# FINAL REPORT 2015/902

## Biorefineries for Profit - A profitable future for Australian agriculture

## SRA CONFIDENTIAL

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| **Final report prepared by:** | Queensland University of Technology |
| **Chief Investigator(s):** | Prof Ian O'Hara, Dr. Mark Harrison, A/Prof Robert Speight, Prof Wiliam Doherty, Dr Tony Vancov, Dr Zhanying Zhang, Dr Stephen Cox, A/Prof Philip Hobson |
| **Research organisation(s):** | Queensland University of Technology, NSW Department of Primary Industries |
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Sugar Research Australia

Australian Government
Department of Agriculture and Water Resources

# Rural R&D for Profit Program

## A profitable future for Australian agriculture: Biorefineries for higher-value animal feeds, chemicals and fuels

## Final Report

**Sugar Research Australia**

**February 2016 – May 2019**

Contributors: I. O'Hara, M. Harrison, R. Speight, W. Doherty, T. Vancov, Z. Zhang, S. Cox, P. Hobson, L. Cao, Z. Amini, L. Gebbie, T.T. Dam, K. Schneider, L. Moghaddam, A. Baker, H. Asad, G. Cai, D. Rackemann, K. Dunn, M. Hassanpour, W. Stolz, J. Te'o, S. Sinclair, S. Salunke, R. Parker, R. Ainscough, J. Strong, S. Bottle, B. Keen, J. Price, K. Mullen, S. Khatun, R. Matindi, P. Darcy, C. Liu, J. Palmer, R. Jones, J. Bartley, S. McIntosh, H. Bakir, K. Masters

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Department of Agriculture and Water Resources
GPO Box 858 Canberra ACT 2601
Telephone 1800 900 090
Web agriculture.gov.au



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For further information on this project, please contact:

Dr Harjeet Khanna
General Manager – Research Funding Unit
Sugar Research Australia
50 Meiers Road, Indooroopilly 4068
Phone: 07 3331 3333
fundingunit@sugarresearch.com.au

Professor Ian O'Hara
Principal Research Fellow
Queensland University of Technology
2 George Street, Brisbane 4001
Phone: 07 31381 1551
i.ohara@qut.edu.au

# Contents

# Plain English summary

Australian agriculture is constantly changing to meet the challenge of rising production costs, climate variability, pests and disease, compliance costs, and changing global patterns of production and consumption. Biorefineries turn primary products, off-specification primary products, and low value or waste by-products from agriculture into higher value bioproducts. The global bioeconomy is creating new markets for agricultural producers and supporting the value of existing crop products and supply chains. Biorefinery development is an important way that Australian agriculture can ensure it remain profitable.

The *Biorefineries for Profit* project began in 2016 with a goal of establishing profitable bioproduct technologies for the Australian sugar, cotton, forestry, and pork industries. The project has developed technologies to turn agricultural waste into high-value products including animal feeds, chemicals, and advanced fuels. The project has also built the value-chain knowledge and human/organisational capacity needed to capture future biorefinery opportunities for Australian agriculture.

The project consisted of seven activities in four areas supported by partners from the sugar, cotton, forestry & wood products and pork industries. Key research areas, activities, outputs and achievements were:

**<u>Animal feeds for greater profitability</u>**

**Activity 2.1 Develop technologies for the use of sugarcane products as animal feed ingredients**

This activity developed new technologies that increased the nutritional value of sugarcane products (primarily bagasse and trash) in animal feeds and increased the potential use of sugarcane by-products as animal feed ingredients. Key outputs and achievements were:

- Development and demonstration of technology to increase digestibility of sugarcane bagasse to the same level as high-quality fodder in cattle rumen fluid trials;
- Identification of a low cost, commercially-available additive to sugarcane bagasse pretreatment that increased the rate of fibre digestion by cattle;
- Development and demonstration of technology to produce sugarcane bagasse-based liquid sugar products with a total sugar content similar to molasses and containing up to 10% prebiotic xylooligosaccharides; and
- Assessment of filamentous fungi and demonstration that they could grow in liquid culture using high-xylose syrup produced from sugarcane bagasse or in solid-state fermentation on raw or pretreated sugarcane bagasse.

**Activity 2.2 Develop feed supplements for enhanced nutritional characteristics of sugarcane based animal feeds**

This activity developed a technology pipeline for the discovery, production and optimisation of animal feed supplements (e.g. enzymes, probiotics) that work with sugarcane based animal feed ingredients to improve their nutritional value in livestock feed applications. Key outputs and achievements were:

- Stored sugarcane bagasse has been demonstrated as a valuable resource for the discovery of novel microbes and enzymes with applications in the industrial biotechnology sector, for example, biofuels, animal feed ingredients, and animal feed supplements;
- The established and characterised microbial collection from sugarcane bagasse created in this project is a novel resource for new and improved enzymes, probiotics, metabolites and/or nutritional supplements;

- From this collection, *Bacillus* strains with probiotic characteristics were identified with the five lead candidate strains cultured in scalable fermentation systems. Results from preliminary poultry feed trials suggest the strains are safe and provide nutritional benefits compared to commercial products; and
- Thermophilic fungi species with lignin degrading characteristics and other species with xylanase and other industrial enzyme activities have been discovered with potential commercial applications.

**Specialty chemicals and fuels**

**Activity 3.1 Develop technologies for the production of chemicals and pharmaceuticals from cotton wastes**

This activity developed new technologies to convert cotton residues into an intermediate chemical product (5-chloromethylfurfual; CMF) and subsequent high value molecules with potential application as pharmaceutical precursors, adhesives and other products. Key outputs and achievements were:

- Development of a reactor for the continuous production of CMF, from cotton gin trash, mote and other biomass feedstocks and the demonstration and optimisation of this process to reduce operating costs of the technology;
- Development and demonstration of a novel process for the recovery, purification and crystallisation of CMF for improved stability, storage and transportability of the product;
- Discovery and development of a new process to produce the valuable product 5-bromo-phalide (a direct pharmaceutical precursor) from CMF; and
- Discovery and demonstration of new pathways to produce commercially relevant plasticisers, adhesives, and flavour and fragrance molecules from CMF.

**Activity 3.2 Develop technologies for producing fermentable sugars and fuel ethanol from cotton gin trash**

This activity developed new technologies for the conversion of cotton gin trash into sugars suitable for fermentation into ethanol and other fermentation products. Key outputs and achievements were:

- Development and optimisation of a process for sequential dilute acid two stage pretreatment of cotton gin trash;
- Development of a fed-batch simultaneous saccharification and fermentation strategy capable of handling higher steam exploded solid loadings with high conversion efficiency; and
- Demonstration of the process at pilot scale exploiting optimised pretreatment conditions and fed-batch simultaneous saccharification and fermentation operations.

**Advanced fuels from sugarcane biomass**

**Activity 4 Develop technologies for the production of advanced fuels from sugarcane biomass**

This activity aimed to develop technology to convert carbohydrates from sugarcane by-products into oils via the use of filamentous fungi fermentation systems and optimise the productivity and yield of oil production for use as fuels. The project demonstrated the production of bio-oils from sugarcane molasses at pilot scale and the upgrading of these oils via hydrothermal liquefaction. Key outputs and achievements were:

- Sugarcane molasses was shown to be a suitable carbon source for the production of microbial oils with only minimal supplementation with inorganic nitrogen and phosphate sources;
- Microbial oil production was successfully scaled-up to 1000 L bioreactor scale at the Mackay Renewable Biocommodities Pilot Plant;
- A hydrothermal liquefaction process was demonstrated to produce high oil yields for processing of the fungal biomass into bio-oils; and

- Several novel yeast strains were identified for the production of microbial oils and co-products from molasses.

**Pathways to biorefinery development**

**Activity 5.1 Assess factors influencing biorefining innovation and adoption in the Australian sugar milling industry**

This activity aimed to assess the factors influencing biorefinery innovation and innovation adoption in enhancing the competitive position of the Australian sugar milling industry within the global value chain. The activity undertook an analysis of the Australian biofuels technological innovation system. The analysis took the form of an historical narrative, grounded in a qualitative event analysis. Key factors influencing the biorefining innovation system were identified and a series of recommendations were made to inform future activities.

**Activity 5.2 Develop opportunities for biorefinery innovation in the forest and wood products industries**

This activity assessed biorefinery value chain opportunities and feasible technologies for biorefinery innovation in the forest and wood product industries. The activity has identified regions in Australia best suited to the profitable integration of biorefineries based primarily upon forestry and wood product feedstocks. A new database has been developed which contains both historical annual primary production (volume of logs harvested) data as well as projected primary production for 2020, 2030 and 2050. The raw data has been collected and the corresponding biomass availability has been estimated on a state-by-state basis. This state based data has been aggregated so that national figures are also available. The potential for biorefining technology based on forest and wood product feedstocks to mitigate against increasing production costs, increased competition from imports and variable construction sector activity has been shown in this study to be significant.

Collaboration with project partners has been critical to the success of the project and offered the opportunity to develop, transfer and deliver outcomes. A key project strength was the ability to access pilot scale facilities through project partners enabling rapid demonstration of technologies.

Extension and adoption activities are critical to ensure adoption of technologies resulting from research outcomes. Communication and extension activities included promotion of project achievements at industry-specific conferences and regional seminars for the cotton, sugar, forestry and pork industries, the broader farming community, external partner organisations and the wider community.

# Project rationale and objectives

## Background

Australian agriculture must continually adapt to remain competitive in an environment of rising production costs, climate variability, pests and disease, compliance costs, and changing global patterns of production and consumption. One of the key pathways to a profitable future for Australian agriculture is to create biorefineries which generate higher value bioproducts from agricultural primary products, off-specification primary products, and low value or waste by-products. The emerging global bioeconomy is creating new market opportunities for agricultural producers while underpinning the viability of existing crop products and supply chains.

Australian agriculture has yet to realise full benefits from production of value-added bioproducts from biorefinery processes and the *Biorefineries for Profit* project addresses the need to develop technologies aligned with Australian feedstocks and supply-chains. The bio-based and renewable chemicals sector in Australia has the potential to be a profitable supplier of biorefinery products into Asia; and Australia's agricultural industries are uniquely placed to satisfy this rising demand. The potential benefits of the biorefinery industry for Australia will be significant, particularly if a 'whole-of-system' approach to residue management is adopted to reduce the costs and enhance the security of supply of agricultural inputs (such as electricity, fertilisers, animal feeds and fuels).

The *Biorefineries for Profit* project aimed to develop technologies to convert Australian agricultural and forestry feedstocks into new value-added products including animal feeds, chemicals, and advanced fuels. In addition, the project aimed to build value chain knowledge and the human and organisational capacity essential to capture future biorefinery opportunities for Australian agriculture.

The *Biorefineries for Profit* project seeks to establish profitable bioproduct opportunities for Australian primary producers and other participants in the sugar, cotton, forestry, and livestock industries, while creating opportunities for these same industries to reduce input costs, such as through lower cost feeds and fuels.

The development of technologies, knowledge, and capacity to increase revenue from existing agricultural, forestry, and animal industries with rapid paths to market will deliver increased productivity and profitability for primary producers and hence contribute to the objectives of the Australian Government's Rural R&D for Profit program.

## Objectives

The *Biorefineries for Profit* project aimed to enhance the profitability of primary producers through establishing new profitable biorefinery opportunities for the sugar, cotton, forest and wood products, and pork industries. The Project objectives were to:

a) Develop technologies for the production of enhanced animal feeds, chemicals and biofuels from sugarcane, cotton, forestry and pork industry residues;

b) Deliver improved pathways for the implementation of new biorefinery industries through improving capacity for technology adoption, supply and value-chain assessment; and

c) Enhance knowledge of biorefinery opportunities across the sugarcane, cotton, forestry and pork industry sectors through promoting project outcomes, industry engagement and research collaboration.

# Method and project locations

The *Biorefineries for Profit* project was administered by Sugar Research Australia, with Dr Michael O'Shea and Dr Harjeet Khanna taking responsibility for overall project administration and reporting. Professor Ian O'Hara from Queensland University of Technology was appointed as the Project Manager.

The project consisted of seven Activities in four major research Areas. Activity Leads were appointed to each activity and were responsible for the achievement of outcomes in each of these Activities. The Project areas, activities and Activity Leads are shown in the following table:

| Area | Activity | Activity Lead |
|---|---|---|
| | 1. Project initiation and management | Prof Ian O'Hara |
| **Animal feeds for greater profitability** | 2.1 Develop technologies for the use of sugarcane products as animal feed ingredients | Dr Mark Harrison |
| | 2.2 Develop feed supplements for enhanced nutritional characteristics of sugarcane based animal feeds | A/Prof Robert Speight |
| **Specialty chemicals and fuels** | 3.1 Develop technologies for the production of chemicals and pharmaceuticals from cotton wastes | Prof Bill Doherty |
| | 3.2 Develop technologies for producing fermentable sugars and fuel ethanol from cotton gin trash | Dr Tony Vancov |
| **Advanced fuel products** | 4. Develop technologies for the production of advanced fuels from sugarcane biomass | Dr Zhanying Zhang |
| **Pathways to biorefinery development** | 5.1 Assess factors influencing biorefining innovation and adoption in the Australian sugar milling industry | Dr Stephen Cox |
| | 5.2 Develop opportunities for biorefinery innovation in the forest and wood products industries | A/Prof Philip Hobson |
| | 6. Communication and extension activities | Prof Ian O'Hara |

The *Biorefineries for Profit* project was established as a multi-party collaboration project that brought together key partners capable of delivering the project objectives. Project partners included Sugar Research Australia, Cotton Research and Development Corporation, Forest & Wood Products Australia, Australian Pork Ltd, Southern Oil Refining, Queensland University of Technology and NSW Department of Primary Industries.

A Steering Committee was formed to provide governance, advice, oversight and strategic direction to the project. The steering committee consists of representatives from each of the partner organisations and included:

- Dr Michael O'Shea, Sugar Research Australia (Chair);
- Prof Ian O'Hara, Queensland University of Technology;
- Dr Allan Williams, Cotton Research Development Corporation;
- Dr Chris Lafferty, Forest and Wood Products Australia;
- Dr Rebecca Athorn, Australia Pork Limited;
- Dr Tony Vancov, New South Wales Department of Primary Industries;
- Mr Ben Tabulo, Southern Oil Refining; and
- Dr Harjeet Khanna, Sugar Research Australia.

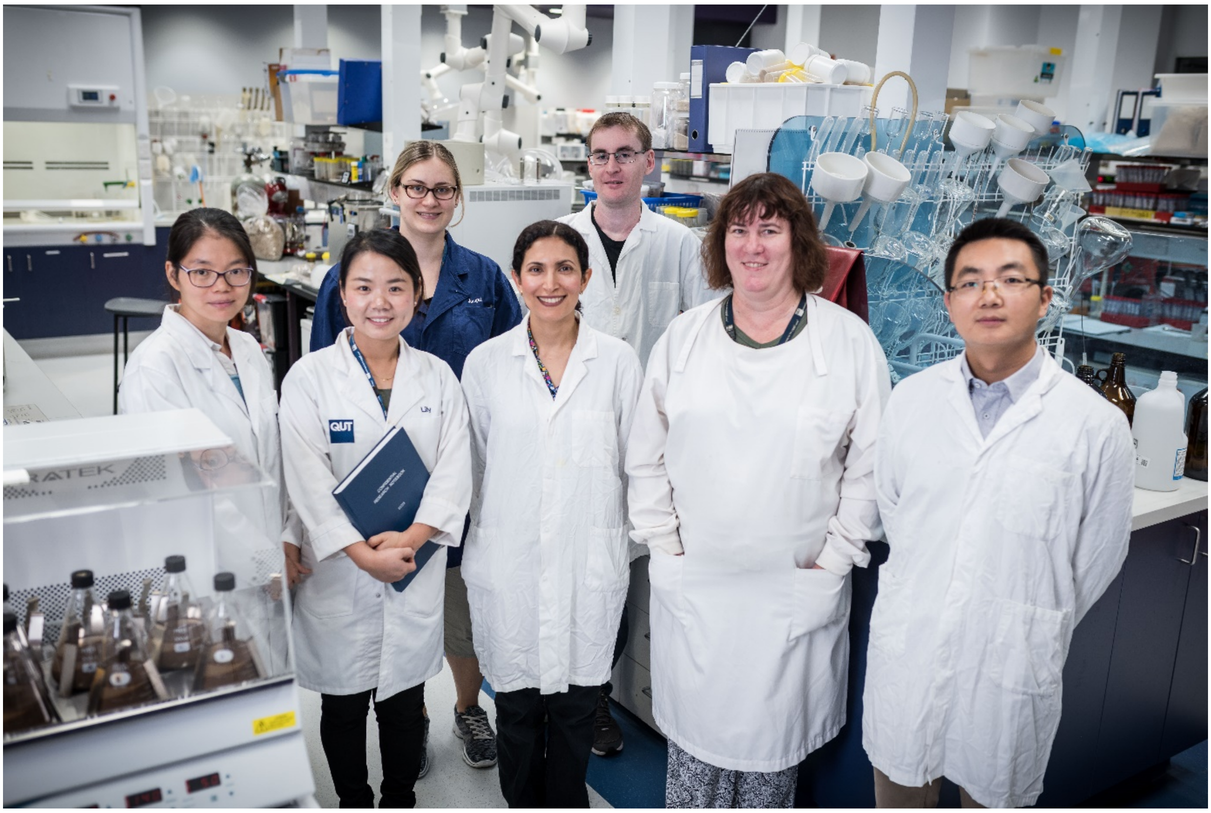
A Science Advisory Group was formed to advise the Steering Committee on technology, industry, commercialisation and adoption strategies. The science advisory group consisted of representatives with a broad range of skills and expertise and included:

- Dr Dianne Glenn, Corelli Consulting;
- Mr Gary Longden, Sugar Research Australia - Research Funding Panel;
- Mr Hywel Cook, MSF Sugar;
- Ms Meagan McKenzie, Queensland Government Department of Agriculture and Fisheries;
- Prof Mark Brown, University of Sunshine Coast; and
- Mr Scott Hogan, Southern Cotton.

Project activities were undertaken in several locations around Australia including:

| Name & type of site (field site, laboratory, project partners site, RDC headquarters) | Street Address | State | Postcode |
|---|---|---|---|
| Queensland University of Technology (laboratory, partner site) | 2 George St, Brisbane City | Queensland | 4000 |
| QUT Banyo Pilot Plant (laboratory and pilot plant site) | 457 Tufnell Rd, Banyo | Queensland | 4014 |
| QUT Mackay Renewable Biocommodities Pilot Plant (laboratory and pilot plant site) | State Route 70, located on the site of the Mackay Sugar Ltd Racecourse Sugar Mill | Queensland | 4740 |
| Wollongbar Primary Industries Institute (laboratory, partner site) | 1243 Bruxner Hwy, Wollongbar | New South Wales | 2477 |
| University of Queensland (laboratory and animal testing facility) | Gatton Campus, Gatton | Queensland | 4343 |
| Cotton Research & Development Corporation (partner site) | 2 Lloyd St, Narrabri | New South Wales | 2390 |
| Forest and Wood Products Australia (partner site) | Level 11, 10-16 Queen Street, Melbourne | Victoria | 3000 |
| Southern Oil Refining (partner site) | 42 Lewington St, Bomen | New South Wales | 2650 |
| Sugar Research Australia (partner site) | 50 Meiers Rd, Indooroopilly | Queensland | 4068 |
| Australia Pork Limited (partner site) | Level 2, 2 Brisbane Avenue, Barton | Australian Capital Territory | 2600 |

The outputs and prospective outcomes from this project are applicable to participants in Australian sugarcane, cotton, forest and wood products and livestock industries. Sugarcane in Australia is concentrated in coastal areas from north Queensland to the northern rivers region of NSW. Major production areas for cotton in Australia are located in the inland regions of northern New South Wales and southern Queensland. Australia has a total of 125 million hectares of forests that covers approximately 16 per cent of Australia's land area. Australia is also among the world's largest and most successful producers of commercial livestock. The potential of turning low-value waste from these industries into high-value products could significantly impact the future business models of the sugarcane, cotton, forest and wood products and livestock industries. Potential impacts from implementation of the project outputs include the development of a new income stream for producers within agricultural industries which can also lead to better utilisation of wastes and value creation through the generation of new waste utilisation business models.



# Project outputs and outcomes

## Project level achievements

Project achievements for each activity including key performance indicators, outputs and prospective outcomes are detailed in the following sections of the report.

## Contribution to program objectives

The *Biorefineries for Profits* project has contributed to the program objectives of achieving productivity and profitability improvements for primary producers. The project has:

**1. Generated knowledge, technologies, products and processes of benefit to primary producers.**

The project has developed several new processes and technologies that, if implemented, will deliver new revenue streams for producers and processors within the agricultural sector. Products targeted in this project including animal feeds, fuels and chemicals can both reduce farm costs and provide new revenue opportunities in the sugar, cotton, forest and wood products and pork sectors. The technologies, products and processes are detailed further in the following sections of the report.

**2. Strengthened pathways to extend the results of R&D.**

The project engaged extensively with agricultural producers and partners to ensure a broad level of awareness of the scope and progress with the project. A detailed list of engagement activities are provided later in the report.

**3. Established and fostered industry and research collaborations.**

This project was the first time that the partner companies representing four sectors of the Australian agricultural industry collaborated to develop new biorefining technologies of broad benefit across the sectors. New collaborations were formed with partner organisations and participants from within industry sectors that are likely to extend into new projects of mutual benefit. A more detailed description of these new collaborations is provided later in the report.

## The evaluation report

A project evaluation report was undertaken by Agtrans Research to analyse the expected and/or demonstrated quantifiable returns on investment of project activities. The impact assessment formed part of the project's end-of-project reporting requirements and a summary of the methodology and results are below. The following summary is an extract from the evaluation report.

**1. Methodology**

The impact assessment used a logical framework approach. This entailed describing the project inputs and then qualitatively identifying and describing the key activities, outputs and outcomes of the investment. Economic, environmental and social impacts then were identified and described.

Following the qualitative descriptions completed using the logical framework, Cost-Benefit Analysis (CBA) was carried out for those impacts identified from the project that could be confidently valued in monetary terms. The CBA therefore focused on economic impacts from the investment where clear, logical pathways could be described from the project activities through to actual or potential future impacts, and for which there were sufficient data available to make reasonable assumptions.

All assumptions made for the impact valuations were described and recorded as part of the impact assessment reporting process. Results of the CBA then were reported and included investment criteria such as the net present value (NPV), benefit-cost ratio (BCR), internal rate of return (IRR) and modified internal rate of return (MIRR).

**2. Findings of impact assessment**

The investment in the joint Biorefineries investment has been successful in that significant findings have been delivered across several areas that may well lead to new uses of what have been traditionally been viewed as by-products or waste products associated with several mainstream rural industries, namely cotton, sugarcane and forestry.

The $6.2 million initial R&D investment (nominal terms) was made over 5 years (years ending June 2015 to 2019) with an additional year of funding of $1.6 million for 2019-2020 to further the most encouraging commercial opportunities discovered to date.

This assessment focuses on the prospective benefits to Australia from the total six years of R&D investment. This assessment is not technical or scientific in nature, but its contribution is in the logical framework it applies to indicate the pathway to future economic impact that the Rural R&D for Profit investment has offered. The activities carried out within the investment are generally in accord with the project plan and a number of findings and outputs have been reported and prospects for their future usage are being progressed. The project has been successful in engaging a number of commercial entities users across a range of industries and sectors.

Future advances are being driven by the extension of the Rural R&D for Profit funding, other research funding outside of Rural R&D for Profit, and interest in potential advancement from a range of commercial groups. Hence, outcomes and impacts are largely prospective and, in the main, their delivery is likely to occur in the future.

Further, this economic evaluation provides an indication that the investment has been worthwhile by valuing only three impacts from the six identified in this report. Despite conservative assumptions and a range of risk factors being accommodated in the three impacts valued, the investment criteria estimated for the R&D investment are positive.

To estimate the investment criteria for this project it was necessary to make a number of uncertain assumptions about potential outcomes and impacts, including the results of the last 1.5 years of the investment in two of the three impacts valued. Given the assumptions made, the investment criteria estimated for total investment in the project of $9.8 million (present value of R&D costs) were positive with an expected present value of benefits of $50.1 million, an expected net present value estimated at $40.4 million, an expected benefit-cost ratio of 5.2 to 1,all estimated using a discount rate of 5% and with benefits estimated over 30 years from the final year of R&D investment (2019/20). The internal rate of return was 11.9% and the modified internal rate of return was 7.3%.

# Animal feeds for greater profitability

Global demand for animal products is increasing, with significant growth in demand in Asia; and Australian agriculture is uniquely placed to satisfy these demands. As a result, there is a need to develop new feed products incorporating ingredients with lower prices, greater availability, and comparable or enhanced feed characteristics. Biomass residues from agricultural crops such as sugarcane have significant potential to be used for animal feeds and are generally cheaper than other feed ingredients. Activities in this area aim to enhance the nutritional value of these lower priced feed ingredients via four strategies that include improving digestibility of the carbohydrates, production of soluble sugar products, microbial protein enhancement, and development of supplement technologies for improved nutrient uptake.

# Activity 2.1 – Develop technologies for the use of sugarcane products as animal feed ingredients

## Introduction and objectives

Global demand for Australian animal products such as meat, cheese, and milk powder is increasing, and our agricultural sector is responding to this demand by increasing production. This means we also need to produce more animal feed. In order to supply growing export markets, there is a real need for feed ingredients that are cheaper, more widely available, and with the same (or better) nutritional value than existing ingredients.

Sugarcane is one of Australia's largest crops and up to 35 million tonnes of sugarcane is grown annually. Sugarcane harvesting and processing generates a range of by-products including 10 million tonnes of bagasse (the fibrous residue left over when the sugarcane is processed at the mill) and trash (the tops and leaves of the sugarcane plant that remain in the field when the stalk is harvested).

Bagasse and trash are poorly digestible in both ruminant and monogastric animals. In the sugar milling process, harvested sugarcane billets are shredded, milled, and washed extensively to remove sugar; this process leaves the bagasse with little, if any, protein or minerals and a very high fibre content. Feeding sugarcane bagasse to ruminants is rarely done and only undertaken when other fibre source are not available.

Trash is a mixture of sugarcane leaves and tops. Sugarcane billets and trash are separated during harvesting, so trash is not processed through the sugar mill. Trash can be collected either during or after harvesting. Despite having a higher protein and soluble sugar content than bagasse, sugarcane trash is, at best, a low-quality fibre source for ruminant feed.

Fibre in bagasse and trash consists of cellulose (a glucose polymer), hemicellulose (a polymer made up of xylose and other sugars), and lignin (a phenolic polymer); as a result, the total sugar content in bagasse and trash fibre is 65%. Despite the high sugar content in bagasse and trash, they are poorly digested by animals because the fibre polymers are inherently stable and the linkages between them are strong.

Pretreatment of crop by-products at elevated temperatures breaks down cellulose, hemicellulose, and lignin in fibre. These changes to the structure and chemistry of the fibre in crop by-products allows rumen microorganisms to more rapidly and completely use the cellulose and hemicellulose in the fibre to fuel their growth. In turn, the increased microbial biomass in the rumen serves as a source of energy and protein to support increased weight gain in the animal.

Pretreatment of crop by-products generates two products; residual solid and liquid hydrolysate. The selection of pretreatment system and conditions determines what proportion of the cellulose, hemicellulose or lignin is dissolved. Hemicellulose is the most susceptible of the fibre polymers to solubilisation and, by controlling the pretreatment conditions, crop by-product pretreatment can produce hydrolysates containing monosaccharides (xylose) and prebiotic oligosaccharides.

Upcycling sugarcane bagasse and trash into animal feed ingredients would allow relatively low value crop by-products to contribute significantly to the income of Australia's 4000 sugarcane farms. The overall objective of Activity 2.1 was to develop new bagasse and trash pretreatment processes that changed their structure and chemistry to improve their nutritional value, and transform them into quality feed ingredients for animals such as cattle, pigs, and chickens.

## Results and discussion

### Benchmarking bagasse digestibility against high-quality fodder

Bagasse was collected from Racecourse Sugar Mill, Mackay, and its nutritional quality was compared to that of high-quality fodders including oaten hay, Lucerne hay, and Vetch (Table 1). High-quality fodders were sourced from a commercial supplier and consist of wind-rowed and baled whole crop.

### Table 1 Nutritional quality of sugarcane bagasse and high-quality fodder

| Fodder | NDF (%) | ADF (%) | Crude protein (%) |
|---|---|---|---|
| Bagasse | 88 | 55 | 1 |
| Oaten hay | 60 | 32 | 7 |
| Lucerne hay | 40 | 29 | 20 |
| Vetch | 36 | 27 | 20 |

Fodder with relatively high neutral detergent fibre (NDF) content will take longer to digest in the rumen, so will be consumed at a slower rate. NDF content in bagasse is at least double that of Vetch and Lucerne hay, and 28% more than that oaten hay. Acid detergent fibre (ADF) content in fodder influences the ability of an animal to digest the fodder. ADF content in bagasse is double that of Vetch and a minimum of 23% higher than oaten hay and Lucerne hay. Crude protein content is a key determinant of both the nutritional and commercial value of fodder. The crude protein content in bagasse is substantially lower than that of high-quality fodders.

The digestibility of bagasse was compared with high-quality fodder using *in vitro* rumen fluid fermentation (Figure 1). Clear differences in initial (T = 0 h) dry matter digestion between sugarcane bagasse and high-quality fodders were observed resulting from the relatively high content of soluble protein and carbohydrates in high-quality fodder. The dry matter digestibility of high-quality fodder (60% – 80%) was significantly higher than that of sugarcane bagasse (40%).

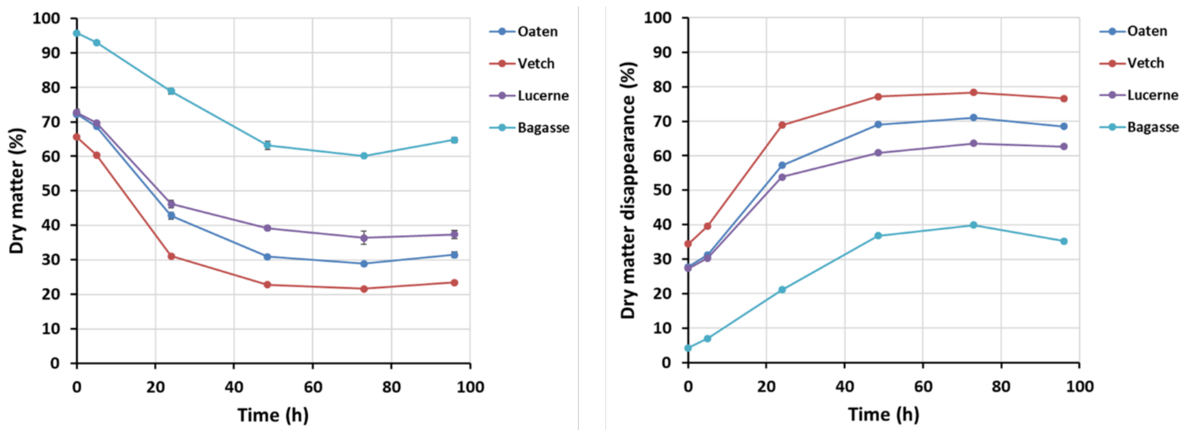



**Figure 1 Fermentation of high-quality fodder and raw bagasse in bovine rumen fluid (left) dry matter (%), (right) dry matter digestibility (%)**

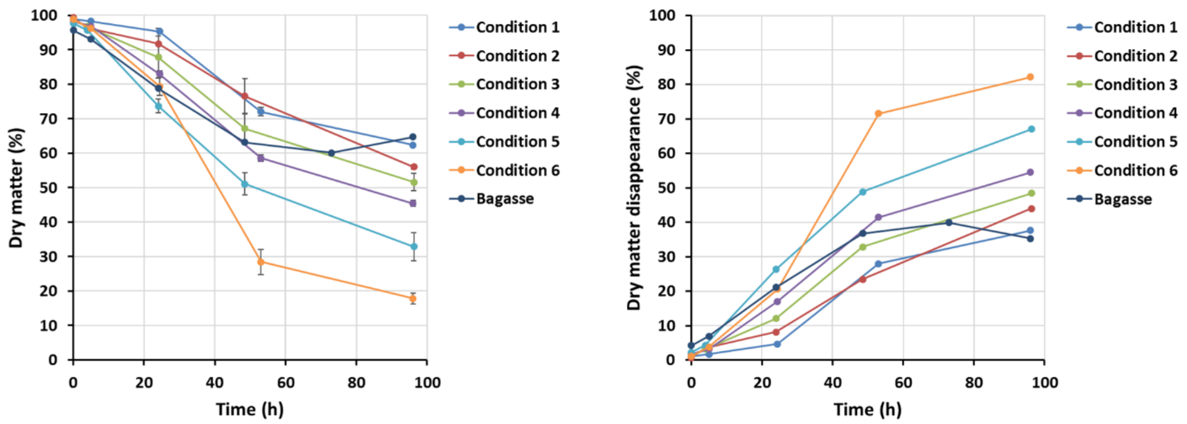
### Enhancing fibre digestibility

To enhance fibre digestibility, bagasse was pretreated using a mild alkali-catalysed system at the QUT Mackay Renewable Biocommodities Pilot Plant. Pretreatment under relatively mild alkaline conditions was considered the most promising technology for the enhancement of fibre digestibility in crop by-products for ruminants because dissolution of the hemicellulose (the cell wall sugar polymer most easily accessible to ruminant microorganisms in fodder) is minimal. The temperature and residence time were chosen to be suitable in a continuous process using existing industrial-scale equipment. The residual solids from pretreatment were analysed for biomass composition (Table 2) and the results showed that cellulose content increased, hemicellulose content was unaffected, and lignin content decreased as the severity of pretreatment increased.

### Table 2 Biomass composition of aqueous alkaline pretreated bagasse

| Condition | Cellulose (%DF) | Hemicellulose (%DF) | Lignin (%DF) |
|---|---|---|---|
| 1 | 37.8 | 23.3 | 26.2 |
| 2 | 39.1 | 22.7 | 24.7 |
| 3 | 39.6 | 22.3 | 25.2 |
| 4 | 44.3 | 23.1 | 22.5 |
| 5 | 47.6 | 23.0 | 19.7 |
| 6 | 51.8 | 23.0 | 15.6 |
| Bagasse | 36.5 | 21.5 | 26.7 |

The digestibility of alkaline pretreated bagasse was compared directly with that of untreated bagasse using *in vitro* rumen fluid fermentation (Figure 2). Dry matter digestibility of untreated bagasse reached a maximum of 40% after 48 h. Under conditions 3 to 6, the digestibility of bagasse increased in proportion to pretreatment severity. Alkaline pretreatment of bagasse under condition 6 resulted in dry matter digestibility of ~70% after 48 h, which is equivalent to that of oaten and Lucerne hay (Figure 1). Further, alkaline pretreatment of sugarcane under condition 6 resulted in dry matter digestibility of 80% after 96 h, which was superior to the high-quality fodders samples tested with the exception of Vetch (Figure 1) despite significantly higher initial dry matter content and low water-extractives.




### Figure 2 Fermentation of residual fibre pretreated in an aqueous alkaline system and raw bagasse in bovine rumen fluid: (left) dry matter (%), (right) dry matter digestibility (%)

## Enhancing fibre digestion rate

The pretreatment processes used in this project substantially enhanced the dry matter digestibility of bagasse; however, it was noted that a greater proportion of the fibre in high-quality fodder was digested in the first 24 h than was observed for pretreated bagasse (Figure 1). If the digestibility of fibre in the first 24 h can be increased, total fodder intake and/or the proportion of the fibrous crop by-products in feed can be increased.

To enhance fibre digestion rate, bagasse was pretreated using the same processes described above in the presence of a relatively cheap, commercially-available additive at the QUT Mackay Renewable Biocommodities Pilot Plant. The digestibility of alkaline/additive pretreated bagasse was compared directly with that of untreated bagasse using *in vitro* rumen fluid fermentation (Figure 3). The additive resulted in minimal increase to the maximum dry matter digestibility of pretreated bagasse relative to aqueous alkaline pretreatment (Figure 2); however, the additive significantly increased in the rate of dry matter digestion of pretreated bagasse in the first 24 h of digestion.

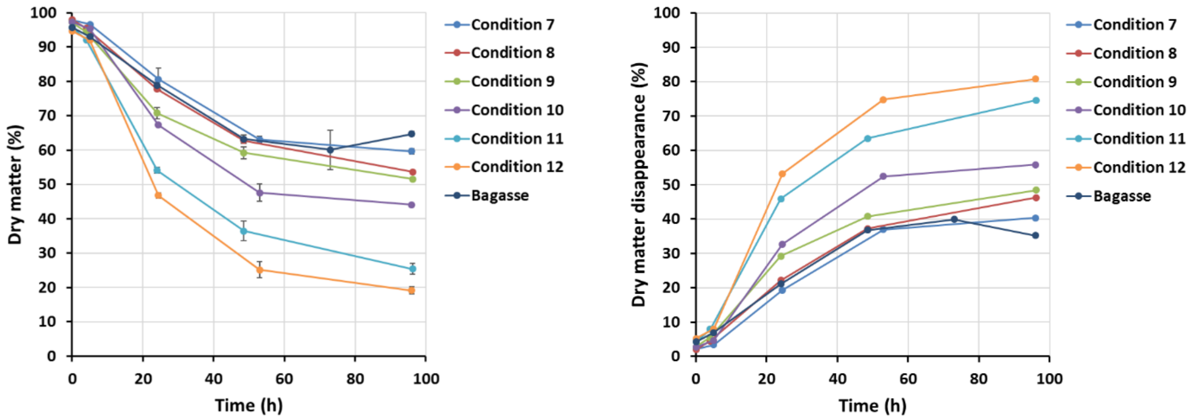



**Figure 3 Fermentation of residual fibre pretreated in an alkaline/co-solvent system and raw bagasse in bovine rumen fluid: (left) dry matter (%), (right) dry matter digestibility (%)**

## Production of sugar syrup

Sugar syrups (like molasses) are a valuable source of energy for ruminants, particularly when high-quality fodder is in limited supply. Masonex is an example of a sugar syrup produced from hardwood using dilute acid pretreatment than can be used in animal feed to supply energy.

To produce sugar syrups from sugarcane crop by-products, bagasse was pretreated using dilute acid under a range of conditions; hydrolysates were concentrated to produce syrups and then analysed for their physical properties and sugar composition in comparison to sugar mill molasses (Table 3). Syrups containing ~70% of the simple sugar content in molasses were produced from sugarcane bagasse.

Probiotics are bacteria that provide health benefits in humans and animals, including pigs and chickens. Prebiotics are indigestible sugar polymers that nourish probiotics and encourage them to function more efficiently. Xylooligosaccharides (XOS) are emerging as valuable prebiotics for both humans and animals, and are produced commercially from crop by-products rich in xylan (like corn cobs) by chemical and enzymatic methods. Bagasse is also rich in xylan. Sugar syrups produced from bagasse by dilute acid pretreatment were shown to contain 10% (w/v) prebiotic xylooligosaccharides (Table 3).

### Table 3 Properties of syrups generated from pretreated bagasse

| Condition | pH | Brix (°Bx) | Total simple sugars (%w/v) | XOS (%w/v) |
|---|---|---|---|---|
| 1 | 5.5 | 28.2 | 4.7 | 2.5 |
| 2 | 6.1 | 50.0 | 13.5 | 9.9 |
| 3 | 6.5 | 58.4 | 28.3 | 3.5 |
| 4 | 6.7 | 44.9 | 12.7 | 8.6 |
| 5 | 7.1 | 49.9 | 12.5 | 8.9 |
| 6 | 6.6 | 57.7 | 27.4 | 1.5 |
| 7 | 6.8 | 47.4 | 20.0 | 2.8 |
| 8 | 6.7 | 54.7 | 15.8 | 8.5 |
| 9 | 6.9 | 61.9 | 25.2 | 3.6 |
| 10 | 6.9 | 62.7 | 30.2 | 3.6 |
| Molasses | 5.11 | 80.5 | 44.1 | - |

### Microbial protein enriched bagasse

The pretreatment processes used in this project substantially enhanced the dry matter digestibility of bagasse but did not improve protein content. Crude protein content in feed is the combination of inorganic nitrogen (like urea) and true protein. True protein is an important component of animal feed and is typically supplied by adding grains. That means that the grain price has a big impact on the cost of feed for animals in intensive production systems like cattle feedlots, pig farms, and chicken farms. Grain supply is variable and there is growing competition between grain production for human consumption and production of animal feed.

Microorganisms contain true protein and they can contain up to 40% true protein by weight, depending on which species is grown and how it is produced. Dry stillage left over from the production of ethanol is one example of a microbial product that contains true protein and can be feed to animals, including cattle and pigs. Another example is filamentous fungi, which have a long history in human food and are able to use the sugars in crop by-products, including bagasse, to grow and produce true protein.

To enhance true protein content, filamentous fungi were grown on bagasse. Species of filamentous fungi were selected based on their previous, safe use in food production and their established capacity to grow on crop by-products from the sugarcane production system. True protein productivity of each strain on bagasse was determined and elite strains were down-selected. To enhance the rate of true protein production even further, each elite strain was grown on bagasse supplemented with hydrolysate from dilute acid pretreatment processes.

## Key findings and achievements

The shortage of conventional animal feeds is well recognised in sub-tropical and tropical regions. Sugarcane bagasse and trash have enormous potential as feed; however, the native structure and composition of these crop harvesting and processing residues limits their inclusion in feed as a source of digestible fibre, soluble sugars, or protein. Activity 2.1 has successfully identified and developed technologies that alter the structure of sugarcane bagasse and trash, and unlock their potential as feed ingredients. Technologies developed included the use of novel pretreatment technologies to enhance the nutritional value of sugarcane bagasse for ruminants and the production of prebiotic xylooligosaccharides from bagasse. The challenges are to now further develop these technologies and demonstrate them at scale so that they can be made available to farmers, apply emerging biomass processing technologies to enhance the value of sugarcane for feed, and develop new technologies to enhance the economics of animal weight gain using the products from Australia's most abundant crop residue.

Key outputs and achievements from Activity 2.1 include:

- Development and demonstration of technology for enhancing digestibility of sugarcane bagasse to a level equivalent to high quality fodders as shown in rumen fluid trials;
- Identification of a low cost, commercially available additive to pretreated sugarcane bagasse to enhance the rate of fibre digestion by ruminants;
- Development and demonstration of technology for the production of sugarcane bagasse-based liquid sugar products with a total sugar content similar to molasses and containing up to 10% prebiotic xylooligosaccharides;
- Assessment of filamentous fungi and demonstration of their capacity to grow in liquid culture using high-xylose syrup produced from sugarcane bagasse or in solid-state fermentation on raw or pretreated sugarcane bagasse.

Key knowledge outputs include:

- An understanding of the effect of room temperature chemical conditioning systems on the structure, composition, and susceptibility of sugarcane bagasse to enzymatic saccharification;
- Establishment of the relationship between severity of pilot-scale acid or base pretreatment and sugarcane bagasse composition, structure, susceptibility to enzymatic saccharification, and rumen fluid digestibility; and
- Establishment of the relationship between severity of pilot-scale aqueous acid pretreatment, yield of prebiotic xylooligosaccharides from sugarcane bagasse, and molecular weight distribution of prebiotic xylooligosaccharides.

# Activity 2.2 – Develop feed supplements for enhanced nutritional characteristics of sugarcane based animal feeds

## Introduction and objectives

Microbial products and enzyme supplements have an established track record of use in the livestock feed market leading to significant economic and environmental advantages in meat production. The use of such products is particularly important when using cheaper and more fibrous feed components. The protein and micronutrient content can be enhanced using microbial whole cells and additional nutrients and energy can be released from plant fibre using hydrolytic enzymes. In some cases, microbial whole cells may be probiotics when they confer health and productivity benefits.

The challenges in implementing effective supplement strategies arise from the need to identify agents with both high efficacy and high stability. Developing enzymes with high efficacy (high activity towards the substrates encountered in feed in conditions found in the relevant parts of the gut) and high stability (to survive the feed pelleting processes and long term storage prior to use) requires the identification of the best candidates from nature, genetic optimisation to improve properties and the generation of high yield production strains and fermentation systems. Similarly, whole cell microbial products and probiotics must display the desired nutrient profiles and probiotic properties in a stable manner and be able to be produced economically in fermentation. Activity 2.2 addressed these challenges for specific applications, including in poultry and pork production.

The objectives of Activity 2.2 were:

1. To establish a pipeline for the discovery and optimisation of animal feed supplements such as microbial whole cells for protein, micronutrient and probiotic applications as well as novel and improved hydrolytic enzymes to aid in the digestion of sugarcane products such as bagasse.
2. To develop the lead targets from objective 1 towards improved performance in specific livestock feed applications (e.g. resistance to degradation during pellet production, enhanced performance in species specific diets, effect on livestock microbiota and feed biofilm formation in the gut).
3. To develop economic, robust and scalable fermentation-based production processes to generate microbial whole cells and enzymes.
4. To demonstrate incorporation of new products in active form in animal feed.

## Results and discussion

In this activity, protocols were successfully established for sampling and identifying the microbes in sugarcane bagasse stockpiles either by culturing and/or direct DNA amplification (Figure 4 and Figure 5). In a first round of screening, 70 microbes were cultured from three depths in a six month old bagasse stockpile located at the Rocky Point Mill and a collection bin at Racecourse Sugar Mill in Mackay. In a second round of screening, 6 months later, microbial growth conditions were modified to specifically target microbes with enzyme activities and other key probiotic performance criteria using new samples from the Rocky Point Mill bagasse stockpile (Figure 5). A collection of 150 microbes isolated from bagasse was then screened for the production of enzymes involved in biomass degradation and animal feed enhancement using protocols developed for high-throughput screening and assaying (Figure 6). Strains were also characterised towards other key probiotic criteria.

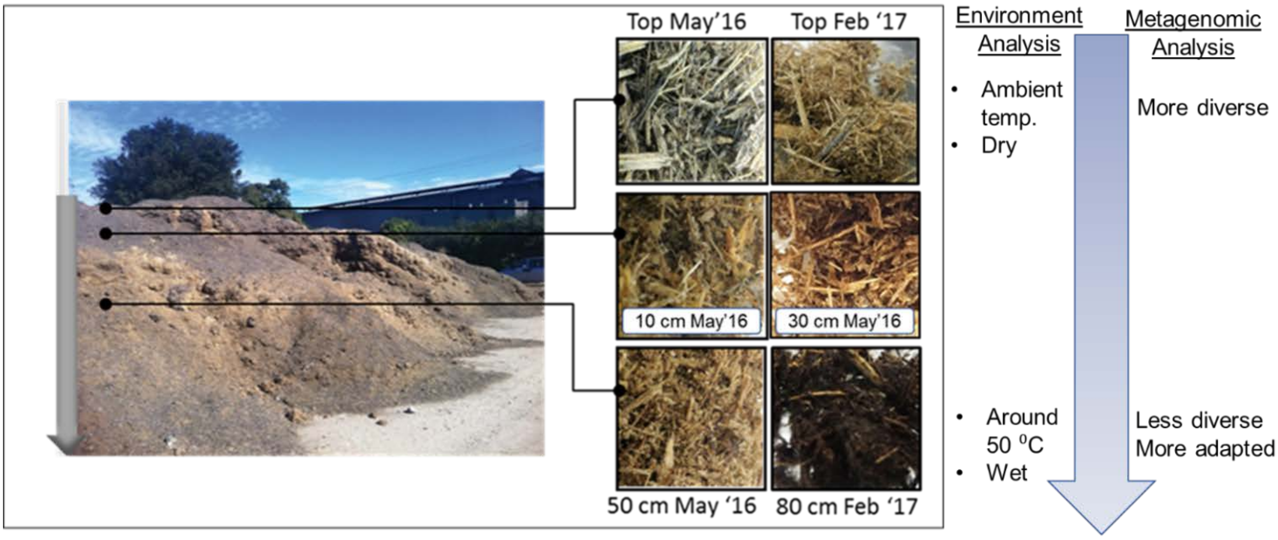



**Figure 4 Bagasse stockpile at the Rocky Point Sugar Mill showing the samples taken and the characteristics of the stockpile**

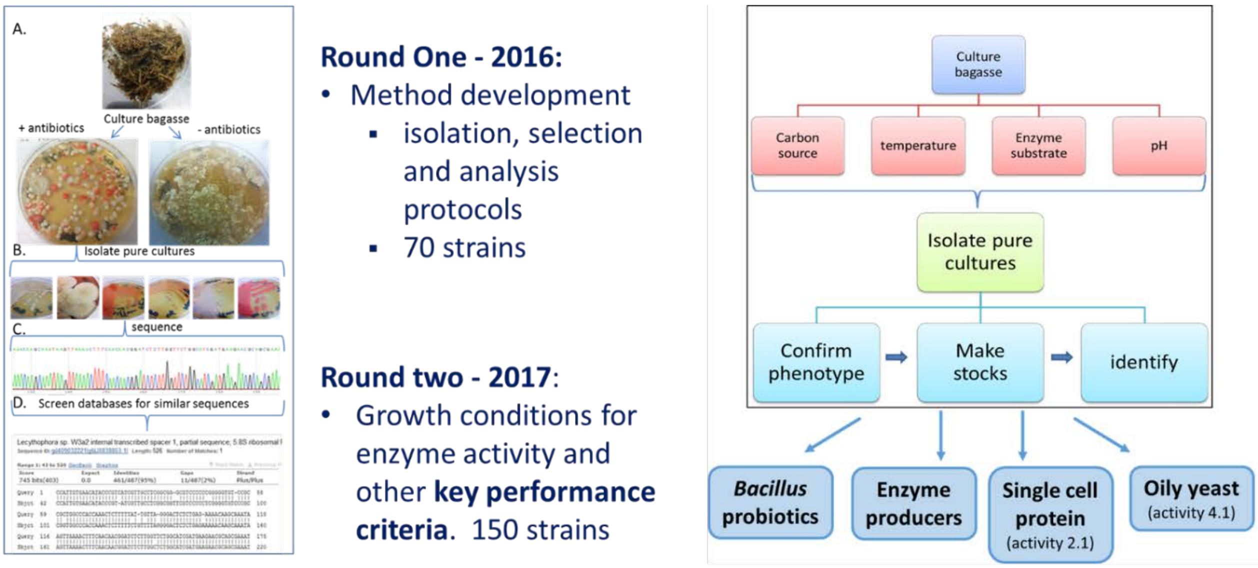



**Figure 5 Summary of methods used to isolate microbes from sugarcane bagasse**

Biomass degrading activity profiles obtained for the microbes isolated from bagasse showed that a large majority (70%) have at least one enzyme activity necessary for degrading the bagasse. When these enzymes were examined in more detail we found that some of the isolates, including potentially novel strains, are excellent hemicellulose degrading xylanase producers. Xylanases are frequently added to animal feed to improve digestion of non-starch polysaccharides, which cause numerous problems in the gut. Sugarcane bagasse is high in xylan, the substrate of this enzyme, so inclusion of this enzyme or probiotic cells that produce this enzyme in bagasse-based feed will be essential.

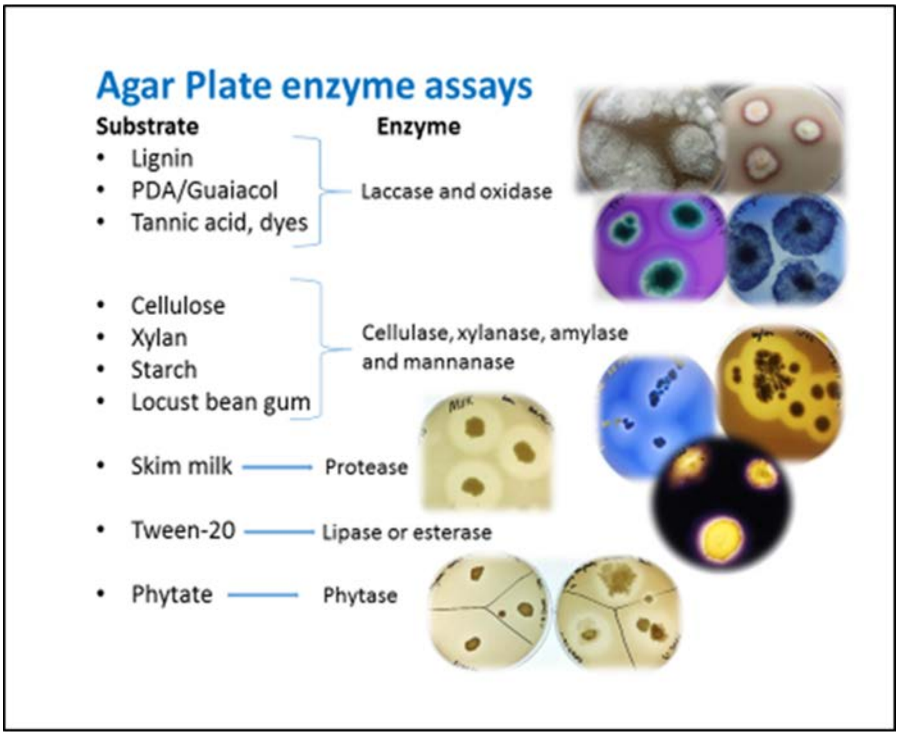



**Figure 6 Examples of the enzyme assays carried out in the study. Zones of clearance or colour changes around microbial colonies indicate enzyme activity**

Numerous isolates of a thermophilic fungal species were obtained from the bagasse. As well as having good cellulose and hemicellulose degrading enzymes, these strains are efficient at degrading lignin using enzymes displaying laccase and peroxidase activities. Industrially, these species and their enzymes could be used to modify the lignin for the production of high value chemicals and feed protein and to remove some of the lignin in bagasse based feeds for improved digestion. The fungus itself, which is non-toxic and high in protein, could be grown on bagasse in solid state fermentation or using the normally recalcitrant lignin as a carbon source for growth. The fungus supplemented bagasse would then be an enriched component of animal feed. Our research has shown that the fungi appear to degrade 50-90% of the lignin.

Thirty-one *Bacillus* strains with probiotic potential were isolated from the bagasse samples. We have assessed these *Bacillu*s strains for their potential as probiotics and enzyme producers to enhance sugarcane bagasse-based livestock feed. The 31 strains were characterised toward key probiotic criteria and compared to two commercial *Bacillus* probiotic products. Five lead candidate *Bacillus* probiotic strains were chosen based on enzyme profiles, positive results towards other probiotic criteria and to represent a spectrum of GRAS (generally regarded as safe) species. The full genomes of these strains were sequence and they were manufactured in scalable fermentation and downstream processing systems (Figure 7).

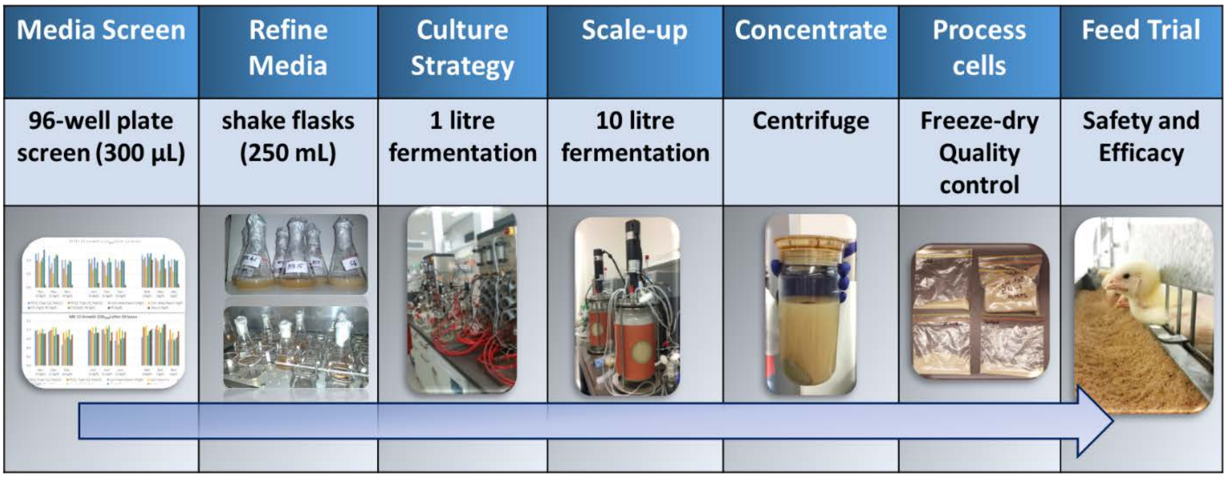



**Figure 7 Summary of the strategy used to manufacture Bacillus spores for adding to chicken feed**

The five lead strains were assessed for growth in different media, their ability to form spores required for product formulation, and for xylanase production in liquid culture, which, as mentioned above, is relevant to digestion of high fibre feeds such as those based on bagasse. High xylanase-producing isolates were cultured in bioreactors for spore production, before freeze-drying to generate a powdered formulation for effective mixing with bulk feeds. The lead candidates were assessed in two preliminary poultry feeding trials, and compared to a commercial probiotic product powder as well as a commercial xylanase enzyme product.

The first poultry feed trial with four novel *Bacillus* strains discovered in this project and a commercial product comparison measured growth performance parameters under optimal conditions and showed that the new strains had no adverse effects on the animal, suggesting they are safe. The second poultry feed trial was set up as a novel bioassay to test whether these new probiotics with high xylanase activity can improve the digestion of high fibre (high xylan content) feed. Positive effects on feed intake, excreta consistency and apparent metabolisable energy were observed with our new strains compared to controls although these differences were not always statistically significant. Further assessment in animal feeding trials is required with larger animals to identify the potential applications and benefits of these new strains.  Whole genome sequencing of the selected *Bacillus* strains suggests that they are novel and do not contain any genes encoding for toxins or antibiotic resistance. Genome sequences of the selected *Bacillus* strains are being used to explore enzymes and other novel phenotypes such as antibacterial or antifungal activity in further detail to understand probiotic effects. Further work is required to explore efficacy, test them with bagasse in feeding trials and to generate data for product registration and commercialisation.

## Key findings and achievements

Enzyme supplements have an established track record of use in livestock feed leading to significant economic and environmental advantages in animal production. Probiotics are a growing sector of the livestock supplement market. Australian agriculture is set to benefit from this project through improved livestock feed supplements leading to reduced costs as well as better utilisation of bagasse as a cheaper livestock feed component. Common industrial/commercial practice is to deposit novel strains in secure culture collections and then patent the strains. One commercialisation path is therefore to legally protect our unique Australian microbial strains discovered in the *Biorefineries for Profit* project, demonstrate manufacturing economics and performance in livestock then commercialise through partners. The initial phases of manufacturing and commercialisation through expanded feeding trials and early stages of commercial sales may also include contract manufacturing at the Mackay Renewable Biocommodities Pilot Plant.

Key project findings and achievements for Activity 2.2 include:

- Stored sugarcane bagasse has been demonstrated as a valuable resource for the discovery of novel microbes and enzymes with applications in the industrial biotechnology sector (for example biofuels (Activity 4), animal feed ingredients (Activity 2.1), and animal feed supplements (Activity 2.2);
- The established and characterised collection of 150 microbes from sugarcane bagasse is a novel resource for new and improved enzymes, probiotics, metabolites and/or nutritional supplements;
- From this collection, *Bacillus* strains with probiotic characteristics were identified with the five lead candidate strains cultured in scalable fermentation systems. Results from preliminary poultry feed trials suggest the strains are safe and provide nutritional benefits compared to commercial products; and
- Thermophilic fungi species with lignin degrading characteristics and other species with xylanase and other industrial enzyme activities have been discovered with potential commercial applications.

# Specialty chemicals and fuels

Agricultural products including sugar, cotton, and forestry products and their by-products are used for a variety of food and fibre applications with minimal additional value-adding. However, many agricultural products and their by-products have the properties to be used as feedstocks for the chemical, plastic and pharmaceutical industries. This project area aimed to develop technologies to produce bio-products from lower-cost feedstocks derived from sugarcane and cotton. Additionally, this area also examined the production of fermentable sugars and second generation biofuels (ethanol) from cotton gin trash. Successful adoption of these technologies will generate significant new revenue streams for cotton ginners and farmers as well as finding better ways of managing cotton industry residues.

# Activity 3.1 – Develop technologies for the production of chemicals and pharmaceuticals from cotton wastes

## Introduction and objectives

The future of the Australian cotton industry is challenged by increasing input costs, increasingly variable climatic conditions, cotton price volatility, and increasing competition from synthetic fibres. The development of new technologies that add significant value to existing by-products is one tool that the industry can use to ensure it remains profitable in the face of these challenges. In Australia, around 210,000 tonnes of cotton gin trash is produced each year and can potentially be used in a wide-variety of ways. However, the majority of these products are of low value compared to cotton fibre. Given the available amount of cotton gin trash and the potential to significantly improve the economic outlook for the industry, new technology to convert cotton gin trash and other residues into high-value products is needed.

Hydrolysis of the carbohydrate portion of biomass (*e.g*., cotton gin trash) can be used to produce a one ring compound called 5-hydroxymethyldurfural (CMF) in high yields. It is a very reactive compound and a valuable intermediate for the production of chemicals and fuels.

CMF is a good precursor for the production of a group of specialty chemicals called benzofurans. Benzofurans are a group of cyclic (or “ring-like”) chemicals with some compounds having anti-inflammatory, immunosuppressive, anti-microbial, and anti-fungal properties. Simple benzofurans are currently used to produce drugs and are traded on the world market at a minimum price of $100 per kg. The value of a benzofuran increases as its structure becomes more complex and complex benzofurans (such as mycophenolic acid, an immunosuppressive drug) can fetch as much as $1000 per g. Increased structural complexity is often achieved by the inclusion of multiple cyclic groups (or “rings”) in the same molecule.

The ability to manufacture benzofuran building blocks is currently lacking in Australia, and Australia compares poorly to other developed countries in this regard (e.g., USA and Germany). The project aimed to establish the cotton industry as a supplier of technology, feedstock and chemicals to the chemical and pharmaceutical industries.

This activity also aimed to build knowledge and expertise in the area of value-adding to cotton biomass. This was manifested through research training for a post-graduate student in an area of importance to the future of the cotton, chemical manufacturing, and pharmaceutical industries in Australia.

The overall objective of Activity 3.1 was to demonstrate value adding to cotton biomass; cotton lint, mote and ginning trash. The high cellulose content in these materials makes them excellent feedstocks for the production of 5-chloromethylfurfural (CMF) and related value-added chemicals. As a useful chemical intermediate with interesting chemical reactivity, CMF, along with other related furanics, serve as attractive building blocks to produce relevant bioactive compounds. To this end, this project explored the development of rational and efficient synthetic methods and strategies for producing and converting biomass-derived CMF to a variety of valuable benzofuran derivatives and/or their direct building blocks. The objectives of this activity were:

1. Develop a continuous process technology to convert cotton gin trash/biomass into CMF;
2. Demonstrate an effective process for the production of high purity CMF and crystalline CMF; and
3. Develop chemical technologies for the conversion of cotton-derived benzofurans to pharmaceuticals, polymer plasticisers, and flavour and fragrance compounds.

The technologies developed aimed to use relatively simple reagents and explore the potential to reduce the number of reaction steps, to reduce overall production cost of the technologies.

## Results and discussion

Currently, industrial processes for the manufacture of platform chemicals typically requires: (i) petrochemical-derived precursors, (ii) high temperatures, (iii) complicated processes, (iv) toxic chemicals, and (v) complicated distillation and purification processes. Traditionally, platform chemicals have been difficult and expensive to manufacture from biomass. In the present study, CMF was produced from cotton gin trash by simple acid hydrolysis. Glucose, fructose, and lignocellulosic biomass (including cotton biomass) were used as feedstocks to produce CMF (Figure 8) continuously in a flow reactor designed during the project. As there is no current commercially established method of producing CMF in a continuous process, this reactor was designed to be 'large laboratory scale' to assist in the design of a demonstration, and future commercial plant.

Results from this project have demonstrated that 400 - 450 g of a CMF-rich bio-oil can be obtained from 1 kg of cotton gin trash, and 700 - 720 g of CMF-rich bio-oil can be obtained from 1 kg of cotton mote. In addition to CMF, the bio-oil contains other valuable compounds like levulinic acid.


### Figure 8 CMF of different purities (a) commercial, (b) different stages of CMF purification, and (c) crystalline

Extensive CMF purification strategies were explored, and resulted in the development of a simple cost-effective process that not only produced high purity CMF, but also crystalline CMF (Figure 8). Various analytical techniques including the use of nuclear magnetic resonance were used to assess the purity of the product. The benefit in producing a very high purity CMF, is that it allows for easy storage and transport, while ensuring product stability. Also, high purity CMF is required for the production of plastics, flavour and fragrance compounds. The purification study also, resulted in development of an adsorbent for the purification of the solvent used in the conversion of cotton gin trash to CMF.

CMF is produced from the carbohydrate component of via fructose as the intermediate. Therefore, the highest yield of CMF is usually obtained from fructose as the starting material. So, it is beneficial to isomerise the glucose that is obtained from the carbohydrate component of the biomass to fructose. The current commercial method used to isomerise glucose to fructose is the slow enzymatic method, involving the use of dilute glucose solution. This project has identified a heterogeneous catalyst derived from nature for glucose-fructose isomerisation at high glucose solid loading.

Similar to the production of the platform chemical, CMF, the production of other valuable chemicals heavily relies on fossil resources as feedstock and complicated reaction steps. The second part of this project focused on the synthesis, through the use of selective reaction pathways, the production of end-user compounds from biomass derived CMF (Figure 9). These involved, application of cyclisation and hydrogenation reactions, oxidation reactions and functional group utilisation which led to the development and demonstration of targeted compounds. Some of the targeted compounds synthesised were based on an oxidative process rather than the reductive process used commercially to produce these compounds.

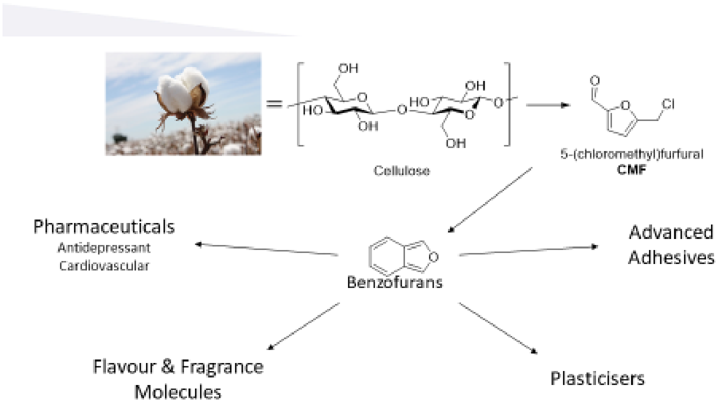



**Figure 9 Chemical pathways for products from cotton biomass developed during this project**

## Key findings and achievements

This project has demonstrated the production of CMF from cotton gin trash and other biomass feedstocks at laboratory scale in a continuous reactor and has developed a technology for making very high purity and crystalline CMF. This technology allows for ready transportation (road, rail, and ship) of the product, for improved CMF stability, and the production of CMF-based polymers. In the second part of this project, synthetic routes have been developed for the production of specialty chemicals from cotton gin trash derived CMF, including pharmaceutical precursors, polymer plasticisers, adhesives and flavour and fragrance compounds. The technologies developed in this project offer the opportunity to replace fossil-fuel derived chemicals with materials derived from sustainable feedstocks. Key activity outputs and achievements include:

- Development of a reactor for the continuous production, rather than batch production, of CMF from cotton gin trash, mote and other biomass feedstocks and the demonstration and optimisation of this process to reduce operating costs of the technology;
- Development and demonstration of a novel process for the recovery, purification and crystallisation of CMF for improved stability, storage and transportability of the product;
- Discovery and development of a new technology for the isomerisation of glucose to fructose;
- Discovery and development of a new process to produce the valuable product 5-bromo-phalide (a direct pharmaceutical precursor); and
- Discovery and demonstration of new pathways to produce commercially relevant plasticisers, adhesives, and flavour and fragrance molecules.

# Activity 3.2 – Develop technologies for producing fermentable sugars and fuel ethanol from cotton gin trash

## Introduction and objectives

This activity advanced the development of technologies producing second generation biofuels from cotton gin trash (CGT). The process of ginning cotton to produce fibre generates up to 230,000 tonne of cotton gin trash annually. Strategies to manage cotton gin trash as a waste material vary but each adds considerable cost to the ginning process. This cost, combined with increasing disposal fees, stricter environmental regulations and emissions and higher fuel prices, is driving interest in transforming cotton gin trash into value added products. Cotton gin trash has many compositional attributes indicating strong potential for use as a feedstock in biorefining. These attributes include high polysaccharide content and concentration at processing sites, consequently minimising costs associated with harvesting and transportation.

Like most lignocellulosic material, conversion of cotton gin trash to sugars for ethanol fermentation entails a pretreatment step to ensure that structural barriers are removed, thereby permitting enzymes to hydrolyse the cellulose. Among the existing physiochemical pretreatment methods for processing cotton gin trash, acid pretreatment with steam explosion is the most frequently reported. Alkaline pretreatment, either stand alone or in combination with dilute organic acids are notable alternatives. Regardless of the approach taken, most pretreatments centre on optimal isolation of fibres with highly digestible cellulose whilst sacrificing pentose sugar recovery. The reasons that this occurs are twofold. Firstly, glucose - which is derived from cellulose - is the most abundant sugar and is easily fermented by *Saccharomyces cerevisiae* yeast and, secondly, pretreatment conditions that are ideal for cellulose recovery differ to those for hemicellulose hydrolysis. This disparity is a fundamental characteristic of all lignocellulosic material and is known as polydispersity of plant biomass recalcitrance. This polydispersity poses problems in developing a single common set of pretreatment conditions for maximum recovery of both cellulose enriched fibres and xylose laden hydrolysates.

Employing a pretreatment process that maximises xylose recovery has the potential to deliver considerable cost savings by providing an easily acquired substrate upon which to cultivate yeast intended for subsequent fermentation. This is economical prudent given that recombinant xylose-utilizing *S.cerevisiae* strains preferentially metabolise xylose by respiration rather than fermentation, and secondly because traditional yeast propagation media (e.g. molasses) is relatively costly.

Thus, the main aim of this activity was to assess and develop processing options for the conversion of cotton gin trash into composite sugars for ethanol fermentation. The project objectives were to:

1. Develop pretreatment strategies for recovery of hemicellulase-laden prehydrolysate liquors with minimal levels of inhibitory/degradation compounds;
2. Develop and optimise pretreatment and hydrolysis conditions for generating glucan enriched fibres which are highly responsive to enzyme digestion;
3. Identify and quantify sugars and compounds released pre, during and post-pretreatment and hydrolysis;
4. Assess the potential of propagating recombinant yeast inoculum – intended for glucose fermentation – in hemicellulose-rich liquors;
5. Investigate fermentation options for the conversion of cotton gin trash sugar hydrolysates to ethanol using commercial and recombinant yeast; and
6. Demonstrate optimised pretreatment and fermentation processes at pilot scale.

## Results and discussion

During this project we developed a sequential dilute acid two-stage pretreatment process to maximise sugar recovery from cotton gin trash. Stage 1 pretreatment conditions were optimised to generate liquors suitable for yeast inoculum propagation intended for subsequent ethanol fermentations, and to serve as a primer for producing glucan enriched and highly digestible fibres during Stage 2. Propagating yeast inoculum in pretreatment liquors was undertaken to reduce costs involved with traditional growth-media based propagation.

Initially, cotton gin trash was subjected to multiple combined severity factors (CSF) conditions with $H_2SO_4$ and temperatures ranging between 1.2% - 15% wt. on solids and 180 °C – 240 °C for 15 min during Stage 1. Fibre recoveries ranged between 39% and 61%. Observed loss of fibre was attributed to solubilisation of carbohydrates into prehydrolysate liquors, generation of degradation products and volatilisation. Raising the pretreatment temperature at higher acid levels diminished C5 (xylose) and C6 (glucose) sugar recovery and increased inhibitor formation (Figure 10). Enzyme saccharification experiments indicated that pretreatment temperatures between 200 °C and 230 °C at low acid levels (0.0-1.0 combined severity factor) resulted in highly digestible fibres. Inclusion of a surfactant (PEG 6000) significantly enhanced the rate of enzyme hydrolysis and quantity of glucose release. Pretreatments corresponding to combined severity factors of 1.0-2.3 (high acid concentrations, low temperatures) produced ideal liquors (with balanced levels of sugars and low inhibitor concentrations) for propagation of the recombinant *S. cerevisiae* strain GSF335. However, highly hemicellulose-laden liquors produced lower than anticipated yields as a consequence of aerobic fermentation of sugars to ethanol (known as the Crabtree effect) and subsequent reassimilation. These routes are energetically inefficient and restrict yeast growth.

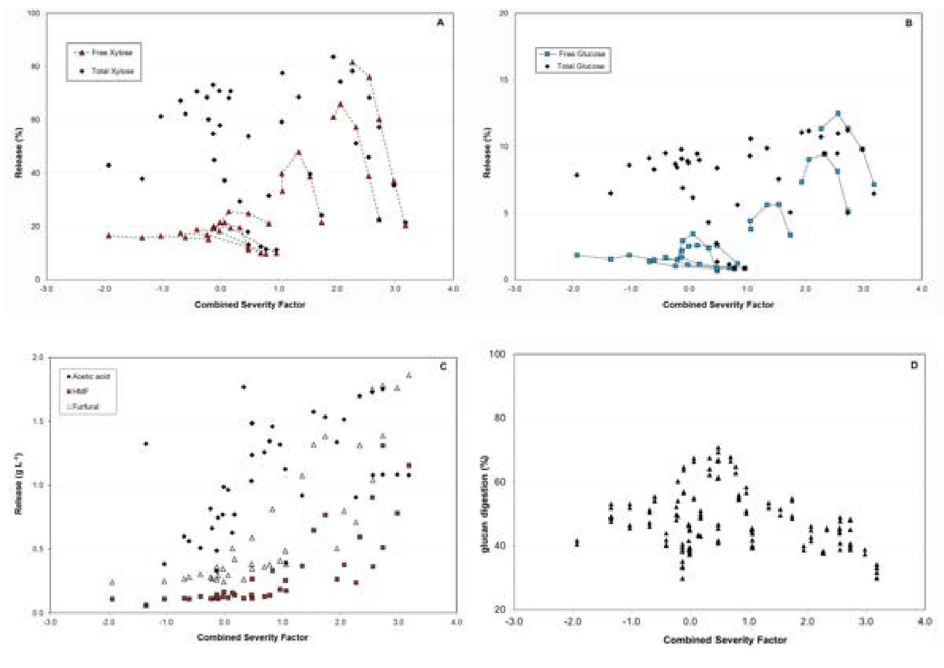



**Figure 10 Release of xylose (A), glucose (B) and inhibitors - furans and carboxylic acids - (C) into prehydrolysate liquors and enzymatic digestion of fibres (D) as a function of pretreatment severity**

***Xylose and glucose are expressed as a percentage relative to xylan and glucan content of pretreated fibre. Dotted lines drawn between discrete data points in A & B denote impact of raising pretreatment temperature at set acid levels.**

Stage 1 pretreatment combinations performed at 180 °C with 9% to 15% acid yielded liquors suitable for yeast propagation (66 g $kg^{-1}$ dry cotton gin trash) with residual acid sufficient for catalysis during Stage 2. Although fibre and glucan content diminished sequentially with processing, digestibility and glucose release per unit base increased. In the presence of the surfactant, second stage pretreated fibres were highly responsive to enzyme hydrolysis yielding 242 g glucose $kg^{-1}$ compared to 200 g glucose $kg^{-1}$ from first stage fibres. Thus optimal two-stage pretreatment conditions identified through bench scale modelling were: 1:6 solids to liquids ratio with 12% $H_2SO_4$ wt. on solids at 180 °C for 15 minutes during the first stage, with pressed unwashed fibres heated to 200 °C for 5 min during the second stage.

These process conditions were scaled-up to kilogram quantities using a Parr stirred reactor. Following a two stage Parr pretreatment process, recovered solids and glucan approached 72% and 35.5%, respectively, which were higher than single stage dilute acid pretreatment yields. Yeast strain *S. cerevisiae* GSF335 - propagated in first stage liquors (59 g $kg^{-1}$ dry cotton gin trash) - was appraised against a commercial strain (Fali®) during separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) trials. Separate hydrolysis and fermentation of second stage pretreated cotton gin trash fibre produced the equivalent of 78 and 79 L ethanol $t^{-1}$ raw cotton gin trash with *S. cerevisiae* strain GSF335 and Fali®, respectively. Simultaneous saccharification and fermentation ethanol yields with *S. cerevisiae* strain GSF335 were similar at 80 L $t^{-1}$ raw cotton gin trash while Fali® produced only 64 L.

Following batch Parr reactor pretreatment experiments, selected pretreatment permutations were evaluated at pilot scale at the Mackay Renewable Biocommodities Pilot Plant (MRBPP). Optimum parameters for recovery of sugars from pilot-scale cotton gin trash treatments were determined as 1:6 solid liquids ratio with 9% $H_2SO_4$ wt. on solids at 180 °C for 15 min during the first stage followed by steam explosion at 200 °C for 5 min during the second stage. Although fibre and glucan recoveries from matching steam explosion and Parr pretreatment conditions were similar, steam exploded fibres were more amendable to enzyme hydrolysis.

The performance of *S. cerevisiae* strain GSF335 (propagated in first stage liquors produced at pilot scale) and Fali® were subsequently compared during simultaneous saccharification and fermentation of pilot scale derived solids. Estimated yields of 101.1 and 99.3 L ethanol $t^{-1}$ raw cotton gin trash were attained for *S. cerevisiae* strain GSF335 and Fali®, respectively. However, ethanol titres for both strains remained below viable commercial targets of >4% w/w. Fed-batch simultaneous saccharification and fermentation strategies with higher steam exploded solid loads were thus formulated and optimised at bench-scale. In the absence of the physical containment certified facilities necessary to evaluate *S. cerevisiae* strain GSF335 at pilot scale, yeast strains Fali® and Summit® were assessed. During fed-batch simultaneous saccharification and fermentation trials up to 34.5% dry solid steam exploded fibre (~11% glucan) was fed over a period of 88 hours, producing titres of 47- 48 g $L^{-1}$ ethanol in 188 hours for Fali® and Summit®, respectively. This represents 88.2 and 89.1% of the maximum theoretical ethanol yield possible based on glucan content $t^{-1}$ raw cotton gin trash for the respective yeast.

For the pilot scale demonstration run, 90 kg of dry cotton gin trash - in 10 kg batches - was pretreated. A total of 104 kg of steam exploded cotton gin trash fibre in a final volume of 150 L - equivalent to 34.5% dry solids – was used for the pilot-scale fed-batch simultaneous saccharification and fermentation. At the commencement, 45.5 kg wet fibre was loaded into a 300 L stainless steel bioreactor with cellulase (22g $kg^{-1}$ wet fibre) and Summit® yeast (1% w/v). After 16 hours at 35 °C, initial viscosities declined to a point allowing for the addition of 12.95 kg wet fibre. Thereafter, the simultaneous saccharification and fermentation was fed at 24 hour increments with 19.5 kg and 2 x 12.95 kg wet fibre up to 88 hours. Cellulase and yeast were added with solids to maintain critical ratios. A final ethanol titre of 41.5 g $L^{-1}$ was attained after 184 hours of fermentation, corresponding to a conversion efficiency of 80.6% with an estimated 115.2 L ethanol $t^{-1}$ raw cotton gin trash.

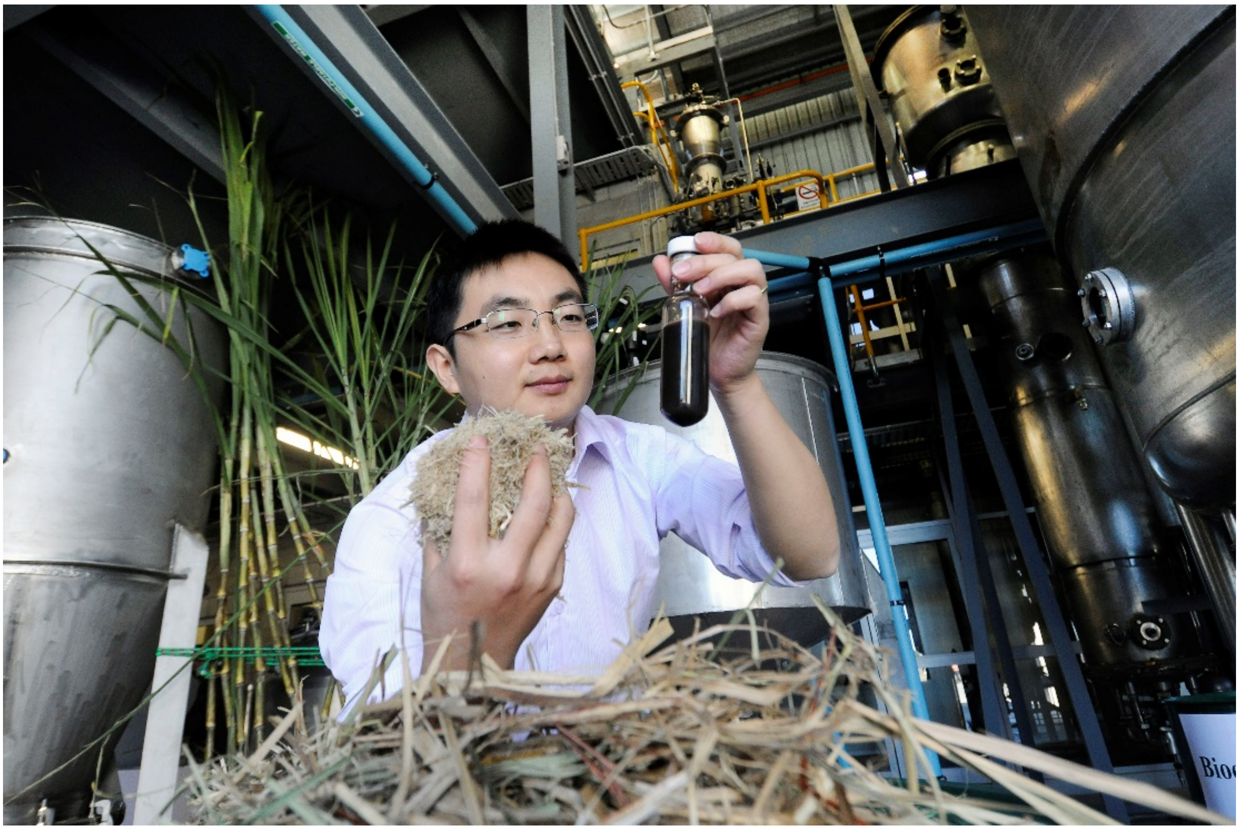
## Key findings and achievements

Most reported cotton gin trash and crop residue pretreatment studies focus on recovering highly digestible cellulose fibres whilst sacrificing the pentose sugar streams. The approach used in this study maximises sugar recovery and valorises both glucose and pentose sugars which is critical in advancing the commercialization of cellulosic biofuels.  Although the intention from the outset was to use *S. cerevisiae* strain GSF335 during pilot-scale demonstration - propagate the recombinant yeast in C5 liquors and use it in ensuing fed-batch simultaneous saccharification and fermentation trials - the lack of adequate physical containment certified pilot scale facilities influenced the choice of yeast during the final trial. The major outputs and achievements from this study include:

- Development and optimisation of a process for sequential dilute acid two stage pretreatment of cotton gin trash whereby first stage conditions:
    - Delivered xylose laden liquors appropriate for yeast inoculum propagation. Production of yeast inoculum on hemicellulose liquors intended for ensuing fermentation of pretreated fibre is economically prudent owing to cost-savings in not having to purchase molasses for preparing yeast inoculum. Furthermore, sale of surplus yeast to other commercial ethanol plants and/or as animal feed supplement would generate additional income; and
    - Served as a primer for producing glucan enriched and highly digestible fibres in stage 2. The prerequisite for high acid during stage 1 resulted in fibres with sufficient residual acid for effective catalysis during second stage
- Development of a fed-batch simultaneous saccharification and fermentation strategy with capable of handling higher steam exploded solid loadings, increasing ethanol titres with high conversion efficiency; and
- Demonstration of the process at pilot scale exploiting optimised pretreatment conditions and fed-batch simultaneous saccharification and fermentation operations.

# Advanced fuel products

Agricultural and livestock industries generate large quantities of biomass by-products and residues. Conversion of these by-products and residues into valuable products has the potential to improve the profitability of these industries. Biofuels are one class of potential value-added products. In recent years, production of high value advanced drop-in biofuels from renewable carbohydrate feedstocks has gained increasing attention worldwide. This activity aimed to produce advanced fuels using sugarcane biomass through a two-stage process. In the first stage, microbial oils were produced by oleaginous microorganisms. In the second stage, advanced fuels were produced through hydrodeoxygenation of either microbial oils extracted from microbial biomass or bio-oils obtained from hydrothermal liquefaction of microbial biomass.



# Activity 4 – Develop technologies for the production of advanced fuels from sugarcane biomass

## Introduction and objectives

Australia has a significant opportunity to create a domestic supply of advanced fuels from renewable biomass. Of particular note are commercial applications for jet and marine fuels and mining and rural applications for diesel. Biomass and lower value agricultural and forestry products can be converted to bio-oils which can be upgraded to produce fractions suitable for use as a fuel in engines. This activity targeted the production of microbial bio-oils from sugars, biomass, and animal waste residues to produce fuel products for commercial applications.

The main aim of this activity was to develop and demonstrate technology for the conversion of sugarcane derived sugars into high energy content fuels. The objectives were:

1. Optimise the productivity and yield of oil production from sugarcane molasses and bagasse-derived sugars, other biomass feedstocks and animal waste residues by filamentous fungi at laboratory scale;
2. Demonstrate the production and extraction of bio-oils from sugarcane juice or molasses at pilot scale; and
3. Demonstrate the upgrading of the bio-oils to low oxygen, high energy content fuels via catalytic hydrogenation.

## Results and discussion

Oleaginous filamentous fungi and yeast are microbial organisms with the potential to produce oil through fermentation. Within this activity, two filamentous fungi, *Mucor plumbeus FRR 2412* and *Mortierella isabellina NRRL 1757* were studied for microbial oil production from sugarcane molasses and bagasse. *M. plumbeus FRR 2412* was firstly used as a model filamentous fungi to develop cultivation and scale-up methods. With *M. plumbeus FRR 2412*, molasses was directly used with supplementation of low levels of ammonium sulphate (1 g/L for 30 g/L sugars) and monopotassium phosphate (0.5 g/L for 30 g/L sugars). Other nutrients were not necessary as molasses was a nutrient-rich carbon source. An inoculation strategy using crushed fungal biomass was developed that improved microbial oil concentrations. Scale-up of *M. plumbeus* production was successfully demonstrated at 1000 L reactor scale at the Mackay Renewable Biocommodities Pilot Plant (Figure 11). However, the overall microbial oil concentration obtained with *M. plumbeus* was low due to the low oil content in cell mass (~25%) and low fungal cell mass concentration (less than 10 g/L) in fermentation broths. In order to improve microbial oil concentration, *M. isabellina NRRL 1757* was obtained and used, which led to the production of ~25.0 g/L microbial oils from ~110 g/L molasses sugars due to the higher oil accumulation capacity (65%-74% in cell mass) and higher cell mass concentration (up to 34 g/L) achievable with this strain (Table 4). The oil concentration achieved with this new strain was 10 fold higher than that produced by *M. plumbeus FRR 2414*.

**Figure 11 1000 L bioreactor at Mackay Renewable Biocommodities Pilot Plant**

**Table 4 Microbial oil production with *M. isabellina***

| Reactor working volume | Initial sugars (g/L) | Biomass concentration (g/L) | Biomass yield (g/g consumed sugars) | Oil content (%) | Oil concentration (g/L) | Oil yield (g/g consumed sugars) |
|---|---|---|---|---|---|---|
| 8 L | 38.4 | 13.6 | 0.36 | 68.2 | 9.3 | 0.24 |
| 8 L | 77.3 | 27.4 | 0.36 | 64.8 | 17.8 | 0.22 |
| 8 L | 111.1 | 33.9 | 0.31 | 73.8 | 24.6 | 0.22 |

In order to reduce the production cost, sugarcane bagasse was also trialled as a potential feedstock. Bagasse was firstly pretreated (to improve the sugar availability for microbial oil production) using an acidified glycerol solvent based process – a previously-reported and highly effective pretreatment method, followed by enzymatic hydrolysis to produce sugars. It was found that residual glycerol in the pretreated solids (up to 5%) did not have significant effects on enzymatic hydrolysis and microbial oil production by *M. isabellina* NRRL 1757. In addition, the nutrients extracted from piggery manure were trialled for microbial oil production. Although the piggery manure could be used as an alternative commercial nutrient source, the high transportation cost, high nutrient recovery cost as well as the biological risk would likely limit its application.

The maximum cell mass of *Mucor* and *Mortierella* may be limited by the filamentous nature of these fungi. Therefore, in addition to filamentous fungi, several yeast strains were also studied for microbial oil production. A yeast strain *Rhodosporidium toruloides* RP 15 was identified as a very productive microbial oil producer from a number of yeast isolated from the sugarcane bagasse stockpile from work undertaken in Activity 2.2 of this project. This strain accumulated up to 60% microbial oils in the cell (dry mass basis) and was able to use glucose and glycerol simultaneously though the glucose consumption rate was higher than the glycerol consumption rate (Figure 12). This strain consumed glycerol faster than the *Mortierella* strain, making it more attractive for microbial oil production using glycerol-pretreated bagasse. In addition, this strain is able to produce carotenoids as a by-product during the microbial oil production process. A biorefining process that co-produces microbial oils (for fuel production) and carotenoids (as a high-value feed additive) is a key strategy for improving the economics of the fuel production process.

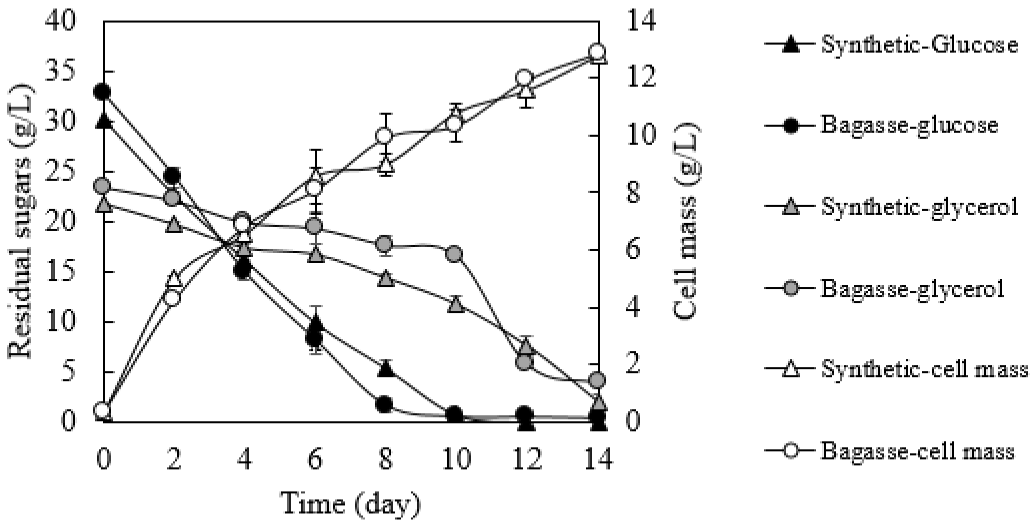



**Figure 12 Co-utilisation of glycerol and glucose by *Rhodosporidium toruloides RP 15***

In order to produce advanced fuels through the process described here, oils are produced, extracted and upgraded to fuels through catalytic hydrogenation. Within this activity, hydrothermal liquefaction technology was used to break down the cell mass for recovery of microbial oils. With *Mucor* biomass, the maximum total bio-oil yield reached 41% which was 15% higher than the microbial oil yield produced through fermentation process alone. The additional 15% of bio-oils produced were attributed to the conversion of non-microbial oil components to oil fractions during the hydrothermal liquefaction process. In addition, the results from hydrothermal liquefaction of *Mortierella* cell mass showed that catalyst was not required for liquefaction of *Mortierella* and direct liquefaction of *Mortierella* biomass led to a bio-oil yield of ~79%, almost 15% higher than the microbial oil content in the fungal biomass (Figure 13). The high bio-oil yield was attributed to the high microbial oil content (64%) and the conversion of fermentation broth components (e.g., phenolics from sugarcane bagasse) to bio-oils.

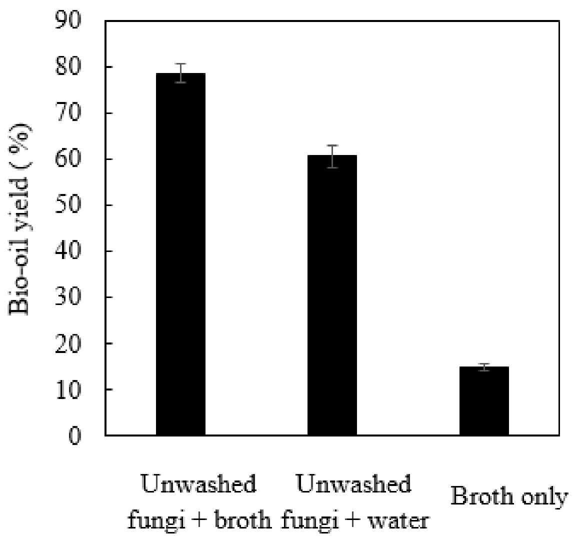



**Figure 13 Bio-oil yield from direct hydrothermal liquefaction of fungal biomass in fermentation broth**

The hydrothermal liquefaction process for recovery of microbial oils from fungal biomass was demonstrated in preliminary trials using QUT's pilot scale continuous liquefaction reactor (Figure 14). Further development work would be required to optimise this process at the pilot scale.

**Figure 14 Continuous hydrothermal liquefaction reactor at QUT Banyo Pilot Precinct**

In an endeavour to investigate the potential for the production of other high value products and co-products from fungi and yeast systems, the production of fructo-oligosaccharides as a high value feed or food additive from sugarcane molasses was investigated. Several yeast strains for the production of fructo-oligosaccharides have been identified. Preliminary results from this work resulted in yields of fructo-oligosaccharides up to 60% were produced in concentrated sugar solutions.

## Key findings and achievements

Product diversification and value-adding to by-products are the two important approaches to improve the profitability and sustainability of the sugar industry. The industry produces approximately 1 million tonnes of molasses and 10 million tonnes of fibre residues per annum. These by-products are potential feedstocks for the production of valuable biocommodities, including biofuels. The aim of this project was to produce advanced fuels from sugarcane residues through microbial oil production from key industry by-products (bagasse and molasses) by oleaginous microorganisms and upgrading of the microbial oils to advanced fuels in a catalytic process. Process economics are likely to be improved by increasing the rate of oil production and through developing high value co-products to support the potential revenue resulting from the process. The key outputs and achievements from this activity are:

- Molasses was shown to be a suitable carbon source for the production of microbial oils with only minimal supplementation of inorganic nitrogen and phosphate sources;
- An inoculation strategy based on the use of crushed fungal cell mass was developed for the cultivation of *M. plumbeus* and improved oil production. With this inoculation strategy, microbial oil production was successfully scaled-up to 1000 L bioreactor scale at the Mackay Renewable Biocommodities Pilot Plant;
- A yeast strain discovered in Activity 2.2 of this project (*Rhodosporidium toruloides RP 15)* was identified as a very productive microbial oil producer. This strain accumulated up to 60% microbial oils in the cell;
- Hydrothermal liquefaction of *Mortierella* without catalysts led to a bio-oil yield of ~79%, almost 15% higher than the microbial oil content in the fungal biomass; and
- Two yeast strains were identified for the production of fructo-oligosaccharides from molasses.

# Pathways to biorefinery development

Critical to the development of biorefinery applications is understanding the characteristics of the feedstocks, value chains associated with products from farm to market, and organisational capacity of the sector to adopt these new opportunities. Underpinning the research was consideration of business models and value chains appropriate for new bioproduct industries in Australia that enable additional flow through of value to primary producers. This capability considered critical issues of organisational capacity and innovation adoption, and the influence of these factors in promoting or constraining the development of biorefinery industry opportunities in Australia. In addition, the project aimed to build comprehensive information on biomass feedstocks from the forestry and wood products industries and identify strategic opportunities for the application of biorefinery technologies in the forest and wood product industries.

# Activity 5.1 – Assess factors influencing biorefining innovation and adoption in the Australian sugar milling industry

## Introduction and objectives

Compared to the USA, Brazil and the EU, Australia has small biofuel and biorefining industries, with ethanol and biodiesel accounting for approximately 1.4% and 0.2% of total gasoline sales and diesel sales respectively. As of 2018, three first generation commercial ethanol plants with a combined capacity of 440 ML supply the market. Despite the development of a biofuels industry having been the focus of multiple government policies since 1979, the industry has remained relatively small in Australia. Since 1979, the Australian biofuels innovation system has attracted around AUS $1.4 billion (AUS $1.6 billion when adjusted to 2017 values) in R&D funds, various forms of industry support, and excise related contributions.

Adjacent to discussions about the development of a biofuel industry within Australia, have been discussions focused on diversification within the sugar industry as one of a range of innovation pathways to increase the productivity of the industry (see, for example, the Sugar Industry Reform Program). While a range of potential alternative products have been articulated including alternative crops, bioproducts and biofuels; the opportunity for investment in ethanol production capacity in particular has been frequently canvassed. Over many years, the sugar industry has generally been supportive of the development of a sugar industry based ethanol industry while acknowledging the potential risks in investment. Despite many companies having actively examined the potential of developing ethanol production capacity, outcomes have been limited.

This brief background begs the question: why have outcomes been so limited? The actions that any industry might undertake to develop biofuel and other diversification opportunities are constrained or enhanced by the technical, industrial, economic, and policy environment in which the industry exists. Internationally, government policy, and most commonly, mandated biofuel use has been instrumental everywhere that significant biofuel markets have developed. As is shown in this report, however, the introduction of a mandate in itself, while important, is insufficient to create demand to support industrial growth and other key factors are relevant to the successful establishment of these new industry opportunities.

The aim of this activity, then, was to examine the history of the Australia biofuels industry to identify explanations for the limited growth of the industry. The lens used to undertake the analysis was the technological innovation system lens, shown in Figure 15 coupled with a qualitative event analysis. The technological innovation systems approach explains how *innovation outcomes*, in particular technology diffusion and industrial development, result from the fulfilment of seven innovation processes, called *functions*. In turn, the functions are enacted within the *structure* of the innovation system by industry, government, and researchers, through the development of effective networks and policies. Adopting a functional innovation system perspective, the aim was to identify why cumulative causation sufficient to develop a biofuels industry has not occurred. This is achieved by collecting evidence of the presence or absence, and appropriateness, of the set of innovation processes known to be formative in the development of a technological innovations system. Where functions are not operating effectively, 'systemic weaknesses', that is, deficiencies in structural elements that are inhibiting the development of a well-functioning innovation system, are identified. As there has been only limited work analysing the Australian biofuel innovation system, this study was necessarily exploratory and did not seek to test specific hypotheses.

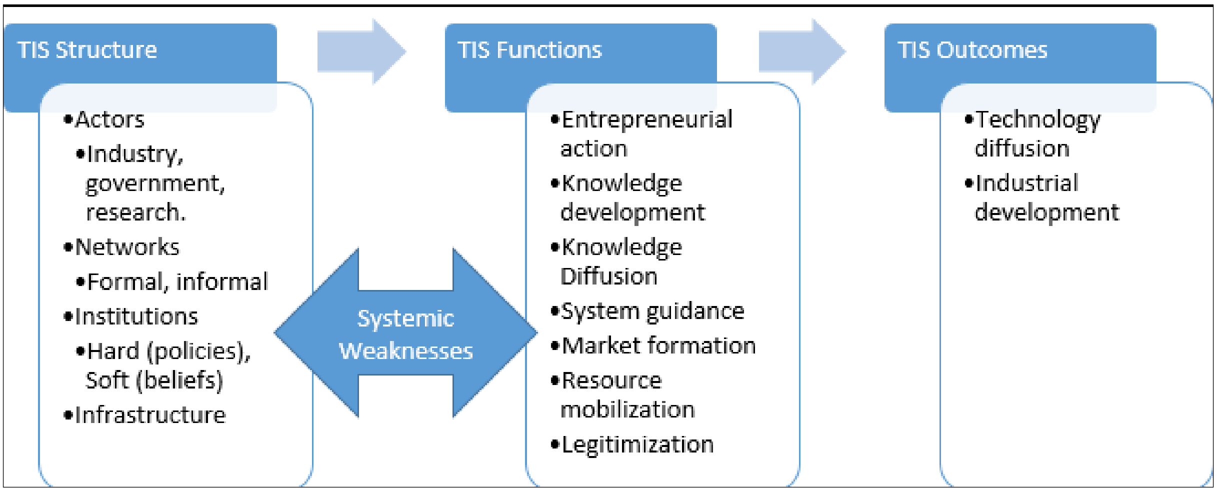



**Figure 15 the technological innovation system lens**

## Results and discussion

The history relating to Australian biofuels progress can be segmented into 7 time periods corresponding to significant changes in biofuel policy at either state or federal level, as shown in Figure 16 below. While not as clean a break as suggested by the diagram, policies and other actions until approximately 2007 primarily focused on first generation biofuels, with an increased focus on a combination of second generation or advanced biofuels, and or biorefining opportunities more broadly since.

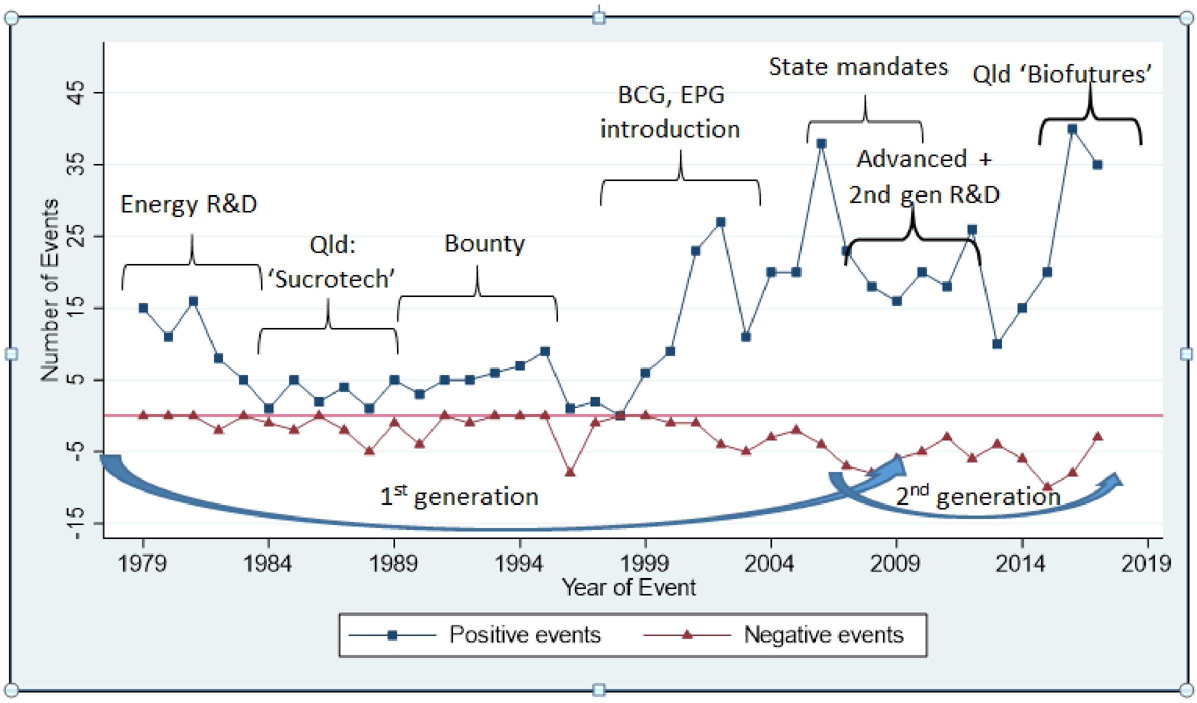



**Figure 16 Number of positive and negative events per year 1979 – 2017, with major periods of innovation system activity. R&D = research and development; BCG = biofuel capital grants; EPG= ethanol production grants**

## Innovation mechanisms

In an attempt to simplify the complexity of the biofuels technological innovation system development, two main innovation mechanisms that have driven the system can be distilled: a science and technology mechanism and an industry growth mechanism, both of which have appeared more than once, but between which there has been limited interaction. Rather, the two mechanisms have developed as the result of different, and in the main, unrelated policy aims and initiatives. Both mechanisms are characterised by periods of increased vitality followed by the emergence of a systemic weakness that has undermined the growth of the system, with a subsequent decrease in the vitality of the innovation system.

## Science and technology mechanism

The science and technology mechanism resembles a traditional innovation focus in which technological innovation and subsequent diffusion are assumed to be the outcome of R&D focused policy and funding. This mechanism was seen in the 1980s, partially in the 1990s, and has reappeared since 2007. External factors motivate a prioritising of biofuel related research, which becomes government policy and guide the direction of the innovation system. In the 1970s, fuel security was the dominant concern; in the 1990s and since 2007 it has been environmental concerns. In this mechanism, research funds are released which leads to a rapid increase in the number of research projects undertaken. Ideally, promising results reinforce the cycle, with more funds released in subsequent funding rounds. Within Australia, in each cycle, the mechanism has appeared to weaken quickly as a result of de-legitimisation of the rationale for funding biofuel related research, that is, a weakening of social and political support for biofuels.

In the 1980s, once the main external factor, fuel security, passed, the rationale for biofuels appeared to be delegitimised by increasing concerns around the cost of production for biofuels and low petrol prices resulting from lower crude oil prices. In the 1990s, the more limited science and technology cycle was associated with funding of the ethanol bounty, and was short lived by design. The development of the ethanol bounty and by association, the research funding, appeared to lack socio-political legitimacy and was dismantled when policy priorities changed.

In the most recent science and technology cycle that commenced in 2007, environmental concerns, specifically climate change, motivated the development of government policy and funding. Unlike the previous science and technology cycles, this cycle has continued despite the complex political environment in Australia relating to climate change and energy policy.

## Industry development mechanism

The second mechanism evinced through the innovation system is an industry development mechanism, albeit a mechanism that has to date been limited in its outcomes. Three periods of activity are represented by this mechanism, two of which focused on first generation biofuels: the introduction of the bounty in the 1990s, and the early 2000s in which the biofuel capital grants (BCG) and the ethanol producer grants (EPG) programs were implemented. The third period in which an industry development mechanism is apparent results from the current Biofutures program in Queensland. All three cycles share similarities in their development and progress.

The industry development mechanism has underpinned the attempted entry of entrepreneurial actors into the innovation system in which the development of the innovation system is driven by entrepreneurs who successfully advocate for government support due to the commercial risk within the still nascent innovation system. To promote further growth, the first movers need to exhibit success, and tap into niche markets.

This entrepreneurial mechanism has been evidenced to a greater or less extent in all three industry development cycles. In both the ethanol bounty and the EPG periods, entrepreneurs entered the innovation system. Government support was successfully negotiated after which additional entrepreneurs attempted entry into the innovation system. The most recent Biofutures cycle differs somewhat in that government policy and mobilisation of resources does not appear to have emerged as a result of entrepreneurial advocacy.

Further development of the innovation system beyond the initial entry of entrepreneurs requires the development of institutions to support market growth. In previous cycles, such a development did not appear to have occurred. Over the past decade, two states have developed policies in the form of biofuel mandates. Despite the introduction of these mandates, however, there has currently been insufficient market growth to enable significant expansion of the industry.

### Market development: A focal systemic weakness

The weak market for biofuels is the focal systemic weakness in the Australian biofuels innovation system. Two possible reasons might explain the weak market formation function in Australia: one a structural coordination issue, the other a consumer acceptance issue. The growth of a biofuels market requires coordination among biofuel producers, and petroleum companies that undertake blending, distribution and wholesaling or retailing of gasoline. Without mandated access, biofuel producers experienced difficulty gaining access to mass markets. The development of the required coordination is likely to depend on government structures and capabilities to undertake such coordination, plus a coordinated industry sector. In NSW and Queensland, actions have been taken to enforce coordination via mandated markets, however, a nationally coordinated policy approach has yet to occur.

The limited market penetration of biofuels even with the introduction of mandates in two states suggest a second problem as well, that of broader public acceptance of biofuels. In contrast to countries that have experienced significant growth in biofuel markets, the policy rationales underlying the introduction of biofuel policies in Australia have been transient. In each period, the legitimating rationales have differed. Variously it has been fuel security, environmental concerns and some combination of agricultural industry support, regional development, or industrial development. While a similar range of legitimating rationales have underpinned actions in countries with more developed biofuel industries, particularly the US, Brazil and some member countries of the EU, notably Sweden and Germany, ultimately a more consistent central legitimating rationale has underpinned action within these jurisdictions. Australia, in contrast, has not prosecuted any policy rationale strongly and consistently. Without such rationale, the capacity to build broad socio-political support for a biofuel industry is restricted.

## Key findings

The history of the Australian technology innovation system for biofuels has been characterised by actions from multiple parties that have not resulted in industry growth. The lack of effective market formation instruments is the most obvious deficiency, but this deficiency is best viewed as an outcome of poor legitimisation. In Queensland, the recent policy approach has altered focus from biofuels to a much broader, inclusive bioeconomy based upon biorefining and bioproducts. The activity undertook an analysis of the Australian biofuels technological innovation system. The analysis took the form of an historical narrative, grounded in a qualitative event analysis. The major findings of this report include:

- It has been beyond the capacity of the Australian sugar industry alone to develop the system-wide elements required for successful diversification into biofuels, however, significant opportunities exist if system wide elements can be successfully aligned;
- The introduction and expansion of a biofuels market in Australia would benefit from improved coordination between the biofuels industry and other industry sectors critical to the introduction and expansion of biofuels;
- Market formation actions hold the most potential for enhancing the biofuel innovation system in Australia and are critical to the successful development of the industry. Market formation actions are

essential in enabling the biofuels industry to gain market access and hence lead to growth in this sector in Australia; and

- Critical to developing a successful biofuels industry in Australia is the need for the development of a coherent, consistent and enduring policy rationale supported by government and industry sectors to build and maintain social legitimacy for the industry.

# Activity 5.2 – Develop opportunities for biorefinery innovation in the forest and wood products industries

## Introduction and objectives

The Australian forestry and wood product industries are challenged to maintain economic viability as a result of increasing production costs, increased competition from imports, and variable construction sector activity. As a result, there is an increasing need to develop biorefinery technologies, knowledge, and capacity that improves revenue from existing forest products and by-products. Integration of value-added bioproducts will be a significant ‘step-change’ for the Australian forestry and wood product industries and offers a genuine opportunity to help these industries mitigate the effects of existing, emerging, and likely future challenges to profitability.

The overall aim of this activity was to identify key opportunities for biorefining to add value to existing forestry products and by-products, and to identify the regions in Australia best suited to profitable integration of biorefineries based upon forestry and wood product feedstocks. The specific objectives were to:

1. Assess key feedstock related factors for biorefinery developments;
2. Assess feedstock use options and product options for biorefinery innovation; and
3. Assess biorefinery value chain opportunities and profitable models of biorefinery innovation in the forest and wood product industries.

## Results and discussion

### Forest and wood product industry feedstocks

In this project, a new database has been developed that contains historical annual primary production (volume of logs harvested) data. Historical data on the production of timber in Australia is available from a number of sources, most of which refer back to data collected by the Australian Bureau of Agricultural and Resource Economics and Sciences (ABARES). For the current study, primary production data for commercially harvested sawlogs and pulplogs over the period 2004 to 2015 was sourced from ABARES. Data was collected on a state-by-state basis for managed native forest, hardwood plantations and softwood plantations. Factors were sought which enabled the amount of residues left behind after logging and during timber processing to be calculated from the volume of commercially harvested logs. Data from previous Municipal Solid Waste (MSW) surveys together with population growth statistics were used to synthesise a set of historical data for urban wood waste generation on a state-by-state basis for the years 2004 to 2015. Finally, diversion factors representing the proportion of harvested feedstock and urban wood waste that could reasonably be recovered for the production of bioenergy or bioproducts production were applied to a detailed breakdown on a state-by-state basis of biomass availability for all forest and wood product feedstocks over a ten year period. Median values of biomass availability by state and biomass type are shown in Figure 17 with error bars representing the seasonally related uncertainty based on the ten years of data previous to 2016.

In 2012, the Department of Agriculture commissioned ABARES to undertake a study of the likely wood demand and supply scenarios over the period to 2050. The resulting report provides data on the estimated availability and use of logs, opportunities for investment and primary processing of wood products from 2011–12 conditions to 2050 for three different economic scenarios. The scenarios are described as: ‘Business-as-usual’, ‘Priority-to-productivity’ and ‘Constrained-wood-production’. A breakdown of the predicted change in forest and wood product biomass type and total availability is given in Figure 19 for the period 2016 - 2050 based on the ‘Business as usual’ scenario. The database was also interrogated to obtain total biomass availability assuming the ‘Constrained wood production’ and ‘Priority to productivity’ scenarios.

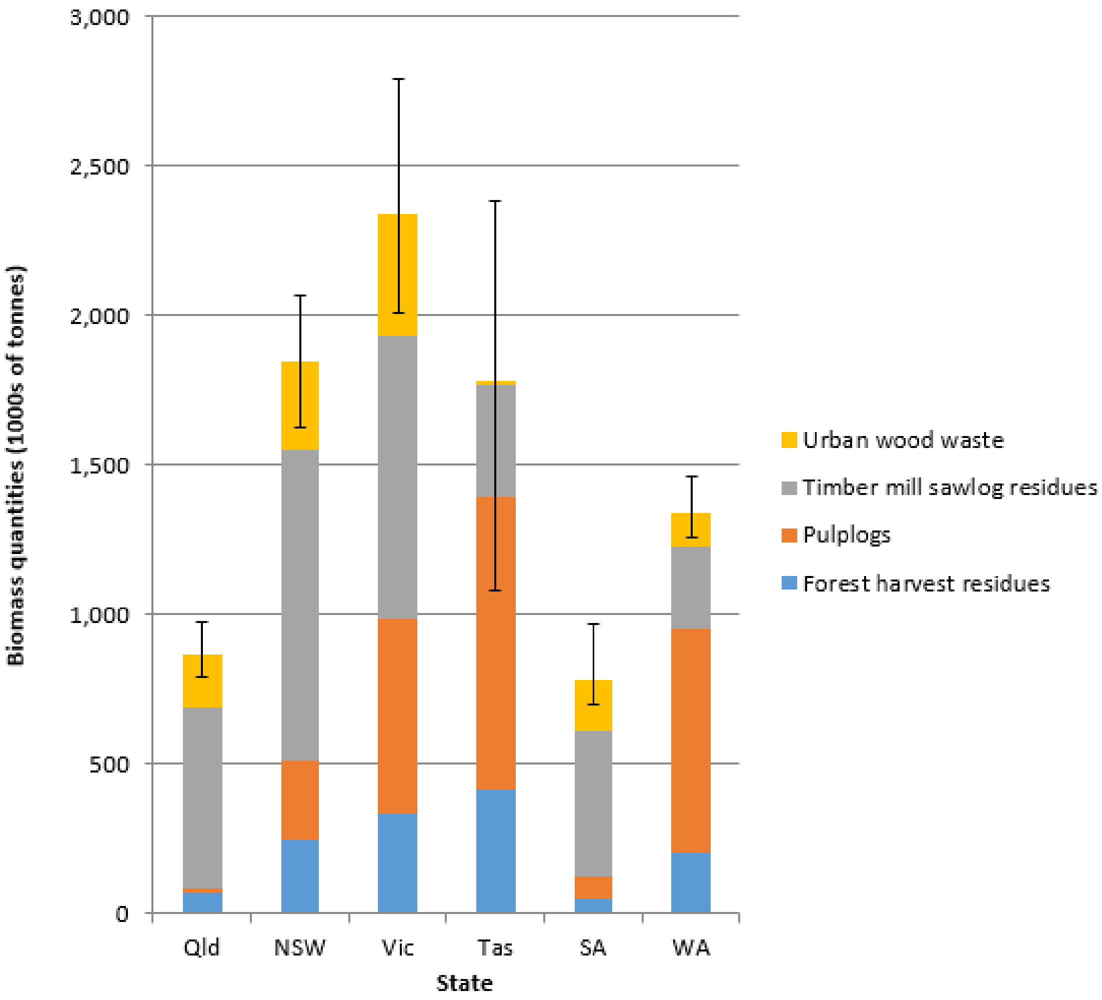



**Figure 17 Biomass availability (dry basis) by state and forest and wood product biomass types**

One of the most significant changes in the mix of forest and wood product derived biomass occurs due to a decrease in the commercial harvesting of native forest with harvest residues from native forest dropping in the year 2030 to just 54% of 2016 levels.

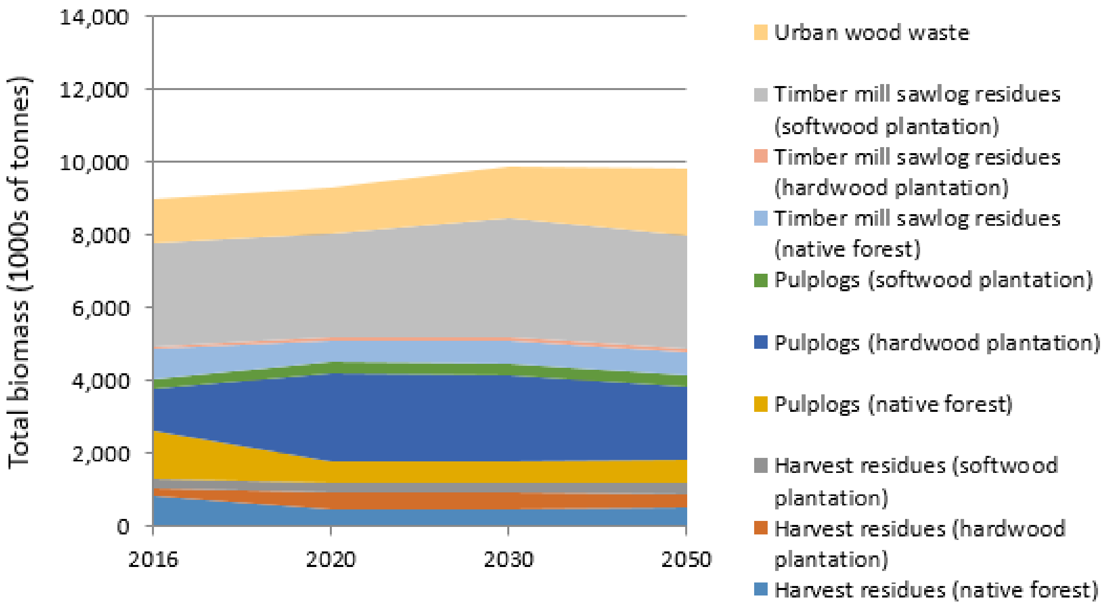



**Figure 18 Estimated change in forest and wood product biomass types with time under the 'Business as usual' forestry industry scenario**

## Biorefinery product options

Potential biorefinery product options have been considered as those derived separately from the lignin and carbohydrate (cellulose and hemicellulose) components of forest and wood product residues.

Review of a number of previous studies resulted in a shortlist of 20 potential fuels and chemicals that can be derived from the carbohydrate component of forest and wood product residues. In addition 10 (predominantly thermochemical) processes and technologies for the production of energy and chemicals from the lignin component of forest and wood product residues were shortlisted. Multi-criteria decision analysis techniques were used to rank the shortlisted products and processes with reference to technical readiness, process productivity, end product market size and compound annual growth rate, process category (i.e. severity in terms of process temperature and pressure), product yield and sale price. Where multiple technologies or processes to produce the same end product existed, these were considered as separate candidate technologies (process technology versions) in their own right and ranked accordingly. The multi-criteria decision analysis clearly identified second generation ethanol and succinic acid as the most commercially promising end products to be targeted when processing carbohydrates (Figure 20). The former (second generation ethanol) is ranked highly primarily because of the relatively high yields of this alcohol and the fact that the technology is close to being market ready. Succinic acid is ranked highly because of the favourable yields, low intensity reaction conditions and the high value of the end product.

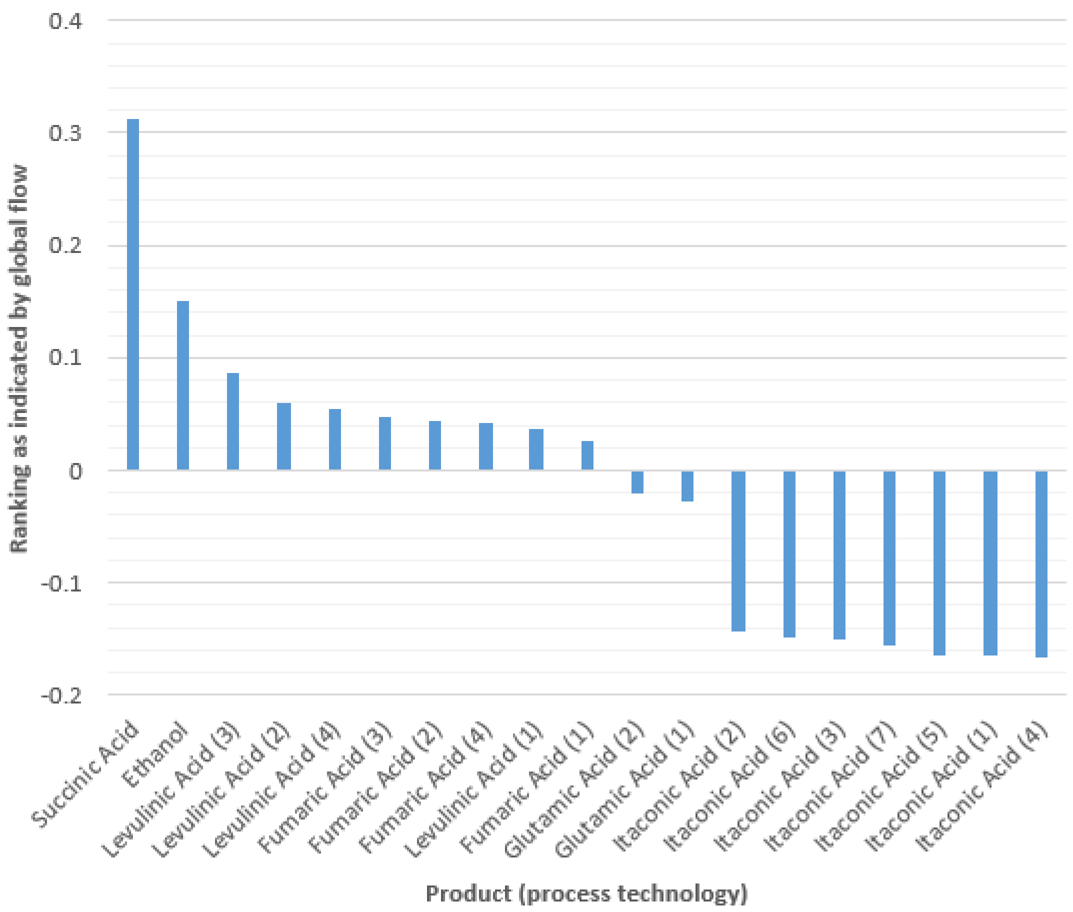



**Figure 19 Ranking of shortlisted products utilising the carbohydrate component of forest and wood product biomass (process technology version in parentheses)**

A significant lack of reliable information on process characteristics and costs associated with lignin derived products led to conventional heat and electricity generation as well as a phenol substitute in the production of phenol formaldehyde resins as the most highly ranked options.

The two or three most highly ranked products and technologies for both carbohydrate and lignin derived products and processes were largely insensitive to the weightings given to the assessing criteria used in the multi-criteria decision analysis.

## Biorefinery value chain opportunities and profitable models of biorefinery innovation

A high level techno-economic analysis of processes in which the most highly ranked carbohydrate and lignin conversion technologies were integrated into a number of different biorefinery options, was carried out. Results were expressed in terms of the factory gate price that could be paid for the forest and wood product residue ($/ tonne dry basis) such that the biorefinery project could deliver an after tax internal rate of return (IRR) of 10%. Analysis of these options indicated that the integrated production of succinic acid and export power (Biorefinery Option 3) added the greatest value to forest and wood product residue ($766 per dry tonne). This biorefinery option is shown schematically in Figure 21. Apart from the high value of the succinic acid product, this biorefinery option performed well as it did not require an initial dedicated pretreatment process to separate the lignin and carbohydrate components of the forest and wood product residues and therefore had low capital and operating (utility) costs relative to other options.

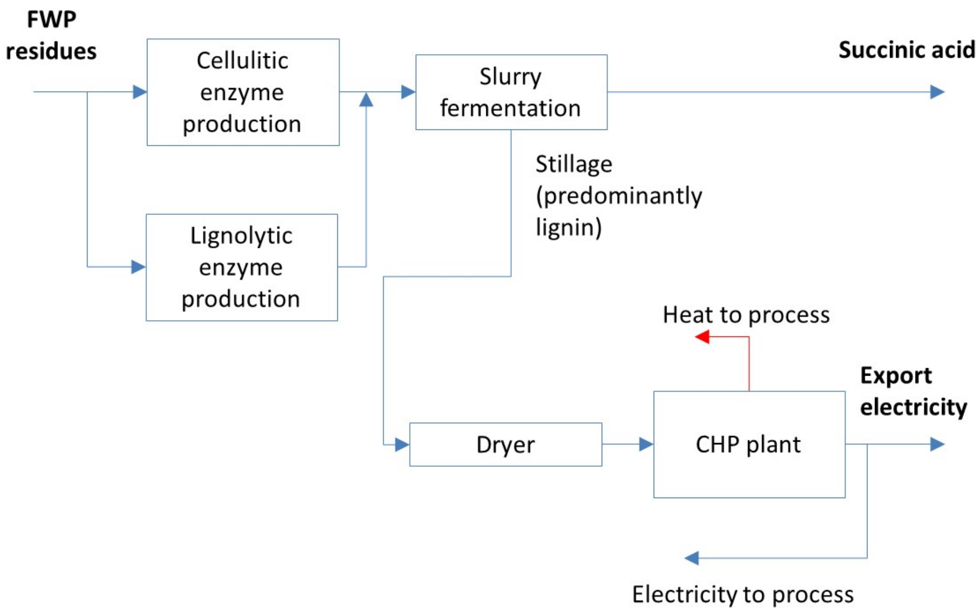



**Figure 20 Schematic of succinic acid and electricity production biorefinery (Biorefinery Option 3)**

Modest net returns ($58 per dry tonne paid for forest and wood product feedstock) were predicted for the integrated production of second generation ethanol and phenol formaldehyde resins (Biorefinery Option 2). The analysis indicated that the integrated production of second generation ethanol and export power (Biorefinery Option 1) was not commercially viable returning a net value of -$28 per dry tonne of forest and wood product residues.

Using the Queensland forest and wood product industry as a case study, preferred biorefinery locations based on spatially specific data available through the Australian Renewable Energy Mapping Infrastructure (AREMI) platform have been obtained. Multi Criteria Analysis and Fuzzy Logic techniques utilising aggregated forest and wood product biomass availability, topographical and infrastructure data layers have identified 22 potential biorefinery sites within Queensland. The total forest and wood product residue resource within a 40 km radius of these 22 potential sites was 4,198,000 dry tonnes annually.

Considering the 22 potential sites identified and using Location-Allocation techniques, optimum biorefinery locations were identified for the three scenarios where a single (p = 1), two (p = 2) and three (p = 3) biorefineries are constructed in Queensland. For these three scenarios, optimum locations were found in the Sunshine Coast, Somerset, Fraser Coast and Rockhampton Local Government Areas (LGA). The single most preferred biorefinery site (p = 1) of the 22 potential sites was located in the Sunshine Coast Local Government Area at which the 282,000 dry tonnes delivered was transported a (mass weighted) mean distance of 70 km on the existing road network (Figure 21).

Estimates were made of the net returns (per tonne of dry residue) for the two most promising biorefinery options (Biorefinery Option 3 and Biorefinery Option 2) when located at these optimum locations. The single optimum biorefinery site (p = 1) gave the highest net returns ($770 per dry tonne of feedstock) at the point of supply of feedstock for the succinic acid/ electricity (Biorefinery Option 3) and $70 per tonne for the ethanol/ phenol formaldehyde resin (biorefinery Option 2) technologies. At this optimum location, Biorefinery Option 3 would produce 69,200 tonnes of succinic acid and 42,600 MWh of export electricity. Alternatively, Biorefinery Option 2 would produce 93 ML of ethanol and 25,200 tonnes of phenol formaldehyde resin.

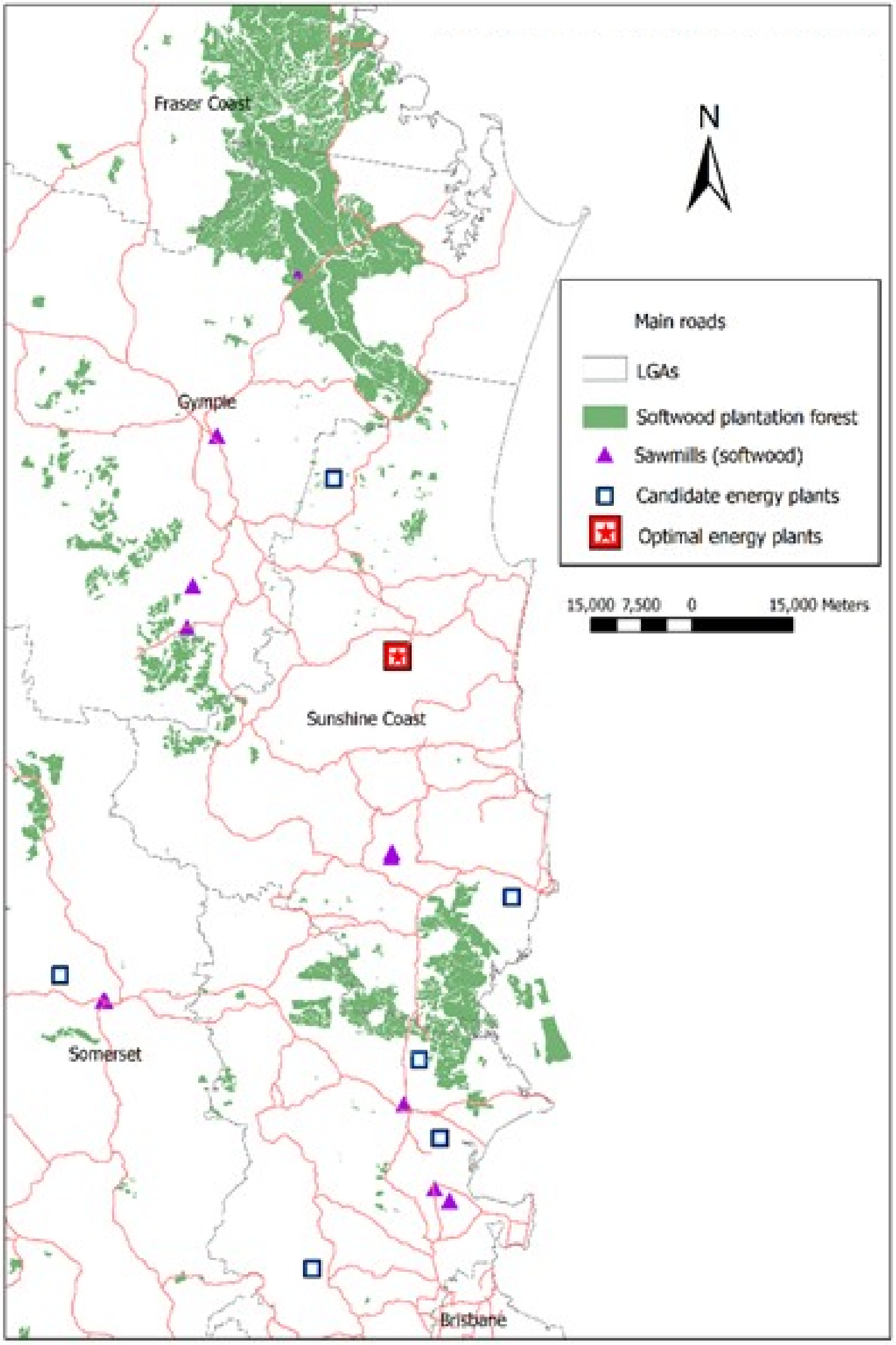



**Figure 21 Location of the single most preferred (p = 1) forest and wood product biorefinery (Queensland case study)**

Poor commercial outcomes are observed for biorefinery Option 2 when the number of optimally positioned biorefineries is increased to three (p = 3). Significantly negative FOB returns per dry tonne are reported for two out of three of these locations. These are due to either high feedstock transport distances (over 670 km for one of these plants) or poor economies of scale (49,000 dry tonnes in another of these plants). Biorefinery Option 3 continues to deliver high net returns of over $600 per dry tonne of residues for all three (of the p = 3) optimum locations.

## Key findings and achievements

Activity 5.2 had the overarching aim of identifying key opportunities for biorefining to add value to existing forestry products and by-products. The activity has identified regions in Australia best suited to the profitable integration of biorefineries based primarily upon forestry and wood product feedstocks. A new database has been developed which contains both historical annual primary production (volume of logs harvested) data as well as projected primary production for 2020, 2030 and 2050. The raw data has been collected and the corresponding biomass availability has been estimated on a state-by-state basis. This state based data has been aggregated so that national figures are also available. The potential for biorefining technology based on forest and wood product feedstocks to mitigate against increasing production costs, increased competition from imports and variable construction sector activity has been shown in this study to be significant. In summary the key findings are:

- The total estimated forest and wood product derived biomass availability at a national level (after making allowances for biomass retained on the forest floor for soil nutrient retention and making assumptions about the amount that can be realistically recovered) is approximately 9 Mt (dry basis) with a seasonally related uncertainty of approximately ± 1.5 Mt;
- Timber mill sawlog residues provide the greatest potential source (42%) of total forest and wood product biomass followed by pulplogs (31%), forest harvest residues (15%) and urban wood waste (12%);
- Within the given estimated seasonally based uncertainties, Victoria indicates the greatest potential (at approximately 25% of the national total) biomass followed by New South Wales and Tasmania (approximately 20% each), Western Australia (15%) and Queensland and South Australia (with approximately 10% each);
- For a 'Business as usual' scenario, forest and wood product derived biomass is estimated to increase from 2016 levels of 9 Mt (d.b.) to a maximum of 10 Mt (d.b.) by the year 2030. It is then expected to level off or even decline slightly by 2050. For the 'Constrained wood production' and 'Priority to productivity' scenarios the maximum production of forest and wood product derived biomass also peaks in the year 2030 at just below 10 Mt (d.b.) and 12 Mt (d.b.) respectively;
- One of the most significant changes in the mix of forest and wood product derived biomass occurs due to a decrease in the commercial harvesting of native forest with harvest residues from native forest dropping in the year 2030 to just 54% of 2016 levels. Pulplog from native forest is similarly expected to decline in 2030 to 43% of 2016 production;
- Forest and wood product derived biomass accounts for an estimated 21% of all major sources of biomass in Australia;
- Review of a number of previous studies and data sources resulted in a shortlist of 20 potential fuels and chemicals that can be derived from the carbohydrate component of forest and wood product residues. Applying multi-criteria decision analysis ranking techniques to these shortlisted products identified succinic acid followed by second generation ethanol as the most commercially promising candidates;
- In addition 10 (predominantly thermochemical) processes and technologies for the production of energy and chemicals from the lignin component of forest and wood product residues have been shortlisted. The use of hydrothermal liquefaction to produce monophenols for phenol formaldehyde resin production followed by conventional heat and power (CHP) generation were the two most highly ranked options; and
- A techno-economic analysis indicated that in terms of integrated biorefinery technologies, the production of succinic acid combined with combined heat and power to provide both process

requirements and electricity export was found to add the greatest value ($766 per dry tonne) to forest and wood product residues.

# Collaboration

The *Biorefineries for Profit* project undertook research, development and demonstration of new technologies to expand biorefinery opportunities across the sugar, cotton, forest and wood product and pork industries. The project developed several new technologies and processes, and generated substantial industry interest in the use of agricultural products, by-products, and wastes to produce high-value commodities. Technologies developed in the project include: (i) chemical and microbial technology for production of high-quality animal feed using lower cost agricultural residues as feedstocks; (ii) identification of new strains of microorganisms that can be used as probiotic feed supplements through high-throughput screening systems, detailed characterisation, and production in high-yielding, economic, and scalable fermentation systems; and (iii) technologies to convert cotton gin trash to intermediate chemicals and pharmaceuticals.

Collaboration with project partners has been critical to the success of the project and offered the opportunity to develop, transfer and deliver outcomes. A key project strength was the ability to access pilot scale facilities through project partners enabling rapid demonstration of technologies.

Collaboration with project partners was enhanced through the formation of a Steering Committee and Science Advisory Group. These committees included representatives from partner and industry organisations and provided a platform for planning, industry engagement and strategic direction.

New industry collaborations have resulted from the project activities, communication and engagement activities. As an example, the project team has developed a new collaboration with an Australian wood products manufacturer to explore aspects of their current production process. The results formed the basis of a successful application to Forest and Wood Products Australia for project funding to further this work.

The project also initiated a new collaboration with the University of Queensland – Queensland Animal Sciences Precinct at Gatton. Collaboration activities included in-vitro and in-vivo animal trials in cattle and chickens.

The project has developed industry linkages to animal feed production companies for commercialisation of novel strains and technologies discovered through the project. Other paths to commercialisation for the probiotics technology continue to be explored. Australian company Rivalea, one of Australia's leading integrated agri-food companies will collaborate in future pig feeding trials.

New collaboration opportunities have also been developed in the scale up demonstration of technology for the production of chemicals from biomass residues. Researchers from the project have formed a collaboration with Mercurius Biorefining to advance the development of a pilot scale CMF production facility in Gladstone, Queensland.

# Extension and adoption activities

Extension and adoption activities are critical within agricultural industries to ensure adoption of technologies resulting from research outcomes. The *Biorefineries for Profit* project allocated significant resources to this activity in generating and implementing the Communication and Extension Plan. Communication and extension activities included promotion of project achievements at industry-specific conferences and regional seminars for the cotton, sugar, forestry and pork industries, the broader farming community, external partner organisations and the wider community.  Key extension activities included:

*Biorefineries for Profit Annual workshop*

An annual *Biorefineries for Profit* project workshop was held each project year. The workshops had approximately 40 participants on average which included members from the project team, researchers, students, Science Advisory Group and the Steering Committee members. The workshop was held to update project stakeholders on project activities, have robust discussions on project deliverables and outcomes and provide recommendations and advice on the future direction of the project.

*Publishing of project achievements*

The project team has participated in and published academic papers, conference and seminar presentations, posters, industry magazine and other media over the course of the project. Journal articles produced were submitted to internationally recognised research publications from a range of disciplines including engineering, poultry science, microbiology and biotechnology fields.

Special attention was given to communication and extension activities within partner sectors, i.e., the pork, forest and wood products, cotton and sugar sectors. This included participating in at least one conference and seminar per industry per project year. This has been successfully achieved and participants in these seminars have expressed a high degree of interest in the project activities and outputs.

*Industry site visits*

Project teams have visited industry sites to further communicate project achievements. Visits were made to a diverse range of company sites including Yarraman cotton gin, Agforce North (Townsville), Rocky Point Sugar Mill, and the Industrial Biotechnology Innovation Centre in Scotland. As the project progressed site visits were also undertaken to progress and advance consideration of pig and chicken feeding trials.

*Media Coverage*

The *Biorefineries for Profit* project participated in several media events including national coverage on television, radio interviews and articles published in The Guardian, SRA Milling Matters magazine and Nature and Health magazine.

On 29th July 2019, the project was featured on ABC Landline. The episode featured Dr Mark Harrison being interviewed at the Mackay Renewable Biocommodities Pilot Plant on his work in turning bagasse into animal feed for use in the livestock industry. Associate Professor Robert Speight and Dr James Strong also explained concepts for the production of probiotics for animal feed applications.

Professor Ian O'Hara's was interviewed on ABC Rural (radio) to discuss project aims, objectives, and potential outcomes for industry.

*Biorefineries for Profit Project website*

The *Biorefineries for Profit* project website was developed to showcase project activities and deliverables on a web platform that easily accessible to public and is updated on a regular basis. The *Biorefineries for Profit* project website contains information that is non-confidential and outlines the project activities, project team and project partners.