

# KNOWLEDGE SHARING REPORT

"Biocatalyst optimization for efficient production of biofuels from non-food biomass and deployment through a global partner corporation"

Written by Geoff Bell. March 2021.

This project received funding from ARENA as part of ARENA's Advancing Renewable Program.

The views expressed herein are not necessarily the views of the Australian Government, and the Australian Government does not accept responsibility for any information or advice contained herein.

Deliveries Unit E2, Lane Cove Business Park 32 Sirius Rd, Lane Cove NSW 2066

**Postal & Visitors** Unit E2, Lane Cove Business Park 16 Mars Rd, Lane Cove NSW 2066

**Tel** +61 2 9420 1244 contact@microbiogen.com

www.microbiogen.com





## Table of Contents

List of Figures and Tablesi
Glossaryii
Executive Summaryiv
Introduction1
Confidentiality 1
Project Success
Background
Why do we need more bioethanol?
Bioethanol as the new "crude oil"
How does the ARENA funded program help make bioethanol the new crude oil?
ARENA program addresses three areas in 2G bioethanol refinery7
Successful ARENA funded program lowers costs as much as 25%
Capital costs could also benefit from an optimised biocatalyst9
Technical Success Criteria to achieve required project outcomes
Technical success benchmarked using full scale hydrolyzates11
Work carried out to benchmark MBG optimized biocatalyst
Successful aerobic fermentation in the MicroBioGen laboratories 12
Optimised Yeast Biocatalyst outperforms Commercial Biocatalyst 15
Optimised yeast outperforms commercial yeast in all scenarios 16
Game Changing Implications for sustainability – LCA Analysis
Massive Positive Implications for Australia – Market Analysis 19
Conclusion 20
References



## List of Figures and Tables

Figure 1: Ethanol fuel production movements by zone, in billions of litres (Source: IFPEN, from FO Licht) 3
Figure 2: Octane number of various fuel octane sources. (Source: RFA, from presentation by G. Cooper) 4
Figure 3: Ethanol as the new "Crude Oil" <sup>7</sup>
Figure 4: Simplified process flow sheet. Typical lignocellulosic biochemical ethanol bio-refinery
Figure 5: Improved yeast catalyst can significantly reduce costs of renewable lignocellulosic ethanol
Figure 6: Aerobic growth trials in MicroBioGen laboratories
Figure 7: MBG yeast biocatalyst morphology post-seed yeast propagation
Figure 8: MBG yeast biocatalyst morphology in main propagation – 1st half
Figure 9: MBG biocatalyst yeast being prepared in a Fluidized bed dryer14
Figure 10: MBG biocatalyst yeast as an Active Dried Yeast (ADY)14
Figure 11: MBG biocatalyst yeast as ADY in vacuum-sealed aluminium packaging 14
Figure 12: TSC vs MBG Yeast and Commercial Strain – Medium Concentration Hydrolyzate
Figure 13: MBG Optimised biocatalyst strain relative to average TSC and benchmark biocatalyst strain 16
Figure 14: Available bagasse resource Australia 19

Table 1: Comparative results for ethanol production using MicroBioGen yeast strains compared to using	
conventional yeast strains	. 18

## Glossary

biocatalyst	a living microorganism that can facilitate various functions, such as converting carbon sources to ethanol and protein
non-food biomass (e.g. bagasse)	the portion of a plant that is not the food portion, for example, in sugarcane, it is the fibre that is left after the sugar juice (sucrose) has been extracted
Commercial in Confidence	a commercial agreement of which the terms cannot be disclosed to the public
lignocellulosic bioethanol	ethanol produced from the sugars released from non-food portion of biomass
organic acid resistance	the ability of a biocatalyst to withstand and survive high concentrations (>1%) of organic acids (e.g. lactic and acetic acid)
C5 sugar	a sugar that has only 5 carbon atoms, such as xylose and arabinose. Most food sugars contain 6 carbon atoms.
mineral and fossil-based fuels (mineral hydrocarbons)	non-renewable fuels that have been extracted from under the earth
first-generation biofuels/technology (1G)	production of biofuels from sugars or fats that could otherwise be used in food
second-generation biofuels/technology (2G)	production of biofuels from biomass that is not suitable for food
substrate	biomass that is utilised by both first and second-generation bioethanol plants to produce bioethanol
dry distillers grains (DDGS)	the residual part of the corn that is left over once the free sugars have been fermented to ethanol
aerobic	fermentation where air is blown into the fermenter to provide oxygen to the biocatalyst
anaerobic	fermentation where oxygen is not supplied, and the biocatalyst must survive in an environment without oxygen
capital cost	the upfront cost to build a 1G or 2G bioethanol refinery



phenotypically stable	a biocatalyst that does not alter its physical characteristics over time
exotic nutrients	compounds added to a fermentation that are not normally added
glycerol	an unwanted by-product of ethanol fermentation
hydrolyzates	the resultant liquid produced once biomass has been pre-treated and broken down into its component parts
LCFS	a low carbon fuel standard that has been applied in various states in the US, incentivising lower carbon emission biofuels

#### **Executive Summary**

Transitioning to renewables is a critical step in the world-wide effort to reduce carbon emissions and build a sustainable future. To combat the worst of the effects of climate change, however, policymakers, businesses and individuals around the world will need to employ all the renewable technologies at our disposal simultaneously. Such technologies include solar, wind, hydrogen, biomass and various biofuels, in particular bioethanol.

Herein, MicroBioGen (MBG) reports that, after more than 15 years and A\$25M in spending, including A\$8M in 3.5 years with ARENA funding, we have successfully completed the final phase in our optimisation of a second-generation yeast biocatalyst that significantly improves the economics and sustainability of bioethanol fuel for transport and other applications. It means that, for the first time, a single biocatalyst can be used to manufacture both fuel and food from non-food biomass. It also means that, if the CO<sub>2</sub> generated from ethanol fermentation were sequestered, then biofuels would replace fossil fuels, add to the food supply and, the more biofuels produced, the more CO<sub>2</sub> would be taken out of the atmosphere. Ultimately, the optimised biocatalyst places biofuels where it can make some of the greatest contributions to fuel and food security and the reduction of CO<sub>2</sub> in the atmosphere.

Of 13 Technical Success Criteria (TSC) that were specified for this project, the optimised biocatalyst achieved a 99% pass rate on average. The success of the program is expected to be a "game-changer" for bioethanol production in the future. Changes compared to a commercial, second-generation yeast biocatalyst include:

- An estimated 25% effective reduction in operating costs.
- High value single-cell protein as a by-product from non-food biomass.
- The more biofuels manufactured the more high-value protein produced.
- If Australia converted just its sugar cane bagasse using the MicroBioGen optimised organism and process, then enough ethanol would be produced to replace nearly 10% of petrol and replace nearly 140,000 tonnes of currently imported protein feed.
- Peer reviewed Life Cycle Analysis (LCA) shows a 29% reduction in CO<sub>2</sub>, 11% decline in fossil energy use and a massive 240% decrease in land use compared to the commercial yeast biocatalyst.



#### Introduction

This is the final report prepared by MicroBioGen (MBG) with respect to the **"Biocatalyst optimisation for efficient production of biofuels from non-food biomass and deployment through a global partner corporation"** project undertaken by MicroBioGen over the last approximately three and a half years.

#### Confidentiality

Due to the confidential nature of the technology developed by MicroBioGen, and actual results achieved in MicroBioGen laboratories, including those by third party trials carried out offshore, the first report that includes data with respect to the Technical Success Criteria (TSC) of the project will also be the final report for this project.

Additional international patents are expected to be lodged based on the work carried out by MicroBioGen over the last three and a half years. These patents will be based on technical work carried out in Australia by MicroBioGen and future offshore work by its global partner. Consequently, some of the data presented in this Knowledge Sharing report will be limited to consolidated data or de-identified data rather than raw data.

In this project MicroBioGen worked very closely with its "Global Partner Corporation". Due to "Commercial in Confidence" issues, some additional data will be consolidated or de-identified to satisfy these contractual commitments.



#### **Project Success**

The entire ARENA funded project was based on the one simple premise – optimise a yeast biocatalyst to produce bioethanol and feed from non-food biomass substrates. While this may appear a simple task, it is one of the most complex tasks that is imaginable in the field of industrial biotechnology and MicroBioGen has been working towards this goal for over 15 years. The primary aims of the project were to lower operating costs in lignocellulosic bioethanol facilities and increase sustainability.

Some core optimisation metrics and their implications are as follows:

- Screater organic acid resistance: higher concentrations of biomass sugars and thus lower costs
- More complete C5 sugar conversions: greater yield and lower costs
- Produce fuel and feed using a single optimised organism: lowering operating costs and increasing sustainability

There were in total 13 confidential Technical Success Criteria (TSC) developed for the project. At the end of the three-and-a-half-year project, which was built upon the previous 10 years or R+D work at MicroBioGen, the optimised yeast achieved between 97% and 99% of the TSC<sup>1</sup>. This compares with the 66% – 83% achieved by the benchmarked commercial yeast biocatalyst.

By almost any measure, the project has been a success, with significant cost and sustainability implications for the production of lignocellulosic bioethanol into the future.



#### Background

#### Why do we need more bioethanol?

The world currently produces approximately 110 billion litres bioethanol per year, with the US supplying the greatest proportion at about 60 billion litres, followed by Latin America at just under 40 billion litres per year (Figure 1)<sup>2</sup>. Most of the current production of bioethanol is derived from either starch substrates such as corn or sugar juice/molasses derived from sugar cane.

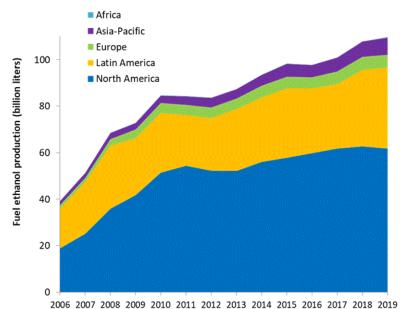


Figure 1: Ethanol fuel production movements by zone, in billions of litres (Source: IFPEN, from FO Licht)

The advantages of bioethanol over mineral and fossil-based fuels are numerous. Bioethanol is cleaner burning, higher in octane (see Figure 2)<sup>3</sup>, sustainable and relatively cheap to produce and competitive with fossil fuels – even without subsidies<sup>4</sup>. Brazil and the US have shown the way in developing renewable ethanol industries that have increased fuel security, reduced poisonous and carcinogenic compounds into the atmosphere through the substitution of aromatic compounds such as benzene, toluene and xylene, reduced CO<sub>2</sub> emissions and helped foster rural industries.



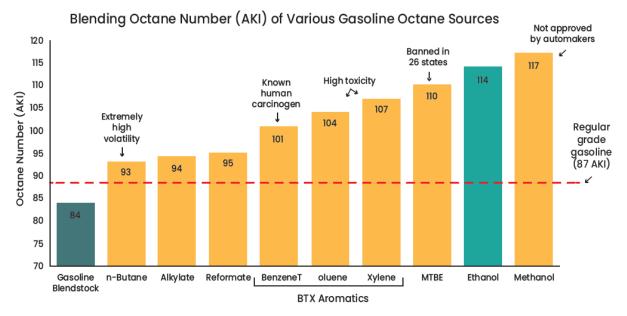


Figure 2: Octane number of various fuel octane sources. (Source: RFA, from presentation by G. Cooper)

While there is still significant potential to continue the growth of bioethanol as an alternative fuel using conventional or first-generation technologies (as above), the next generation of biofuels (second-generation) based on non-food biomass has the greatest potential to deliver the sort of quantities of fuel to meaningfully replace mineral hydrocarbons in the fuel supply. In the US alone, studies by the US Department of Energy have shown that there are more than a billion tonnes of biomass available for conversion to biofuels in the US without negatively impacting current uses of biomass<sup>5</sup>. Around the world there are tens of billions of tonnes of biomass that would be suitable for conversion to biofuels<sup>6</sup>.



#### Bioethanol as the new "crude oil"

Crude oil is not just used for the production of diesel and petroleum<sup>7</sup>. It is also used to produce kerosene for aviation fuels, plastics, detergents, packaging, tyres, toys and the heavier elements can be used for large marine transport and road construction amongst others. Crude oil is critical for the global economy and will not easily be replaced. Ethanol (including lignocellulosic ethanol) can be used as the base molecule to replace most applications of crude oil (see Figure 3) and if produced in sufficient quantities at a low enough cost, it has the potential to be the new "Crude Oil" – but renewable and cleaner.

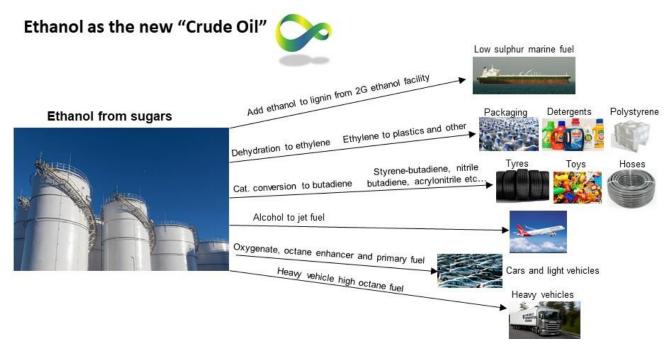


Figure 3: Ethanol as the new "Crude Oil"7



#### How does the ARENA funded program help make bioethanol the new crude oil?

Lignocellulosic bioethanol holds the promise of delivering huge quantities of ethanol into the global market for use as a fuel, an octane enhancer and as a precursor to a wide range of products that the world depends upon for high standards. However, despite its relatively low substrate costs, lignocellulosic bioethanol presents several challenges. Some of these are:

- First-generation (1G) ethanol is primarily based on corn and sugar from sugar cane. This is a mature and straightforward technology leading to relatively low costs – despite relatively high substrate costs.
- Second-generation (2G) bioethanol is based on highly recalcitrant biomass<sup>8</sup> (difficult to break down into component sugars) and the technology is immature.
- The technology to convert the liberated biomass sugars is still under development with significant improvement opportunities yet to be achieved.
- Ethanol using corn delivers multiple products. High value corn oil as well as valuable by-products such as dry distillers grains (DDGS) and more recently high protein yeast concentrates<sup>9</sup>. At present there are few if any valuable by-products that are produced from second-generation biofuel facilities.
- Presently, costs are significantly higher for 2G versus 1G bioethanol<sup>10</sup>.

MicroBioGen has been working consistently for the last 15 years to develop and optimise the genetics required to significantly reduce the costs of 2G bioethanol and at the same time increase its renewable credentials. Over the last 15 years, MicroBioGen has spent in excess of A\$25M in its development program, successfully completed 2 Federal Government supported R+D grants and has now successfully completed the third and potentially final program to bring a superior biocatalyst to the industry.



The ARENA funded program has been designed to address the cost and sustainability issues through three key technical and commercial optimisation areas. These are:

- Increase the robustness of the yeast biocatalyst so that it can withstand and thrive in the more difficult and inhibitory conditions associated with 2G bioethanol refineries.
- Enhance the same yeast that thrives in the difficult process conditions so that it can "clean" the waste streams that are found at the back end of the process.
- Develop a unique yeast biocatalyst that, once grown on the lignocellulosic bioethanol facility, can be used in the primary anaerobic fermentation to convert all available sugars to ethanol. Once this same yeast biocatalyst has grown on the "waste stream", any excess yeast can be sold as a high value, high protein by-product.

The simplified flow diagram below (Figure 4) shows the key areas that MicroBioGen has targeted in order to lower operating costs, enhance sustainability and develop and new by-product sales stream. The areas in focus are in RED boxes.

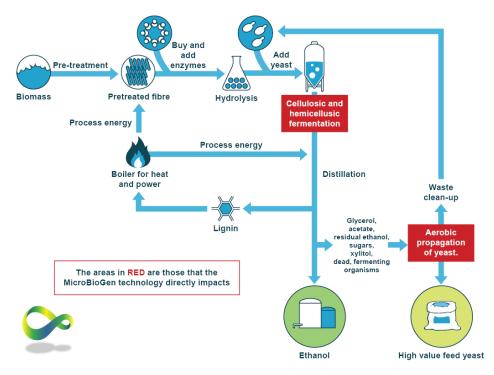


Figure 4: Simplified process flow sheet. Typical lignocellulosic biochemical ethanol bio-refinery.

## Successful ARENA funded program lowers costs as much as 25%

There is very little data available with respect to "real world" operating costs in second-generation bioethanol facilities. Typically, any cost data is commercially confidential and not available to the public. Nevertheless, a lot of work has been carried out by Government and independent agencies over the years in determining the cost of second-generation bioethanol from biochemical pathways.

In determining the potential benefits of an optimised yeast biocatalyst developed by MicroBioGen, an estimate was made with respect to costs of a "typical" second-generation facility. It is possible that real costs could be significantly higher, but it is unlikely to be significantly lower. Based on modelling by MicroBioGen, it was determined that the potential impact on "effective" operating costs (after by-product revenues – high value yeast), the various benefits are presented in the chart below (Figure 5).

Figure 5 is a greatly simplified representation of the potential benefits of an MBG optimised yeast biocatalyst. All 2G bioethanol facilities are run differently, with most facilities using different biocatalysts. Each plant is also optimised towards the substrate that is being utilised, pre-treatment process and the strengths and weaknesses of the particular biocatalyst. While subject to variation, the benefits of an optimised yeast biocatalyst presented below are expected to be in the "ballpark" and may actually be understating the benefits.

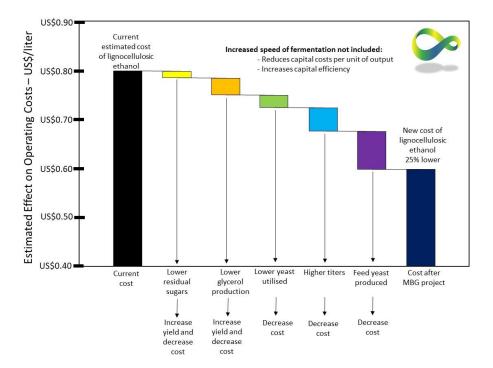


Figure 5: Improved yeast catalyst can significantly reduce costs of renewable lignocellulosic ethanol



## Capital costs could also benefit from an optimised biocatalyst

An optimised yeast strain developed by MicroBioGen holds the promise of faster fermentations, thus reducing the size of the equipment required to ferment and distil the resulting ethanol rich slurry.

By increasing the tolerance to organic acids and ethanol, the MBG developed yeast will also allow higher solids concentrations through the hydrolysis process, reducing water throughout the process and again, reducing the capital requirements.

Another benefit of increased acid and hydrolyzate resistance is reducing the need for any detoxification of the hydrolyzate. This is expected to benefit both operating costs and potentially lower initial capital costs.

One area where there will be an increase in capital costs is in the area of aerobic fermentation. In the MBG process flow, an aerobic propagator is required to grow the yeast on the low value waste stream. The capital cost to incorporate an aerobic propagator is expected to at least be partially offset by a lower capital cost requirement for waste-water management as well as greater water recycling possibilities using the MBG developed process flow. This ignores the significant benefit of converting low value or negative value waste-water into a high value, high protein single cell feed.

In all, the MBG developed process flow is not expected to add significant capital costs to a project – if any at all.

## Technical Success Criteria to achieve required project outcomes

MicroBioGen's global partner identified 13 criteria that needed to be achieved for the project to be considered a complete success. The precise details are of the 13 criteria are confidential, however, each of the criteria can be broadly identified as follows:

- High conversion of glucose to ethanol
- > High uptake of xylose into the biocatalyst cell
- High conversion of the xylose to ethanol
- Low level of glucose at the end of fermentation
- ➢ Biocatalyst must be able to reach a high level of ethanol in the fermentation
- Biocatalyst must be phenotypically stable
- Requires a small pitch at the start of the fermentation (i.e. little yeast required per fermentation)
- Fermentation time cannot exceed 50 hours
- Successful fermentation requires no exotic nutrients (saves costs)
- Siocatalyst can perform with high levels of the organic acid acetate
- Must be able to be produced/grown on standard carbohydrates
- Once grown, the yeast biocatalyst can be formulated as a stable dry yeast
- Can grow efficiently on non-sugars such as glycerol and organic acids



#### Technical success benchmarked using full scale hydrolyzates

The optimisation project was carried out over a three-and-a-half-year period. The original plan was to have the best optimised strains trialled at full scale in an operating second-generation bioethanol facility. However, at the beginning of the third year of optimisation the Covid-19 pandemic arrived and this severely limited opportunities for large scale trials.

MicroBioGen was able to secure real world hydrolyzates from a full-scale operation and use this hydrolysate material as the basis for trialling and benchmarking the biocatalyst yeast. It should be noted that, unlike other projects where scale-up is a significant risk, this is not the case with biocatalysts developed by MicroBioGen.

MicroBioGen has demonstrated through its 1G biofuels programs that it can mimic large scale fermentations (as much as 4 million litres) in laboratory fermentations with only 20 grams of material. This is the equivalent of a scale-up factor of 200 billion times. Given the success of the 1G development programs, MicroBioGen is confident that the 2G laboratory trials will also scale up accurately.

## Work carried out to benchmark MBG optimized biocatalyst

#### Successful aerobic fermentation in the MicroBioGen laboratories

One of the key success criteria was to demonstrate that the optimised yeast biocatalyst could be produced using industrial protocols on carbohydrate substrates. This photo shows actual trials underway.



Figure 6: Aerobic growth trials in MicroBioGen laboratories

Figure 7: MBG yeast biocatalyst morphology post-seed yeast propagation

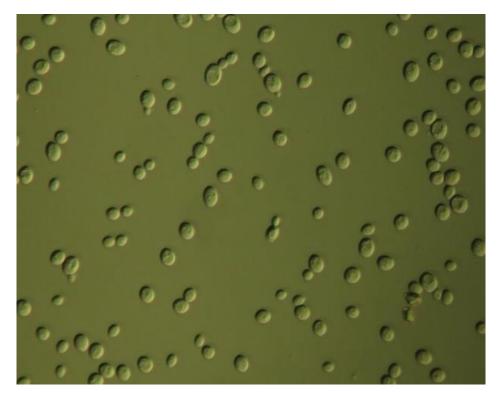


Figure 8: MBG yeast biocatalyst morphology in main propagation – 1st half



Figure 9: MBG biocatalyst yeast being prepared in a Fluidized bed dryer

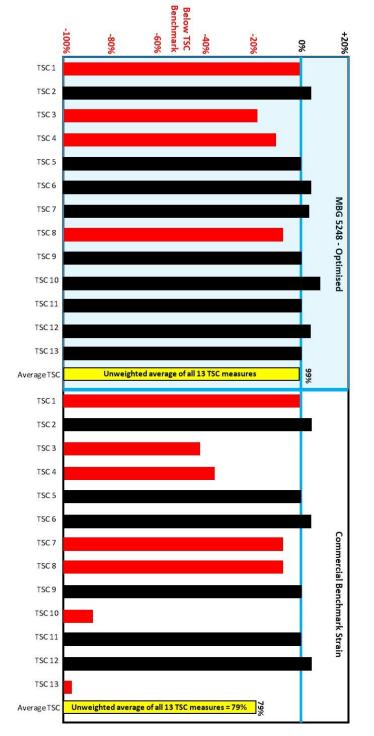


Figure 10: MBG biocatalyst yeast as an Active Dried Yeast (ADY)



Figure 11: MBG biocatalyst yeast as ADY in vacuum-sealed aluminium packaging





#### Optimised Yeast Biocatalyst outperforms Commercial Biocatalyst

Figure 12: TSC vs MBG Yeast and Commercial Strain – Medium Concentration Hydrolyzate

Figure 11 shows that the MBG Optimised Strain (represented in the top half) achieved an average of 99% of the 13 different TSC criteria, compared to just 79% for the Commercial Benchmark Strain (represented in the bottom half). A red bar indicates the biocatalyst did not achieve the TSC whereas a black bar represents the biocatalyst did reach the TSC.



## Optimised yeast outperforms commercial yeast in all scenarios

The Optimised MBG Biocatalyst strain was trialled against the 13 Technical Success Criteria (TSC) under 3 conditions. That is, high concentration, medium concentration and low concentration hydrolyzates. The benchmark commercial strain was trialled under the same conditions. Key points with respect to the trial results:

- The higher the concentration of hydrolyzate, the better the commercial outcome so long as the biocatalyst employed can handle the conditions and complete or nearly complete sugar conversion.
- > As concentrations increase, the greater the outperformance of the MBG optimised biocatalyst
- Since the MBG optimised yeast can grow on its own waste streams, even if extra sugars are left behind in the high concentration conditions, all sugars are converted to high value product – ethanol or single cell protein.
- The results achieved are outstanding and under all circumstances, the MBG yeast achieved an average with 3% of the TSC.

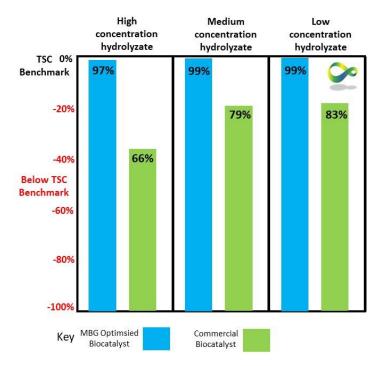


Figure 13: MBG Optimised biocatalyst strain relative to average TSC and benchmark biocatalyst strain



#### Game Changing Implications for sustainability – LCA Analysis

An LCA analysis has been carried out by Lifecycles – an independent expert group based out of Melbourne Australia that specialize in Life Cycle Analysis (LCA)<sup>11</sup>.

Life Cycle analysis is important as it calculates the estimated impact of any project on a range of important sustainability measures such as abatement of CO<sub>2</sub>, fossil fuel use (increase/decrease), water use and land use amongst others.

At the end of the 3.5 year ARENA funded Optimisation program, Lifecycles was engaged by MicroBioGen to analyse the impact of the optimised yeast biocatalysts from an LCA perspective. In order to have full confidence in the LCA analysis, the LCA was "peer reviewed" by a leading expert in the field – Annette Cowie.

MicroBioGen is an industrial biotechnology company and has no expertise in the field of LCA. However, it was clear to the scientists at MicroBioGen that optimizing a yeast biocatalyst with the following properties would likely have positive implications for LCA if deployed into industry. The core optimised benefits include the following:

- ➢ An ability to successfully ferment sugars from lignocellulosic sources at higher concentrations.
- > To be less impacted by higher organic acid levels found in lignocellulosic biochemical process.
- An ability for the yeast biocatalyst to grow on low value waste streams.
- A single biocatalyst that can convert sugars to ethanol (without air) and convert waste stream to protein (with air)

These seemingly simple characteristics required over 15 years of development and close to A\$30M in R+D. Having achieved the key characteristics, the LCA implications are game changing. A summary of the LCA result is presented below in Table 1:



	CLIMATE CHANGE	Fossil energy USE MJ NCV	PARTICULATE MATTER g PM2.5	EUTROPHICATION	CONSUMPTIVE WATER USE L	Land use m <u>².year</u> 1
	kg CO2 eq.					
Proposed project	1.32	22.2	-0.02	0.83	4.6	-2.32
Business-as- usual	1.85	25.0	0.25	1.66	18.8	1.66
Comparative results	-29%	-11%	-108%	-50%	-75%	-240%

Table 1: Comparative results for ethanol production using MicroBioGen yeast strains compared to using conventional yeast strains.

The LCA results are outstanding. That is, for the first time there is now a single biocatalyst that reduces  $CO_2$  by 29% against one of the leading current industry biocatalysts, reduces fossil energy use by 11% and importantly, reduces land use requirements by 240%. As a consequence of the ARENA project and MicroBioGen optimising the yeast biocatalyst, biofuels in the future will actually add to the food supply as well as the fuel supply. If CCS (carbon sequestration) is utilised on the  $CO_2$  produced during the fermentation stage, then biofuels will also be able to extract  $CO_2$  from the atmosphere – that is negative  $CO_2$  emissions - even when taking into account the utilization of the clean burning, high octane fuel.

MicroBioGen | Biocatalyst optimization for biofuels from non-food biomass



## Massive Positive Implications for Australia – Market Analysis

Through the three and a half year Grant program, MicroBioGen engaged independent outside experts to review both the implications of the technology (LCA analysis) and some of the commercial implications (Export and Market analysis). These independent reports showed that:

- The Asia Pacific was a potential export market for ethanol produced in Australia utilising the MicroBioGen Optimised yeast biocatalysts.
- The most beneficial market with respect to price is the Californian market with its LCFS program in place. The Californian LCFS model has been highly successful in generating the right conditions for penetration of biofuels into the fuel supply chains. This concept could be adopted in Australia.
- Australia has an excellent opportunity to utilise the MBG technology to develop an industry that would be capable of delivering enough bioethanol for a 10% replacement of fossil derived fuels (utilising sugar cane waste only) and at the same time produce over 130,000 tonnes of high value, high protein feed yeast for the domestic market – replacing a significant portion of current protein imports.
- The MicroBioGen optimised yeast would convert the biofuel industry into one that would significantly add to both fuel security and food security at the same time as adding regional job opportunities and increasing diversification of rural communities.
- To develop this market opportunity will require some Government support to lever open fuel supply chain with biofuels mandated to ensure market penetration. Successful models from other countries could be utilised as the basis for consideration.

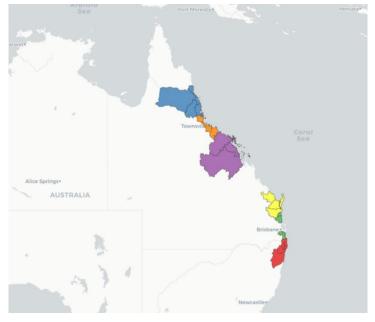


Figure 14: Available bagasse resource Australia



#### Conclusion

At the end of 3.5 years of optimising a commercial yeast strain supported through an A\$8M ARENA funded program, MicroBioGen achieved almost all its goals.

Based on the average of the 13 Technical Success Criteria, MicroBioGen was able to average between 97% and 99% of the key benchmark targets. The implications of the successful program are significant. Some of the implications include:

- An estimated 25% effective reduction in operating costs.
- High value single cell protein as a by-product from non-food biomass.
- The more biofuels manufactured the more high-value protein produced.
- If Australia converted just its sugar cane bagasse using the MicroBioGen optimised organism and process, then enough ethanol would be produced to replace nearly 10% of petrol and replace nearly 140,000 tonnes of currently imported protein feed.
- Peer reviewed Life Cycle Analysis (LCA) shows a 29% reduction in CO<sub>2</sub>, 11% decline in fossil energy use and a massive 240% decline in land use compared to the commercial yeast biocatalyst.

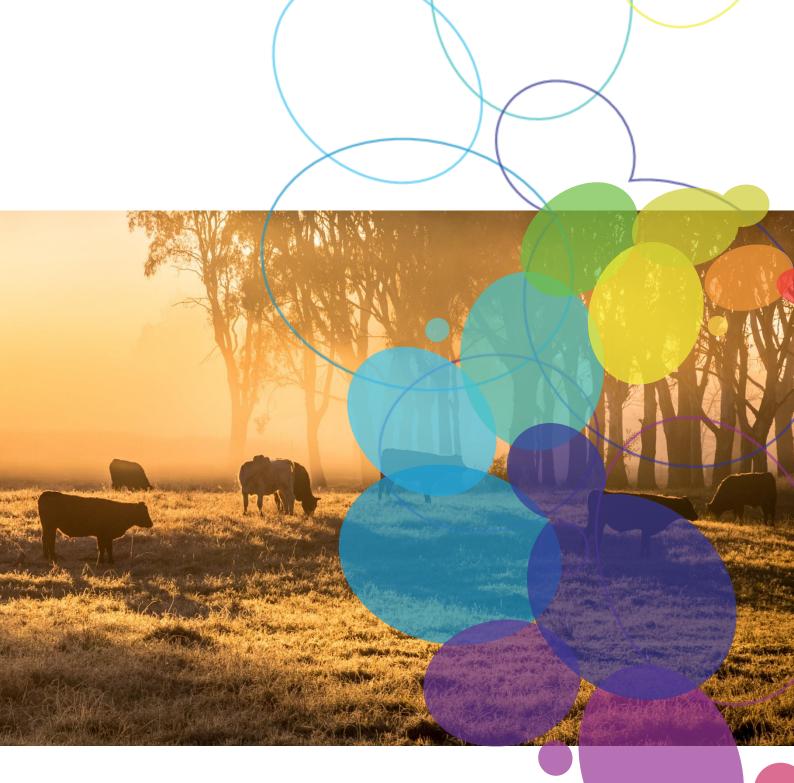
In the current environment where it appears that many believe that the future is all electric, the results achieved by MicroBioGen show that an "all of the above" approach makes more sense. The ability to lower CO<sub>2</sub> emissions faster, diversify risk away from a "single" technology approach, adding to the food supply and holding out the potential to have biofuel production extract CO<sub>2</sub> from the atmosphere are important issues to consider outside of just trying to reduce emissions. It presents Australia with the potential to reduce expensive protein imports, develop new export markets and develop and alternative supply to industries such as the military, heavy marine transport and heavy road transport amongst other opportunities.

MicroBioGen would not have been able to complete the program without the significant Federal Government support over the last 15 years. Previous grants allowed MicroBioGen to develop the base genetics and technology required for the successful optimisation program and the final A\$8M project allowed the completion to an optimised commercial product. Of course, the R+D will not stop where it is. The optimisation program highlighted areas of further opportunity to lower costs and increase efficiencies even further. As the MicroBioGen logo suggests, the possibilities with yeast are almost infinite.



- 1. MicroBioGen. 2021. ARENA Milestone 5.4 Report CONFIDENTIAL.
- 2. IFPEN. 2020. Economic Outlook Biofuels Dashboard.
- 3. Renewable Fuels Association. 2017. Ethanol and the Economics of Octane: The Superior Solution. Presented by Cooper, G.
- 4. Minnesota Biofuels Association. 2016. Ethanol and octane for beginners.
- 5. US Department of Energy. 2016. Billion ton report.
- 6. Tripathi, N. *et al.* 2019. Biomass waste utilisation in low-carbon products: harnessing a major potential resource. *npj Clim Atmos Sci* 2, 35. https://doi.org/10.1038/s41612-019-0093-5
- 7. Bell, G. 2020. Biobased Chemicals: Potential Opportunities for Queensland. Presented as part of the IEA Task 42.
- 8. Yang, H. *et al.* 2019. Overcoming cellulose recalcitrance in woody biomass for the lignin-first biorefinery. *Biotechnology for biofuels*, 12, 171. https://doi.org/10.1186/s13068-019-1503-y
- 9. Schill, S.R. 2018. Hatching Higher Protein. *Ethanol Producer Magazine*. April 2018 edition.
- 10. Gyekye, L. 2017. Second-generation biofuels 'more cost-effective' than first-generation biofuels, new study suggests. Biofuels International.
- 11. Lifecycles. 2021. Life Cycle Assessment of ethanol production from bagasse using MicroBioGen yeast strains.

Page | 21



Deliveries Unit E2, Lane Cove Business Park 32 Sirius Rd, Lane Cove NSW 2066

**Postal & Visitors** Unit E2, Lane Cove Business Park 16 Mars Rd, Lane Cove NSW 2066

**Tel** +61 2 9420 1244 contact@microbiogen.com

www.microbiogen.com



© 2021 Microbiogen Pty Ltd. ABN 91 096 059 700.