final report

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Manufacture of the Industrial Enzyme γ-glutamyltranspeptidase from bovine kidney

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Executive Summary

A method of purifying γ-glutamyltranspeptidase from beef kidneys has been developed which may be used in any abattoir where appropriate equipment is available.

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1 Background

A key step in the GGC manufacturing process is the use of the enzyme, gamma glutamyl transpeptidase (GGT). The richest natural source of this enzyme is bovine kidney. Currently, the GGT enzyme is commercially available in low volume (mg) as a research reagent from various animal sources, bovine, porcine and equine. Biospecialties Australia's new GGC manufacturing process requires the use of GGT as an industrial enzyme and, as such, it must become available at large volume (tonnes) and at low cost.

The current pilot scale process for the manufacture of GGC requires approximately 2.8 kg of bovine kidney to supply sufficient enzyme to produce 1 kg of GGC in a batch process, where the enzyme is not immobilised and is not recycled. For industrial scale, it would be preferable to immobilise the enzyme so that it can be recycled for use in subsequent reactions. The use of immobilised enzyme not only offers the benefit of reduced manufacturing costs but also improved processing and GGC purification. Immobilised enzymes can normally be recycled for between 10-50 reactions before the specific activity diminishes below a suitable threshold level for the manufacturing process. The number of reactions possible with an immobilised enzyme is dependent on the reaction conditions, the stability and structure of the particular enzyme, and the nature of the immobilising matrix.

At full industrial scale, it is envisaged that the production of the GGT enzyme would involve an abattoir based extraction, concentration and stabilisation of the GGT from harvested healthy kidneys. Ideally, the subsequent processing technology to purify and immobilise the enzyme would also be suitable for operation by normal abattoir staff. If not, the crude extract could be shipped and sold to BSA, which would conduct the final processing steps.

2 Outcomes:

The process development for the production of the enzyme γ-glutamyltranspeptidase has been completed. The process flow diagram is detailed in figure 1. Provided suitable equipment is available the process could be conducted on site at an abattoir.

2.1 Homogenisation

If frozen, kidney is cut into cubes of approximately 5cm length. The cubes are then placed in a blender with a buffer consisting of sodium bicarbonate and sucrose. Unfrozen kidney may be added directly. In the blender, the kidney is then homogenized for 2 minutes at high speed, 4 times. This produces a slurry of homogenized kidney in buffer. The mixture is diluted further with buffer, if required, to ensure the slurry flows easily.

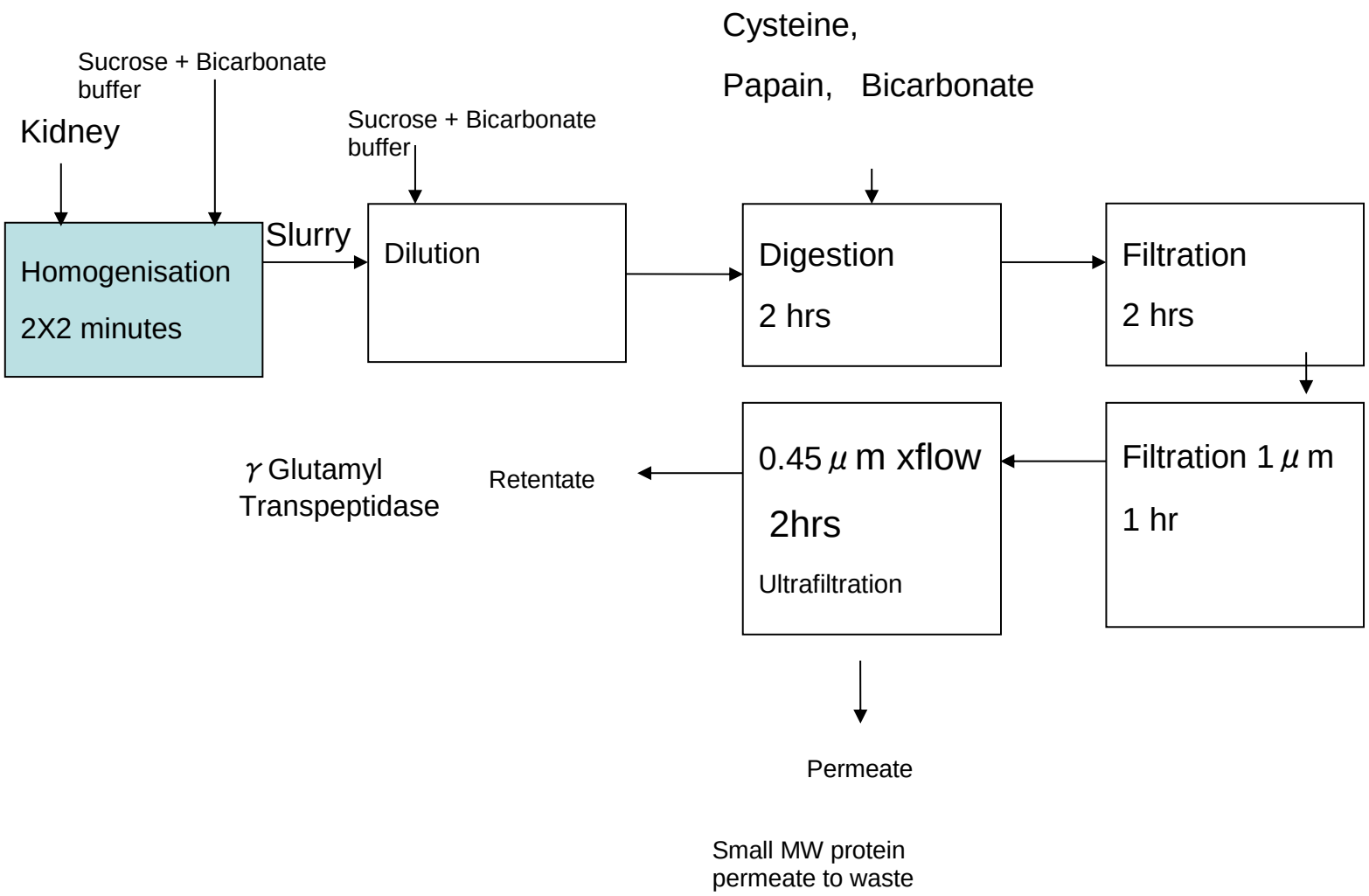

Figure 1: Flow Diagram of Process for γ-glutamyl transpeptidase production

2.2 Digestion

The slurry is transferred to a jacketed stirred vessel. Papain concentrate, cysteine and sodium bicarbonate are added. The solution temperature is raised to 45°C, mixed thoroughly and allowed to stand at temperature for approximately 2 hrs. This step cleaves the active portion of the enzyme away from the cellular membranes, leaving the now soluble γ-GT in solution.

2.3 Cooling/Filtration

The digested enzyme solution is chilled to 15°C. The slurry is pumped to the Filter Press at 3 bar pressure. The first filtration is through a 1 µm dead end filter, then followed by a cross flow 0.22µm hollow fibre filter.

2.4 Ultrafiltration

The recovered enzyme solution is subjected to ultrafiltration using a 10,000 D membrane to remove the ammonium sulphate and lower molecular weight protein contaminants. The permeate contains water and low molecular weight contaminants with the enzyme remaining in the retentate. The membrane is then washed with a sodium bicarbonate buffer solution.

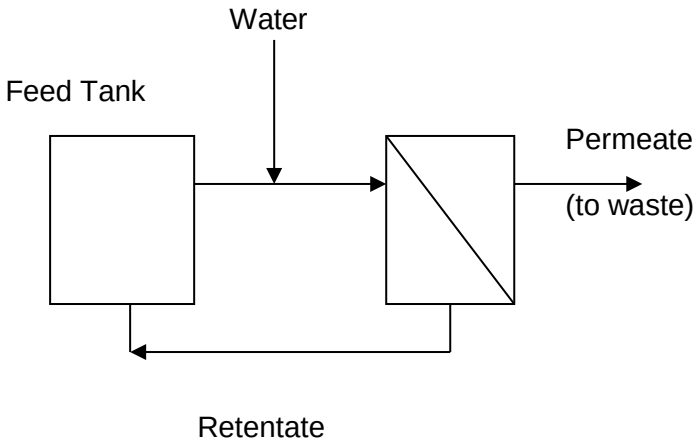

Figure 3: Schematic of Ultrafiltration setup

The retentate is further concentrated using the same ultrafiltration membrane to produce an enzyme solution containing 100 – 200 units per ml.

Table I: Inputs per batch.

Inputs	weight kg	Vol(L) added	Accumulated vol(L)	Step
Kidney	30			Homogenisation
Sucrose	0.542			
$NaHCO_3$	0.212			

RO H_2O		60	90	
RO H_2O		90	180	Dilution step
Papain	0.027		180	Digestion step
Cysteine.HCl.H_2O	0.221			“
RO H_2O		180	360	Dilution
1um GAF				Clarification
0.2um microfiltration/wash		4x50(200)	560	
			30	Ultrafiltration/
$NaHCO_3$	0.365	100	130	Concentration

3 Plant Requirements

Table II lists plant requirements for each step. The rest of the report details the requirements in further detail.

Table II: Summary of Process Equipment

Process Step	Equipment required
Homogenisation	Meat Saw, Blender
Papain digestion	Stirred jacketed vessel
Filtration	Filter Press, GAF filter, pumps.
Ultrafiltration	10,000 Molecular weight cutoff membrane, housing, pumps.
Storage	Freezer or Fridge
Water Purification	Reverse Osmosis system

3.1 Water Purification

Purified water is used in the enzyme production. The water purification system used at BSA comprises first through 10μm and 1μm inline filters, an activated carbon filter, softening via

resin, then a reverse osmosis system using brackish water membranes. This generates high purity water with a conductivity of ~ 1µS/cm.

Figure 4: Water Purification System, including two reverse osmosis units.

3.2 Homogenization

A Nolex Junior Model meat saw is used to cut the kidney into cubes. Any suitable meat saw could be used.

Figure 5: Meat saw used to cut frozen kidney

For homogenization, a blender with a large bowl is required for this step. Biospecialties uses a robot coupe® vertical cutter/ mixer blender with a 60L bowl and two blade assembly. Any suitable appropriately sized blender could be used.

Figure 6: Blender used in kidney homogenisation.

For digestion, a stainless steel reactor vessel with internal heating coils and external insulation was used. A glycol-water heat transfer fluid was used to provide heating and cooling as necessary. A Lightnin air driven mixer is used for agitation- as isopropanol is flammable, a flameproof motor is required. The model shown here is a 0.25 horsepower mixer. The vessel chosen must be easy to clean, corrosion resistant and be temperature controllable.

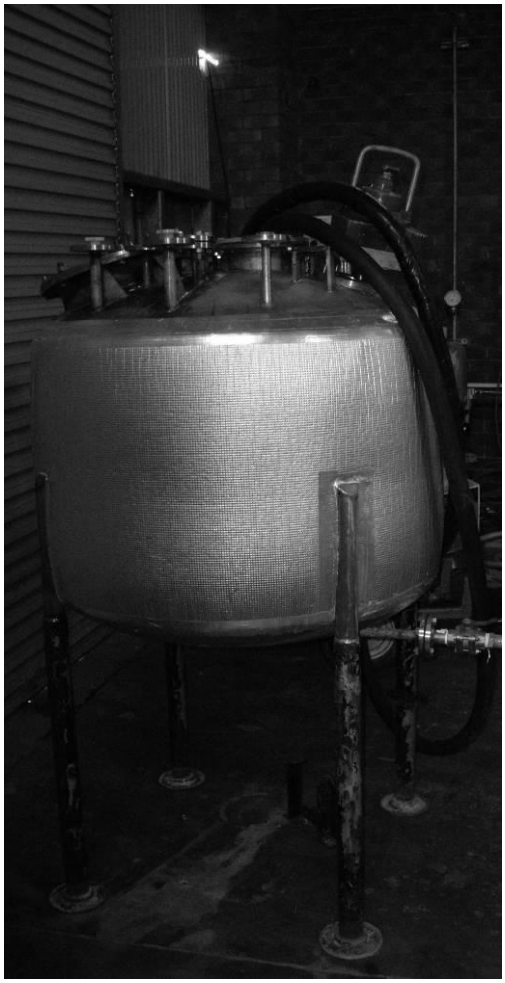

Figure 7: Reactor vessel used in papain digestion. The digestion is allowed to stand in this vessel. On the top right, the top of an air driven mixer may be seen.

The first step uses a Filter Press followed by a GAF filtration system with a 1.0µm bag. The second filtration step uses a Memtec 0.22µm hollow fibre cross flow system. Any suitable 0.22 cross flow filtration system could be used.

Figure 8: GAF filtration setup. A bag held in place by a stainless steel mesh is placed inside the filtration apparatus.

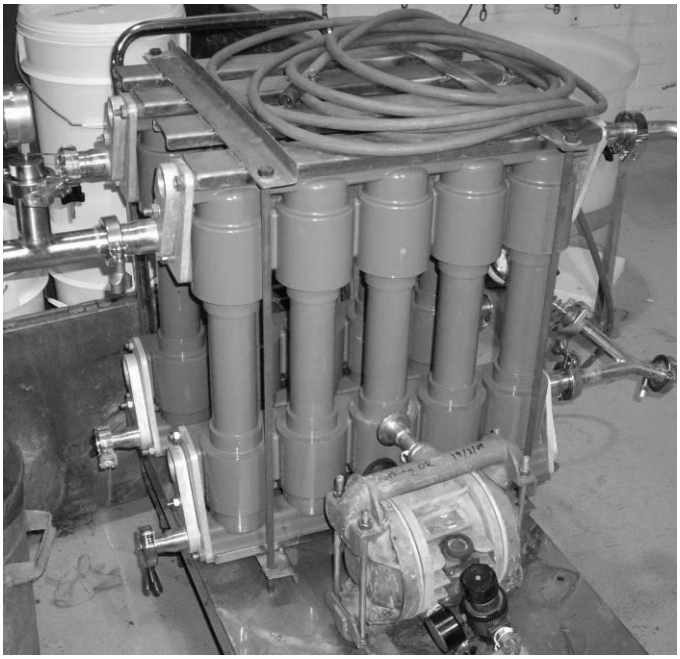

Figure 9: MEMTEC bank of hollow fibre membranes. The diaphragm pump shown below is used to provide pressure.

Ultrafiltration uses an AMI M-U2540PES polyethersulfone 10,000 molecular weight cutoff membrane. Any suitable 10,000 molecular weight cutoff membrane would be suitable for ultrafiltration, along with a suitable pump, tanks piping and so on.

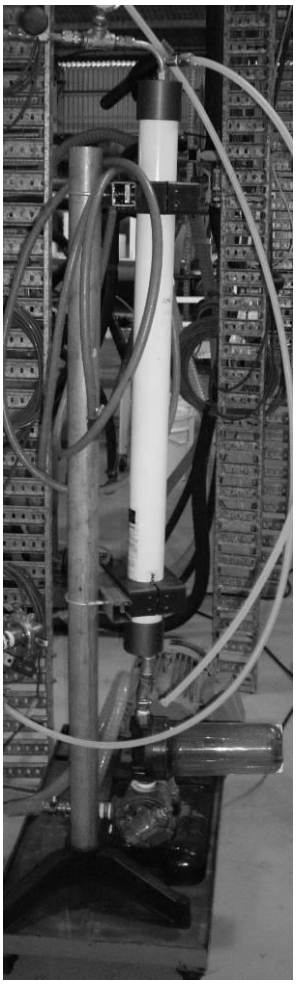

Figure 10: Membrane used for Ultrafiltration.

3.3 Processing conditions

Equipment should be clean and in working order prior to commencing enzyme production. Kidney may be used frozen or used fresh. Purified water must be available in plentiful amounts at any abattoir where γ-GT is used.

Conclusion

The process described above was successful in producing an enzyme solution of desired activity. The pilot plant had a footprint of about 10m2 , processed 30kg liver and produced 5kg of concentrated extract containing aproximately 1 million units of enzyme per batch over a 24 hour period.The process could, with suitable changes to chemicals and other variables, be adapted to the extraction of other components such as bioactives and alternative enzymes from red meat tissues and coproduct streams.