final report

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Commercialisation of bioactives

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Executive summary

Lycopodium Process Industries Pty Ltd (Lycopodium) was engaged by Meat and Livestock Australia (MLA) to complete a cost / benefit analysis (CBA) of a process for the production of Alkaline Phosphatase (AP). This study was completed in accordance with the document *'MLA Guide to Value Propositions and Cost Benefit'.* The approach taken was to develop a process engineering economics model tailored to the unique data developed by Flinders University for the over-expression of AP in bovine tissue. A number of assumptions were made to generate the model, with the assumptions having the greatest influence on the economic viability of the project being the non-distributor margin, 150 batches per annum of AP are sold, and that AP production (that the scaled up AP yields are the same as at the smaller scale and that the yield from purification is 61%). The table below shows the findings of the economic analysis. This data shows that there would be a risk in the investment never being paid back for the novel production system. The traditional process is the most promising production option.

Scenario		Results			
Scale and Yield	Non-Distributor Margin [%]	DPP [yrs]	NPV [mil $ over 10 years]	Value add [$ / head]	Annual Net Benefit [mil $ pa]
17.6 kg, traditional, no-disaggregation, 411 U/ml.	40%	1.36	45.1	31.29	4.5
17.6 kg, traditional, no-disaggregation, 411 U/ml.	20%	2.85	9.2	6.42	0.92
17.6 kg, traditional, no-disaggregation, 411 U/ml.	15%	10.34	0.29	0.20	0.03
0.50 kg, novel, non-automated, 1975 U/ml.	40%	2.38	10.4	7.25	1.04
0.50 kg, novel, non-automated, 1975 U/ml.	23.96	11.00	0.0	0.00	0.00
0.50 kg, novel, non-automated, 1975 U/ml.	20%	N.A.	-2.6	-1.79	-0.26
0.50 kg, novel, non-automated, 1481.3 U/mL	40%	4.2	4.0	2.75	0.42
0.50 kg, novel, non-automated, 1481.3 U/mL	31.9%	11.00	0.0	0.00	0.00
0.50 kg, novel, non-automated, 1481.3 U/mL	20%	N.A.	-5.8	-4.03	-0.58

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1 Purpose and description

Overall, the purpose of this consultancy is to provide data essential to making informed and rationale commercial decisions and to the development of the adoption strategy. High value bioactive co-products of red meat have the potential to improve the profitability of the red meat industry. MLA has an extensive programme investigating various options for the production of bioactive products. One project (A.BIT0008) aims to develop new bioprocesses by amplification of the yields of commercially valuable bioactive products from cultured animal organs. In phase 1 of this project, up to 43-fold amplification of the levels of certain bioactives was reported in small-scale laboratory trials. Phase 2 has focused on scale-up to commercially competitive levels using alkaline phosphatase (AP) as a model bioactive. The external consultant, Dr Gareth Forde, has analysed the production process and generated an engineering-based economic model and sensitivity analysis for the manufacture of bioactives using the organ culture method developed. This analysis was conducted after phase 1 of the project. It is now timely to refine and further develop this model incorporating:

1. Updated technical and financial data (yield, production time, COGS, CAPEX, etc.) generated during phase 2,
2. More accurate data on the transfer price within the supply chain, and
3. Bioactives, other than alkaline phosphatase, that are co-stimulated and are of commercial value.

Additionally, the project will provide data on the market size (at various points in the supply chain), market dynamics (growth, contraction, prospect of disruptive technology) and market need to enable a SWOT analysis. This project will also develop separate analyses identifying the costs and benefits accruing to:

1. The red meat industry based on the "*MLA Guide to Value Propositions and Cost/Benefit*", and
2. A distributor of the bioactive products which will be used in a pitch of this technology to these organisations (customers for the technology).

The project will identify gaps in the technical data required for the development of an adoption strategy. It is important to identify and fill such gaps before the technical aspects of the project are complete.

2 Refinement of process engineering economic model

2.1 Yield Data

Data source	Data and findings	Volumetric yield used for process engineering economics model
Dr J. Adams estimate, 2010.	Volumetric AP activity in 1700 ml of crude culture: Initial conc: 411 U/ml. Final conc. 1386 U/ml. Assuming 10,000 U/mg protein, gives 0.1386 mg AP / ml and hence 235.62 mg AP per 500 g wet weight bovine batch following stimulation.	1386 U/ml
PM 5.1	Chemical stimulants and rocking did not increase production of the bioactive AP in ovine liver above the level previously demonstrated in unstimutated minced organ cultures (Milestone 4.2). Optimal AP production was obtained within 16hrs of culture in the conditioned medium without changing the media. Unable to increase the production of AP above the level that we reported in milestone 4.2.	
PM 5.3	W1: 1997 U/ml; W2: 1975 U/ml. At the same mince:medium, production of AP per ml and per gm were similar in both the bioreactor and 6-well plate.	1975 U/ml.
PM 6.1	WAVE#2 sample resulted in an AP-activity of 1481.3+/-122.5 U/mL, 178.04 +/-14.03 U/mg of protein. Activity of Alkaline Phosphatase per mg of total protein resulted in a 17.5-fold increase for the WAVE#2 sample.	1481.3 U/mL
PM 6.3	No consideration of AP – PM scaled-up the process in a laboratory-scale bioreactor system for disaggregated cells testing cell adherence and survival.	

Trial	6 well plate	B1-A	B1-B	B2	W1	W2
Reactor type	6 well plate	Stirred tank	Stirred tank	Stirred tank	Wave	Wave
Inoculum (g)	1.5	500	500	150	150	375
Volume (ml)	2	666	1666	1000	500	500
Mince/Medium ratio (g/ml)	0.75	0.75	0.3	0.15	0.3	0.75
Air source	5% CO2	air	air	air	5% CO2	5% CO2
Air flow rate (L/min)	NA	1	1	0.6	0.1	0.1
Stir speed	NA	120 rpm	120 rpm	107	NA	NA
Rock speed	NA	NA	NA	NA	24 rpm/7º	24 rpm/7º
AP (U/ml) at 48hrs	1615	NA	635	313	1997	1975
AP (U/g) at 48hrs	1211	NA	187	63	599	1481

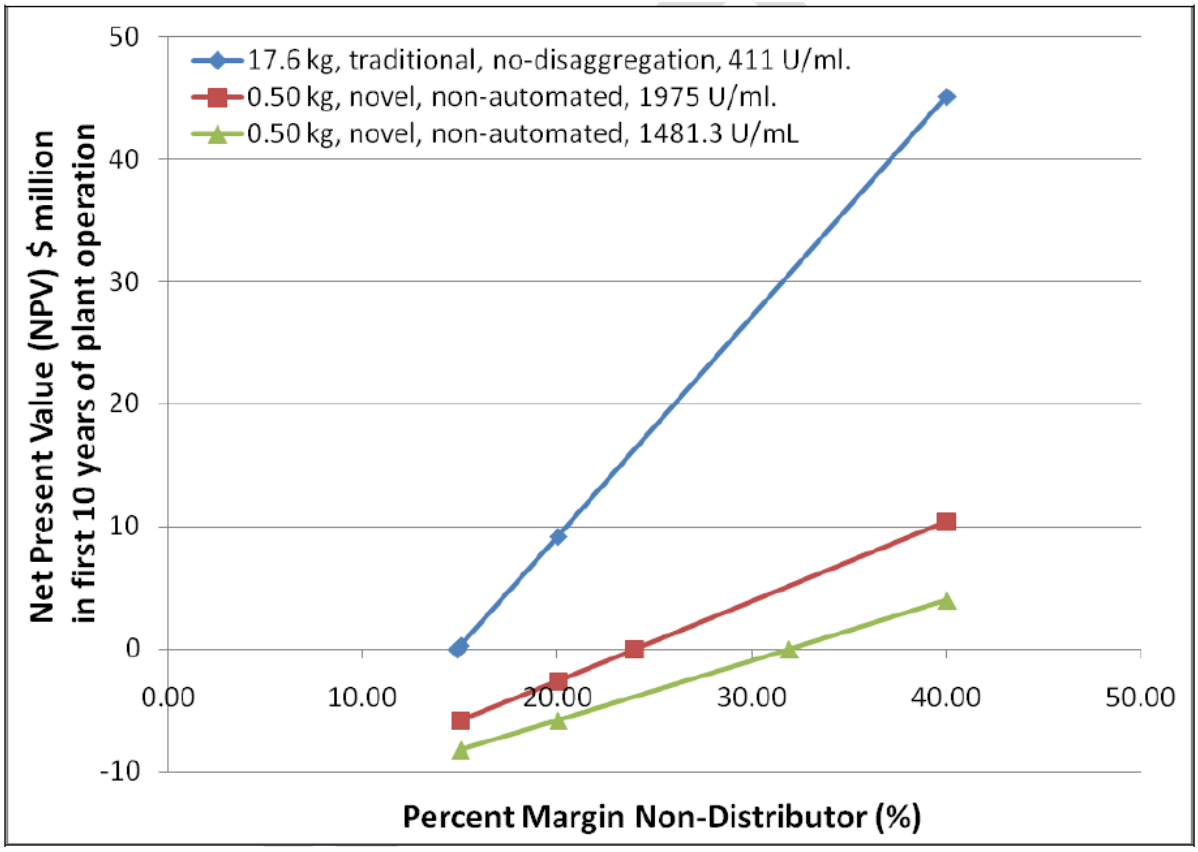
Table 1. Bioreactor conditions and yields

2.2 Refinement of model

Two base cases were used: a non-automated, traditional extraction facility processing 17.6 kg of tissue per batch and a 0.50 kg non-automated facility making use of the novel process. The main parameters varied were the margin being returned to the producer of the bulk bio-molecules and the yield, as per the following table:

	Scenario		Results			
	Scale and Yield	Non-Distributor Margin [%]	DPP [yrs]	NPV [mil $ over 10 years]	Value add [$ / head]	Annual Net Benefit [mil $ pa]
1	17.6 kg, traditional, no-disaggregation, 411 U/ml.	40%	1.36	45.1	31.29	4.5
2	17.6 kg, traditional, no-disaggregation, 411 U/ml.	20%	2.85	9.2	6.42	0.92
3	17.6 kg, traditional, no-disaggregation, 411 U/ml.	15%	10.34	0.29	0.20	0.03
4	17.6 kg, traditional, no-disaggregation, 411 U/ml.	14.84%	11.00	0.00	0.00	0.00
5	0.50 kg, novel, non-automated, 1975 U/ml.	40%	2.38	10.4	7.25	1.04
6	0.50 kg, novel, non-automated, 1975 U/ml.	23.96	11.00	0.0	0.00	0.00
7	0.50 kg, novel, non-automated, 1975 U/ml.	20%	N.A.	-2.6	-1.79	-0.26
8	0.50 kg, novel, non-automated, 1975 U/ml.	15%	N.A.	-5.8	-4.05	-0.58
9	0.50 kg, novel, non-automated, 1481.3 U/mL	40%	4.2	4.0	2.75	0.42
10	0.50 kg, novel, non-automated, 1481.3 U/mL	31.9%	11.00	0.0	0.00	0.00
11	0.50 kg, novel, non-automated, 1481.3 U/mL	20%	N.A.	-5.8	-4.03	-0.58
12	0.50 kg, novel, non-automated, 1481.3 U/mL	15%	N.A.	-8.2	-5.73	-0.82

Using the large scale approach for 17.6 kg of tissue for a non-automated bioprocess facility, ensures that for non-distrubuto margins of 14.8% and above the cost to construct and operate the plant will be realized within 10 years of the plant being operational. The figure below shows the graphical output of the above table.



3 Downstream purification

The Milestone Number 6.2 results created the following information of per tinent relevance to this project:

- AP was able to be purified in a two stage process (precipitation an anion exchange chromatography) from minced organ culture medium

The most successful purification achieved a 44% recovery of activity and a 60 fold increase in AP concentration. The economic model assumed that three purification stages would be required (e.g. anion exchange, gel filtration (size), and hydrophobic interaction chromatography) to obtain pharmaceutical grade AP. It was further assumed that each stage resulted in a yield of 85%, that is 85% of the active units of enzyme that are applied to the chromatography column are able to be captured in the purified and concentrated target fractions. Based on the data provided in the first pass Milestone 6.2, results it is a reasonable assumption that after 6 – 18 months of downstream purification R&D that the assumed purity and yields would be able to be achieved.

4 MLA guide to value propositions and cost benefit analysis methodology

This section outlines some of the decisions that were made in relation to implementationof the document *'MLA Guide to Value Propositions and Cost Benefit'.* The *'MLA Guide to Value Propositions and Cost Benefit'* assesses whether MLA should invest in a particular line of research and development (R&D). The area most pertinent to this project is "Developing new products" [2.3], hence the MLA Annual Operating Plan (AOP) KPI associated with this area is to "Develop Technologies and capabilities along the supply chain, capable of meeting consumer demand resulting in a net increase in the worth of the carcass by $5/head for cattle…from value added red meat products and bioactives". In keeping with section 2.3, this report meets the requirements for the R&D stage CBA. The enterprise level is that of a bio-actives manufacturer from tissue collection to the point where the 'bulk' purified bioactive is sold to a distributor. The calculations are based on a single plant positioned immediately adjacent to an existing abattoir that collects and processes the tissue on-site. The facility is assumed to have a life span of 10 years.

5 Market dynamics and analysis

5.1 Market Dynamics

Specialty Medical Enzymes represents the largest product segment for specialty enzymes. Enzymes including Asparaginase, Alkaline Phosphatase and Peroxidase among others are projected to demonstrate the fastest compound annual growth rate (CAGR) of 5.6% from 2010 to 2015. The global market for Specialty Enzymes is forecast to reach US$4.3 billion by the year 2015. Pharmaceuticals & Diagnostics represents the largest as well as the fastest growing end-use segment of specialty enzymes. Source: Specialty Enzymes – A Global Strategic Business Report, Global Industry Analysts, Inc., Oct 2010. http://www.prweb.com/releases/specialty_enzymes/specialty_medical_enzymes/prweb4714354.htmIn 1993, the global market for immunoassays using AP, horseradish peroxidase, luciferase and galactosidase was US$25mil. Hence, the value of AP would be expected to be in the vicinity of US$6.25 million assuming a even distribution. This equates to $9.73 million. Based on this information, the large scale production would equate to 43.9% of the global market. The following table outlines producers of AP:

Accurate Chemical and Scientific Corporation	USA
Advanced Technology &Industrial Co. Ltd	Hong Kong, China
Apollo Scientific Ltd.	UK
Chemos GmbH	Germany
Discovery Fine Chemicals	UK
Lee Biosolutions, Inc.	USA
New England Biolabs	MA, USA

ProteoChem, Inc.	USA
Sigma-Aldrich	USA
Thermo Fisher Scientific (Milwaukee) LLC	USA
USB / Affimetrix	CA, USA
Worthington Biochemical Corporation	NJ, USA

The following table outlines the changes in the various prices of Bovine Sourced AP from Sigma-Aldrich. Of greatest concern is the release of a recombinant protein expressed in a yeast that is only 2.65 the cost of intestinal mucosa sourced AP in its first year.

	Tissue Source			**$ / Unit as supplied**				
YEAR	Intestnial Mucosa	Bovine Liver	Bovine Kidney	Intestinal Mucosa	Bovine Liver	Bovine Kidney	Recombinant Bovine - *E. coli. P4252-1KU*	Recombinant Bovine - *Pichia pastoris. D5319*
2007	$1,295.40	$627.30	$583.10	0.026	2.509	0.583	1.239	NA
2009	$1,347.00	$592.00	$606.50	0.027	2.368	0.607	1.146	NA
2010	$1,529.55	$829.15	$771.47	0.031	3.317	0.771		
2011	1622.4	$878.80	$817.44	**0.032**	3.515	0.817		
2012	1653.79		$833.70	**0.033**		0.834	1.738	0.088