final report

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Blood derived adsorbents for large scale industrial applications

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Background:

Blood is an undervalued by-product of bovine and ovine slaughter. In essence, it is a suspension of red blood cells (together with lower levels of other cell types) in a clear fluid, called plasma. Both the cells and the plasma are rich in protein. These proteins find various industrial applications, including inter alia roles as valuable additives in manufactured food and pet food products, with the ability to perform the same roles as, and sometimes better than, egg white and soy protein in terms of their emulsifying capacity, foaming attributes, water retention capability, rheological properties and nutritional characteristics.

The separation of the plasma proteins from red blood cells requires further processing and incurs extra cost, so currently the red blood cells are either sent to rendering, end up in low value blood meal or are added directly to food. In the latter case, direct addition of red blood cells to many food products is limited because of the intense red-black colour and metallic taste associated with the high iron content of the haemoglobin in the cells. However, the opportunity exists to concomitantly derive at least two value-added co-products from the red blood cells, which could together offset the cost of processing more effectively than if only a single product was targeted. The first product is the abundant and quite functional globin proteins and the second product is the haem (the substance with co-ordinatively bound iron) which is responsible for the intense colour and oxygen transport characteristics. It is this bovine haem (BH) and the derived protoporphyrin IX (PIX) that were the subject of the feasibility study carried out as Project A.BIT.0022.

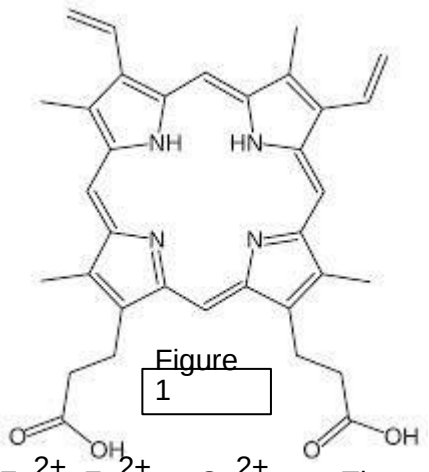

Figure 1

Haem biosynthesis in mammals employs the enzyme ferrochelatase, which converts protoporphyrin IX into heme b (i.e. to Fe(II)-protoporphyrin IX) whilst in plants the corresponding chlorophyll biosynthesis utilises the enzyme magnesium chelatase, which generates Mg(II)-protoporphyrin IX. Protoporphyrin IX (Figure 1), itself is derived via several cytoplasmic and mitochondrial enzymatic reactions from succinyl CoA. Protoporhyrin IX is a tetrapyrrole-containing molecule, with methyl, propionic acid and vinyl pendant side chains, which give it the capability of binding in solution to various metal ions, such as Fe^{2+}, Zn^{2+} or Cu^{2+}, etc. The metal-binding capability of immobilised haem or its metal free form, protoporphyrin IX (and its analogues), can thus in principle be used to

generate new classes of resins/ adsorbents for use in selective applications, including, but not limited to, e.g. the capture of heavy metals, toxic metals, transition metals, rare earth metals and precious/semi-precious/noble metals from diverse sources via metal chelating mechanisms, or various charged, polar or non-poalr substances present in effluent streams from manufacturing processes via pseudo-ion exchange or mixed mode adsorption mechanisms.

Project Approach and Strategy:

One of the key chemical properties of protoporphyrin IX is thus its ability to bind strongly to metal ions, especially iron and other borderline and hard metal ions, to generate metallo-macrocyclic compounds, either in the free-state or as immobilised complexes. The objective of this feasibility study was two-fold. Firstly new approaches were investigated to determine whether it is technically and commercially feasible to harvest more efficiently the haem, or the corresponding form lacking the iron – protoporphyrin IX. Secondly, new approaches were investigated to determine whether these molecules could be more efficiently attached to suitable support materials to generate low cost resins with the capability to adsorb or trap as a commercially viable process other metal ions or other contaminants, such as those found in waste streams in the mining, the chemical or food manufacturing industries.

The Project was structured to be carried out in two discrete stages, the first dealing with an examination of refinements to the extraction of haem from bovine blood and the generation of the protoporphyrin IX, and the second dealing with the development and evaluation at a laboratory scale of new types of adsorbents capable to trapping metal ions. Because of their chemical structures haem and protoporphyrin IX have the potentiaql to be immobilised via their carboxylic acid groups (of the propanoic acid side chains) or vinyl groups onto solid supports such as activated cellulosic or other polysaccharidcxe materials, synthetic polymers, inorganic, ceramics or other composite materials to create re-usable adsorbents for use in multiple cycles of selective metal ion capture or other types of applications. When recyclability to reasonable cycle numbers is achieved, then these new adsorbents would clearly provide significant economic advantages over traditional methods of capture employing, e.g. activated charcoal which are limited to single use applications and which do not readily allow the selective recovery of specific metal ions or other bound compounds through optimised elution regimes. To achieve these ends, 6 technical tasks were experimentally undertaken sequentially as part of this project as follows:

- **Task A:** - Optimisation of the isolation of bovine haem (BH) and protoporphyrin IX (PIX) from bovine blood
- **Task B:** - Examination of the binding / displacement properties of the isolated BH and PIX with various metal ions in free solution

- **Task C:** - Evaluation of alternative strategies for the immobilisation of purified BH onto a range of solid supports to produce new classes of adsorbent materials
- **Task D: -** Evaluation of alternative strategies for the immobilisation of purified PIX onto a range of solid supports to produce new classes of metal-binding adsorbent materials or adsorbent materials with other binding attributes such as pseudo-ion exchange or mixed mode features.
- **Task E:** - Evaluation of the binding performance of newly prepared metal-binding adsorbent materials with different metal ions in batch adsorbent systems
- **Task F:** - Examination of the feasibility to employ the immobilised BH or PIX adsorbents in alternative pseudo-ion exchange or mixed mode binding / displacement modes of adsorption.

Project Outcomes:

Successful completion of these tasks has confirmed that

(a) it is feasible to extract at laboratory scale the haemoglobin from bovine blood erythrocytes using new cost efficient procedures under mild, solvent free conditions;

(b) it is feasible to produce the haem from the haemoglobin under mild, solvent free conditions that involved a number of 'in stream' process modifications and product characterisation studies, which also enable the globin component to be recovered and used in other applications;

(c) it is feasible to produce in excellent yields the protoporphyrin IX from the haem with no subsequent loss of iron binding functionality as demonstrated through product characterisation studies, associated batch binding studies and acquisition of data on the respective metal binding stability constants;

(d) it is feasible to immobilise the haem and the derived protoporphyrin IX onto commercially available support materials appropriate for large scale applications;

(e) the recovered protoporphyrin IX once immobilised retains the ability to bind iron and other ions; and

(f) the immobilised haem and protoporphyrin IX once immobilised retain the ability to also function as pseudo-ion exchange and mixed mode adsorbents with the capability to bind to other value-adding components in waste production streams.

Economic Modelling by Lycopodium

Based on the experimental data and results obtained in the Feasibility Study on new processes for the manufacture of haem (bHb) and protoporphyrin IX (bPIX) using bovine blood (bB) as the feedstock, the basis for the deployment of the technologies was refined, scaled to an industrial process and analysed in accordance with Meat and Livestock Australia's (MLA's) "Guide to Value Propositions and Cost/Benefit Analysis v1.0".

The products from this Feasibility Study have relevance to the following MLA AOP KPI's:

- 2.3: Developing new products
- 3.2: Increasing cost efficiency and productivity – off farm

Various potential uses for bPIX in specific products were considered, such as applications in separation science, therapeutic uses and as bio-active dietary supplements. The major assumptions made for this analysis of bHb and bPIX production from bB were based on an Earnings Before Income Tax (EBIT) scenario, 7% discount rate, a batch production procedure employing a process volume of approximately 1000 L per unit operation, and a payback period considered from the date when full production commences (i.e. with the assumption that the total capital investment (TCI) and all start-up costs are expended at the start of the first year of full scale production).

A large number of alternative scenarios were considered. The cost benefit analysis was performed at a processor enterprise level (i.e. the production of bHb and bPIX from bB at a specific abattoir site) and at a final product level (i.e. conversion of bHb or bPIX to a final product suitable for the intended end-use within different targeted markets by a third party manufacturer or alternatively through a joint venture partnership). Cost estimation for construction works, where appropriate, was made in accordance with Rawlinson's Construction Handbook (2011, indexed to 2012). Other cost estimations were in accordance with standard industry cost estimation techniques, such as the AACE International Recommended Practice No. 18R-97, "Cost Estimate Classification System – As Applied in Engineering, Procurement, and Construction for the Process Industries". From this analysis, the economic results were found to be most sensitive to the value of bPIX sold by the manufacturer (or the mass of PIX sold), followed by the operating cost, and least sensitive to the total capital investment (TCI).

This assessment also considered the scale of and associated economics for conversion of the laboratory scale process derived from this Feasibility Study to proposed industrial scale with a scaling factor >30. Alternative block flow diagrams for different scaling alternatives consistent with these objectives were considered.

Based on this analysis, a base case for the deployment of the technologies at the proposed industrial scale was derived and a ranking of economically viable options established, based on the application of bHb and bPIX in different application fields. This analysis indicated that for a processing facility with the capacity to handle the collection of blood from 200,000 cattle per annum (12.0 L BB per head) and 1 million lambs per annum (3.0 L blood per head), equating to 5.4 million L per annum of blood, the proposed facility using 1000 L per batch would consume approximately 3.2% per annum of the available blood volume and generate in excess of 600 Kg bPIX per annum if only a single operational batch cycle installation was employed.

In accordance with the criteria set out in the document “MLA Guide to Value Propositions and Cost/Benefit Analysis v1.0”, the context of the MLA AOP KPI 3.2 (Increasing cost efficiency and productivity – off farm), the base case results in a simple value add per each animal of $3.15, but if the target was $3.50 / head the bPIX would need to be sold by the manufacturer at ca 15% higher value. Because in the scenario of 1000 L per batch, the bPIX production requires a relatively small amount of feedstock: e.g. only 3.2% of the available BB from a 5200 head per day meat processing facility, the ROI could be further improved by relatively small increases in the value of the bPIX on a per head basis although even from with this 1000 L per batch scenario the discounted payback period was excellent (< 1 year). Alternatively, the analysis indicates that reduction in operating costs of the order of 12.5% through the use of larger process scales would results in the required threshold of $3.50 / head being met. Not unexpectedly, the sensitivity analysis confirmed that the economic viability of the technology was sensitive to revenue generated, which is a direct function of bPIX value and amount of bPIX sold.