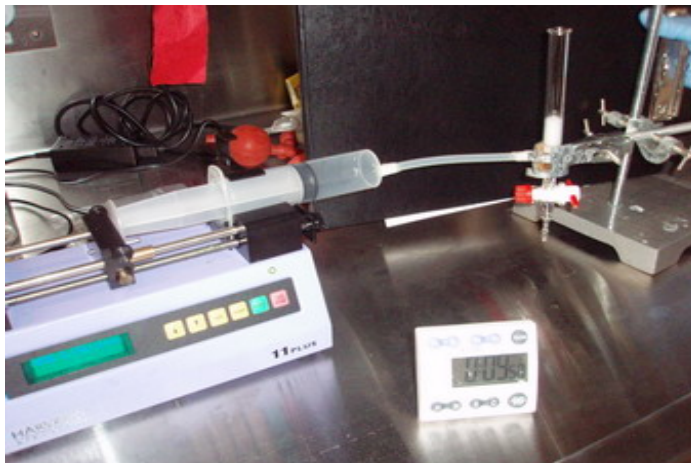


Figure 4.2 Experimental set up of foaming tests

In parallel, LAS tests were performed under identical conditions for the Pepfactant® tests. Solution (0.5 ml) of LAS with a concentration of 2 mM was prepared in 25mM (HEPES) to ensure a pH of 7.4. It was pipetted onto the glass frit through the top opening in the tube. Air was also bubbled through the solution at a rate of 0.2 ml/min and timed from when the first bubble appeared in the solution. In the same principle, at the end of 25 min, air bubbling was stopped and foam height of the solution was measured. After the first test, 6 further tests on LAS solution were carried out, with 50% reduction in concentration – 1 mM, 0.5 mM, 0.25 mM, 0.125 mM, 62.5 μM and 31.25 μM . All of the measures were undertaken at room temperature.

In order to reduce contamination which is likely to influence the foaming results of solution, between the tests conducted on AM1 and LAS solution, it was necessary to clean the foam tube after one measurement was finished. The clean required a single rinse 1 with Milli-Q water, rinse with EDTA, rinse 3 times with 50% ethanol, rinse 7 times with distilled water and finally rinse 2 times with acetone followed by air drying.

4.4 Results

Foam is an important aspect of detergent products, and surfactants are mainly responsible for foam generation. Simple foam height measurements have commonly been used to compare various surfactants present in different formulations. According to the concentration dependence of foam height, which is proportional to the foam volume, as mentioned in the Porous Filter Method, it is practical to make rapid and simple estimates of the foaming ability of surfactants.

➤ *Pepfactant*[®] AM1

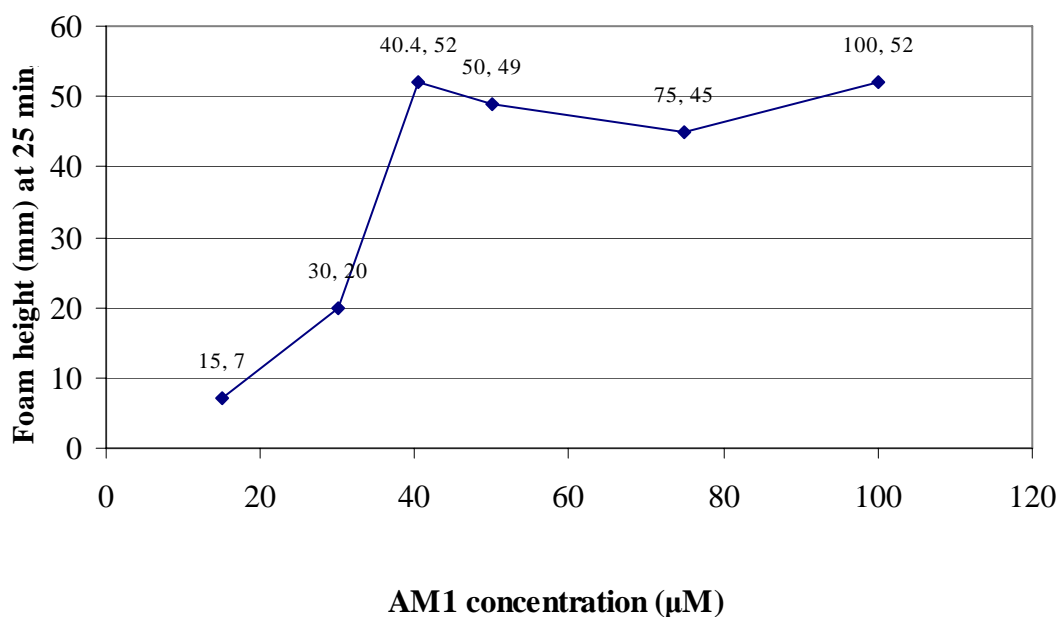


Figure 4.3 Concentration dependence of Pepfactant[®] AM1 foams (25mM HEPES pH 7.4 200µM Zn)

Fig 4.3 shows results of the range of the foam height for AM1 solution under different concentrations. At the same flow-rate of aeration and during the same period, between 15 µM and 40 µM, the foam height increases sharply, from 7 mm to over 52 mm. When the concentration is higher than 40 µM, the variations of foam

height is negligible, except the deviation (45 mm) appeared on 75 μM solution. Hence, for AM1 solution, to get the maximum foam height at an air flow rate of 0.2 ml/min, the minimum concentration requirement was approximately 40 μM .

➤ *Chemical surfactant LAS*

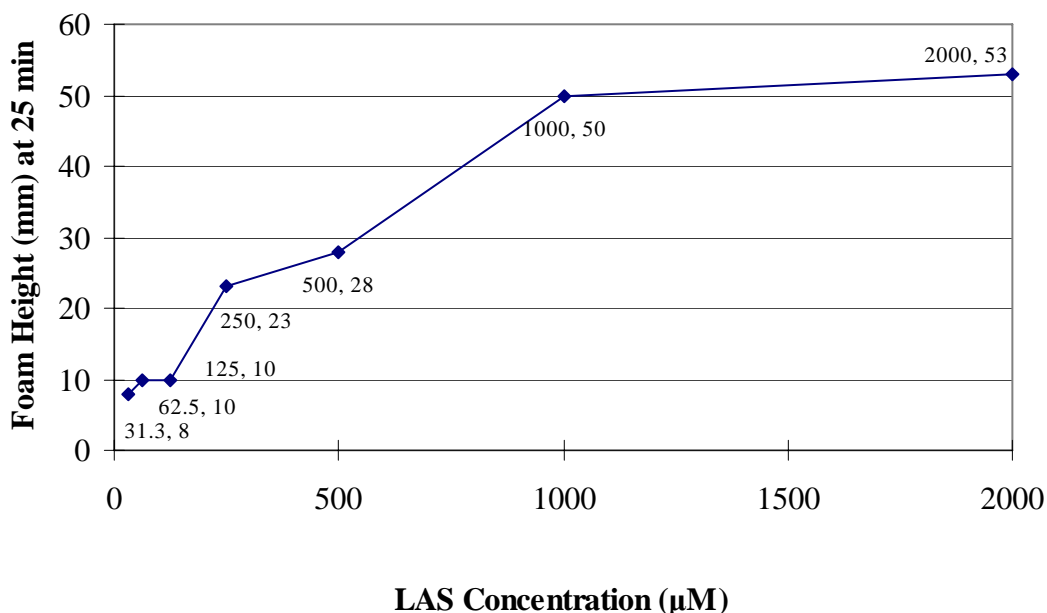


Figure 4.4 Concentration dependence of LAS foams (25mM HEPES pH 7.4)

Fig 4.4 displays the foam height outcomes of LAS solutions. As seen, the asymptotical trend remains the same in the presence of the LAS solution. Overall, within the tested concentrations, the foam height of LAS solution increases rapidly between 31.25 μM and approaches an asymptote after 1 mM. For the LAS solution, to get the maximum foam height at this air flow rate (0.2 ml/min), the minimum concentration required is approximately 2 mM.

4.5 Conclusions

With the foam height test results of surfactant solution at different concentrations, it is feasible to compare the foaming ability of Pepfactant[®] AM1 and chemical surfactant LAS. Principally, the foaming ability of both surfactants had a similar

exponential relationship with solution concentrations. However, as the concentration increases, the foam height of AM1 solution raises more rapidly than that of the LAS solution. It was shown that the minimum concentration of AM1 solution to generate the maximum foam height within the tested foam height range is 40 μM while for LAS solution the minimum concentration is 1 mM. Therefore, under identical conditions, one mM of AM1 use has the same foaming ability as 25 mM of LAS use. Combined with the LCA comparison, 1 tonne of AM1 products have the foaming ability of approximately 25 tonne LAS products.

However, it should be noticed that the laboratory scale foaming ability test of AM1 and LAS is only expected to give a qualitative indication of the relative foaming ability of the two surfactants. To achieve further quantitative information, which would require pilot tests on them, is not required for this LCA comparative purpose.

5 RESULTS and DISCUSSION

In this chapter, the Life Cycle Assessment (LCA) comparison between Pepfactant[®] and conventional surfactant manufacture is discussed. On the perspective of the sustainability design for industrial scale process, the LCA comparison results are beneficial for developing the current simulated Pepfactant[®] manufacturing process. In addition, the comparison will also cover the foaming test results of Pepfactant[®] AM1 and chemical surfactant LAS under identical conditions in order to predict the quantitative substitution for LAS with AM1.

5.1 Comparison Results towards LCA Profiles

5.1.1 Raw Materials

Based on the Life Cycle Inventory in Chapter 3, the data shows that following the quantitative consumption level from high to low, the materials required for Pepfactant[®] AM1 manufacture include: nitrogen, water, oxygen, ethanol, glucose, NaOH, HCl, NH₃, Salts, TEVP and other media trace elements; for LAS manufacture they consist of: water, naphtha, salt mineral, salts, LAB, kerosene, benzene, NaOH, and sulphur. This is presented in Fig 5.1 and Fig 5.2 by compositions and quantities of the raw material requirements for Pepfactant[®] AM1 and chemical surfactant LAS production.

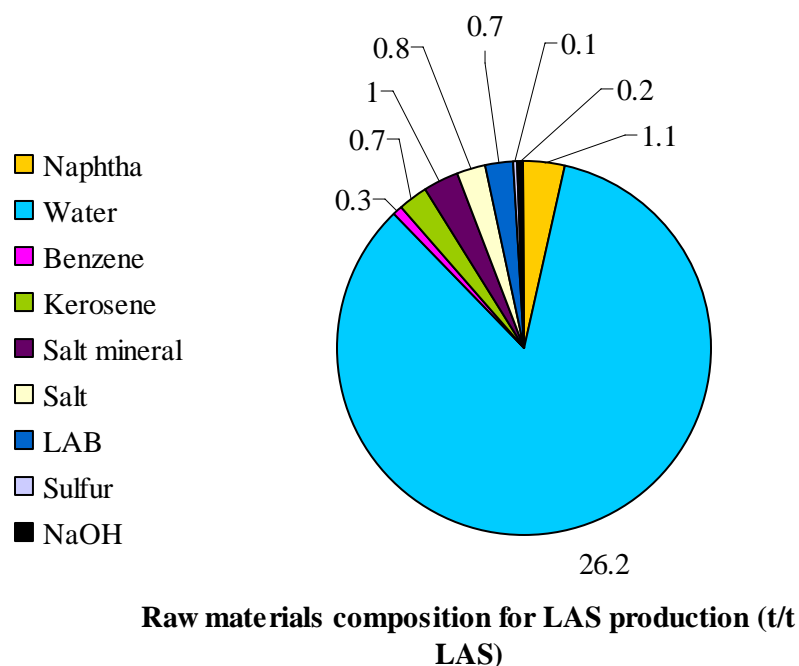
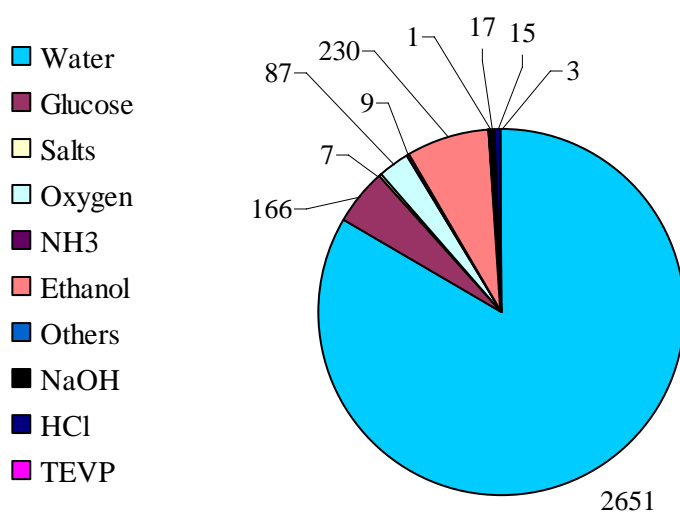


Figure 5.1 Raw materials depletion for Pepfactant[®] AM1 production

The composition of raw materials for producing Pepfactant[®] AM1 is presented in Fig 5.1. In AM1 manufacture, water is consumed considerably and is the most essential material due to the use for media in biomass fermentation and the high demand of washing water to prevent contamination in the biochemical process. It was estimated that 2,651 tonnes of water would be required to produce 1 tonne Pepfactant[®] AM1. On this point, it is necessary to enhance water reuse or recycle. Apart from water, Pepfactant[®] production needs great amount of glucose, estimated to be 166 t/t AM1, which provide the carbon source in the main fermentation. In fermentation, the key unit process to generate biomass, a high quantity of oxygen (87 t/t AM1) is also consumed as a vital ferment source. It is noted that ethanol consumption is high for use at precipitation, estimated to be 230 t/t AM1 although in the simulation model. The vacuum distillation was adapted and is hypothetically able to recycle 98% of ethanol.



Raw materials composition for AM1 production (t/t AM1)

Figure 5.2 Raw materials depletion for LAS production

The composition of raw materials for producing LAS is presented in Fig 5.2. Compared with AM1 production, only 26 tonnes water was consumed by 1 tonne LAS production. In fact, other material requirements of LAS manufacture are also at much lower levels in comparison with AM1 manufacture. As shown in Fig 5.2, the materials include naphtha 1.1 t/t LAS for Benzene production, salt mineral 1 t/t LAS for salt mining, kerosene 0.7 t/t LAS for alkylation, salt 0.8 t/t LAS for NaOH production, LAB 0.7 t/t LAS, NaOH 0.2 t/t LAS and sulphur 0.1 t/t LAS for LAB sulphonation. For LAS manufacture process, alkylation is a material-consuming operation in the entire process, requiring raw materials totalling up to 13.6 t/t LAS (51.7% of the total amount demanded by the process).

The results for raw material requirements of AM1 and LAS manufacture show the great differences between materials demanded, either in quantities or compositions. The essential reason for this difference is the dissimilarity of the manufacture approaches for nano-technological peptide and chemical surfactant. The only common materials required for both surfactants' production is water, taking a major proportion, 83% for AM1 production and 84% for LAS production. Overall, the

Pepfactant AM1 manufacture consumes much larger quantity of materials, especially highly amounted on water, ethanol and glucose.

5.1.2 Energy Requirements

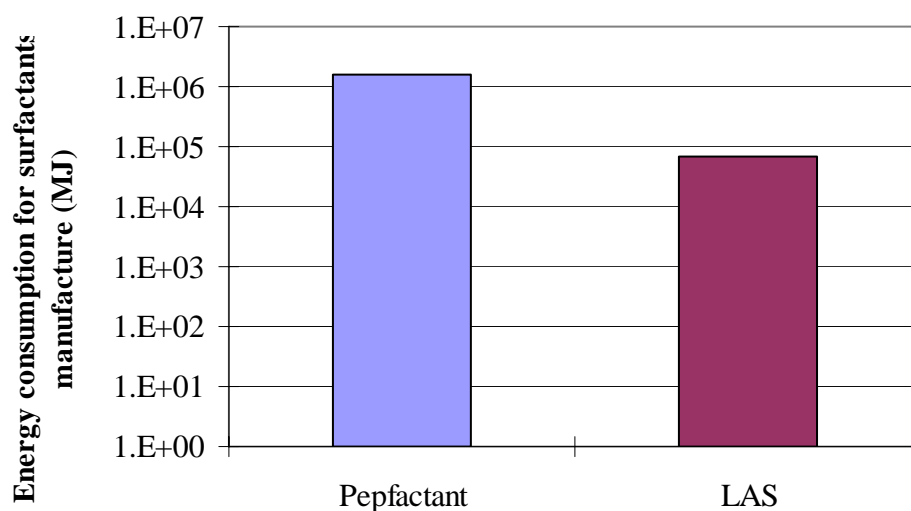


Figure 5.3 Energy requirement comparison between Pepfactant[®] AM1 and LAS production

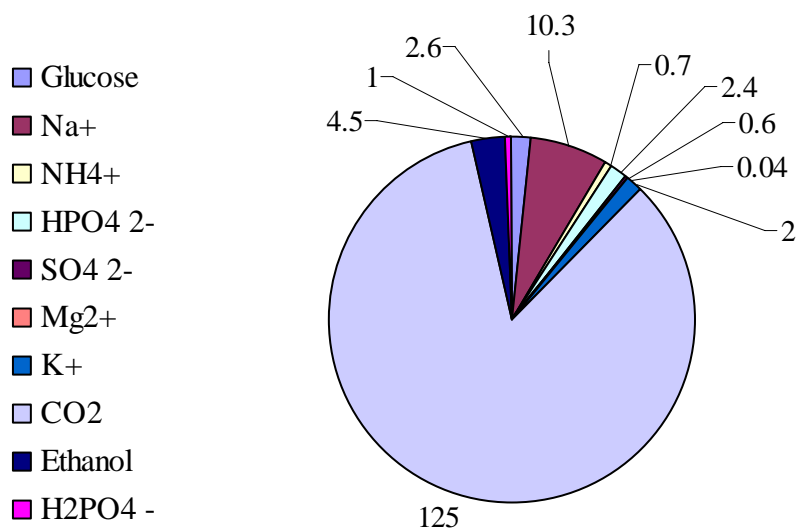
To compare the energy requirements of both surfactants production, as shown in Fig 5.2, the direct energy consumption of Pepfactant[®] and LAS production was estimated. For Pepfactant[®] AM1 manufacture, the direct energy was estimated by investigation of electrical power which was essentially employed to operate the entire process. For LAS production, investigated by the early work of Pittinger *et al* (1993), the energy consumed was aggregated on different forms, including electricity, fossil fuels, and combustion steam or heat.

According to the calculation and estimation on 1 tonne baseline in system boundary, it was noted that the electrical power requirement of the current simulated Pepfactant[®] manufacture process was probable to result in greater energy depletion (1,564,000 MJ/t AM1), compared with the estimated energy consumption on LAS production (69,870 MJ/t LAS).

Within the unit operations during manufacturing Pepfactant[®] AM1, Spray drying needs a high proportion of electrical energy (637,200 MJ/t AM1) under the proposed production scale. The electricity consumed by spray drying is almost equal to the half of that used for the entire manufacture process.

5.1.3 Atmospheric and Aqueous Emissions

Based on the Life Cycle Inventory in Chapter 3, Fig 5.4 and Fig 5.5 display the compositions and quantities of the atmospheric and aqueous emissions from Pepfactant[®] AM1 and chemical surfactant LAS production. Following the emission quantity from high to low, the atmospheric and aqueous emissions from AM1 manufacture include: CO₂, Na⁺, ethanol, glucose, HPO₄²⁻, K⁺, H₂PO₄⁻, NH₄⁺, SO₄²⁻ and Mg²⁺; for LAS manufacture the emissions contain: hydrocarbons, Cl, SO_x, CO, COD, NH₃, HF, NO_x, S, phenol, F and Cr.



Atmospheric and aqueous emissions composition (t/t AM1)

Figure 5.4 Atmospheric and aqueous emissions for Pepfactant[®] AM1 production

As shown in Fig 5.4, the overall emissions from Pepfactant® AM1 manufacture were estimated to be higher than compared with those released from LAS production. Importantly, it is clear to notice that Pepfactant® AM1 manufacture was possible to emit a large amount of green house gas (125 t CO₂/t AM1). This is because CO₂ is the primary co-product in fermentation, which makes the entire process present an unacceptable environmental compatibility in the assessed boundaries. The high percentage of Na⁺ ions were caused by main nutrient salts use and CIP releases – the neutralised NaOH with HCl solution. Indeed, other mineral emissions and ion releases were generated from the excess addition of the nutrient salts in fermentation. The purpose of additional use of these nutrient salts was to ensure the high production of the biomass. In future design, it is crucial to optimise the nutrient addition and the biomass production ratio, aiming to reduce release from processing.

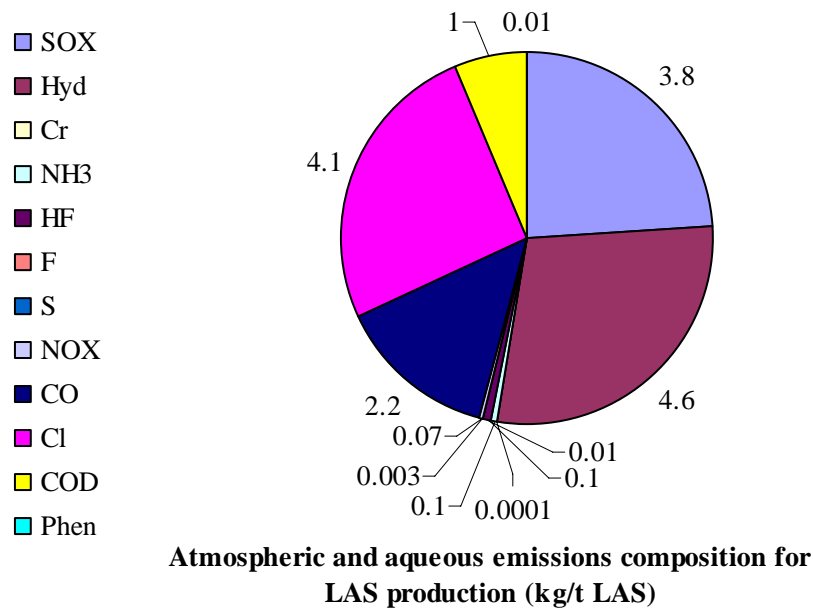


Figure 5.5 Atmospheric and aqueous emissions for LAS production

As shown in Fig 5.5, in comparison, on LAS production, there are no estimated direct CO₂ emissions from the unit operations. But, a more complex composition of the adverse substances involved in a wider range of environmental impacts are

generated from the process, including SO_x (0.004 t/t LAS), NO_x (0.00077 t/t LAS), NH₃ (0.0001 t/t LAS), Hydrocarbons (0.0046 t/t LAS), CO (0.0022 t/t LAS), Cl (0.0041 t/t LAS), HF (0.0001 t/t LAS), and slight quantity of phenol, F, Cr, BOD, COD.

The results on atmospheric and aqueous emissions from AM1 and LAS manufacture indicate the great difference between materials demanded by them, either in quantities or in qualities. Quantitatively, compared with LAS production, Pepfactant[®] AM1 manufacture creates a much larger amount of releases to the air or water, in particular greenhouse gas – CO₂. Qualitatively, the air or water borne emissions from Pepfactant[®] AM1 do not cover as a wide range as compared with LAS production.

5.1.4 CO₂ Emissions (for energy requirements)

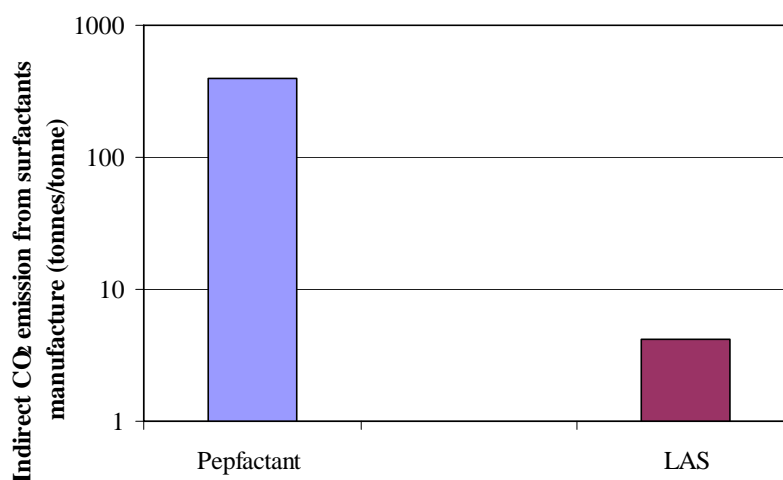


Figure 5.6 CO₂ emissions for energy requirements

As an important criteria to evaluate greenhouse effects, the indirect CO₂ emissions were investigated in manufacturing Pepfactant[®] AM1 and chemical surfactant LAS. Fig 5.6 displays the comparison results based on the estimation.

In the assessment boundary of manufacture phase, the indirect CO₂ emissions were focused on the CO₂ generated by the energy depletion or consumption. For Pepfactant[®] production, as electricity is used as the primary energy source, the CO₂ emissions were estimated from consumption of purchased electricity production. The calculated result – 397 t CO₂/t AM1 was achieved by using average CO₂ emissions factors on consumption of purchased electricity by end users (Department of the Environment and Heritage, Australia, 2006) to multiply the converted electrical power requirement of 1 tonne AM1 product.

In comparison, LAS production creates lower amounts of indirect CO₂ emissions – 4.2 t/t LAS, calculated in the boundary of the manufacture phase by Patel *et al* (1998), without covering product utilisation and degradation phases. In this work, the CO₂ emissions were estimated with combining the fossil fuel (derived from gas, oil,

or coal) and non-fossil fuel (hydro, nuclear, solar, wind energy sourced) related energy and feedstock consumption.

5.1.5 LCA Comparison

Using the LCA method CML (Heijungs, 1992), the environmental impacts from Pepfactant[®] and LAS production in the manufacture phase are characterised and quantified. The LCA comparison results between these two surfactants production are summarised in Fig 5.7. Quantitatively, the comparison was carried out between the characterisation scores of possible environmental impacts from the production of the two surfactants. This was conducted by converting the impact category indicators to the baseline of 1 tonne product, involving raw material depletion, global warming potential (GWP), nitrification potential (NP), human toxicity potential (HT), photochemical oxidant formation potential (POCP), ecotoxicity potential (EP), acidification potential (AP), odorous air and solid waste release.

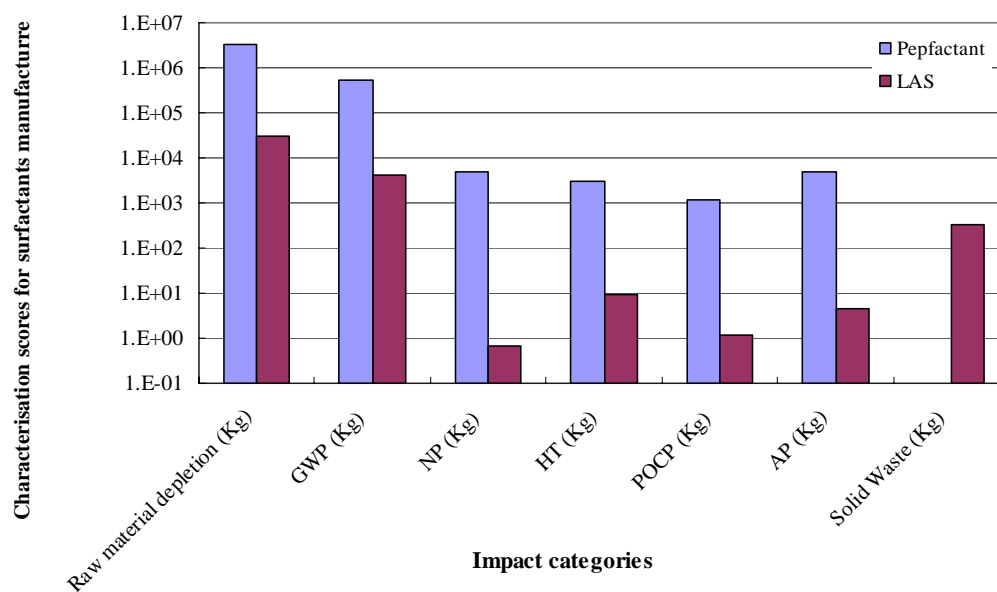


Figure 5.7 LCA comparison between Pepfactant[®] AM1 and LAS production

Specifically, raw material and energy depletion of both surfactants production has been mentioned. Global warming potential covers the direct CO₂ emissions as the co-product and the indirect CO₂ emissions associated with energy consumption. Pepfactant[®] manufacture has a higher GWP than LAS manufacture due to the high emissions of both direct and indirect CO₂. The high potential of human toxicity, eutrophication and acidification from Pepfactant[®] manufacture primarily results from the releases of the excess nutrients and salts added in the media, which can be tackled by lowering down the use when optimising the reaction conditions. Photochemical oxidant formation potential (POCP) is exclusively caused by the ethanol waste, which requires a well designed precipitation and recycle system to reduce the ethanol use or waste. Furthermore, the consumption of NaOH with HCl in neutralisation in CIP operation also has effects on the high potential of human toxicity. As discussed in Chapter 3 (3.4.2), the impacts of ecotoxicity and odour potential are dropped out in this LCA comparison for the reason that it is difficult to assemble the sufficient LCI data for Pepfactant[®] AM1 production under the simulation.

Although LAS manufacture generates a wider range of atmospheric and aqueous emissions, the amount is lower compared with Pepfactant[®] manufacture and thus the emissions have fewer contributions to environmental problems. In fact, the emissions of SO_x, NO_x, CO, NH₃, HF, hydrocarbons from LAS manufacture are mainly responsible for the potential impacts of human toxicity, nutrification, acidification and photochemical oxidant formation.

Currently, it is still disputable if there is superiority for Pepfactant[®] AM1 manufacture compared with LAS manufacture due to the lack information on substances affecting on ecotoxicity and odorous air potential. As Pepfactant[®] AM1 manufacture adopts biomass accumulation and cleavage approach, there is a great deal of cell debris (67.9 t/t AM1) co-produced in the process containing minor capacity of DNA (0.7 t/t AM1). However, these co-products are highly biodegradable and recyclable materials so that the environmental impact of solid wastes from their releases was ignored.

5.2 Foaming Ability Comparison

After implementation of a LCA on both surfactants production and comparison of the environmental impacts from their manufacture processes, it was straightforward to outline the environmental footprints of Pepfactant[®] manufacture. For this early large-scale Pepfactant[®] AM1 manufacture process model, it was located at a lower level of environmental compatibility and sustainability compared with LAS manufacture. This finding is suggestive to direct Pepfactant[®] designers to consider the weakness and optimisation for the process in the issue of sustainability.

The function of Pepfactant[®] is discussed here and is as a significant part of an extended LCA that could involve the application of Pepfactant[®] as foaming agents. For foaming agents, the foam height is an ideal parameter to indicate the basic function – foaming ability. In order to determine the superior foaming ability, the comparison was conducted on Pepfactant[®] AM1 solution and LAS solution.

As the bench experiment results shown in Fig 4.2 and Fig 4.3, the presence of 25 mM HEPES, 0.5 ml 40 μ M AM1 solution (pH 7.4) can achieve the 50 mm foam height at the end of 25 minutes when aerated at 0.2 ml/min. However, LAS at 25 mM HEPES, 0.5 ml 1mM (pH 7.4) generated 50 mm foam height using the same duration at the same aeration period. Additionally, within the same range of foam height, the magnitude of AM1 solution concentration is significantly less than LAS solution concentration, 0-100 μ M AM1 against 0-2 mM LAS. Besides, the increasing trend of concentration dependence of AM1 foams is less obvious than that of LAS foams. Specifically, the results show that the minimum concentration of AM1 solution to generate the maximum foam height within the tested foam height range is 40 μ M while that of LAS solution is 1 mM.

Therefore, under the same conditions, AM1 solution has a superior foaming ability compared with LAS solution. Approximately, to produce the maximum foam height, the concentration of LAS solution was 25 times higher than the concentration of AM1 solution. In a quantified comparison converted to the functional unit of AM1, 1 tonne of AM1 has the potential foaming ability to replace 25 tonnes of LAS.

This comparison result could be regarded as an important trade-off for the environmental impact comparison for the production of both surfactants production. The quantified functional attribute comparison indicates that AM1 has great potential to substitute the conventional chemical surfactant as highly demanded industrial surfactant products. Using the LCA result comparison with the same form as Fig 5.7, Fig 5.8 is the updated version reflecting the difference of environmental impacts from Pepfactant® AM1 and LAS production, in which the LCA boundaries were extended to usage of both surfactants. As displayed in Fig 5.8, the 25-time lower usage rate of Pepfactant® AM1 causes minor environmental impacts of energy requirement and human toxicity compared with LAS as well as decreases the differences of other environmental impacts between them.

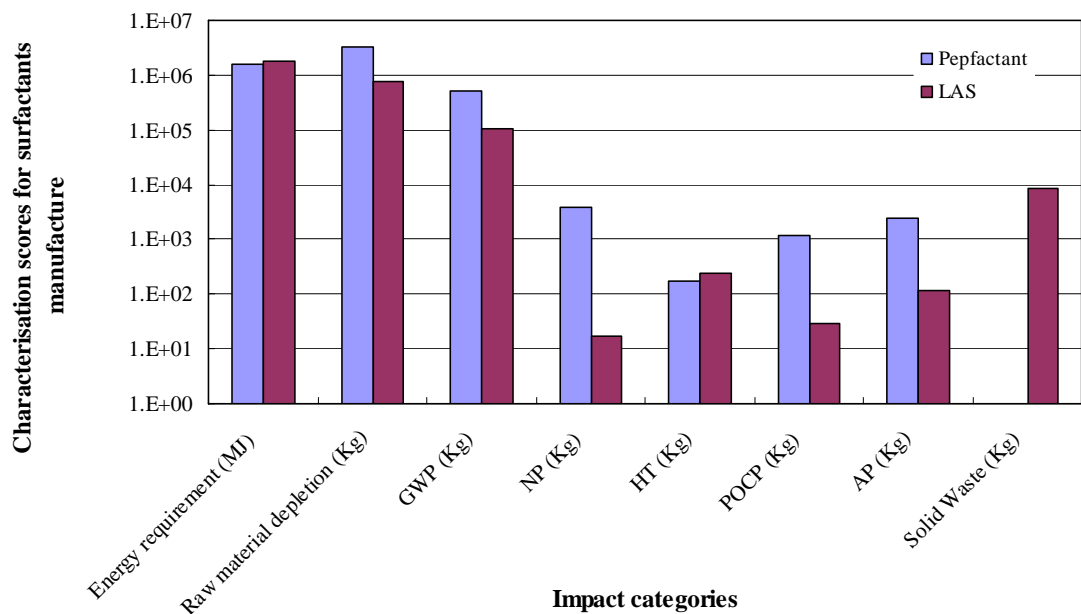


Figure 5.8 Extended LCA comparison between Pepfactant® AM1 and LAS taking account of usage

5.3 LCA Improvement

Based on the LCA comparison restricted on manufacturing process between Pepfactant[®] AM1 and chemical surfactant LAS, the newly proposed industrial scale manufacture of Pepfactant[®] AM1 is weak in terms of environmental compatibility and sustainability. The partial LCA outcomes imply that much greater amounts of raw materials and energy are required to product AM1 and higher magnitude of emissions from AM1 manufacture that have environmental impacts on water and the atmosphere.

Nevertheless, it is vital to note that limited data availability at the design stage leads to the lack of LCA system boundaries for Pepfactant[®] products. In other words, the extension of system boundaries for achieving a complete LCA would potentially improve the LCA results. As analysed in Chapter 3, when the raw material and energy acquisition were regarded, the major environmental impact of AM1 manufacture could be of less importance. Namely, global warming potential of AM1 manufacture could be improved by feedstock – sugarcane’s capability of absorbing massive CO₂. Also, providing that the non-fossil generated fuel was used to produce the leading energy – electricity during manufacturing AM1, the reduced CO₂ emissions would cause a favourable result of global warming potential.

In fact, when the LCA is extended to cover the full cycle of products, the results would be more meaningful. That is why the applicable surfactant function – foaming ability were compared and quantified as an element needing to be involved in the LCA between Pepfactant[®] and chemical surfactant. To draw a more complete view, although the manufacture of Pepfactant[®] AM1 displays inadequate environmental sustainability compared with chemical surfactant LAS, the application of AM1 has considerable potential to substitute LAS, i.e., approximately 1 tonne AM1 replace 25 tonne LAS functionalising as a foaming agent. This finding makes the LCA comparison practical between Pepfactant[®] and chemical surfactant.

Therefore, the system boundaries should be extended so that the LCA can be as complete and accurate as possible. Subsequently, LCA results could be improved by

taking account of each life cycle phase - raw material and energy acquisition, manufacturing, application, and recycle.

6 CONCLUSIONS

6.1 Conclusions

As the real large scale Pepfactant[®] AM1 production has not been established, this gate-to-gate LCA undertaken on AM1 mainly focused on the manufacturing phase. To compile the life cycle inventory of the AM1 manufacture, a series of process assumptions have been advised and the calculations have been carried out on raw material and electrical energy requirements as well as atmospheric, aquatic and solid emissions. In comparison, the life cycle impact assessment was conducted based on the life cycle inventory of LAS production in the published work of Pittinger *et al* (1993) and Patel *et al* (1998). In order to transit to a cradle-to-grave LCA, the assessment boundaries were extended, by using a comparable parameter to indicate the foaming ability difference between AM1 and LAS.

With the CML assessment method (Heijungs, 1992), the characterisation results of Pepfactant[®] AM1 and chemical surfactant LAS manufacture process led to the following conclusions:

- The current system boundaries were set on the manufacture process of Pepfactant[®] AM1 and chemical surfactant LAS production and the functional unit was on the base of 1,000 kg product. This is logical, as characterisation is a phase that occurs after a life cycle inventory is made, which serves for the quantification of environmental involvements associated with the evaluated process. For gate-to-gate LCA as performed in this research, it is critical to identify the specific system boundaries. In order to enable the comparison, it is necessary to maintain unified functional units between the two surfactants manufacture though the assessment procedures.
- Raw material requirements for Pepfactant[®] AM1 and chemical surfactant LAS was aggregated with the net raw materials requirement – 3,186 t/t AM1 against 31.2 t/t LAS. Specifically, water took a great proportion among the materials for surfactants manufacture, 83% for AM1 and 84% for LAS.
- Energy requirement for AM1 and LAS manufacture was traced from different sources. The former depends primarily on the electrical power, much higher

(1,564,000 MJ/t AM1) than the latter - accumulated from the various forms (69,870 MJ/t LAS).

- Carbon dioxide (CO₂) was the main cause of green house gas emissions and responsible for global warming potential during manufacturing AM1 and LAS. For AM1 processing, the CO₂ emissions included the direct emissions (solely from fermentation) and indirect emissions (co-produced from the coal-fired power generation in Australia), totalled up to 522 t/t AM1 compared with 4.2 t/t LAS, estimated for average LAS production in Europe.
- The potentials of nitrification, human toxicity and acidification were characterised on the base of emissions to the water and air according to the inventory of AM1 and LAS manufacture. The remainders of excess nutrients in the media and the release of NaOH washing neutralised with HCl were the main cause of these impacts during AM1 processing. The same impact indicators for LAS manufacture were significantly less.
- For AM1 manufacture, as the biomass releases were expected to be completely able to be reused for animal feedstock, the solid wastes were nil amounted. For LAS manufacture, the solid wastes were totalled up to 0.33 t/t LAS, without specification on the sources and clarifications.
- The ecotoxicity and odours air potential could be estimated from LAS manufacture (quantified effects of 1.36 kg/t LAS and 0.26 kg/t LAS respectively), but from AM1 manufacture because of the lack of the current LCI data,
- For Pepfactant® AM1, when the LCA boundary was extended to feedstock resources, energy generation and the scenario of water recycle, the severe environmental impacts from its manufacture – global warming potential (GWP) and water requirement would be significantly diminished.
- Laboratory scale experimentation results show that AM1 has 25 times higher than LAS foaming ability under identical conditions. The superior foaming ability of AM1 reduces the environmental impacts of the production by 25 times when it comes to the extended LCA involving both surfactants' usage.

6.2 Future Work

At the early design stage of Pepfactants[®] technology, it is of vital importance to propose the appropriate scale-up approaches and optimisation for the production process. Environmental sustainability is a critical concern when designing and optimising the process for new products. Therefore, this project aimed to assist the Pepfactants[®] designers to understand the environmental footprints of proposed large scale Pepfactant[®] AM1 manufacture, analysed with partial LCA and compared with a widely used chemical surfactant LAS.

However, it is necessary to notice that innovation of Pepfactants[®] technology lies on the novel functions and properties applied as surfactants – switchibility and reversibility between foam and detergent states. A thorough LCA or environmental sustainability study of Pepfactants[®] should be performed from the cradle to grave, incorporating an industrial application, disposal and even recycle phases once the large scale production is fulfilled in the future. Particularly, the environmental imprints of great water and other material consumptions e.g. NaOH would be reduced by appropriate recycle technology as well as the tremendous CO₂ emissions would be offset with the regards of the glucose source – sugarcane’s intake.

In that case, the assessment boundaries would be extended further to more comprehensive upstream and downstream processes and additional parameters would be tested and considered during the assessment. Furthermore, the functional unit could be incorporated with the greater foaming ability, such as “tonne or kg of oil removed by a surfactant” in terms of use as cleaning agents. Through the full LCA upon the entire life cycle of Pepfactants[®], it is evident to justify the environmental sustainability of these new products. In addition, it is helpful to predict the potential to replace the conventional surfactants for these novel nano-biomaterial based surfactants.

References

Abbott N.L., 2001, New horizons for surfactant science in chemical engineering, *American Institute of Chemical Engineers Journal*, **47**: 2634

Ajay S., Jonathan D., Van H. and Owen P.W., 2006, Surfactants in microbiology and biotechnology: Part 2. Application aspects, *Biotechnology Advances*, **100**: 100

Akers R.J., 1976, *Foams*, Academic Press, New York

ATSE (Australian Academy of Technological Sciences and Engineering), 2004, Water recycling in Australia, www.atse.org.au/index.php?sectionid=597, viewed on 5th/10/2007

Azapagic A., 1999, Life cycle assessment and its application to process selection, design and optimisation, *Chemical Engineering*, **73**: 1

Badger P.C., 2002, Ethanol from cellulose: a general review, *Trends in new crops and new uses*, edited by Janick J. and Whipkey A., ASHS Press, Alexandria, US

Bare J.C., Norris G.A., Pennington D.W., McKone T., 2003, TRACI: The Tool for the Reduction and Assessment of Chemical and Other Environmental Impacts, *Journal of Industrial Ecology*, **6**:49

Bessler D.U., 1983, Demulsification of Enhanced oil recovery produced fluids, Report of Petrolite Corporation U.S.A.

Bikerman J.J., 1953, *Foams, theory and industrial applications*, Reinhold, New York

Bos M.A., Vliet van T., 2001, Interfacial rheological properties of adsorbed protein layers and surfactants: a review, *Advanced Colloid Interface Science*, **91**: 437

Christmann L.J., 1928, Method and apparatus for measuring froth, US Patent 1,866,296

Ciambrone D., 1997, *Environmental life cycle analysis*, CRC Press, Boca Raton,

CSIRO (Australian Commonwealth Scientific and Industrial Research Organisation), 1998, Upright sugarcane produces more sugar, <http://www.csiro.au/files/media/Release/mr1998/UprightSugarcaneProducesMoreSugar.htm>, viewed on 13th/10/2007

Davidson A.S. and Milwidsky B., 1987, *Synthetic detergents*, Longman Scientific & technical, Essex,

Delta Electricity Australia, 2006, Delta Electricity Sustainability Report 2006, <http://www.de.com.au/default.aspx?FolderID=37&ArticleID=244>, viewed on 11/10/2007

Dexter A.F. and Middelberg A.P.J., 2007, Switchable peptide surfactants with designed metal binding capacity, in press,

Dexter A.F., Malcolm A.S., Middelberg A.P.J., 2006, Reversible active switching of the mechanical properties of a peptide film at a fluid-fluid interface, *Nature materials*, **5**: 502

Engelbrecht C., 2007, Purification strategies for functional peptides, *Masters Thesis*, Australian Institute for Bioengineering and Nanotechnology, The University of Queensland

Fairman R., Chao H.G., Mueller L., Lavoie T.B., Shen L., Nonotny J. and Matsueda G.R., 1995, Characterisation of a new four-chain coiled-coil: Influence of chain length on stability, *Protein Science*, **4**: 1457

Gallardo B.S. and Abbott N.L., 1997, Active control of interfacial properties: a comparison of dimeric and monomeric ferrocenyl surfactants at the surface of aqueous solutions, *Langmuir*, **13**: 203

- Garti N. and Bisperink C., 1998, Double emulsions: progress and applications: Food colloids, emulsions, gels and foams, *Current Opinion in Colloid and Interface Science*, **3**: 657
- Gauthier C., 2005, Measuring corporate social and environmental performance: The extended life-cycle assessment, *Journal of Business Ethics*, **59**: 199
- Goedkoop M., Effting S., Collignon M., 2000, The Eco-indicator 99 (A damage oriented method for life cycle impact assessment) Manual for Designers, <http://www.pre.nl/eco-indicator99/>, viewed on 11th/11/2006
- Graus W.H. J., Voogt M. and Worrell E., 2007, International comparison of energy efficiency of fossil power generation, *Energy policy*, **35**: 3936
- Griffith J.E., Cox R.A., Dennis J.L. and Waugh B.K., 2002 Apparatus and method for foaming fluids and for testing foamed fluids, US Patent 6484568B1
- Haigh S.D., 1996, A review of the interaction of surfactants with organic contaminants in soil, *The Science of the Total Environment*, **185**: 161
- Harrison R.G., Todd P., Rudge S.R. and Petrides D.P., 2002, *Bioprocess design in: Bioseparation science and engineering*, Oxford University, Oxford
- Heijungs R., 1992, *Environmental life cycle assessment of products – Guide*, CML (Centre of Environmental Science), TNO (Netherlands Organisation for Applied Scientific Research) and B&G (Fuels and Raw materials bureau), Leiden
- Hong Y.S., Lau L.S., Legge R.L. and Chen P., 2004, Critical self-assembly concentration of an ionic-complementary peptide EAK, 16-I, *Journal of Adhesion and Adhesives*, **80**: 913
- Huang D.D.W., Nilolov A. and Wasan D.T., 1986, Foam stability in the presence of oil, *Langmuir*, **2**: 672

Hunt R.G., Sellers J.S. and Franklin W.E., 1992, Resource and environmental profile analysis: A life cycle environmental assessment for products and procedures, *Environmental Impact Assessment Review*, **12**: 245

International Organisation for Standardisation, 2006, *ISO 14044: 2006 Environmental management-Life cycle assessment-Life cycle impact assessment*

Itsubo N., 2004, Weighting Across Safeguard Subjects for LCIA through the Application of Conjoint Analysis, *International Journal of LCA*, **9**: 196

Jolliet O., Margni M., Charles R., Humbert S., Payet J., Rebitzer G., Rosenbaum R., 2003, IMPACT 2002+: A New Life Cycle Impact Assessment Methodology, *International Journal of LCA*, **8**: 324

Jones T.J., Neustadter E.L. and Whittingham K.P., 1978, Water-in-crude oil emulsion stability and emulsion destabilisation by chemical demulsifiers, *Journal of Canadian Petroleum Technology*, **17**: 100

Jong L.I. and Abbott N.L., 2000, A chemodegradable surfactants system based on oxidation of disulfide bonds using hypochlorite, *Langmuir*, **16**:5553

Josa A., Aguado A., Cardim A. and Byars E., 2007, Comparative analysis of the life cycle impact assessment of available cement inventories in the EU, *Cement and concrete research*, **37**: 781

Kanidky J.R., Poniatowski A.F. and Mehta N.R., 2000, Cooperativity among molecules at interface in relation to various technological processes: Effect of chain length on the pK (a) of fatty acid salt solutions, *Langmuir*, **16**: 72

Lanunier L.S., Elaine N.S. and Marangoni D.G., 1999, Surfactants and their applications, *Annual Reports on the Progress of Chemistry*, **99**: 3

Latham P.W., 1999, Therapeutic peptides revisited, *Nature Biotechnology*, **17**: 755

Lee G.H., Cooney D., Middelberg A.P.J. and Choe W.S., 2006, The economics of inclusion body processing, *Bioprocess Biosystems Engineering*, **29**: 73

Leja J., 1982, *Surface Chemistry of Froth Flotation*, Plenum Press, New York

Liu Y., Mu L., Liu B.H. and Kong J.L., 2005, Controlled swithable surface, *Chemical Engineering Journal*, **11**: 2622

Lodish H., Baltimore D., Berk A., Zipursky S.L., Matsudaira P., and Darnell J., 1995, *Molecular Cell Biology*, 2nd edition, Scientific American Books Inc.

Lucassen-Reynders E.H., 1981, *Anionic surfactants physical chemistry of surfactant action*, Dekker, New York

Malhotra A.K. and Wasan D.T., 1998, Interfacial rheological properties of absorbed surfactant films with applications to emulsions and foam stability, *Thin liquid films*, Dekker, New York

Malcolm A.S., Dexter A.F. and Middelberg A.P.J., 2006, Foaming properties of a peptide designed to form stimuli-responsive interfacial films, *The royal society of chemistry – Soft matter*, **2**: 1057

Malysa K., Lunkenheimer K., Miler R. and Hartenstein C., 1981, Surface elasticity and frothability of n-octanol and n-octanoic acid solutions, *Colloids surface*, **3**: 329

Martin A.H., Grolle K., Bos M.A., Stuart M.A. and van Vliet. T., 2002, Network forming properties of various proteins adsorbed at the air/water interface in relation to foam stability, *Journal of Colloid interface Science*, **254**: 175

McClements D.J., 2004, Protein-stabilised emulsions, *Current Opinion in Colloid and Interface Science*, **9**: 305

Merrifield R.B., 1963, Solid phase peptide synthesis. I. The synthesis of a tetrapeptide, *Journal of American Chemical Society*, **85**: 2149

Middelberg A.P.J. and Jones D.B., 2003, Interfacial protein networks and their impact on droplet breakup, *American Institute Chemical Engineers Journal*, **49**: 1553

Miettinen P. and Hamalainen R.P., 1997, How to benefit from decision analysis in environmental life cycle assessment (LCA), *European Journal of Operational Research*, **102**: 279

Mikitenko V.S., Maskaev A.K. and Lebedev E.V., 1977, Improvements in Lubricant Quality and in Efficiency of Lubricant Use, *Tsnihteftekhim*, **10**: 101

Mikitenko V.S., Maskaev A.K., Lebedev E.V., Dul'neva L.L. and Dobrova O.A., 1982, Comparative evaluation of methods for determination of foaming tendency of lubricating/cooling fluids, *Khimiya Tekhnologiya Topliv Masel*, **8**: 42

Milner S.T., 1991, Polymer brushes, *Science*, **251**: 905

Miyazaki N., Siegenthaler C., Schoenbaum T., Azuma K., 2003, 21st century COE Monograph Series 7, Social Science Research Institute of International Christian University, Tokyo

Monson L.T. and Stenzel R.W., 1946, *Colloid Chemistry*, edited by Alexander J., Rheinhold Publishing Corp., New York

Morreale G., Lee G.E., Jones D.B. and Middelberg A.P.J., 2004, Bioprocess-Centered Molecular Design (BMD) for the effective production of an interfacially active peptide, *Biotechnology and Bioengineering*, **87**: 912

Mukherjee S. and Kushnick A.P., 1989, Enhanced oil recovery and production stimulation, *Oil-field chemistry*, edited by Borchardt J.K. and Yen T.F., American Chemical Society, Washington D.C.

Muller-Wenk R., 1994, The Ecoscarcity Method as a Valuation Instrument within the SETAC-Framework, *Integrated Impact Assessment into LCA*, SETAC-Europe, Brussels 1994: 115

NLWRA (Australian National Land and Water Resources Audit), 2002, Australia's natural resources 1997-2002 and beyond, Canberra, http://audit.ea.gov.au/ANRA/docs/final_report/final_report_looking_ahead.cfm, viewed on 22nd/10/2007

Painter H.A., 1992, Anionic surfactants, *Handbook of environmental chemistry*, edited by Hutzinger O., Springer verlag, Berlin,

Patel M.K., Therib A. and Worrell E., 1998, Surfactant production and use in Germany: resource requirements and CO₂ emissions, *Resources, Conservation and Recycling*, **25**: 61

Penteado J.C.P., Seoud O.A.E. and Carvalho L.R.F., 2006, Linear alkylbenzene sulfonates: Chemistry, environmental impact and analysis, *QUIMICA NOVA*, **29**: 1038

Peregrina C.A., Lecomte D., Ariabosse P., and Rudolph V., 2006, Life cycle assessment (LCA) applied to the design of an innovative drying process for sewage sludge, *Process safety and environmental protection*, **84**: 270

Petka W.A., Harden J.L., McGrath K.P., Wirtz D. and Tirrell D.A., 1998, Reversible hydrogels from self-assembling artificial proteins, *Science*, **281**: 389

Pittinger C., Sellers J.S., Janzen D.C., Koch D.G., Rothgeb T.M. and Hunnicutt M.L., 1993, Environmental life-cycle inventory of detergent-grade surfactant sourcing and production, *Journal of the American Oil Chemist's society*, **70**: 1

P&G (Procter Gamble) Scientists, 2002, Ingredient safety information - Phys-chem, Environmental Hazard and Fate Data - Linear Alkylbenzene Sulfonate (LAS), http://www.scienceinthebox.com/en_UK/pdf/LAS.pdf, viewed on 11th/06/2007

- Porter M.R., 1991, *Handbook of surfactants*, Blackie & Sons. Ltd., London
- Preston W.C. and Richardson A.S., 1929, The surface properties of soap solutions, *Journal of American Chemical Society*, **10**: 1021
- Pugh R.J., 1996, Foaming, foam films, antifoaming and defoaming, *Advanced Colloid Interface Science*, **64**: 67
- Ross J. and Miles G.D., 1941, An apparatus for comparison of foaming properties of soaps and detergents, *Oil and soap*, **18**: 99
- SAIC (Scientific Applications International Corporation), 2006, *Life cycle assessment: Principles and practice*, American National Risk Management Research Laboratory, Reston
- Schneider J.P., 2002, Reversible hydrogels from the intramolecular folding and self-assembly of a designed peptide, *Journal of American Chemical Society*, **124**: 15030
- Schramm L.L., 2001, *Dictionary of Colloid and Interface Science*, John Wiley and Sons, New York
- Schramm L.L., 1994, *Foams fundamentals and applications in the petroleum industry*, American Chemical Society, Washington D.C.
- Schramm L.L. and Green W.H.F., 1992, An absolute differential maximum bubble pressure surface tensionmeter employing displaced capillaries, *Colloid Polymer Science*, **270**: 694
- Sengupta T., Yate M. and Papadopoulos L.D., 1999, Metal complexation with surface-activity with surface-active Kemp's triacid, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **148**: 259

Sengupta T. and Papadopoulos K.D., 1994, Kemps triacid at oil-water interfaces, *Journal of Colloid Interface Science*, **163**:234

Shin J.Y. and Abbott N.L., Using light to control dynamics surface tensions of aqueous solutions of water soluble surfactants, *Langmuir*, **15**: 4404

Smith V.H. and Arnold K.E.,1992, *Petroleum Engineering Handbook*, edited by Bradley H.B., Society of Petroleum Engineers, Richardson, Texas

Smith B.F., Robinson T.W., Cournoyer M.E., Wilson K.V., Sauer N.N., Mullen K.I., Lu M.T. and Jarvinen J.J, 2004, Polymer Filtration: A new technology for selective metals recovery, *Plating and surface finishing*, edited by American Electroplaters and Surface Finishers Society, California

Standards Australia and Standards New Zealand, 1998, *AS/NZS ISO 14040:1998 Environmental management-Life cycle assessment-Principles and framework*

Standards Australia and Standards New Zealand, 1999, *AS/NZS ISO 14041:1999 Environmental management-Life cycle assessment-Goal and scope definition and inventory analysis*

Steen B., 1999, A systematic Approach to Environmental Priority Strategies in Product Development (EPS). Version 2000 – Models and Data. *Chalmers University of Technology, Centre for Environmental Assessment of Products and material Systems (CPM) Report 1999:5*

Tambe D., Paulis J. and Sharma M.M., 1995, Factors controlling the stability of colloid-stabilised emulsions: 4. evaluating the effectiveness, *Journal of Colloid Interface Science*, **1**: 463

Todd J.A. and Curran M.A., 1999, Streamlined life-cycle assessment: A final report from the SETAC north America streamlined LCA workgroup, Society of Environmental Toxicology and Chemistry (SETAC) and SETAC Foundation for Environmental Education,

<http://www.cnpl.cl/documentos/doc%5Cdisse%C3%B1o%20medioambiental%5CStr>

eamlined%20Life-

Cycle%20Assessment.%20A%20Final%20Report%20from%20the%20SETAC%20North%20America%20Streamlined%20LCA%20Workgroup.pdf., viewed on 8th/03/2007

Venhuis S.H., Mehrvar M., 2004, Health effects, environmental impacts, and photochemical degradation of selected surfactants in water, *International Journal of Photoenergy*, **6**: 115

Weier K.L., 1998, Sugarcane fields: sources or sinks for greenhouse gas emissions? *Australian Journal of Agricultural Research*, **49**: 1

Wenzel H., Hauschild M.Z., Alting L., 1997, *Environmental assessment of products.1 – Methodology, tools, techniques and case studies*, edited by Chapman & Hall, Kluwer Academic Publishers, Hingham, MA, USA

Whidhab E., Dressier M., Feigl K., Fischer P. and Megias-Algualcil D., 2005, Emulsion processing –from single-drop deformation to design of complex processes and products, *Journal of Chemical Engineering Science*, **60**: 2101

Whitesides G.M., 1995, Self assembly and nanotechnology, conference proceedings of The fourth foresight conference on molecular nanotechnology, Palo Alto

Wilde P.J., 2000, Interfaces: their role in foam and emulsion behaviour, *Current Opinion in Colloid and Interface Science*, **5**: 176

Wilde P.J., Mackie A., Husband F., Gunning P. and Morris V., 2004, Proteins and emulsifiers at liquid interfaces, *Advances in Colloid and Interface Science*, **108**: 63

Yadav S.S., 2006, Production and purification of the smart peptide: AM1, *Masters Thesis*, Australian Institute for Bioengineering and Nanotechnology, The University of Queensland

Ying G.G., 2005, Fate, behaviour and effects of surfactants and their degradation products in the environment, *Environment International*, **32**: 417

Zaki N.N., Abdel-Raouf M.E. and Abdel-Azim A.A.A., 1996, Polyoxyethylenated bisphenol-A for breaking water-in-oil emulsions, *Polymers for Advanced Technologies*, **127**: 1239

Appendix A Chemical Surfactants Production

This section provides the detailed information about the traditional approaches for different chemical surfactants production, referred to text 1.3.2.

1. Anionic surfactants

- a) **Soap** is produced from triglyceride through a reaction with sodium hydroxide.
- b) **Alcohol sulphate**, FAS: sulphatation of fatty alcohol and it is indicated that bleaching may be used, most probably with hydrogen peroxide
- c) **Alcoholesulphate**, FESL: a fatty acid alcohol is ethoxilated by ethane oxide and sulphated. During production, by-products such as alcohol sulphate, alcoholesulphate and 1, 4-dioxane are formed, these compounds are not considered problematic.
- d) **Alkylbenzenesulphonate**, LAS: A paraffin is first chlorinated. Then it reacts with benzene in the presence of hydrochloric acid or hydrofluoric acid to produce alkylbenzene. Sulphonation is achieved in a continuous process with sulphur trioxide or sulphuric acid to give the corresponding sulphonic acid. This is then neutralised to give the desired salt, most often sodium. By-products are dialkyltetraline sulphonates and dialkyl indanesulphonates.
- e) **Secondary alkanesulphonates**, SAS: a paraffin is sulphonated with a dilute stream of vaporised sulphur trioxide in a continuous thin film reactor. The olefin is obtained by wax cracking or ethylene polymerisation, using a Ziegler-type catalyst, some discoloration occurs. By-products are disulphonate and sodium sulphate.
- f) **α - Olefinsulphonate**, AOS: a α - olefin is sulphonated with sulphur trioxide. This is an exothermic reaction that causes colouring, requiring bleaching, if not controlled. Performing the reaction in thin films solves the problem. By-products are disulphonate and olefin.
- g) **α - methylestersulphonate**, MES: methylester of a fatty acid is sulphonated with sulphur trioxide. This reaction is complicated, giving a mix of α -methylestersulphonate (80%), disodium salt of the fatty acid (17%) and the soap of the fatty acid (3%).

- h) **Isothionates** are produced through a reaction with a fatty acid and sodium isothionate. The sodium isothionate is produced by a reaction between sodium disulphide and ethane oxide.

2. Non ionic surfactants

- a) **Alcholethoxilate**, AEO: these are produced from an alcohol of fossil or renewable origin and ethene-oxide.
- b) **Alkylphenoethoxilate**, APEO: these are produced through a reaction of an olefin with phenol and further with ethene-oxide.
- c) **Alkylpolyglycoside**, APG: this is produced by a reaction between a saccaride and a fatty acid. Saccarides may be of both mono and disaccaride type.
- d) **Fatty acid ethoxilate** is produced through a reaction with a fatty acid and ethene oxide or polyethylene glycol. The product includes diesters and polyethylene glycol.

3. Cationic surfactants

Quaternary ammonium salts are made through reaction of a tertiary amine and methyl chloride or dimethylsulphate. The amine may be a fatty-alkyl-dimethyl- or di-fatty-alkyl-methylamine. In order to increase the biodegradation of these compounds low molecular amines are used. These are quarternised by reaction with methylchloride or dimethylsulphate, before esterification with fatty acids. 2,3-dihydroxypropanetrimethyl-ammoniumchloride and methyltriethanolamine are the two most common quaternary amines.

4. Amphoteric surfactants

- a) **Betain** is produced through the reaction between a tertiary amine and monochloroacetic acid together with sodiumhydroxide. This produces an inner salt with a positive nitrogen group and a negative carbonyl group. The amine is generally alkyldimethylamine, alkylamidopropyldimethylamine or alkyl-bis(2-hydroxyethyl) amine.

- b) **Sulphobetain** is produced through the addition of epichlorhydrin to tertiary amine (alkyldimethylamine) with a subsequent sulphatation by sodium sulphide.
- c) **Glycinates** and propionates are the names describing amphoteric surfactants. Alkylpolycarboxyglycinates (APAC) is the general name of compounds with one nitrogen molecule which can react with monochloroacetic acid, acrylic acid or chloropropionic acid.

Appendix B Applications of Chemical Surfactants

This section presents basic information about some among the diverse applications of chemical surfactants, referred to text 1.3.3.

1. Ore Flotation

The flotation of solid particles on liquid medium depends on the contact angle, which can be changed by the addition of surfactants (Launier *et al*, 1999). One of the key steps of mineral processes is treating crude mineral ores by flotation, for which a small amount of collector is added during the grinding and slurring process. The collector, which is an anionic, cationic, or non-ionic surfactant, acts to alter the wettability of the ore particles. In practice, a foaming agent is usually added so when air is blown through the suspension, the ore particles attach to the air bubbles. The ore particles then float to the surface where they are recovered by skimming (Leja, 1982). Flotation of this type is also used as purification for sludges and effluents.

2. Surfactants and Detergency

Detergency is defined as “the action of surfactants that causes or aids in the removal of foreign material from solid surfaces by adsorbing at interfaces and reducing the energy needed to effect the removal” (Schramm, 2001). Usually wetting agents that rapidly diffuse and adsorb at appropriate interfaces are most effective. An instance of traditional detergent, soap is produced by the saponification of glyceride oils and fats with NaOH or KOH, with glycerol generated as a by-product. However, more synthetic detergents are manufactured and have replaced soaps, with the bulk of manufacturing deriving from alkyl sulfates, alkyl-aryl sulfonates and non-ionic polyethylene oxide.

An efficient detergent must be a good wetting agent, possess the ability to displace soil materials into the washing fluid, be a good solubilising agent, and be a reasonable anti-redeposition agent (Launier *et al*, 1999). The best detergents are made of surfactants which effectively adsorb at the air-water and solid-water

interfaces, the mechanism of which can be explained as outstanding detergents able to form surfactant micelles.

3. Foams and froths

A mixture of gas with either oil or water makes practical foams and the gas phase turns up in the form of bubbles dispersed within the liquid. Foam drainage is an important element in the formation and early development of foam (Weaire *et al*, 1998). Industrial occurrences of foams are long-standing and widespread. In fact, foams may be applied or encountered at all stages in the processing industries and have important properties that may be desirable in some process contexts and undesirable in others (Schramm, 1994; Bikerman, 1953; Akers, 1976; Bikerman, 1973).

Although many factors such as film thickness and adsorption behaviour have to be taken into account, the ability of a surfactant to reduce surface tension and contribute to surface elasticity are the most important features of foam stabilisation. The relationship between foam stability and surface elasticity can answer the questions about why some surfactants will act to promote foaming while others reduce foam stability, while others prevent foam formation in the first place (Malysa *et al*, 1981; Lucassen-Reynders, 1981; Schramm *et al*, 1992; Huang *et al*, 1986).

4. Defoaming

There are such agents preventing foam formation in the first place (foam preventatives or foam inhibitors) while sometimes reducing the foaming stability of a system (Hong *et al*, 2003). Although these agents are not straightforward surfactants, their relevance to surfactants is that in all cases the cause of the reduced foam stability can be traced to various kinds of changes in the nature of surfactant-stability interface (Launier *et al*, 1999).

When adding the defoamers to a foaming system, they act by co-solubilisation or by replacement of the original surfactants into the interface. They may decrease dynamic foam stability if the substance acts against the formerly present stabilising

factors. Defoamers tend to be adsorbed at the gas/liquid interface, displacing foam promoting surfactant and breaking or inhibiting foam with very slight solubility in water. Instead, foam can be destroyed by adding a chemical that actually reacts with the foam-promoting agents. Foams may also be destroyed or inhibited by the addition of certain insoluble substance (Launier *et al*, 1999; Pugh, 1996).

5. Emulsions

Emulsions present a dispersed phase of the mixture of oil and water, which is a continuous phase with the droplets dispersed within the different phases. The typical range of the diameter of the droplet is between 0.1 and 100 μm . But it can be as low as down to nanometers or up to hundreds of micrometers. While most common types of emulsion are used on the phases of oil-water or water-oil, they can be applicable for multi-type, oil-water-oil, water-oil-water or more complex circumstances. Emulsions play an active role in the processing industries and have important properties that could be desirable in some process contexts and undesirable in others. They have versatile applications in cosmetics, agriculture, food, photography, leather, and drug-delivery (Garti *et al*, 1998).

6. Demulsification

Not all of the processes need emulsions, in some process industries chemical demulsification is frequently used to separate water from oil in order to produce a fluid suitable for further processing. The primary step in systematic emulsion breaking is to characterise the emulsion in terms of its nature (oil-water, water-oil, or multiple emulsion), the number and nature of immiscible phases, the presence of a protective interfacial film around the droplets and the sensitivity of the emulsifiers (Malhotra *et al*, 1998; Jones *et al*, 1978; Tambe *et al*, 1995).

Based on an emulsion characterisation, a chemical addition can be prescribed to neutralise the effect of the emulsifier, followed by mechanical means to complete the phase separation. A wide range of chemical demulsifiers are available in order to effect this separation (Tambe *et al*, 1995; Bessler, 1983; Mukherjee *et al*, 1989). The classification of surfactants as demulsifiers usually depends on chemical structure.

Demulsifier surfactants include soaps, glyceryl esters, fatty acid esters, fatty alcohols, and alkylphenol ethoxylates; alkyl sulfonates, alkyl aryl sulfonates, and alkyl aryl sulfates; alkylpolyoxyethylene glycol ethers and alkylphenol (ethylene oxide) ethers; derivatives of alkyltrimethylammonium salts and alkylpyridium salts, and polyester amines and others (Smith *et al*, 1987; Beger *et al*, 1987; Mouson *et al*, 1946; Grace, 1992; Zaki, 1996)

Appendix C Short Description of LCIA Methods

1. CML Guide

CML is the Centre of Environmental Science at the University of Leiden, Netherlands. CML guide is a systematic methodology proposed by the research group of CML in early 1990's to accelerate the targeted product policy measures, a part of The Netherlands National Environmental Policy Plan. The method described in this manual implement LCA as instrument to support the product policy and also is a philosophy. The method can also be used as a tool for ecological product development and improvement in industry, as a regulatory instrument for government and as an instrument to inform consumers. CML guide essentially comprises two parts: Guide and Backgrounds.

The Guide describes a method which can be used to carry out an environmental assessment of the life cycle of one or more products. Hence, it is largely aimed at those who actually undertake environmental product assessments. There are likely to be consulting engineers, scientific institutes and departments of large companies. The Backgrounds describe the reasoning behind the method described in the guide. The reasons for certain choices are explained and compared to methods used elsewhere. The guide which is intended for the implementation of life cycle assessment is divided into three sections: the summary which includes all guidelines, the report itself and the appendices (Heijungs *et al*, 1992).

2. Eco-indicator 95/99

The Eco-indicator '95 methodology is being used very often by designers but is criticised by environmental experts at the same time because some environmental aspects were not accounted for in the method. The new Eco-indicator 99 method includes many more aspects and is therefore more complex than the 95 version but the resulting Eco-indicators are still the same user-friendly units. The weighing system between the different environmental aspects - the core of the Eco-indicator method - has also been changed. The 1995 Eco indicator used the so-called Distance-

to-Target approach. This method was criticised because there was no clear-cut objective way to define sustainable target levels. This problem is in the present Eco-indicator method avoided by introducing a damage function approach. The damage function presents the relation between the impact and the damage to human health or to the ecosystem. The Eco-indicator 99 does reflect the present state of the art in LCA methodology and application. This of course does not mean that all problems are solved. Further developments in environmental science, material technology and LCA methodology will take place and should result in future improvements of the Eco-indicator (Goedkoop *et al*, 2000)..

3. EDIP 97/ 2003

EDIP97 is a thoroughly documented midpoint approach covering most of the emission-related impacts, resource use and working environment impacts (Wenzel *et al.*, 1997, Hauschild and Wenzel, 1998) with normalization based on person equivalents and weighting based on political reduction targets for environmental impacts and working environment impacts, and supply horizon for resources. Ecotoxicity and human toxicity are modeled using a simple key-property approach where the most important fate characteristics are included in a simple modular framework requiring relatively few substance data for calculation of characterization factors.

Update through EDIP2003 methodology (Hauschild and Potting, 2003, Potting and Hauschild, 2003) supporting spatially differentiated characterization modeling which covers a larger part of the environmental mechanism than EDIP97 and lies closer to a damage-oriented approach. This part of the general method development and consensus programme covers investigations of the possibilities for inclusion of exposure in the life cycle impact assessment of non-global impact categories (photochemical ozone formation, acidification, nutrient enrichment, ecotoxicity, human toxicity, noise) (Wenzel *et al*, 1997).

4. EPS 2000d

The EPS 2000d impact assessment method is the default impact assessment method in the EPS system. It is developed to be used for supporting choice between two product concepts. Category indicators are chosen for this purpose, i.e., they are suitable for assigning values to impact categories. Category indicators are chosen to represent actual environmental impacts on any or several of five safeguard subjects: human health, ecosystem production capacity, biodiversity, abiotic resources and recreational and cultural values. The characterization factor is the sum of a number of pathway-specific characterization factors describing the average change in category indicator units per unit of an emission, e.g. kg decrease of fish growth per kg emitted SO₂. An estimate is made of the standard deviation in the characterization factors due to real variations depending on emission location etc. and model uncertainty. This means that characterization factors are only available, where there are known and likely effects. Characterization factors are given for emissions defined by their, location, size and temporal occurrence. Most factors are for global conditions 1990 and represents average emission rates. This means that many toxic substances, which mostly are present in trace amounts, have a low average impact. Weighting factors for the category indicators are determined according to people's willingness to pay to avoid one category indicator unit of change in the safe guard subjects (Steen, 1999).

5. Impact (2002)+

The IMPACT 2002+ life cycle impact assessment methodology proposes a feasible implementation of a combined midpoint/damage approach, linking all types of life cycle inventory results (elementary flows and other interventions) via 14 midpoint categories to four damage categories. For IMPACT 2002+ new concepts and methods have been developed, especially for the comparative assessment of human toxicity and eco-toxicity. Human Damage Factors are calculated for carcinogens and non-carcinogens, employing intake fractions, best estimates of dose-response slope factors, as well as severities. The transfer of contaminants into the human food is no more based on consumption surveys, but accounts for agricultural and livestock production levels. Indoor and outdoor air emissions can be compared and the intermittent character of rainfall is considered. Both human toxicity and ecotoxicity effect factors are based on mean responses rather than on conservative assumptions.

Other midpoint categories are adapted from existing characterizing methods (Eco-indicator 99 and CML 2002). All midpoint scores are expressed in units of a reference substance and related to the four damage categories human health, ecosystem quality, climate change, and resources. Normalization can be performed either at midpoint or at damage level (Jolliet *et al*, 2003). The IMPACT 2002+ method presently provides characterization factors for almost 1500 different LCI-results, which can be downloaded at <http://www.epfl.ch/impact>.

6. JEPIX

This method is developed and applied by the JEPIX Forum, a voluntary initiative of several organizations and private persons from Environmental Accounting, Environmental Management, Eco-Rating and Life Cycle Impact Assessment in Japan. Inspired by the Swiss EcoScarcity method, JEPIX is based on the distance-to-target principle, but in many respects takes different approaches to derive Ecofactors for the weighting of interventions. The method puts more emphasis on a transparent, simple and understandable, but trend-consistent description of the political situations rather than on the preciseness of natural science based modelling. It is designed to indicate, where political pressure is high and therefore new legal requirements are likely to occur and hence to rise environmental costs for industry. Therefore it is considered as complementary to existing LCIA methods, which indicate damage to environment and/or society.

A first version of JEPIX was published in 2003 as a draft focusing on emissions and addressing 11 focal subjects of Japanese environmental legislation. It provides weighting factors for some 1050 interventions. For substance bound legislation, the weighting is based on annual flows (actual and target), whereas for effect oriented legislation midpoint models such as GWP, ODP, Human Toxicity or POCP are used to derive national flows. As the environmental situation varies substantially across Japan, the weighting factors for some 150 substances are scaled to reflect the situation in each of the 47 prefectures as well as for some 100 rivers, 15 lakes and 3 inland sea areas/bays.

The draft version was published in 2003 with support of the Japan Environmental Ministry (MoE), the Ministry for Economy Trade and Industry (METI) and the Ministry for Education and Technology (MEXT). Since 2003 some 40 leading Japanese Companies (including Komatsu, Canon, TEPCO, Suntory, Fuji Film, All Nippon Airways, J-Power, etc.) are applying this method to evaluate and communicate their environmental performance data and to conduct LCA of products and services. Under the Centre of Excellence Program of the Japanese government, the method will be enhanced based on their experience. The final version of JEPIX is expected for publication in 2006. An integration of resources as well as the adoption of newly available data on chemicals is already under development.

7. LIME

LCA National Project of Japan has conducted a study aimed at the development of a Japanese version of the damage oriented impact assessment method called LIME (Life-cycle Impact assessment Method based on Endpoint modeling). In LIME, the potential damage on socio economic impact caused by the utilization of abiotic resources, increase of extinction risk and loss of primary production caused by mining of resources are measured as main damages of resource consumption. Modeling socio-economic impact was based on the concept of user-cost, which accounts for the equity of future generations. The procedure to measure damages on ecosystem is based on studies estimating the risk of extinction of specific species in the field of conservation biology. Lists of damage factors of mineral resources, fossil fuels and biotic resources like wood material have already prepared and released to the public. The development of these factors enables us to compare and integrate with the damages derived from the other impact categories like global warming and acidification without value judgment of ordinary people (Itsubo *et al*, 2004).

8. Swiss Eco-Scarcity

The method of environmental scarcity – sometimes called Swiss Ecopoints method – allows a comparative weighting and aggregation of various environmental interventions by use of so-called eco-factors. The method supplies these weighting factors for different emissions into air, water and top-soil/groundwater as well as for

the use of energy resources. The eco-factors are based on the annual actual flows (current flows) and on the annual flow considered as critical (critical flows) in a defined area (country or region).

The eco-factors were originally developed for the area of Switzerland (see references below). There, current flows are taken from the newest available statistical data, while critical flows are deduced from the scientifically supported goals of the Swiss environmental policy, each as of publication date. Later, sets of eco-factors were also made available for other countries, such as Belgium and Japan.

The method has been developed top-down and is built on the assumption that a well established environmental policy framework (incl. the international treaties) may be used as reference framework for the optimization and improvement of individual products and processes. The various damages to human health and ecosystem quality are considered in the target setting process of the general environmental policy; this general environmental policy in turn is then the basis for the 'critical flows'. An implicit weighting takes place in accepting the various goals of the environmental policy. The ecopoints method contains common characterization/classification approaches (for climate change, ozone depletion, acidification). Other interventions are assessed individually (e.g. various heavy metals) or as a group (e.g. NM-VOC, or pesticides).

The method is meant for standard environmental assessments, e.g., with specific products or processes. In addition, it is often used as an element of environmental management systems (EMS) of companies, where the assessment of the company's environmental aspects (ISO 14001) is supported by such a weighting method.

The method was first published in Switzerland in 1990 (Muller-Wenk, 1994).

9. TRACI

TRACI is an impact assessment methodology developed by the U.S. Environmental Protection Agency that facilitates the characterization of environmental stressors that have potential effects, including ozone depletion, global warming, acidification, eutrophication, tropospheric ozone (smog) formation, ecotoxicity, human health

criteria-related effects, human health cancer effects, human health noncancer effects, and fossil fuel depletion. TRACI was originally designed for use with life-cycle assessment (LCA), but it is expected to find wider application to pollution prevention and sustainability metrics.

To develop TRACI, impact categories were selected, available methodologies were reviewed, and categories were prioritized for further research. Impact categories were characterized at the midpoint level for various reasons, including a higher level of societal consensus concerning the certainties of modeling at this point in the cause-effect chain. Research in the impact categories of acidification, smog formation, eutrophication, human health cancer, human health noncancer, human health criteria pollutants was conducted to construct methodologies for representing potential effects in the United States. Probabilistic analyses allowed the determination of an appropriate level of sophistication and spatial resolution necessary for impact modeling for each category, yet the tool was designed to accommodate current variation in practice (e.g., site-specific information is often not available). The methodologies underlying TRACI reflect state-of-the-art developments and best-available practice for life-cycle impact assessment (LCIA) in the United States (Bare *et al.*, 2003).