Optimisation Of An Enzyme Treatment Process For Sheepskins

A thesis presented in partial fulfilment of the requirements for the degree of Master of Engineering in Bio-Process Engineering

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List of Abbreviations and Terms Used:

LASRA – Leather and Shoe Research Association of New Zealand

1SCrossC – 1 step cross-current process

2SCrossC – 2 step cross-current process

3SCrossC – 3 step cross-current process

4SCrossC – 4 step cross-current process

2SCounterC – 2 step counter-current process

3SCounterC – 3 step counter-current process

Slats – Processed sheepskins just after washing stages of pre-tanning operations

Pelts – Processed sheepskins just after pre-tanning operations

Green skin – Raw sheepskins with the wool still not depilated

Beamhouse operations – Current industry standard pre-tanning operations using sulphide and lime depilant along with the 'beam' knife

Fellmongery – Industrial facility in which pre-tanning/beamhouse operations are conducted

Fellmongering/Pre-tanning operations – Industrial process of removing valuable wool from raw (or green) wool skins to convert sheepskins into pickled pelts

Zone 1 – Washing stages of the LASRA process

Zone 2 – Enzyme treatment stages (including neutralization process) of the LASRA process

LASRA process – LASRA enzyme treatment process to be optimized and improved in this work

Float – Solution/Suspension of liquid (water and solids) which makes up the aqueous phase in pre-tanning operations

Product – Processed sheepskins

Bating – A form of enzyme treatment process (using a different enzyme as the LASRA process) following the deliming process in conventional pre-tanning operations

Process operation – A single stage/part of pre-tanning operations e.g. washing stage process operation or enzyme treatment process operation,

List of Nomenclature Used

$M_{x\text{-}yz}$ (units' kg)

Where;

x - Insoluble solids (IS)
- Total Dissolved Solids (TDS)
- Total Solids (TS)
- Moisture Content (W)
- Grease (G)
- Sulphide (S2-)
- Enzyme Dissolved Solids (EDS)
- Total Kjeldahl Nitrogen (TKN)
- Total (T)

And;

y - Product stream (P)
- Solvent stream (S)
- Effluent stream (E)

And;

z - 1 to 5 (for stream S and E)
- 0 to 5 (for stream P)

$C_{x\text{-}yz}$ (units' kg_x/kg)

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ABSTRACT

An enzyme treatment process for the pre-tanning of sheepskins has been previously reported by the Leather and Shoe Research Association of New Zealand (LASRA) as an alternative to current industry operations. The newly developed process had marked benefits over conventional processing in terms of a lowered energy usage (73%), processing time (47%) as well as water use (49%), but had been developed as a "proof of principle". The objective of this work was to develop the process further to a stage ready for adoption by industry. A process ready for adoption by industry is one that is able to generate good quality products (good quality products are those that has solids removed to industry standards, without visible damages to grain surface, evenly dyed etc.), require minimal changes to plant or operation layout and reduce usage of resources (e.g. water and chemicals).

Mass balancing was used to investigate potential modifications for the process based on the understanding developed from a detailed analysis of preliminary design trials. Results showed that a configuration utilising a 2 stage counter-current system for the washing stages and segregation and recycling of enzyme float prior to dilution in the neutralization stage was a significant improvement. Benefits over conventional processing include a reduction of residual TDS by 50% at the washing stages and 70% savings on water use overall. Benefits over the un-optimized LASRA process are reduction of solids in product after enzyme treatment and neutralization stages by 30%, additional water savings of 21%, as well as 10% savings of enzyme usage.

The optimized (new) LASRA process uses existing equipment and requires no additional outlay of capital. The process is now developed to a point where it should be trialled at industrial scale.

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The LORD God made garments of skin for Adam and his wife and clothed them.

Genesis 3:21 (NIV 2005)

1.0 GENERAL INTRODUCTION

The preliminary stage of ovine leather processing is designed to condition the sheepskins for subsequent tanning operations. This is conventionally achieved by sulphide depilation of wool from sheepskins followed by a series of washing stages with freshwater and strong alkali to remove wool and unwanted protein components. This leaves behind the collagen and elastin proteins required in finished leather. A novel enzyme treatment process was recently developed by the Leather and Shoe Research Association of New Zealand (LASRA) as an alternative to current industry standard beamhouse (pre-tanning) operations. This is described in more detail in chapter 2 but the key features are summarized below in order to describe the context of this work. A block diagram of the general concept of the LASRA process is seen in Figure 1.1 below;

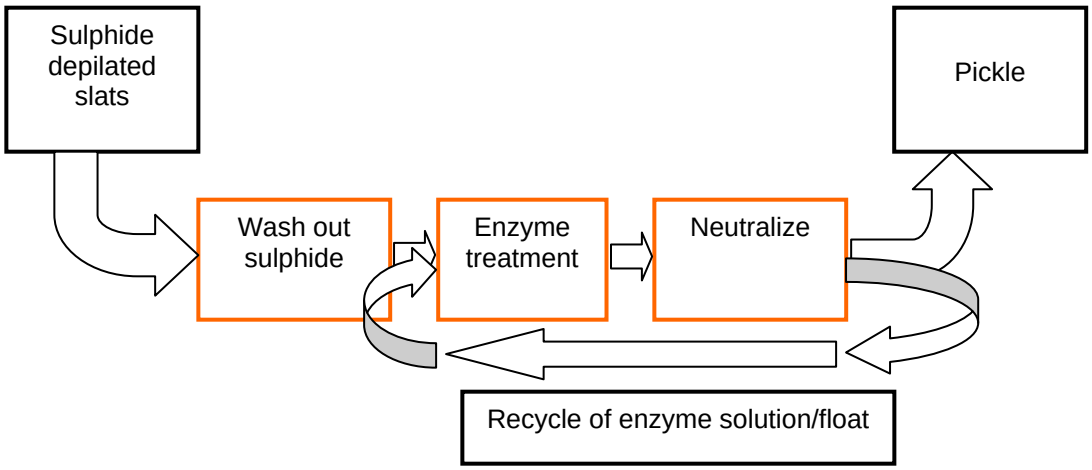


Figure 1.1: Block diagram of the LASRA enzyme treatment process

The main feature of the LASRA process is the replacement of strong alkali washes and bating steps in conventional processing with a single enzyme treatment process. This new process reduces alkali use with a significant increase in processing rates, reducing typical processing times from 26 to 14 hours (Allsop 2007). In each stage, the skins were contacted with a solvent stream (containing e.g. water or enzyme solution) in a rotary drum for a set length of time (i.e. a

semi-batch process). The float is then drained after processing and the skins are then contacted with the solvent stream of the following process operation.

The starting point of this work was the process developed by LASRA as seen in more detail in Figure 1.2. It may be divided into 2 sections which will be optimized individually.

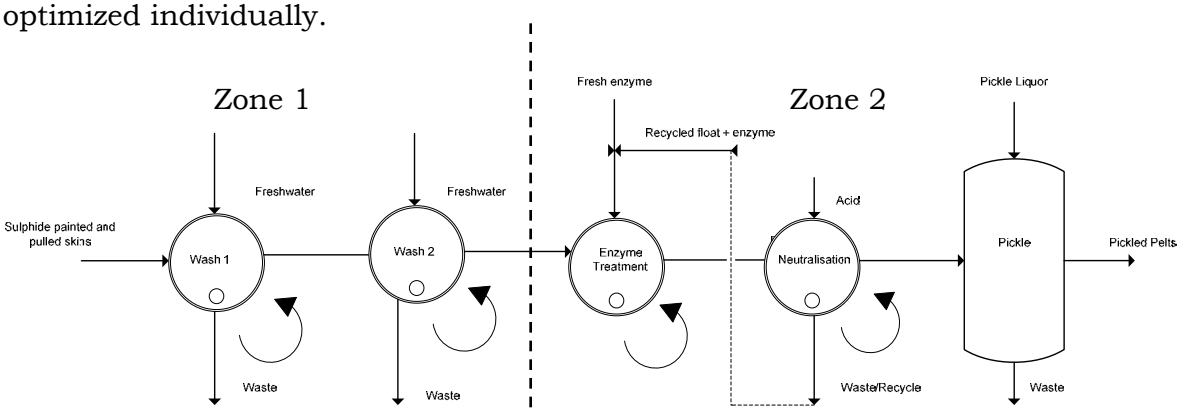


Figure 1.2: Flow diagram of the LASRA enzyme treatment process. Zone 1 are the washing stages while zone 2 are the enzyme treatment stages to be detailed in chapters 4 and 5 respectively.

The first section is two co-current washing stages which were tasked primarily to remove traces of sulphide and lime depilation paint and other soluble material from the sheepskin. The washing stages section of the LASRA process is known as Zone 1 in this work. After the washing stages, the skins undergo enzyme treatment and a neutralization process (known as zone 2 in this work). The enzyme treatment solubilises unwanted insoluble solids (mainly protein) leaving the important collagen and elastin proteins. The neutralization process arrests the proteolytic activity of the enzyme and prepares the sheepskins for the pickling operations by lowering the pH to a neutral range from strongly alkaline conditions. The major benefits of the LASRA process are;

1. It delivers acceptable quality pickled pelts. This is of primary concern as industry is unlikely to adopt a new process unless it can achieve the quality of product currently produced by conventional processing.
2. Reduction of processing time by 47% compared to conventional processing. This was achieved by a reduction of the number of process stages overall.

3. Energy usage reduction by 73%. This was achieved through the reduction of processing time in rotary drums as a result of reduced process stages. Savings were also delivered as a result of the removal of heating systems which was an important feature of the conventional process.
4. Water savings by up to 49%. This was achieved through a reduction of process stages especially washing stages.
5. Reduced chemical usage. This was possible through the implementation of an enzyme treatment stage (with the LASRA process) which displaced the liming stages and reduced overall quantities of alkali used. Savings on enzymes used in bating with conventional processing was offset by the use of proteolytic enzymes in the enzyme treatment process of the LASRA process.
6. Reduced environmental impact. This was achieved by a reduction of the use of chemicals that were known hazardous and environmental pollutants such as sulphide and lime.

Despite the benefits, the process was developed by LASRA as a proof of principle. Further work was required to optimize the process and make it more suitable for industrial application. A process suitable for ready uptake by industry must be able to;

- Generate good quality products (pickled pelts). Good quality products are skins that have adequate solids removed (to industrial standards), do not have any visible damages to grain and skin surface in addition to it being evenly dyed. This is especially important for tanneries found in countries such as New Zealand which places importance on higher quality products which translates to higher market price. Pelt quality is dependent on removal of non-collagenous protein components without damaging the key collagenous proteins required in the finished leather. Of secondary importance is the effectiveness of the process at washing soluble material from the skin. LASRA have shown that their proof of principle process can produce pelts of acceptable quality using their identified enzyme and

processing conditions (time). As such, optimisation for quality of finished product can be based on improving the efficiency of washing the skins.

- Use existing capital equipment. Trialling and adoption of the process in established tanneries will be much easier and less expensive if minimal or no changes were required to capital equipment and operating systems.
- Reduce resource use. A reduction of resource use will translate to cost savings for tanneries. On a global perspective, savings of natural resources especially, is of high importance to maintain sustainability of the resources and reduce waste generation.
- Minimize energy use. Reduction of energy usage will result in costs reduction as well as reduced energy generation.
- Reduce or at least maintain status quo of tanning industry's environmental impacts. With one of the main aims of sheepskins processing being to remove as much unwanted solids as possible, the better process is one which maintains environmental discharges as close as possible to only those originating from skins.

The purpose of this work was to apply process engineering principles to optimise the proof of principle process developed by LASRA (Figure 1.2) and present a modified process ready for adoption by industry. The optimization in this work was done on the basis of both improvements of washing effectiveness (quantified by residual total dissolved solids on processed sheepskin) and reduction of resource (water and chemical/enzyme) use. Potential improvements to finished leather quality through changes to enzyme selection and treatment conditions was a separate LASRA research project and was outside of the scope of this work.

At the conclusion of the work, the following specific objectives were required to be met;

- Presentation of an optimized version of the LASRA process that will meet the requirements of a pre-tanning process suitable for industry.

- Evaluation of the benefits of this optimized process in comparison with both conventional processing and the LASRA proof of principle (as seen in Figure 1.2).

2.0 LITERATURE REVIEW

To identify processing strategies and technologies that might be used to improve the LASRA enzyme treatment process, a thorough review of the literature on conventional fellmongery operations and attempts to improve or replace these processes was required. The following was to be achieved through a review of literature;

1. A detailed review of the conventional pre-tanning (beamhouse) process currently used in industry.
2. Problems associated with conventional pre-tanning operations in the form of raw materials use (with the impacts of their discharge) and with its general operations were to be identified.
3. A review of the research work on providing solutions to the problems of conventional pre-tanning process operations.
4. Reports on the design and effectiveness of the enzyme treatment process developed by LASRA were also explored in more detail in this chapter.

2.1 Tanning Process for Sheepskins

One of the oldest crafts known by men is the skill of leather tanning. Even in the book of Genesis in the bible, it talks about God giving Adam and Eve (the first of humankind forefathers from a biblical worldview) animal skins to clothe them (NIV 2005). Through paintings on cave walls, it can be seen clearly that men and women during the Stone Age have also already been using leather or at least hides of animals as clothing. A little more recently, proofs from archaeology have found that ancient Hebrews and Egyptians from a few thousands of years ago have used leather that are so well manufactured and preserved that some are even found in good shape today (Churchill 1983). Some of the most primitive methods of leather (essentially processed and preserved animal hides) tanning were by smoking the animal hides or use of vegetable tannins (Churchill 1983, Crispim & Mota 2003). Other tanning methods discovered later (mostly through trial and error) have been found to preserve leather for longer periods of time as well as to result in a softer and more flexible texture.

Facilities which perform beamhouse operations are known as fellmongeries. These may be found together with a slaughterhouse or as an independent facility, some even with attached tanneries (Carrie & Woodroffe 1960, European Commission 2001). Currently, the majority of the sheepskins produced in New Zealand are conditioned by pre-tanning operations (by removal of unwanted proteins and other solids) and pickled to be sold to European and Asian markets where tanning operations are performed (Allsop 2008, European Commission 2001). The following sub-sections will provide a thorough overview of the conventional pre-tanning operations.

2.1.1 Conventional pre-tanning process operations

Sheepskins are usually a by-product from the meat processing industry. The skins that are obtained from the slaughterhouse are usually cured (by a drying method or brine treatment) first to reduce the progress of bacterial and biochemical decomposition. Before commencement of pre-tanning operations, the skins are re-hydrated and washed to remove any traces of dirt or faecal matter (Churchill 1983, Thorstensen 1993). Figure 2.1 shows the pre-tanning operations for the processing of sheepskins to pickled pelts through the conventional way;

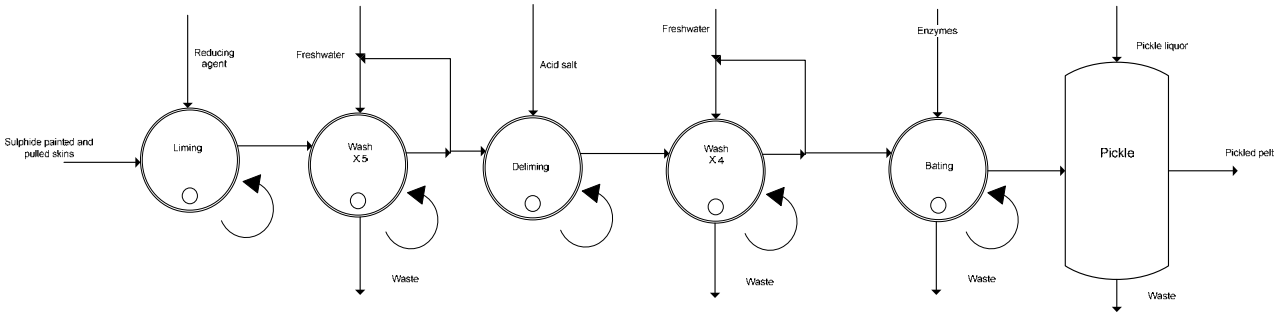


Figure 2.1: Schematic diagram of the typical conventional pre-tanning process operations

From Figure 2.1, it can be seen that the process is typically made up of 6 distinct stages. First is the liming process of sulphide painted sheepskins, then a set of washing steps, followed by a de-liming or bating process, another set of wash steps followed finally by a pickling operation (Rao *et al.* 2003a & 2003b). Each of these key processes is explained in further detail in the sub-sections to follow.

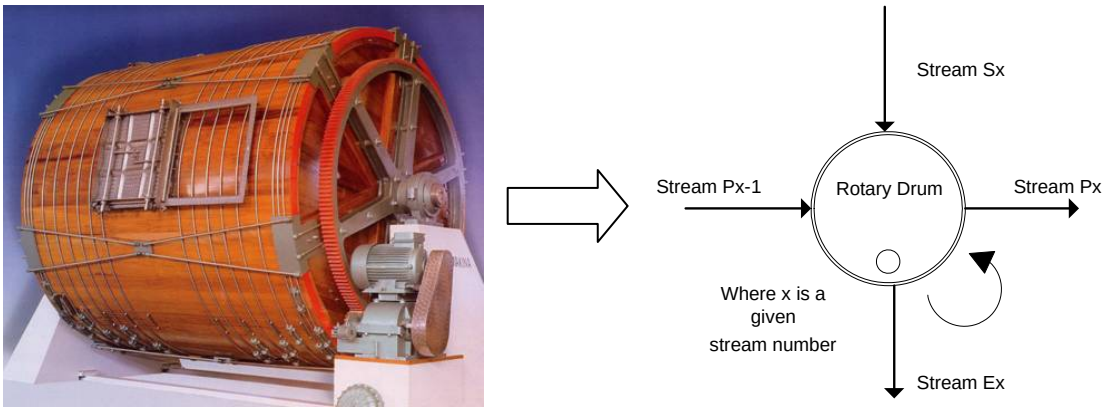


Figure 1.2: Shows a rotary drum (Hisar 2009) commonly used in the tanning industry represented as a P&ID symbol that was used throughout this work.

Figure 2.2 shows the rotary drum commonly used in the tanning industry and its P&ID representation that will be used throughout this work. Although a representation of the pre-tanning operations indicate a continuous line of processing from one drum to another (as seen in Figure 2.1), the process is in fact conducted in a semi-batch manner. For a given process operation, sheepskins are contacted with solvent water (or at times with an added chemical mixed) in rotary drums as pictured in Figure 2.2. The skins could be transferred into subsequent processing vessels or remain in the drum with different solvents used to carry out the next step.

2.1.1.1 Depilation step

Prior to the main conventional processing operations shown in Figure 2.1 is a depilation step. The choice of depilatory agent is crucial as it ultimately determines the quality of the leather produced, as well as the ease of un-wooling and removal of unwanted particles from skin (Carrie & Woodroffe 1960). The main objectives of the depilation step are listed below (Carrie & Woodroffe 1960);

- break the cysteine double bonds found in keratin (which is a protein found on the surface of the flesh that needs to be removed during pre-tanning operations)
- facilitate chemical penetration into the fibres by swelling the collagen fibres and fibrils.
- facilitate easier removal of the wool by weakening the wool roots structure and inducing swelling of the skin.

Some of the common chemicals used in the depilation or hair (although fellmongeries in New Zealand focus on wool breed sheep, research work that is based on the removal of hair from skin for pre-tanning operations are also applicable) unloosening step in the fellmongeries are; sodium sulphide, sodium hydroxide or modified starches. The painting mixture commonly used in industry in New Zealand is usually composed of a mixture of sodium sulphide, lime and solvitose (a paint thickening agent) (Allsop 2008). Painted skins are usually then put through a dewooling machine before the liming process. For some small scale industries, dewooling and unhairing may also be done by hand with a beam knife (Thorstensen 1993).

2.1.1.2 The liming process

At the first main step of the conventional process, sulphide painted slats are placed in a rotary drum and reacted with an excess of lime and an additional quantity of sodium sulphide. The drum is generally left rotating between 18 to 24 hours. Rao *et al.* (2003a & 2003b) reported a lime and water combination of 10% and 300% (relative to slat weight) respectively with treatment time of 5 minutes per hour for 6 hours before the slats were left overnight in a bath of the chemicals. New Zealand industry sources reported the use of 4% lime with 100% water on a weight

basis with a total liming time of 18 hours at a pH of about 13 and temperatures of about 25^{o}C (Allsop 2008).

Main objectives of the liming process are reviewed below (Carrie & Woodroffe 1960, Thorstensen 1993);

a) **Removal of hair/wool from the slats.** The depilation step removes most of the wool from the slats, but even in ideal conditions and with careful wool pullers, there will be some wool left on the skin. If any residue wool is left after the depilation step, the wool will become a permanent fixture on the processed skins even after the pickling stage which downgrades the quality of the lamb pelts. The liming action ensures that any residue wool is eliminated from the skin before further processing.

b) **Removal of epidermis** – the epidermis of the slats mainly consists of keratin. Strong sulphide paint would normally remove most if not all of the epidermis also known as 'scud'. The liming action removes any remaining traces of it. This occurs through the combined action of a reducing agent (sodium sulphide) and an alkali (lime) breaking down keratin while leaving the needed collagen on the slats.

c) **Causing the swelling of the slats** – swelling is necessary for the opening of the fibre structures to allow the removal of residual wool/hair. The highly alkaline condition of slats together with the presence of a reducing agent enables the collagen in the slats to absorb a lot of water especially the collagen found on the grain side of the slat. It also should be noted that swelling has to be limited to avoid a series of irreversible slat defects.

d) **Removal of natural fats and fat-cell membranes** – the action of alkali during the liming operation effectively collapses the

membrane walls of the fat-cells before removing the physiological fats such as lecithin and cephalin. Triglycerides of fatty acids with higher melting points are generally removed as much as possible with the use of higher processing temperatures bearing in mind that too high a temperature may cause drastic effects to slat properties.

The discharge of lime sludge (as a result of its use) with its high content of sulphide is polluting to the environment and a hazard. Lime sludge is associated with the build-up of unwanted solids waste while its sulphide content has been known to be hazardous to general health, particularly in its oxidised state (Rao *et al.* 2003a, Thorstensen 1993).

2.1.1.3 Washing off the sulphide and lime

After the sheepskins have had as much hair removed as possible, it is then washed between 4 to 5 times. This is done to remove traces of the sulphide paint and lime which remain on the surface of the skins by using freshwater in a rotary drum (Ahmed & Gasmelseed 2003, Cassano *et al.* 1997). Each washing step is about 20 minutes, run at 15 ˚C. At each step, freshwater is used at amounts equal to 100% of slat weight.

2.1.1.4 The de-liming and bating processes

The processes of deliming and bating are two processes with different functions, run continuously one after the other. The de-liming step is done to remove/neutralize most of the lime found on/in the slats (which then reduces the swelling in the skins) as well as to remove all the organic skin matter decomposed through the action of lime into the lime liquor (Carrie & Woodroffe 1960).

It is important that the slats are thoroughly de-limed to the point that most or if not all the swelling has subsided. This is so that the slats will be able to withstand higher temperatures and mechanical action downstream of the process. Some ways of determining if the de-liming process has been completed is to use chemical indicators such as phenolphthalein or thymol blue or to observe the differential swelling and fibre distortions of the slats (Thorstensen 1993, Rao *et al.* 2003a). There are a variety of common acidification chemicals used in the de-liming process such as ammonium salts (e.g. ammonium chloride) (Rao *et al.* 2003a) and carbon dioxide (Allsop 2008).

Immediately following the de-liming process is the bating process. The bating process is essential in the degradation of non-collageneous and globular proteins to soften hide structure and remove traces of excess hair, unwanted epidermis and fat residues, thereby preparing the hide for the tannery. The bating process finishes what was started in the depilation step of the process, especially with the more chemically resistant fibrous proteins. This is so that the lamb slats will have a smoother, softer and fuller surface finish (Carrie & Woodroffe 1960, Crispim & Mota 2003).

Up until about a century ago, the common agent used in the bating processes was animal manure. However, as its use was unhygienic with highly fluctuating results, it eventually gave way to the use of the proteolytic enzymes used today. Much research has been done to find the best enzymes and optimize their use. Some of these enzymes have gone on to be tested for their use to potentially replace lime and sulphide (Crispim & Mota 2003, Ahmed & Gasmelseed 2003). This will be discussed in further detail in sub-section 2.3.1.2.

Conventional bating is done by adding 0.02% carbon dioxide, 100% freshwater and 0.0005% enzyme (relative to initial slat input weight) into a rotary drum and run for about an hour and a half for an average size industrial fellmongery (Allsop 2008). This process is done at a much higher temperature then the rest of the process (35^{o}C). This is to provide optimal working conditions for the enzymes as well as to facilitate removal of decomposed organic matter from the slats which have not been removed using cold water. A sheep slat that is fully bated has a characteristic smooth and silky feel and will retain that impression even when pinched (Carrie & Woodroffe 1960).

2.1.1.5 Washing step after de-liming/bating

This step is basically carried out in a similar fashion as the previous series of washing steps. Usually done in 3 to 4 stages, the purpose of this series of washing steps is to remove as much of the chemicals used as well as halt the actions of the enzymes on the sheep slats (Thorstensen 1993, Rao *et al.* 2003a).

2.1.1.6 Pickling

The final stage of conventional processing is the pickling step. Its sole objective is to preserve the skins from the putrefying action of moulds and decay while awaiting leather tanning. This is usually done by immersing the sheep skins (known as sheep pelts at that stage) in a solution of extreme pH. By this stage, the pelts are mostly a network of hide proteins. To avoid damage to the pelts, an extreme acidic solution is normally used over an extreme alkaline solution (Carrie & Woodroffe 1960, Thorstensen 1993). A pickling solution is normally composed of 20% salt and 1% sulfuric acid of the weight of skins to give a solution pH of less then 1 (Allsop 2008).

2.2 Alternative Pre-tanning Options to Conventional Processing

In this section, alternative pre-tanning options to conventional processing are explored with the exception of the LASRA enzyme treatment process which will be outlined in detail in section 2.3. Other than research being done to improve and optimize single unit operations of the conventional process, not much literature has been found which highlights a whole new novel process which bypasses the liming/de-liming and bating processes. Most of the work found was focused on either replacing or lowering use of lime to aid removal of hair/wool from sheepskins. As mentioned previously, lime and sulphide used in pre-tanning operations is hazardous and polluting to the environment (Mäntele & Bridle 1994) so other alternatives have been investigated for use in place of these conventional chemicals. Potential alternatives should be less hazardous and polluting to the environment but yet still able to generate similar depilation and conditioning effects on sheepskins (Cassano *et al.* 1997, Rao *et al.* 2003a).

Some of the chemicals investigated by other researchers include hydrogen peroxide (H_2O_2) and sodium hydroxide (NaOH). Marsal and his colleagues (1999) looked into the use of hydrogen peroxide in an alkaline medium to replace conventional un-hairing methods. Although the overall results were satisfactory, they found that hydrogen peroxide caused total destruction of the hair and also gave rise to contaminated wastewater almost to the level of using lime. It was concluded that the use of hydrogen peroxide may not be particularly useful as it is a hazardous chemical as well as a hindrance to hair recovery. The destruction of hair, particularly sheep hair or wool is not desirable for the fellmongeries as wool is still a valued commodity for the textile and apparel industry (Thorstensen 1993). The use of NaOH to replace the conventional chemicals of lime and sulphide alkali appears promising although there have been significant physical differences reported in the slats pre-tanned using NaOH and hydrogen peroxide after they have been chrome tanned (Valeika *et al.* 1997). However, mechanical properties (such as tensile strength and elongation) of the leather pre-tanned using NaOH were reported to have been comparable with known standards (Valeika *et al.* 1998 & 2000).

A group of researchers had also revisited the possibility of using cheaper and locally available materials (such as cow pancreas) abandoned decades ago for its inconsistent results (Ahmed & Gasmelseed 2003). Frendrup (2000) outlined the use of a few more sheep slat un-hairing agents. Most of the un-hairing agents outlined by Frendrup only seek to assist conventional un-hairing methods with only one or two found to be more environmentally friendly than conventional chemicals. Key points from Frendrup's report are summarised in table 2.1 below;

Table 2.1: Table shows some alternatives of lime and sulphide replacement for use in pre-tanning operations (Compiled from Frendrup 2000)

1.	**Un-hairing with organic sulphur compounds**	➢ Types of sulphur compounds used are; mercaptoethanol, salts of mercaptoacetic acid, formamidinesulphinic acid ➢ Able to considerably reduce amount of sulphide consumed and discharged to wastewater ➢ High cost associated with their use so may not be a economically viable method in industry
2.	**Enzymatic un-hairing**	➢ High cost associated with its preparation and use ➢ Very applicable if high quality wool/hair recovery is needed ➢ Used in combination with sulphide depilation or with lime-sulphide mixtures
3.	**Lyotropic un-hairing (breaking of hydrogen bonds)**	➢ Known use in industry with acetic acid and autolytic enzyme base ➢ Method is most suitable when grain layer is not used ➢ Slats treated in this manner still needs some treatment with lime-sulphide mixture
4.	**Amine un-hairing**	➢ Normally mixed with dimethylamine and sodium hydroxide to accelerate un-hairing in conjunction with straight lime ➢ High potential to generate carcinogenic nitrosamines in beamhouse air, so this technique is not used anymore ➢ Non-nitrosamine generating amino compounds such

		as hydroxylamines used widespread in un-hairing auxilliaries

2.2.1 Novel 'whole process' approaches to conventional pre-tanning operations

From literature, two alternatives were found which were novel 'whole process' alternatives to conventional pre-tanning operations.

The first is the work of Cassano *et al.* (2000) using enzymatic agents to replace conventional chemical un-hairing methods together with the use of an ultrafiltration technique. The enzyme used was a proteolytic enzyme Erhavit MC (TFL Italia S.p.A., Brescia, Italy). An ultrafiltration unit (with a cut-off at 20 kDa and an operating pressure range of 1-6 bar) was used to recycle the enzymatic float, removing all the proteins and other coarse particulates stripped from the sheepskins found in float while retaining most of the enzymes and chemicals to be used in subsequent runs. Figure 2.2 below shows a schematic diagram of the enzymatic treatment of sheepskins with ultrafiltration of spent floats as proposed by Cassano *et al.* (2000).

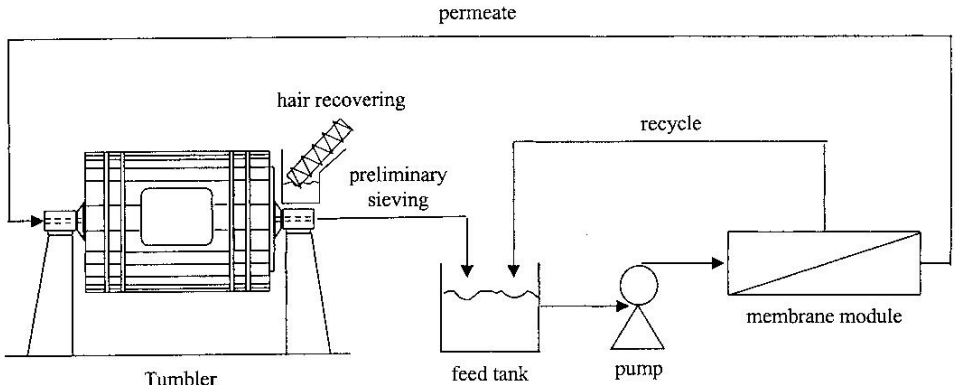


Figure 2.3: Schematic diagram of the proposed enzymatic treatment process of sheepskins with recycle of spent floats through an ultrafiltration membrane technique (Cassano *et al.* 2000).

However, the skins were still sulphide painted to facilitate hair/wool removal and some lime was added together with the enzymes in the process. The major benefits of this process were reduction of pollutants such as sulphide and lime as well as reduction of the long process time associated with the conventional un-hairing process with lime.

The other process was the 3-step tanning process introduced by Rao *et al.* (2003a & 2003b). This novel method of tanning skins, although tested on goatskins, provided a viable alternative to the tanning of sheepskins. This method consists of three main operations; enzymatic dehairing (enzyme from Biodart, SPIC), fibre opening of the skin using sodium hydroxide (NaOH) and chrome tanning of the pelts. Figure 2.3 below shows the proposed 3-step tanning process of Rao *et al.* (2003a & 2003b) highlighting the main stages;

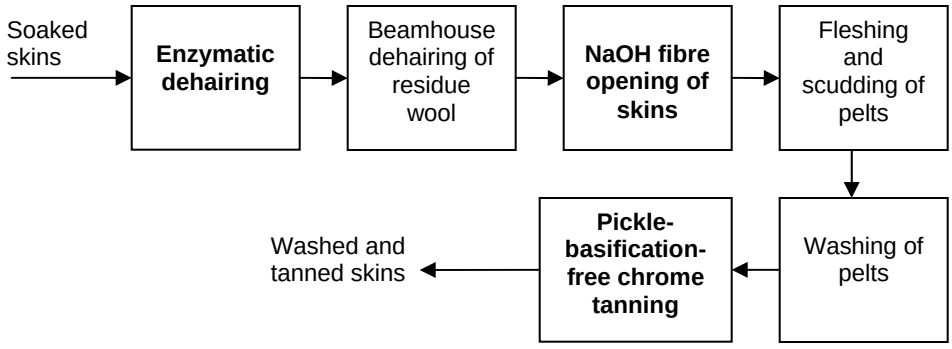


Figure 2.4: Block diagram shows the 3-step tanning process proposed (with the 3 main processes emphasised in bold) by Rao *et al.* (2003a & 2003b). Pre-tanning operations are until just before chrome tanning operations.

Although this process still employs the conventional sulphide painting method to facilitate hair/wool removal, it totally eliminates the liming and bating steps by merely treating the sheep slats/skins with a combination sodium hydroxide, enzyme and freshwater. Advantages of this process include a reduction of hazardous chemicals and environmental pollutants used conventionally (e.g. lime and sulphide alkali) coupled with its lowered processing time and costs.

2.3 The LASRA Enzyme Treatment Process

This section looks into the details of the LASRA enzyme treatment process to be optimized. Despite still utilizing sulphide in its process, it is a progress towards the total elimination of sulphide and lime in pre-tanning operations. The LASRA process also utilizes much of existing equipment in fellmongeries and does not require drastic changes in processing (Allsop 2008). As a result of that, the optimization of the LASRA process may focus on enhancing existing benefits over conventional processing (e.g. further reduction of freshwater use).

The process may be considered novel in many ways although it has to be noted that the process has its origins in a 3-step tanning process developed by Rao (2003a & 2003b). Due to different environmental conditions and the nature of New Zealand raw materials (e.g. sheepskins), this has resulted in the novelty of the LASRA enzyme treatment process in a local context. The process may be seen below as figure 2.4.

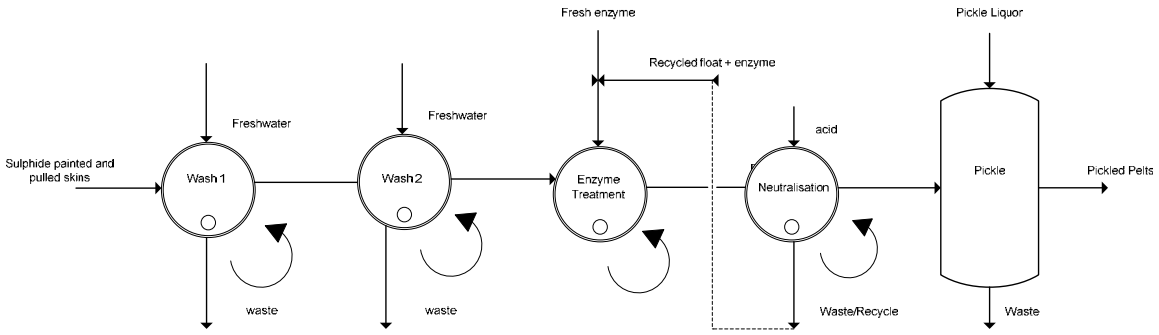


Figure 2.5: Schematic diagram of the LASRA enzyme treatment process with recycle. The float of the neutralization process is recycled into the enzyme treatment process of a subsequent run together with a top-up of enzyme to required enzyme activity.

2.3.1 Proposed enzyme process overview

The new pre-tanning process developed by LASRA is known as the single step enzyme based process. Although it is comprised of 5

distinct steps, the name was derived from the enzyme treatment in that it is the only protein hydrolysis step in the process. An enzyme treatment process replaces the need for alkali and lime washes along with the bating process (Allsop 2006).

The sulphide painted sheepskins (entering the first step) and pickling of sheep pelts (entering the last step) are the same as with the conventional version of the process. This has been explained in detail in sub-section 2.1.1.1 previously. However, while the sulphide painting steps are much the same for both processes, there are fewer washing stages in the LASRA enzyme treatment process. This is because the sulphide levels could be reduced sufficiently in two washing steps to allow effective enzyme treatment. Further washes could be employed to remove more soluble material and therefore an optimum number of washing stages should be determined by considering washing effectiveness together with the processing time and resource usage.

2.3.1.1 Washing out the sulphide

After the sheepskins have had as much wool and hair removed as possible (facilitated by the sulphide painting) the depilated sheepskins are then washed twice to remove traces of sulphide and washable solids found on the skins. This is carried out using freshwater in a rotary drum. Each washing step is half an hour (Allsop 2008). This is a similar operation and processing time as used by Rao *et al.* (2003a).

2.3.1.2 Enzyme treatment process

Enzyme treatment is used in place of the (conventional) liming and bating processes to remove protein compounds not required in the finished product. Rao *et al.* (2003a) used a proprietary enzyme

from Biodart an Indian biotechnology firm. Other enzymes identified by Crispim & Mota (2000) as potential hair-save possibilities included *Riberzyme* MPX (a bacterial protease) and *Ehravit* MC (also a protease). Pre-tanning trials conducted using these enzymes were found to be successful at the pilot scale level but further testing is needed to gauge the enzymes industrial applications. Although Frendrup (2000) reported similar enzymes being used in the industry (with companies such as Cromogenia based in Spain and Novo Nordisk in Denmark being at the forefront of this effort), this was still used in conjunction with traces of lime, sulphide and other hazardous chemicals. In this instance, the enzyme was just used to improve bating operations and/or reduce hazardous chemicals use.

LASRA have also conducted investigations of its own to find the enzyme which will function best in its system and given environmental conditions (e.g. operating pH and temperature). The enzyme needs to have peak activity in mildly alkaline pH (8-10) and at low temperature (~20^{o}C). The same enzymes that were used by Rao *et al.* and others were also trialled at LASRA. However, the successful results obtained by the above international researchers were found to be unrepeatable on New Zealand sheepskins and processing conditions (Allsop 2008). After further investigative studies, it came down to three potential enzymes which could be applicable in the New Zealand context (Allsop 2006). These were Purafect 400L (Genencor International Ltd.), Novolime (Novozymes A/S) and Maxizyme SEM (Textan Chemicals P Ltd.).

All three enzymes were analyzed for their temperature and pH activity profile and then tested on small samples of slats. The enzyme which gave the most satisfactory result was then taken through the whole pre-tanning operations with a larger batch of slat. Through this, it was found that Maxizyme SEM (Textan

Chemicals P Ltd.) showed satisfactory conditioning of the slats with sufficient removal of alkali-soluble proteins and only minor breakdown of collagen. The pelt quality (before it was pickled) was just as good as or even better in some aspects than conventionally produced pelts (Allsop 2006).

Table 2.2 below summarizes the properties of the slats manufactured by the LASRA process, compared with the industrial standards set for sheep pelt quality. The total protein and soluble protein values (an important leather quality measure) lie just outside industrial standards. As such the improvement of the LASRA process to lower these values with minimum additional resource use is required.

Table 2.2: Properties of slats processed by the LASRA enzyme treatment process compared with known industry standards of quality (Allsop 2006).

Property	Values	LASRA guideline values
Moisture (%)	62.2	60 - 70
Total protein (%)	19.2	~ 18
Soluble protein (%)	1.2	0.9 – 1.0
Protein ratio	2.4	7.5 max
Shrinkage temperature (^{o}C)	58.2	57 - 58
Pickle pH	0.84	0.8 – 0.9
Free acid (%)	0.54	0.5 – 0.6
Salt (%)	11.9	11 - 13

The enzyme treatment unit operation was carried out in a rotary drum at about 20^{o}C and between pH 9 and 10, ideal for optimized enzyme activity. A cooling/heating system was installed throughout the process to maintain the skins at that temperature in the winter (Allsop 2008). An appropriate enzyme treatment time has to be maintained so that the sheepskins are sufficiently conditioned but yet not overly treated to the point where the

enzymes have begun to break down the collagen contained in the skin, subsequently causing damage of the final leather (Cassano *et al.* 1997, Rao *et al.* 2003a).

Due to the susceptibility of enzymes to environmental conditions such as pH and temperatures, it is relatively easy for enzymes to be denatured hence losing their effectiveness. A 20% enzyme loss has been reported in the LASRA enzyme treatment process during enzyme recycling and this is needed to be made up with fresh enzyme for the next run (Allsop 2007). As enzymes generally have a high cost, it is essential to be able to recover as much enzyme as possible. Some ways in which the loss of enzyme activity may be reduced would be by using more stable heating/cooling system and a narrower pH range. Depending on the molecular size of the enzymes, it may be recovered prior to reuse using techniques such as ultrafiltration (Cassano *et al.* 2000).

2.3.1.3 Neutralisation process

Following enzyme treatment, the pH of the skin is reduced from pH 11 to 9-10. This is to halt the enzyme activity and prevent further proteolysis and damage to the proteins essential for leather structure. Neutralisation also prepares the skins for the pickling process. By adjusting the pH of skins to a neutral level from high levels of alkalinity, this ensures no grain damage occurs in the pickling operations which are conducted at very low pH levels.

At LASRA, the process was carried out in a rotary drum with dilute sulphuric acid (approximately 0.5%) on a 1:1 basis. Sulphuric acid was used as it was a widely available and cheap acid found in tanning industry circles (Edmonds 2008). The acid was first added into the drum and the contents were drained, leaving the skins to be pickled after allowing an hour for the

neutralisation to take place (Allsop 2006). The neutralization of the enzyme float in this manner was to ensure rapid pH changes to the skins did not occur as this can cause damage to skins quality.

For pickling operations, additional amounts of acid (up to 1%) and salt (up to 20%) are added to prevent undesirable swelling in pelts and preserve the pelts for transportation to tanning facilities.

2.3.1.4 Float recycling

An implementation of a recycling system would produce savings on associated enzyme costs. Direct recycling of the neutralised enzyme float at both 100% and 50% strength was investigated by Allsop (2007);

a) **100% float strength test** (all the float of a given run was used in the enzyme treatment process of a subsequent run). Top-up of enzyme was done to account for loss of activity due to dilution of float and denaturation as a result of environmental pH changes. Dilution of float occurred as the volume of recycled float was twice the amount needed.

b) **50% float strength test** (50% of the float of a given run was used and it's volume was made up with freshwater). Top-up of enzyme to original operational levels was again required for the same reasons as the previous test.

It was found that the leather processed by recycled enzyme floats had marginally weaker physical attributes than the leather from enzyme process without a recycling system but still within acceptable industry limits. The weaker attributes of leather could be due to the increased soluble proteins content found in the skins shortly after it was pickled (pickled pelts).

It was also noted that the pickled skins processed with the enzyme process with recycling had a significantly 'dirtier' appearance. Products of enzyme treatment process using a 50% strength recycled float were markedly 'cleaner' than the products processed using 100% strength recycled float though not as 'clean' if run without any recycle of float (Allsop 2007).

While the issue of weakened pickled pelts is within accepted industrial standards (Allsop 2008), the 'dirtier' pickled pelts processed by the LASRA process with recycling may be overcome by incorporating a filtration step into the recycling system to separate out the soluble/insoluble proteins, wool/hair and other particles from the enzyme as done by Cassano *et al.* (2000). A filtration step could remove solids materials through use of an ultrafiltration or microfiltration unit depending on the size of the molecules that need to be separated.

Edmonds (2009a) recommends the soluble hide substance ratio (SSHR) as a measure of solids content (particularly protein) in pre-tanned sheepskins. It can be shown that higher values of SSHR in pre-tanned sheepskins are undesirable in subsequent tanning operations and are an indication of incomplete pre-tanning operations. For example, a higher degree of unwanted solids material removal can facilitate improved penetration and distribution of chemicals used downstream of pre-tanning operations to improve the elastic and flexible characteristics of leather (Allsop & Passman 2003). As such, the degree to which solids (particularly soluble components) are washed from the skin can be used as a measure of the process effectiveness.

2.3.2 Summary of benefits of the enzyme process

Some significant advantages of the process developed by LASRA, as compared with the conventional process, are highlighted below (Allsop 2006);

1. **Processing time of slats was reduced by 47%**. This occurred as a result of the reduction in the number of process operations that there were with the LASRA enzyme treatment process over the conventional. This was especially so with the reduced amount of washing stages compared with the conventional process. Trials were conducted by LASRA demonstrating this. The total processing time by the conventional method was about 26 hours while the LASRA process had a 14 hour processing time. Specifically, instead of having a liming process of 18 hours and at least 4 washing steps following that, the process is now reduced to a 2 washing steps of half an hour each. From this, savings of up to 10 hours (making up the bulk of time savings) of processing time was readily possible despite taking into account the 7 hours needed for enzyme treatment.

2. **Energy use was reduced by 73%**. The savings occurred as the LASRA process had a reduction of processing time associated with a reduced number of process operations and the absence of the need for any heating of the process floats. Conventional pre-tanning operations required heating of washing stages just after liming as well as the process float of de-liming/bating processes.

3. **Water consumption was decreased by 49%**. This occurred as a result of there being less process operations with the

LASRA enzyme treatment process over the conventional process. Fewer process operations translated to a reduced quantity of freshwater that was needed.

4. **Biochemical Oxygen Demand (BOD_5) values were decreased by 40% and Total Kjeldahl Nitrogen (TKN) values were reduced by 31%**. The reduction of those two pollution indices indicated that a reduced quantity of material was being removed from skins. While these developments may be beneficial to the environment, it may also mean that a lower quality pelt is being produced. However, as presented in table 2.2, the pelts manufactured by the new LASRA process were within industrial standards.

2.4 Freshwater Usage Minimization Options for Pre-tanning Operations

One other major area of which optimization and improvement work had been conducted on current industry standard pre-tanning operations is the area of freshwater usage (and hence effluent discharge) minimization. Although not always a prime concern of water rich countries such as New Zealand, freshwater availability is of high importance to tanneries found in countries which have limited supply of this resource. Tanneries in these locations may face high charges for their freshwater use or have their supply rationed by their local authorities. It is of vital importance then that the tanning industry in these countries explores novel methods for minimization of water use. This includes finding ways in which the water used in the process may be reused or recycled (Kunyu *et al.* 2007).

Countries such as India and China which currently house major tanning industries, face an ever increasing risk of not being able to provide freshwater to their population (which collectively make up almost a third of the global population) (Kunyu *et al.* 2007, Water Woes 2009). For example, the dry northern region water table of China is being depleted at a rate of a meter a year due to over pumping (Water Woes 2009). Many parts of East Asia have also been reported to be unable to provide freshwater to almost 20% of their country's population (Gleick *et al.* 2009).

Kunyu and his colleagues (2007) have recently demonstrated that it is possible to recycle the water used at selected streams of the process to be used in the pickling step of the pre-tanning process without any compromise of final leather produced. This was demonstrated by comparing skins that were processed using freshwater and those that utilized water taken from effluent streams at various points of the process. All investigation work done by Kunyu *et al.* (2007) was on a pilot scale basis. The end result was that the leather produced by utilizing recycled water was found to be just as good and in some instances better than

those processed using freshwater in terms of the leather's strength properties and handling characteristics.

Another water recycling technique was outlined by Cassano *et al.* (1997) looking into the possibility of incorporating an ultrafiltration unit (UF) to filter all the process water so that it may be recycled back into the process. With all the large molecular weight particles and wool being filtered out, the water may be recycled as many times as it is needed. In addition to that, traces of sulphide may also be recycled from the permeate provided it is recharged back up to its required concentration before it is re-used in the sulphide painting of sheepskin (Molinari *et al.* 1995, Long 1995).

A possible modification to the LASRA enzyme treatment process is that water used downstream in the process may be used in a process operation upstream. For example, instead of using two streams of freshwater into both the first and second wash of the sulphide painted skins, one may instead recycle the water used in the second wash into the first wash. This is known as multistage counter-current extraction. As it can potentially provide savings of raw materials (e.g. freshwater) it could be a more economical choice over a co-current system (Seider *et.al.* 1999). Geankoplis (2003) defines the methodology to be carried out in order to design such a system through determining the optimum number of stages needed and the minimum amount of solvent to be used. An optimized multistage counter-current extraction system is akin to the requirements of pre-tanning operations as the objective of pre-tanning operations is to extract un-wanted solids from skins to be manufactured into leather.

2.5 Literature Review Summary

The following was observed through a review of existing literature of the tanning industry, with focus on the process operations found in a fellmongery (pre-tanning operations);

a) Problems associated with conventional pre-tanning operations had been identified (through an in-depth overview of conventional processing). This included a need for the reduction of freshwater used and chemicals that are hazardous and polluting to the environment as well as optimization work.
b) The LASRA process (developed as an alternative to conventional pre-tanning operations) which was to be the focus of optimization work does not require much capital to purchase new equipment or drastic changes in processing. This is advantageous as optimization work may then focus on enhancing existing benefits (e.g. further reduction of freshwater use) over conventional processing.
c) The work of international researchers to resolve the problems of the conventional process operations had been highlighted. The work done on individual aspects of conventional processing and the pre-tanning operations as a whole had both been explored. In particular water and enzyme reuse could be adopted to improve the LASRA process performance. The use of filtration could also be used to facilitate reuse of these solvent streams without compromising the skin quality.
d) Solids content (particularly soluble solids material) was an identified quality indicator of pre-tanned sheepskins. Optimization of the LASRA process to increase removal of solids may be done as it is an industrially known gauge of the effectiveness of pre-tanning operations.
e) The LASRA enzyme treatment process as an alternative of the future to conventional process operations had been thoroughly explored giving details on the purpose and objective of individual process operations.

The following chapter will look to fully characterize the existing LASRA enzyme treatment process using mass balancing. Flow parameters will also be identified to assist characterization work and also to be used in the evaluation of modifications to the process using the knowledge base and overall understanding established in this chapter.

3.0 ENZYME TREATMENT PROCESS CHARACTERIZATION

The previous chapter reviewed current conventional pre-tanning operations and the LASRA enzyme treatment process. Also included in the review were possible solutions that researchers have investigated in an effort to transform the pre-tanning industry into one that uses non-hazardous substances and which are more efficient. It is evident that there are opportunities for implementation of optimization and improvement measures such as recycling process streams, utilization of multi-stage processing and in-situ clean-up of process streams, which may improve the performance of the LASRA proof of principle enzyme treatment process.

The goals of this research were to investigate these process modifications primarily through mass balance modelling of the process. As a basis for constructing mass balances for these alternative process configurations, it was first necessary to understand the skin composition and how it changes during processing. This may be done.

The specific aim of this chapter was to characterize the enzyme treatment process through a detailed mass balance of measured experimental data for the LASRA proof of principle process. This included;

1. Identification of what compositional parameters would be used to characterize the various streams of process components.
2. Define the relationships that link the input and output compositions in each unit operation (e.g. how much skin solubilisation is caused by the enzyme?).
3. Define key parameters to describe the operation of the process (e.g. how well the drums are drained after each stage) and the constraints in these variables (eg. What is the minimum amount of water needed to ensure sufficient operation of the drums?).

3.1 Mass Balance as a Tool for Process Characterization

The main tool used in the work of characterizing the LASRA enzyme treatment process, was a mass balance. By being able to identify the input and output streams from individual unit operations within the process and ultimately the process as a whole, the constraints of the system may be identified for optimization and improvement work. Constraints within the process could include practical substrate to solvent ratios (e.g. skin to fresh water ratios) and quality parameters required of the final product (e.g. pickled pelts total solids and moisture content) or behaviours of individual unit operations of the LASRA enzyme treatment process (e.g. has equilibrium state been reached?). Future optimization work of constructing forward mass balances to evaluate configuration alternatives requires the ability to predict all outputs from the known inputs. The following sub-section focuses on defining the input and output process parameters together with explanations for the practical ranges selected for future characterization of the various streams found in the mass balance.

3.1.1 Identification of components to balance

The most appropriate components to balance need to be first identified. This is so that an understanding of the input and output streams of individual process operations of the LASRA process may be gained. To do this, all the data available collected by LASRA for the proof of principle process was collated and used to build a mass balance. Important criteria in choosing appropriate compositional parameters to describe the process in mass balances are;

- Must be applicable to each stream

- Able to be traced throughout the process; from the first inputs (sheepskins and solvent water), all the way to the final outputs (final processed pickled pelts and effluent).
- They must be conserved over a unit operation (i.e. inputs must always equal outputs).

From data available at LASRA, the flow parameters that meet these requirements are; total flow, moisture/water content and total solids (TS), which can be further divided into total dissolved solids (TDS) and insoluble solids (IS). Insoluble solids are predominantly the insoluble components of the skin but also include particles that might be washed out and could be present in the skin or float. Other parameters that met the requirements were grease, sulphide (S^{2-}) content, and total Kjeldahl nitrogen (TKN). Enzyme dissolvable solids (EDS) were also defined for latter parts of the process due to conversion of IS to TDS.

A mass balance comprising mainly of solids and water content would be ideal for this work as the mass balance will characterize both the effluent water quality as well as the composition of sheepskins as it is being processed through the various process operations. Solids composition is a widely known method used to characterize the quality of physical material such as sheepskins.

One other available data set which did not meet the requirements for inclusion in a mass balance was the chemical oxygen demand (COD). It is often used to characterize output effluent streams but not used to describe the composition of sheepskins entering into a process. It is also not clear whether COD is conserved or changed as a result of chemical reactions that occur in the processes. However, as COD is a good measure of general effluent quality, it may be used to evaluate process float quality in terms of impact on receiving waters. This widely used effluent quality measure can

provide alternative descriptions of process floats to compliment results obtained by a solids description. This may only be done if a correlation between the compositional parameters used in the mass balances and COD could be found. This issue is discussed in further detail in section 3.5 of this chapter.

Although readings of volatile total solids (VTS), volatile insoluble solids (VIS) and sodium content (Na) were known to be used to characterize effluents of fellmongeries in industry, they were not used in the mass balance. Pilot scale trials data from LASRA on these flow parameters were not available. For the given reasons, characterization of the processes through mass balances was done using TS, TDS, IS, S^{2-}, grease, and TKN.

3.2 Pilot Scale Experimental Trials

A set of data from eight trials conducted by LASRA on the proof of principle process were available for detailed analysis. The trial runs were done at a pilot scale level, each utilizing 6 sheepskins of varying total mass between 12 and 14 kg. For each trial the total quantities of each stream were measured and the separated floats were measured for total solids, insoluble solids, moisture/water content, grease, sulphide and total Kjeldahl nitrogen. Samples from the neck section of representative incoming slats and processed skins were taken and analysed for their total solids and total Kjeldahl nitrogen content, although it was not possible to do this for each trial specifically.

Each run begins with the entry of depilated sheepskins (slats) into the washing stage 1 through to the pickling stage of the process. The different experimental runs were also conducted in groups of 4 processes each (1 – 4 and 5 – 8) with successive runs within a particular group of 4 processed by recycling the neutralization float of the previous experimental run. For example, the float of the neutralization stage of run 1 is provided to the enzyme treatment stage of run 2 while the float of the neutralization stage of run 4 is discarded.

Process runs 1 to 4 were conducted at LASRA by the standard configuration of the LASRA enzyme treatment process. Two wash runs to wash off the depilation paint from the slats was followed by an enzyme treatment stage and a neutralization step after that. Following the neutralization of the slats was a pickling step. A schematic diagram showing the layout that is process runs 1 to 4 may be seen below as Figure 3.1. The difference between process run 1 and run 2, 3 and 4 is that the waste of the previous process run neutralization step (E4) is channeled to the fresh enzyme (S3) input of the following process run enzyme treatment step. This was done after the addition of extra enzyme to account for any dilution effects.

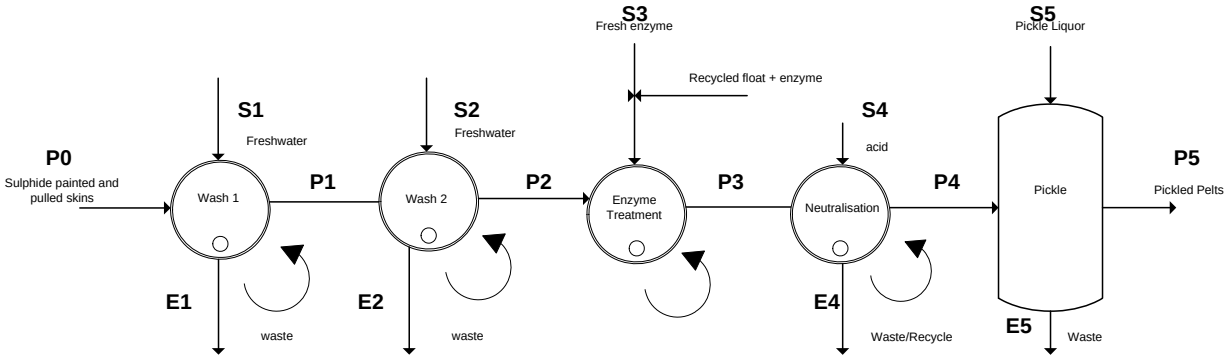


Figure 3.1: Representing process runs 1 to 4 a schematic diagram of LASRA enzyme treatment process standard configuration. Nomenclature used include: S = solvent stream, P = slat stream, E = effluent (drained float) stream. The usage of 'E4' following effluent stream 'E2' was to allow future references to the effluent stream of the enzyme treatment as 'E3'.

Process runs 5 to 8 were also conducted at LASRA. These runs were configured with a slight modification to the standard LASRA enzyme treatment process. In runs 5 to 8 there was an industrial fibre filter installed at the waste of the neutralization step. As such, the waste of the previous neutralization step is filtered from large solids (e.g. wool) before it is channelled to the enzyme treatment step of the following process run. A schematic diagram showing the layout of process runs 5 to 8 may be seen below as Figure 3.2. For the purpose of explaining the workings of the mass balance, process run 5 was used. This was because run 5 was the first process run that had complete characterization of all the stream compositional parameters identified in section 3.1 to be investigated for use in future mass balances.

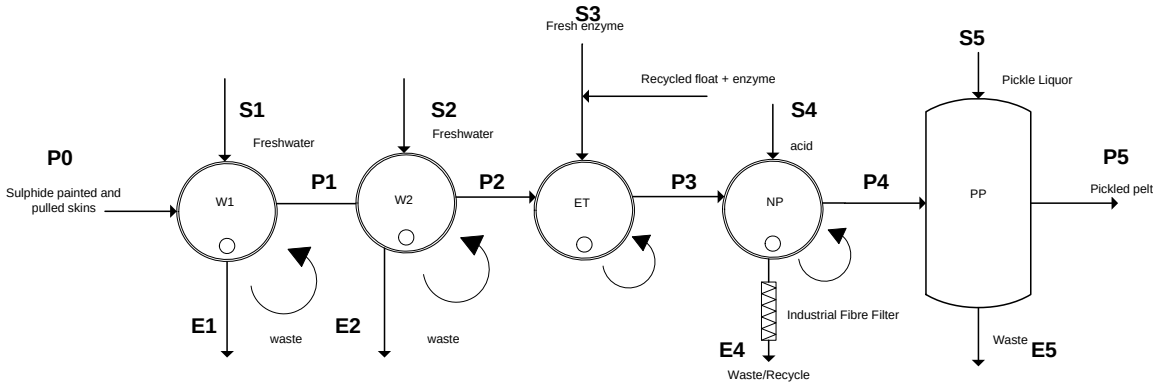


Figure 3.2: Schematic diagram of the modified LASRA enzyme treatment process of process runs 5 to 8. Stream labels are bold in black. Nomenclature used include: W1 & W2 = washing stages, ET = enzyme treatment stage, NP = neutralization process, PP = pickling process

Below is a list of the defined (in sub-section 3.1) stream composition parameters used together with how they were measured and a description of the form of the raw measurement. Also included are details of how the raw data were rearranged into a form suitable for use in the mass balance.

a) **Total solids (TS)** – Values were found as a concentration (C_{TS}) of dry solids in the total mass (units: kg dry solids/kg total mass). For the mass balance, this value was also converted to a mass (M_{TS}) through multiplication with the total mass (M_T). This value was measured using an oven drying method conducted between 103 - 105^{o}C for an hour. For more details on the methodology of obtaining TS value, refer to Standard Methods of Total Solids Dried (1998).

b) **Insoluble solids (IS)** – Values were reported as a concentration (C_{IS}) (units: mg insoluble solids/kg total mass). By the assumption that the density of float is 1000 kg/m^3, this raw measurement value was converted from a per litre float to per total mass basis. This value is represented on the mass balance also as a mass value (M_{IS}) through multiplication with total mass. This value was obtained through the oven drying method conducted between 103 - 105^{o}C on the residue of filtered float. For more details on the methodology of obtaining IS value, refer to Standard Methods of Total Suspended Solids Dried (1998).

c) **Total Dissolved solids (TDS)** – Values (units: kg dissolved solids/kg total mass) were calculated from the measured mass of TS and IS ($M_{TS} - M_{IS} = M_{TDS}$). Although there are methods of obtaining the TDS value as a measured quantity, this was not done to prevent over-definition of this value. On the mass balance, TDS is displayed as both a mass value (M_{TDS}) and a percentage of total mass or concentration value (C_{TDS}).

d) **Moisture content** – Values (units: kg water/kg total mass) are calculated from TS of a dried solid sample ($M_{MC} = M_T - M_{TS}$). On the mass balance, moisture content is displayed as both a mass (M_{MC})

value and a percentage of total mass or concentration value (C_{MC}). This value was obtained through the oven drying method conducted at around 40^{o}C.

e) **Total Kjeldahl Nitrogen (TKN)** – Values (units: kg TKN/kg total mass) were obtained from LASRA and measured by the Kjeldahl digestion method of a sample of sheepskin or sheep slat. By the assumption that the density of float is 1000 kg/m^3, this value is represented on the mass balance as a mass (M_{TKN}) value through multiplication of total mass with the percentage or concentration of TKN (C_{TKN}) in the sample. For more details on the methodology of obtaining TKN values, refer to Standard Methods of Total Nitrogen by Macro-Kjeldahl Method (1998).

f) **Sulphide content (S^{2-})** – Values (units: ppm – parts per million) were obtained from LASRA and measured by the ion-selective electrode method of float samples. The sulphide content in the depilation paint used on sheepskins was determined to obtain the initial sulphide value. This value is represented on the mass balance as concentration of sulphide (C_{S2-}) in sample float. For more details on the methodology of obtaining S^{2-} values, refer to Standard Methods of Sulphide by Ion Selective Electrode (1998).

g) **Grease and fat contents** – Values (units: kg Grease/kg total solids) were obtained from LASRA and measured by the Soxhlet extraction method of a sample of sheepskin/sheep slat or float. This value was also represented on the mass balance as mass (M_G) value through multiplication of total solids (M_{TS}) value with an estimated percentage of grease in dry slat of 15%. For more details on the methodology of obtaining grease values, refer to Standard methods of Grease by Soxhlet Extraction Method (1998).

Tables 3.1a & 3.1b show the raw data provided by LASRA for each of the process runs used as system inputs for the mass balance;

Table 3.1a: System inputs for the mass balance up until enzyme treatment stage. These values were obtained from LASRA

	P0					S1							E1								S2								S3	
Trial	M_T kg	C_{TS} kg/kg	C_{TKN} kg/kg	M_G kg	C_{S2-} ppm	M_T kg	M_T kg	C_{IS} kg/kg	C_{TS} kg/kg	C_{TKN} kg/kg	M_G kg	C_{S2-} ppm	COD kg/kg	M_T kg	M_T kg	C_{IS} kg/kg	C_{TS} kg/kg	C_{TKN} kg/kg	M_G kg	C_{S2-} ppm	COD kg/kg	M_T kg	M_{IS} kg
1	12.65	34.4%	8.5%	n.a	n.a	12.65	9.6	1.34%	5.9%	2.0%	n.a	n.a	0.046	12.65	13.4	0.32%	3.0%	0.9%	n.a	n.a	0.024	12.65	0.01
2	13.5	34.4%	8.5%	n.a	n.a	13.5	10.7	0.59%	5.7%	0.2%	n.a	n.a	0.043	13.5	14.0	0.67%	2.8%	0.7%	n.a	n.a	0.026	13.5	0.01
3	12.8	34.4%	8.5%	n.a	n.a	12.8	9.9	1.56%	5.4%	1.9%	n.a	n.a	0.048	12.8	13.5	0.86%	2.7%	0.9%	n.a	n.a	0.025	12.8	0.01
4	13.9	34.4%	8.5%	n.a	n.a	13.9	11.0	1.51%	5.6%	1.6%	n.a	n.a	0.049	13.9	14.0	0.36%	2.8%	0.8%	n.a	n.a	0.017	13.9	0.01
5	12.7	34.4%	8.5%	0.65	33202	12.7	9.4	1.42%	5.6%	2.0%	0.005	5782	0.058	12.7	13.0	0.47%	3.0%	0.8%	0.003	5813	0.024	12.7	0.01
6	13.9	34.4%	8.5%	0.72	33202	13.9	9.5	1.44%	5.7%	2.1%	0.004	5570	0.059	13.9	14.7	0.65%	2.9%	1.0%	0.003	5238	0.031	13.9	0.01
7	13.0	34.4%	8.5%	0.67	33202	13.0	10.1	2.08%	5.8%	1.6%	0.006	6263	0.053	13.0	12.9	0.54%	2.3%	0.7%	0.002	6299	0.021	13.0	0.01
8	12.8	34.4%	8.5%	0.66	33202	12.8	10.6	1.88%	6.6%	1.8%	0.006	7558	0.049	12.8	12.6	0.63%	2.4%	0.8%	0.004	7734	0.026	12.8	0.01

Table 3.1b: System inputs for the mass balance of neutralization to pickling process. These values were obtained from LASRA

	S4			E4					S5				E5					P5			
Trial	M_T kg	C_{TDS} kg/kg	M_T kg	C_{IS} kg/kg	C_{TS} kg/kg	C_{TKN} kg/kg	M_G kg	C_{S2-} ppm	COD kg/kg	M_T kg	C_{TDS} kg/kg	M_T kg	C_{IS} kg/kg	C_{TS} kg/kg	C_{TKN} kg/kg	M_G kg	C_{S2-} ppm	COD kg/kg	M_T kg	C_{TS} kg/kg	C_{TKN} kg/kg
1	12.65	2.0%	23.1	0.47%	2.5%	0.8%	n.a	n.a	0.016	12.65	21.0%	19.8	0.08%	10.4%	0.2%	n.a	n.a	0.007	8.9	30.7%	3.42%
2	13.5	2.0%	22.1	0.96%	2.3%	1.2%	n.a	n.a	0.024	13.5	21.0%	22.3	0.15%	10.6%	0.3%	n.a	n.a	0.007	10.8	30.7%	3.42%
3	12.8	2.0%	22.8	1.33%	2.6%	1.3%	n.a	n.a	0.027	12.8	21.0%	20.8	0.08%	10.4%	0.3%	n.a	n.a	0.006	10.05	30.7%	3.42%
4	13.9	2.0%	24.2	1.29%	2.4%	1.4%	n.a	n.a	0.031	13.9	21.0%	23.7	0.07%	10.5%	0.7%	n.a	n.a	0.006	10.65	30.7%	3.42%
5	12.7	2.0%	24.6	1.02%	2.4%	0.9%	0	3490	0.018	12.7	21.0%	18.8	0.08%	10.5%	0.2%	1E-04	0	0.005	8.95	30.7%	3.42%
6	13.9	2.0%	27.3	1.37%	2.4%	1.2%	0	4022	0.028	13.9	21.0%	21.7	0.14%	10.7%	0.2%	2E-04	0	0.006	10.2	30.7%	3.42%
7	13.0	2.0%	24.5	1.00%	2.5%	1.2%	0	3762	0.024	13.0	21.0%	20.1	0.15%	10.3%	0.3%	2E-04	0	0.006	10.65	30.7%	3.42%
8	12.8	2.0%	23.8	1.17%	2.8%	1.4%	0	4469	0.028	12.8	21.0%	20.0	0.08%	10.7%	0.2%	1E-04	0	0.007	9.45	30.7%	3.42%

* All M_x values have units of kg

** All C_x values have units of kg_x/kg total mass

3.3 Overall Mass Balance for LASRA Enzyme Treatment Process

Figure 3.3 shows the overall make-up of the LASRA enzyme treatment process including all unit operations with inputs and outputs into the system. To demonstrate an overall mass balance of the system, processes 5 to 8 were used. The block of 4 processes were related through a recycling loop which has the neutralization float (E4) of the previous trial run being recycled into the enzyme treatment process (S3) of the following trial run.

This section aims to present the issues associated with the whole pre-tanning process as a single entity from its first input (sulphide painted depilated sheepskins) to its last output (pickled sheepskins also known as pelts). Abbreviations used here are defined below;

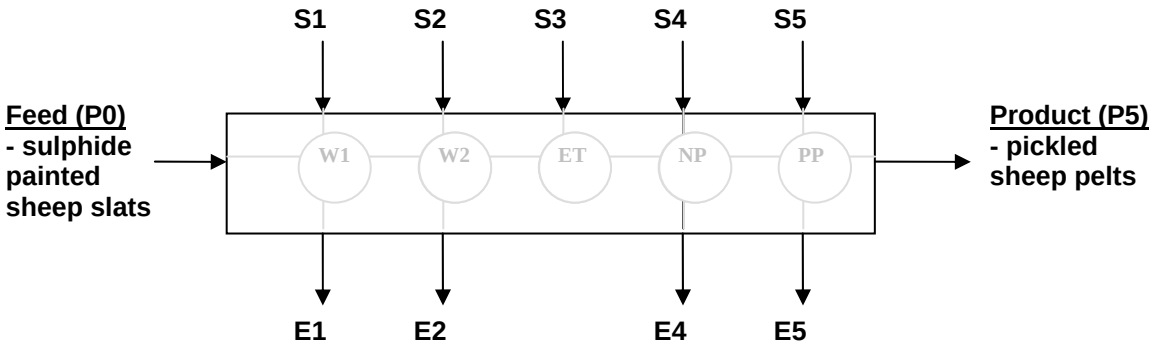


Figure 3.3: Single block diagram of the LASRA enzyme treatment process.

Tables 3.2 to 3.5 below show the overall mass balances of a block of four experimental runs of the LASRA enzyme treatment process.

Table 3.2: Total mass balance for process run 5

		IN		TOTAL	OUT		TOTAL	% ERROR
		Feed	Solvent	IN	Product	Effluent	OUT	
M_T	(kg)	12.7	63.5	76.2	8.95	65.75	74.7	1.97%
M_{TS}	(kg)	4.37	2.94	7.31	2.75	3.48	6.23	14.77%
M_{TKN}	(kg)	1.08	0.00	1.08	0.31	0.55	0.86	20.37%
M_{TDS}	(kg)	Not possible to calculate						
M_{IS}	(kg)	Not possible to calculate						
M_G	(kg)	0.653	0.00	0.653	0.306	0.172	0.478	26.80%
C_{S2-}	(ppm)	33202	0.00	33202	0.00	15085	15085	54.57%

Table 3.3: Total mass balance for process run 6

		IN		TOTAL	OUT		TOTAL	% ERROR
		Feed	Solvent	IN	Product	Effluent	OUT	
M_T	(kg)	13.9	69.5	83.4	10.2	73.1	83.3	0.12%
M_{TS}	(kg)	4.78	3.21	7.99	3.13	3.94	7.07	11.51%
M_{TKN}	(kg)	1.18	0.00	1.18	0.35	0.72	1.07	9.32%
M_{TDS}	(kg)	Not possible to calculate						
M_{IS}	(kg)	Not possible to calculate						
M_G	(kg)	0.717	0.00	0.717	0.351	0.127	0.478	33.33%
C_{S2-}	(ppm)	33202	0.00	33202	0.00	14830	14830	55.33%

Table 3.4: Total mass balance for process run 7

		IN		TOTAL	OUT		TOTAL	% ERROR
		Feed	Solvent	IN	Product	Effluent	OUT	
M_T	(kg)	13	65	78	10.65	67.5	78.15	-0.19%
M_{TS}	(kg)	4.47	3.01	7.48	3.27	3.56	6.83	8.69%
M_{TKN}	(kg)	1.11	0.00	1.11	0.36	0.61	0.97	12.61%
M_{TDS}	(kg)	Not possible to calculate						
M_{IS}	(kg)	Not possible to calculate						
M_G	(kg)	0.671	0.00	0.671	0.366	0.108	0.474	29.36%
C_{S2-}	(ppm)	33202	0.00	33202	0.00	15085	15085	54.57%

Table 3.5: Total mass balance for process run 8

		IN		TOTAL	OUT		TOTAL	% ERROR
		Feed	Solvent	IN	Product	Effluent	OUT	
M_T	(kg)	12.8	64	76.8	9.45	66.95	76.4	0.52%
M_{TS}	(kg)	4.4	2.96	7.36	2.9	3.81	6.71	8.83%
M_{TKN}	(kg)	1.09	0.00	1.09	0.32	0.66	0.98	10.09%
M_{TDS}	(kg)	Not possible to calculate						
M_{IS}	(kg)	Not possible to calculate						
M_G	(kg)	0.66	0.00	0.66	0.325	0.142	0.467	29.24%
C_{S2-}	(ppm)	33202	0.00	33202	0.00	19761	19761	40.48%

Below are the equations used to calculate the various components of tables 3.2 to 3.5. Superscripts indicate stream of origin while subscripts indicate stream component parameter;

Feed IN – the amount of component *x* in the feed is given by;

$$M_x = C_x^{P0} \times M_T^{P0} \quad \textbf{(Equation 3.1)}$$

Solvent IN – the amount of component *x* in the combined solvent inputs is given by;

$$M_x = \sum_{i=1}^{5} C_x^{Si} \times M_T^{Si} \quad \textbf{(Equation 3.2)}$$

Product OUT – the amount of component *x* in the product leaving the process is given by;

$$M_x = C_x^{P5} \times M_T^{P5} \quad \textbf{(Equation 3.3)}$$

Effluent OUT– the amount of component *x* in the combined effluent streams is given by;

$$M_x = \sum_{i=1}^{4} C_x^{Ei} \times M_T^{Ei} \quad \textbf{(Equation 3.4)}$$

*where *x* could refer to TS, TKN, TDS, IS, G or $S2^-$

To be able to construct a mass balance over a particular component, information on it for all inputs and outputs to the process had to be available. For some flow component values used in the detailed mass balance, sufficient information was un-available. For example, from table 3.2, it is seen that a mass balance for the components TDS and IS were not able to be constructed as actual observed values for them in the product stream were un-available. As a consequence of this, further work from component balances around each unit operation was required to estimate these. This is described in later sections of this chapter.

It can be seen that there is some error between the input and output of the total mass flow, total solids and TKN balances for processes 5 to 8 (seen in tables 3.2 to 3.5). This was calculated by equation 3.5 as seen below;

$$\textbf{\% ERROR} = \frac{\left(M_x^{Feed} + M_x^{Solvent} - M_x^{Product} - M_x^{Effluent}\right)}{\left(M_x^{Feed} + M_x^{Solvent}\right)} \times 100\% \quad \textbf{(Equation 3.5)}$$

For the total mass flow, the error is quite small and hence may be attributed to minor measurement and sampling errors. Looking across the four tables a maximum of approximately 2% unaccounted for mass was found for a given trial run. For the other components of the mass balance such as total solids (TS) and TKN, errors of between 10-20% were recorded across processes 5 to 8. These losses are significant and are of concern. The sampling and analysis of effluent flows including the final product values are likely to be relatively accurate, in comparison to the input values. This is justified by the fact that effluent and product values were process specific unlike the initial input values which were values measured for representative samples of depilated slats. The reason for representative slats being analyzed instead of process specific slats is because sampling requires analysis of a piece of the slat itself. Because the measurements are destructive, there would be no sample left to process. Sampling of a representative piece of slat will introduce slat to slat variation errors and also errors due to the variability across the slat.

Error margins in the grease and sulphide balance were found to be significantly high. This is unexpected as they are likely to be conserved during washing and enzyme processing (i.e. not changed by reaction). The error present in the balance must be a result of uncertainty in the measurement of these properties in the experimental work (i.e. measurement at the effluent streams of individual unit operations). Another reason for the large error percentage found for grease, may be attributed to the inability of the current measurement procedures used, to account for all grease or fat contents in all streams. There are various types of solid materials found on sheepskin that may be considered as ‘grease’ but methods of quantifying them accurately were unavailable at LASRA. The large percentage error found for the sulphide balance may be also due to the fact that significant quantities of sulphide are lost to the environment due to the chemical conversion of sulphide into hydrogen sulfide (H_2S). This gas evolves to the environment throughout the time a sheep slat is processed.

The method in which the discrepancies for each of the mentioned input-output classes were rectified is highlighted below;

a) The unaccounted total mass was spread proportionally over the effluent masses as those were considered to be the most prone to measurement errors (being relatively large).

b) The mass balance for TS and TKN was rectified using a solver function adjusting the initial values to match the final one. This was done as the initial concentration values obtained from LASRA used were generic representative values of slats. Properties of slats vary from one slat to another and also across different parts of it.

This shows that a more detailed analysis of each unit operation was required for a number of reasons;

a) To estimate the levels of IS and TDS and the changes that occur to these ratios due to the enzyme treatment on the slat as it progresses through the process.

b) To understand the assumptions that may be made regarding the partitioning of these components in each unit operation.

c) To define the processing parameters for each unit operation. For example; how much float can be added to the skins? How well is the slat drained after each unit operation?

A more detailed analysis of the LASRA enzyme treatment process and mass balance on it is provided in the following section to achieve these aims.

3.4 Detailed Mass Balance of LASRA Enzyme Treatment Process

Figure 3.4 presents a schematic representation of the LASRA enzyme treatment process without any optimization measures being implemented. By carrying out a detailed mass balance of the process a better understanding of the general workings of the process operations and its constraints can be obtained. The mass balance will later be used to aid the construction of other modified models of the mass balance in an attempt to optimize and improve the process (chapters 4 and 5). In the washing stages (W1 and W2) it is expected that soluble materials will be washed out of the slats depending on how thoroughly it is drained. The enzyme treatment stage (ET) will result in a conversion of insoluble material in the slats to being soluble and hence washable from the skin. It is also possible that insoluble particles such as cells, wool or follicles may be loosened by the enzyme and this material may be washed out into the solution phase.

The neutralization process (NP) will dilute the enzyme and soluble solids concentration in the float. The addition of the slats to pickling liquor at the pickling process (PP) would be the last step in the manufacture of the majority of exported sheepskins. In this work however, the slats were subsequently drained from the pickle to allow further processing to become tanned sheepskin. For each stage (unit operation) of the LASRA enzyme treatment process, the following components were balanced; Total flow (of a given stream including all solids and water), total solids (TS), insoluble solids (IS), total dissolved solids (TDS), water and, total Kjeldahl nitrogen (TKN)

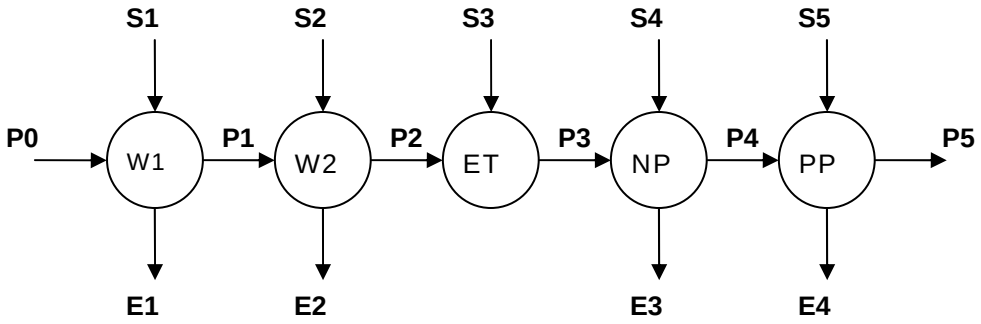


Figure 3.4: Simple process block diagram of the pre-tanning operations used in the detailed mass balance.

3.4.1 General approach and key assumptions made in the mass balance

Data from experimental trials (see table 3.1a & 3.1b in section 3.2) was used to construct the full mass balance. It is clear from the raw experimental data that not all inputs were well characterized. As such the mass balance was required to estimate some of those input compositions. In addition, the mass balance was used to identify the various assumptions that could be made over each unit operation. Some of the assumptions made during the construction of the mass balance were;

a) The density of all effluent streams was approximated as the density of water (1000 kg/m^3).

b) The process investigated to construct the mass balance (run 5) was considered to be a representative process for all other LASRA enzyme treatment process pilot scale runs. Consequently, this would mean that observed and calculated values of all other process runs in general would be within 'ball park' range of the values obtained from

process run 5.

c) As the values of enzyme treatment float were taken at the 6th hour (a reasonably long period of time), it is assumed that the process had reached equilibrium. Equilibrium was also assumed in the washing stages (i.e. the concentration of soluble material is the same in the free water associated with the slat and the separated float). This would allow estimation of product quality through the composition of the effluent of a given process. If there is less soluble material in the effluent in equilibrium with the finished skin, there will be less soluble material remaining in the water associated with that skin.

d) No other enzyme digestion occurs after the neutralization step. This means that all the enzyme dissolved solids were generated only in the 6 hours of the enzyme treatment process.

e) All enzyme dissolvable material stays dissolved throughout the whole process (e.g. no solubilisation or precipitation of components occur at the neutralization process).

f) There is a finite quantity of insoluble particles that can be washed from sheepskins.

g) There is a finite quantity of insoluble material loosened from the skin during enzyme treatment that can subsequently be washed out.

h) Washable insoluble material reaches an equilibrium concentration between free water associated with the slat

and the separated float.

i) There is a quantity of water bound to the protein components of the skin which is not able to act as a solvent to soluble material to suspend insoluble particles.

Together with these assumptions it was required to define some parameters to characterize the operation of the process. The solvent (that is freshwater) to sheep slats ratio is a convenient way to define the quantity of the solvent used in each step. This means that for every kilogram of raw slats used in the process, a specified mass of solvent will be used in each washing step. It is important to know the limits and capacity of the drums for future optimization work, as it is possible to alter the number of washing steps, its configuration as well as the sheep slats to solvent ratio to come up with a more efficient process. In this instance it is also important to monitor the swelling of slats as large volumes of highly alkaline float has the potential to drive swelling to the point where the quality of final product comes under threat of irreversible damage.

The known limits for solvent to slat ratio are between 0.6 and 2.0 for practical operation of fellmongery drums used in industry. Any less than 60% solvent may result in insufficiently washed sheep slats and extra burden on the drums used in terms of wear and power draw. If more than 200% water is used, besides the issue of drum capacity, more power will also be needed to agitate the drum due to the increase weight of contents. In the experimental trials this ratio was 1:1.

Table 3.6 – Table showing the ratios of total float drained from the skins relative to total amount of input across individual unit operations or LASRA enzyme treatment process

	Effluents to total input ratio (kg Ex/kg Px + Sx)
Wash 1	0.37
Wash 2	0.45
Neutralization	0.61
Pickle	0.67

Table 3.6 summarizes the ratio of total float drained from the skins (E_x) relative to the total amount of input, which includes the mass of skins (P_x) and solvent (S_x) added for the given unit operation. From table 3.6 it can be seen that the ratios increases from one unit operation to another. Under normal circumstances, one might expect this ratio to be similar throughout the process.

However washing step 2 has a higher ratio then washing step 1 because, in washing step one, the raw sheep slats fed into the drum are relatively dry and thus have a high capacity for liquid holdup. The sheep skins expand to absorb water and thus have filled most of liquid holding capacity in the first washing step. This phenomenon is reduced or not seen at all in the second washing step, hence more effluent (mostly solvent) is found for the second washing step.

In the neutralization step, a higher ratio again was observed compared with both preceding unit operations. This is because of the addition of two solvent streams (enzyme liquor in the enzyme treatment stage and the neutralization liquor in the neutralization process stage). The final stage pickling step had the highest effluent to total inputs ratio. This is due to the fact that the pickle liquor is a hypertonic solution which causes all the liquid from sheep pelts to be discharged into the solution and drained from the skin. In addition to the physico-chemical changes occurring to the skins affecting this ratio, the amount of effort or degree to

which the drums are drained will also be of importance. Thorough draining would require more time but would result in more complete removal of washed materials from the skin. It is likely that the most thoroughly drained unit operation would be after pickling in preparation for further tanning operations.

3.4.2 Bound water content of sheepskin

An important property of sheepskins that needs to be considered is the bound water ratio of the sheepskins to be used in the mass balance. The presence of water associated with the solids matrix of the slat which is not readily removed under normal draining conditions will affect the workings of the mass balance if ignored. Lewis (1947) describes the presence of both 'bound water' (water that does not behave like a solvent as it is bound to other moieties which affect its inherent properties) and 'free water' in sheep fleece. From Yates (1969), data on moisture absorption at 20°C by sheepskins is seen in figure 3.5. This has been used to estimate monolayer moisture content, where the first layer of moisture associated with the solids is present and indicative of bound water.

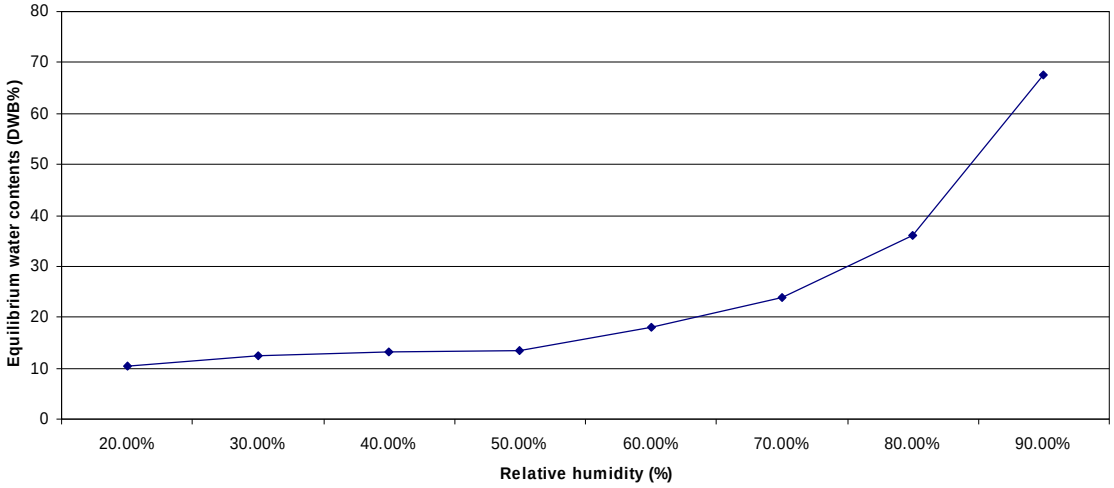


Figure 3.5: Plot showing the bound water content of sheepskins (value at free water limit) (Yates 1969)

A model was needed that could determine the monolayer value of the sheepskin samples from the data reported by Yates. Hui *et al.* (2007) found that the GAB model (equation 3.6) was best as it was robust by being able to account for a wide range of water activities (0.1 to 0.9).

$$M = \frac{M_o c_g f_g aw_g}{\left[\left(1 - f_g aw_g\right)\left(1 + \left(c_g - 1\right) f_g aw_g\right)\right]}$$

Equation 3.6: GAB model of sheepskins equilibrium water contents (Hui *et al.* 2007). Values are - M_o = 7.98, c_g = 20, f_g = 0.98

This equation was fitted to the data by non-linear regression using Excel. From this study, the bound water was found to be 0.0798 kg bound water/kg dry skin (taken from the Mo value of the GAB model). This was comparatively lower than the bound water of human *stratum corneum* (which is the top most layer of the epidermis) which was found from un-freezable water measurements to be between 0.3 – 0.35 kg bound water/kg dry skin by Walkley (1972).

To validate these results an experiment was conducted to measure the bound water of sheepskin found at various parts of the skin and stages along the pre-tanning operations. An outline of the methodology can be found in appendix 2. The basic principle behind the experiment was to measure the fraction of freezable water (bound water being the unfreezable fraction) in samples using differential scanning calorimetry (Perkin-Elmer DSC7 – PerkinElmer USA). The mass of dry solids of a sample slat together with the enthalpy of the endothermic heating of approximately 10 mg of sample of the same slat was used to produce an experimental value of the bound water ratio (Brown 1988). The endothermic heating method was done by first cooling the sample from room temperature to -80^{o}C at a rate of 50^{o}C/minute followed by a heating sequence of the sample back to room temperature at

a rate of 5°C/minute. The steps taken to calculate a single bound water ratio can be seen below;

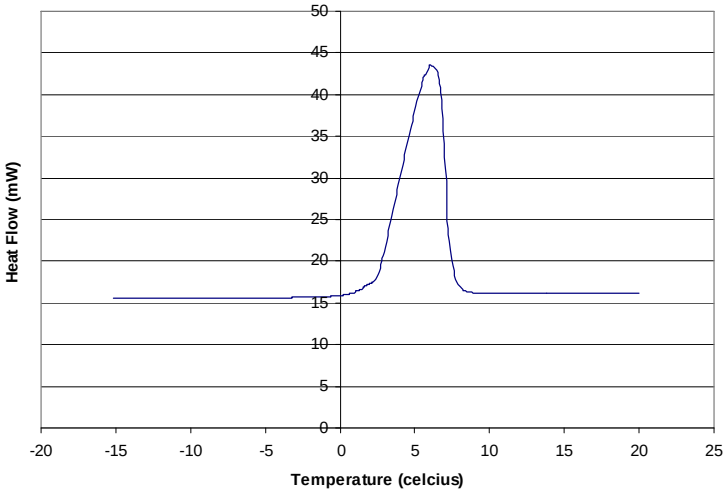


Figure 3.6a: Heat flow graph showing the heating section of the cooling and heating of reference sample (RO water). Area under curve is the enthalpy of given sample.

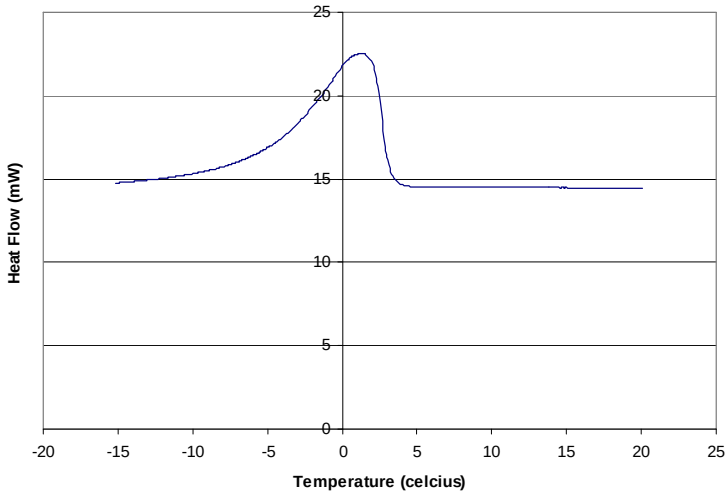


Figure 3.6b: Heat flow graph of the heating section of the cooling and heating of depilated sheepskin sample. Area under curve is the enthalpy of given sample.

The bound water ratio was then calculated according to Eq 3.7.

$$\textbf{Mass of freewater (kg)} = \frac{Enthalpy(Figure-3.6b)}{Enthalpy(Figure-3.6a)} \times M_T \text{ (depilated sheepskin mass)}$$

Mass of boundwater (kg) = (Depilated sheepskin mass × Percentage moisture content in M_T) – Mass of freewater

Bound water ratio (kg bound water/kg dry solids – Bw)

$$= \frac{Boundwater(kg)}{M_T \times C_{TS}^{*}} \qquad \textbf{(Equation 3.7)}$$

*Where C_{TS} is the percentage total solids in the depilated sheepskin sample

The bound water (Bw) ratios calculated by equation 3.7 are found below in table 3.7;

Table 3.7: Bound water values of various parts of skin and at various stages of pre-tanning operations

Sample	kg average wet sample	% average water content in sample	Bw ratio (kg bound water/kg dry skin)	Average Bw ratio (kg bound
Depilated skin	2.03	71.8%	0.51, 0.43	0.47
Green skin flank	1.35	73.9%	0.55, 0.43	0.49
Green skin neck	1.62	74.0%	0.59, 0.59	0.59
Limed neck	2.10	84.1%	1.31, 1.39	1.35
Limed flank	2.16	83.8%	1.32, 1.09	1.21
Limed butt	2.13	82.4%	1.14, 0.99	1.06
Delimed/bated neck	2.14	81.0%	1.52, 1.79	1.65
Delimed/bated flank	2.25	79.9%	1.47, 2.00	1.73
Delimed/bated butt	2.10	78.8%	1.27, 1.36	1.32
pickled neck	2.10	63.2%	1.06, 1.35	1.20
pickled flank	2.11	65.8%	1.58, 1.23	1.40
pickled butt	2.07	60.5%	1.23, 1.29	1.26

The sheep slat samples at various stages of pre-tanning operations were taken from a conventional process run (with samples from the LASRA enzyme treatment process un-available due to time and resource constraints). For the purpose of mass balancing, it was assumed that the bound water of the pelts would be the same for the LASRA and the conventional process at comparable stages of the process even though this may not in fact be the case. This is a reasonable assumption as the pH conditions and water contents of

samples of both the LASRA and conventional process (at comparable stages) were similar (Edmonds 2008). The limed samples may be applied to the LASRA enzyme treatment processed slat at the washing stages while the delimed/bated samples would be similar to the enzyme treated and neutralized samples of the LASRA enzyme treatment process. As the procedure for pickling sheep slats are the same for both processes, the values in table 3.7 would apply for both processes.

Looking at table 3.7, it can seen that the results of the experimental work is significantly different from the bound water values of sheep and human skin found in the studies of Yates (1969) and Walkley (1972). However, the high values of bound water seen from the results of experimental work were found in the work of other researchers investigating other biological and organic matter. Some examples include whey protein which had a bound water range between 0.5 – 1.2 kg bound water/kg dry solids across different types (Berlin *et al.* 1976) and the cortex of eye lenses which had bound water values of up to 0.61 kg bound water/kg dry solids (Bettelheim *et al.* 1986).

Subsequent analysis showed that a bound water value was needed to complete the mass balance around the first unit operation (washing stage 1) of the LASRA enzyme treatment process. The average bound water ratio of limed samples of 1.21 kg bound water/ kg dry skin derived from the values seen in table 3.7 was used in the mass balance. This was because the limed samples bound water values may be assumed to be the same as the bound water value of sheepskins of the LASRA enzyme treatment process at its washing stages.

3.4.3 Solids composition of slats at individual unit operations of the process

A full mass balance of the LASRA enzyme treatment process was carried using Microsoft Excel. A summary for the example process (run 5) data is given in Table 3.8 below.

Table 3.8: Detailed mass balance of process run 5. The highlighted values are system inputs as seen in Table 3.1a & 3.1b. All M_x values have units of kg solids while all all C_x values have units of kg_x/kg total mass.

		P0	S1	E1	P1	S2	E2	P2	S3	P3	S4	E4	P4	S5	E5	P5
M_{IS}	(kg)	**2.63**	**0.00**	**0.19**	**2.44**	**0.00**	**0.058**	**2.38**	**0.01**	**0.86**	**0.00**	**0.128**	**0.730**	**0.00**	**0.013**	**0.72**
C_{IS}	(kg/kg total)	20.71%	0.00%	1.96%	15.46%	0.00%	0.44%	15.64%	0.06%	3.08%	0.00%	0.51%	4.72%	0.00%	0.07%	8.04%
M_{TDS}	(kg)	**0.74**	**0.00**	**0.35**	**0.40**	**0.00**	0.34	**0.06**	**0.01**	**0.06**	**0.25**	**0.48**	**1.36**	**2.67**	**2.01**	**2.03**
C_{TDS}	(kg/kg total)	5.83%	0.00%	3.64%	2.53%	0.00%	2.57%	0.39%	0.06%	0.21%	2.00%	1.91%	8.81%	21.00%	10.45%	22.68%
M_{EDS}	(kg)	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**1.53**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
C_{EDS}	(kg/kg total)	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	5.48%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
M_{TS}	(kg)	**3.37**	**0.00**	**0.54**	**2.83**	**0.00**	**0.40**	**2.43**	**0.02**	**2.45**	**0.25**	**0.60**	**2.10**	**2.67**	**2.02**	**2.75**
C_{TS}	(kg/kg total)	26.54%	0.00%	5.60%	17.92%	0.00%	3.00%	15.94%	0.16%	8.77%	0.00%	2.38%	13.57%	21.00%	10.50%	30.70%
M_w	(kg)	**9.33**	**12.70**	**9.07**	**12.95**	**12.70**	**12.85**	**12.81**	**12.68**	**25.49**	**12.45**	**24.56**	**13.38**	**10.03**	**17.21**	**6.20**
C_w	(kg/kg total)	73.46%	100.00%	94.40%	82.01%	100.00%	97.00%	84.06%	99.84%	91.23%	98.00%	2.40%	86.43%	79.00%	89.50%	69.30%
M_{TKN}	(kg)	**0.87**	**0.00**	**0.19**	**0.67**	**0.00**	**0.11**	**0.57**	**0.00**	**0.57**	**0.00**	**0.23**	**0.34**	**0.00**	**0.04**	**0.31**
C_{TKN}	(kg/kg total)	6.82%	0.00%	2.00%	4.27%	0.00%	0.80%	3.72%	0.00%	2.03%	0.00%	0.90%	2.20%	0.00%	0.20%	3.42%
Total Mass - M_T	(kg)	**12.70**	**12.70**	**9.61**	**15.79**	**12.70**	**13.25**	**15.24**	**12.70**	**27.94**	**12.70**	**25.16**	**15.48**	**12.70**	**19.23**	**8.95**

In trial run 5, the total mass of each solvent stream was equal to the total mass input of P0 as seen in table 3.8. No TDS and TKN were present in the washing stage in-going solvent streams.

Table 3.1a showed that stream P0 was experimentally characterized in terms of M_T, C_{TS} and C_{TKN}. To complete the characterization of stream P0, either its concentration or mass of total dissolved solids (TDS) was to be found to be able to complete Table 3.8. This was done through a TDS balance around the first washing stage. The system inputs values used in these calculations are seen as highlighted values in Table 3.8. Calculations may be seen below. Concentration values are labelled as 'C' while mass values are 'M' (value in subscript is indicated flow parameter while superscript is indicated stream of origin);

Calculation of TDS concentration at stream P0: C_{TDS}^{P0}

Equation 3.8 was formulated from a balance of dissolved solids in each stream. The free water present in the washed slat (*P1*) was calculated using the bound water ratio discussed above (*Bw*). This free water was assumed to have the same concentration of dissolved solids as the effluent stream (*E1*).

$$C_{TDS}^{P0} = \frac{M_T^{E1} \times C_{TDS}^{E1} + \frac{\left(M_T^{E1} \times C_{TDS}^{E1} \left(M_W^{P1} - \left(M_{TS}^{P1} \times Bw\right)\right)\right)}{M_W^{E1} - (M_{TS}^{E1} \times Bw)}}{M_T^{P0}} \qquad \textbf{(Equation 3.8)}$$

Where;

$M_W^{P1} = M_W^{P0} + M_W^{S1} - M_W^{E1}$

$M_{TS}^{P1} = M_T^{P0} \text{ x } C_{TS}^{P0} + M_T^{S1} \text{ x } C_{TS}^{S1} - M_T^{E1} \text{ x } C_{TS}^{E1}$

Bw = bound water/un-freezable water (kg/kg dry skin) defined in section 3.4.2

$C_{TDS}^{E1} = C_{TS}^{E1} - C_{IS}^{E1}$

$M_W^{E1} = M_T^{E1} - (M_T^{E1} \text{ x } C_{TS}^{E1})$

Following the calculations above, all the unknown variables (concentration and mass values of IS, TDS, TS, water content, total mass, and TKN) around the first washing stage (of streams P0, S1, E1 and P1) could be determined. Calculation of the unknown concentration or mass values of flow parameters beyond the first washing stage was a straightforward matter. This may be done by equating the inputs and outputs as seen below for any given flow parameters for a given stage *x*, as seen in equation 3.9 below.

$$M_T^{Px} \text{ x } C_x^{Px} + M_T^{Sx} \text{ x } C_x^{Sx} = M_T^{Ex} \text{ x } C_x^{Ex} + M_T^{Px+1} \text{ x } C_x^{Px+1} \qquad \textbf{(Equation 3.9)}$$

It also has to be noted that calculations of flow parameters by equation 3.9 above may deviate from values calculated by equation 3.8 through the assumption that the process is in equilibrium. Due to the length of the processing time (6 hours), this assumption is reasonable and the difference may be attributed to errors found in system input values. This was investigated by sensitivity analysis and it was found that errors of up to 15% in the measured value of $C_{TS\text{-}Ex}$ (concentration of TS in a given effluent stream) may account for the discrepancies between the two methods of deriving unknown flow parameters.

3.4.4 Determination of enzyme dissolved solids generated

Enzyme dissolved solids (EDS) refer to the proteineous materials in the skin or essentially insoluble solids (IS) that are made soluble (to form TDS) by hydrolytic action with proteolytic enzyme. This is seen in Table 3.8 for the mass balance of process run 5. To simplify representation of the solid characterization values on the mass balance, EDS values following the enzyme treatment process were consolidated together with TDS.

In order to construct a predictive mass balance of the process, an estimate of the quantity of skin solids that are solubilized by the enzyme was required. The EDS value used in the forward mass balances (detailed in further chapters) was derived from the average EDS on a total solids (TS) basis value for trial runs 1 and 5. The way in which EDS would be calculated would be by mass balancing of the total dissolved solids (TDS). This is demonstrated in sub-section 3.4.4.1.

3.4.4.1 Mass balancing of the total dissolved solids (TDS)

The EDS value to be used in predictive forward mass balances was found by a TDS balance over the whole of zone 2 of the LASRA enzyme treatment process. It was assumed that the process floats were at equilibrium and hence had the same solids concentrations for both effluent and product stream. The steps in which the EDS was to be calculated is highlighted below;

Total TDS flowing out of zone 2 LASRA enzyme treatment process;

M_{TDS}^{E4} (kg) = $M_{TS}^{E4} - (C_{IS}^{E4} \times M_{T}^{E4})$

M_{TDS}^{P4} (kg) = $(M_{TDS}^{E4} \div M_{W}^{E4}) \times (M_{W}^{P4} - M_{TS}^{E4} \times Bw)$

Total TDS output (kg TDS) = $M_{TDS}^{P4} + M_{TDS}^{E4}$

*Where all M_{W}^{E4} is free water.

*Bw = bound water ratio (kg/kg dry skin)

Total TDS flowing into zone 2 LASRA enzyme treatment process;

M_{TDS}^{S3} (kg) = $M_{TS}^{S3} - (C_{IS}^{S3} \times M_{T}^{S3})$

M_{TDS}^{P2} (kg) = $(M_{TDS}^{E2} \div M_{W}^{E2}) \times (M_{W}^{P2} - M_{TS}^{E2} \times Bw)$

Total TDS input (kg TDS) = $M_{TDS}^{S3} + M_{TDS}^{P2}$

Therefore;

EDS generated (kg EDS) = Total TDS out – Total TDS in

The calculation of EDS through this method was carried out on process runs 1 and 5 which gave EDS values of 0.34 kg and 0.30 kg respectively. Although process runs 5 to 8 were of a different configuration, the first run may still be used as it does not have a recycle of solids affected by a change in process configuration. For process runs 2, 3 and 4, the EDS could not be determined accurately. These runs included a recycle of float from a prior

process and are subject to errors in the estimation of solids composition of float recycled. A possible reason for this is the occurrence of partial sedimentation of solids in float.

Due to the reasonable agreement of EDS values of process run 1 and 5, an EDS value to be used in forward and predictive mass balances could be derived by an average of both values – 0.21 kg of EDS. Placed on the basis of the amount of TS input into enzyme treatment process (stream P2) and able to be used for any given process runs, EDS is 0.08 kg EDS/kg TS in P4.

3.4.4.2 Enzyme released insoluble solids

The amount of enzyme released insoluble solids generated as a result of the enzymatic action in the enzyme treatment process may be assumed to be negligible and hence not included in the any of the mass balances. This was concluded through a mass balance analysis being conducted around the enzyme treatment process which found no extra insoluble solids generated as a result of enzyme activity.

3.5 Quantifying Chemical Oxygen Demand (COD) in Effluent Streams

The mass balances developed in this chapter were carried out in such a way that each component was conserved. For that reason, the flow parameters of TS, TDS, IS and TKN were selected to characterize the process streams as detailed in the previous sections of this chapter. There are a number of other measures commonly used to characterize process streams, especially with regard to effluent waste streams. Some of these give an idea of the environmental quality of a given effluent waste stream. One widely used measure is the Chemical Oxygen Demand (COD) analysis.

In process optimizations done in this work, it was of value to be able to compare processes by the COD quality of process floats as well. As process float quality can be linked to product quality, COD results can compliment the results obtained through a direct evaluation of product by a solids evaluation. As pointed out before, the conservation of COD was not observed in the mass balance but could potentially be calculated by correlating COD to the flow composition parameters used in the mass balance. This was done by exploring potential correlations between one or more independent variables such as Insoluble Solids (IS), Total Solids (TS) and Total Kjeldahl Nitrogen (TKN). If a good correlation was found, the value of COD could then be predicted as a function of these given independent variables.

Trials 1 to 8 provided data for COD as well as all the component concentrations used in the mass balances. The grease and sulphide concentrations were also available. However, these values were not considered in the mass balances due to the magnitude of errors in the measurement or loss to volatilization making balances of these measures difficult. A simple linear regression was carried out to relate the measured COD from the outlet streams (E1, E2 and E4) and also using values for float composition collected for each hour of enzyme treatment. Effluent streams from the pickling stage were not used due to the large differences in composition due to addition of 'pickling chemicals' at that stage of pre-tanning

operations. In addition, with an increasing number of tanning operations being done offshore away from New Zealand, pickle (which is a pre-tanned animal hide preservative) effluents are now less likely to be produced in New Zealand fellmongeries. The resulting correlation relating COD and the variables found on the mass balance is seen as equation 3.10 below;

$$COD = 3.63 \times C_{IS} + 0.0006 \times C_{TS} \quad \text{(Equation 3.10)}$$

The coefficient of determination (R^2 value) of this correlation to COD was found to be 0.95. An R^2 value of this magnitude indicates a strong correlation between the measures of IS and TS with COD. Figure 3.6 compares the predicted COD using equation 3.6 with the observed COD values;

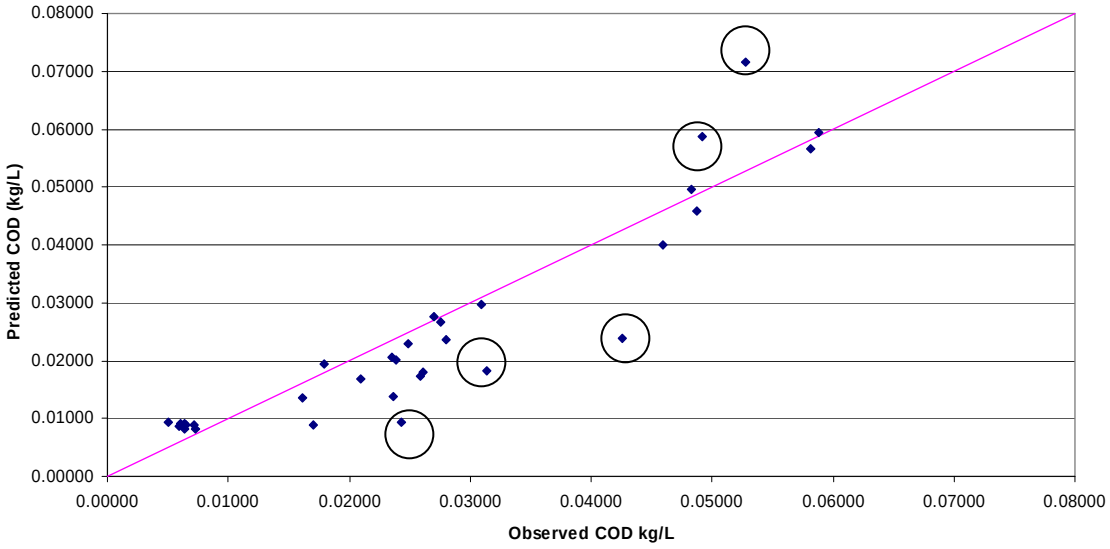


Figure 3.7: Plot of predicted COD calculated using equation 3.10 against observed COD. Circled data points are some clear outliers of the data set. The line found on the plot is a y=x line.

From Figure 3.7 it can be seen that equation 3.10 closely predicts COD values. Some noticeable outlying data points are circled on figure 3.7. These data points were all washing stage effluent samples. This may be due to the fact that washed slats still had much foreign organic matter on it which may have contributed to COD readings but were not accounted for by any of the 2 independent variables used. Some of examples of foreign matter unaccounted for by the correlation of

equation 3.10 include grease and sulphide, which may be present in significant enough quantities

to be detected by the COD analysis. The use of equation 3.10 will be demonstrated in the following chapters of the thesis.

3.6 Process Characterization Summary

A summary of the things achieved in this chapter are seen below;

a) Mass balances were used to characterize all process runs of the LASRA enzyme treatment process that were used in this chapter. A solver function was also employed to offset errors between the initial input and final output compositions.
b) Flow composition parameters that were used to characterize input and output streams of each mass balance were identified. This was done by identification of mass balance flow parameters which were explicitly seen to be conserved throughout the process.
c) Relationships that existed between various input-output variables used in the mass balance were defined for each unit operation.
d) The bound water ratio of sheepskins was established through experimental work by Differential Scanning Calorimetry (DSC) and its feasibility confirmed by comparison with literature values. This is an important input variable for the mass balance to ensure that the processes depicted by mass balancing are as close as possible to real time processes.
e) The assumption of achieving equilibrium in each unit operation was investigated and found to be valid with minor deviations accounted for by measurement of uncertainty.
f) The value of EDS used for the operation of forward mass balances to be used for investigation of various alternatives to the LASRA enzyme treatment process was defined on a total solids basis (i.e. the average amount of insoluble solids made soluble by the enzyme). This value is 0.08 kg EDS/kg TS stream P2.
g) Chemical oxygen demand (COD) may be used to compliment a solids description of process float quality. Through linear regression, an equation was derived to be able to predict COD for a given process stream from values used in the mass balance.

Although there is uncertainty in some of the process parameter estimates, they can still be used to predict the output stream composition for each stage in future work to investigate process alternatives and allow optimization work to be done on each. Chapter 4 will address the washing stages (zone 1) of the LASRA enzyme treatment process while chapter 5 continues into the optimization of the enzyme stages (zone 2) of treatment and neutralization.

4.0 OPTIMIZATION AND IMPROVEMENT WORK OF THE WASHING STAGES OF LASRA ENZYME TREATMENT PROCESS

The previous chapter presented a thorough overview of the prototype LASRA enzyme treatment process by means of mass balances. This chapter now looks into the various ways in which the washing stages of the LASRA enzyme treatment process (zone 1) may be modified so that its raw resource use and the slat quality before it enters enzyme treatment may be improved and optimized. The analysis discussed in this chapter is based on employing existing batch rotary drums. Other alternatives such as continuous counter current washing were not investigated as they would require significant changes to existing capital equipment available in fellmongeries and hence would be unlikely to be adopted. A schematic diagram of zone 1 may be seen as figure 4.1;

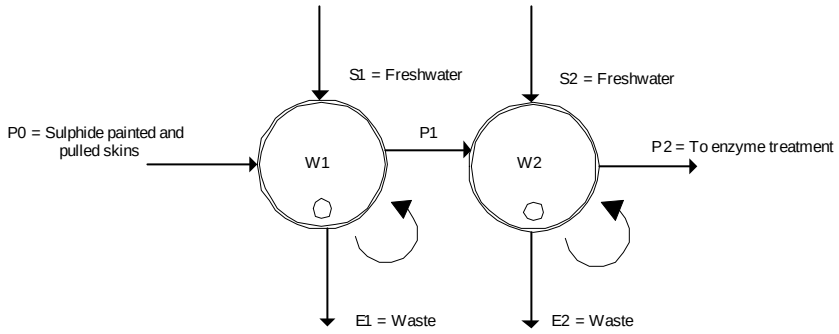


Figure 4.1: Schematic diagram of zone 1 of the LASRA enzyme treatment process

The parameter optimized was freshwater use. Water savings and hence effluent minimization was highlighted by Rao *et al.* (2002) and Thanikaivelan *et al.* (2004) as being an important process driver. In many parts of the world, possible reductions to water use, while attaining the same measure of leather quality using any different process configurations would be very much welcomed. However, for application in a New Zealand context, water use may not be so much of an issue (Foster 2008). According to Allsop (2008) it was more important for

the optimized process to be able to produce better quality washed slats while still maintaining equal amounts of water used in the process overall. By having more thoroughly washed or 'cleaner' sheep slats entering the enzyme treatment, this could set the slats up for a higher market value as previously highlighted in sub-section 2.3.1.4. Cleaner sheep slats were defined as those in which a greater proportion of the soluble and washable insoluble solids had been removed during the washing stages. In the previous chapter, it was shown that the assumption of equilibrium between the soluble solids concentrations in the effluent and remaining free water in the skins was appropriate. As such, it was then possible to use the composition of the float separated from the last washing stage as a direct measure of washing effectiveness.

The following sections highlight various possibilities for process modifications including an optimization based on both scenarios. In optimising for resource use, the washed slats composition/quality (after the two washing stages) was fixed (to that obtained by LASRA process) while the amount of freshwater used was varied according to a given process configuration. The optimization was based on the assumption that the composition/quality currently attained by the LASRA process is an acceptable standard to be achieved. In the second optimisation scenario, the total amount of freshwater used was fixed at the total amount of water currently used by the LASRA process. The optimization objectives would be to produce improved quality washed slats in terms of more complete washing of soluble and particulate solids from the skins.

A number of modifications are possible to enable potential reductions in solvent usage or more effective washing. The approach taken in the work on zone 1 was on optimization of process configuration and possible introduction of minor equipment. These would include using additional washing stages, using counter current washing operations and filtration of recycled floats. In addition, process operation variables such as float to skin ratio can also be adjusted. Comparison of these alternatives was achieved through the use of mass balances as it allowed detailed investigations to be conducted without the need for large numbers of

time consuming experimental trials. The modifications of zone 1 of the process were carried out, based on the understanding gained from the previous chapter.

4.1 Assumptions and Constraints Used in Mass Balance Development

The construction of a mass balance for the different modifications to the LASRA enzyme treatment process required a forward mass balance. The sheep slat and solvent input streams were defined while the effluent and product stream were determined by making appropriate assumptions of the process, drawn from the understanding of the current LASRA enzyme treatment process developed in Chapter 2. The assumptions made during the construction of the forward mass balance were as follows;

a) The sheepskin input composition (Insoluble solids - IS, total dissolved solids - TDS, total solids - TS, water content and total Kjeldahl nitrogen - TKN values) used, was assumed to be representative of all sheepskins and hence kept constant throughout the testing of various modifications to the LASRA enzyme treatment process. The trial run used in this instance was run 5 (see table 3.1a & 3.1b, section 3.2 for summary of raw data). The values to be used are seen below in table 4.1;

Table 4.1: Table of sheepskin initial composition taken as representative of all sheepskins. All the values were used in investigating alternative process. Mass of initial skin input for run 5 was 12.70 kg

Stream Parameters	Initial Input Values (kg/kg initial skin input)
Insoluble solids (M_{IS})	0.20
Total Dissolved Solids (M_{TDS})	0.06
Total Solids (M_{TS})	0.26
Total Mass (M_w)	1.00
Total Kjeldahl Nitrogen (M_{TKN})	0.07

b) The values of process run 5 were used to construct forward mass balances. Run 5 values were used as it had values for all flow parameters (which will be analyzed in latter parts of this chapter). The zone 1 configuration was the same as zone 1 of the standard LASRA enzyme treatment process.

c) The bound water content of the sheep slat was 1.21 kg bound water/kg dry skin, calculated during the construction of the LASRA enzyme process mass balance.
d) All water in the effluent stream was considered to be free water.
e) The entrained (carried over) water/moisture in the product stream after draining the drum was found to be 5.6 kg water/kg IS initial input. This was found as an average value of all the values of various trial runs of the LASRA enzyme treatment process.
f) Insoluble solids of the sheep slats were divided into two types. The first is the insoluble solids which is un-washable unless a process works on it (such as an enzyme treatment) by altering its physical properties. The other was classified as washable insoluble solids. Washable insoluble solids (WIS) on the sheep slat were found to be 0.031 kg WIS/kg initial sheepskin total mass. This was found by adding up all the insoluble solids that were washed out of sheep slat by the LASRA enzyme treatment process across all washing steps and taking an average value of the available trial runs.
g) The total Kjeldahl nitrogen to total solids ratio of the product stream was found to be 0.23 kg TKN/kg TS. This was again found as an average value of all the values of various trial runs of the LASRA enzyme treatment process.
h) The amount of water used in the solvent stream may not exceed 200% or be less then 60% of the initial sheepskin input mass. The upper level is based on the capacity of the drums used in processing (without reducing processing throughput). The lower limit is based on having enough float for effective contact with the skin during processing (Allsop 2008).
i) The minimization of freshwater solvent use was based on producing a product of required total dissolved solids (TDS). This was because TDS was the flow parameter which was most subject to variation in process configuration and inputs value (e.g. water) as compared to the other possibilities such as IS, TS and TKN. The target TDS in washed skin was 0.017 kg/ kg initial slat weight based on the quality achieved in pilot scale

trials 1 to 8 (table 3.1a & b) which were found to produce skins of acceptable quality post tanning.

j) The optimization based on maximizing quality (minimum TDS in washed skin) was based on a total solvent usage of 2 kg/kg slat weight. This was selected based on the total amount of freshwater used for runs 1 to 8 which had 2 washing stages utilizing 1 kg freshwater/kg slat weight each.

4.2 Development of Forward Mass Balances

Following the definition of the process variables to be used in forward mass balances and the identification of process constraints in the previous section, potential modifications to zone 1 can then be evaluated. Figure 4.2 shows an example of a forward mass balance around a single washing stage of the LASRA enzyme treatment process. Values characterising stream P0 have been identified as seen in table 4.1 while the solvent stream S1 is freshwater at a ratio of 1:1 with total mass of stream P0 (all values in these streams are system inputs).

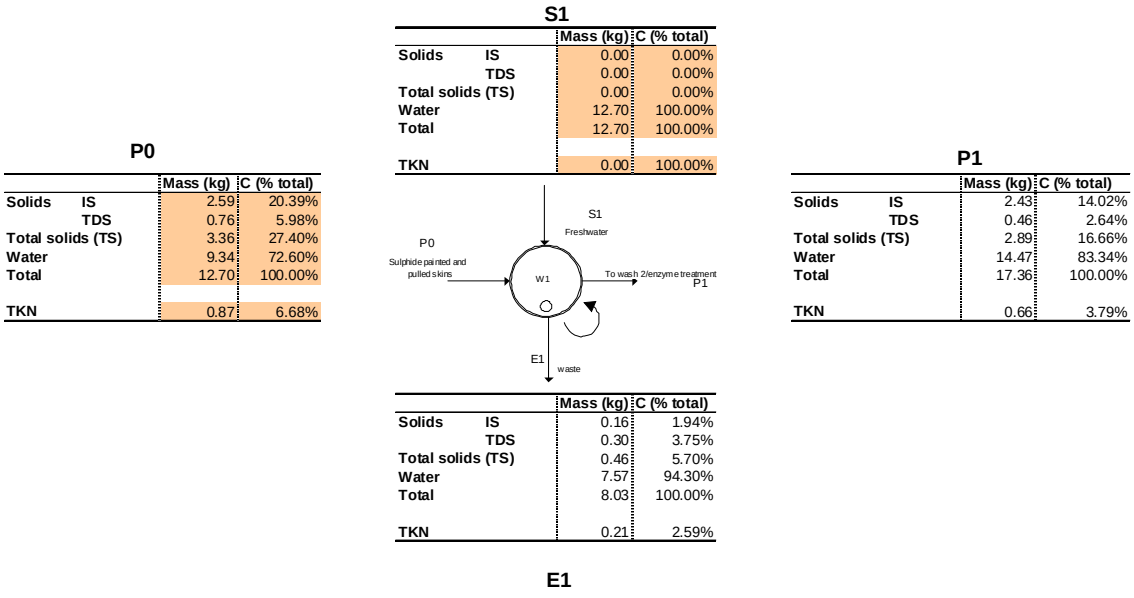


Figure 4.2: Mass balance around the first washing stage of the LASRA enzyme treatment run 5. Stream P0 and S1 are pre-defined system inputs and are highlighted.

The derivation of effluent stream E1 and product stream P1 are given below, calculated using the assumptions and constraints defined in section 4.1. First of all, the water content of the product outlet (P1) is dependent on how thoroughly drained the drum is. This is characterized by the moisture/IS ratio defined in section 4.1. Therefore,

Moisture (water) content of stream P1 (M_W^{P1}) =>

M_W^{P1} = Moisture/IS ratio X M_{IS}^{P0} **(Equation 4.1)**

A total moisture balance can then be used to determine the amount of moisture present in the effluent stream E1.

Moisture content of stream E1 (M_W^{E1}) =>

$$M_W^{E1} = M_W^{P0} + M_W^{S1} - M_W^{P1} \quad \textbf{(Equation 4.2)}$$

An insoluble solids balance over the stage can be done from the assumption that the concentration of washable insoluble solids (WIS) in the free water associated with the washed skin is the same as the concentration of IS (as all are assumed to be WIS) in drained effluent. This is through the assumption that floats are in equilibrium.

Insoluble solids of stream P1 (M_{IS}^{P1}) =>

$$M_{IS}^{P1} = M_{IS}^{P0} + M_{IS}^{S1} - M_{IS}^{E1} \quad \textbf{(Equation 4.3)}$$

To obtain the insoluble solids value of stream P1 by equation 4.3, the only unknown input variable is the mass of insoluble solids in the effluent stream of the stage and this may be calculated as seen below;

Insoluble solids of stream E1 (M_{IS}^{E1}) =>

$M_{IS}^{E1} = C_{IS}^{E1}$ X M_W^{E1} **(Equation 4.4)**

The concentration of insoluble solids in stream E1 may be found from solving the WIS balance (equation 4.5) seen below, using the quadratic formula of equation 4.6. It is assumed that all insoluble solids in solvent and effluent streams are all WIS.

WIS balance =>

WIS on sheep slat on basis of initial slat input mass $= M_T^{P0} + M_{IS}^{S1} = M_{IS}^{E1} + \text{Fw X } C_{IS}^{E1}$ **(Equation 4.5)**

Where;

$\text{Fw} = M_W^{P1} - \text{Bw* X } M_{IS}^{P1}$

Solving for concentration of insoluble solids of stream E1 in washing stage 1 (W1) from equation 4.5 =>

$\frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$ = Mass fraction of IS in W1 **(Equation 4.6)**

Where;

$a = \text{Bw X } M_W^{E1}$

$b = M_W^{E1} + M_W^{P1} - \text{Bw X } (M_{IS}^{S1} + M_{IS}^{P0})$

c = - (Sheep slat WIS on basis of initial slat input mass X $M_T^{P0} + M_{IS}^{S1}$)

Bw* = bound water/un-freezable water (monolayer moisture content)

While previously the bound water values were described on a dry solids basis (Bw), to simplify the mass balance the total solids were assumed to be equal to the insoluble solids basis when estimating the bound water content (Bw*). This may be done as most of the dry solids in sheepskin are insoluble solids (IS) and this simplification added negligible errors to the calculations.

The concentration of Total Dissolved Solids (TDS) in the effluent stream in W1 was calculated by a similar method

Concentration of TDS of effluent stream in W1 (C_{TDS}^{E1}) =>

$M_{TDS}^{P0} + M_{TDS}^{S1} = (M_W^{P1} + M_W^{E1} - \text{Bw* X } M_{IS}^{P1}) \text{ X } C_{TDS}^{E1}$ **(Equation 4.7)**

$$\Rightarrow C_{TDS}^{E1} = \frac{\left(M_{TDS}^{P0} + M_{TDS}^{S1}\right)}{\left(M_{W}^{P1} + M_{W}^{E1} - Bw^{*} \times M_{IS}^{P1}\right)}$$

Once the concentration of TDS of the effluent stream had been determined, the amount of TDS in the effluent stream could be calculated. The TDS in the product stream can then be calculated by a TDS balance over the stage.

Total dissolved solids of stream E1 (M_{TDS}^{E1}) =>

$$M_{TDS}^{E1} = C_{TDS}^{E1} \text{ X } M_{W}^{E1} \qquad \textbf{(Equation 4.8)}$$

Total dissolved solids of stream P1 (M_{TDS}^{P1}) =>

$$M_{TDS}^{P1} = M_{TDS}^{P0} + M_{TDS}^{S1} - M_{TDS}^{E1} \qquad \textbf{(Equation 4.9)}$$

Through summation of the insoluble solids and total dissolved solids of the product stream (P1) and the effluent stream (E1) respectively, the mass of total solids (TS) of both streams may be determined.

Total solids of stream P1 (M_{TS}^{P1}) =>

$$M_{TS}^{P1} = M_{TDS}^{P1} + M_{IS}^{P1} \qquad \textbf{(Equation 4.10)}$$

Total solids of stream E1 (M_{TS}^{E1}) =>

$$M_{TS}^{E1} = M_{TDS}^{E1} + M_{IS}^{E1} \qquad \textbf{(Equation 4.11)}$$

The summation of the TS and moisture content of stream P1 and E1 will also result in the total mass for both streams.

Total of stream P1 (M_{T}^{P1}) =>

$$M_{T}^{P1} = M_{TS}^{P1} + M_{W}^{P1} \qquad \textbf{(Equation 4.12)}$$

Total of stream E1 (M_T^{E1}) =>

$$M_T^{E1} = M_{TS}^{E1} + M_W^{E1} \quad \text{(Equation 4.13)}$$

Through a study of the total Kjeldahl nitrogen (TKN) of process runs 1 to 8, a TKN to TS ratio of product stream P1 was derived. This ratio was then used to calculate the TKN in product stream.

Total Kjeldahl Nitrogen of stream P1 (M_{TKN}^{P1}) =>

M_{TKN}^{P1} = TKN to TS ratio of product stream P1 X M_{TS}^{P1} **(Equation 4.14)**

Following that, the TKN in effluent stream E1 may be calculated by the TKN balance over the stage.

Total Kjeldahl Nitrogen of stream E1 (M_{TKN}^{E1}) =>

$$M_{TKN}^{E1} = M_{TKN}^{P0} + M_{TKN}^{S1} - M_{TKN}^{P1} \quad \text{(Equation 4.15)}$$

Following this, all subsequent wash stages streams were completed using the same set of equations as shown above.

4.2.1 Sensitivity analysis of forward mass balance

The use of the mass balance for the optimization of various possible configurations to zone 1 of the LASRA enzyme treatment process requires understanding of its sensitivity to the underlying constraints and assumptions made in its construction. For example are the predictions sensitive to the bound water ratio or the extent to which the skins are drained prior to further processing. If these values do significantly influence the predictions, more specific or robust common values and ratios must be obtained from an analysis of all available LASRA enzyme treatment process mass balances. For that reason, a sensitivity

analysis was conducted on the values, based upon a set of assumptions, input values and the process characterization values and ratios used in the mass balance.

A list of values used in the mass balance that have been derived in chapter 3 are seen below.

1. The bound water ratio (kg bound water/kg dry solids) of sheep slats in product stream.
2. The total washable IS in skin, which is IS that may be dislodged through washing the slats with water and/or agitation. The additional washable IS that may be dislodged as a result of the enzyme treatment are considered separately in the next chapter.
3. The input value of IS for the sheepskins coming into the pre-tanning process.
4. The input value of TDS of the sheepskins entering the pre-tanning operations.

To assess the influence of changing any one of the values above, the variation in the predicted total dissolved solids (TDS) concentration in the product stream water after wash 2 was investigated. This value was chosen as the dependent value as it (especially the one after the washing steps of pre-tanning operations) is one of the measures of float quality and washing capabilities of the current sheep slat washing system. The process configuration used in the sensitivity analysis test was the standard two washing stages of the LASRA enzyme treatment process.

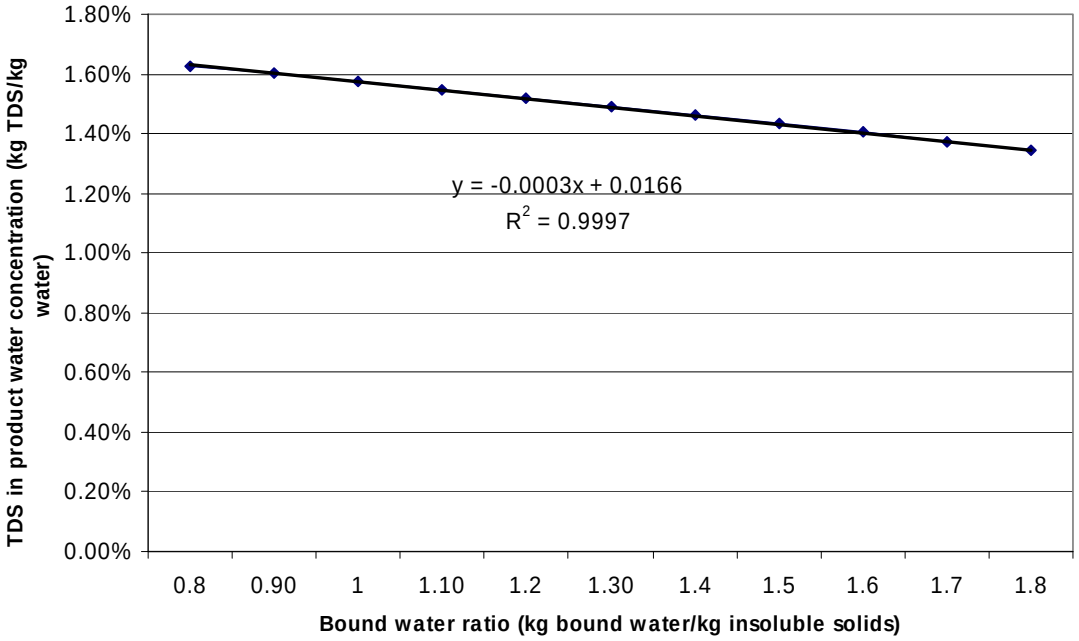


Figure 4.3: Plot of concentration of TDS in product water (in stream P2) against bound water ratio (insoluble solids basis)

Figure 4.3 shows an example of one of the 6 variables (bound water ratio as derived from experimental work seen in chapter 3) listed above plotted against the TDS concentration across a range of its possible values (plots of other independent variables listed against TDS concentration may be found in appendix 3). The range of values (between 0.8 and 1.8) used was to account for all possible bound water ratios observed on currently available data.

From the plot in figure 4.3, it can be seen that through the spectrum of possible ratios of the bound water ratio, there will be a variation of the TDS concentration of no more then 0.4% (between 1.3% and 1.7%) TDS in the float of the product stream from wash 2 (stream P2). Other plots can be found in appendix 3, for the other independent variables listed previously. From these plots the variation of the TDS concentration caused by these variables is similar or less than in the given example above. Hence it may be concluded as follows;

1. The use of the values in the construction of the mass balance derived from assumptions and averages taken from observations of previously conducted processes is valid and robust for use.
2. The mass balance spreadsheet constructed is fairly robust as it can account for variability of the characteristics of sheepskins used. Should an optimization on the water use in the process be done with varying characteristics of sheep skin, even then the mass balance can still be trusted to produce accurate results.

4.3 Modification Options for Zone 1 LASRA Enzyme Treatment Process

Through mass balances, two major types of modifications to the LASRA enzyme treatment process will be detailed in the following sub-sections. They were the;

1. Number of cross-current washing stages
2. Employment of counter-current configuration to the washing stages

4.3.1 Cross-current washing stages configuration for zone 1 of the LASRA enzyme treatment process

The number of washing stages (arranged in a cross-current configuration) may be a determinant to the quality of washed slats. Up to 4 washing stages arranged in a cross-current configuration for zone 1 were considered (note, the LASRA process had 2 washing stages). For each modified configuration of the process, the same set of equations as presented in sub-section 4.2 were used to define the composition of the individual streams (solvent, product or effluent streams) of the washing stages. Figure 4.4 shows a schematic diagram of a multiple stage sheepskin washing configuration.

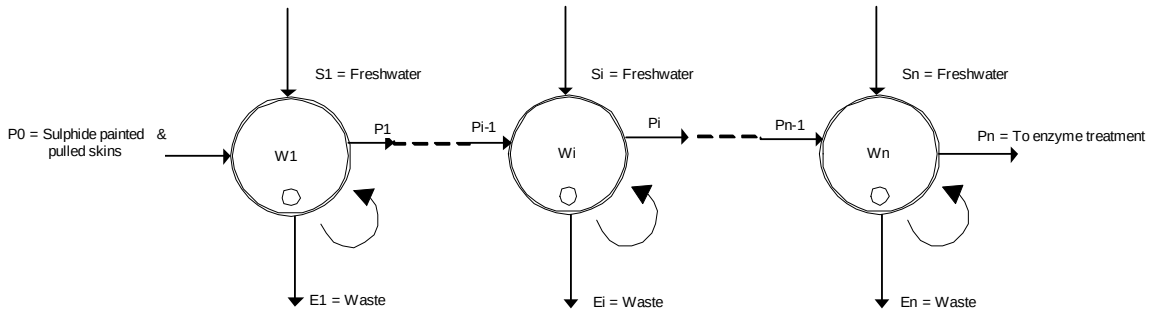


Figure 4.4: Schematic diagram of multiple washing 'i' stages beyond the first washing stage. The 'nth' stage refers to the last washing stage of the given configuration.

The optimisation based on the least amount of water required to achieve the specified target of 0.017kg TDS/kg initial skin weight are seen in table 4.2 below;

Table 4.2: Results of optimization to freshwater usage based on TDS values. The percentage water use is based on the initial sheepskin input of process run 5 which was 12.7 kg. A water use of 100% would indicate 12.7 kg freshwater used.

Process	Total Water Used (% initial skin input)	TDS of sheep slats (kg/kg initial skin weight)
1 stage cross current	281%	0.017
LASRA process (2 stage)	200%	0.017
3 stage cross current	170%	0.017
4 stage cross current	154%	0.017

The 4 stage cross-current configuration of zone 1 was not considered any further despite it needing the least amount of water used to achieve its objective. Given the requirement of a minimum 60% solvent (of initial depilated sheepskin weight) needed for each washing stage; this option may not be feasibly implemented given the quantity of water used to achieve its optimization objective.

In a similar manner, from table 4.2, it can be seen that it is not possible to implement a single stage washing configuration due to the constraint of feasible solvent to skins ratio. Despite foreseeable benefits of a single stage system in terms of reduced processing time and related costs, its implementation would result in a compromise of productivity (i.e. processing less skins in a drum) which is obviously undesired by the pre-tanning industry.

An investigation was also made into which zone 1 configuration could produce the best quality of washed slats with a fixed amount of water (to the total amount used in zone 1 of LASRA process). With two washing stages, each using a 100% solvent to initial depilated sheepskin weight, the total amount of water used with

the LASRA process was 200% solvent to initial depilated sheepskin weight. Table 4.3 below summarizes the results of the findings.

Table 4.3: Results of optimization to slat quality based on TDS values. The percentage water use was also based on the initial sheepskin input of process run 5 which was 12.7 kg.

Process	Total Water Used (% initial skin input)	TDS of sheep slats (kg/kg initial skin weight)*
1 stage cross current	200%	0.022
LASRA process (2 stage)	200%	0.017
3 stage cross current	200%	0.013
4 stage cross current	200%	na

For the 4 stage cross current process (4SCrossC), no solution may be obtained because of the constraint of needing to have at least 60% solvent to initial depilated sheepskin weight in each washing stage.

Tables 4.2 and 4.3 shows that the water use and slat quality improves as additional stages were used. The 3SCrossC process appears to be the best option because it presents a lowered freshwater usage in achieving standard slats quality as well as improving the quality of washed slats should the same amount of water be used in standard methodologies. The impact on overall processing time (up to 50% longer total washing time) must be weighed up together with these advantages in performance.

4.3.2 Counter-current wash configuration as a modification to zone 1 of LASRA enzyme treatment process

In counter current washing, a freshwater input is only used for the last washing step while preceding steps use the effluent water from that next downstream step (see Figure 4.5). The term ‘counter-current’ suggests that the sheepskins move in the opposite direction to the flow of wash water. With that, the

cleanest skins will be washed with the cleanest wash water while the 'dirtiest' skins will be washed with the 'dirtiest' water. This mode of operation is a commonly used technique in industry (Tervola 2005). For the purpose of this study, up to three counter-current washing stages were investigated. The basic layout of a multistage counter-current washing configuration can be seen in Figure 4.5 below;

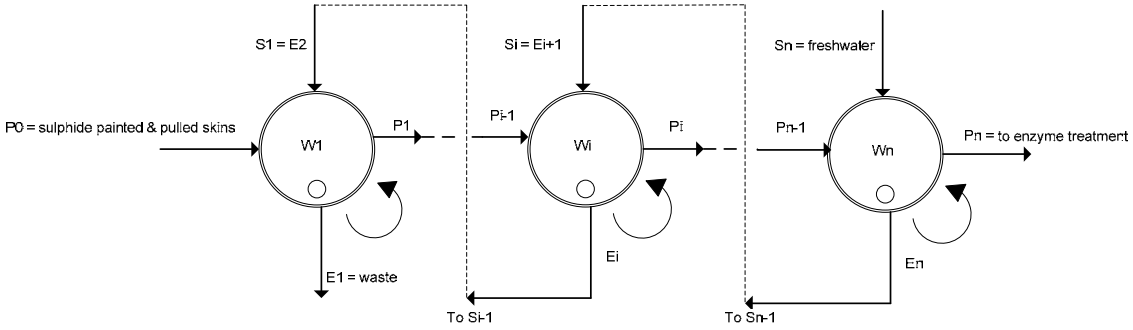


Figure 4.5: Schematic diagram of multiple counter-current washing 'i' stages beyond the first washing stage. The 'nth' stage refers to the last washing stage of the given configuration.

Optimization was again done to two bases; water usage and washed slat quality. As before, the set of equations as listed in sub-section 4.2 was used to define all the flow parameters for all the streams of all washing stages. The only exception being that, the effluent stream of a downstream washing stage was used as the solvent stream input of the preceding washing stage. To produce a steady state solution of the counter-current mass balance, up to 10 iterations were done (trialled using an active mass balance spreadsheet) for both the two stage configuration (2SCounterC) and three stage configuration (3SCounterC). Through iterations of the mass balances, steady state was deemed to have been achieved when the effluent stream flow parameters of the last washing stage converged with the solvent stream of the washing stage just before.

For the optimization based on water minimization, the quality of washed slats was fixed to the quality obtained by the LASRA process. Results of the investigation may be seen in table 4.4 below;

Table 4.4: Results of optimization towards freshwater usage to TDS for counter-current modifications to LASRA enzyme treatment process. 0.017 kg TDS per kg initial skin weight was the quality achieved by the LASRA process.

Process	Total Water Used (% initial skin input)	TDS of sheep slats (kg/kg initial skin weight)
LASRA process (2 stage)	200%	0.017
2 stage counter-current	135%	0.017
3 stage counter-current	95%	0.017

The results obtained show that multistage counter-current configuration yields significant reductions in freshwater use while achieving its required objective. The addition of extra counter-current washing stages does further reduce water usage. However, as can be seen from the results for 2 stage counter-current (2SCounterC) and 3 stage counter-current (3SCounterC) processes, these reductions are decreasing in magnitude as compared to the initial reduction by employing a counter-current configuration. Further reductions could be achieved with four or more counter-current stages but limitations on the minimum float volume (of at least 60% initial slat weight) and an increase of processing time would limit the practicality of such initiatives.

For the second optimization scenario, the freshwater input was set at 200% freshwater to skins ratio. The aim of this optimization method was to investigate the improvements on slat quality that may be achieved in each counter-current configuration using the same amount of freshwater as the LASRA process. The results are seen in table 4.5 below;

Table 4.5: Results of optimization of slat quality to TDS for counter-current modifications to LASRA enzyme treatment process

Process	Total Water Used (% initial skin input)	TDS of sheep slats (kg/kg initial skin weight)*
LASRA process (2 stage)	200%	0.017
2 stage counter-current	200%	0.009
3 stage counter-current	200%	0.003

From table 4.5, it can be seen that reduction of TDS values of even the basic counter-current configuration (2SCounterC) was up to 50%. The 3SCounterC process in turn suggested a potential reduction of up to 80% of TDS should it be implemented as a modification to the LASRA enzyme treatment process. This would result in more thoroughly washed slats which would translate to improved quality pickled pelts with lower levels of solids such as unwanted protein.

4.3.3 Overall comparison across the proposed modifications to zone 1 of LASRA enzyme treatment process

Table 4.6 summarizes the overall results for optimizing water use (for required slat TDS composition) together into one table and similarly table 4.7 summarizes slat composition optimization for residual TDS on slats (when water usage is maintained equally for all processes).

Table 4.6: Summary of results of water optimization to TDS for all possible modifications to Zone 1 of the LASRA enzyme treatment process

Process	Total Water Used (% initial skin input)	TDS of sheep slats (kg/kg initial skin weight)
1 stage cross current	281%	0.017
LASRA process (2 stage)	200%	0.017
3 stage cross current	170%	0.017
4 stage cross current	154%	0.017
2 stage counter-current	135%	0.017
3 stage counter-current	95%	0.017

Table 4.7: Summary of results of slat quality optimization to TDS for all possible modifications to Zone 1 of the LASRA enzyme treatment process

Process	Total Water Used (% initial skin input)	TDS of sheep slats (kg/kg initial skin weight)*
1 stage cross current	200%	0.022
LASRA process (2 stage)	200%	0.017
3 stage cross current	200%	0.013
4 stage cross current	200%	na
2 stage counter-current	200%	0.009
3 stage counter-current	200%	0.003

It can be seen that the 3 stage counter-current configuration (3SCounterC) gives the best results for both optimization objectives, followed by the 2SCounterC washing configuration. However, based on the practicalities involved should the modifications be implemented in industry, the 3SCounterC configuration would have significant disadvantages due to a longer processing time for the sheepskin to be washed. This is a result of having one extra wash step over the LASRA process two washing steps thus potentially offsetting some savings on time obtained by the LASRA process. For industrial processes, time is of high importance hence the faster throughput rates the better. This would mean that the 2SCounterC configuration is more likely to be adopted by the industry.

Another process improvement option for the washing stage is filtration of floats prior to recycle. This would further reduce freshwater use and/or improve slat quality. The implementation of a filtration technique will however significantly increase capital costs. It will also require the use of a technology not widely used in the tanning industry. As the counter-current washing process achieved good efficiencies, the filtration option was not considered any further.

Overall, the 2SCounterC configuration would be the most ideal option of modification for zone 1 of the LASRA process. It can be shown that this process results in significant improvements to the quality of the washed slats over that produced by the LASRA process if water usage is maintained. More solids being removed will provide savings of resources used downstream of the washing process as less enzyme and freshwater would be required to condition the leather to achieve the same desired quality of washed slats (as discussed in sub-section 2.3.1.4). These gains would be achieved without a need to invest in extra capital equipment as well as maintaining the savings in processing time achieved by the LASRA process originally. Moreover, if product quality is maintained, the amount of freshwater used is reduced (over the LASRA process) to achieve the target quality. Optimization to this basis may be desirable for tanneries found in parts of the world where availability of freshwater resource is an issue.

By employing a 2SCounterC process the water savings gained by the LASRA process would increase from 40% to 70% total freshwater savings over the conventional process.

4.4 Validation

The previous analysis suggested that the two stage counter current (2SCounterC) washing configuration was the most suitable to employ for Zone 1 of the LASRA enzyme treatment process. To validate the predicted results, experimental trial runs were conducted to compare slat composition of the 2SCounterC washing configuration with that of the regular process using the same quantity of water. This section outlines this validation experiment.

4.4.1 Experimental design

The objective of the validation experiments on Zone 1 of the LASRA enzyme treatment process was to confirm and validate the theoretical study which showed that the 2SCounterC configuration for washing sheepskins will produce better quality skins that are passed on into the enzyme treatment stage. As previously defined in the mass balance trials, better quality washed skins were defined as having a higher fraction of total dissolved (soluble) solids removed from sheepskins.

The 2SCounterC washing configuration was run at maximum drum volume capacity (that is, 200% wash water as per mass of skins). This was done so that a comparison of sheepskin quality processed using the benchmark 2 stage cross-current process and the 2SCounterC configuration could be demonstrated. To reduce experimental error, the skins were cut lengthwise into 2 pieces of equal characteristics as best as possible with each half used in a different washing technique. In this way, potential variations from one piece of sheepskin to another and even different parts of the same skin could be minimized. With that, it was assumed that the concentrations (per kg skin) of various parameters (of flow parameters used in mass balance – IS, TDS etc.) analyzed were the

same for all skins. To compensate for slight variations in skin masses and to ensure consistency of the different wash configurations tested, solvent stream water inputs were adjusted accordingly.

A difficulty in the experimental design was due to the requirement that the solvent stream of the first washing stage of a given run was to be the recycled float from a previous trial run, which does not exist to begin with. As such, a series of washes of sheepskins were carried out, through use of E2 effluent of a previous trial run for S1 solvent stream of a subsequent run. This is pictured in Figure 4.6 below in a set of 3 runs.

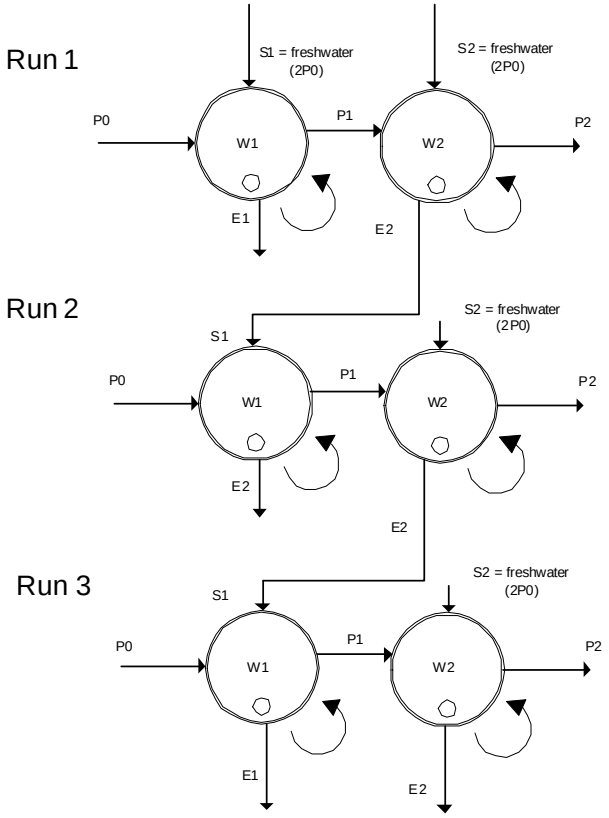


Figure 4.6: The 2SCounterC washing system to be trialled experimentally. Up to 3 runs will be done to ensure that the E2 stream used as the S1 stream of subsequent run is as close as steady as possible

In addition to that, a series of washes were also required to ensure that the E2 stream being used as S1 had reached steady state. As

mentioned in sub-section 4.3.2, steady state of the float was deemed to have been achieved if flow parameter values of an effluent stream were the same as the values of the solvent stream of the washing stage just before. To determine the number of washing stages to achieve a float in equilibrium for validation work, the measure of TDS concentration in its given stream was used. The agreement between TDS concentration in an effluent stream (E2 for a 2 stage counter-current configuration) and the solvent stream (S1) of a washing stage before was measured. The outcome conducted over 5 iterations/runs (to 2 decimal points) may be seen in Figure 4.7 below;

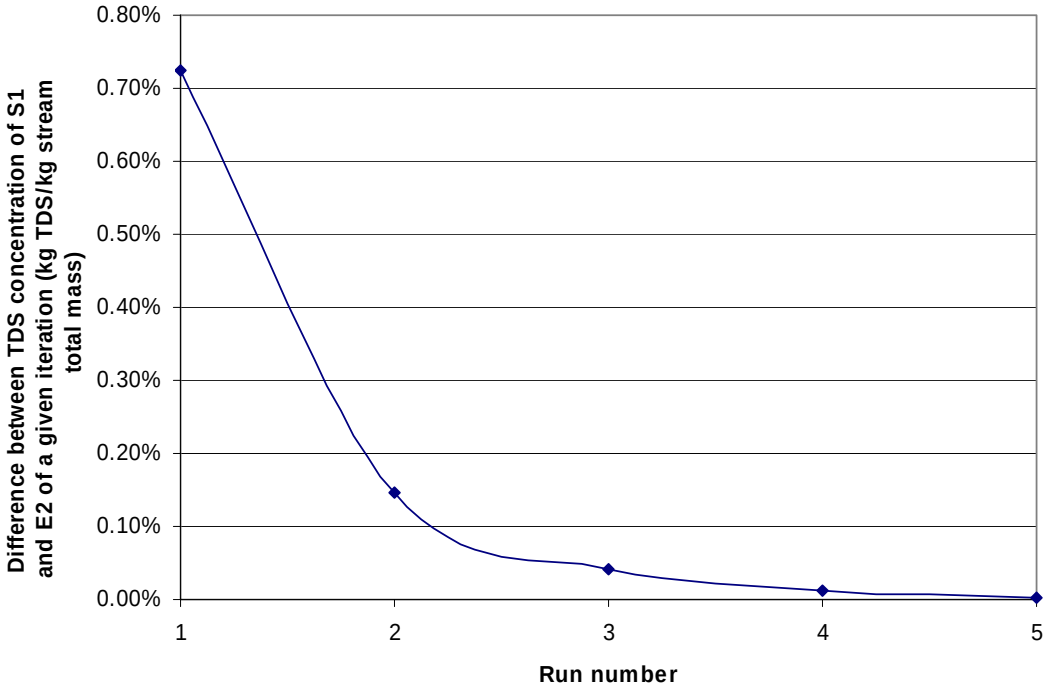


Figure 4.7: Predicted progression towards equilibrium measured by the difference between TDS concentration of streams E2 and S1.

From Figure 4.7, it can be seen that equilibrium would have been reached beyond 5 iteration/process runs. However, it was also found that increasing the number of runs used to produce a float, resulted in reducing total float volume (in actual experimental trials) and there was a risk of the float used in the actual 2SCounterC run not being sufficient to run the process. This is

due to the fluctuating weights of the half skins used. Hence a balance was needed between the number of runs required to create a float that was in equilibrium and to ensure that there was enough float for the actual 2SCounterC process (which is the last run). This issue, coupled with resource constraints of both time and sheepskins availability, a compromise of 3 runs of the 2SCounterC washing configuration would be conducted in validation work with each run processing 2 half skins at a time. As seen Figure 4.7, the TDS concentration agreement between E2 and S1 for 3 runs was only different by 0.04%

Float samples were taken from the effluent streams of both washing stages with the following parameters being monitored for comparison of the two processes; IS (Insoluble solids), TDS (dissolved solids), TS (total solids), moisture content and TKN (total Kjeldahl nitrogen). These parameters were chosen as the experimental techniques are relatively easy to conduct and analyze yet able to accurately reflect sheepskin quality and hence the better configuration to wash skins in the pre-tanning operations. Comparisons could then be made based on the amount soluble or insoluble solids washed out of sheepskins. The full list of methodologies used in this experiment can be seen in appendix 4. Results were also compared against the predictions formed during the study on various modifications stated in section 4.3 before.

4.4.2 Results and discussion

Table 4.8 shows the raw data (results) of the validation work conducted at the LASRA pilot scale fellmongery.

Table 4.8: Raw data (results) of the validation work comparing the LASRA enzyme process standard configuration (2SCrossC) and the proposed modification of its washing stages (2SCounterC). The runs of 2SCounterC R1 and R2 were runs used to generate a float to be used in R3 that was as close to equilibrium as possible (as detailed in sub-section 4.4.1 before).

Process run name	IS (mg/l)	Average IS	% TS (dry solids basis)	Average % TS	% TKN (w/w total protein)	Average % TKN	COD (gm-3)
2S CrossC R1W1	16120	15700	6.95	6.71	2.38	2.32	59740
	15280		6.46		2.26		
2S CrossC R1W2	3960	4140	3.03	3.20	1.32	1.30	34740
	4320		3.37		1.28		
2S CounterC R1W1	20920	12090	7.53	4.87	2.33	1.53	24351
	3260		2.21		0.73		
2S CounterC R1W2	7120	4210	2.68	1.80	1.28	0.80	12662
	1300		0.91		0.31		
2S CounterC R2W1	30800	19500	10.04	6.96	3.57	2.46	112338
	8200		3.87		1.34		
2S CounterC R2W2	4960	3400	3.83	2.57	1.61	1.05	42208
	1840		1.31		0.49		
2S CounterC R3W1	7640	7880	5.07	4.74	1.81	1.79	13961
	8120		4.41		1.77		
2S CounterC R3W2	1640	1630	1.37	1.38	0.59	0.58	58442
	1620		1.38		0.57		

Looking at the COD readings found on table 4.8, it can be seen that these may not be reliably used in the analysis of validation work. Discrepancies can be seen in readings of 2SCounterC R2W1 which shows an unusually high COD value. Particularly of more concern in this work is the value of the 2SCounterC R3W2 COD value which is almost 4 times higher than that of 2SCounterC R3W1. With a multistage counter-current washing system, it is usually the first washing stage which should wash out the larger amount of organic material (solids) from sheepskins.

Table 4.9 shows the summarized results of the validation work, comparing both the 2SCrossC and 2SCounterC configuration through the measures of IS, TDS, TS, and TKN (2SCrossC is the 2-step cross-current washing configuration while 2SCounterC is the 2-step counter-current washing configuration).

Table 4.9: Summary of the comparison between the 2SCrossC and 2SCounterC processes using IS, TDS, TS, and TKN as measure

	2SCrossC (% washed out per skins input weight)	2SCounterC (% washed out per skin input weight)
IS	1.22%	1.08%
TDS	5.13%	5.41%
TS	6.34%	6.49%
TKN	2.35%	2.45%

Table 4.9 showed (with the exception of IS) that the counter-current process extracted more TDS, TS and TKN than the cross-current process. These results validate the initial findings that the 2SCounterC configuration does yield improved quality skins. However, the improvements of the 2SCounterC over the 2SCrossC were not to the level predicted by a mass balance study. It has to be noted that the prediction generated by a mass balance study was for ideal conditions and that stringent pilot plant procedures may not have been exercised in the validation work. Also, with the measure of TDS used being a value of small magnitude, systematic and random errors may have resulted leading to a difference in results between the mass balance study and validation work.

Despite there being a small reduction of IS being washed out through the 2SCounterC, this was offset by the fact that more TDS was washed out leading to an increase of TS washed out overall.

To further emphasize the advantages of the 2SCounterC configuration as shown by the validation work, table 4.8 showing the results of the validation work, shows that there is a higher concentration of IS, TS and TKN discharged with the 2SCrossC process over that discharged by the 2SCounterC process (for the second washing stage). For example, the concentration of TS in the effluent stream of washing stage 2 for 2SCrossC was 3.2% while for 2SCounterC it was 1.4%. With the assumption that the process floats were in equilibrium, this meant that these concentrations were consistent between the effluent and product streams. This

would mean that there is a higher concentration and also amount of IS, TKN and hence TS associated with the slats produced by the 2SCrossC configuration (with the assumption that the slats of 2SCrossC and 2SCounterC configurations had been drained to similar levels of slat 'dry-ness' at the end of their respective trial runs).

Overall, it is recommended that industrial scale runs be conducted in a similar fashion as was done in the validation work to test the benefits of the 2SCounterC configuration of zone 1. The results obtained would be more reflective of the potential of 2SCounterC as larger scale processing would provide more meaningful values for comparison.

4.5 Summary of Optimization Work on Zone 1 of LASRA Enzyme Treatment Process

To end the chapter, a summary of the things achieved are seen below;

a) Forward mass balances were established in order to predict flow parameter outcomes given inputs. These forward mass balances were then used to evaluate potential modifications to the current washing step configuration (2SCrossC) of the LASRA enzyme treatment process.
b) The 2 stage counter-current washing configuration (2SCounterC) was found to be the more ideal modification to 2SCrossC
c) Through the mass balance study done on the 2SCounterC configuration, a freshwater usage reduction of up to 40% may be achieved. In addition to existing benefits of the un-optimized LASRA process, total freshwater savings over conventional is 70%.
d) Through the mass balance study done on the 2SCounterC configuration, a reduction of total dissolved solids (TDS) on the sheep slat departing the washing stages of up to 50% may be achieved (when compared with the LASRA process).
e) The finding of c) has since been validated through a trial run (although not to the magnitude predicted by mass balance). It is recommended that an industrial scale study be conducted in a similar fashion to the validation work done to confirm findings.

However, it has to be noted that for the 2SCounterC configuration to be implemented in the industry, this may require the expansion of additional capital resources to upgrade existing pre-tanning facilities such as storage drums to store the float to be recycled. In the following chapter 5 , similar approaches, conducted to find a suitable modification to Zone 2 of the LASRA enzyme treatment process are outlined.

5.0 OPTIMIZATION OF THE ENZYME TREATMENT STEP

After washing the depilated sheepskins through zone 1 of the LASRA enzyme treatment process, the slats are processed to remove material not required in the finished leather. This is carried out through the use of enzymes which hydrolyze most proteinaceous components, leaving key collagenous and other key proteins intact. The general process operation can be seen in Figure 5.1 below. This part of the process is referred to in this thesis as zone 2 of the LASRA enzyme treatment process.

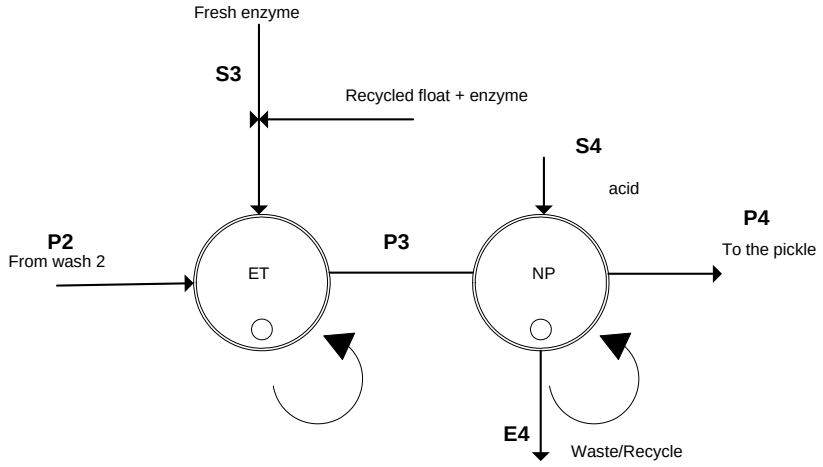


Figure 5.1: Schematic diagram showing zone 2 of the LASRA enzyme treatment process (configuration of trial runs 1 to 4)

In the previous chapter, alternative processes for the washing of depilated sheepskins to remove soluble and particulate materials were evaluated. This was achieved by understanding the process constraints and assumptions that could be made during the washing stages. From this, mass balances were developed for possible processing regimes. This allowed comparison of alternative configurations of the LASRA enzyme treatment process in terms of resource use (e.g. water) and process effectiveness. This same approach was adopted in the

optimization and improvement work of zone 2 of the LASRA enzyme treatment process.

For fellmongeries which do not have a tanning facillity, a pickling process operation is carried out. However, this was not considered as part of the make up of zone 2 (as seen in Figure 5.1), but rather as a latter stage process largely unchanged from conventional processing.

The two approaches to modifications of zone 2 of the LASRA enzyme treatment process that were considered in this work were; recycling of the enzyme treatment float prior to the neutralization process and in situ cleanup options of process float before it is recycled from a given point of zone 2. Implementation of these modifications to the existing system has the potential for savings in terms of enzyme, chemicals and freshwater use. For the purpose of constructing the forward mass balances to explore the various modifications, values from trial run 1 were used as this run was representative of standard LASRA enzyme treatment process trial runs prior to any optimization and improvement measures being carried out. Process run 5 which was used as the representative run in chapter 4 is now being used to investigate the implementation of a particular modification to zone 2, detailed in latter parts of this chapter.

In order to effectively assess each option, the next section of this chapter addresses the following issues;

1. Processing constraints and assumptions of zone 2 of the process.
2. The extent of enzyme activity at different parts of zone 2 (i.e. how much additional soluble material results from the enzyme treatment).
3. Factors which contribute to the change of activity in enzymes and the nature of impurities.

After exploration of these issues, various modification alternatives could be implemented and evaluated by forward mass balance in a similar fashion to that demonstrated in chapter 4.

5.1 Process Identification and Constraints

Forward mass balances are required to analyze possible modifications to the LASRA enzyme treatment process. This section focuses on laying out the various assumptions and process constraints (derived from an understanding of zone 2 of the LASRA enzyme treatment process) that can be used in the construction of the forward mass balance. Figure 5.2 below (a detailed version of Figure 5.1) shows the measured aspects of zone 2 of the LASRA enzyme treatment process.

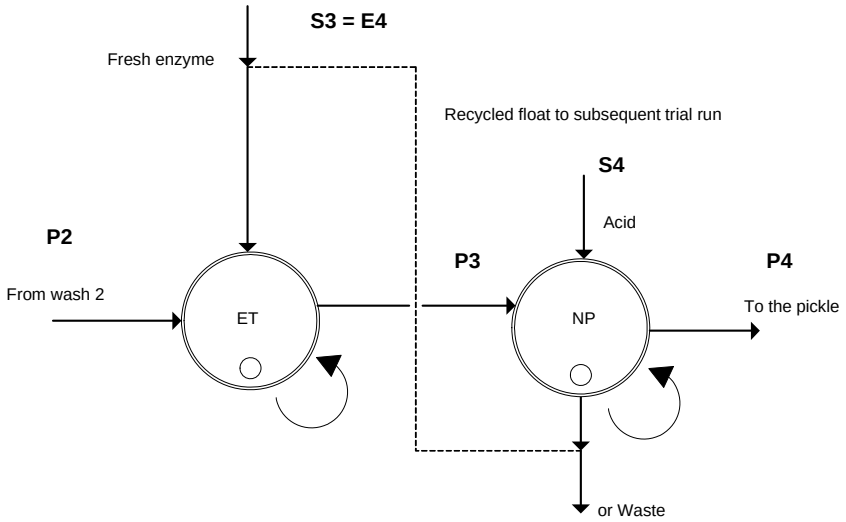


Figure 5.2: Schematic diagram showing zone 2 of the LASRA enzyme treatment process with measured process flow characteristics (trial runs 1 to 4)

For the construction of forward mass balances, only the inputs into zone 2 (stream P2) and solvent streams (S3 and S4) were specified. The characterization of stream P2 used values from a representative trial run while streams S3 and S4 were fixed to be on a 1:1 ratio with initial total mass of depilated sheepskins used in the process run. To simplify calculations, the flow characteristics of effluent and product streams were predicted through 'common values and ratios', drawn from results of LASRA pilot scale runs. The flow characteristics of the product stream were calculated by a total balance over a given process operation. Some of the assumptions that were used to predict the effluent stream characteristics of the effluent stream E4 may be seen below;

a) The percentage of insoluble solids (IS) inputs into the neutralization process at stream P3 that was found to be washable by nature is 0.22% (kg washable IS in neutralization stage/kg IS input into neutralization process at stream P3). Even if there was a recycle of IS from a prior run, this amount is very small. All IS discharged at the effluent stream of neutralization stage are of this type - washable by nature. This percentage value was averaged across trial runs 1 to 4.
b) The water content of product stream P4 was taken as the ratio of moisture relative to IS found in sheepskins at product stream P4. The water content of P4 was found to be 7.11 times the IS found in the sheepskins product of stream P4. This value was taken as an average of the values obtained from trial runs 1 to 4.
c) The amount of enzyme dissolved solids (EDS) generated was the average value calculated between runs 1 and 5 represented as a function of total solids of the same stream (product stream of enzyme treatment that is, stream P3). The value to be used in forward mass balance is 0.08 kg EDS/kg TS stream P2. Details on the way this was derived may be seen in chapter 3.
d) The float in both the enzyme treatment and neutralization stage of zone 2 is assumed to have achieved equilibrium by the time the process ends. This would mean that the washable insoluble solids and dissolved solids concentrations of the effluent and product streams of both process operations would be the same.
e) All total Kjeldahl nitrogen (TKN) found in effluent streams are liquor TKN (liquor TKN was defined previously in chapter 3).
f) The density of float was assumed to be the same as water (1000 kg/m^3).
g) The total Kjeldahl nitrogen (TKN) of stream E4 was taken as the percentage amount of total solids (TS) that were nitrogenous in nature. The value was found to be 48.1% (kg TKN in E4/kg TS in E4). This value was taken as an average of the values obtained from trial runs 1 to 4.
h) All EDS were assumed to be proteinaceous in nature.
i) Process trial 1 was considered to be a representative of all other LASRA enzyme treatment process pilot scale runs as these had similar

configurations. Values from this run were used in constructing forward mass balances of zone 2.

j) No other enzyme digestion occurs after the neutralization step. This means that all the enzyme dissolved solids were generated only in the 6 hours of the enzyme treatment process.

k) All enzyme dissolvable material stays dissolved throughout the whole process. This is despite the fact that it is possible that dissolved solids in the enzyme treatment step could precipitate due to the changing pH during neutralization stage.

5.1.1 Extent of enzyme activity

Although enzymes are catalysts and hence should not be depleted during a reaction, partial loss of enzyme activity can occur for a variety of reasons as given below (Allsop 2007, Perry *et al.* 2007);

a) Changes in the enzymes immediate environment (i.e. temperature and pH)
b) Auto-proteolytic activity of the enzyme
c) Deactivation through adsorption of enzyme onto insoluble particles and thermal de-naturation
d) Dilution of surrounding float
e) Loss through residual liquor associated with the skin going into product stream

For these reasons, partial loss of enzyme activity in the process float (E4) may occur should the enzyme be used repeatedly and over time, without a top-up of fresh enzyme at periodic intervals. The enzyme used by LASRA (Maxizyme SEM) was chosen over others as it had the highest activity in mildly alkaline (pH 8 to 10) conditions and low temperatures (~20^{o}C) (Allsop 2006).

To investigate this, the enzyme activity was monitored using enzyme activity assays with skin powder azure (SPA). Figure 5.3 below shows the changes in the activity of the proteolytic enzyme throughout zone 2 (including pickling operations in this instance). Enzyme activity values in Figure 5.3 below are recorded as relative to the original enzyme starting activity.

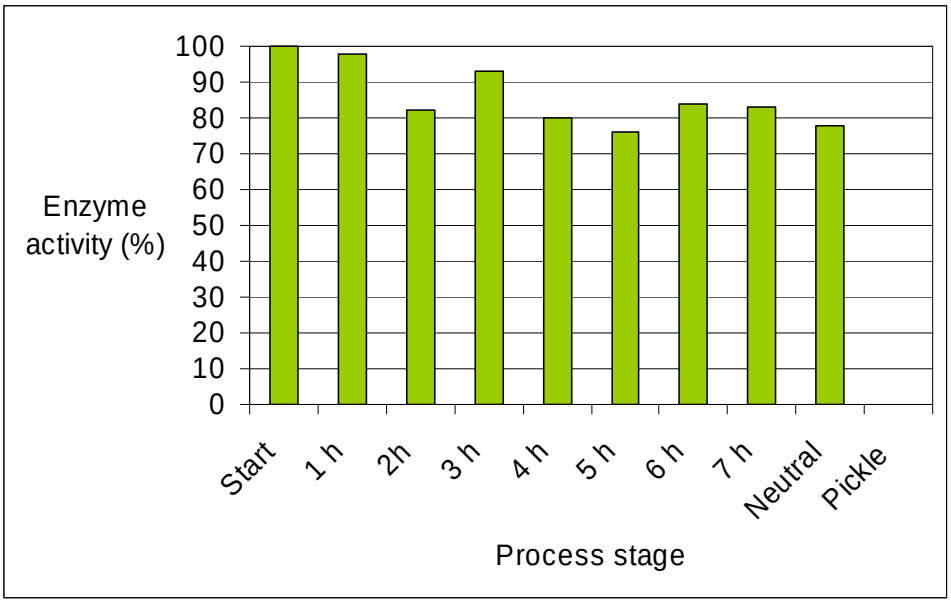


Figure 5.3: Graph showing the variation of relative enzyme activities across process operations of zone 2 and hours of enzyme treatment – taken from Allsop (2007). It has to be noted that these activities were corrected for the dilution of the float within neutralization process.

As no active temperature control had been used by Allsop (2007) in an effort to reduce the power consumed by the LASRA enzyme treatment process, any temperature fluctuations would explain the fluctuating activities of the enzyme activity as seen in Figure 5.3. However, the non-usage of temperature control is justified as part of standard operating procedures of the LASRA process by overall high levels of enzyme activity. The pH changes imposed between the enzyme treatment and neutralization process can also cause the enzyme activity fluctuations (note that the activity in the neutralized enzyme solution was corrected for the dilution affects of the added dilute sulphuric acid).

Given the processing conditions of the LASRA process and the nature of enzymes, this section shows that the enzyme used does maintain acceptable levels of activity throughout a single run. When a recycle of enzymatic float was done, enzyme activity levels were restored to original operating levels by addition of fresh enzyme.

5.1.2 Effect of carried over solids on enzyme treatment

Another possible cause for the loss of enzyme activity may be due to an increase in more readily available substrate through the recycling of float in subsequent batches where solubilised protein levels are likely to accumulate. With 'carry-over' of dissolved solids found in the recycled float of the previous process and even more solids coming in with the slats, the enzyme may encounter solids for which it has a higher affinity compared to those it is required to digest. Despite the fact that on each trial run, up to 50% enzyme was added to account for the dilution that occurred during neutralization, this may not be enough to counter-act the loss of enzyme efficiency due to the reasons stated above. An experiment was conducted to test this effect.

This was carried out by measuring the enzyme activity in the presence of controlled samples. The testing was conducted in a controlled environment (i.e. using enzyme in freshwater and a defined substrate in the form of skin powder azure – SPA). This was done in preference to the actual operating conditions of LASRA enzyme treatment process float to reduce the possibility of other currently unknown factors affecting the outcome of the experiments. Enzyme activity was measured from the rate of dye release from SPA after it was contacted with enzyme solution. SPA is powdered sheepskin that has been covalently bonded to an azure dye. As the proteins are hydrolyzed the dye is released. Step

by step preparation of the substrate (SPA) used in enzyme activity measurements may be seen in appendix 6 while the preparation of the enzyme assay is outlined in appendix 5.

The control experiment involved the measurement of enzyme activity over time using just a specific amount of SPA of 4.5 mg SPA/ml solution. Test samples were evaluated for enzyme activity similarly to the control, with the addition of un-dyed skin protein (to simulate the presence of insoluble solids in general other than intended substrate) or bovine serum albumin (BSA - to simulate presence of dissolved protein solids). Should the enzyme activity be significantly less than that of the control, it can then be shown that the enzyme treatment of the LASRA enzyme treatment process may be made less efficient with the accumulation of solids due to recycling the process floats

To simulate the conditions in the actual pre-tanning operations as close as possible, the un-dyed substrate was added in similar concentrations to that in the recycled float added to enzyme treatment stage of zone 2 (approximately 10% extra solids coming in through the recycle stream from the preceding trial run) as well as a spectrum of varying concentrations. The results of the experimental work may be seen in Figure 5.4a & b below;

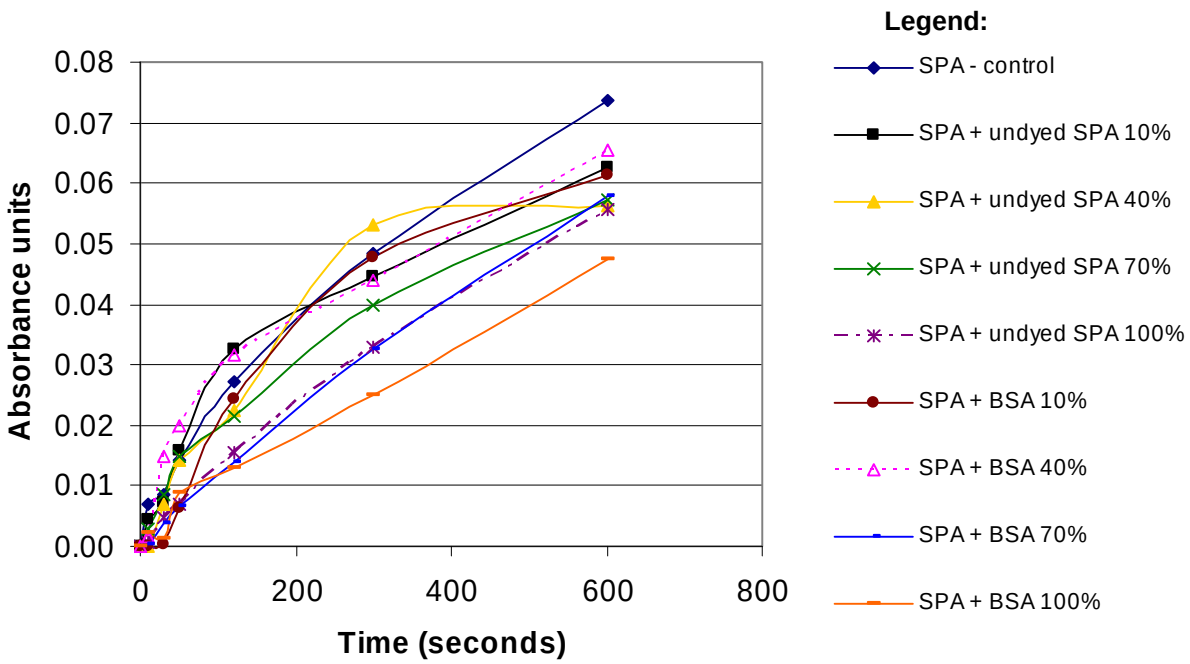


Figure 5.4a: Results of the enzyme assay experiment. Addition of representative total and dissolved solids were as per kg of SPA added.

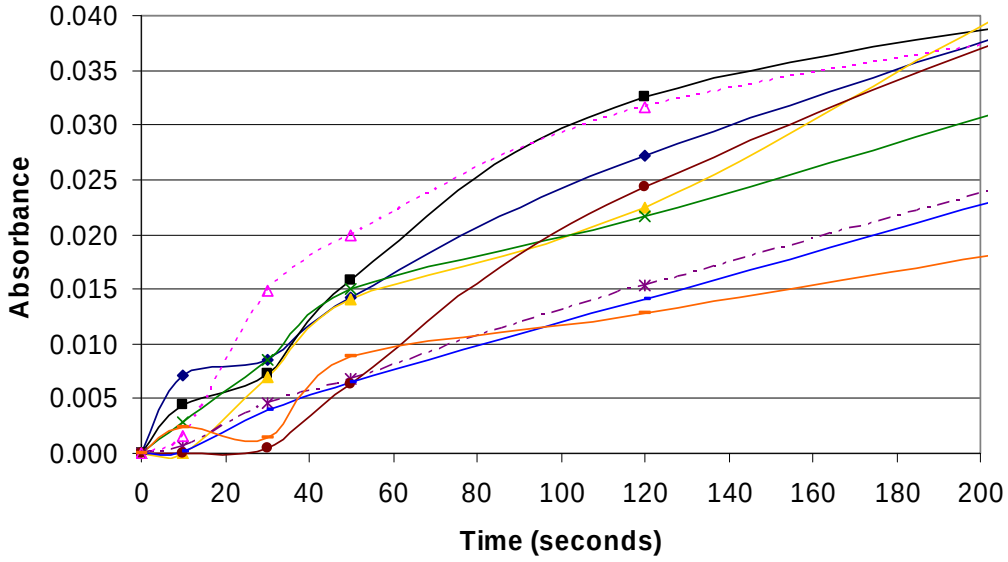


Figure 5.4b: Magnified region of Figure 5.4a between the times of 0 to 200 seconds.

Figure 5.4a & b shows no significant decline of enzyme activity overall with the addition of representative total and dissolved solids (un-dyed skin protein and BSA respectively) at varying concentrations. Even when comparing the control enzyme assay

activity with the enzyme assay experiment which yielded the lowest activity (with 100% representative dissolved solids added), there was not a marked decline.

By calculating the initial slopes of plots representing their respective experimental run, the enzyme activities of each run may be obtained. The enzyme activity of the control experiment was found to be 1.19×10^{-4} (unit absorbance - abs per second) while the enzyme activity of the assay which had a 100% addition of BSA was 7.71×10^{-5} (abs/second). The solids input through recycling of float add only 10 – 15% of total solids in the enzyme treatment process. As such, the loss of activity incurred is negligible. The reason for this is likely due to enzyme being present in excess quantities from the beginning, in the enzyme treatment process of the first trial run in a block of trial runs. Furthermore, with up to 50% of the original enzyme added as top up fresh enzyme in subsequent trial runs of the LASRA enzyme treatment process, this would be more than enough to replace the loss of activity.

The investigative work shows that carried over solids have a negligible effect on enzyme activities in the event of recycled float being used at zone 2. The results also suggest that it may be possible to reduce the enzyme concentrations in the process without compromising processing time or performance. Further investigations on this should be carried out but were outside the scope of this project.

5.1.3 Temperature and pH conditions of zone 2 of LASRA enzyme treatment process

The enzyme used was a variant of the proteolytic enzymes used in washing detergents. This enzyme has suggested operating conditions of moderate alkalinity pH (between pH 8 and 9) and in warm temperatures (LSBU 2009). With the enzyme treatment process having an operating pH between 10.5 and 12.5, pH conditions of float are able to maintain sufficient enzyme activity despite being less then ideal for optimum activity. Washed slats in float with traces of sulphide are passed into the enzyme treatment process at a pH between 11.5 and 13.5. This residual alkalinity is able to maintain the high pH in the enzyme step even after the addition of the neutralized recycled enzymes from a previous trial run. Dilution of the highly alkaline float using the enzyme solution was also not enough to bring down the enzyme treatment float to the desired pH range. However, implementation of pH control measures may result in un-necessary cost and therefore should be thoroughly researched to balance their benefits with the potential costs (this study was not covered in the work done).

Due to the ability of the enzyme to function in low temperatures (~20^{o}C), temperature control was only used in winter at the enzyme treatment stage of the process. This contributed to a significant reduction of energy usage (which would translate to savings in operating costs) of the LASRA process over conventional processing as highlighted in chapter 2. Due to the nature of the enzyme used it may be concluded that the LASRA enzyme treatment process is currently run at near optimum pH but at less than an ideal temperature (as enzymes generally have higher activities at higher temperatures) conditions.

5.1.4 Enzyme de-activation options

Following the enzyme treatment process operation, there is a need to de-activate the enzyme before the slats are progressed into the tanning operations (or pickled) to ensure that no damage to the slats occurs. This may be done efficiently by altering the surrounding pH or temperature of the enzyme. With the LASRA enzyme treatment process, this was done by the addition of dilute acid to alter the pH of the float. This method is more suitable as temperature inactivation by the addition or removal of heat would increase operating costs. Heating would also potentially damage the skins due to collagen denaturation and if the enzyme is largely inactivated the potential for recycling of the enzyme is no longer viable.

5.1.5 Float recycling system at zone 2 of LASRA enzyme treatment process

Another feature of the LASRA enzyme treatment process is that a recycling system is used. The float of the neutralization stage was recycled into the enzyme treatment stage of the following trial run. The recycle of float into subsequent trial runs was done only up to 3 runs which brings the total number of trial runs in a single block of runs to 4. After 4 recycles of float, the sheep pelts post neutralization process began to have visible traces of surface dirt. Although the surface dirt on the pelts may be washed off by an additional washing step before the pelts are progressed into tanning operations, the loss of aesthetically pleasing features of the pelt may be detrimental to its market value (sheepskin at various stages of tanning operations are inspected routinely). The occurrence of surface dirt on the pelts may be due to solids

accumulation that occurs as a result of the recycling of float from the previous trial runs.

Figure 5.5 shows the insoluble solid contents of floats across all processes of pre-tanning operations including the solids levels at each hour of enzyme treatment. Data was taken from the results of trial runs 1 to 4 which were processed using the standard LASRA enzyme treatment process configuration and values were normalized with respect to the original depilated sheepskins input mass.

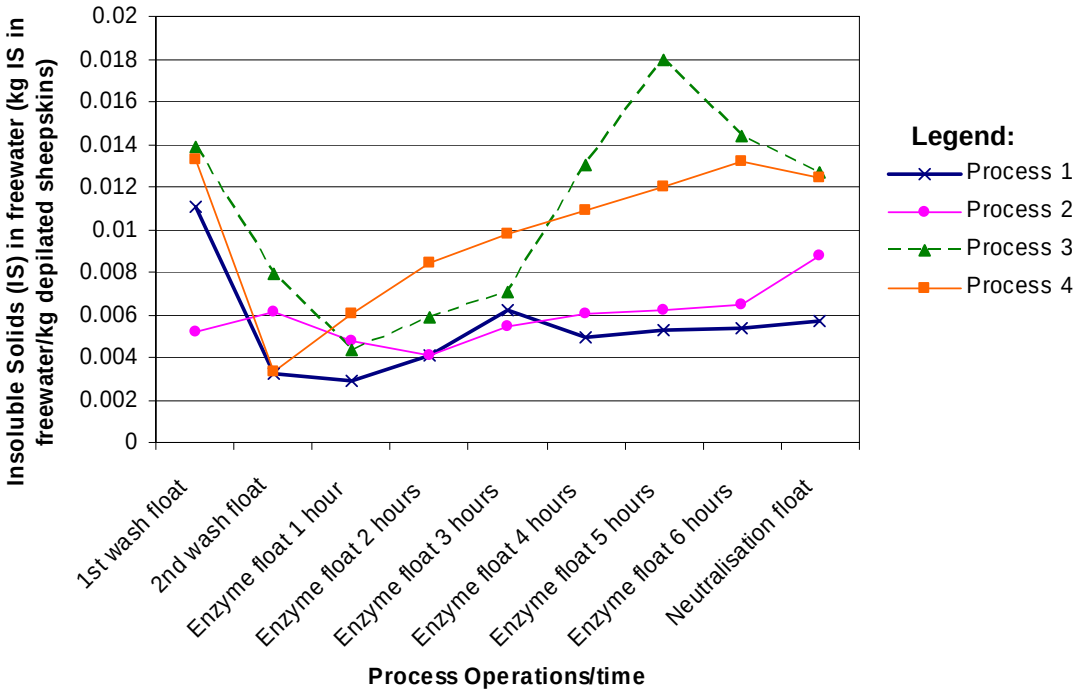


Figure 5.5: Degree of insoluble solids (IS) accumulation over pre-tanning process operations and enzyme treatment time (process 1 - 4) - normalized with original sheepskin mass

From Figure 5.5, it can be seen that there is a certain degree of solids accumulation occurring in the process floats across individual trial runs in recycled floats. To reduce the risk of the decline in the commercial value of the pelts and to avoid additional use of water to wash the 'dirty' pelts, an improvement to the enzyme treatment could be possible. Removal of insoluble solids

by centrifugation or filtration or an additional washing stage could be adopted.

5.2 Development of Forward Mass Balances

Figure 5.6 shows zone 2 of the LASRA enzyme treatment process. The equations following Figure 5.6 demonstrate the way in which each stream was calculated from given inputs of stream P2 and S3. The method in which effluent stream E3 (normally not present hence drawn faintly on Figure 5.6) was calculated is also shown (to allow the consideration of the modification of drainage of float before neutralization stage to be detailed in latter sections of the chapter).

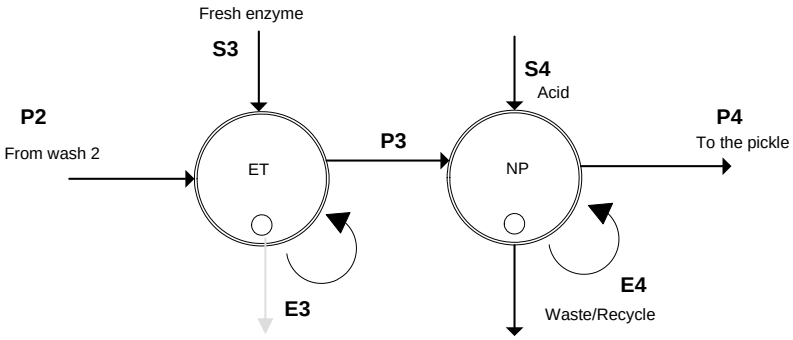


Figure 5.6: Zone 2 of the LASRA enzyme treatment process with stream labels. As only a straight run will be considered, there is only fresh enzyme input into the enzyme treatment process. Stream E3 is drawn faintly as it is not part of standard zone 2 but will be considered as part of modification work in latter parts of this chapter

Stream P2 and S3 system inputs were defined with the following flow parameters which will were defined or calculated from zone 1 calculations outlined in the previous chapter.;

M_{IS} (C_{IS}), M_{TDS} (C_{TDS}), M_{TS} (C_{TS}), M_W (C_W), M_T (C_T), M_{TKN} (C_{TKN})

Stream E3 (all the following values will be zero if effluent stream 3 was not used or considered)

The first flow parameter to be derived is the amount of moisture found in stream E3. This may be done by multiplying the value 7.11 (which is ratio of moisture to IS at stream P3) with the amount of IS in ET to obtain the moisture content of stream P3. The amount of moisture in E3 is obtained by subtracting the amount of moisture of P3 from the total amount of water.

(Equation 5.1)

$$M_W^{P3} = 7.11 \text{ x } (M_{IS}^{P2} + M_{IS}^{S3} - M_{EDS})$$

$$M_W^{E3} = M_W^{P2} + M_W^{S3} - M_W^{P3} \quad \textbf{(Equation 5.2)}$$

The derivation washable insoluble solids (IS) mass was done by multiplying the percentage of washable IS (0.22%) with the total amount of IS found in ET (combined IS of stream P2 and S3). By the assumption that the concentration of washable IS at stream E3 was the same as in the free water component of P3, the mass of washable IS may be divided according to the composition of water at each stream (E3 and P3).

$$M_{IS}^{E3} \text{ x } (M_W^{P3} + M_W^{E3}) = [0.22\% \text{ x } (M_{IS}^{P2} + M_{IS}^{S3})] \text{ x } M_W^{E3} \quad \textbf{(Equation 5.3)}$$

$$M_{IS}^{E3} = \frac{\left[0.22\% \times \left(M_{IS}^{P2} + M_{IS}^{S3}\right)\right] \times M_W^{E3}}{\left(M_W^{E3} + M_W^{P3}\right)}$$

The quantity of TDS discharged at E3 was found by the assumption that the concentration of TDS at stream E3 was the same as in the water component of P3 and thus may be divided accordingly based on the composition of water at each stream.

$$M_{TDS}^{E3} \text{ x } (M_W^{E3} + M_W^{P3}) = (M_{TDS}^{P2} + M_{TDS}^{S3}) \text{ x } M_W^{E3} \quad \textbf{(Equation 5.4)}$$

$$M_{TDS}^{E3} = \frac{\left(M_{TDS}^{P2} + M_{TDS}^{S3}\right) \times M_W^{E3}}{\left(M_W^{E3} + M_W^{P3}\right)}$$

In a similar fashion, the mass of EDS generated at ET ($*M_{EDS\text{-}E3}$) may be divided between stream E3 and P3 as was done with TDS.

$$*M_{EDS}^{E3} = \frac{0.08 \times M_{TS}^{P2} \times M_W^{E3}}{\left(M_W^{E3} + M_W^{P3}\right)} \quad \textbf{(Equation 5.5)}$$

$*M_{EDS}^{E3}$ was generated as a result of M_{IS}^{E3} being converted to M_{TDS}^{E3} or also known as M_{EDS}^{E3}. 0.08 kg EDS/kg TS stream P2 was calculated in chapter 3 (sub-section

3.4.4.1) as the amount of enzyme dissolved solids (EDS) that may be used in forward mass balances

The TS found in this stream (E3) may then be calculated by the summation of IS, TDS and EDS values.

$$M_{TS}^{E3} = M_{IS}^{E3} + M_{TDS}^{E3} + M_{EDS}^{E3} \quad \textbf{(Equation 5.6)}$$

The total flow (T) of E3 is calculated by the summation of TS and water content of E3.

$$M_{T}^{E3} = M_{TS}^{E3} + M_{W}^{E3} \quad \textbf{(Equation 5.7)}$$

Stream P3

The water content of stream P3 was calculated by equation 5.1 while the amount of IS found in P3 may be calculated by performing an IS balance around ET taking into account the IS that had been converted to EDS.

$$M_{IS}^{P3} = M_{IS}^{P2} + M_{IS}^{S3} - M_{EDS}^{P3} - M_{EDS}^{E3} - M_{IS}^{E3} \quad \textbf{(Equation 5.8)}$$

The amount of TDS was calculated by a TDS balance around ET.

$$M_{TDS}^{P3} = M_{TDS}^{P2} + M_{TDS}^{S3} - M_{TDS}^{E3} \quad \textbf{(Equation 5.9)}$$

The amount of EDS found in this stream may be obtained by subtracting the amount of EDS in E3 from the total amount of EDS generated in ET.

$$M_{EDS}^{P3} = (0.08 \text{ x } M_{TS}^{P2} + M_{TS}^{S3}) - M_{EDS}^{E3} \quad \textbf{(Equation 5.10)}$$

The amount of TS may be calculated by the summation of IS, TDS and EDS values.

$$M_{TS}^{P3} = M_{IS}^{P3} + M_{TDS}^{P3} + M_{EDS}^{P3}$$ **(Equation 5.11)**

The total flow (T) of E3 is calculated by the summation of TS and water content of E3.

$$M_{T}^{P3} = M_{TS}^{P3} + M_{W}^{P3}$$ **(Equation 5.12)**

* If stream E3 was not used or considered $M_{X}^{E3} = 0$

Stream E4

The first flow parameter to be derived is the amount of moisture found in stream E4. This may be done by multiplying the value 7.11 (which is ratio of moisture to IS at stream P4) with the amount of IS in ET to obtain the moisture content of stream P4. Amount of moisture in E4 is obtained by subtracting the amount of moisture of P4 from the total amount of water.

$$M_{W}^{P4} = 7.11 \text{ x } M_{IS}^{P3}$$ **(Equation 5.13)**

$$M_{W}^{E4} = M_{W}^{P3} + M_{W}^{S4} - M_{W}^{P4}$$ **(Equation 5.14)**

The derivation of washable IS mass was done by multiplying the percentage of washable IS (0.22%) with the total amount of IS found in ET (combined mass of IS in stream P3 and S4). By the assumption that the concentration of IS at stream E4 was the same as P4, the amount of IS made washable by enzymatic action at ET may be divided according to the composition of water at each stream (E4 and P4).

$$M_{IS}^{E4} \text{ x } (M_{W}^{P4} + M_{W}^{E4}) = [0.22\% \text{ x } (M_{IS}^{P3} + M_{IS}^{S4})] \text{ x } M_{W}^{E4}$$ **(Equation 5.15)**

$$M_{IS}^{E4} = \frac{\left[0.22\% \times \left(\mathrm{M}_{\mathrm{IS}}^{\mathrm{P3}} + M_{IS}^{S4}\right)\right] \times M_{W}^{E4}}{\left(M_{W}^{E4} + M_{W}^{P4}\right)}$$

As EDS are essentially TDS, these were calculated together with TDS at the neutralization process. The quantity of TDS discharged at E4 was found by the assumption that the concentration of TDS at stream E4 was the same as P4 and thus may be divided accordingly based on the composition of water at each stream.

$$M_{TDS}^{E4} \text{ x } (M_W^{E4} + M_W^{P4}) = (M_{TDS}^{P3} + M_{TDS}^{S4}) \text{ x } M_W^{E4} \quad \textbf{(Equation 5.16)}$$

$$M_{TDS}^{E4} = \frac{\left(M_{TDS}^{P3} + M_{TDS}^{S4}\right) \times M_W^{E4}}{\left(M_W^{E4} + M_W^{P4}\right)}$$

The amount of TS and T at E4 may then be calculated by the summation of its respective components.

$$M_{TS}^{E4} = M_{IS}^{E4} + M_{TDS}^{E4} \quad \textbf{(Equation 5.17)}$$

$$M_T^{E4} = M_{TS}^{E4} + M_W^{E4} \quad \textbf{(Equation 5.18)}$$

Stream P4

The water content of stream P4 was calculated by equation 5.13 while the amount of IS found in P4 may be calculated by performing an IS balance around the neutralization process.

$$M_{IS}^{P4} = M_{IS}^{P3} + M_{IS}^{S4} - M_{IS}^{E4} \quad \textbf{(Equation 5.19)}$$

The amount of TDS may be calculated by a TDS balance around the neutralization process.

$$M_{TDS}^{P4} = M_{TDS}^{P3} + M_{TDS}^{S4} - M_{TDS}^{E4} \quad \textbf{(Equation 5.20)}$$

The amount of TS and T at P4 may then be calculated by the summation of its respective components.

$$M_T^{P4} = M_{IS}^{P4} + M_{TDS}^{P4}$$ **(Equation 5.21)**

$$M_T^{P4} = M_{TS}^{P4} + M_W^{P4}$$ **(Equation 5.22)**

5.3 Alternative Processes for Zone 2 of LASRA Enzyme Treatment Process

The previous section of 5.2 focused on outlining various aspects of zone 2 of the LASRA enzyme treatment process such as the various assumptions that have been made in order to have a forward mass balance constructed, constraints and boundaries for possible modification work of the available system. Using the information found, this section explores some alternatives for modification of zone 2 of the LASRA enzyme treatment process.

5.3.1 Coarse filtration of neutralization float

With coarse filtration of neutralization float, most of the larger particles such as wool may be removed from the float before it is recycled to the following trial run. Despite that, most of the smaller solid particles would remain in the float. A particle size distribution was conducted on 10% concentration of float samples from the enzyme treatment process (although the float to be filtered was to be of the neutralization process, it is reasonable to assume that the float of enzyme treatment had similar compositions). This was done using the Malvern Mastersizer S (Malvern Instruments UK) using the 300RF lens (size range between 0.05 - 878.7μm), with the 30HD presentation on a polydispersed model. The results may be seen in the plot as below (the full methodology on the way in which the particle size distribution test was done may be seen in appendix 7);

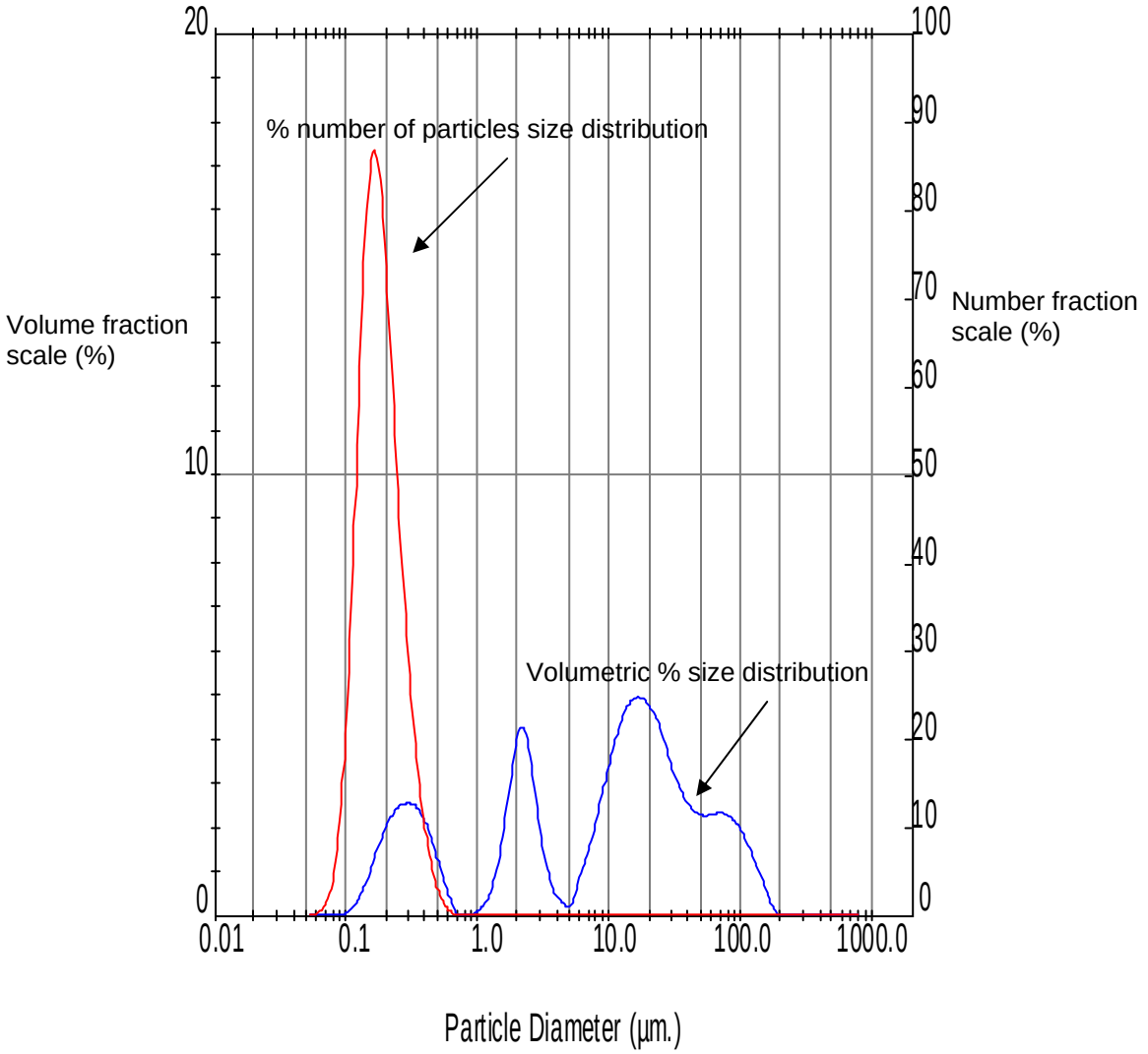


Figure 5.7: Plot of particle size distribution of solids in a sample enzyme treatment float represented as a volumetric percentage size distribution and as percentage number of particles size distribution.

From Figure 5.7 it can be seen that there is a large quantity of small particles of approximately 0.1 to 0.5 µm in diameter and an almost negligible amount of large particles of approximately 10 to 100 µm in diameter in the enzyme treatment float. One method of recovering these suspended (insoluble) particles would be to use a fine filter which has a particle size cut-off of about 0.1 µm to remove most of the solids in the enzyme treatment float before it is recycled to the following trial run. Ulrich (1984) gives a few possible techniques to remove these fine particles such as the use

of centrifuges (helical conveyor or also known as solid bowl centrifuge) or process filters such as the plate and frame filter press.

A coarse filtration technique could be used to remove the small quantity of large particles (based on the particle size distribution results as seen in Figure 5.7). A filter which has a size cut-off of between 1 to 10 μm may be used in this instance. Ulrich (1984) suggests filtration techniques which could facilitate solids separation of the indicated size region includes bag and cartridge filters. However, the selection of filters to be used in filtering pre-tanning operations effluent float is beyond the scope of this work.

Should the results of the particle size distribution (PSD) test be taken into account and used (i.e. a coarse filtration technique implemented to remove larger particles leaving those found by the PSD test) this will produce a cleaner float. At best, this modification would be able to slow the process of solids accumulation and occurrence of 'dirty pelts'. With that, more recycle runs may be able to be done before the resulting pelts appear with significant surface dirt. A schematic diagram showing zone 2 of the LASRA enzyme treatment process with this modification is shown in Figure 5.8 below.

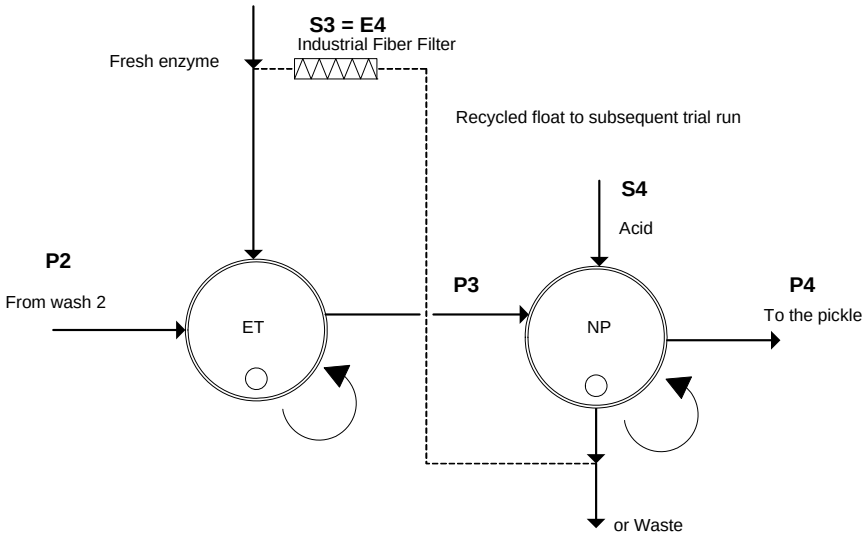


Figure 5.8: Schematic diagram showing zone 2 of the LASRA enzyme treatment process with measured process flow characteristics (trial runs 5 to 8)

For the construction of the forward mass balance for this modification, the following assumptions were used (relating to the behaviour of filtered float before its recycle) in addition to the prior highlighted assumptions stated in section 5.2. The filter used here was a coarse industrial fibre filter (with an approximate size cut-off between 1 and 50 μm). This would mean that a lot of the fine solids are retained in the filtrate and recycled into following trial runs (values are an average of the values taken from trial runs 5 to 8 on a volumetric fraction basis).

1) The average amount of float (solids and water content combined) remaining after filtration through an industrial fibre filter is 99.7% (kg float in filtrate/kg float in neutralization process).
2) The average amount of insoluble solids (IS) remaining after filtration through an industrial fibre filter is 50.0% (kg IS in filtrate/kg IS in neutralization process).

3) The average amount of total Kjeldahl nitrogen (TKN) remaining after filtration through an industrial fibre filter is 95.6% (kg TKN in filtrate/kg TKN in neutralization process).
4) All moisture passes through the industrial fibre filter.

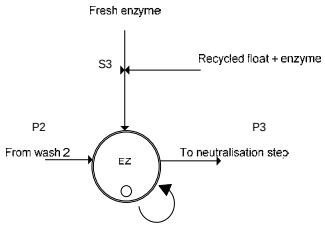
5.3.1.1 Mass balance analysis of coarse filtration of neutralization float

P2

		Mass (kg)	C (%)
Solids	IS	2.41	16.46%
	DS	0.07	0.51%
Total solids (kg)		2.48	16.97%
Water		12.14	83.03%
Total		14.62	100.00%
TKN	Total	0.53	3.62%

S3

		Mass (kg)	C (%)
Solids	IS	0.01	0.06%
	DS	0.01	0.06%
Total solids (kg)		0.02	0.12%
Water		12.63	99.88%
Total		12.65	100.00%
TKN	Total	0.00	0.00%

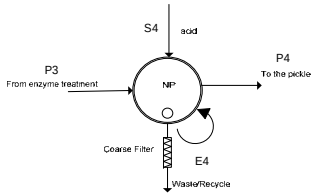


P3

		Mass (kg)	C (%)
Solids	IS	2.20	8.08%
	DS	0.08	0.30%
	EDS	0.21	0.77%
Total solids (kg)		2.50	9.15%
Water		24.78	90.85%
Total		27.27	100.00%
TKN	Total from	0.53	1.94%

S4

		Mass (kg)	C (%)
Solids	IS	0.00	0.00%
	DS	0.25	2.00%
Total solids (kg)		0.25	2.00%
Water		12.40	98.00%
Total		12.65	100.00%
TKN	Total	0.00	100.00%



E4

		Mass (kg)	C (%)	Filtered
Solids	IS	0.003	0.01%	0.001
	DS	0.31	1.44%	0.25
Total solids (kg)		0.32	1.46%	0.25
Water		21.50	98.54%	21.50
Total		21.81	100.00%	21.75
TKN	Total	0.15	0.70%	0.15

P4

		Mass (kg)	C (%)
Solids	IS	2.202	12.16%
	DS	0.23	1.27%
Total solids (kg)		2.43	13.43%
Water		15.68	86.57%
Total		18.11	100.00%
TKN	Total from	0.38	2.08%

Figure 5.9: Schematic diagram showing zone 2 of the LASRA enzyme treatment process with measured process flow characteristics (trial runs 5 to 8). The EDS value of stream E4 and P4 onwards have been combined with TDS values. The coarse filter was applied at the effluent stream E4.

The forward mass balance of zone 2 (figure 5.9) was derived in a similar fashion as described in section 5.2 with the exception of effluent stream E4. The assumptions on the functionality of the industrial fibre filter as described prior to this were applied to effluent stream E4 flow parameters as described below;

Filtered Stream E4 (equations referred to in this sub-section can be found in section 5.2).

For stream E4, the water content may be calculated by equations 5.13 and 5.14. The mass of IS in E4, was calculated as seen with equation 5.15. As highlighted before, it was assumed that the industrial fibre filter will be able to retain 50% of IS on a volume basis to obtain the filtered mass of IS in E4 (as it will be able to trap most of the large IS). With that, the mass of IS in filtered E4 may be derived by equation 5.23.

$$M_{IS}^{E4}(\text{filtered}) = M_{IS}^{E4} \text{ x } 50\% \qquad \textbf{(Equation 5.23)}$$

The mass of TS may be calculated as seen with equation 5.17 before while it's filtered version is calculated by equation 5.24 as seen below.

$$M_{TS}^{E4}(\text{filtered}) = M_{T}^{E4}(\text{filtered}) - M_{W}^{E4}(\text{filtered}) \qquad \textbf{(Equation 5.24)}$$

The mass TDS (inclusive of EDS) in E4 can then be calculated as seen with equation 5.16 while a filtered version may be calculated by equation 5.25.

$$M_{TDS}^{E4}(\text{filtered}) = M_{TS}^{E4}(\text{filtered}) - M_{IS}^{E4}(\text{filtered}) \qquad \textbf{(Equation 5.25)}$$

With total flow (T) being calculated as seen with equation 5.18, it was then assumed that 99.7% of the total flow of E4 will pass through the filter (i.e. only 0.3% of E4 will be retained by the

filter). With that, the filtered version is then calculated by equation 5.26

$$M_T^{E4}(\text{filtered}) = M_T^{E4} \times 99.7\% \qquad \textbf{(Equation 5.26)}$$

Figure 5.10 shows the comparison between the standard configurations of zone 2 compared with the modification to zone 2 explored in this sub-section using the mass balance. The concentration of IS in the free water fraction of stream E4 was used to evaluate the two systems. This form of evaluation was chosen as the effectiveness of a filtration method is dependent on its ability to filter out insoluble solid material.

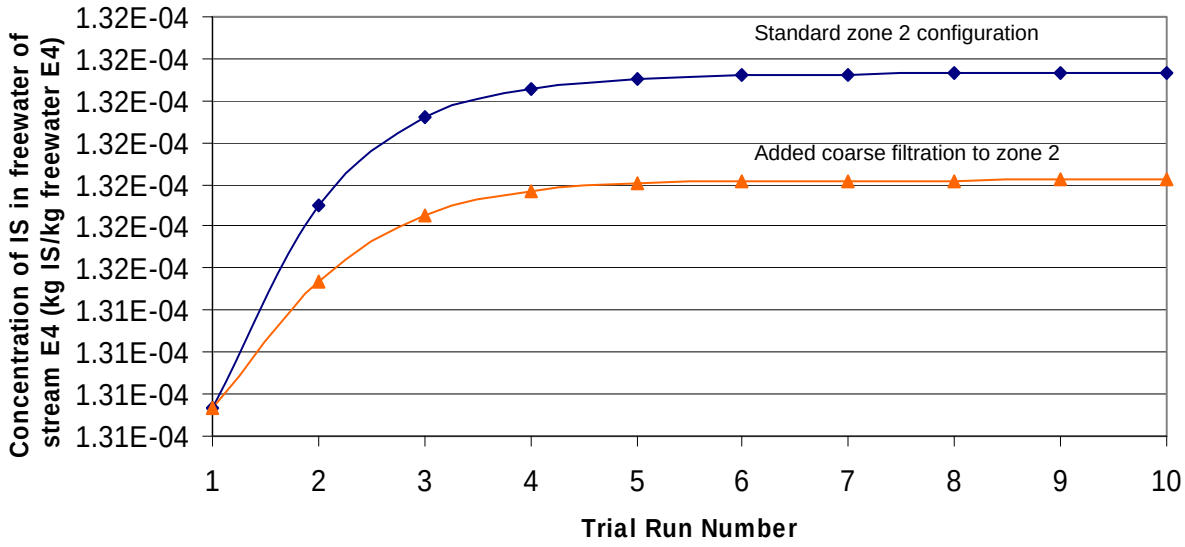


Figure 5.10: Graph showing the comparison between the IS accumulation in float of the standard zone 2 configurations and the coarse filtration modification to zone 2 as predicted by the mass balances.

Looking at Figure 5.10, it can be seen that there is a minute reduction in the concentration of IS in the free water fraction of stream E4. This indicates that a coarse filtration method may not be effective enough to be justified as a viable modification to zone 2 of the LASRA process. For more effective filtration of float, a

more elaborate filtration technique is needed. One such technique is highlighted in the following sub-section.

5.3.2 Microfiltration of neutralization float

Cassano *et al.* (2000 & 2001) conducted trials that showed the feasibility of using a microfiltration technique such as ultrafiltration to filter out all the unwanted macro and micro particles from process floats. In theory, this technique will succeed in the separation of the bulk of un-wanted macro and micro particles found in the process float from the enzyme process. This will in effect drastically lower the occurrence of 'dirty pelts' which usually occurs as a result of solids accumulating in the process float. To achieve that, a suitable membrane with the right cut-off size had to be chosen to perform this separation.

To do this, the molecular weight range of proteins in the float and the enzyme needed to be determined. This was done by Edmonds (2008a) using an SDS-PAGE (gel electrophoresis) technique at various buffer concentrations and conditions. The results of the experimental work are shown in Figure 5.11. For the trial run which resulted in Figure 5.11 seen below, a gradient gel of 4-14% Polyacrylamide (PA) was used (detailed methodology on the way this was prepared may be seen in appendix 8). Recycled float samples (500 µL) were first centrifuged at 14100 G for 5 minutes. Following that 200 µL of clear supernatant was desalted using chloroform methanol. The pellet was washed with water and freeze dried to remove traces of methanol before it was made up in 20 µL sample buffer. Samples were boiled, cooled and centrifuged for 5 minutes before it was placed on the lanes (10 µL portions). The samples were diluted to a few different dilution levels for clarity of

results. Table 5.1 shows the contents of each lane of the gel of figure 5.11.

Table 5.1: Lane contents of the gel electrophoresis (SDS-PAGE) of figure 5.11

Lane	Content of gel of Figure 5.11
1	Recycle float (cycle 1) 1:2 dil.
2	Recycle float (cycle 1) 1:4 dil.
3	Recycle float (cycle 1) 1:8 dil.
4	Recycle float (cycle 1) 1:16 dil.
5	Recycle float (cycle 2) 1:2 dil.
6	Recycle float (cycle 2) 1:4 dil.
7	Recycle float (cycle 2) 1:8 dil.
8	Recycle float (cycle 2) 1:16 dil.
9	Protein Marker
10	Pure enzyme solution

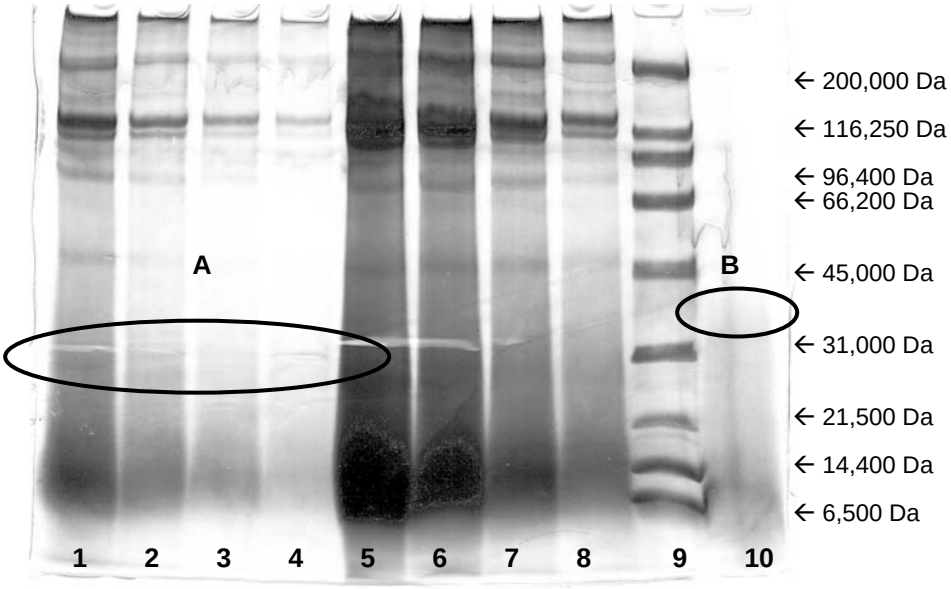


Figure 5.11: Shows an SDS-PAGE analysis of process float with lane numbers stated along with the size indicators of the marker proteins.

Looking at Figure 5.11 the largest molecular weight band on lane 10 was 31,000 Daltons (marked B). A faint band was also found in the lanes of float sample of the first cycle (marked A). This suggests the presence of enzymes which have a molecular weight of approximately 31,000 Daltons as there can only be enzyme particles found in the pure enzyme solution. With that, should a

membrane filtration process be adopted a filter with a slightly larger than 31,000 Daltons cut-off may be trialled.

5.3.2.1 Mass balance analysis of microfiltration of neutralization float

The forward mass balance for the study of microfiltration the neutralization float may be constructed in a similar way as was done with the forward mass balance for the coarse filtration study. The difference between this study and for coarse filtration is the characteristics of the filter used. Given the nature of the use of a microfiltration technique, solids retention of up to 60% of suspended (insoluble) solids may be feasibly attained. As for dissolved solids, Vinovations (2009) report being able to retain 25% of retentate concentration using reverse osmosis with a particle size cut-off of approximately 1000 Daltons. To increase the processing speed, a compromise of solids retention of about 40% of the retentate concentration may be sufficient and this was applied to the forward mass balance.

Figure 5.12 below compares the standard configuration of zone 2 with the system which has microfiltration of the neutralization float using the mass balances. A marked improvement can be seen in the reduction of solids in the effluent stream of E4.

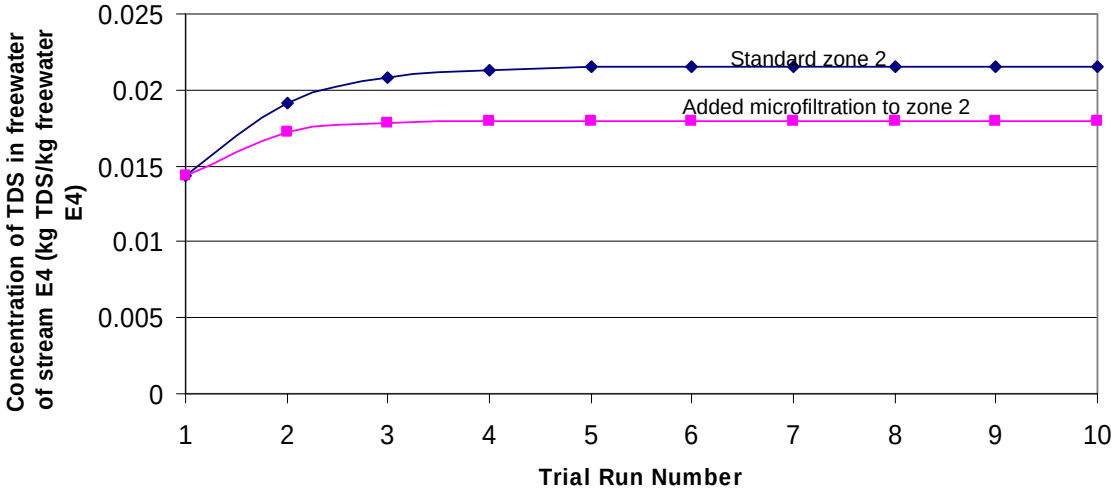


Figure 5.12: Graph showing the comparison between the TDS accumulation in float of the standard zone 2 configurations and the microfiltration modification to zone 2.

To ensure that the desired concentration is achieved, a cross-flow configuration as seen in Figure 5.13 would have to be employed. To achieve their retentate concentration of 25%, Vinovations (2009) reported the usage of a similar cross-flow system.

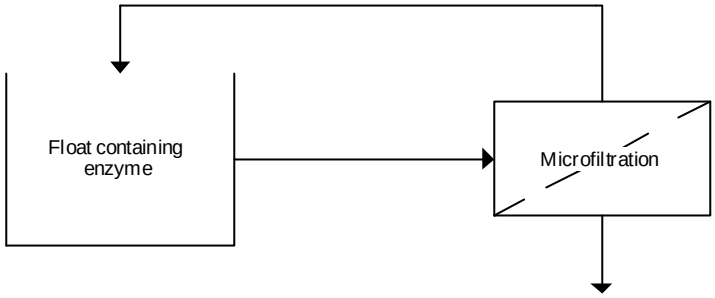


Figure 5.13: Cross-flow filtration system that could be used to achieve optimum microfiltration results.

However, some disadvantages of this modification include the added processing time needed in order to retrieve as much enzyme as possible. To achieve the desired retentate (containing recovered enzyme) concentration, multiple runs will be needed through the cross-flow configuration seen in Figure 5.13 to optimize the enzyme recovery. This will add to total processing time. In addition because the enzyme will pass through the membrane (its size being smaller than the molecular weight cut off) its concentration

will be constant throughout the float in the cross-flow system and will result in unwanted enzyme wastage. The fact that 40% retentate remains after filtration (in the compromised scenario) means that up to 40% of enzyme will be discarded. This could compromises the original intention of this modification to save as much enzyme as possible (even in the best case scenario as described in literature, up to a quarter of enzymes will be discarded). To further increase enzyme retention would be to further increase the processing time and cut back on original time and energy savings in this area as detailed in chapter 3.

Based on the results of the forward mass balance study, the option of a microfiltration recovery system of enzyme for zone 2 was not considered any further. The minimal savings of enzyme achieved and the potential of processing time savings lost through microfiltration does not justify this modification to zone 2.

5.3.3 Float separation before neutralization stage

One of the key problems with the standard configuration of zone 2 is the dilution of the enzymes that occurs during the neutralization process. To remedy this, an investigation was made into a modification that first separates the float of the enzyme treatment stage before filtration and recycling to the enzyme treatment stage of the following trial run. Through this configuration, any build-up of surface dirt on sheep pelts as a result of using recycled float will be removed through the neutralization stage (which now acts as an extra washing stage although its primary function was to halt enzyme activity). In theory, this modification should be able to achieve the following;

a) Reduce loss of enzyme activity as the enzyme is now recycled before dilution and being subjected to a drop of pH through the neutralization stage. With that, less enzyme top-up is needed in following trial runs.
b) Reduced use of acid to deactivate the enzyme still remaining on slats as with the drained skins, the pH buffering capacity will be lowered.

This modification to zone 2 would be able to achieve its optimal results if the float of the enzyme treatment process is drained fully and the neutralization process conducted in the presence of as little moisture as possible (i.e. concentrated acid is used to neutralize the remaining enzyme found on the slats which only contains entrained water surrounding it). This would mean that an optimal amount of acid may be used and freshwater use reduced. However, this sudden pH change to the pelts may cause undesired damage to the surface of the sheep slats. A comprise would be to still drain all the float used at the enzyme treatment stage but use acid diluted in a stream of freshwater of the same volumetric ratio as other solvents streams. Zone 2 of the LASRA enzyme treatment process with this alternative of modification applied is seen in Figure 5.14 below;

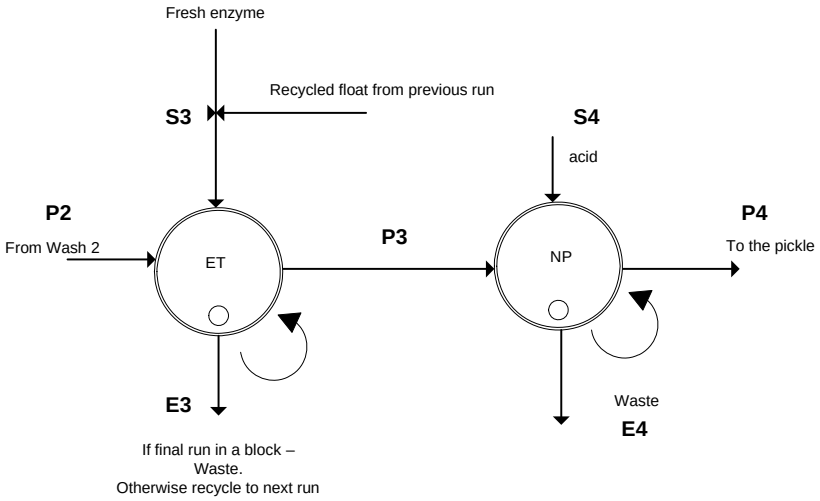


Figure 5.14: Schematic diagram showing the draining of process float being done before the neutralization process

A forward mass balance was also used to investigate the benefits of this modification to zone 2. It was constructed in a similar fashion as the ones used in previous two modification alternatives. The only difference with this configuration of zone 2 is that the effluent stream E3 was now present and this was calculated using the equations shown in section 5.2.

Figure 5.15: Graph showing the comparison between the TS accumulation in float of the standard zone 2 configurations and the float separation before neutralization stage modification to zone 2.

Figure 5.15 shows the results of a forward mass balance study done comparing the standard configuration of zone 2 with the system with one which has the float drained before the neutralization process (i.e. at the enzyme treatment stage). Figure 5.15 shows a reduced amount of solids at stream P4 and hence a cleaner float for up to 10 recycling trials investigated than the standard configuration. The rate of accumulation of solids was also significantly lowered as the float is recycled from one trial run to another.

One of the possible disadvantages with this modification is that there may be an increase of enzyme being discarded. From initial testing with mass balances, it can be seen that the concentration of enzymes in water associated with the skin going from the enzyme treatment process to the neutralization process is higher with this modification as compared with the standard configuration. This loss is in proportion to the ratio of water associated with the skins to overall water found in the stage. Percentage enzyme loss may be calculated using equation 5.27 below;

$$\%\,enzyme \quad loss = \frac{Water \quad in \quad P_i}{Water \quad in \quad P_i + Water \quad in \quad E_i} \times 100\%$$

(Equation 5.27)

*Where 'i' denotes stream number. For the standard configuration, 'i' is the number '4' while for the modified configuration; 'i' is the number '3'

Using equation 5.27, it was found that the enzyme losses were 40% and 60.0% for the standard (values used were from run 1) and modified configuration (values were from run 9) of zone 2 respectively for 1 process run.

Although the enzyme loss in the standard configuration is lower than that of the modified configuration, only half of the recycled float of the standard configuration may be used at a given time (i.e. only up to half the concentration of recycled enzyme may be used at a given time). Due to the dilution that occurs by adding the neutralisation liquor, approximately twice as much process float is available to recycle into the next batch. With each successive recycle of float, there will be added losses of enzyme being 'left behind' in unused float as well as the burden of having to handle and store process floats. In this regard, the modified configuration is in actual fact an improvement over the standard recycle configuration. In a series of process runs where the process float is recycled, the modified configuration of zone 2 may save approximately 10% more enzyme over the LASRA process configuration. This is because the losses from the LASRA process zone 2 configuration over a series of recycle runs is 70% (if the dilution losses are considered), while its still 60% for the modified configuration.

With this modification to Zone 2 of the LASRA enzyme treatment process, it may also be possible to recycle the float of the neutralization process to incur further savings on freshwater. As the concentration of acid used in the neutralization process is quite low (~0.5%), a recycle of neutralization float would require a top-up of acid to the required strength. However, this option was not considered in a forward mass balance as a recycle of float at this stage would offset any reduction of solids accumulation acquired through the separation of float before the neutralization process.

5.3.4 Summary of alternative configurations to zone 2

Comparing all three possible modifications to zone 2 of the LASRA enzyme treatment process using the forward mass balance tool, it would appear that the separation of float before the neutralization process is the best option. This is because it showed the largest reduction of solids over 10 runs investigated when each of the modification alternatives was compared with the standard configuration of zone 2. Section 5.4 highlights details of experimental runs conducted to validate the findings of this section.

5.4 Validation of the Modification Options to LASRA Enzyme Treatment Process

Experimental work to validate the various modifications to the LASRA enzyme treatment process as listed below will be highlighted in this section of the chapter;

a) Coarse filtration of neutralization float (Process run 5 which was used as the representative run in chapter 4 is now used to validate the findings of the implementation of a coarse filtration modification along with runs 6, 7 and 8)

b) Drainage of float before neutralization stage

5.4.1 Coarse filtration of neutralization float

This modification to the LASRA enzyme treatment process was incorporated into trial runs 5 to 8 by adding an industrial fibre filter to the neutralization liquor before the float was channelled to the following process. This was primarily to remove large particulate materials that accumulate in ongoing recycles and has an impact on the quality of the pelts produced (sheep slat after the neutralization process) by giving them a 'dirty' appearance. Despite an indication from the mass balance studies which showed that such an effort would yield only minute improvements, such a filtration technique was trialled to see if a small fraction of large particulate material would affect pelt quality (or 'dirty-ness'). The results may be seen in figure 5.16 below compared with figure 5.5 (reproduced for comparison).

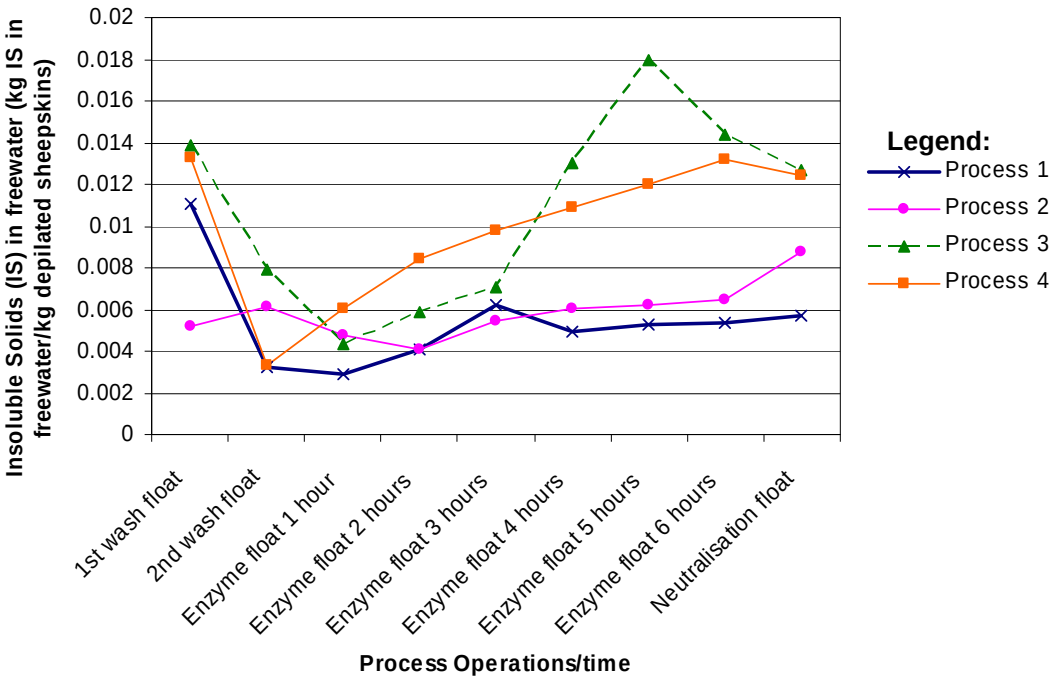


Figure 5.5: Degree of insoluble solids (IS) accumulation over pre-tanning process operations and enzyme treatment time (process 1 - 4) - normalized with original sheepskin mass

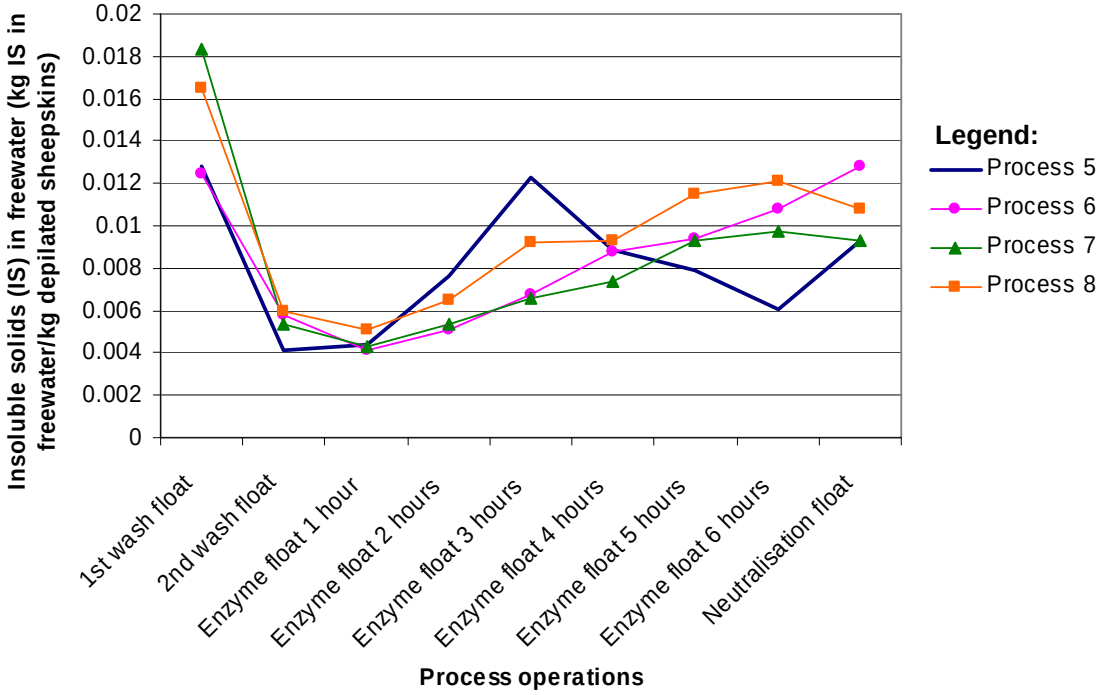


Figure 5.16: Degree of insoluble solids (IS) accumulation over pre-tanning process operations and enzyme treatment time (process 5 - 8) - normalized with original sheepskin mass

Comparing figure 5.5 and 5.16, the results of the mass balance study was confirmed that there was only a minute reduction in the accumulation of solids for the configuration utilizing a coarse filtration technique at the effluent stream of the neutralization process.

However, the evaluation of pickled pelts indicated a more marked effect between the two techniques. The pelts produced by trial runs of 5 to 8, showed less indication of surface dirt as compared to the pelts processed through trial runs of 1 to 4 (Allsop 2008). This would suggest that the added filtration did clean the recycle float to a certain extent through the removal of a portion of IS thus delaying the effects of solids accumulation and occurrence of surface dirt on the processed pelts. This also suggests that even though the coarse particulate sized fraction is small, it is a significant contributor to the occurrence of 'dirty' looking pelts.

5.4.2 Drainage of float before the neutralization stage

Drainage of the float before the neutralization process was incorporated into trial runs 9 to 12. The raw data for these trial runs can be seen in table 5.2a and 5.2b below;

Table 5.2a: System inputs for the mass balance of process runs 9 to 12 up until enzyme treatment stage. These values were obtained from LASRA

	P0			S1	E1				S2	E2				S3		E3			
Trial	M_T kg	C_{TS} kg/kg	C_{TKN} kg/kg	M_T kg	M_T kg	C_{IS} kg/kg	C_{TS} kg/kg	C_{TKN} kg/kg	M_T kg	M_T kg	C_{IS} kg/kg	C_{TS} kg/kg	C_{TKN} kg/kg	M_T kg	C_{TS} kg/kg	M_T kg	C_{IS} kg/kg	C_{TS} kg/kg	C_{TKN} kg/kg
9	13.4	34.4%	8.5%	13.4	8.9	0.16%	5.7%	1.6%	13.4	14.2	0.05%	3.2%	1.0%	13.4	0.12%	12.3	0.02%	3.4%	1.9%
10	16.0	34.4%	8.5%	16.0	8.9	0.14%	5.6%	1.7%	16.0	17.0	0.07%	3.2%	0.9%	16.0	0.12%	15.0	0.05%	4.4%	2.6%
11	13.4	34.4%	8.5%	13.4	10.8	0.22%	6.3%	1.9%	13.4	13.3	0.05%	3.0%	1.0%	13.4	0.12%	11.7	0.06%	5.7%	3.2%
12	14.0	34.4%	8.5%	14.0	10.2	0.14%	5.6%	1.4%	14.0	15.2	0.07%	3.0%	0.8%	14.0	0.12%	11.3	0.06%	5.3%	3.0%

Table 5.2b: System inputs for the mass balance of process runs 9 to 12 of the neutralization process and pickle. These values were obtained from LASRA

Trial	S4		E4				S5		E5			
	M_T kg	C_{TDS} kg/kg	M_T kg	C_{IS} kg/kg	C_{TS} kg/kg	C_{TKN} kg/kg	M_T kg	C_{TDS} kg/kg	M_T kg	C_{IS} kg/kg	C_{TS} kg/kg	C_{TKN} kg/kg
9	13.4	2.0%	15.7	0.01%	1.1%	0.5%	13.4	21.0%	21.0	0.003%	11.1%	0.1%
10	16.0	2.0%	17.9	0.01%	1.3%	0.6%	16.0	21.0%	25.4	0.004%	10.2%	0.2%
11	13.4	2.0%	13.9	0.02%	1.5%	0.7%	13.4	21.0%	21.2	0.004%	10.8%	0.3%
12	14.0	2.0%	14.9	0.01%	1.7%	0.8%	14.0	21.0%	21.7	0.003%	n.a.	0.2%

For these processes, the float was drained and filtered before it was channelled into subsequent process runs with the float of process run 12 discarded.

Figure 5.17 shows the IS accumulation in trial runs 9 to 12;

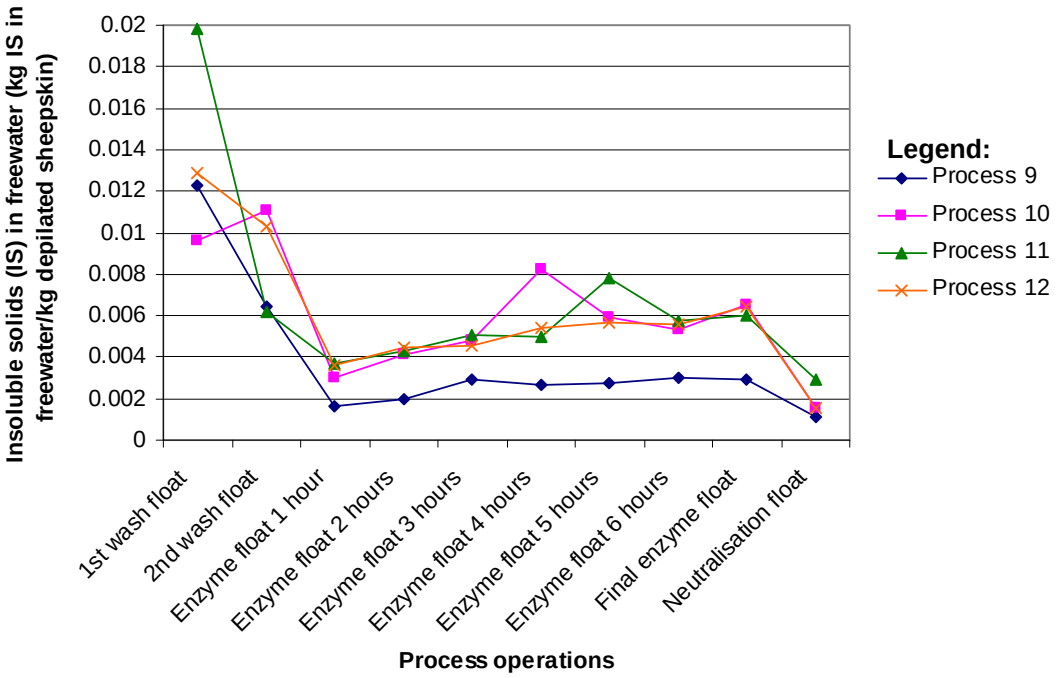


Figure 5.17: The degree of solids accumulation over pre-tanning process operations and enzyme treatment time (process 9 - 12) – normalized against original sheepskin mass

Contrasting Figure 5.17 with 5.5 (of trial runs 1 till 4 which do not have any implemented modification measures) it may be seen that

there is a marked improvement through the implementation of this modification to the LASRA enzyme treatment process.

Figure 5.18 shows the TS accumulation in trial runs 1 to 4 while Figure 5.19 shows the TS accumulation in trial runs 9 to 12;

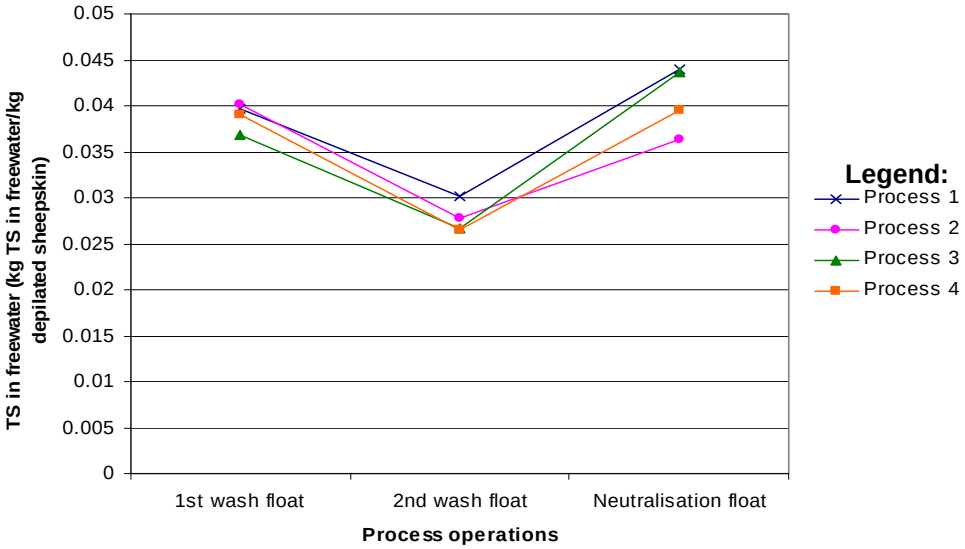


Figure 5.18: The degree of total solids (TS) accumulation over pre-tanning process operations (process 1 - 4) – normalized against original sheepskin mass

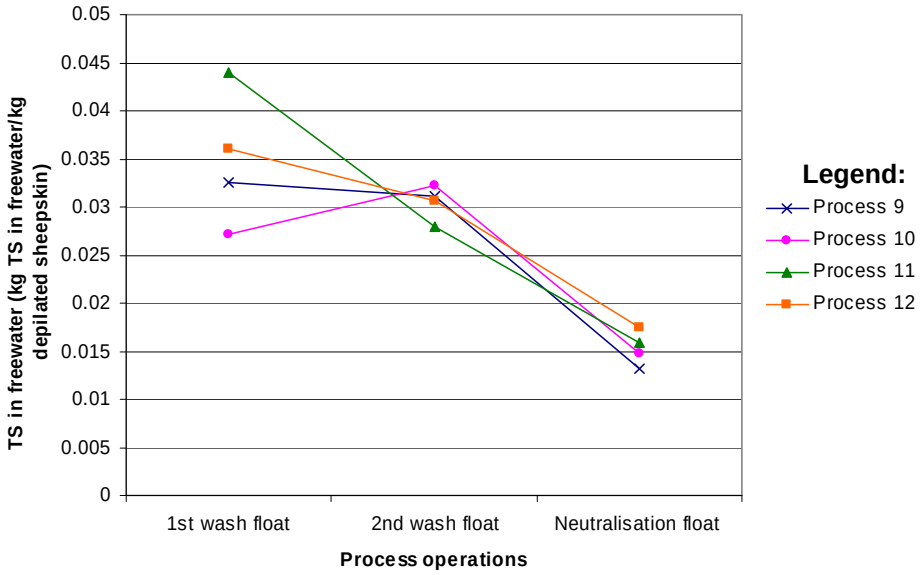


Figure 5.19: The degree of total solids (TS) accumulation over pre-tanning process operations (process 9 - 12) – normalized against original sheepskin mass

The TS comparison seen in Figure 5.18 and Figure 5.19 confirms the findings of the IS results as seen earlier. There appears to be a marked improvement of float quality with the implementation of this modification. The accumulation of solids from one trial run to another as seen in trial runs 1 to 4 was not detected at all for trial runs 9 to 12 in the results of IS and TS study. In addition to that, the graph of Figure 5.17 and Figure 5.19 also suggests that this modification to the LASRA enzyme treatment has also been successful in keeping solids quantity to the minimum despite the build-up that could easily occur as a result of maintaining a float recycle system. This ensures that pickled pelts are processed in a relatively 'clean' float as compared to other trial runs. This validation work confirms the results obtained by a mass balance study which earlier indicated an improvement of float quality through a TDS analysis.

Visual evaluations of pickled pelts of validation work were conducted by noting the accumulation of surface dirt on pelts. Results indicated that the pelts produced from the trial runs of 9 to 12 were significantly 'cleaner' than those produced by the standard LASRA enzyme treatment process procedure (Allsop 2008). This would suggest that the addition of the filtration step at the enzyme treatment process was strategic to ensure that accumulated solids were adequately removed before the float was channelled to the following trial run thus significantly delaying the effects of solids accumulation and occurrence of surface dirt on processed pelt.

5.5 Summary of Modification Work on Zone 2

It may be concluded that to drain the enzyme treatment process float and filter the float before it was channelled to the following trial run would be the best modification to zone 2 of the LASRA enzyme treatment process. Not only was this method able to produce 'cleaner' pre-tanned pelts through minimization of solids accumulation in the float with a float recycle system in place, it had potential to significantly reduce enzyme activity loss (with no enzyme being subjected to pH change in the neutralization process) as well as acid use (with decreased buffering capacity of float with lower volume).

It may be demonstrated that this modification of zone 2 of the LASRA process in fact saved at least 10% more enzyme over the un-optimized configuration. As the enzyme top-up in subsequent trial runs is currently done in excess, the loss of enzyme activity on slats processing may not be noticeable at all. Alternatively, a reduction in enzyme concentration could be investigated in future work.

Other options for modification such as coarse filtration of the neutralization float were also done. Although able to produce 'cleaner' pre-tanned pelts for tanning operations, it did not have the other benefits of reducing enzyme activity loss and reduction of acid use. When using the aesthetic quality of pickled pelts as a yardstick for comparison, the various configurations were rated as seen below;

Trial run 9 to 12 (separation and filtration of float before neutralization process) > Trial run 5 to 8 (filtration of neutralization float) > Trial run 1 to 4 (basic configuration)

The separation and filtration of float before of enzyme treatment float before neutralization is the best modification option for the LASRA enzyme treatment process.

6.0 CONCLUSIONS AND RECOMMENDATIONS

The main objective of this investigation was to take the pre-tanning process developed by LASRA as a proof of principle (Figure 1.2) and to optimize it through the application of engineering principles, to recommend a refined process suitable for application in industry. As discussed in Chapters 1 and 2, a process suitable for industry must be able to generate good quality products, require minimal changes to plant or operation layout and reduced usage of resources (e.g. water and chemicals). The process identified in this work employed a two stage counter-current washing configuration and segregated the effluent streams drained from the enzyme treatment process and neutralisation stages. The enzyme float was also recycled in subsequent batches. Figure 6.1 shows a flow diagram of the resulting optimized enzyme treatment process;

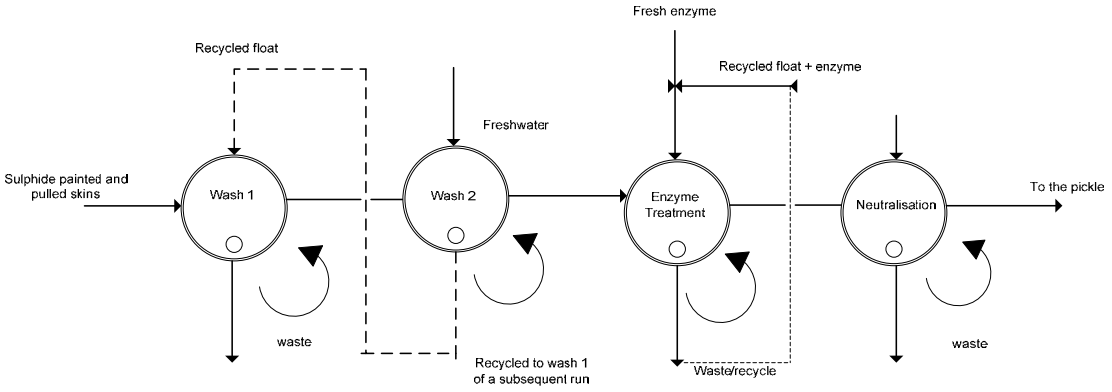


Figure 6.1: Flow diagram of the optimized LASRA enzyme treatment process. As detailed in the work, the pickling stages were not part of optimization work

Two bases for optimization were used to arrive at this process. One was to reduce freshwater use by maintaining current product quality standards. Product quality was evaluated from the effectiveness of the process at removing residual total dissolved solids from sheepskins going on to the pickle. Savings on freshwater has impacts on the tanning and pre-tanning industries particularly those in locations where water shortages occur.

This study also optimized the process on the basis of improving product quality (through reduction of residual total dissolved solids) while using existing

quantities of resources. A process that can more effectively remove unwanted soluble or enzyme solubilised components from the skin is more desirable by the industry as it provides benefits in post-processing tanning operations.

The principle tool used to fulfil the objectives of the study was mass balances. These were first developed over the original proof of principle process configuration. This was done to provide an understanding of underlying mechanisms for separation of unwanted solids from skins and raw material characterization. For example, the applicability of the assumption of equilibrium stage separations was tested. Similarly the extent of solubilisation of the skin by the enzymes was evaluated. The understanding of the standard process developed in this way was then used to generate common values and ratios to be used in the construction of forward or predictive mass balances where output streams were predicted from known inputs. Analytical methods such as the investigation of the bound water of sheepskins at different stages of the pre-tanning operations using the Differential Scanning Calorimetry (DSC) were also employed at this stage to aid the construction of mass balances.

The work done on zone 1 of the process, found that a 2 step counter-current (2SCounterC) configuration of the washing stages provided the most benefits. The 2SCounterC configuration was able to utilize a markedly reduced freshwater usage of up to 40% to produce the same quality slats as the standard process. Together with existing savings of the un-optimized LASRA process, the total savings of the optimized process was 70% freshwater. It could also produce improved quality slats (reduction of up to 50% less total dissolved solids) through use of the same quantities of resources as in the original LASRA process. These findings were validated through experimental studies on a pilot scale plant of the 2SCounterC configuration. These benefits were delivered without additional washing stages being employed. As a consequence, there was no additional capital equipment requirement, processing time or energy demands as compared with the original LASRA process.

The work on zone 2 found that the separation of float during the enzyme treatment stage (instead of doing so at the neutralization process) had the lowest solids accumulation during repeated enzyme solution recycling. For enzyme solution recycling of 10 trial runs, the solids accumulation for the optimized process was up to 30% less than the original LASRA process. This is due to the fact that with the enzyme treatment float drained, the neutralization process now acts as an additional washing step to further clean the pelts of any soluble or washable insoluble material. It was also found that this modification also incurred enzyme savings of up to 10%. Reduced enzyme use, reduces the overall cost of the process.

Visual monitoring of the sheepskins used in validation studies conducted by LASRA of the modification alternatives has since confirmed these findings. The validation tests also showed that the relatively small amount of larger particles had an impact on pelt quality and may be removed through the use of a coarse filtration technique. The implementation of the separation of enzyme treatment float prior to neutralisation resulted in a marked reduction of solids accumulation in the finished skins of 37% less than the original LASRA process over 4 runs.

Table 6.1 shows a relative comparison of the benefits of the optimized LASRA process with the original LASRA process as well as conventional processing.

Table 6.1: Comparison between the conventional process, original LASRA process and the optimized LASRA process. Values were based on the processing of 6 sheepskins of a total mass of 12.65kg. * The enzyme use in conventional processing is different than what is used in the enzyme based processes.

Quality Parameter/Process Description	Conventional Pre-tanning Operations	LASRA process	Optimized LASRA process
Total dissolved solids in processed sheepskin	0.77 kg	0.77 kg	0.72 kg
Number of stages	13 stages	5 stages	5 stages
Processing time	26 hours	14 hours	14 hours
Relative water usage (relative to conventional)	100%	51%	30%
Energy usage (relative to conventional)	100%	27%	27%
Enzyme usage* (kg enzyme/kg sheepskin - %)	0.0005%*	0.12%	0.108%

From table 6.1, it can be seen that the optimization of the LASRA process has resulted in a refined process that offers many advantages over conventional processing. The process now meets the requirements for a process ready for uptake by the industry. It is recommended that the optimized LASRA enzyme treatment process is now tested at an industrial scale. Due to the nature of the process there is minimal investment required to carry out the trials in an existing factory, so setting up such a trial should not be difficult. From these trials, the benefits of the process and its ability to produce high quality skins will be evident and facilitate its more wide spread adoption in the industry.

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APPENDIX 1

Other nomenclature used

a_w	Water activity	
c_g	GAB constant (related to monolayer heat of sorption)	
f_g	GAB factor (related to heat of sorption of multilayer)	
M_o	GAB monolayer moisture content	$kg_{H2O}/kg_{dry\ solid}$
M	Moisture content	$kg_{H2O}/kg_{dry\ solid}$
Bw	Boundwater/Un-freezable water	$kg_{H2O}/kg_{dry\ solid}$
Fw	Freewater/Freezable water	$kg_{H2O}/kg_{dry\ solid}$
COD	Chemical Oxygen Demand	kg/L

APPENDIX 2

Measure of bound water in sheepskin by Differential Scanning Calorimetry (DSC7)

1. Pieces of sheepskin of 10cm x 7cm in measure of the following samples were taken and frozen overnight in a freezer.
   - green (sheepskin before depilation) sheepskin from the neck, flank and butt
   - depilated sheepskin
   - limed sheepskin (now known as slat) from the neck, flank and butt
   - delimed/bated slat from the neck, flank and butt
   - pickled sheepskin (now known as pelt) from the neck, flank and butt

2. To find out the enthalpies of the respective samples, pieces of sheep skin/slat/pelt to be run in the Differential Scanning Calorimetry (Perkin-Elmer DSC7 – PerkinElmer USA). Samples we cut out from the main piece and placed into DSC sampling capsules. Mass of all samples must be between 0.004 grams and 0.008 grams. A pair of reverse-osmosis (RO) water samples was also made.

3. Samples used to find out dry masses were made by placing pieces of the sample onto metal drying dishes and dried in an oven at 40^{o}C overnight. These samples must weigh between 70-100% of the mass of the metal drying dishes. To obtain wholly dried samples, similar procedures were applied as when finding out insoluble solids. Refer to Standard Methods of Total Suspended Solids Dried (1998).

4. The enthalpy of pure water was established by running the pair of RO water samples in the DSC7.

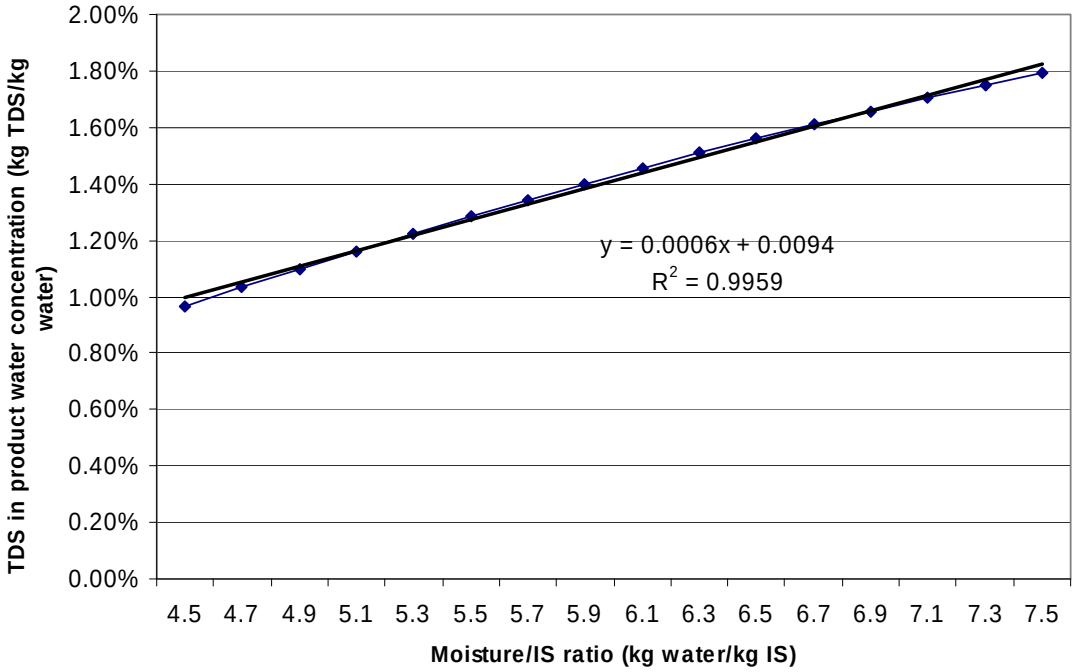
5. Following that skin samples were run in the DSC together with a sample of pure water.

6. All samples were processed through the following methodology; samples were cooled from room temperature to -80^{o}C at a rate of 50^{o}C/minute followed by a heating sequence of the sample back to room temperature at a rate of 5^{o}C/minute.

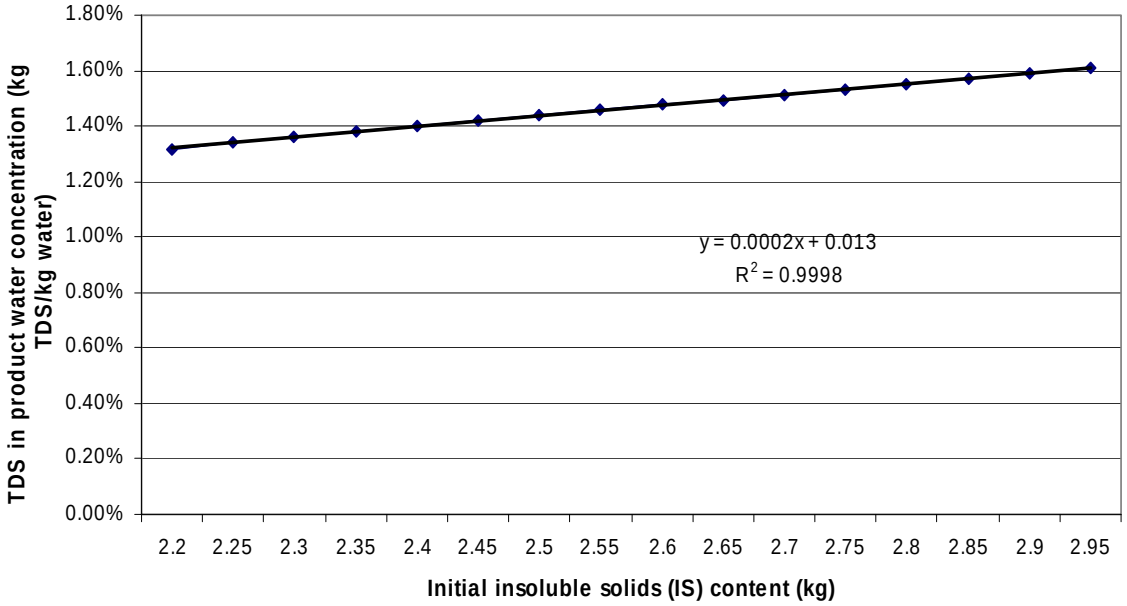
7. Calculations of bound water values from the values found through step 1 to 6 are found on section 3.4.2 of the thesis.

APPENDIX 3

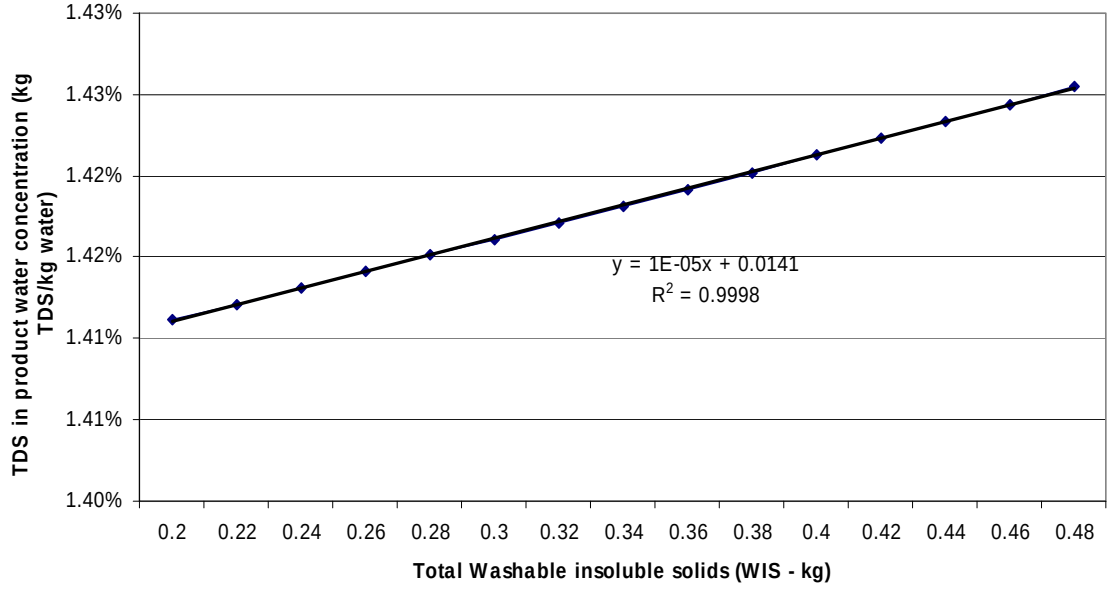
Sensitivity analysis of variables used in the forward mass balance of Zone 1



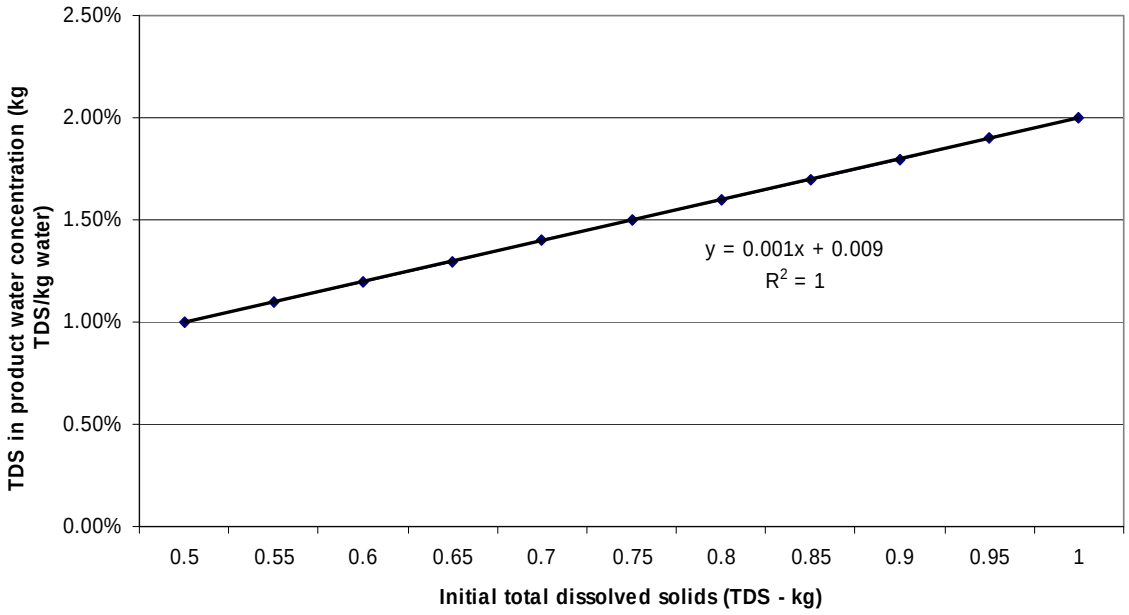
Appendix 2.1: Shows the minimal variation of the concentration of TDS values across possible values of moisture/IS ratio. The variation of TDS concentration was no more then 0.9% (between 0.9% and 1.8%)



Appendix 2.2: Shows the minimal variation of the concentration of TDS values across possible values of initial insoluble solids (IS) of sheepskin. The variation of TDS concentration was no more then 0.5% (between 1.1% and 1.6%)



Appendix 2.3: Shows the minimal variation of the concentration of TDS values across possible values of total washable insoluble solids (WIS). The variation of TDS concentration was no more then 0.02% (between 1.41% and 1.43%)



Appendix 2.4: Shows the minimal variation of the concentration of TDS values across possible values of initial total dissolved solids (TDS) content in sheepskin. The variation of TDS concentration was no more then 1.0% (between 1.0% and 2.0%).

APPENDIX 4

Methodology for the validation experimental run of the 2SCounterC modification to Zone 1

2SCrossC process run

1. A total of 2 sheepskins were taken from the freezer and each split evenly into 2 portions.
2. One half of each skin was replaced back into the freezer while the other 2 halves had Quikpul overnight depilation paint applied
3. The next day, all the wool was pulled out of the skins before it was placed into the first wash for 1 hour with 100% (wt/wt) freshwater.
4. After wash 1, the float was sampled, analyzed for SS, TDS, TS, water content, TKN and COD (Chemical Oxygen Demand) before it was dumped.
5. The skins were then placed into wash 2 for 1 hour with 100% freshwater.
6. After wash 2, a similar analysis that was conducted on the float of wash 1 was done on the float of wash 2.

Run 1 (2SCounterC process)

1. A total of 4 sheepskins was taken out of the freezer and split evenly into 2 portions
2. The 8 half portion of skins were then randomly arranged into groups of 2 skins each to reduce experimental error.
3. All skins had Quikpul overnight depilation paint applied onto it.
4. The next day, all the wool was pulled out of the skins.
5. The first group (of 2 randomly assigned half skins) was then placed into the first wash of the countercurrent process for 1 hour with 200% freshwater.

6. After wash 1, the float was sampled, analyzed for SS, TDS, TS, water content, TKN and COD (Chemical Oxygen Demand) before it was dumped.
7. The skins were then placed into wash 2 for 1 hour with 200% freshwater.
8. After wash 2, a similar analysis that was conducted on the float of wash 1 was done on the float of wash 2.
9. The float of wash 2 was then stored to be used as the solvent stream input for the first wash of the next run.

Run 2-4 (2SCounterC process)

Repeat the above steps (step 1-9) always using the wastewater of wash 2 of the previous run as the solvent stream input (adjusted to 200% of the mass of sheepskins being processed) in wash 1 of the current run along with another group of 2 half skins.

Run 5 (2SCounterC process)

1. The 2 half skins that had been stored away during the running of the 2SCrossC was thawed and had Quikpul overnight depilation paint applied onto it.
2. The next day, the wool was pulled out from the skins and placed into the first wash using the float of wash 2 of run 4 as its solvent stream input.
3. After wash 1 (with 200% wastewater as solvent stream), the float was sampled, analyzed for SS, TS, TS, water content, TKN and COD (Chemical Oxygen Demand) before it was dumped.
4. The skins were then placed into wash 2 for 1 hour with 200% freshwater.
5. After wash 2, a similar analysis that was conducted on the float of wash 1 was done on the float of wash 2.

APPENDIX 5

Methodology for the preparation of skin powder azure (SPA) substrate (Edmonds 2009).

1. Fresh lambskin was shaved then frozen at –10°C. The frozen skin was shaved again to remove all remaining wool that projected from the surface of the skin.

2. The frozen skin was lyophilised then ground in a Wiley mill #2 mill with 3mm mesh size.

3. The ground material was then degreased in a Soxhlet with dichloromethane for 6 hours at 6 changes of solvent per hour.

4. The skin was then dried at 40°C for 16 hr in an oven.

5. The colorimetric substrate was then prepared from the ground degreased skin by the following method; 60g of ground degreased skin was fully wetted in a solution of 400 mL of 0.45% sodium chloride under vacuum. The solution pH was then adjusted to pH 11 using 0.5M tri-sodium orthophosphate and 6g of Remalzol Brilliant Blue 'R' (Sigma) was added. The mixture was then stirred for 30 minutes at 40°C. Then either 800 mL of water at 60°C was added and the mixture stirred for another hour at 45°C (designated low temperature, SPA < 70) or 800 mL boiling water was added and the mixture stirred at 75°C for 5 minutes (designated high temperature, SPA>70).

6. The mixtures were cooled and settled over 1 hour and following that the supernatant water was siphoned off and another 800 mL of cold water was added and mixed. These washes continued until the supernatant water appeared clear and showed no peak of absorbance at 595nm.

7. The resultant “blue” slurry was rinsed with 20 successive volumes of acetone and the subsequently dewatered solids were then dried at 40°C overnight.

8. The dry solids were ground in a coffee grinder in the presence of solid carbon dioxide and sieved and the solids fraction between 90-200 mesh sizes were kept.

9. The resultant “skin powder azure” was then stored at room temperature.

APPENDIX 6

Preparation of enzyme activity assay

1. 100 mg of skin powder azure substrate (SPA) was measured into conical flasks – one for each analysis. 20 ml of Tris/HCL buffer (adjusted to pH 7.8) was added to SPA and the solids were suspended through swirling the flask.

2. Enzyme solution was prepared just before use to a 0.5% solution through the addition of 0.5 mg of enzyme pellets in 100 ml water.

3. The flask was then placed in a shaking water bath at 35^{o}C for at least 5 minutes to allow the solution to equilibrate before addition of enzyme protease (Maxizyme SEM, NPN Ltd.).

4. 8 plastic 10 ml centrifuge tubes were prepared for each sample by discharging 2 ml of 5% Trichloroacetic acid (TCA) in each. TCA was used to deactivate the enzyme activity in its respective tube. The tubes was to contain samples of enzyme activity at time intervals; 0, 10, 30, 50, 120, 300 and 600 seconds.

5. To obtain zero time reading, 1 ml of buffer was taken from its flask and discharged into the centrifuge tube marked zero time. Following that, a 2 ml bate solution was discharged into flask and timer was started.

6. At designated times; sample was drawn from the flask and discharged into its represented enzyme activity time tube. After all tubes had been filled, these were vortexed to ensure even mixing of the enzyme solution and substrate.

7. All test tubes were then centrifuged at 4000 rpm for 30 minutes.

8. The supernatants found in each tube were measured at 595 nm and calibrated against a water blank sample.

APPENDIX 7

Particle Size Distribution by the Malvern Mastersizer S (Downey 2009)

1. Turn on the Sizer Unit (red button at the side, left hand bottom position). The button should glow.

2. Turn on the laser by turning the key next to the red button clockwise (a green light should glow). The unit should ideally be turned on an hour before use.

3. Check tubing is orientated for sample to flow through the Small Volume Sample Unit. Turn on the Small Volume Sample Unit (switch is on the back – a green light should glow).

4. Empty the sample unit by moving the lever on the right hand side to open the drain and then refill the unit with RO water. Turn the propeller inside the unit by using the dial on the front. Run the sample for a few seconds to remove air bubbles from the tubing and turn down the propeller to allow air to rise up out of the solution. We normally have the dial turned to the 1 o'clock position when measuring samples. Do not leave sample unit running for long periods as this will over heat the unit.

5. Rinse the sample unit with RO water several times to clean it. The sample unit should be filled to within 1 cm of the top of the bowl.

Computer Programme – first sample

1. Open Sizer programme –Go to **Setup/Presentation** to determine type of analysis. In this instance, the 30HD presentation was chosen.

2. Go to **Setup/Hardware** to make sure correct lens is selected. This will normally be 300RF. Open the Mastersizer door covers to check which lens is in place. *Please see Mark Downey if you need it to be changed.*

3. Go to **Setup/Analysis** to ensure the correct model is used for your analysis. This can be changed after you have measured a sample so you can test the effect of different models. Polydisperse is a good default option.

4. Align the laser with the stirrer on (icon with xyz plot – Ctrl A). You will also find this command under 'Measure' in the drop down menus. The Mastersizer S will align automatically within a few minutes. Stop and start the stirrer to ensure there is no air trapped under the propeller blades. Laser power has to be at >50% and within the green zone. If the laser power is less, you may need to wash the sample unit with water or give the laser more time to warm up. Alignment only needs to be done when starting the first run. Close this window.

5. Open Documentation (icon with hand holding pen over paper – Ctrl N) and put information required in (you can also press F4 to start an automatic sequence).

   Mark ☒ Copy to current sample, OK – only if the current sample is the file you wish to save in.

6. Measure the background (Ctrl B) with laser power >50% and the stirrer turned to about 1 o'clock. Pause at each stage – mark the box. You may proceed through each stage for making a measurement by using the space bar or the mouse cursor

7. To Start Measuring: in the Inspect Window

   Add your sample (process float at 10% of original concentration) until Obscuration is in the green zone. About 18-20% is a good target. The amount required varies from 1 drop for cream to entirely filling the sample unit for thin protein suspensions. Allow the obscuration

reading time to settle (10 seconds). Try to be consistent. If you go over the obscuration level, add RO water to dilute the sample.

8. Go to the next window "Measure" and start measuring.

9. Analyse. Check you have used the correct presentation i.e. 3OHD, if not you may re-analyse. If your data is saved, you can re-analyse as well, so you don't need to repeat measurements.

10. To save **(very important step)**
    a. File/Save as – to change directory double click on, e.g. "e:\" (for USB drive) or "[:]" (You can save the Mastersizer file on the computer)
    b. File/Open Sample
    c. File/Sample Records – open

    From now on the programme will automatically save each run to this file. There is no known limit to the amount of runs that can be saved in each file.

11. Check that the data is valid by going to **View/Data (Ctrl D)** and **View/Fit (Ctrl F). Data** will show the difference between your data and the background (there should be a big difference) and **Fit** will show how well the Mastersizer fitted the data. A good fit will show a low residual. Too high an obscuration or the wrong presentation can cause a poor residual. The small sizes are hardest for the Mastersizer to fit.

12. Go to **Edit/Copy** to copy data to an excel file. **Copy/Result will copy the %volume within each size range, whilst Copy/Sizes** will copy the size ranges themselves.

13. Wash out Sample Unit 3 to 5 times, stop the stirrer to remove air bubbles, start with stirrer at 1 o'clock position.

14. Repeat steps 5-13 for subsequent replicates and samples.

The Mastersizer unit was cleaned and turned off as specified by the latest available user guide dated February 2009 (Downey 2009) after use.

APPENDIX 8

Gradient gel (7 – 14% Polyacrylamide, 2 small format gels example) (Edmonds 2008)

1. Half the volume of gel to be poured is made from the light fraction (as define below) formulation and the other half is prepared from the heavy fraction (as defined below) formulation.

2. The two formulations are then mixed on a linear gradient mixer to produce a linear gradient between the two.

3. A pump is used to pump the mixture from the gradient mixture which is then carefully poured into a gel from the bottom of the gel working up. The presence of sucrose in the heavy fraction helps the layering of the polyacrylamide with the concentration going from that of the heavy fraction to that of the light fraction.

Light fraction

Milli Q water	2.840 mL
Acrylamide (40% solution)	1.093 mL
Tris buffer (1.5 mol/L, pH 8.8)	1.333 mL
Ammonium persulphate (10% solution)	48 uL
(Prepared fresh, daily, from dry stock stored under vacuum)	
Sodium dodecyl sulphate (10% solution)	53 uL
Temed	2 uL

(Actually Temed was 1 uL added directly to the gradient mixer column)

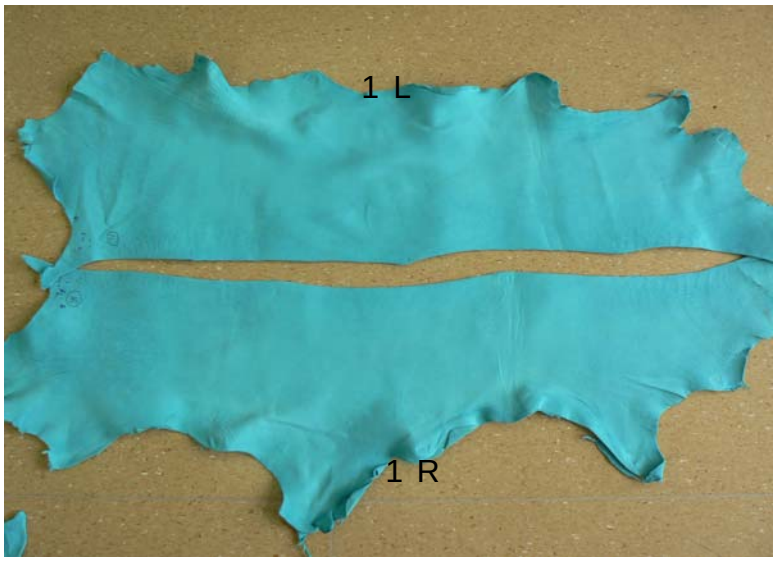
Heavy fraction

Milli Q water	1.661 mL
Acrylamide (40% solution)	2.582 mL
Tris buffer (1.5 mol/L, pH 8.8)	1.666 mL
Ammonium persulphate (10% solution)	28 uL
(Prepared fresh, daily, from dry stock stored under vacuum)	
Sucrose	0.96 g
Sodium dodecyl sulphate (10% solution)	64 uL
Temed	2 uL

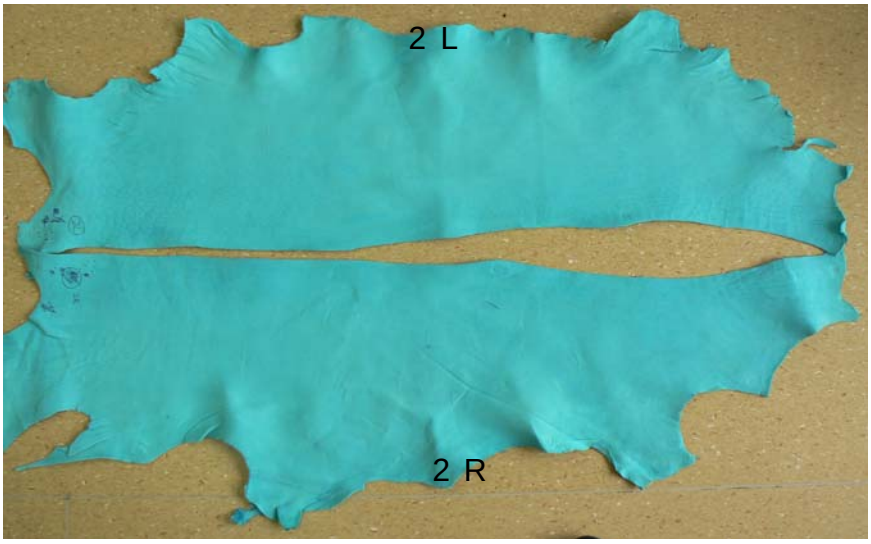
(Actually Temed was 1 uL added directly to the gradient mixer column)

APPENDIX 9

Photos showing the comparison of sheepskins washed by the standard washing stages configuration against those by 2SCounterC



Appendix 8.1: Shows half sheepskins 1L and 1R. Half sheepskin 1L was washed by through 2SCrossC while 1R by 2SCounterC. Both half sheepskins have seen been tanned and dyed blue.



Appendix 8.2: Shows half sheepskins 2L and 2R. Half sheepskin 2R was washed by through 2SCrossC while 2L by 2SCounterC. Both half sheepskins have seen been tanned and dyed blue.

Soli Deo Gloria!