Non-Confidential Final Report

CONVERSION OF INDUSTRIAL CO_2 EMISSIONS INTO BIOFUELS AND CHEMICALS

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Abstract

Capturing one megaton of GHG emissions in Alberta per annum and recycling the carbon into valuable products is the goal of the CCEMC Grand Challenge. Current methods of carbon capture are cumbersome and extremely expensive to implement, making this process today a large cost center. To address this need, Oakbio proposes a novel biotechnology that captures large amounts of CO_2 at point source and converts the carbon into biofuel and chemical intermediates. Oakbio partnered with the F.R. Tabita lab at the Ohio State University to engineer its flue gas adapted microbial strains to produce the chemical *n*-butanol. *n*-Butanol currently serves a $9billion market as a chemical intermediate and is also an effective biofuel, with high octane level and low engine corrosion. In this project, we demonstrate the proof-of-concept for the conversion of CO_2 captured from Alberta industrial flue gases and conversion into *n*-butanol. Initial validation of the butanol producing strains is made using laboratory gas and cement flue gas. The most performing strains are then grown on raw flue gas samples collected in Alberta at a coal-fired cement plant and at an oil refinery, thank to industrial collaborations developed under this grant. In both cases, the production of *n*-butanol is validated. The process is successfully scaled-up to bench scale. Our life cycle analysis shows realistic potential to achieve the Grand Challenge GHG reduction goals when *n*-butanol is used as a gasoline replacement. At scale, and with the availability of abundant and inexpensive H_2 supply, like it is the case in Alberta, our techno-economic model calculates that profitable CO_2 capture can be achieved. The next step in process development is improving *n*-butanol concentration towards the commercial goal. Fermenter process design, media optimization and further synthetic biology are proven tools to enable it. Oakbio's results fuel the fundamental belief that effective and profitable methods of large scale carbon capture can be developed and have a significant impact on Climate Change.

Key Words

Carbon capture, carbon conversion, carbon utilization, *n*-butanol, biobutanol, butanol, biofuels, biocatalysts, CCEMC, Grand Challenge, Alberta, Oakbio

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Executive Summary

Project Description

The economy of Alberta, Canada, is dependent on heavy industry, including cement, chemicals manufacturing, oil and natural gas production and upgrading, all of which emit large quantities of carbon dioxide (CO_2). Anthropogenic CO_2 emissions are now recognized as a significant contributor to Climate Change. The government of Alberta places high value on finding and developing technology solutions that enable its core industries to continue production while, at the same time, reducing GHG emissions. The goal of the CCEMC Grand Challenge is to fund the development and installation of those technologies that have the potential to decrease net GHG emissions by 1 MT GHG per annum in the province of Alberta.

In Round I of the CCEMC Grand Challenge, Oakbio and the Prof. R. Tabita lab propose to develop a process that is able to capture CO_2 from Alberta's industrial flue gas emissions and convert it into the chemical *n*-butanol. *n*-butanol serves a $9B market as a chemical intermediate, and can be used as an effective transportation fuel. The key milestone of the round 1 work was the achievement of the proof-of-concept at bench scale, including an economic and GHG benefits evaluation for a commercial scale. At commercial scale, the use of Oakbio's *n*-butanol as a replacement for petroleum-derived gasoline is expected to provide a net GHG emissions reduction meeting the goal of the Grand Challenge.

Oakbio Inc., a Sunnyvale, California company, has been developing CO_2 capture and conversion systems since 2009. At the core of Oakbio's gas fermentation technology are microorganisms capable of using CO_2 as carbon feedstock and converting it into chemical products. Oakbio developed several strains of microorganisms, which are superior at capturing CO_2 and converting it to useful chemicals. In this project, Oakbio validated its technology on different flue gas sources and demonstrated production of commercially attractive chemicals.

Over the past several years, research in the F. R. Tabita lab at the Ohio State University has concentrated on elucidating and determining the mechanisms by which Hydrogen (H_2) bacteria regulate the biochemistry and genetic capacity for the conversion of CO_2 to the needed organic constituents required for cell growth. Gene engineering technology is a staple of the Tabita lab, which recently embarked on using synthetic biology principles to co-opt normal metabolic pathways so that the resulting engineered strains may produce desired products, such as liquid biofuels.

Outcomes & Learnings

Currently available technologies for carbon capture and sequestration (CCS) are largely non-biological. They are also cumbersome and extremely expensive to implement. Novel carbon capture and *conversion* (CCC) technologies, such as the OakBio technology, allow production of valuable chemicals from captured CO_2, which can re-enter the value chain, henceforth recycling the carbon. Revenues from product sales offset the cost of carbon capture and may even, as is the case for Oakbio, create a profitable business. Unlike the algal photosynthesis platforms used by some companies to capture carbon, Oakbio's process does not require an input of light

energy; therefore, it can operate 24/7, at all latitudes, and in high volume and high density, without the real estate requirements of photosynthetic systems.

Initial proof-of-concept of *n*-butanol production using cement flue gas from California was achieved thanks to Oakbio's existing collaboration with a major international cement producer, at one of its facilities in California. As part of the CCEMC grant, Oakbio was able to develop two important collaborations with industrial partners in Alberta: a large cement producer and a major oil facility, both subsidiaries of large international groups. These two industries are core to the local economy of Alberta and represent major sources of CO2 emissions in the province. Both were readily willing to cooperate with Oakbio under the CCEMC grant, however, it took perseverance and time before we were able to obtain gas samples, due to heavy regulations in the oil industry. The collected gas was shipped to California to run experiments at the Oakbio's laboratory in California.

In the CCEMC grant, Oakbio and the Tabita lab demonstrated for the first time production of *n*-butanol using CO2 from unadulterated Alberta flue gas as the sole carbon source. A new microbial strain able to convert industrial flue gas CO2 into *n*-butanol was created by combining one of Oakbio's proven flue gas-resistant microbial strains with the Tabita lab's ability to engineer it to produce *n*-butanol. After screening 2-3 generations of engineered microbes from the F. R. Tabita Lab, a mutant strain that produces a significantly higher *n*-butanol titer when fed cement flue gas from Alberta has been discovered. Additional engineered strains display improved biomass production from CO2 and can generate even higher titers of *n*-butanol when cultivated on lab gas.

Oakbio demonstrated significant *n*-butanol production on both Alberta cement flue gas and on gas samples provided by the oil refiner. Each new generation of engineered microbes showed incremental improvement in productivity. The system was validated up to 20L scale. In the next development stage, improvements in media composition, gas fermentation process engineering and further microbe engineering will make it possible to significantly increase process yield and overall productivity towards commercial profitability. Oakbio is also poised to build on its existing collaborations in Alberta in the next phases of the CCEMC Grand Challenge.

GHG / non-GHG Impacts

Butanol is currently used mainly as a chemical feedstock, priced at $7-8/gal in a $9 billion market. *n*-Butanol is also as an effective biofuel substitute for gasoline and for ethanol. *n*-Butanol has higher energy content than ethanol, enabling higher gas mileage for the same amount of fuel. *n*-Butanol can also be blended to higher concentration with gasoline than ethanol with no ill effects; since *n*-butanol does not absorb water, it is not as corrosive as ethanol to internal combustion engines.

The Alberta fuel market grew at a consistent annualized rate of 2.33% from 1993 – 2012. By 2019, the first year of commercialization of Oakbio's proposed technology, this annual market growth will result in a demand for more than 7 billion liters of fuel. (Ref: Statistics Canada, Table 405-0002 – Gasoline and Other Petroleum Fuels Sold, CANSIM database. http://www5.statcan.gc.ca/cansim/a05). If produced at large scale and at competitive cost, *n*-butanol could capture the 5% market share currently occupied by ethanol as part of the mandatory renewable alcohol blend. When *n*-butanol production cost becomes competitive against gasoline, then Oakbio product can penetrate the larger share of the transportation fuel

market. Oakbio's carbon life cycle analysis estimates that the company's *n*-butanol product has the potential to reduce GHG emissions by 1.8 kg of CO_2e/L of gasoline (produced from Alberta's oil sands) replaced. Based on the above assumptions, Oakbio's projected CO_2 emissions reductions at commercial scale could represent 1.04 Mt p.a. by 2028, increasing to 1.5 Mt p.a. by 2033.

Hydrogen is the source of energy used by Oakbio's microorganism to fix the carbon from CO_2. At large scale, H_2 is currently produced from steam reformation (SMR) of natural gas, which has become inexpensive, due to increased availability and current low price of natural gas resources. At the same time, technologies of H_2 production from electrolysis of water are being developed with the goal to make it price competitive with SMR. Increasing amounts of green electricity are also available from wind, solar, hydro-electric and other processes. If H_2 is derived from renewable sources, the proposed process can be entirely GHG emissions free. For a project located in Alberta, we considered that H_2 to be supplied by a H_2 pipeline or a co-located SMR, both of which are located near each other in the province.

Based on Oakbio's techno-economic model, a 12.5 million L fermenter could capture 1 million ton of CO_2/year and convert it into *n*-butanol at a profit of $49 per tonne of CO_2 captured. Profit is significantly higher if the *n*-butanol produced is sold as a chemical intermediate rather than as a gasoline replacement. The Oakbio's profit figures compare very favorably with losses of $40-$70/tonne CO_2 captured expected from current amine-based methods of carbon capture, with additional costs accrued for CO_2 compression, purification, transportation and disposal costs. Some estimates range as high as $80-$120/t CO_2 when all Capex and Opex costs are added.

It is also important to note that several other possible value streams can be generated from Oakbio's process. These include sales of secondary products (residual biomass and other valuable products contained in it) but also remediation of non-GHG pollutants commonly found in industrial flue gases (H_2S, NOx, SOx, CN). Some amounts of these toxic components could be fixed by Oakbio's microbes.

It is hoped that Oakbio's demonstrated platform has the potential to help Alberta's energy sector emerge as the nexus for technology innovation in carbon capture, create jobs locally, and foster economic activity in related fields (construction, engineering, and transportation sectors).

Overall Conclusions

1. Proof of concept on lab gas and flue gas: Oakbio and the F.R. Tabita lab succeeded in creating a microbial strain that consumes CO_2 directly from raw flue gases and converts it into the gasoline replacement *n*-butanol. Initial proof of concept was achieved on lab gas and on cement flue gas collected in California.
2. Oakbio partnered in Alberta with two core industries that are CO_2 emitting: a cement plant and an oil refining facility. Samples of gas emissions were collected at both sites in Alberta and the compressed gas cylinders were shipped to Oakbio for testing.
3. Proof of concept production of butanol from the Alberta cement flue gas demonstrated a significant titer during lab-scale fermentation.
4. Proof of concept production of butanol from the Alberta oil refinery flue gas demonstrated an even higher titer during lab-scale fermentation.

5. The fermentation process was successfully scaled-up to 20L fermenter on both lab gas and Alberta flue gas. A twenty liter fermenter is considered to be a large size at bench scale, and a necessary milestone before scaling-up to pilot size. Oakbio then mapped-out the path for further process scale-up, towards pilot and commercialization.
6. Life cycle analysis on GHG reduction using the Oakbio process shows realistic potential to achieve the goal of reducing Alberta's GHG emissions by >1Mt per year. The modeling is based on *n*-butanol partially replacing gasoline as transportation fuel, with the result that GHG emissions will be reduced by 1.8 kgCO_2e/L gasoline replaced.
7. At scale, and in the presence of an inexpensive source of H_2, Oakbio's technology can transform carbon capture from a cost center to a profit making business. Our techno-economic modeling calculates that, at commercial scale, profitability is achieved. An Oakbio plant could capture 1 M metric tonnes of CO_2 per year, produce 493ML *n*-butanol and make a profit of $208M/year.

The commercial ramifications of these results are significant. Government regulators are setting up production caps based on carbon emissions ceilings /site and are increasing carbon taxes beyond that level. Oakbio's successful proof of concept for profit-driven CO_2 capture opens an attractive value proposition to CO_2 emitters. In Alberta, the availability of inexpensive natural gas is particularly advantageous, as it allows for the production of inexpensive H_2 through SMR, which significantly increases the profitability of Oakbio's method of CCC. Oakbio's process does not need carbon credits to be profitable, and so the credits can be transferred back to the CO_2 emitter partner.

Driven by forthcoming stricter regulations on carbon emissions, cement manufacturers have expressed more interest in Oakbio's process potential for reducing GHG emissions at a profit or neutral cost than by the manufacture of a new line of chemical products. Hydrogen can be supplied through pipeline or by setting up an SMR on site. SMR emits CO_2, and so the major net GHG reductions are obtained through petrochemical displacement by Oakbio's products.

Oil upgraders are chemicals producers and already possess SMR unit(s) on site. In our work, we successfully used SMR gas from an Alberta oil upgrader to grow microbes that produced *n*-butanol. Most of the feed gas to the SMR is coming from different offgas steams from their hydroconversion process, the rest being purchased natural gas. With Oakbio's technology, this inexpensive SMR gas (which contains CO, CO_2, H_2 and CH_4) could be used as a feedstock for *n*-butanol production, and more broadly, as an alternative to bitumen for the production of a portfolio of valuable chemicals. The presence of SMR units on site, the desire to mitigate the CO_2 release from the gasification of asphaltines and other SMR waste gases, all make Oakbio's technology an attractive value proposition to explore for an oil upgrader.

Next Steps

In the next stage of development, Oakbio's will validate the process at pilot scale with the following goals:

1. Increase *n*-butanol concentration
2. Scale-up the process to ~1,000+L fermenter size

The risk of this next phase of development is largely mitigated by the fact that several other companies in related fields have been successful following a similar path, and that Oakbio will leverage a number of proven technologies in its endeavor.

This stage will not only validate the process scientifically, but it will also confirm its GHG reduction potential and economic attractiveness.

Increasing *n*-butanol production

A butanol concentration of 5g/L is recognized as the threshold at which *n*-butanol harvesting becomes economically feasible. Methods of butanol extraction are well defined as biological production of butanol (with acetone and ethanol) was one of the largest industrial fermentation processes early in the 20th century. As opposed to current Oakbio's gas fermentation, these earlier microbial processes used sugar-based fermentation.

Typically, strain improvement programs focus on achieving a certain titer first and then work on improving productivity towards a desired commercial goal. Such improvements in titer and productivity (representing, for example, a 100- to 200-fold improvement) are realistically achievable goals. Companies such as Lanzatech, Coskata and Amyris have all scaled up their original lab scale synthetic biology platforms by implementing continuous incremental improvements in yield, titer, productivity, etc by 250-fold or more. Oakbio has already improved its production of *n*-butanol from Alberta flue gas sources over 15-fold during the course of this CCEMC grant. The methods to achieve these improvements are well established and can easily be applied to Oakbio's platform:

1. **Pathway engineering**. The Tabita lab at the Ohio State University will continue constructing new mutant strains that build upon growth data and butanol production data on flue gas obtained by Oakbio.
2. **Strain engineering**. Random and targeted mutagenesis will be used to improve the overall performance of the host strain outside of the pathway of interest. Oakbio has in-house expertise for enzyme engineering, gene shuffling and other microbial synthetic biology methods acquired at Intrexon, Maxygen and Codexis.
3. **Medium optimization.** For instance, Oakbio developed an improved fermentation medium for variants cultivated on flue gas, resulting in cells reaching production phase faster than before.

Process Engineering and Scale-Up to 1,000+ L

The next stage for the gas fermenter system is to scale-up from current lab scale (20L) to pilot scale (~1,000+L fermenter size) or a 50 fold increase. Gas fermentation at scale is not a novel science, it has been pioneered by Russian scientists 30 years ago, and more recently, companies such as Lanzatech and Coskata have been scaling up their process to commercial size. Lanzatech gas fermentation process also uses microbes, albeit different than Oakbio's, to convert steel mill off-gas or other syngas (containing CO, H2, CO2 and CH4) to produce ethanol. Current plan with their steel mill partner includes a 34 million gal/yr ethanol production plant.

Introducing better control of the fermentation environment through automation and analytics will permit more efficient conversion of gas feedstocks into product. A very large production enhancement could potentially be attained by improving the fermentation conditions.

Oakbio has already designed a custom 100L gas-fed bioreactor aimed at optimizing process parameters relevant for industrial applications. If awarded a CCEMC Grand Challenge round 2 grant, Oakbio plans to locate a 1,000+L pilot at an incubator in northern Alberta and another one at a second CO2 emitter site. A neutral location would be more accessible physically and offer more flexibility (such as the ability to test multiple sources of collected flue gas samples), whereas co-location strengthens the bond with the industrial partner.

Communications Plan

Oakbio and the team from the F. Robert Tabita lab at the Ohio State University plan to author and submit a peer reviewed publication on the subject of CO2 capture from industrial flue gas using data collected during this work. Additionally, Oakbio will submit an article to *Cement World*, an industry journal which covers the cement industry.

We will also give company presentations, which include some of our research findings at conferences, such as "The Advanced Biofuels Leadership Conference" (ABLC) held in November 2015 in San Francisco. We could not identify an appropriate conference in Alberta over the next few months, but will ready when such an opportunity occurs.

We will report the results obtained in this grant to our industrial partners in Alberta, the cement plant and the oil upgrader, to engage them into the next phases of our development. We will broaden our reach to other companies in the field and to potential investors in order to secure the funds necessary to bring this promising new technology to commercialization.

1. Project Description

1.1 Goal of the Grand Challenge

The Province of Alberta has taken a world-leading position in adopting legislation to address climate change, beginning in 2003 with the passage of the Climate Change and Emissions Management Act (Statutes of Alberta, 2003). This Act first established the Province's goal of reducing its GHG emissions to 50% of 1990 levels by 31 December, 2020.

In 2007, Alberta implemented its Greenhouse Gas Reduction Program in support of this overall goal. The Program requires large GHG point sources (emitters of more than 100,000 tonnes CO2e per year) to reduce their GHG emissions by 12% currently, with the potential to grow to 20% by 2017. Emissions over this cap will be charged $30 per tonne (Alberta Greenhouse Gas Reduction Program, 2015).

The goal of the CCEMC's Grand Challenge is to fund development of carbon utilization technologies that can reduce GHG emissions from Alberta's major emitters by more than 1 million tonnes per year.

1.2. Methods for Reducing Point-Source GHG Emissions

A number of technologies are currently proposed for reducing GHG emissions from large point sources such as oil refineries or coal-fired or natural gas-fired power plants. While a complete review of these technologies is outside the scope of this document, it is important to note that they fall broadly into one of two categories: "CO2 Capture and Sequestration", and "CO2 Capture and Conversion" (CCC), also called "CO2 Capture and Utilization" (CCU).

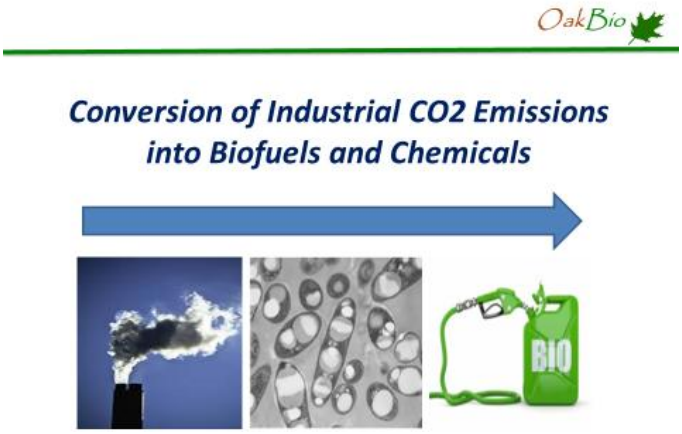
CO2 Capture and Sequestration
CO2 Capture and Sequestration (CCS) technologies separate CO2 from industrial flue gases before emission into the atmosphere, and then store that separated CO2 underground or in the deep ocean in perpetuity. Limitations of CCS detailed in Appendix 1.

CO2 Capture and Utilization
In contrast to CCS, CO2 Capture and Utilization uses industrially-emitted CO2 as the feedstock for production of a carbon-based value-added product, such as plastics or fuels. These technologies regard carbon dioxide (CO2) emissions as a widely-available, inexpensive manufacturing feedstock that they can convert into value-added products.

Utilizing CO2 as a carbon resource, rather than storing it in perpetuity, is economically advantageous. It eliminates the issue of CO2 storage and transportation costs, and provides an economic incentive by generating a marketable product. The end product can sequester the capture carbon for long or short periods of time depending on its use. When the end product is used to replace an otherwise petroleum-derived product, it also creates a GHG reduction.

1.3 The Oakbio/FRT Lab Proposal

Figure 1: Schematic of the Oakbio Process



Oakbio and the FRT Lab jointly proposed to convert carbon dioxide (CO_2) from Alberta's industrial emissions into a transportation fuel for use by Alberta's citizens using a specially-developed microbial carbon capture and utilization (CCU) process. Oakbio believes that the proposed technology will be attractive to major industrial emitters in Alberta and worldwide because it will utilize their GHG emissions to produce value-added chemical products. Carbon emissions will be transformed from a cost center to a profit center. On a life-cycle basis, the proposed technology will reduce net GHG emissions by replacing gasoline produced from crude oil with *n*-butanol, an effective transportation fuel.

More specifically, in Round I of the Grand Challenge, Oakbio and the FRT Lab will demonstrate proof-of-concept of their technology, the technical feasibility of a novel microbe-based process for capture and conversion of flue gas CO_2 to the gasoline replacement *n*-butanol. Technically, the project goals include:

1. Combine Oakbio's pre-existing flue gas-resistant, CO_2-consuming microbial strain with Tabita Laboratory's ability to engineer strains to produce *n*-butanol from CO_2.
2. Demonstrate initial proof-of-concept production of *n*-butanol using laboratory gas and cement flue gas sourced from a cement plant located in northern California.

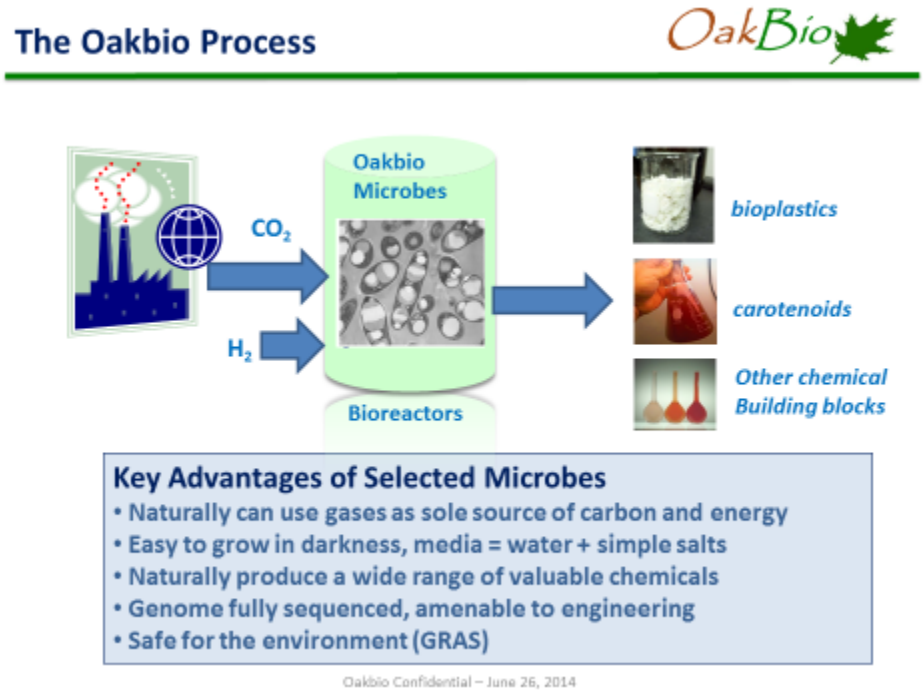
2. Technology Background

2.1. Oakbio Technology

At the heart of Oakbio's technology are carefully selected microbes that can directly consume CO2 as their sole source of carbon and H2 as their sole source of energy. This is referred to as "chemoautotrophy". Chemoautotrophy is a well-known type of microbial metabolism, studied in detail since the mid-1970's (Volova, 2009). It is similar to photosynthesis, the process through which plants and certain bacteria fix CO2 using light energy, except that H2 takes the place of light. Oakbio's chemoautotrophic microbes provide several key advantages over fermentative organisms used currently for industrial processes:

- They naturally use inexpensive gases as their sole source of carbon and energy
- They grow in darkness around the clock, and require only water and simple salts in their growth media.
- They naturally produce a wide range of valuable chemicals.
- Their genomes are fully sequenced, so they are amenable to genetic engineering.
- They are derived from strains that are Generally Recognized As Safe (GRAS) by the US FDA, so they are safe for the environment.

Figure 2: Schematic of the Oakbio Process



Carbon monoxide can also act as a possible energy source and as a carbon source, but CO generally works best in a mix with H_2 and/or CO_2 and/or O_2, due to its toxicity to microbes (Volova, 2009). Oakbio's CO2 Capture and Conversion technology represents the first to use

industrial CO_2 from flue gas and H_2 for chemoautotrophic production, converting CO_2 to chemical value. (More detail on Oakbio's technology development is provided in Appendix 2).

Oakbio-Lehigh Southwest Cement Plant Field Laboratory

For the past three years, Oakbio has collaborated with the Lehigh Southwest Cement Plant in Cupertino, CA, to operate an on-site lab to monitor microbial growth (shown in Figure 3). This field lab allows Oakbio to test the ability of its microbes to grow on unadulterated cement flue gas as their sole carbon source, and H2 as their sole energy source. The field lab is contained in a large steel container:

- The environmental testing enclosure was provided by the Lehigh plant.
  - A vertical pipe leading into the container transports cement flue gas directly from the kiln to Oakbio's fermenters.
  - The flue gas is cooled from >100°C to ambient temperature (~30°C). No other alterations are made before it is bubbled into Oakbio's fermenters.
- In this way, Oakbio is able to grow multiple cultures at 1L and 20L scale, capturing CO2 from cement flue gas and converting it into biomass and bioplastics.
- Oakbio has utilized this field lab as a test-bed for testing the growth of a number of different bacterial strains in the presence of actual cement flue gas.

2.2. Tabita Laboratory Technology

Over the past several years, research in the FRT Lab has concentrated on elucidating and determining the mechanisms by which H_2 bacteria (including photosynthetic varieties) regulate the biochemistry and genetic capacity for the conversion of CO_2 to the needed organic constituents required for cell growth. Gene engineering technology is a staple of the Tabita Laboratory. They recently embarked on using synthetic biology principles to co-opt normal metabolic pathways so that engineered strains may produce desired products, such as liquid biofuels. Using genes obtained from other organisms, they have engineered *R. eutropha* strain H16 to produce butanol from CO_2 feedstocks, via the protocols illustrated in Figure 4.

Figure 4: Production of *n*-butanol from CO2 and H2.

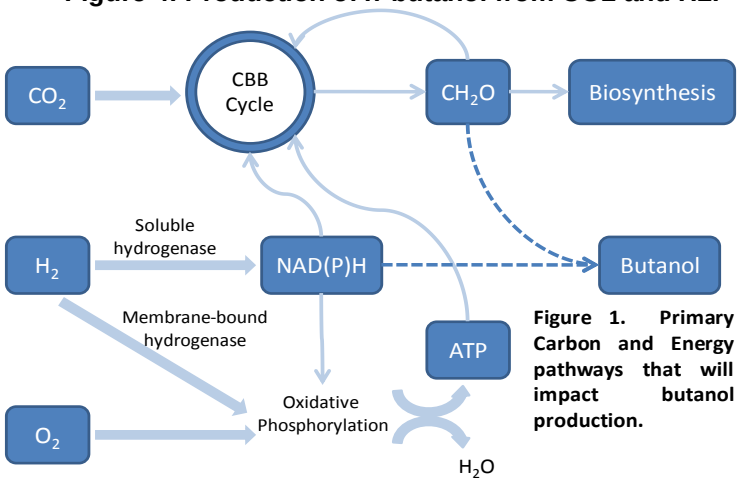


Figure 1. Primary Carbon and Energy pathways that will impact butanol production.

3. Work Scope

3.1. Objective

The objective of this project is to demonstrate proof-of-concept production of *n*-butanol from Alberta industrial flue-gas. The ultimate goal is to capture and convert a large amount of CO2 in Alberta. Positive results and techno-economic analysis will further validate the Oakbio/FRT Lab integrated process as a viable, scalable industrial platform.

3.2. Tasks (more detail in Appendix 3)

Table 1: Production of *n*-butanol from CO2 and H2.

Task		Description	Duration (by quarter)					
1	Oakbio	Administration						√
2	Tabita Lab	Creation of engineered microbes for n-butanol production						√
3	Oakbio	Screen engineered strains on gas mixtures using lab and flue-gas and perform analytics						√
4	Oakbio	Demonstrate process scale-up to 20L bioreactors						√
5	Oakbio	Demonstrate production of n-butanol from Alberta flue-gas						√
6	Oakbio	Estimation for GHG reduction and techno-economic analysis						√

3.3. Milestones

The Milestones listed below were achieved by executing iterative experiments, in which the Tabita Laboratory created chemoautotrophic, *n*-butanol producing microbial constructs using Oakbio's OB311 strain as background, Oakbio analyzed the productivity of each on laboratory gas and on at least one industrial flue gas, and the data were used to improve the Tabita Laboratory's next generation of constructs. This iterative cycle was executed five times.

- Mid-Project Milestone:
  - Tabita Laboratory: provide multiple strains of *n*-butanol producing microorganisms
  - Oakbio: complete testing of best strains of *n*-butanol producing microorganisms on laboratory gas mixtures and on cement flue gas.
  - Oakbio: scale up promising strains to 20 L bioreactors.
- End of Project Milestone:
  - Oakbio: demonstrate *n*-butanol production from at least one of the Tabita Laboratory engineered organisms on at least one of the Alberta collected flue gases
  - Oakbio and Tabita Laboratory: prepare report on strain and process engineering progress, estimate GHG capture based on product yields of Tabita Laboratory/Oakbio system and associated techno-economic analysis.

4 - Experimental Methodology

See Appendix 4 for a full literature review of scientific topics related to this proposal.

4.1. Microbe engineering – F.R. Tabita Lab

There are two major approaches taken with respect to strain modification of flue gas utilizing hydrogen bacteria to maximize product formation from CO_2. These include:

(1) enhancing carbon uptake capabilities in test strains.

(2) using synthetic biology approaches to construct strains that will enable substantial titers of butanol to be produced.

These are ongoing objectives as considerable strain modification is required. We are guided in these studies by prior successful modification of the widely used industrial organism, *Escherichia coli*, to produce substantial titers of butanol without the need for expensive antibiotics and inducer molecules [Laguna et al., Metabolic Engin.. Commun. 2, 6-12 (2015)]. Aspects (1) and (2) will eventually be combined to construct strains that maximally convert CO_2 from flue gas into butanol.

4.2 Chemoautotrophic Growth – Oakbio

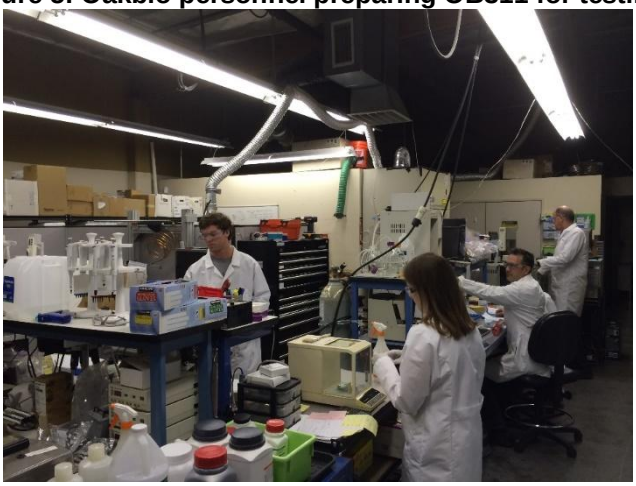
Oakbio has received and tested five "generations" of *n*-butanol-producing strains from the FRT Lab. Each generation has consisted of multiple variants. Each strain that Oakbio received from the FRT Lab was initially tested simply for growth on lab gas. This was done to ensure that the strain construction process had not interfered with the microbe's ability to metabolize gases (as opposed to sugar). Any microbe that had lost a significant portion of its ability to grow utilizing CO2 as its sole carbon source and H2 as its sole energy source was not further characterized for *n*-butanol production.

The FRT Lab identified two of these variants as top butanol producers in *R. eutropha* H16. The FRT Lab then incorporated these pathways into Oakbio's industrial flue gas-resistant strain OB311, and these strains were then tested for growth on gas at Oakbio.

For 'lab gas' cultures, a mixture of commercial gases (a CO2-H2-O2 mixture) was supplied to the culture bottles. For cement 'flue gas' cultures, the flue gas obtained from the plant (which contains CO2, O2 and trace combustion gases) was mixed with commercial H2 and then supplied to the cultures.

Cells were cultured with continuous bubbling and agitation in a temperature-controlled incubator at 30°C. At regular intervals, small cultures samples (~1.5 ml) were aseptically removed. The optical density of each culture was recorded in duplicate by an ICN Titertek microplate reader by measuring the absorbance of 200 ul samples at 620 nm. The dry weight of each culture can also be determined by centrifuging the culture, washing and resuspending the pellet in the same volume of water, transferring the washed cell suspension to a pre-weighed test tube, drying the cells in a lyophilizer, and determining the net weight of the lyophilized cells.

Figure 5: Oakbio personnel preparing OB311 for testing.



Assays for production of *n*-butanol were performed on the remainder of each culture sample described above. The remaining volume of each sample was centrifuged to remove the cells and debris, and then the *n*-butanol content of the supernatants was analyzed in triplicate. In this way, the *n*-butanol production data can be compared with the growth curves for each variant.

These experiments can be carried out in the same way by substituting different OB311 variants and/or cultivating them on different sources of flue gas to determine whether the cells tolerate and grow on a given source of gas, as they have already been shown to do on other flue gases we have tested.

4.3 Testing on Various Gases

Oakbio has used three distinct gas mixtures (Table 3) for the CCEMC experiments:

1. **Oakbio Lab Gas** – a mixture of pure CO_2, oxygen (O_2), nitrogen (N), and H_2. This mixture is used to set a baseline level of growth and production performance for Oakbio's microbes and for comparison with industrial gas sources.
2. **Alberta Cement Plant Flue Gas** – unadulterated flue gas collected immediately prior to emission into the atmosphere from a major Alberta cement plant. As can be seen from Table 3, this gas contains environmental contaminants such as NOx and SOx at ppm levels; these can also affect microbial growth. H_2 was mixed into this gas at Oakbio's Sunnyvale, California laboratory, no other changes were made.
3. **Alberta Oil Refinery Gas** – specifically, "Pressure Swing Absorber (PSA) inlet gas" from normal operations of a Hydrogen Manufacturing Unit (HMU) at a major Alberta oil upgrader and refinery. PSA inlet gas is an ideal microbial feedstock because it already contains the appropriate ratio of H_2:CO_2 desired by Oakbio's microbes.

Note that H_2 must be added to the cement flue gas, diluting the %CO_2 and %O_2 reported in Table 2 from their original compositions. In contrast, the gas produced by SMR contains sufficient quantities of H_2 for Oakbio's microbes to grow on their own.

The Alberta cement flue gas contains CO, NOx and SOx in measurable amounts (on the order of parts per million) and may even contain hydrogen sulfide (H_2S). This will be the case when coal is burned to provide energy for the cement manufacturing process. At the time the cement flue gas was collected for these experiments, the plant was burning coal to provide approximately

50% of its energy requirements. Therefore, the cement flue gas creates a challenging and industrially-relevant environment for testing the CCC capabilities of Oakbio's microbes.

A note about the Alberta oil refinery gas: this is a gas mixture produced by an HMU owned and operated by a major multinational oil conglomerate in the Province of Alberta. Briefly, this HMU consists of an SMR (which cracks CH4 into H2, CO2, and CO) and a PSA (that purifies the H2 for use in oil upgrading and refining). "PSA inlet gas" was collected after the SMR and before the PSA. Therefore, the gas used here is a mixture of H2, CO2, methane (CH4), and carbon monoxide (CO). A small amount of air was mixed in with this gas at the Sunnyvale, California laboratory to provide O2 for Oakbio's microbes; no other alterations were made.

Sourcing Unadulterated Flue Gases from California Industrial Partners

The experiments described above were performed using laboratory gas mixtures as control experiments, and using unadulterated flue gases from Oakbio's industrial partners. Collection of these unadulterated flue gases for use in growth and *n*-butanol production experiments is shown in the two Figures below.

Figure 6: Collection of flue gas from a power plant.

Dr. Erika Segraves, Oakbio's Principal Investigator, collecting flue gases for testing from a local power plant.

Figure 7: Oakbio cement plant field laboratory.

Oakbio's on-site field laboratory at the Lehigh Southwest Cement Plant. Note the direct feed of flue gas into the field laboratory (vertical pipe).

Note that Figure 6 illustrates gas collection at a local (California-based) natural gas-fired power plant, and Figure 7 shows Oakbio's on-site field laboratory at the Lehigh Southwest Cement Plant located in Cupertino, CA.

Oakbio developed the "next generation" of its mobile gas-collection apparatus especially for the CCEMC Grand Challenge.

Sourcing Unadulterated Flue Gases from Alberta Industrial Partners

The gas collection system was shipped to a cement plant located in the Province of Alberta, where Oakbio personnel used it to collect enough flue gas to perform several months' worth of gas-fermentation experiments.

Development of Alberta Industrial Relationships – Oakbio

Immediately after the kick-off meeting in April, 2014, Oakbio began its efforts to establish collaborations with Alberta industrial installations in order to source flue gas for its work with the CCEMC. Two Alberta flue gas emitters emerged as collaborators:

1. An oil upgrading facility
2. A cement manufacturing plant

These two plants represent industries that are core to the local economy of Alberta but that also emit large volumes of CO_2 in the province. Both companies were readily willing to cooperate with Oakbio under the CCEMC grant, however, time, patience and perseverance were required before Oakbio was able to collect gas samples on their sites. These delays can be expected when working with heavily regulated industries in Alberta or elsewhere.

These two sites were selected as having high potential for collaboration:

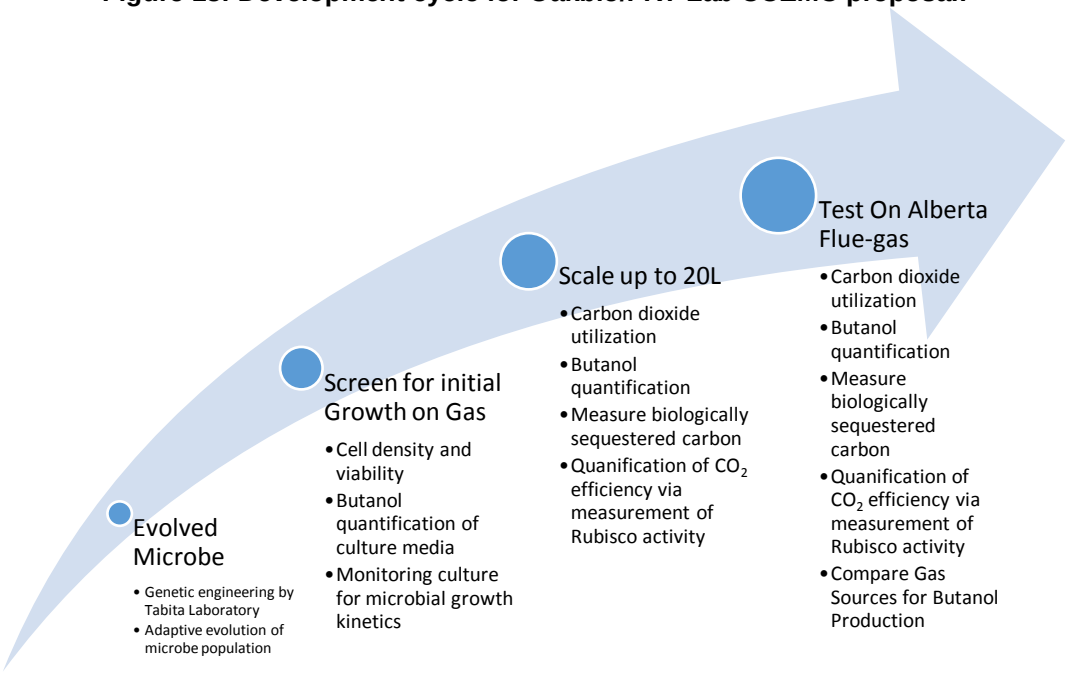
- The oil upgrading facility represents a strong potential commercialization path as it is one of Alberta's core industrial sectors.

  The facility generates a product gas that consists of a high percentage of H_2, along with a significant portion of CO_2. This gas mixture is ideal for Oakbio's flue gas-adapted microbe because it utilizes H_2 as its energy source and CO_2 as its carbon source. At scale, utilizing a product gas that includes H_2 would significantly decrease the cost of production of any end products.

- The Alberta cement plant uses a similar process to that of Oakbio's current, California-based collaborator, but utilizes different combustibles to heat the kiln.
  - Oakbio already has a relationship with a cement manufacturer in Cupertino, CA. Therefore, Oakbio's technology has significant potential for success.
  - The Alberta cement plant is located in a high-profile site, and is therefore under increased public pressure to address climate change. In fact, this plant was suggested as a possible project site by one of Oakbio's reviewers at the CCEMC Grand Challenge kickoff conference for precisely this reason.
  - This cement plant utilizes coal as part of its process to heat the kiln, which was not the case for our cement collaborator in California. This opportunity gives Oakbio a chance to test its micro-organisms to the presence of coal combustion gas.

5. Scientific Outcome

Figure 13: Development cycle for Oakbio/FRT Lab CCEMC proposal.



Task 2 – Engineer microbes to produce n-butanol (FR Tabita lab)

Strategy:
Inactivation of Competing Pathways
Insertion of Improved Enzymes for Butanol Production Pathway
Enhancement of Intrinsic CO2 Fixation Capabilities

Outcome: Over the course of the duration of this grant, and thank to gas culture feedback loop with Oakbio, the Tabita lab delivered to Oakbio five generations of engineered microbes, with an average of four microbe variants in each generation. The result of their growth and butanol production is highlighted in the next paragraphs.

Task 3 – Screen engineered microbes on lab gas and cement flue gas

Results:

Growth of engineered strains on lab gas and Lehigh cement flue gas was demonstrated at small scale and at 20L bioreactor scale.

Significant production of n-butanol by some of the mutants was demonstrated for both types of gas.

Task 4 – Scale-up gas fermentation process to 20L

Results:

Growth of engineered strains on Alberta cement flue gas was demonstrated at 20L bioreactor scale and significant n-butanol production was measured.

Task 5 – *n*-butanol production from Alberta flue gas

Results:

Growth of strains engineered for improved carbon uptake and improved butanol production was demonstrated on lab gas, Alberta cement flue gas, and Alberta oil refinery upgrader gas.

Significant production of n-butanol by some of the mutants was demonstrated for both types of gas.

The newest engineered strains showed a 10x increase in butanol production on flue gas compared to previously tested strains.

Overall Scientific Outcome:

1. All of the strain OB311 mutants can be cultivated on CO2-H2-O2 feedstock gas at scales ranging from 0.25 to 20 liters.
2. OB311 mutants can be successfully cultivated on raw cement flue gas and upgrader gas from Alberta.
3. Enhanced carbon uptake mutants generally show enhanced production of biomass from CO2.
4. The new butanol production mutants show significant improvement in *n*-butanol titers compared to the previous generations of mutants.

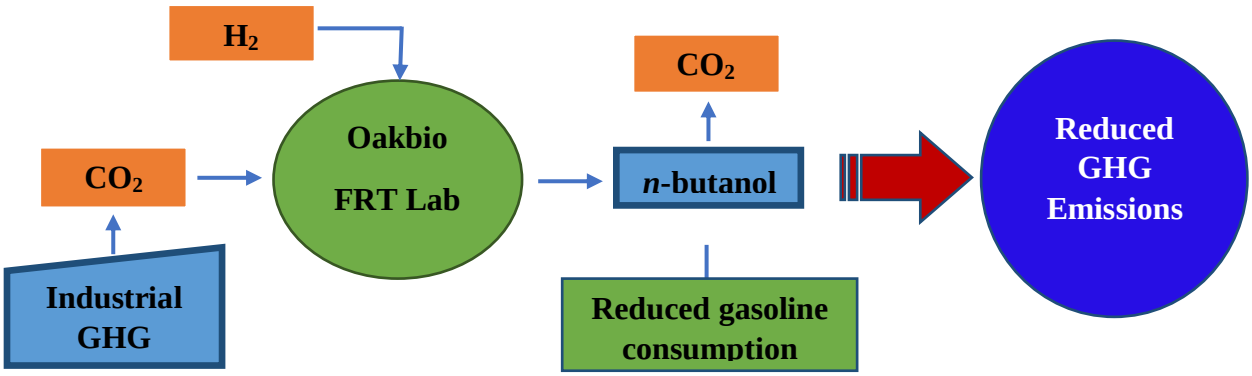
6. GHG Reductions and Other Benefits

6.1 Approach

Oakbio's technology will reduce GHG emissions by converting industrial CO2 into *n*-butanol, a light-duty transportation fuel that can replace gasoline. The goal is to demonstrate opportunity to reduce Alberta's net GHG emissions by >1 MT per year.

While *n*-butanol is being used as a chemical feedstock, demand for fuel in Alberta is significantly larger than worldwide demand for *n*-butanol as a chemical intermediate. For example, worldwide demand for *n*-butanol as a chemical feedstock in 2001 was roughly 2 gigaliters (OECD SIDS initial assessment report, 2001); demand in Alberta alone for light-duty transportation fuel that year exceeded 4.5 gigaliters (Statistics Canada, Table 405-0002).

Figure 22. Schematic diagram of Oakbio/FRT Lab GHG emissions reductions.



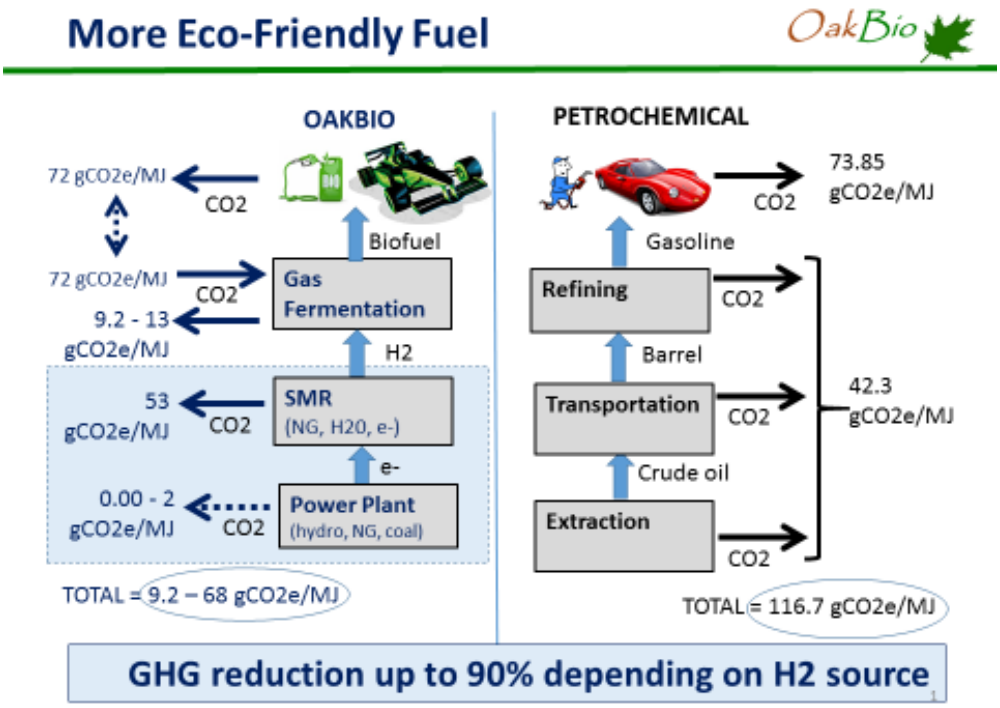
For Oakbio's *n*-butanol manufacture, the upstream materials are H_2, small amounts of inexpensive mineral salts, and carbon-containing industrial flue gases. The principal source of GHG emissions due to the Oakbio/FRT Lab proposal is projected to be from the supply for H_2, depending on the source. In our GG model, the source of GHG emissions reduction will be gained from substitution of petro-chemically derived gasoline by the Oakbio n-butanol.

The flow of carbon through Oakbio's *n*-butanol production process, as well as the baseline petrochemical production process for gasoline, is illustrated schematically in Figure 22. Note that calculations are in units of grams CO2e per MJ of fuel to facilitate comparison. As shown on the left side of Figure 23, this analysis breaks the proposed Oakbio process into four steps, each with associated GHG emissions. Total GHG emissions reduction is achieved after step 5, replacement of gasoline.

Power Plant : The power plant may be a coal- or natural gas-fired, nuclear, hydroelectric, solar, or wind generator with associated GHG emissions. It refers to the generation of electricity required in order to operate the proposed process. Oakbio's microbes do *not* capture and convert CO2 at this stage.

H_2 can be obtained from many sources, each affecting the life-cycle GHG emissions of associated products.

Figure 23. GHG emissions reductions from Oakbio's renewable *n*-butanol.



Steam Methane Reformation SMR refers to the generation of H2 gas from reformation of CH4. SMR is currently the most economical and immediately applicable technology for H2 production. SMR demands for electricity and consumes natural gas (CH4) and water to H2 and CO2. In order to calculate the life-cycle GHG impact of this generation method, the source of CH4 must be considered: if the CH4 comes from anaerobic digestion of municipal waste, or would otherwise be released into the atmosphere, the process is technically net GHG-negative, due to CH4's GHG intensity of 21 and CO2's GHG intensity of 1.

In the future, novel greener technology for H2 production will likely become more competitive to SMR. Electrolysis of water has a GHG impact directly related to the method of generation for the electricity used. If that electricity is generated from wind, solar, geothermal, or hydroelectric resources, the H2 produced can be nearly carbon neutral.

Gas Fermentation: It captures and converts CO2 from the industrial partner into *n*-butanol and residual biomass. It requires electricity for operation (accounted for in #1 above).

Combustion of *n*-butanol: Carbon temporarily sequestered in *n*-butanol (in #3 above) is combusted and released back into the atmosphere.

Replacement of gasoline: Gasoline production from Alberta's oil sands and consumption in Alberta provides the baseline GHG emitting process used for comparison in this model.

Petrochemical baseline emissions (right side Figure 23) were estimated by combining published emissions factors for production and consumption of gasoline from Alberta's tar sands.

The "per megajoule" analysis outlined above was then combined with gasoline consumption data for the Province of Alberta from 1993 to 2012 to generate an estimate of total annual GHG emissions reduction potential (Statistics Canada, Table 405-0002). Two market penetration scenarios were considered in order to estimate *n*-butanol production volumes for the Province of Alberta from project inception through 2032.

6.2 GHG Modeling

The following key components of GHG emissions were considered for our modeling and are fully detailed in Appendix 6:

- SMR– Sourcing and consumptions of Methane
- Power Plant– Electricity for SMR and for Gas Fermentation
- Gas Fermentation– Capture and Conversion of CO2 from Flue Gas
- Combustion of n-Butanol
- Baseline - Life Cycle Analysis of Alberta Gasoline

GHG Emissions Reductions per Liter from Replacing Gasoline with n-Butanol

Table 5 illustrates estimated GHG emissions for production of one liter of *n*-butanol by the proposed technology, GHG emissions for one liter of gasoline produced from bituminous sands in Alberta, and the potential GHG emissions reduction of replacement. For the purpose of estimating this volumetric reduction, the emission intensities of production and consumption of gasoline have been multiplied by 0.84, to account for the 16% difference in energy content of *n*-butanol and gasoline.

Table 4 Comparison of life-cycle GHG emissions from n-butanol and gasoline manufacture and consumption

Overall GHG Emissions Change, kg CO_2e per liter *n*-butanol	-1.54
GHG Emissions, Gasoline Consumption, kg CO_2e per liter	3.98
Correction for *n*-Butanol Lower Energy Content than Gasoline	3.34
GHG Emissions, *n*-butanol Manufacture, kg CO_2e per liter	1.80

Calculation of Total Annual GHG Emissions Reduction

The total annual GHG emissions reduction due to the proposed technology at commercial scale is estimated by multiplying the GHG Emissions Change calculated in Table 4 by the number of liters of *n*-butanol produced at commercial scale.

Target Market for Oakbio n-butanol

Currently, Alberta's RFS Regulation mandates that fuel contain 5% renewable alcohol; this requirement is met by blending ethanol into the fuel supply. But ethanol is a less-than-desirable additive because it contains only 66% of the energy of gasoline on a volumetric basis, and it can be mixed up to a maximum ratio of 10%. *n*-butanol is advantaged as a fuel additive over ethanol because it contains 84% of the energy of gasoline, increasing gas mileage for equivalent blends, and because *n*-butanol may be mixed in higher ratios with gasoline than ethanol without damage to North American internal combustion engines (US EPA).

The Alberta fuel market is expected to demand slightly more than 7 billion liters of fuel by 2019. This projection is based on a consistent 2.33% annual growth rate of the Alberta fuel market from 1993 – 2012. These data are also consistent with the reference case developed by MK

Jaccard and Associates for refined petroleum product consumption in the Transportation sector; demand for refined petroleum products increases from 413 petajoules in 2010 to 677 petajoules in 2050 (see Table 8, page 27, Jaccard and Associates, 2007).

According to the US Energy Information Administration, global gasoline demand has grown consistently as well, from an average of 16 million barrels per day in 1986 to slightly more than 22 million barrels per day in 2010. This is a 2010 daily demand of almost 3.5 trillion liters. There is no reason to expect that worldwide demand for transportation fuels will level off between now and 2032. It is clear from these numbers that the Alberta and global gasoline markets are easily large enough to absorb the volumes of product necessary to achieve meaningful GHG emissions reductions.

6.3 Results and Discussions

Overall Results

Our calculations estimate that the goal of GHG reduction of 1 Mt annually will be achieve between 2025 and 2030, and could reach up to 2.1 Mt annual GHG emissions reduction in 2032, depending upon market conditions.

We project a 2019 market launch for our *n*-butanol product, which is in line with the development time allowed by the CCEMC Grand Challenge. Market penetration for our product will be primarily influenced by three interrelated factors: renewable fuels standard regulations, cost-economics and rate of technology deployment. Figure 24 shows GHG reductions obtained under two market penetration scenarios.

Market Penetration Scenarios

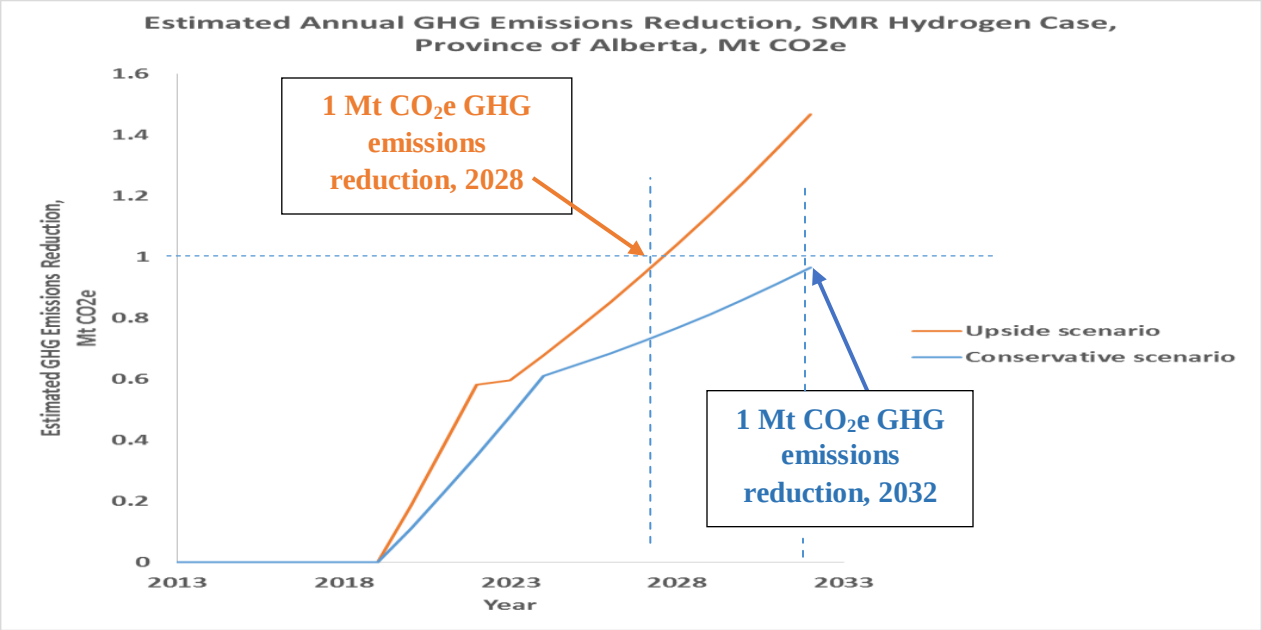


Figure 24. Comparison of GHG emission reductions under two scenarios.

- *Conservative Scenario*

The blue line in Figure 24 results from the assumption that the proposed technology will achieve 5% market penetration in 2024, or five years after completion of the CCEMC Grand Challenge. Market share then grows much more slowly to approximately 6.5% by 2032. This market penetration scenario is driven by rapid adoption of Oakbio *n*-butanol as a superior blendstock to ethanol for purposes of Alberta's RFS, and then slow adoption thereafter. GHG emissions reductions continue to increase after 2024 because of the 2.33% annual growth rate of Alberta's transportation fuel market.

- *Upside Scenario*

The orange line in Figure 24 results from the assumption that Oakbio/Tabita Laboratory's *n*-butanol will achieve 5% market share by 2023. This assumption is driven by renewal of the RFS Regulation in 2020 and by *n*-butanol's superiority to ethanol as a fuel additive. Market penetration accelerates after achieving cost-parity with gasoline in 2024. Market penetration is projected to reach 10% in 2032.

6.4 Potential Future non-GHG Benefits

The proposed technology offers a number of non-GHG benefits to the Province of Alberta:

Profitable GHG Regulation Compliance Strategy
Oakbio's process of CC and conversion to *n*-butanol has the potential to be profitable at commercial scale. More importantly, this profitability can be achieved independently of existing GHG emissions regulations, taxes, or caps. This compares favorably to the >$1 Billion capital expenditure required to add a CCS system to one existing coal-fired power plant in Saskatchewan, which implies a cost of $90 per tonne of CO2 captured by that system.

Remediating non-GHG Pollutants
OB311 is derived from *Ralstonia eutropha* H16, a well-known microbe with high potential for bioremediation. *R. eutropha* is known to degrade a large list of chloro-aromatic compounds and chemically-related pollutants. For example, it can degrade the herbicide 2,4-dichlorophenoxyacetic acid, dioxin, benzene, diesel fuel, acetone and organic acids. In gas streams, *R. eutropha* is to reduce major contaminants such as SOx, NOx, CN, and H2S. It is important to note that Oakbio's proprietary strain OB311 is more resistant to flue gas containing these compounds than is the original *R. eutropha* H16 strain from which it was derived. This indicates that these remediation activities and potential are also much greater in OB311.

Advantaged Biofuel for Renewable Fuel Standard
Oakbio and FRT Lab *n*-butanol will facilitate RFS compliance for Alberta's fuel producers, and improve the energy content of the blended fuel supply. *n*-butanol is superior to ethanol as a renewable alcohol:

- Ethanol is subject to a 10% "blend wall" due to its corrosive potential; *n*-butanol is not.
- *n*-butanol is compatible with existing fuel infrastructure and internal combustion engines (EPA/British Petroleum joint study). Even under current US Environmental Protection Agency regulations, 60% more *n*-butanol may be blended into the US fuel supply than ethanol.

- *n*-butanol contains nearly 30% more energy per unit volume than ethanol (EPA/British Petroleum joint study).

Jobs and Economic Activity

The proposed project and its results will expand opportunities for Alberta-based businesses by providing income for construction, chemical manufacturing, and transportation companies.

- Construction: As of 2011, Alberta was home to 147 industrial installations that each reported GHG emissions of more than 50,000 tonnes (Alberta Environment and Sustainable Resource Development, 2013). The technology resulting from this project will be developed into an independent add-on that can be constructed at each of these locations and will convert CO2 into the gasoline substitute *n*-butanol.
- Chemical Manufacturing: Each of these add-ons constructed on-site will require staff with multiple skillsets, including chemical engineering, heavy equipment maintenance and operation, and site management.
- Transportation: The *n*-butanol produced at each location will need to be transported to commercial fuel blenders to be incorporated into Alberta's fuel supply. This will provide business for rail and trucking companies.

7. Techno-Economic Analysis

7.1 Approach

Oakbio has developed a techno-economic forecasting model to evaluate the performance of its process at scale. This techno-economic evaluation includes the following aims:

- Calculate CO2 capture performance
- Estimate revenues/profit generation from conversion of gas feedstock into products
- Determine technical performance thresholds that determine financial viability
- Identify and evaluate key sensitivities / risk towards economic performance

The scope of this exercise is to determine CO2 capture potential and its cost at a representative CO2-emitting site at commercial scale. We assume a representative industrial site emitting 1 million ton of CO2 per annum.

The Excel program was used to develop a flexible techno-economic model. The model inputs include active cells for key parameters covering the manufacturing process, technical productivity, feedstock criteria, product(s) value and financial assumptions. Values in these cells can be changed to determine technical and economic sensitivities for each variable. The model output includes calculations of CO2 capture performance, revenues from products converted from CO2 and overall financial viability performance criteria for the Oakbio process.

The model assumes that Oakbio technology has been fully developed to scale; R&D costs are sunk cost and are not included into this economic analysis. Oakbio achieved commercial scale production metrics, exhibited linear scalability of its process from pilot and demonstration scale. The model also evaluates the system performance of the process independently of who is operating it, whether it be Oakbio, Lehigh, a JV between the two companies, or a third party.

To populate the model, we built a base case representative of what we estimate is a realistic value for each of the inputs. We then performed a sensitivity analysis to identify the key variables most impacting the techno-economic performances and studied their individual impact of overall system performance based on an estimated range of possible values with references provided.

All figures and assumptions utilized in the forecasting model are determined by analysis of comparable processes whenever applicable, published scientific studies, other public-information, by using current Oakbio data and by leveraging personal discussions with industry experts with references attached in Appendix 9.

7.2 Results and Discussions

Based on Oakbio's assumptions, a 12.5 million L fermenter can capture 1 million tonnes of CO2/year and convert it into n-butanol at a profit of $49 per tonne of CO2 captured. This calculation assumes a projected n-butanol productivity of 4 g/L/hr, an H2 price of $1.00/Kg and gasoline retail price of $1.15/L.

Table 5 Key inputs and output figures from Oakbio techno-economic modelling

Fermenter capacity	12,500,000	L
Capex.	$319,338,181	L/yr
Amt. of N-Butanol Produced	492,594,593	kg/yr
Revenues from n-butanol	$375,577,222	$/yr
Total Amt. of CO2 Required	1,007,270,612	kg/yr
Total Amt. of H2 Required	85,289,450	kg/yr
Cost of H2	$85,289,450	$/yr

Oakbio's carbon capture value is calculated by dividing overall net present value (NPV from free cash flow analysis) by the quantity of CO2 fixed by Oakbio process and converted into n-butanol. It is difficult to compare Oakbio value with those of other CCC technologies, as the methods of profitability calculations vary significantly and are sometimes opaque. For reference purposes, current methods of CO2 capture estimate a cost basis at $40-$70/t, with additional costs accrued for CO2 compression, purification and storage. Some estimates for the initial demonstration projects have ranged as high as $80-$120/t CO2. While most of cost calculations are based on Capex, operating costs can also be significant, at roughly $5-$7/tonne in variable costs and $1-$5/tonne for fuel and power costs. As a rule of thumb, industry observers have estimated that fitting a 900MW coal power plant with carbon capture & sequestration technology would cost roughly $1Bn, which would represent a 40-60% increase in the capital cost of the plant. This figure excludes downstream investments in infrastructure to transport and store the CO2.

Additional value streams could be generated from Oakbio's CCC plant. These were not included in our modeling:

1. Revenues from additional sales of cement or other core product to the emitting partner if plant output was capped by a certain level of carbon emissions
2. Sales of secondary products (residual biomass and likely other products contained in it)
3. Increasing value of tax credits / tax savings (base case: only $15/tonne CO2, planned for $30/tonne)
4. Extra-value, if any, given by market for "green(er) core product of Oakbio's industrial partners"
5. Others (remediation of toxic component, less use of combustibles, etc.)

Sensitivity Analysis

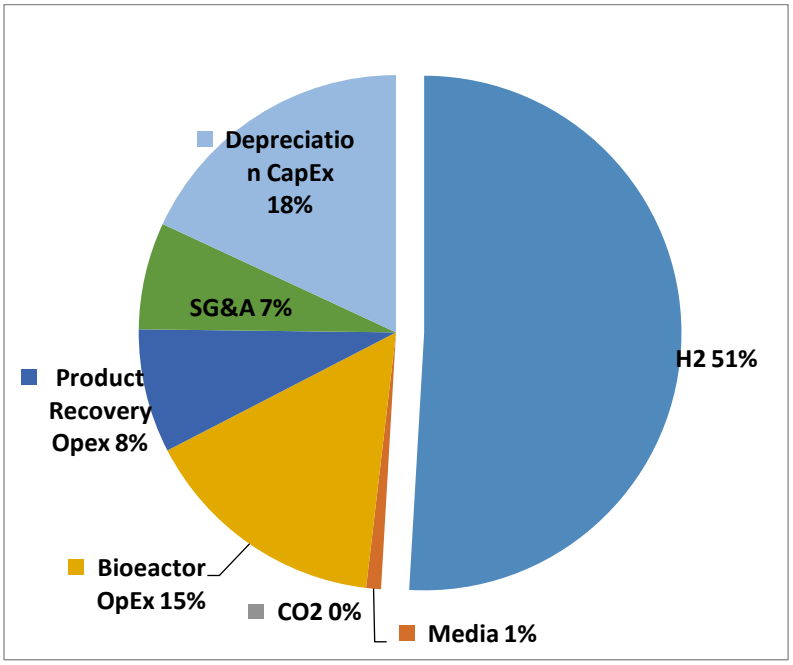
Economic performances are most affected by:

1) H2 cost

The cost of H2 needed to fuel the process, which is linked to the price of natural gas supply. Alberta has a lot of resources in natural gas. Near Edmonton, H2 is available from a pipeline or from SMR installed and operated under long-term contract. Our H2 pricing and H2 access feasibility in Alberta originate from private discussion with a local representative of a major gas distributor. Our base case assumes H2 price of $1/Kg.

In other geographical areas, natural gas might be less abundant and more expensive. A high H2 price of $2.0/kg would bring the profit to only $17/ tonne CO2. Please note that even a zero cost CCC process is still a very attractive proposition compared to current alternatives.

Figure 1: Modeled Project Expenses at Commercial Scale.



2) Price of crude oil and gasoline.

Variation in the price of these products will affect the value n-butanol as a replacement fuel. The prices of crude oil and gasoline have varied significantly over the last year and half since this grant's kick-off. Some experts believe that the current low price of crude oil is driven more by political reasons than by economic fundamentals and is therefore only temporary. Nevertheless its impact on gasoline price and on the price of greener replacement fuel has been dramatic. Our base case models an average retail price at CAN$1.15 /L in August 2015. When this figure is lowered to CAN$1/gal the CC value goes down to $21/tonne CO2 captured but when the price of gasoline goes up to CAN$1.3/L then CC value increases to $45/tonne CO2.

Overall Factors Affecting Commercialization of Proposed Technology

Renewable fuels mandate: The proposed technology will generate a product superior to ethanol, the current fuel additive of choice for the Renewable Fuels Standard Regulation. This mandate is set to expire January 31, 2020. A renewal of this mandate beyond that timeline would provide additional support for rapid adoption of the proposed technology. It would give Oakbio/Tabita Laboratory and its industrial partners time to reduce costs while scaling to a commercial process, so that n-butanol may be sold at prices competitive in the broader gasoline market.

Techno-economics: Key variables for production cost linked to our technology include H2 cost, yield of microbial growth and overall efficiency of system achieved through process engineering.

We expect H2 cost to continue its current downward trend, and one of the primary goals for Rounds 2 and 3 of the Grand Challenge will be to improve process yield and system efficiency. Another factor that will influence economics is the presence of tax incentives such as the blender tax credit in the US ($0.60 per gallon through 1990, $0.51 through 2008, $0.45 from 2009).

Rate of technology deployment: The rate of adoption of our technology will also be influenced by GHG regulations in place in Alberta at the time our technology is ready for commercial roll-out. A technology offering the advantage of converting CO2 into a valuable product instead of being buried underground offers an attractive solution for carbon capture. Current solutions for carbon capture such as such as amine-based methods are very expensive to deploy and operate. It is of note that production of transportation fuels at the site of CO2 capture is attractive as it eliminates the cost of distribution. Several of the major GHG emitters in Alberta such as mineral extraction are located in remote locations and incur large expenses to transport fuels to their plants. This in-situ use of our biofuel alleviates early pressure to produce fuels at cost-competitive price to gasoline when technology is not yet fully optimized.

8. Overall Conclusions

Oakbio and the Tabita Laboratory have achieved proof-of-concept of a novel technology capable of capturing CO2 from unadulterated industrial flue gases collected in Alberta and converting it into the gasoline replacement *n*-butanol. At scale, this technology is projected to reach the Grand Challenge goal of >1 megatonne GHG emissions reduction between 2025 and 2030 with very favorable economics. Oakbio/F.R.Tabita lab has the potential to transform Carbon Capture from a cost center into a profitable chemical manufacturing business.

Proof-of-concept was demonstrated by:

1. Engineering multiple strains of OB311, Oakbio's pre-existing CO2-consuming microbe, to produce *n*-butanol from H2 and CO2. Multiple strains were produced in order to test different metabolic pathways and optimize OB311's CO2 uptake and *n*-butanol productivity.
2. Initial proof of concept was achieved on lab gas and on cement flue gas collected in California.
3. Oakbio partnered in Alberta with two CO2 emitting industries: a cement plant and an oil upgrading facility. Samples of gas emissions were collected at both sites in Alberta and the compressed gas cylinders were shipped to Oakbio for testing.

   Proof of concept production of butanol from the Alberta cement flue gas was demonstrated.

   Proof of concept production of butanol from the Alberta upgrader plant flue gas was demonstrated.
4. The fermentation process was successfully scaled-up to 20L fermenter on both lab gas and Alberta flue gas. A twenty liter fermenter is considered to be a large size at bench scale, and a necessary milestone before scaling-up to pilot size.
5. Life cycle analysis on GHG reduction projects achieving the goal of reducing Alberta's GHG emissions by >1Mt per year between 2025 and 2030. An upside and downside scenarios of market penetration were considered. The modeling is based on *n*-butanol partially replacing gasoline as transportation fuel, with the results of reducing GHG emissions by 1.8 kgCO2e/L gasoline replaced.
6. Techno-economic analysis calculates that at scale, the proposed technology has the potential to transform Carbon Capture from a cost center into a profitable chemical manufacturing business. Hydrogen is the main cost for Oakbio's n-butanol production and Alberta is rich in natural gas, which supplies H2 through Steam Methane Reformation (SMR). At targeted productivity of 4g/L/hr, a 12,5 ML Oakbio plant could capture 1 M metric tonnes of CO2 per year, produce a large quantity of *n*-butanol, and make a substantial profit.

The commercial ramifications of these results are significant. Governments' regulators are setting up production caps based on carbon emissions ceilings /site and are increasing carbon taxes beyond that level. Oakbio's successful proof of concept of profit-driven CO2 capture at point source and conversion into products opens an attractive value proposition to CO2 emitters. In Alberta, the availability of inexpensive natural gas is particularly advantageous as it allows the production of inexpensive H2 through SMR, which significantly increases the

profitability of Oakbio's method of CCC. Oakbio's process does not need carbon credits to be profitable, the credits can be transferred back to the CO_2 emitter partner.

Driven by forthcoming stricter regulations on carbon emissions, cement manufacturers have expressed more interest in Oakbio's process potential of reducing GHG emissions at a profit or neutral cost than by the manufacture of a new line of chemical products. Hydrogen can be supplied through pipeline or by setting up an SMR on site. SMR emits CO_2 so the major net GHG reductions are obtained through petrochemical displacement by Oakbio's products.

Oil upgraders are chemicals producers and already possess SMR unit(s) on site. In our work, we successfully used SMR gas from an Alberta oil upgrader to grow microbes, which produced n-butanol. Most of the feed gas to the SMR is coming from different offgas steams from their hydroconversion process, the rest being purchased natural gas. With Oakbio's technology, this inexpensive SMR gas (which contains CO, CO_2, H_2 and CH_4) could be used as a feedstock for n-butanol production, and more broadly, as an alternative feedstock to bitumen for the production of a portfolio of valuable chemicals. The presence of SMR units on site, the desire to mitigate the CO_2 release from the gasification of asphaltines and from other waste gases which can be processed by SMR, make Oakbio technology an attractive value proposition to explore for an oil upgrader.

9. Scientific Achievements & Communications Plan

Oakbio and the team from the F. Robert Tabita lab at the Ohio State University plan to author and submit a peer reviewed publication on the subject of CO_2 capture from industrial flue gas using data collected during this work. The plan is to submit this publication to the top scientific journals. Additionally, Oakbio will submit an article to "Cement World", an industry journal which covers the cement industry.

We will also give company presentations, which include some of our research findings at conferences, such as "The Advanced Biofuels Leadership Conference" (ABLC) held in November 2015 in San Francisco. We could not identify an appropriate conference in Alberta over the next few months, but will ready when such an opportunity occurs.

We will report the results obtained in this grant to our industrial partners in Alberta, the cement plant and the oil upgrader, to engage them into the next phases of our development. We will broaden our reach to other companies in the field and to potential investors in order to secure the funds necessary to bring this promising new technology to commercialization.

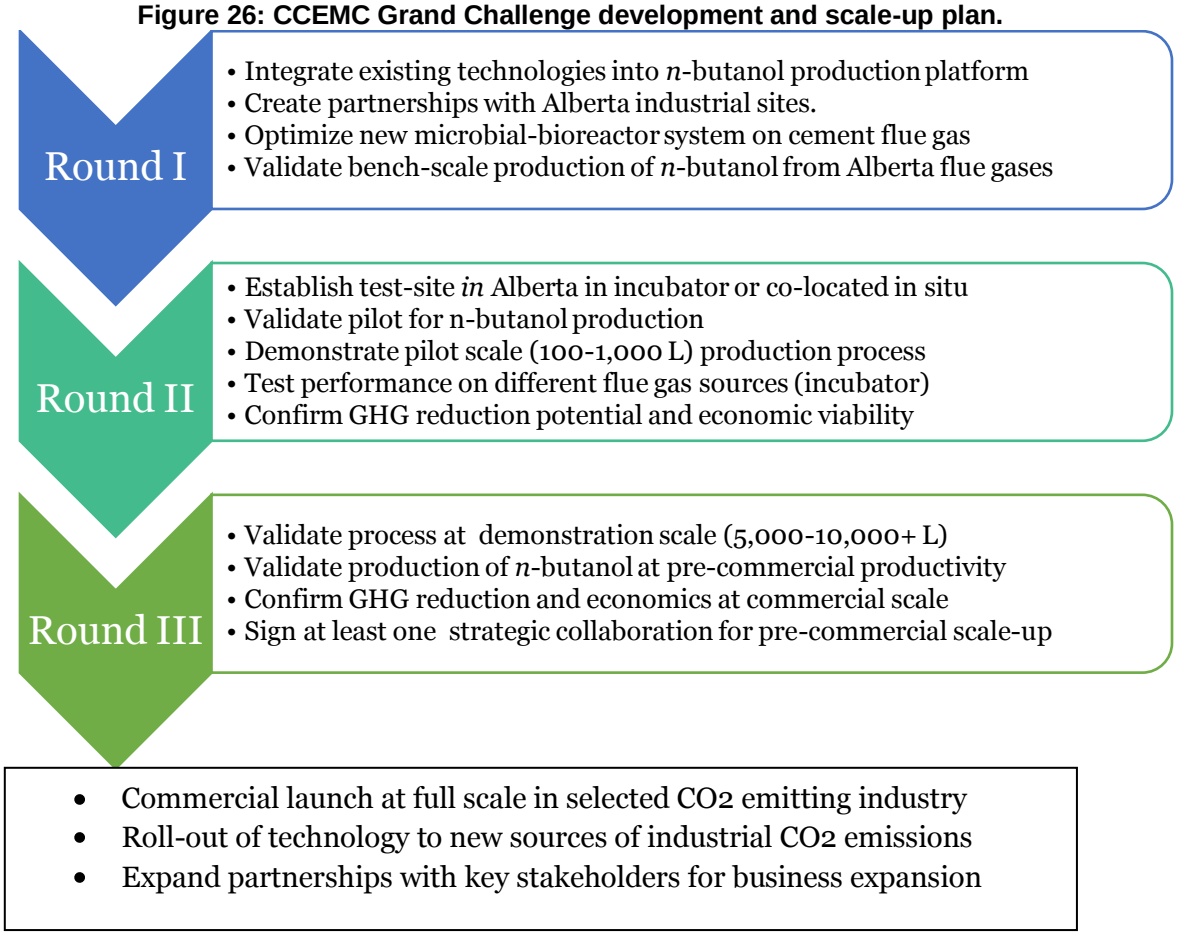
10. Next Steps

10.1 Process Scale-Up and Commercialization

Oakbio and FRT Lab's work for Round I of CCEMC's Grand Challenge entailed creation and validation at bench scale of the *n*-butanol production on Alberta flue gas. Round II will encompass scale-up and validation of the Oakbio/Tabita system at pilot scale (100+L) in a field lab located on an Alberta CO_2-emitting industrial site, for production of both *n*-butanol and bioplastics. Round III will be demonstration scale (5,000+L) for technical validation, confirmation of economics and assessment for GHG reduction capability. Full-scale launch and roll-out of the system in several industries will follow.

The proposed technology proposed is part of a profit-driven approach to GHG capture and utilization to produce chemicals, fuels and plastics. These products will be sold primarily on wholesale markets to large companies that process them further. Because of the large product volume these processes are intended to produce, and the capital expenditures required to build a large-scale biorefinery, it is critical to engage potential industrial partners early and they will not only become tomorrow's customers but also financial partners to help fund the more expensive stages of manufacturing scale-up.

Figure 26: CCEMC Grand Challenge development and scale-up plan.



Oakbio has already engaged with a number of large chemical companies who have expressed interest in becoming clients and partners of Oakbio. Additionally Oakbio has engaged with H_2 and electrical power producers, the suppliers of the energy which drives our process, and is our major operating expense. There has been a very positive response as they understand, particularly in the case of fuels, that the ability to store electrical or hydrogen energy in dense products such as *n*-butanol provides a long term large market for their products.

10.2 Strain and Process Development

Oakbio will simultaneously pursue three distinct technology development strategies to improve OB311's CCC process performance:

- Synthetic biology and strain improvement
- Media and feedstock development
- Fermentation process design

Each of these strategies may improve OB311's performance, which will maximize Oakbio's chances of developing a profitable CCC process. It should be noted that iterative cycles of laboratory-based strain and fermentation improvements at the pilot scale will be performed on new mutants constructed to improve n-butanol production, since the data from production runs can be used to inform the next round of laboratory work. In this way, iterative cycles of strain development and process development can continuously improve the final output at the plant.

Simultaneously pursuing each of these three technology development strategies can reasonably be expected to generate a CCC performance improvement of 500- to 1000-fold, based on peer-reviewed studies and the published materials of comparable companies.

Oakbio has already improved the production of *n*-butanol from Alberta flue gas sources over 10-fold during the course of this CCEMC grant, and there are still six months to go before the submission of the Phase II proposal. However, higher numbers will certainly be required for commercial operation. Strain improvements designed to increase the product yield of a microbial process to commercial scale invariably require a multi-faceted approach. The combination of many hundreds of enhancements over time has been shown to significantly raise the titer, yield, and productivity. This broad approach is how many companies such as Amyris, Gevo, etc. have achieved successful commercial scale activity. Oakbio will use these same methods to improve its OB311-based butanol production strains, as well as to improve the overall fermentation process. The methods include:

1. **Pathway engineering**. The Tabita laboratory at Ohio State University will be constructing new mutant strains that build upon the success already achieved to date with the "fifth generation" variants. Enzyme engineering and metabolic pathway engineering will be used to improve the productivity and selectivity of the enzymes that generate butanol from the central carbon pathways of the organism. Competing pathways that generate undesired products will be reduced or eliminated. The growth data and butanol production data on flue gas obtained by Oakbio will be used to inform the design of new mutants.
2. **Strain engineering**. Random and targeted mutagenesis will be used to improve the overall performance of the host strain outside of the pathway of interest. Oakbio has already employed adaptive evolution to generate the flue-gas resistant host strain OB311

that grows much faster than the parent strain, and techniques such as this can be used to further improve the microbial host for specific feedstocks.

3. **Medium optimization and feedstock delivery.** Creating a medium that is customized to the precise needs of the microbes as they grow and produce *n*-butanol will also be essential to future improvements. We will continue to improve the medium and the gas feedstock delivery system as we scale-up the process.

10.3 Bioreactor System Development

Oakbio aims to advance its microbial system for industrial waste CO2 Carbon Capture and Conversion (CCC) technology towards the production of commercial quantities of *n*-butanol. The goal is to optimize its bioreactor (gas fermentation) system, to improve feedstock supply and utilization, and increase biofuel productivity. It involves three related parts:

- Bioreactor design
- Monitoring System Design
- Control System Design

Next step is to build one or several 100L bioreactor test beds. In order to achieve this goal we propose a 100L gas driven fermenter system which has appropriate controls for measurement and control of gas mixtures and delivery combined with integrated sensing and measurement of dissolved CO2, dissolved H2, pH, temperature, microbial growth optical density measurements (OD 600), and dissolved O2 at multiple points in the bioreactor vessel.

Process engineering and scale-up.

Oakbio anticipates building 1,500 liter fermentation systems in both Alberta and Sunnyvale to pursue fermentation process improvements at a larger scale. Introducing better control of the fermentation environment through automation and analytics with permit more efficient conversion of gas feedstocks into product. These enhancements will play a crucial role in the R&D scale-up strategy for Round 2 of the Grand Challenge.

APPENDIX

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Appendix 1 – Project Description

Alberta Industrial Operations and GHG Emissions

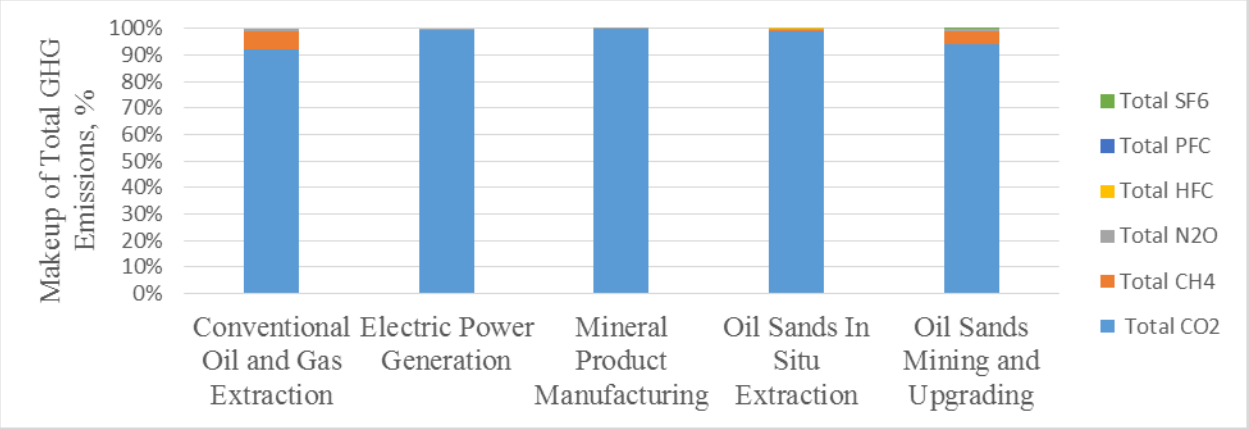
According to Statistics Canada, more than 38% of Alberta's $364.5 Billion Gross Domestic Product (GDP) in 2014 was produced from three economic sectors (Highlights of the Alberta Economy, 2015):

- Energy – 25.5% (production of fossil energy sources including crude oil and natural gas)
- Manufacturing – 6.9% (includes petroleum refining, and production of chemicals and cement)
- Transportation & Utilities – 5.7% (including generation of electricity)

At the same time, these economic sectors were also responsible for more than 80% of Alberta's 2011 reported GHG emissions (Alberta Environment Report, 2013):

As the Figure below shows, more than 90% of the GHG emissions from each of these industrial operations consists of CO2 (data from Alberta Environment Report, 2013).

Figure 1: Composition of Alberta industrial GHG emissions.



Achieving the Greenhouse Gas Reduction Goal

Alberta's primary GHG emitters are key contributors to Alberta's economy. For example, naively reducing the output of the sectors discussed above by 20% would also reduce Alberta's GDP by more than 7%. Given the Province's average growth rate of 3.5% between 1994 and 2014 (Business in Alberta, 2014), such a reduction in GDP would be economically disastrous.

Climate Change and Emissions Management Corporation

Alberta's Climate Change and Emissions Management Corporation (CCEMC) was created in 2009 to help address this conflict. The CCEMC is an arms-length, independent entity whose mission is to "participate in funding initiatives that reduce GHG emissions and improve [Alberta's] ability to adapt to climate change" (CCEMC annual report, 2014). In 2013, the CCEMC unveiled its Grand Challenge for Innovative Carbon Uses, an ambitious technology development and commercialization competition to "recognize the most innovative ideas for carbon uses the world has to offer" (CCEMC annual report, 2014).

Current Status of Commercial Carbon Capture Technology

Currently available technologies for carbon capture and sequestration (CCS) are largely non-biological. They are also cumbersome and extremely expensive to implement. For example, a non-biological capture and sequestration system installed as part of a new coal gasification technology ('TRIG') by the Southern Company at its Kemper County, MS power plant contributed to construction cost increases of over $1.2 billion dollars in just the past four years (http://www.forbes.com/sites/williampentland/2015/02/09/southern-companys-discomfort-what-kemper-and-vogtle-plants-say-about-competitive-power-markets/1/).

These cost increases will largely be passed on to the electricity rate payers, and have driven the total cost of construction to over $6 billion. Moreover, current carbon capture technology adds large additional operating costs to the power generation process (Rubin & Zhai, 2012).

Novel carbon capture and *conversion* (CCC) technologies allow production of valuable products from captured CO2, instead of simply burying it underground. Revenues from product sales offset the cost of carbon capture and may even, as is the case for Oakbio, create a potentially profitable business, using a more cost-effective process for capturing CO2 than current CCS technology.

Reported limitations of CCS:

- CCS **increases capital expenditures**.
  - This technology is difficult to retro-fit, and can add 50-100% to the cost of installed capacity for a power plant (Equity Research Americas, 2014).
- This process is **energetically and economically expensive** to operate.
  - The best-understood CCS technology, methyl ethanolamine-based post-combustion capture (MEA-CC), may consume as much as 25% of the total power produced by a power plant in order to capture CO2 before it is emitted into the atmosphere (Equity Research Americas, 2014).
  - Implementing this technology could increase the cost of electricity to the consumer by 64% (Equity Research Americas, 2014). For example, the cost of the Southern Company's newly constructed Kemper coal-fired power plant with CCS technology more than doubled from $2.6 billion to $5.5 billion (Brewer, 2014).
  - Closer to Alberta, SaskPower recently spent $1.2 billion to retrofit its existing 110-megawatt coal-fired generating unit at the Boundary Dam plant in Saskatchewan, Canada (Foreign Policy, 2014).
    - Capturing CO2 produced by the retrofitted plant is expected to cost as much as $90 per tonne.
- Separated **CO2 must be transported** to geological sequestration sites.
  - Sequestration requires construction of new gas pipelines to transport high-pressure CO2 from power plants or industrial installations to the sequestration site (Equity Research Americas, 2014).
- Separated **CO2 must be stored underground** in oil and gas reservoirs or coal seams (Equity Research Americas, 2014) and monitored for potential leaks forever, with ongoing risk of catastrophic damage to terrestrial and marine ecosystems.

Appendix 2 – Technology Background

Chemoautotrophy for Carbon Capture and Conversion

"Chemoautotrophy" refers to a metabolism that derives both energy and carbon from inorganic chemical sources. Chemoautotrophic H2 microbes such as *R. eutropha* can grow using molecular hydrogen (H_2) as an energy source and CO_2 as a carbon source. Unlike the algal photosynthesis platforms used by some companies to capture carbon, Oakbio's process does not require an input of light energy; therefore, it can operate 24/7, at all latitudes, and in high volume and high density, without the real estate requirements of photosynthetic systems. Also note that the CO_2 that can be separated from flue gases via other industrial methods represents an ideal feedstock for Oakbio's chemoautotrophic organism.

Background of H2/CO2-Utilizing Bacteria

Studies of the "Knallgas" bacteria, which utilize hydrogen and oxygen (as well as carbon dioxide and organic carbon) for growth were first conducted many decades ago by environmental microbiologists (Schwartz & Friedrich, 2003). Some of the best known hydrogen-utilizing bacteria come from the genus *Ralstonia*, named after the American microbiologist Ericka Ralston (Ralston *et al.*, 1972). One of these bacteria, *Ralstonia eutropha* strain H16, has been very extensively investigated for possible use in commercial fermentation (using H2, CO2 and O2 as feedstock gases; Volova, 2010). Its metabolism includes the Calvin-Benson-Bassham (CBB) cycle, which utilizes the RuBisCO enzyme to efficiently capture CO2 from the atmosphere and convert it into multi-carbon molecules. The complete genome sequence is published (Pohlmann *et al.*, 2006), and a variety of broad host range expression systems are available (Laguna *et al.*, 2015). This bacterium is well-known for producing bioplastics (polyhydroxyalkanoates, or PHAs) at up to 75% of its cell dry weight, and naturally has a very high flux of carbon directed toward 4-carbon products.

Commercial Exploitation of Chemoautotrophic Metabolism

Oakbio has been developing CO2 capture and conversion systems since 2009. During that time, Oakbio developed several strains of microorganisms which are superior at capturing CO2 and converting this to useful chemicals. In order to achieve this goal, Oakbio employed a step-wise process to develop a robust CO2 capture platform, as shown in Figure 1.

First, the company selected the best microbe for carbon capture and conversion using H2 as an energy source. *Ralstonia eutropha* strain H16 was chosen because it is a chemoautotrophic bacterium that lives naturally in freshwater sloughs, and has previously been shown to be an excellent platform for CO2 capture (see Figure 2 a and b below).

Development of Oakbio Microbe OB311

Oakbio selected the naturally-occurring chemoautotrophic microbial strain *R. eutropha* H16 for further development into an industrial platform organism, named OB311, based on early screening results obtained at the Lehigh field lab.

Figure 1: Oakbio development flowchart

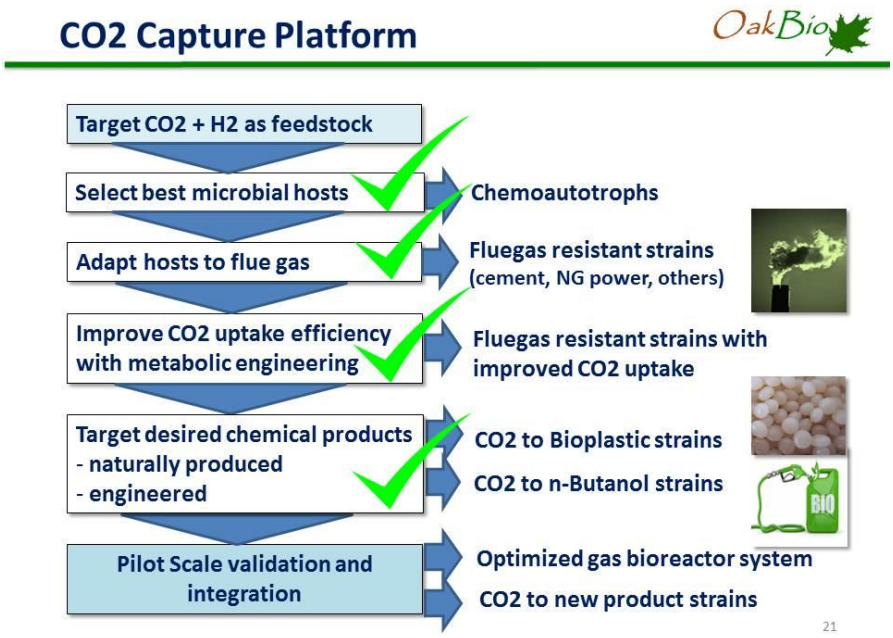


Figure 2a. Growth on flue gas showing the Oakbio OB311 flue gas-resistant strains (top curves) versus wild-type (bottom).

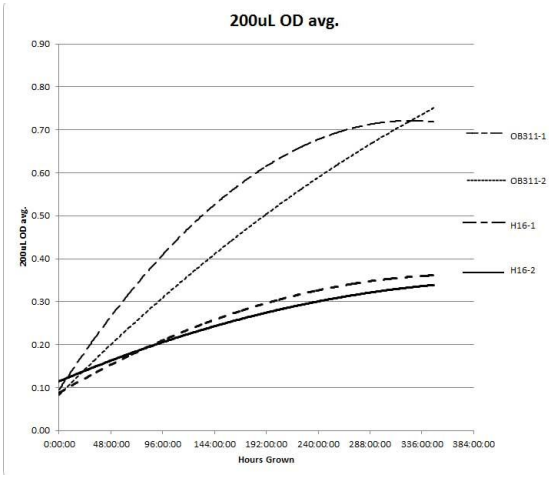


Figure 2b. Reduction in flue gas CO2 to near atmospheric levels by OB311 in Oakbio bioreactor.

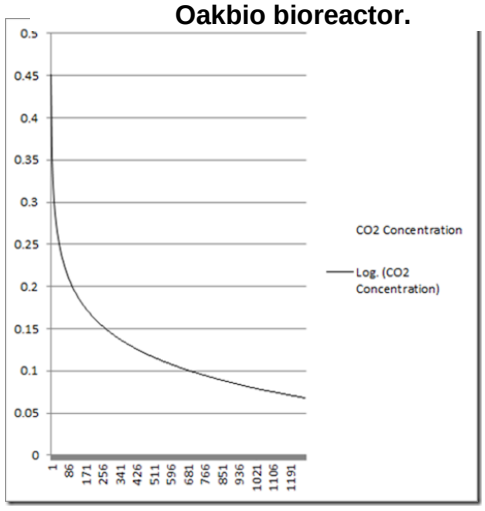
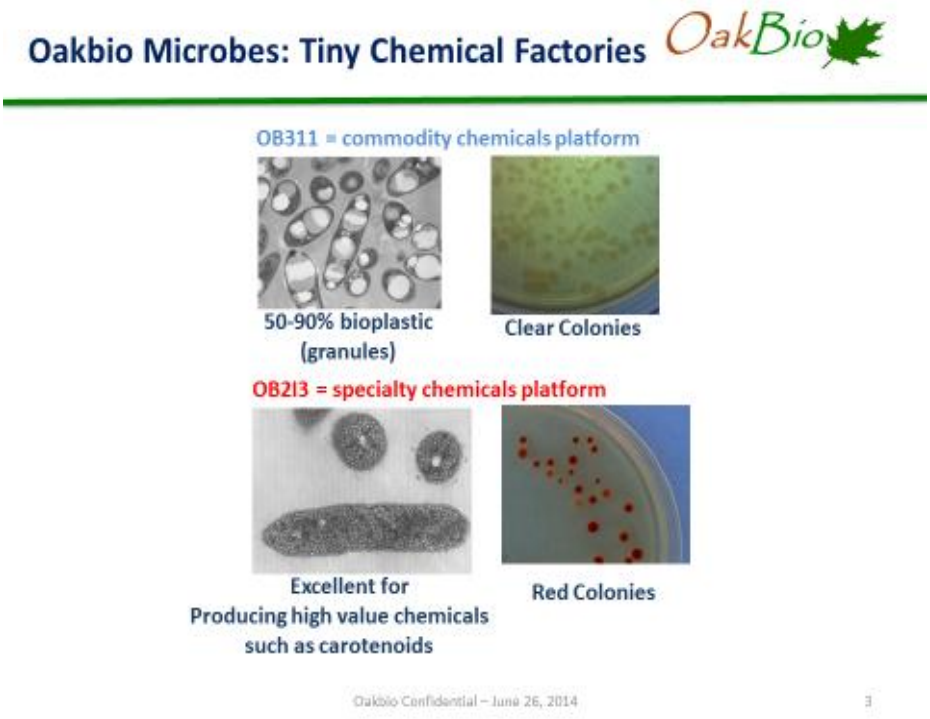


Figure 3 clearly shows that *R. eutropha* H16, the parent strain of OB311, and *R. capsulatus*, the parent strain of OB213, were the only naturally-occurring chemoautotrophic strains to grow. Based on such results, *R. eutropha* H16 and *R. capsulatus* were chosen as the starting points for development as Oakbio's biomanufacturing platform organisms. Continuous culture of these microbes over the course of two years was then used to adapt these microbes for robust growth on cement flue gas.

Some of the characteristics and advantages of strains OB311 and OB213 are shown in Figure 4, below.

Figure 4: Oakbio's OB311 and OB213 Microbes



Source: Oakbio internal document.

OB213 was selected for its high density of internal membranes, its ability to produce carotenoids and other high-value chemical products, as well as its ability to synthesize small amounts of PHB, all while growing on cement flue gas. The internal membrane structure of the microbe, and its ability to produce high-value compounds like carotenoids in addition to bioplastics, make OB213 an excellent platform microbe for production of specialty chemicals.

Note that no work was performed with *R. capsulatus* for the present grant. *R. capsulatus* will not be discussed further in this report because it is best suited for production of small amounts of high-value chemicals, and therefore not suited to CCEMC's goal of reducing GHG emissions by >1 Mt per year.

The primary chemical products naturally produced by OB311 are biopolymers called polyhydroxyalkanotes (PHAs). Naturally-occurring PHAs in OB311 are polymers of the 3-hydroxybutyrate (3HB) monomer. 3HB is a basic chemical building block that can be converted to hundreds of chemical products, including C4-fuels like n-butanol (Ishizaki, et al., 2001).

Microbes that can fix CO_2 have been reported to produce PHAs up to 70-90% of biomass under ideal conditions (Volova, 2009). Also, demand for biobased plastics is growing. Both PHA and 3HB on their own have the potential to grow into markets of many millions of tons annually, driving profitable capture of millions of tons of CO_2 (Reportsnreports, 2014).

CO_2 Capture Capability

Oakbio has also demonstrated (under a grant from the California Energy Commission) that strain OB311 can reduce the concentration of CO_2 in cement flue gas by up to 40% after one pass through a bioreactor. This result has been verified by independent third-party testing.

Recycling the headspace gas for an additional 20 minutes in a closed system increases the carbon capture efficiency to 70%.

Butanol Production from CO2 and H2

Butanol is an attractive four-carbon product to make from waste CO2 and H2, since it can be used not only as a chemical feedstock, but also as an effective biofuel substitute for gasoline and for ethanol. H2 is an inexpensive gas widely available from a variety of sources, including steam reformation of natural gas and electrolysis of water. When H2 is derived from renewable sources, the process can be entirely sustainable. Oakbio's technology takes advantage of the growing number of sources for production of H2. Increasing amounts of green electricity are available from wind, solar, hydro-electric and other clean sources, which can be transformed to H2 and then to liquid fuels using Oakbio's technology.

As a drop-in fuel, *n*-butanol is currently an exceptionally attractive product, as described above. Consequently, *n*-butanol and isobutanol are under extensive investigation in the biofuels and renewable chemicals spaces. The potential benefits of manufacturing an appreciable portion of Alberta's yearly light transportation fuel requirement directly from the waste streams of Alberta's heaviest industries are manifest.

Engineering of *R. eutropha*

As previously described, there are two major approaches taken with respect to strain modification of flue gas utilizing hydrogen bacteria to maximize product formation from CO_2. These include: (1) enhancement of intrinsic carbon uptake capabilities in test strains (2) using synthetic biology approaches, construct strains of *R. eutropha* that will enable substantial titers of butanol to be produced. These are ongoing objectives as considerable strain modification is required. We are guided in these studies by prior successful modification of the widely used industrial organism, *Escherichia coli*, to produce substantial titers of butanol without the need for expensive antibiotics and inducer molecules (Laguna *et al*., 2015). Aspects (1) and (2) will eventually be combined to construct strains that maximally convert CO_2 from flue gas into butanol.

Scale-up of OB311 CCC Process

The key bacterium that comprises the platform for Oakbio's CCC process is *Ralstonia eutropha* (now *Cupriavidus necator*) strain H16, referred to as the "wild-type" strain. This common environmental bacterium is a chemoautotroph, meaning that it can "fix" C1 carbon, such as CO2, into multi-carbon compounds using H2 (hydrogen) as an energy source (Volova, 2009). Oakbio's proprietary strain of this bacterium, designated as OB311, has been specifically adapted to grow on raw industrial flue gas, which contains various toxic components (e.g., CO, SOx, NOx) that inhibit the growth of most bacteria, including the wild-type H16 strain. These compounds also strongly inhibit the growth of most microalgae (Cheah *et al*., 2014). This adaption to grow on flue gas was not achieved by directed genetic engineering but by repetitive culture, taking advantage of spontaneously arising mutations. The best organisms were selected to grow in the given conditions.

Another key feature of this bacterium is that it naturally converts a very high percentage of the CO2/H2 feedstock into bioplastic, known as polyhydroxyalkanoates (PHAs), during a nitrogen-limited cultivation regime. PHA production occurs at the expense of other biomass. PHAs are typically composed of polymers of beta-hydroxybutyrate, derived from 3-hydroxybutyryl-CoA, which is also a useful starting point for manufacturing other four-carbon products, such as *n*-butanol. By favoring the natural production of butyrate while simultaneously limiting or

eliminating other metabolic pathways that consume the key intermediate acetyl-CoA, one can maximize the cellular flux directed toward making the desired alternative product.

The purpose of the present grant is two-fold:

1. Redirect the tremendous carbon flux of Oakbio's flue gas-resistant microbe OB311 from PHA to the more industrially- and commercially-relevant chemical, *n*-butanol.
2. Demonstrate that this engineered OB311 produces *n*-butanol from unadulterated, Alberta-sourced industrial CO2 emissions.

Appendix 3 – Work Scope

Task 1 – Administration
Oakbio will be the interface with the CCEMC Project Manager, which include interaction with CCEMC project manager, accounting and progress reports. Inputs for these will be

Task 2 – Creation of engineered microbes for *n*-butanol production

- Will begin as soon as funding is received and will continue for 12 months
- Iterative engineering of microbes for n-butanol production
- Perform analytics on culture samples, enzymology, genetics

Task 3 – Screen engineered strains on laboratory and flue gas
For each microbial construct, bioreactors will be inoculated and aspirated with CO_2, O_2, and H_2 for a minimum of 50 hours in a temperature-controlled incubator. These constructs will be screened on laboratory and flue gas mixtures for growth viability, flue gas resistance and *n*-butanol production.

Oakbio will collect samples during pre-log, logarithmic, and steady-state growth and quantify growth kinetics and *n*-butanol production over time. These traits will be used to determine which constructs advance to testing in 20-liter bioreactors. Success factors are:

a) Set up experimental systems and controls.
b) Grow cultures on multiple gas mixtures.
c) Perform analytics on culture samples to determine production over time.
d) Select at least one *n*-butanol producing construct for validation in a 20L bioreactor.
e) Additional tests on key enzyme activities.

Task 4 - Demonstrate production of *n*-butanol in 20-liter bioreactors using lab and flue gas
Construct(s) identified in Task 3 will undergo validation for the production of *n*-butanol in 20-liter bioreactors. Success factors are:

a) Successful growth at 20-liter scale.
b) Perform analytical tasks as defined in Task 3 above.
c) Comparison of scale between 1-liter and 20-liter reactors.

Task 5 – Production of n-Butanol (and PHAs) on Alberta Flue-gas.
Construct(s) identified in Task 3 will undergo validation for the production of *n*-butanol on Alberta and Lehigh flue gases. Success factors are:

a) Obtain flue gas samples from at least one Alberta industrial site. Oakbio will target flue gases from power plants, combined heat and power co-generation plants, and cement manufacturing facilities. For example, Heidelberg/Lehigh Cement Group owns Inland Cement in Edmonton, Alberta, using a process identical to that of the Lehigh Southwest Cement plant in Cupertino. Additionally, we plan to interface with other efforts at carbon capture which use electro-reduction, MEA, or Carbonic Anhydrase (CO_2 Solutions Inc.) to capture CO_2 for sequestration. In this way we can also serve CO_2 re-utilization of

GHGs captured from remote operations such as Oil Sands extraction and processing, at a centralized plant.

b) Test selected strains using Alberta flue gas. Oakbio has an existing system and procedure for this in which bioreactors are housed in an incubator and supplied with flue gas from collection containers and hydrogen from a hydrogen generator, with pass through gas safely vented into a Labconco fume hood.

Task 6 - Estimation of GHG reduction and techno-economic analysis

a) Estimate GHG reduction at bench scale. Oakbio will utilize data collected in the course of Tasks 3 and 4 to estimate GHG reduction per liter of butanol produced.
b) Provide estimated trajectory for GHG reductions at pilot and commercial process scales.
c) Provide a techno-economic analysis of *n*-butanol production costs at commercial scale.

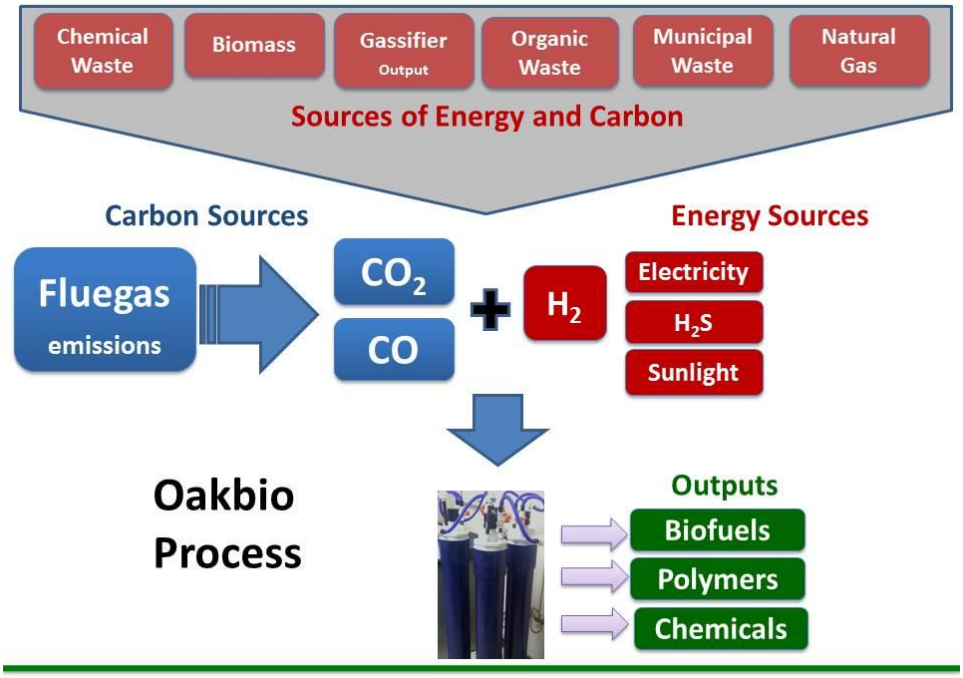
Appendix 4 - GHG Reduction and Other Benefits

4.1 Oakbio Flexible Carbon Capture Platform

In order to make a significant contribution to GHG capture and conversion, any carbon capture and conversion/utilization technology must be focused on creating products which satisfy markets of very large scale. This is why Oakbio is working to create a biomanufacturing platform capable of addressing multiple large markets. Bioplastics and *n*-butanol as biofuel are the first two of hundreds of potential products addressable by this platform technology. Plastics and transportation fuels are also two of the largest volume carbon based products currently used globally, and their petrochemical manufacture and consumption are major contributors to GHG emissions in Alberta and worldwide.

The ultimate robustness and applicability of the Oakbio/FRT Lab system will be at least partially determined by its flexibility. Oakbio/FRT Lab microbes offer flexibility of carbon sources, of power sources and of outputs to make a robust, adaptable production platform (see Figure 1).

Figure 1. The Oakbio process is flexible in terms of energy, carbon and product output.



Please note that in addition to projected use as a biofuels, *n*-butanol is currently utilized in a number of higher value chemicals markets, in which the carbon stay sequestrated for longer period of time (see discussion in main report).

4.2 Detailed GHG Model Assumptions

GHG Emissions from H2 Production

H_2 can be obtained from many sources, each affecting the life-cycle GHG emissions of associated products. The model presented here considers H2 obtained from steam reformation of methane (SMR) and H2 obtained from electrolysis of water utilizing renewable electricity. We first describe SMR because it is currently the most economical and immediately applicable to the pilot and production facilities that will be utilized in Rounds II and III.

SMR is a commercial H2 production technology currently seeing wide use. It creates demand for electricity and consumes natural gas (CH4) and water to H2 and CO2. It is the lowest-cost technology currently available for H2 production at scale. In order to calculate the life-cycle GHG impact of this generation method, the source of CH4 must be considered: if the CH4 comes from anaerobic digestion of municipal waste, or would otherwise be released into the atmosphere, the process is technically net GHG-negative, due to CH4's GHG intensity of 21 and CO2's GHG intensity of 1.

Electrolysis of water has a GHG impact directly related to the method of generation for the electricity used. If that electricity is generated from wind, solar, geothermal, or hydroelectric resources, the H2 produced can be nearly carbon neutral.

The GHG impact of these different H_2 sources is summarized in Table 1. This proposal assumes that H2 for input into the Oakbio/FRT Lab process will be produced by SMR. SMR is modeled in this proposal because it represents the worst-case scenario for GHG impact of the proposed technology.

Source of H2 Gas	kg CO2e per liter of *n*-butanol
SMR of Pipeline Natural Gas	(<2 kg CO2e per liter of n-butanol or >1 kg CO2e
SMR of Methane from Anaerobic Digestion	15.0 captured
Electrolysis of Water using Renewable Electricity	0.00 released

Table 1 GHG emissions due to hydrogen gas generation from different methods. Pipeline natural gas causes the release of 1.4 Kg per liter of n-Butanol. A mix of 5% waste gas plus 95% pipeline gas is GHG neutral. Pure waste gas accounts for a net reduction of 15

Power Plant GHG Emissions – Electricity for SMR

The GHG emissions from generating electricity required to produce hydrogen as a feedstock for the proposed technology will reach 0.06 kg CO2e per liter of *n*-butanol produced. The electricity required for SMR is assumed to be obtained from Alberta's grid. Therefore, the emissions intensity of generating electricity is calculated as a weighted average of the percentage of power generated from coal, natural gas, and renewable sources in the Province of Alberta, multiplied by their respective emissions intensities. This calculation is detailed in Table 2 below.

Power required for H2 generation via SMR	0.569 kW-hr per kg H_2 (US DoE model)
Emissions intensity of lignite coal	2.18 lbs CO_2e per kW-hr generated (US EIA)
Emissions intensity of natural gas	1.22 lbs CO_2e per kW-hr generated (US EIA)
Emissions intensity of renewable electricity	0 lbs CO2e per kW-hr generated
Alberta electricity generation from coal	43% (Alberta Energy website, http://www.energy.alberta.ca/electricity/682.asp)
Alberta electricity generation from natural gas	40% (Alberta Energy website)
Alberta electricity generation from renewables	17% (Alberta Energy website)

Alberta emissions intensity of electricity generation	1.43 lbs CO2e per kW-hr generated (calculated)

Table 2 Activities and emissions factors, with references, for calculation of GHG emissions due to hydrogen gas generation

Power Plant GHG Emissions – Electricity for Gas Fermentation

- Oakbio has estimated the GHG emissions due to generating electricity for its gas fermentation process at commercial scale by modifying a model published by the US National Renewable Energy Laboratory (*Process Design and Economics for Biochemical Conversion of Lignocellulosic Biomass to Ethanol*, NREL, 2011).
- This detailed model studies all facets of construction and operation of a 61 MMGPY ethanol fermentation plant, including electricity requirements for each portion of the manufacturing process.
    - The model assumes that sugar is the microbes' feedstock, and that sugar is obtained from the breakdown of cellulosic biomass.
- This is a feasible starting point because it models a commercially-relevant biofuels production facility using microbial fermentation as its production strategy.
- Electricity consumption for this cellulosic biomass to ethanol process as reported by NREL is reproduced in the table below:

Total NREL ethanol production requirement	1.03 kW-hr per liter ethanol produced
NREL Breakdown of Ethanol Process by Stage	**% of Total Energy Use**
Pretreatment & Conditioning	21%
Enzymatic Hydrolysis & Fermentation	10%
Cellulase Enzyme Production	20%
Distillation & Solids Recovery	8%
Wastewater Treatment	26%
Boiler/Turbogenerator	5%
Utilities	10%

Table 3: Electricity consumption reported for NREL cellulosic biomass to ethanol conversion process.

- The Oakbio/FRT Lab proposed process differs from this model in four significant ways:
    - It does not require Pretreatment or Conditioning of its microbes' feedstock.
        - The industrial flue gas that is the microbial carbon source is unadulterated.
    - It does not require separate Cellulase Enzyme Production.
        - Oakbio/FRT Lab microbes grow solely on unadulterated industrial flue gas and H2 and produce all required enzymes themselves.
    - It has significantly lower Wastewater Treatment requirements, because all feedstocks are gases, instead of dissolved chemicals.
        - May reduce electricity requirement by half, or more.
    - It performs only Fermentation of the gaseous feedstocks, and not Enzymatic Hydrolysis.
        - Enzymatic Hydrolysis is the final feedstock-preparation step before sugars derived from cellulosic biomass can be consumed by ethanol-producing microbes.
- These differences are reflected in the Table below, showing Oakbio/FRT Lab's estimated process electricity requirements.

Table 4: Estimated electricity requirements for Oakbio process

- This analysis results in an estimated reduction of process electricity requirements from 1.03 to 0.54 kW-hr per liter of biofuel produced.
    - Note that this process electricity requirement has been adjusted (according to the same procedure described elsewhere in this document) for *n*-butanol's higher energy content than ethanol.
- The estimated electricity requirement of 0.54 kW-hr per liter of *n*-butanol produced translates to GHG emissions of 0.35 kg CO2 per liter of *n*-butanol produced. This calculation relies on the weighted-average emissions intensity calculation for electricity generated in Alberta described in the previous section.

SMR GHG Emissions – Sourcing of Methane

Natural gas is a required feedstock for production of H2 by SMR. Recovery, processing, and transmission (collectively, "sourcing") of methane require energy, so there are GHG emissions associated with this step of Oakbio's proposed process.

The GHG emissions associated with sourcing of CH4 were estimated using the Argonne National Laboratory GREET 2014 Life-Cycle Analysis package. This software estimates the CO2 emissions associated with recovery, processing, and transmission of natural gas for Canadian electricity production to be 3.264 kg CO2 per 1 MMBtu. Converting this to kg per liter of *n*-butanol produced results in an emissions estimate of 0.076 kg CO2e per liter of *n*-butanol produced.

SMR GHG Emissions – Consumption of Methane

SMR converts one molecule of methane to four molecules of H2 and one molecule of CO2 in a two-step process requiring additional energy input. The GHG emissions of this process were estimated by first calculating the amount of H2 needed to produce one liter of *n*-butanol, and then estimating the associated electricity requirements for SMR, and the amount of CO2 emitted via consumption of natural gas.

The GHG emissions from consumption of natural gas for producing H2 are estimated to be 0.75 kg CO2e per liter of *n*-butanol. This calculation assumes a 90% energy conversion efficiency from methane to H2. More importantly, this calculation assumes that natural gas is sourced directly from a pipeline or other major producer. If waste sources of methane are utilized as feedstock for H2 production, GHG emissions due to SMR will drop considerably. For example, H2 production via SMR would technically be GHG-neutral if 5% of the methane used was sourced from compost heaps, municipal waste, or other non-fossil fuel sources. This is due to methane's Kyoto multiplier of 21, relative to CO2's Kyoto multiplier of 1.

Gas Fermentation GHG– Capture and Conversion of CO2 from Flue Gas

- Oakbio/FRT Lab estimate that this life-cycle stage will temporarily capture and convert 1.92 kg CO2 per liter *n*-butanol produced.
- The amount of CO2 captured and converted into *n*-butanol as a result of the Oakbio/FRT Lab's technology is back-calculated from the well-known and well-understood amount of carbon present in one liter of *n*-butanol. The calculation is straightforward because the sole carbon source provided to Oakbio/FRT Lab's microbes is CO2 in the form of unadulterated industrial flue gases; therefore, the carbon present in any *n*-butanol produced this way will necessarily have come from industrially-emitted CO2.

Combustion of n-Butanol - GHG Emissions

- As a biofuel, the *n*-butanol produced through this proposed technology will eventually be combusted in internal combustion engines.
    - This will convert the *n*-butanol back into CO2 and H2O.
- Therefore, this life-cycle stage will emit 1.92 kg CO2 per liter of *n*-butanol produced.
- These emissions cancel the capture and conversion of the previous stage, resulting in net-zero GHG emissions specifically for capture, conversion, and combustion.

GHG Emissions Baseline - Life Cycle Analysis of Alberta Gasoline

Gasoline serves as the standard light-duty transportation fuel for the Province of Alberta and much of the developed world. In Alberta it is produced from crude oil derived from bituminous sands – a GHG-intensive process. Gasoline is typically "disposed of" by combustion in an internal combustion engine, emitting GHG in the process.

The amount of GHG emitted over the life-cycle of a liter of gasoline produced and consumed in Alberta is detailed in *Life Cycle Assessment Comparison of North American and Imported Crudes*, a study prepared by Life Cycle Associates and Jacobs Engineering for the Alberta Energy Research Institute (Life Cycle Associates, 2009). Specifically, the total well-to-wheel GHG emissions for crude oil produced from bituminous sands utilizing steam-assisted gravity drainage, and upgraded to gasoline in a coke-fired upgrader, is 115.7g CO_2e per MJ of gasoline. This breaks down to an average of 72.85g CO_2e per MJ from combustion, 0.8g CO_2e per MJ from CH_4 and N_2O, and 42.3g CO_2e per MJ from manufacturing and production (see Table 8-5, Life Cycle Associates, 2009). These numbers translate to 1.5 kg CO_2e emitted per liter of gasoline produced, and 2.5 kg CO_2e emitted per liter of gasoline combusted.

GHG Emissions Reductions per Liter from Replacing Gasoline with n-Butanol

Table 5 illustrates estimated GHG emissions for production of one liter of *n*-butanol by the proposed technology, GHG emissions for one liter of gasoline produced from bituminous sands in Alberta, and the potential GHG emissions reduction of replacement. For the purpose of estimating this volumetric reduction, the emission intensities of production and consumption of gasoline have been multiplied by 0.84, to account for the 16% difference in energy content of *n*-butanol and gasoline.

Overall GHG Emissions Change, kg CO_2e per liter *n*-butanol	-1.54
GHG Emissions, Gasoline Consumption, kg CO_2e per liter	3.98
Correction for *n*-Butanol Lower Energy Content than Gasoline	3.34
GHG Emissions, *n*-butanol Manufacture, kg CO_2e per liter	1.80

Table 5 Comparison of life-cycle GHG emissions from n-butanol and gasoline manufacture and consumption

Calculation of Total Annual GHG Emissions Reduction

The total annual GHG emissions reduction due to the proposed technology at commercial scale is estimated by multiplying the GHG Emissions Change calculated in Table 5 by the number of liters of *n*-butanol produced at commercial scale. Estimation of this total production number constitutes the remainder of this section.

Target Market for Oakbio/FRT Lab Proposal

The Oakbio/FRT Lab partnership has identified the Alberta light-duty transportation fuel market as its target for distribution of *n*-butanol as a fuel additive. Currently, Alberta's RFS Regulation mandates that fuel contain 5% renewable alcohol; this requirement is met by blending ethanol into the fuel supply. But ethanol is a less-than-desirable additive because it

contains only 66% of the energy of gasoline on a volumetric basis, and it can be mixed up to a maximum ratio of 10%. *n*-butanol is advantaged as a fuel additive over ethanol because it contains 84% of the energy of gasoline, increasing gas mileage for equivalent blends, and because *n*-butanol may be mixed in higher ratios with gasoline than ethanol without damage to North American internal combustion engines (US EPA).

The Alberta fuel market is expected to demand slightly more than 7 billion liters of fuel by 2019. This projection is based on a consistent 2.33% annual growth rate of the Alberta fuel market from 1993 – 2012. These data are also consistent with the reference case developed by MK Jaccard and Associates for refined petroleum product consumption in the Transportation sector; in this reference case, demand for refined petroleum products increases from 413 petajoules in 2010 to 677 petajoules in 2050 (see Table 8, page 27, Jaccard and Associates, 2007).

According to the US Energy Information Administration, global gasoline demand has grown consistently as well, from an average of 16 million barrels per day in 1986 to slightly more than 22 million barrels per day in 2010. This is a 2010 daily demand of almost 3.5 trillion liters. There is no reason to expect that worldwide demand for transportation fuels will level off between now and 2032.

It is clear from these numbers that the Alberta and global gasoline markets are easily large enough to absorb the volumes of product necessary to achieve meaningful GHG emissions reductions.

4.3 Market Penetration Scenarios

Two market penetration scenarios are presented in Figure 19. The Figure shows projected percent market share of Alberta's light-duty transportation fuel market as a function of time.

Conservative Scenario
The blue line in Figure 2 results from the assumption that the proposed technology will achieve 5% market penetration in 2024, five years after completion of the CCEMC Grand Challenge. Market share then grows much more slowly to approximately 6.5% by 2032. This market penetration scenario is driven by rapid adoption of Oakbio *n*-butanol as a superior blendstock to ethanol for purposes of Alberta's Renewable Fuel Standard, and then slow adoption thereafter. GHG emissions reductions continue to increase after 2024 because of the 2.33% annual growth rate of Alberta's transportation fuel market.

Upside Scenario
The orange line in Figure 2 results from the assumption that Oakbio/Tabita Laboratory's *n*-butanol will achieve 5% market share by 2023. This assumption is driven by renewal of the RFS Regulation in 2020 and by *n*-butanol's superiority to ethanol as a fuel additive. Market penetration accelerates after achieving cost-parity with gasoline in 2024. Market penetration is projected to reach 10% in 2032.

Figure 2. Projected penetration of Oakbio's n-butanol into Alberta's gasoline market

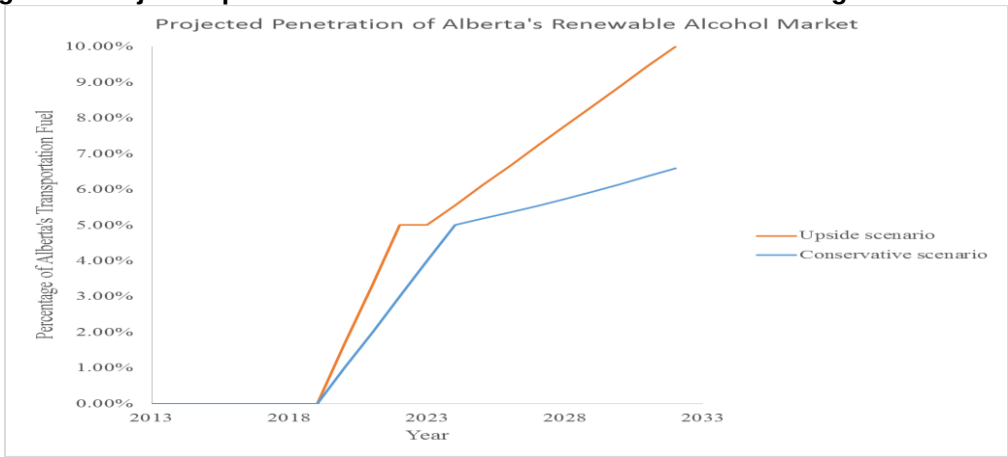
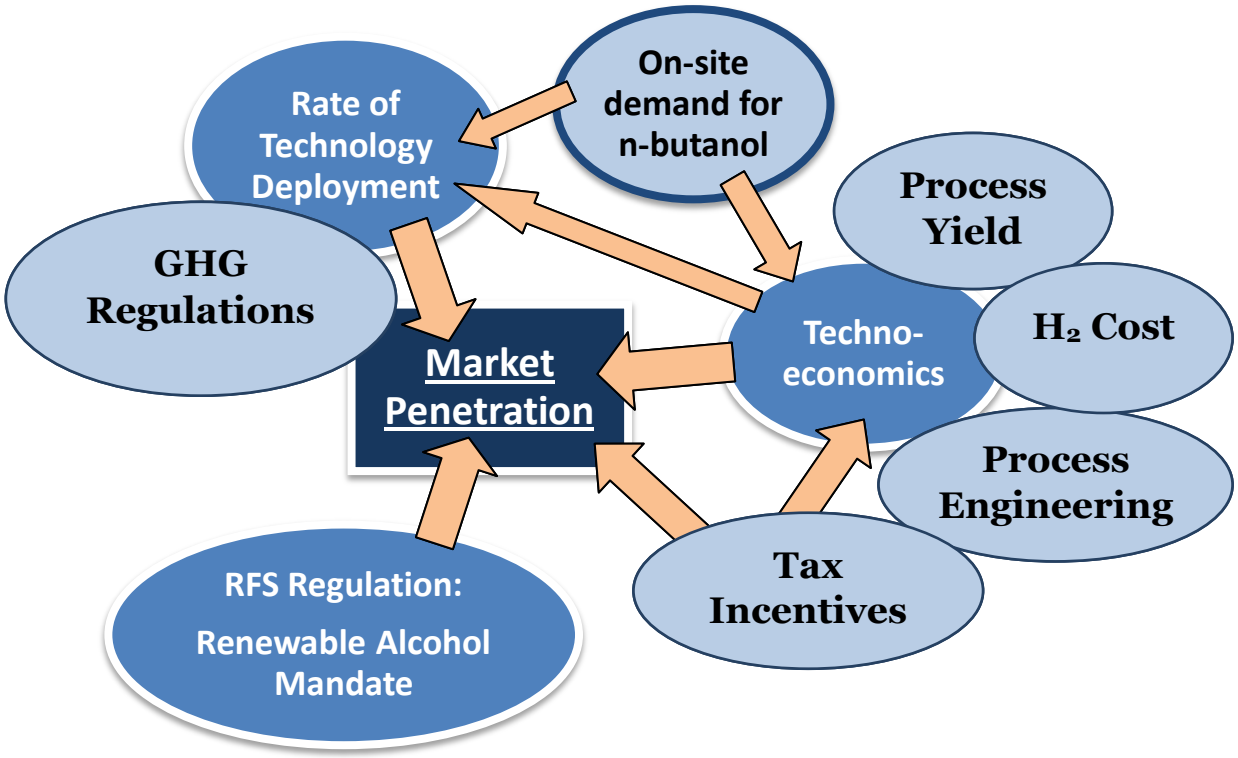


Figure 3. Factors affecting market penetration of Oakbio/Tabita Laboratory proposed technology



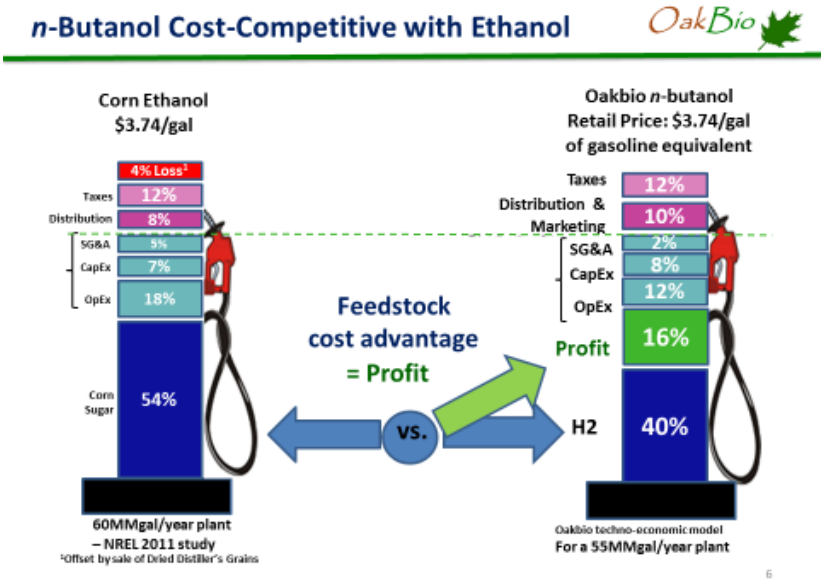
4.4 Future Non-GHG Benefits

N-butanol better than ethanol

Figure 4 (below) compares the costs (raw materials, capital expenditure, operational expenditure, and SG&A) required for Oakbio to produce *n*-butanol from CO2+H2 and for current biofuels manufacturers to produce ethanol from corn (ethanol cost model from National Renewable Energy Laboratory study, 2011; *n*-butanol cost model based on Oakbio internal modeling). All costs and volumes have been adjusted for energy equivalence.

In this case, H2 for Oakbio's process is supplied via SMR (steam methane reforming) of natural gas, costing ~$1.50 per gallon of gas equivalent (40% of the price at the pump). At the same time, a standard ethanol plant will have to spend ~$2.00 per gallon of gas equivalent on corn as a raw material (54% of the price at the pump) to make the same amount of fuel.

Figure 4. Techno-economic comparison of corn ethanol and Oakbio n-butanol production costs.



This advantage in raw materials cost leads directly to a profit of ~$0.60 per gallon of gas equivalent for Oakbio, as opposed to a loss of ~$0.15 per gallon of gas equivalent for the ethanol manufacturers. Note that corn ethanol plants cover this loss by selling other, non-biofuel products.

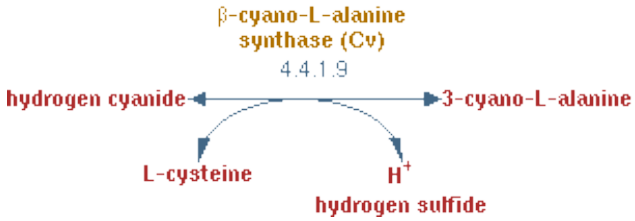
Bioremediation of other toxic gas components

OB311 is derived from Ralstonia Eutropha H16, a well known and studied microbe with high potential for bioremediation. *OB311* is able to degrade a large list of chloroaromatic compounds and chemically related pollutants for example, the microbe can degrade the herbicide 2,4-dichlorophenoxyacetic acid, Dioxin, benzene, diesel fuel, acetone and organic acids.

In gas streams, the microbe is capable of reducing the major contaminants, SOx, NOx, CN, H2S and other compounds. It is important to note, that OB311 is more resistant to flue gas sontaining these compounds than is the original Ralstonia Eutropha H16 strain from which it was derived, and this indicates that these remediation activities and potential are also much greater in OB311. Remediation of compounds other than CO2 and CO, was not part of this grant work, however we are aware of the high potential of this organism to remove other fluegas contaminants and plan to make this a focus of future work.

Cyanide

OB311 is resistant to, and capable of uptake and breakdown of cyanide (CN) present in flue gas. The key enzymes poisoned by cyanide in many microbe species are of a cyanide resistant form in OB311. Cyanide is a key compound employed by the microbe as a functional part of several key enzymes and is converted from its toxic gaseous form, hydrogen cyanide, to the non-toxic form 3-cyano-alanine rapidly by the cell.



Hydrogen Sulfide

OB311 will uptake and breakdown hydrogen sulfide to synthesize the amino acids cysteine and homocysteine via the metabolic pathways:

cysteine biosynthesis I : *O*-acetyl-L-serine + hydrogen sulfide -> L-cysteine + acetate + H^+

homocysteine biosynthesis : *O*-acetyl-L-homoserine + hydrogen sulfide -> L-homocysteine + acetate + H^+

SOx uptake and breakdown

In a related pathway, H16 will uptake SOx which is converted to sulfite in solution and convert this to H2S which will the undergo the above reactions to form the noted amino acids:

For the example of SO2, the reaction $SO_2 + H_2O \rightleftharpoons HSO_3^- + H^+$

occurs when SO2 is a dissolved gas in water, such as occurs in our bioreactors, and the produced HSO_3^- , or sulfite, then undergoes the following reaction to produce H2S which is converted to the essential nutrient sulfate.

sulfite + 3 NADPH + 5 H^+ ← 3 $NADP^+$ + hydrogen sulfide + 3 H_2O

NOx uptake and breakdown

Denitrification. A complete denitrification pathway allows the organism to exploit alternative electron acceptors such as NO_3^- and NO_2^-.

OB311 is a denitrifying microorganism able to uptake and metabolize nitric oxide (NO), nitrous oxide (N20) and Nitrogen dioxide (NO2) to produce the critical nutrient nitrate (NO3). Nitric oxide is not only an essential respiratory substrate of the denitrifying cell but constitutes in nanomolar concentrations a key signal for the expression of nitrite reductase and NO reductase. Because of this, the concentration of NO is continually maintained at this minute concentration by the microbe cells.

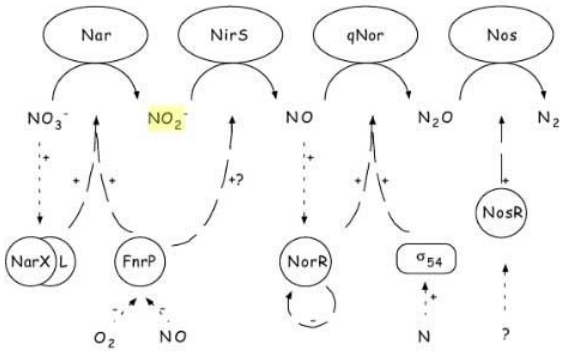
The primary chemical reactions performed by the microbe are:

Nitrous Oxide to Nitric Oxide

N2O [membrane] + 2 an oxidized c-type cytochrome[membrane] + H2O[membrane] ← 2 NO [membrane] + 2 a reduced c-type cytochrome[membrane] + 2 H+[membrane]

Nitric Oxide to Nitrogen

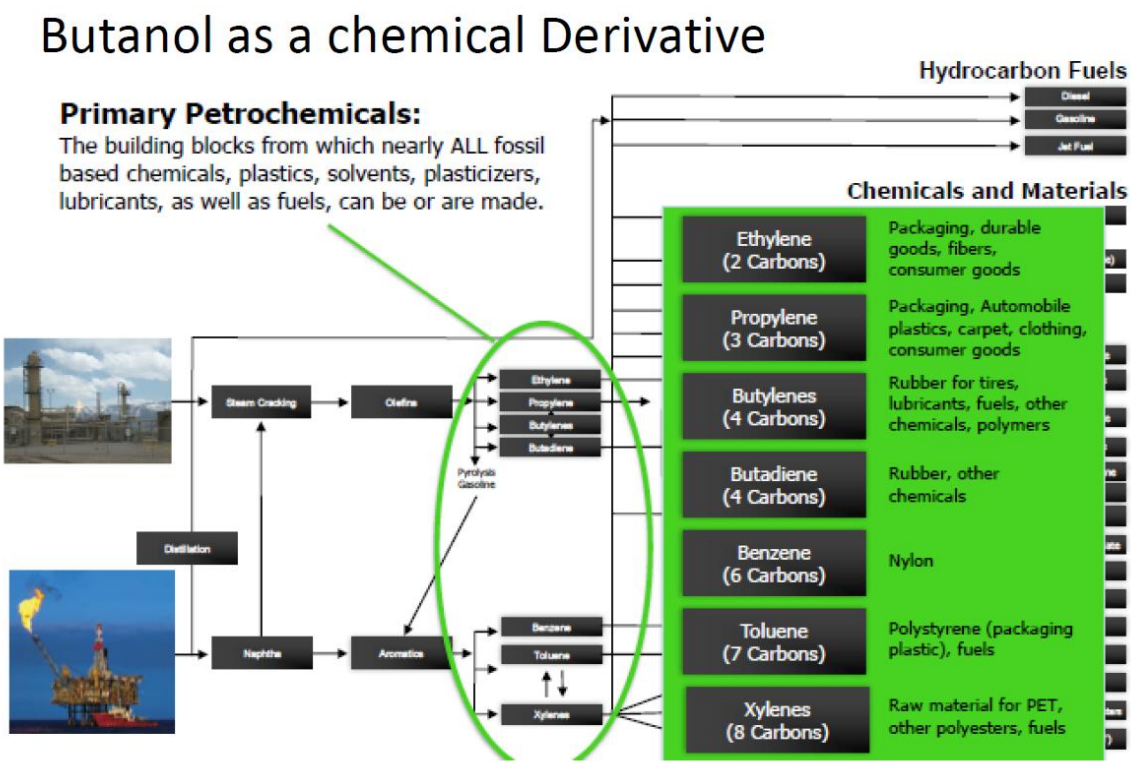
$2NO \rightarrow N2 + oxygen$



OB311 NItrogen Metabolism

Nar (nitrate reductase), NirS (nitrite reductase), qNor (Nitric Oxide reductase), and Nos (nitrous Oxide Reductase) convert the various forms of nitrogen to the essential metabolite Nitrate (NO3-) which is used to make proteins, amino acids and other critical biochemicals.

It should be noted that these reactions are an evolutionary adaptation to provide nitrate (NO3-) and the culture methods used by Oakbio are nitrate limited, thus greatly favoring the conversion of the various nitrogen oxides to nitrate.



Appendix 5 – Process Scale-Up and Commercialization

5.1 Process Development Timeline

The general timeline of Oakbio's technology development plan, incorporating the three strategies discussed above, is shown in Figure 1. This plan requires a significant expansion of Oakbio's current scientific and engineering capabilities. As such, the work it describes will commence once Oakbio has successfully raised additional financing.

Oakbio envisions a 3-4 year technology development process that will scale-up its technology from the laboratory through a successful commercial demonstration. This four-year development period will encompass two major process scales defined by the fermentation capacity studied in each stage:

1. Pilot scale 100-1,000L
2. Demonstration scale 5,000+L

The first two years of technology development will occur primarily at lab (100+L) and pilot scales (1000L). During this time, Oakbio will improve the performance of its bacterial strains utilizing synthetic biology techniques, and optimize the microbe's growth medium and nutrition. Both optimizations are iterative processes, in which new microbial CCC strains and growth conditions will continually be created and tested. This work is done at lab-scale for faster cycle times and to minimize expenses. Eventually, the highest-performing strains will be tested in pilot- and demonstration-scale bioreactors.

At end of year 2/beginning of year 3, Oakbio will utilize the data it has collected from two years of technology development to begin in-depth design work on its CCC process. An entire demonstration-scale CCC plant will be designed, including fluid- and gas-handling systems, bioreactors, and associated machinery.

In concert with these efforts, Oakbio will begin to develop strategic partnerships with major CO2 emitters seeking to reduce their GHG emissions levels, and with major chemical and materials manufacturing companies seeking alternatives to petroleum as a feedstock for their processes. The requirements of these strategic partners will directly influence development of Oakbio's demonstration facility.

At the beginning of year 3, Oakbio will begin testing of its highest-performing microbial strains at demonstration scale. The performance data gained from this phase of the technology development plan will be used to further improve the CCC process, and to prove the commercial potential of Oakbio's technology.

Commercial implementation of Oakbio's technology will commence at the end of the four-year development timeline. A full commercial CCC plant using Oakbio's technology will operate with a fermentation capacity >1,000,000L, and will be co-located with a major point-source of CO2 emissions, such as a cement or power plant. Each of Oakbio's installations will capture >100,000 metric tons of CO2 and convert it into profitable petroleum replacements (see model discussion below).

5.2 Synthetic Biology and Strain Improvement

Objective: optimize OB311's metabolism by using synthetic biology to eliminate non-productive uses of carbon and energy. Improve OB311's resistance to flue gas contaminants through continued adaptive evolution.

Examples of industrial strain improvement programs that have significantly increased product yields and reduced costs:

- **Lanzatech** improved the butanol titer of their engineered Clostridium strain (growing on steel mill waste gas) to over 1.5 g/L (25.66 mM) by overexpression of certain butanol synthesis pathway genes (Kopke *et al.*, 2013; Shen, 2013).
- **Coskata**'s high-throughput screening lab in Warrenville, IL, has been pursuing a strategy of "guided mutation and selection" to improve their production strain (Coskata website, 2014). According to the company, their anaerobic high-throughput screening system has a capacity of 150,000 new strains per year. They claim to have been able to "improve the overall productivity of [their] micro-organisms a thousand-fold and minimize the requirements for additional nutrients." Since anaerobic organisms, such as Coskata's, are inherently slower-growing and more difficult to handle than OakBio's aerobic bacteria, we anticipate being able to achieve significantly better results in a shorter time with OB311.
- **Amyris, Inc**. (Emeryville, CA) has employed a sophisticated high-throughput system for screening yeast variants generated by random mutagenesis or rational design. For production of artemisinin (an anti-malarial drug), this effort, combined with substantial fermentation development, culminated in a 250-fold increase in production of amorpha-4,11-diene to 40 g/L concentration (Westfall *et al.*, 2012). Amyris is now exploiting their artemisinin platform to develop an efficient farnesene production platform for the biofuels market. In 2013, the company reported that they had used their metabolic engineering and screening technology to improve the activity of the rate-limiting synthase 8-fold above the wild-type enzyme, and that they anticipated a further 3-fold improvement. (Amyris 2013 Q1 10-Q Exhibit) Oakbio's platform development, which can now take advantage of the lessons learned by these other companies, is expected to proceed in much the same way.

5.3. Media and Feedstock Development

Optimizing composition of growth medium and gas feedstocks will increase biomass generation, decrease batch duration, and increase n-butanol as a percentage of biomass by providing the precise nutrients that the microbe needs for fastest possible growth.

5.4 Fermentation Process Design Improvements

Objective: optimize the design of the fermentation vessel and associated fluid- and gas-handling systems, and increase the overall scale of the fermentation system.

- Process improvement: Up to a several thousand-fold increase in production is possible via classical process improvement techniques (Parekh, *et al.*, 2000). These steps may include strain improvement, medium optimization, metabolic flux analysis, and the like. These techniques have been used, for example, to improve the production of penicillin

4,000-fold over the original parent (Peberdy, 1985). We will apply these methods to the systematic improvement of OB311 performance.

- Feedstock recycling: Volova (2009) and others have shown that *Ralstonia* is an excellent organism to grow using both gas- and medium- recirculation (to improve feedstock utilization). These methods will help to greatly improve conversion efficiency and reduce costs. We will seek to incorporate these procedures into our process design.
- Improvements in downstream processing: Current butanol extraction methods comprise various steps, such as: separating the fermentation broth from the cells and removing the *n*-butanol from the aqueous phase by pervaporation, liquid-liquid extraction or gas-stripping. Distillation is not a feasible option because the boiling point of butanol is higher than that of water (Kaminski *et al.*, 2011). Optimizing the extraction process will improve the overall process yield. Currently, pervaporation appears to be the most economically attractive method.

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