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FINAL PROJECT REPORT

CARBON DIOXIDE CAPTURE AND CONVERSION TO CHEMICAL PRODUCTS

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- The California Energy Commission.
    - The California Energy Commission's Public Interest Energy Research program, for funding the key proofs of concept described in this document.

- The Lehigh Southwest Cement Plant, Cupertino, California.
    - Lehigh has partnered with Oakbio for the past three years to provide space for an on-site field laboratory with a continuous supply of unadulterated cement flue gas. This has been indispensable in Oakbio's biological carbon capture and utilization R&D.

- The Gateway Generating Station in Antioch, California, a natural gas-fired power plant owned and operated by the Pacific Gas & Electric Company.
    - PG&E has given Oakbio access to unadulterated natural gas-fired flue gases from its Antioch power plant. These samples are key to proving the general applicability of Oakbio's technology.

PREFACE

The California Energy Commission Energy Research and Development Division supports public interest energy research and development that will help improve the quality of life in California by bringing environmentally safe, affordable, and reliable energy services and products to the marketplace.

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Carbon Dioxide Capture and Conversion to Chemical Products is the final report for the Carbon Dioxide Capture and Conversion to Chemical Products project (contract number PIR-11-019) conducted by Oakbio, Inc. The information from this project contributes to Energy Research and Development Division's Energy-Related Environmental Research Program.

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ABSTRACT

Increasing levels of carbon dioxide (CO^2) emissions from human sources have been directly linked to climate change, which has resulted in more frequent and severe weather events. Oakbio demonstrated that a novel microbe-based technology could transform the economics of carbon capture into a profit making business. Oakbio adapted microorganisms that consume CO^2 directly from unadulterated industrial flue gas and use molecular hydrogen (H^2) as their only energy source. These microbes grow in liquid medium using specially designed fermentors and consume flue gas CO^2 and H^2 as their feedstocks. Oakbio selected two proprietary microbes, strains OB311 and OB213, which naturally produce polyhydroxyalkanoate (PHA), a biodegradable plastic from these feedstocks. This process was tested at an operating test system at a California cement plant. Additional industrial flue gas was collected at a local electric power plant powered by natural gas combustion to test this system with a different source of CO^2. Key performances were measured including CO^2 capture, microbial production and bioplastics production.

Oakbio found that OB311 was the more successful of the two microbes, showing that a large percentage of CO^2 present in a cement flue gas stream can be captured in one pass through solution. Under ideal conditions, OB311 accumulated up to 75 percent of its dry biomass weight as PHA. OB311 was also shown to grow on CO^2 from natural gas-fired power plant emissions.

Projections based on detailed technical and economic analysis show that Oakbio technology has significant potential to become a profitable method of carbon capture. The next step in developing Oakbio's technology will be to set-up pilot facilities at several CO^2-emitting locations in California to further validate the technology at scale.

Keywords: Oakbio, carbon capture and conversion, carbon dioxide, hydrogen, biological conversion, microbe, chemoautotrophy, fermentation, bioreactor, bioplastic, polyhydroxyalkanoate, biomass, cement flue gas, natural gas-fired power plant, coal-fired power plant, industrial carbon emissions, greenhouse gases, climate change

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EXECUTIVE SUMMARY

Introduction

Increasing levels of carbon dioxide (CO2) emissions from human sources have been directly linked to climate change, which has resulted in more frequent and severe weather events. In 2012, California emitted 459 million metric tonnes of CO2 equivalent - emissions from passenger vehicles accounting for 36 percent, 21 percent from electric power generation and industrial emissions at 19 percent.

There are a number of technologies currently proposed for reducing greenhouse gas emissions from large point sources such as oil refineries, or coal-fired and natural gas-fired power plants - CO2 capture and sequestration or CO2 capture and conversion. CO2 capture and sequestration technologies separate CO2 from industrial flue gases before emitting into the atmosphere and then store the separated CO2 underground or in the deep ocean for perpetuity. This technology is costly and potentially poses environmental challenges since the CO2 must be transported to the geological formations used as sequestration sites.

New technologies for carbon capture from industrial flue gas are emerging, the most attractive of which involve converting the captured CO2 into commercial products that recycle the carbon into consumer markets and further reduce carbon emissions by replacing petroleum-derived products. This process is called CO2 capture and conversion (CCC).

Project Purpose

Oakbio developed a novel CCC technology which uses microorganisms that can remove CO2 from flue gas emissions and convert it into bioplastics, biofuels and other products. To validate this technology, Oakbio collaborated with the Lehigh cement plant in Cupertino, California. Lehigh is a subsidiary of the Heidelberg group, the second largest cement producer in the world. Lehigh provided Oakbio with an onsite field laboratory and direct flue gas feed. In addition, PG&E provided access for flue gas collection from their natural gas combustion Gateway Generating Station in Antioch, California. Oakbio also investigated the technical and economic feasibility of their novel microbe-based process on an industrial large-scale facility.

Oakbio has adapted its microorganisms to consume CO2 directly from unadulterated industrial flue gas. This gas is the only carbon source and molecular hydrogen (H2) is the only energy source, making these microorganisms "chemoautotrophic". Oakbio's selected microorganisms are considered "Generally Recognized As Safe" for the environment, naturally produce a range of chemicals, and represent a good platform for metabolic engineering. Finally, unlike photosynthetic microbes which require light as a source of energy, these microbes (chemoautotrophic) may be grown at large densities in the dark, 24/7, in a conventional fermenter. These characteristics provide a significant advantage for industrial process development and commercial-scale production.

Project Process

For the current project, Oakbio selected two proprietary bacterial strains (OB311 and OB213) which naturally produce polyhydroxyalkanoates (PHA), a well-studied class of biodegradable

plastics. Both OB311 and OB213 were measured for CO2 capture potential and PHA production when these strains were fed industrial flue gas and H2. Benchmark experiments were also conducted in the Oakbio laboratory, where these microbes were fed "lab gas", a commercial gas with a fixed CO2 and H2 composition. Other benchmarking performance criteria were conducted using wild type organisms (the parents of Oakbio's improved strains).

Oakbio also developed a roadmap to scale its process up to a commercial level. The technical performance and economics of the Oakbio process was evaluated at commercial scale when co-located at a representative CO2-emitting industrial site. Carbon capture potential, product revenues from carbon conversion and overall economics of the process are then discussed, with added insights from a sensitivity analysis on key performance variables.

Project Results

OB311 grows faster and to higher cell density than OB213 and its parent microbial strain on cement flue gas. This assessment was supported by culture dry weight measurements. Oakbio's adaptation of OB311 to cement flue gas conditions has created a microbe well-suited for industrial CCC. Faster growth means faster process cycle time. Higher cell density means higher productivity per unit volume. Shorter process cycle time and higher productivity per unit volume increase the efficiency and profitability of a fermentation process.

OB311 consumes approximately 40 percent of the CO2 present in cement flue gas after one pass through a fermenter, and an additional 30 percent after 20 minutes of recycling the gas through a closed bioreactor system. The result shows that a large percentage of CO2 present in a cement flue gas stream can be captured in one pass through solution. With a large enough bioreactor, a significant percentage of total plant CO2 emissions could be captured and converted to chemical products, especially when such a gas recycling system is installed. A gas recycling system will also smooth the variation in gas composition inherent to cement production process.

For OB311 cultures that were fed cement flue gas, PHA accounted for a minimum of 24 percent of total biomass after 3-7 days in stationary phase. Under more ideal conditions (lab gas, larger gas fermenter, longer fermentation time), PHA accumulated to 75 percent of total dry biomass weight. A next step in process development should include full characterization of OB311's bioplastic production, including the maximum amount of time a culture may spend in stationary phase before process costs of continuing the batch culture outweigh the value of productivity gains.

OB311 was also shown to grow on CO2 from natural gas-fired power plant emissions at more than twice the rate it grows on cement flue gas. While naturally-occurring chemoautotrophic microbes grow well on optimized lab gas, the impurities and toxins (such as CO, SOx, NOx) present in Lehigh cement gas have a significant deleterious effect.

Future Commercial Scale

Oakbio's CCC system at commercial scale has the potential to capture 50 percent and more of the carbon emissions for a cement plant. The captured CO2 could then be converted into bioplastics and sold to generate additional revenues and profit.

Oakbio's modeling (which accounts for process technical performance as well as economic factors) estimates a positive carbon capture and conversion value of $51/metric tonne CO2. Although this value compares favorably with figures provided by other emerging methods of CCC, it is difficult to derive any meaningful conclusions, as the methods of calculations are most often different. For reference purposes, current methods of CO2 capture estimate a negative value (cost) of $40-$70/metric tonne, with additional costs accrued for CO2 compression, purification and storage. Some estimates for the initial demonstration projects have ranged as high as costs of $80-$120/metric tonne CO2.

The initial cost of installing a scaled-up Oakbio's system at a representative CO2-emitting site is significant, but, with a PHA product margin estimated at 36 percent, break-even on the initial capital expenditure can be achieved after three years of operation. Overall economic performance is sensitive to the cost of the H2 supply, which represents the bulk of feedstock cost for the Oakbio system. Economics are also sensitive to the market value of PHA.

When evaluating the overall GHG reduction performance of the Oakbio system, the carbon cost of all components in the process value chain needs to be considered. The source of H2 plays a major role in net GHG reductions. H2 can be obtained from many sources, including from renewable sources, such as solar and wind.

Finally, and importantly, in addition to the production of chemicals converted from CO2 capture, the Oakbio system offers several other benefits, such as carbon tax credits, potential increase in cement production over emission limits set by government, potential price premium on "green" cement product, and bioremediation linked to Oakbio's microbial activity. All of these benefits represent strong incentives for CO2-emitting industries.

Benefits to California

Oakbio successfully demonstrated that its technology can reduce the cost of CO2 compliance by generating revenues from CO2 capture. This was achieved by converting captured CO2 into PHA bioplastics, a commodity chemical. Oakbio also determined the technical and economic feasibility of a novel microbe-based process for capture and conversion of flue gas CO2 to bioplastics, using an already operating test system at a Cupertino cement plant. Key quantitative indices of system performance were measured, including CO2 capture, microbial biomass production and bioplastics production. The final report, including an analysis model, informs the cement industry and the CO2-emitting energy industry on the economic feasibility of the Oakbio system at large scale.

Oakbio's technical success could lead to commercial scale development of its process for CO2 Capture and Conversion in the cement and power generation industries. The project demonstrated a profitable process for producing valuable chemicals at a lower cost that will encourage further investment in California technology process development, create local jobs, reduce CO2 release from critical local industries, and could dramatically expand the bioplastics industry.

CHAPTER 1:
Project Background

In 2006, California passed Assembly Bill 32, becoming one of the first states in the US to require sharp reductions in greenhouse gas (GHG) emissions compared to a 1990 baseline (AB32, 2006). The California Energy Commission has funded this work through its Public Interest Energy Research (PIER) program in response to California State Assembly Bill 32 and the accompanying Executive Order S-3-05.

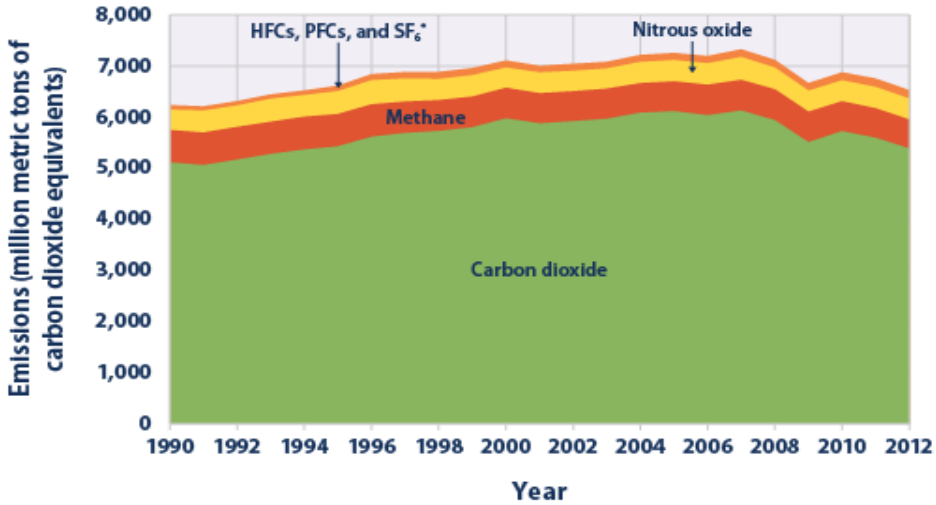
In 2011, Oakbio, Inc., a Sunnyvale, California-based company, received funding from the Energy Commission's PIER program to investigate a biological technology for ***Carbon Dioxide Capture and Conversion to Chemical Products***. This project determined the technical and economic feasibility of a novel microbe-based process for capture and conversion of flue gas carbon dioxide (CO2) to bioplastics, using an already operating test system at the Lehigh Southwest Cement Plant in Cupertino, California. Measurements of key quantitative indices of system performance were preformed, including CO2 capture, microbial biomass production and bioplastics production.

If commercialized, this technology will enable production of value-added chemicals, such as biodegradable plastics, directly from industrial flue gases.

1.1 Industrial CO2 Emissions

The US alone has emitted at least 6 billion metric tonnes of GHG every year since 1990 (Figure 1.1). Carbon dioxide (CO2) makes up the vast majority of these emissions, followed in decreasing order by methane, nitrous oxide, and various others.

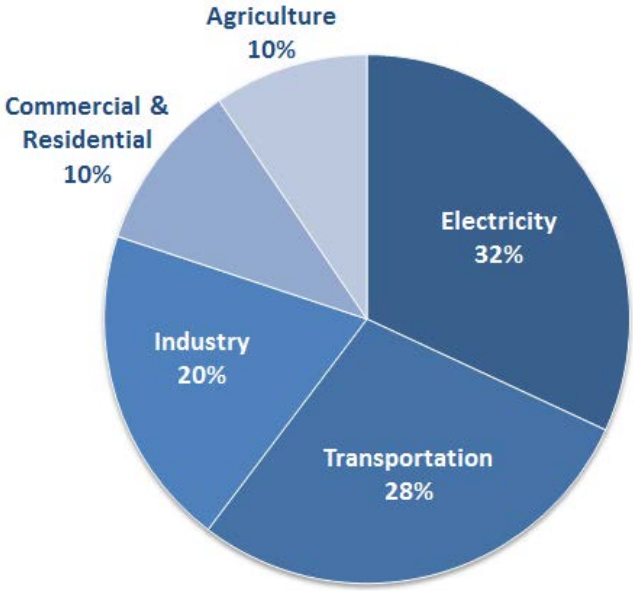
Figure 1.1: Total US Greenhouse Gas Emissions, 1990-2012



Source: US Environmental Protection Agency, http://www.epa.gov/climatechange/science/indicators/ghg/us-ghg-emissions.html

Three sectors of the US economy account for 80% of the CO2 emissions: generation of electricity at 32%, transportation (through combustion of liquid fuels) at 28%, and industry (through CO2-emitting industrial processes such as calcination of cement) at 20% (Figure 1.2).

Figure 1.2: Breakdown of US Greenhouse Gas Emissions by Sector, 2012



Source: US Environmental Protection Agency, http://www.epa.gov/climatechange/ghgemissions/sources.html

1.1.1 California State Greenhouse Gas Emissions

California emitted a total of 459 million metric tonnes of CO2-equivalent in 2012. Combustion of liquid fuels in passenger vehicles accounted for 36% of this total, followed by generation of electric power with 21%, and industrial emissions at 19% (California Air Resources Board, 2012).

California's primary CO2 emitters in the electric power category are natural gas-fired power plants, accounting for nearly 46 million metric tonnes of CO2 in 2012. In the industrial category, oil & gas extraction and refining together account for GHG emissions of almost 47 million metric tonnes of CO2 in 2012; much of these emissions are due to combustion of natural gas to generate combined heat and power. Finally, the cement manufacturing industry in California was responsible for emitting nearly 7 million metric tonnes of CO2 from 31 sites in 2012 (California Air Resources Board, 2012).

1.2 Reducing California's GHG Emissions

There are a number of technologies 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, or CO2 Capture and Conversion.

1.2.1 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.

- 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).
- 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.

1.2.2 CO2 Capture and Conversion (CCC)

In contrast to CCS, CO2 Capture and Conversion utilizes industrially-emitted CO2 as the feedstock for production of a carbon-based value-added product, such as plastics or fuels. Thus, in CCC the CO2 is never sequestered, but instead converted into a non-GHG chemical form, reducing GHG emissions at an industrial point source. There are a number of existing companies pursuing CO2 Capture and Conversion utilizing a variety of chemical and biological technologies. Examples include:

- Green Plains Renewable Energy is currently growing algae on CO2 emitted by ethanol plants for use in advanced biofuel production, animal feed, and as biomass for energy production (Green Plains Renewable Energy, 2014).
- Skyonic Corporation will electrolytically convert CO2 into baking soda, and limestone for use in the construction industry (Skyonic Corporation, 2014).

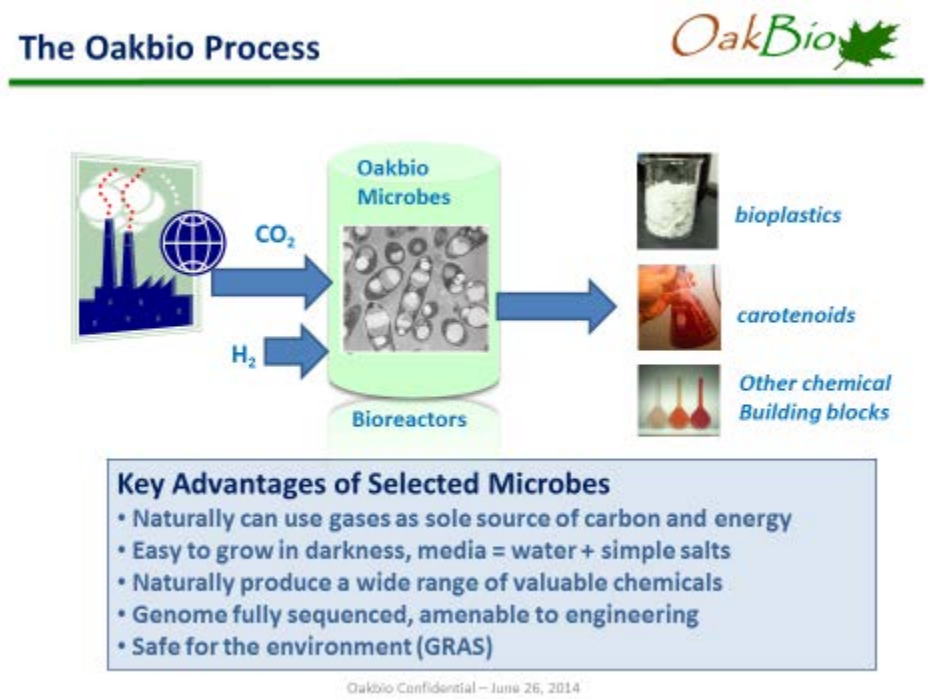
- Liquid Light will catalytically convert purified CO2 from industrial activity into value-added chemicals such as ethylene glycol (Liquid Light, 2014).

Utilizing CO2 as a feedstock in this way, rather than storing it in perpetuity, is economically advantageous. It eliminates CO2 storage and transportation costs from the industrial process, and provides an economic driving force by generating a marketable product.

1.3 The Oakbio CO2 Capture and Conversion (CCC) Process

Oakbio's proposed CCC process is illustrated below. At its heart are carefully selected microbes that can directly consume CO2 as their sole source of carbon and H2 as their sole source of energy.

Figure 1.3: Schematic of the Oakbio Process



Source: Oakbio internal document.

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.

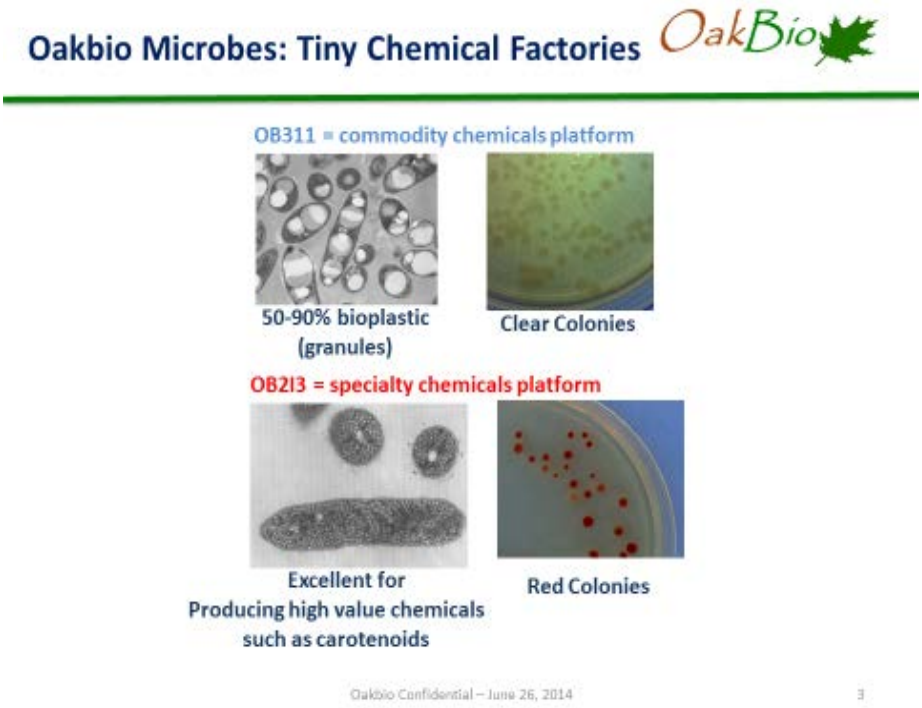
Carbon monoxide (CO) can also act as a possible energy source and as a carbon source, but CO generally works best in a mix with H2 and/or CO2 and/or O2, due to its toxicity to microbes (Volova, 2009). Oakbio's CO2 Capture and Conversion technology represents the first to use industrial CO2 from flue gas and H2 for chemoautotrophic production, converting CO2 to chemical value.

1.3.1 The Oakbio Microbes, OB311 and OB213

Oakbio selected two naturally-occurring chemoautotrophic microbial strains for further development into industrial platform organisms, named OB213 and OB311, based on early screening results obtained at the Lehigh field lab. 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.

The purpose of the work funded by the Energy Commission and reported in this document is to validate OB311 and OB213 as platform organisms for CO2 Capture and Conversion to chemical products. Some of the characteristics and advantages of strains OB311 and OB213 are shown in Figure 1.4.

Figure 1.4: 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.

OB311 was selected for its ability to store up to 90% of its dry cell weight as a biodegradable plastic called polyhydroxyalkanote (PHA). PHA is expected to accumulate in the two bacteria Oakbio has adapted to grow in cement gas. To assess overall chemical value, the company will measure the amount of PHA produced and also the amount of its primary constituent monomer, 3-hydroxybutyrate (3HB). This four-carbon (C4) monomer is obtained by breakdown of the PHA biopolymer. 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).

There are several reasons why PHAs are a desirable product for conversion of CO2 to chemical products. Microbes that can fix CO2 have been reported to produce PHAs up to 75% of biomass when grown on pure lab gases (Volova and Voinov, 2004; Volova, 2009). Also, demand for biobased plastics is growing. Both PHA and 3HB have the potential to grow into markets of many millions of tons annually, driving profitable capture of millions of tons of CO2 (Reportsnreports, 2014).

1.4 Unmet Needs in Carbon Capture - Conclusions

Increasing levels of carbon dioxide (CO2) emissions from human sources have been directly linked to climate change, which has resulted in increased frequency and intensity of severe weather events. Current methods of CO2 capture in industrial flue gases are costly, and captured CO2 needs to be disposed of, with underground sequestration potentially posing new environmental challenges.

New technologies for carbon capture from industrial flue gas are emerging, the most attractive of which involves converting the captured CO2 into commercial products that can not only recycle the carbon into consumer markets but also further reduce carbon emissions by replacing petroleum derived products. Oakbio has developed such a Carbon Capture and Conversion (CCC) technology. At the core of Oakbio's technology are microorganisms capable of removing CO2 from flue gas emissions and converting it into bioplastics, biofuels and other products.

CHAPTER 2:
Project Overview – Tasks and Methods

2.1 Project Purpose

Oakbio's objectives are to determine the technical and economic feasibility of a novel microbe-based process for capture and conversion of flue gas CO2 to bioplastics. Key quantitative indices of system performance will be measured including CO2 capture, microbial biomass production and bioplastics production. This final report, including a techno-economic model, will inform the cement industry and the CO2-emitting energy industry on the likely economic feasibility of the company's system at larger scale.

2.2 Key Tasks

- Establish On-Site Gas Fermentation Laboratory at Cement Plant
- Test Oakbio Microbes on Cement Flue Gas
    - Compare the performance of two cement gas-adapted bacterial strains, OB213 and OB311, at the cement plant with controls run in parallel using synthetic gas mixtures at the Sunnyvale Laboratory.
- Test Oakbio Microbes on Natural Gas and Coal Flue Gas
    - Compare the performance of cement gas-adapted microbes (OB213 & OB311) using gas from coal- and natural gas-fired power plants in California. These experiments are of necessity performed in the Sunnyvale laboratory.
- Assess Technology Development and Commercialization Path
    - Apply data from Tasks 2 and 3 to Oakbio's techno-economic model to describe the economic performance of its technology. From identification of the variables that most affect economic performance, Oakbio provide to the Energy Commission a techno-economic evaluation of feasibility of this CO2 capture and chemical conversion process.

2.3 Experimental Strategy

Oakbio pursued four goals in order to systematically compare OB311 and OB213's growth and CO2 capture performance on various flue gases and on lab gas:

- Establish on-site gas fermentation laboratory at Lehigh Southwest Cement Plant.
- Grow OB311 and OB213 on Cement Flue Gas.
- Measure CO2 concentration in flue gases before and after exposure to microbial cultures.
- Measure the bioplastic content of microbial cultures.

Establish On-Site Gas Fermentation Laboratory

- Obtain consistent access to unadulterated cement flue gases.
- Establish growth and analytics capabilities.

Grow Oakbio Microbes on Flue Gases

- Measure growth over time by optical density (OD).
- Weigh mature cultures that have been carefully dried to measure the total dry weight of biomass produced.

Measure CO2 Uptake from Flue Gases

- Observe change in gas concentration of gases pre- and post-culture using in-house analytics.
- Obtain third-party gas analysis of samples of flue gases pre- and post-culture by gas chromatography from the environmental sampling company Curtis & Tompkins.

Measure Bioplastic Content of Microbial Cultures

- Submit mature, dried culture samples for third-party Combustion Analysis by Elemental Analysis, Inc., of Lexington, Kentucky.
    - Combustion analysis accurately and precisely measures the amount of carbon present in a given sample. All carbon present in each submitted culture must have been fixed from carbon dioxide because each culture was grown with lab or cement gases as its sole source of carbon.
- Utilize the R-Biopharm AG D-3-hydroxybutyric acid (3HB) test kit to quantify the Bioplastic Content of biomass. This kit provides a fast, sensitive measurement of concentration of PHA bioplastics.

2.4 Tasks and Methods - Conclusions

This report describes the work done under a grant from the California Energy Commission to validate Oakbio's technology as a valuable CCC technology and as a broad bio-manufacturing platform for the profitable production of chemicals.

2.4.1 Establish On-Site Gas Fermentation Laboratory

Oakbio's main industrial collaborator on this grant was the Lehigh Southwest Cement Plant located in Cupertino, California. Lehigh is a subsidiary of the Heidelberg group, the second largest cement producer in the world.

2.4.2 Grow Oakbio Microbes on Flue Gases

For the present grant project, Oakbio selected two proprietary bacterial strains, named OB311 and OB213, which naturally produce polyhydroxyalkanoates (PHA), a well-studied class of biodegradable plastics. Lehigh provided Oakbio with access to an onsite field laboratory and

direct flue gas feed. In addition, PG&E's Gateway Generating Station in Antioch, CA, agreed to collaborate with Oakbio under this grant and provided access for flue gas collection. The Gateway Generating Station uses natural-gas combustion to produce electricity.

2.4.3 Measure CO2 Uptake from Flue Gases

Oakbio has adapted its microorganisms to consume CO2 directly from unadulterated industrial flue gas. They use this gas as their sole carbon source and molecular hydrogen (H2) as their sole energy source. Therefore, all carbon present in biomass and bioplastic was converted from CO2. These chemoautotrophic properties make them different from obligatory photoautotrophic microbes, which require light as a source of energy, and use photosynthesis to fix carbon.

Oakbio's selected microorganisms are safe for the environment (GRAS), naturally produce a broad range of chemicals, and have also been studied and sequenced, so that they represent a good platform for metabolic engineering. Finally, unlike photosynthetic microbes, these microbes may also be grown at high density in the dark, 24/7, in a fairly conventional fermenter, which provides a significant advantage for industrial process development and scale up.

2.4.4 Measure Bioplastic Content of Microbial Cultures

Oakbio has determined the bioplastic content of microbial cultures grown on flue gases as their sole source of carbon. This was done using accurate spectrophotometric methods, and enhanced by third-party combustion analysis results that determine the total amount of carbon present in a given sample.

2.4.5 Final Conclusions

Oakbio has demonstrated the potential of its microbes for CCC from industrial flue gases at the laboratory scale. The company accomplished this by developing collaborations with two industrial emitters located in the San Francisco Bay Area, building the required gas handling and analytical capabilities, and executing its research plan for this Energy Commission grant. The results of this research are presented in the following two chapters.

Chapter 3:
Testing the Oakbio Process on Cement Flue Gas

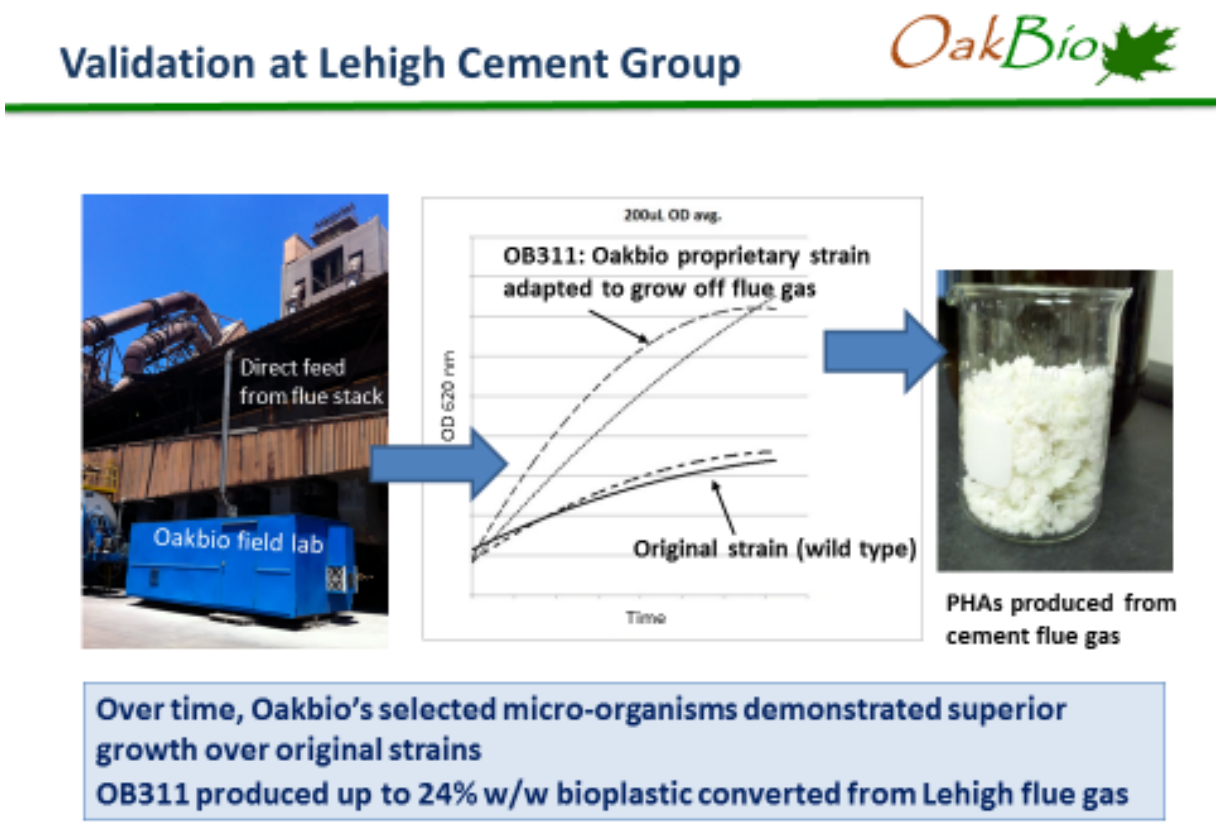
3.1 Primary Objectives

Establish on-site gas fermentation laboratory at the Lehigh Southwest Cement Plant. Compare the performance of two cement gas-adapted microbes, OB213 and OB311, at the cement plant with controls run in parallel using synthetic gas mixtures at the Sunnyvale Laboratory.

3.2 Oakbio Field Laboratory at the Lehigh Southwest Cement Plant

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 3.1). 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 the large blue container pictured below.

Figure 3.1: Oakbio Lehigh Field Laboratory Description and Background



Source: Oakbio internal document.

- The blue environmental testing enclosure was provided by the Lehigh plant.
    - The 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.

3.2.1 Growth and Analytical Capabilities at the Lehigh Field Lab

Figure 3.2: Cement Plant Field Lab Set-Up

H2 generators providing H2 for microbial growth at Oakbio's on-site laboratory at the Lehigh Southwest Cement Plant.

Oakbio's 1L microbial growth incubator. Fully loaded, this incubator can support up to six microbial cultures at one time.

Source: Oakbio internal document.

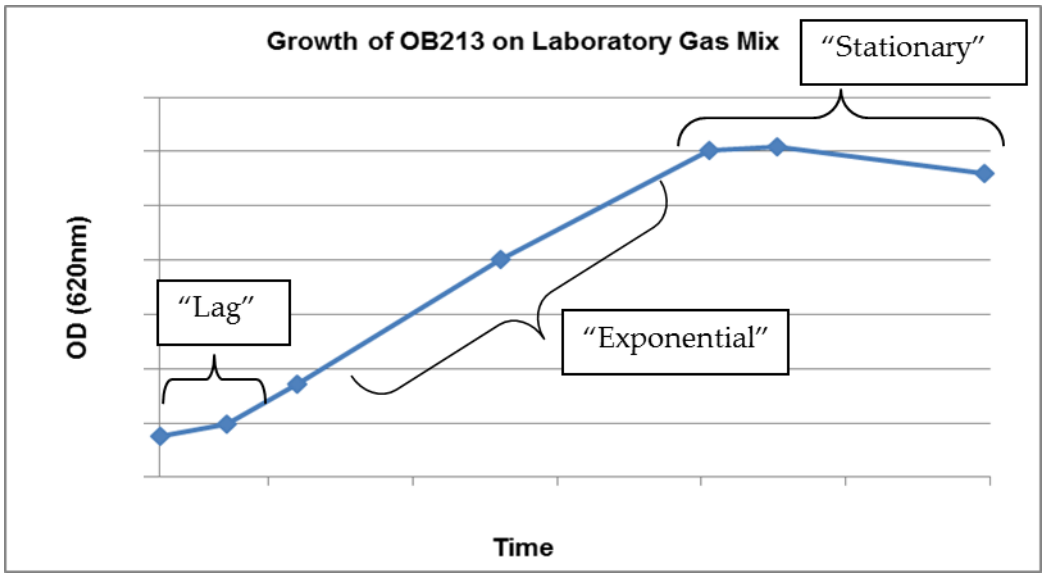
The Lehigh field lab also includes gas-handling equipment, and spectroscopic equipment such as plate readers for measuring microbial growth

3.3 Brief Introduction to Microbial Growth Curves

Throughout this document, microbial growth curves are presented as a first method for understanding the general health of a particular strain, its sensitivity to environmental factors such as toxins, and for comparing the general health or suitability of two or more strains in a given set of conditions. One such curve collected under ideal conditions is shown in Figure 3.3, below.

The growth curve above roughly approximates a standard S-curve. This standard curve consists of three phases, illustrated in the figure.

Figure 3.3: Representative Growth Curve of OB213 on Laboratory Gas Mix



Source: Oakbio internal data.

- Lag phase: the period early in growth of the microbial culture, during which the culture adjusts to its environment but does not multiply.
- Exponential phase: the well-known period of geometric multiplication that all microbial cultures with access to sufficient resources undergo.
    - When comparing microbes, the strain with the higher slope during exponential phase is said to have a higher growth rate, and will likely reach stationary phase sooner. Higher growth rates are desirable as chemicals of interest tend to be produced during "stationary" phase.
- Stationary phase: occurs once the microbe's population has reached its highest sustainable level given available resources. If the microbial population overshoots this "carrying capacity", there may be a slight decrease in population, as seen in the figure above.
    - The OD at which a microbial population enters stationary phase indicates that culture's final concentration at maturity. A higher stationary phase OD (higher concentration at maturity) is desirable because chemicals of interest tend to be produced during stationary phase.

This figure presents the growth of Oakbio's microbe OB213 under a set of idealized conditions:

- This OB213 culture is being grown on a calculated ideal mixture of pure gases, CO2:O2:H2:N2.
- There are no impurities, inhibitors or toxins present.
- This is referred to as "lab gas".
- In contrast, industrial flue gases often contain inhibitors such as carbon monoxide, and toxins such as SOx and NOx.

The OB213 culture's growth is quantified by measuring its "optical density at 620nm" (OD 620nm) at various timepoints over the course of the experiment.

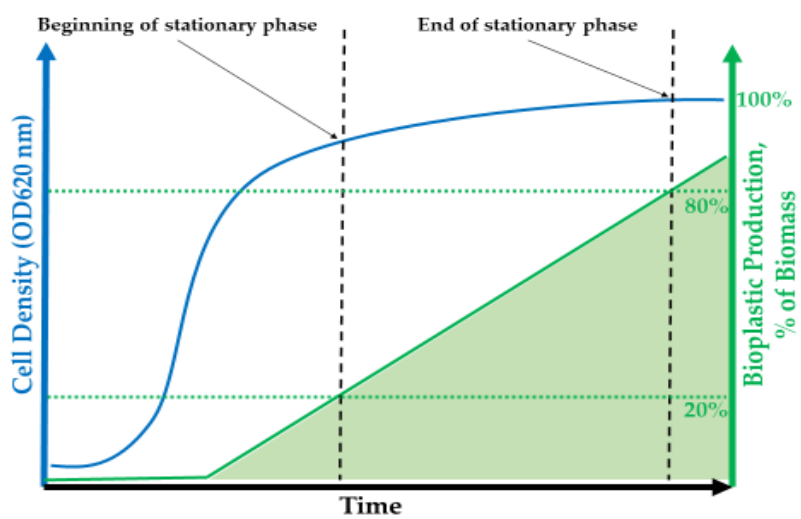
- OD 620nm is an indirect measurement of the culture's cell density.
- OD 620nm is the industry standard method for observing microbial growth due to its speed, ease of use, and accuracy.

The general objective of any microbial fermentation technology is to maximize growth rate and stationary-phase culture density. Such maximization enables highly reproducible processes with high productivity.

3.3.1 Microbial Production of Biodegradable Plastics for Energy and Carbon Storage

Below is an idealized diagram illustrating the relationship between growth stage and bioplastic production for the parent strain of OB311. Bacterial cultures are not expected to produce bioplastic during the lag and exponential phases; bioplastic is instead generated as the culture spends time in stationary phase. Therefore, cultures that have spent less time in stationary phase will have produced less bioplastic than cultures that have enters the stationary phase.

Figure 3.4: Schematic Diagram of Microbial Growth and Bioplastic Production



Source: Oakbio internal graphic.

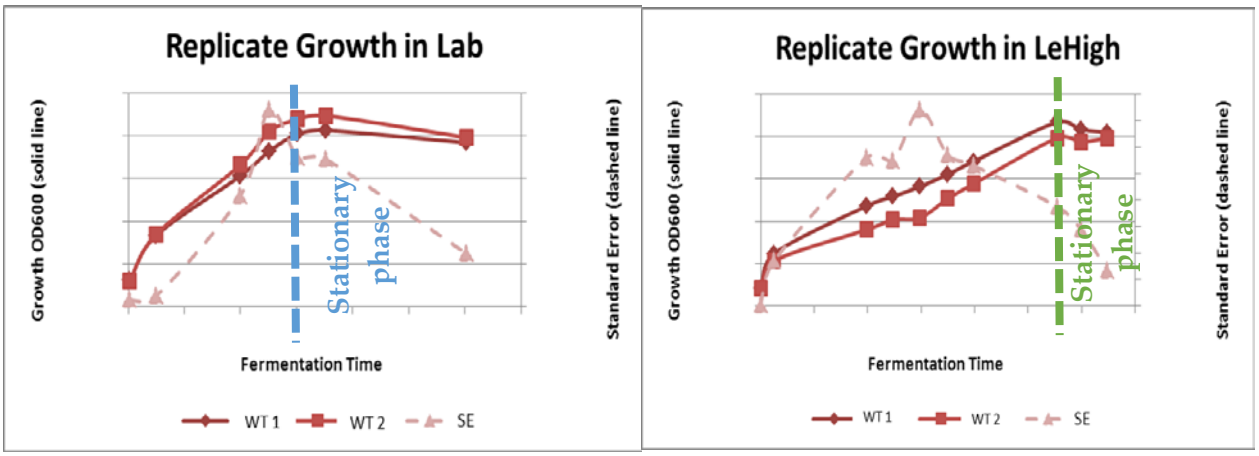
This behavior is controlled by the concentration of ammonium nitrate in the culture medium. Ammonium nitrate supplies the nitrogen that cells require to synthesize new proteins (they cannot use atmospheric nitrogen, N2); once the supply of ammonium nitrate in the culture medium runs out, the cells cannot divide and they are forced into stationary phase (Volova, 2009).

When provided with an abundance of CO2 and H2, and a dearth of nitrogen, the bacterium will begin to accumulate PHB as an energy and carbon storage strategy. This behavior is exploited for the bioplastic production phase of Oakbio's CO2 Capture and Conversion technology.

3.4 Grow Oakbio Microbes on Cement Flue Gas

Figure 3.5 (below) shows the results from two experiments conducted at the same time, comparing replicate growths of OB311's parent strain on a laboratory gas mixture and on Lehigh flue gases.

Figure 3.5: Comparison of OB311 Growth on Lab Gas and on Lehigh Flue Gas



Source: Oakbio internal data.

Identical pairs of experiments were examined under lab gas at the Sunnyvale Laboratory (Figure 3.5, left panel) and under cement flue gas at the Lehigh field lab (Figure 3.5, right panel) in order to quantitate experimental variation within the system ("SE" = "standard error").

The data show that the parent strain grown on lab gas reaches stationary phase after several days (dashed blue line in Figure 3.5, left), while the parent strain grown on Lehigh cement gas grows at approximately half that rate (x-axes are identical between graphs; dashed green line in Figure 3.5, right).

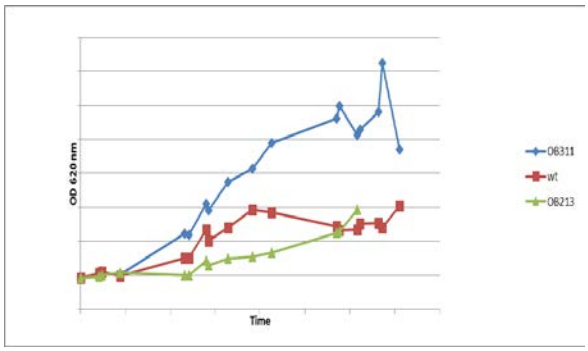
Growth Comparison of Oakbio Microbes and Parent Strain on Cement Flue Gas

Figure 3.6, below, compares the parent strain and Oakbio's flue gas-adapted microbes OB311 and OB213, all growing at the same time on Lehigh cement gas.

When grown under these conditions, OB311 demonstrates higher growth rate during exponential phase *and* reaches a higher cell density before entering stationary phase than its parent strain (wild-type). OB311 also reaches approximately twice the cell density of strain OB213. This is because OB213 is just entering the exponential phase of growth while OB311 is entering stationary phase.

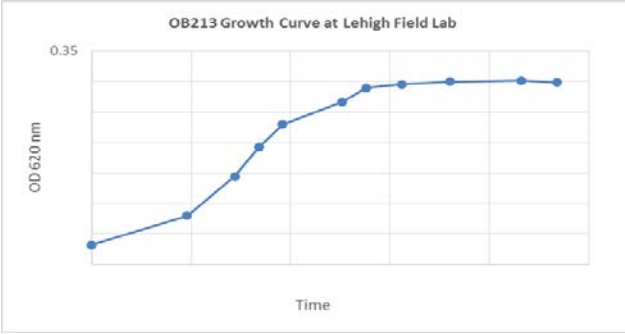
The right panel of Figure 3.6 illustrates OB213's full, slower growth pattern (~twice as long as OB311 for OB213 to reach stationary phase). OB213 growing on cement flue gas requires significantly more time to reach stationary phase compared to OB311.

Figure 3.6: Comparison of OB213, OB311 and WT Parent Strain Growth on Lehigh Flue Gas



OB311 (blue) grows faster and to higher density on cement flue gas than its parent strain (red) or OB213 (green).

Source: Oakbio internal data.



OB213 grown on cement flue gas (blue, above) requires nearly twice as long to reach the same cell density as OB311.

This experiment shows that OB311 was better-adapted to grow on cement flue gas relative to the WT parent strain, and that OB311 grows significantly faster than OB213. This improvement is the result of extensive strain engineering, which has probably introduced random beneficial mutations into its genome. These changes appear to have improved the ability of the bacterium to at least partially tolerate otherwise toxic flue gas components, such as NOx and Sox, that can be detrimental to microbial growth. Nevertheless, strain OB311 will still grow faster, and to higher cell density, when it is fed lab gas rather than industrial cement gas (see Figure 3.5).

It is unclear whether engineering of OB311 has better adapted it to chemoautotrophic growth in general, or made it more robust toward the presence of impurities and toxins in particular. Growth of OB311 on other sources of industrial flue gases, with presumably different impurity profiles, will provide more information on this question.

3.5 Measure CO2 Uptake from Cement Flue Gas

Oakbio measured the amount of CO2 taken up from cement flue gas by the OB311 and OB213 microbes in three ways:

- "One-Pass" experiment
- "Headspace Recycling" experiment
- Combustion analysis

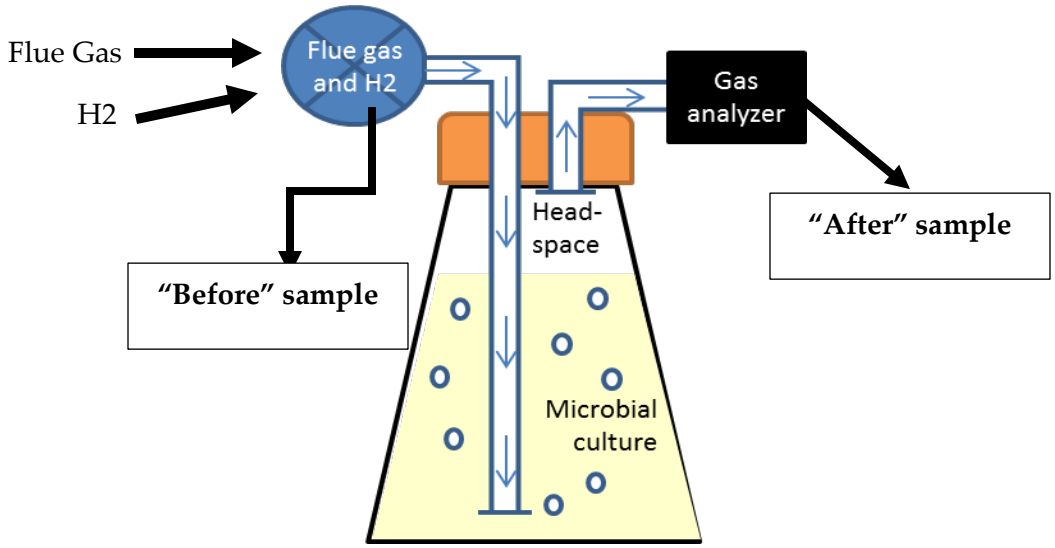
The "One-Pass" experiment measures the ability of a given culture to utilize CO2 from a flue gas stream at one point in time. The ability of that same culture to utilize CO2 throughout a given time window, during which the experimental system is sealed, is measured by the "Headspace Recycling" experiment. Finally, combustion analysis provides an accurate measure of the total mass of carbon contained in biomass, carbon which can only have come from CO2 contained in flue gas.

3.5.1 "One-Pass" and "Headspace Recycling" Experiments

Both of these experiments measure the decrease in %CO2 of a gas stream after passing through a bioreactor. The "One-Pass" experiment measures this decrease after one pass (Figure 3.7). "Headspace Recycling" measures this decrease over 20 minutes of recycling the gas stream through the fermenter, while the fermenter is sealed.

Samples were analyzed at Oakbio, and were also analyzed by Curtis & Tompkins Analytical Laboratories, for third-party confirmation of Oakbio's internal results.

Figure 3.7: Schematic Diagram of "One-Pass" CO2 Uptake Experiment



Source: Oakbio internal document.

"One-Pass" and "Headspace Recycling" Experiments with OB311

Table 3.1 compares representative results of Oakbio's in-house measurements of the decrease in concentration of CO2 in Lehigh cement gas after passage through a 1L fermenter containing OB311.

Table 3.1: Relative CO2 Uptake by OB311 from Cement Flue Gas

Culture (1L Volume)	%CO2 Decrease from Gas Analysis (Oakbio)	%CO2 Decrease from Gas Analysis (Third Party)
OB311	40%	43%

Source: Oakbio internal data and attached report from Curtis & Tompkins Environmental Testing Agency.

The OB311 culture consumed approximately 40% of the CO2 present in the Lehigh cement gas stream. This is confirmed by third-party gas analysis, which measured a 43% decrease in CO2 concentration.

Oakbio observed an *additional* 30% decrease in CO2 concentration of the headspace gas when the bioreactor system was sealed, and the gas recycled for 20 minutes. Results from "one-pass" and "headspace recycling" experiments on the same microbe may be added, because the "one-pass" experiment effectively measures the microbe's initial ability to consume CO2 from a given gas mixture, and the "headspace recycling" experiment measures the microbe's ability to consume CO2 from that same gas mixture after initial exposure, over time. Thus, the OB311 culture above was able to reduce the concentration of CO2 in a sample of cement flue gas by a total of 70% after 20 minutes. Note that these experiments were conducted with small volumes of cement flue gas, and 1L cultures.

This measurement of a 40% decrease in CO2 gas concentration in cement flue gas after one pass, and an additional 30% after 20 minutes of gas recycling, shows OB311's potential as a CCC platform microbe, since a large amount of CO2 can be captured in only one pass through solution. This result suggests that, given a large enough bioreactor, a significant percentage of CO2 contained in total plant emissions could be captured and converted into chemical products.

- Oakbio has observed in preliminary experiments that much higher relative CO2 uptake rates may be achieved if hydrogen is supplied in overabundance (data not shown).
- This result highlights the need for optimization of the H2:CO2 ratio, and of H2 and CO2 feed rates, in order to create a commercially viable process.

Based on these results, there are a number of additional bioprocess parameters that should be explored:

- Larger fermenter volumes.
    - The efficiency of microbial fermentation is affected by fermentation volume. While traditional, sugar-based fermentations tend to perform worse at larger volumes due to slow diffusion of dissolved feedstocks, Oakbio expects its process to perform equivalently, if not better, as fermentation volume is increased.
- Higher gas flow rates/larger gas amounts.
    - Achieving similar CO2 capture results at commercial scale will require innovative process and fermenter designs.

Minimum Required CO2 Concentration for Microbial Consumption

Oakbio conducted an additional experiment to examine the large decrease in CO2 concentration in cement flue gas that could be achieved by combining the "One-Pass" and the "Headspace Recycling".

By supplying H2 in large excess to an OB311 culture, along with CO2 starting at a lower concentration than before, Oakbio was able to determine that the CO2 concentration of a gas stream could be reduced from a few percent to approximately atmospheric levels. For comparison, OB311 cultures are normally grown at ~10% CO2.

This ability to utilize CO2 even at atmospheric concentrations will add resilience and flexibility to any industrial process utilizing OB311 for production of chemicals. In the event of equipment downtime, the culture can be stabilized and supported using atmospheric gas.

3.5.2 Carbon Content of Microbes Grown on Cement Flue Gas

The carbon content of a given organic sample may be determined by combustion analysis, in which the sample is combusted and the amount of resulting CO2 is carefully measured. All carbon in the resulting CO2 must have been present in the sample, which allows calculation of the mass of carbon in the sample.

<u>A note about these results</u>: the mass of assimilated CO2 reported here is larger than the total biomass generated. This is due to three facts:

1. The mass of a hydrogen atom is one-sixteenth of the mass of an oxygen atom.
2. Carbon atoms in biological molecules are more often bound to hydrogen atoms than they are to oxygen atoms. Determining this frequency would require a specific and detailed accounting of the relative populations of each biological molecule present in each culture, which is beyond the scope of this study.
3. Carbon atoms in CO2 are always bound to two oxygen atoms. Taking into account this difference in atomic weights, the masses reported here are entirely reasonable.

The collected results of Oakbio's carbon content determinations are presented in Table 3.2. These results are discussed in the following sections.

Table 3.2: CO2 Converted from Cement Flue Gas into Biomass

Culture (L)	wt% Carbon
parent strain (1L)	43%
OB213 (1L)	38%
OB311 (1L)	42%
OB311 lab growth (18L)	50%

Source: Oakbio internal data

Carbon Content of OB311 Grown on Cement Flue Gas

Strain OB311, when grown on Lehigh flue gas using Oakbio's standard protocols, contains a large percentage of carbon by weight. If allowed to grow for an additional three days under bioplastic-producing conditions, the weight% of carbon of the culture further increases, while the weight% of nitrogen decreases and the weight% of hydrogen stays roughly constant (data not shown).

Interestingly, when the parent strain and OB311 are both grown for an extended period of time on Lehigh cement gas, the carbon content of the cultures matches very well. This is not necessarily expected because OB311 captured and converted many times more CO2 (in absolute terms) than its parent strain. These results suggest that, at least up until early stationary phase, growth rate does not affect the %carbon content of the microbe. Carbon from CO2 is fixed into both cellular biomass as well as well as bioplastic.

- The carbon content of each culture measured here can only have come from CO2 originally emitted by the Lehigh cement plant. The cultures utilized for this experiment were never fed sugars or pure CO2.
- The parent strain shows higher atomic nitrogen levels than OB311, indicating that the parent has the carbon sequestered in protein, whereas OB311 has utilized it to manufacture non-nitrogen containing molecules, such as bioplastic.
- OB311 lab growth shows higher weight% carbon, similar weight% hydrogen, and lower weight% nitrogen
    - This result implies that the strain has produced a large amount of molecules that do not contain nitrogen, consistent with bioplastic production during stationary phase.
- These data will be combined with the culture dry weight discussed above to calculate the absolute amount of CO2 uptake.

This is precisely the reason that Oakbio created the flue gas-resistant strain OB311 in the first place, and it is an encouraging result. Oakbio intends to pursue this opportunity by testing OB311's growth on a variety of additional flue gases, starting with those from coal- and natural gas-fired power plants, discussed in Chapter 4.

Carbon Content of OB213 Grown on Cement Flue Gas

Strain OB213, grown on Lehigh flue gas using Oakbio's standard protocols, contains a substantial fraction of carbon by weight. This carbon content is ~5% lower than that of the OB311 parent strain and OB311 itself.

The ~5% lower carbon content of OB213 cultures as compared to OB311, along with OB213's lower total biomass generation, suggests that OB213 is a less-ideal commercial-scale carbon capture and utilization platform microbe than is OB311. However, OB213 is nevertheless potentially interesting for its ability to chemoautotrophically produce carotenoids and other high-value specialty products.

3.6 Measure Bioplastics Content of Microbial Cultures

Oakbio also characterized the bioplastic production abilities of its microbes grown on cement flue gas. Together with the measurements of CO2 uptake previously discussed, these data complete the proof of concept of Oakbio's microbial CCC process as being able to consume CO2 from unadulterated cement flue gas and convert it into products.

Bioplastic Content of OB311 Grown on Cement Flue Gas

Table 3.3: Bioplastic Content of OB311 Grown on Cement Flue Gas

Culture (1L Volume)	Bioplastic, % of Total Biomass	Time in Stationary Phase (days)
parent strain	12.3%	N/A
OB311	>24%	3-7

Source: Oakbio internal data

Table 3.4: Bioplastic Content of Parent Strain Grown on Lab Gas

Culture (from literature)	Bioplastic, % of Total Biomass	Time in Stationary Phase (days)
parent strain	70-75%	3-5

Source: Volova and Voinov, 2004; Volova, 2009

The data in Table 3.3 show a significant increase in bioplastic as a percent of total biomass when OB311 is grown on cement flue gas, as compared to its parent strain. The parent strain grown on lab gas may accumulate up to 75% of its dry cell weight as PHA (results from literature). This illustrates the potential for significant improvement of OB311's bioplastic productivity when growing on industrial flue gases.

As has already been discussed, OB311 grows significantly faster and to higher cell density on cement flue gases than its parent strain. Consequently, under these conditions, it is no surprise that the bioplastic content of OB311 is 2 times higher than the bioplastic content of the parent (as illustrated in the schematic diagram of bioplastic accumulation with growth in Figure 3.4).

It can be seen from Table 3.3 and Table 3.4 that OB311 is able to produce at least 24% of its culture dry weight as bioplastic if cultured in stationary phase on cement flue gas. This result can be compared to the 70% level of bioplastic production observed by Volova *et al*. in their wild-type strain when fed lab gas over a period of 4 days in a 1600L pilot-scale facility (Volova, 2009). Volova *et al*. obtained this higher bioplastic productivity over a shorter growth time due to several differences in experimental and bioreactor set-up:

- Volova's team fed their strain with lab gas in an effort to obtain the highest possible growth rates. Oakbio's objective is to utilize CO2 directly from flue gases containing actual impurities and inhibitors, therefore growth is expected to be slower.
- Volova's experimental system also included specialized gas-mixing and recycling capabilities.
    - As discussed before, Oakbio expects that improved control of CO2:H2 ratio as well as delivery rates for both gases will improve microbial growth rates and productivities in its strains.
- The work reported by Volova was conducted in a 1600L, specially-designed bioreactor.
    - As discussed before, Oakbio expects to achieve improved productivity at increased fermenter volume because of the gaseous nature of its feedstocks-- in contrast with sugar-based fermentations.

On a different note, one potential reason that the PHB content of OB311 grown on cement flue gas is significantly lower than the PHB content of OB311 grown on lab gas is the intermittency of Lehigh's gas supply (described in-depth in Section 3.7). Because PHB serves as OB311's storage molecule for energy and carbon, OB311 will consume it when adequate carbon or energy is not available from the environment. This may lead to OB311 consuming its stores of PHB during Lehigh's unanticipated shutdowns.

These results point out several next steps for development of Oakbio's CCC technology:

- Bioreactor and process engineering to optimize gas delivery and maximize gas utilization.
- Improving gas-mixing and recycling capabilities.
- Fully characterizing OB311's bioplastic productivity during stationary phase.
- Comparing growth of saturated OB311 cultures at Lehigh on cement flue gas, and at the Sunnyvale lab *on cement flue gas* stored in tanks and continuously supplied to the culture. This will isolate the effect of intermittency in industrial operations on OB311's PHB production.

Progress in each of these areas will improve overall process design and contribute to creating a more efficient and economical bioplastics production process.

Bioplastic Content Measurement for OB213

In contrast to OB311's performance in producing bioplastics, OB213 generated a much smaller amount of biobased polymer.

This result completes the picture of OB213 as an inferior microbial CCC platform to OB311. OB213 grows more slowly, to lower cell density, has lower internal carbon content, and produces much less bioplastic than OB311. For purposes of commercial carbon capture and conversion into bioplastic, OB311 is the clear winner between the two.

Bioplastic Produced per Culture Volume per Unit Time

Similar to the calculations above, each culture discussed here was grown at the Lehigh field lab, on Lehigh cement gas, in a 1L fermenter. The rate of bioplastic production/volume/time can be calculated from the culture's mass of bioplastic produced, culture volume, and culture growth time as specified in the Method Description for "Monitor Growth and Health of Cultures by Optical Density".

Cultures that had reached stationary phase were harvested as needed for further characterization. There is no reason that OB311 could not have spent more time in stationary phase growing on lab gas – the timing of the stationary phase timepoints are not biologically imposed.

Both the OB311 parent strain and strain OB213 produce PHA bioplastic at roughly 50-fold smaller rates than OB311 when grown on cement flue gas. This is expected, given the two strains' growth and biomass generation performance discussed above.

It is important to note that OB311's bioplastic productivity increased from the level observed with several days spent in stationary phase on cement flue gas to a much higher level after several additional days spent in stationary phase on lab gas. This suggests that increasing fermentation volume, along with increasing culture time in stationary phase will have a significant positive impact on bioplastic production. Note that Oakbio has twice attempted to grow OB311 at the Lehigh field lab on cement flue gas, in 18L bioreactors for a longer growth period. Unfortunately, both experiments failed due to unplanned cement plant shutdowns.

These results provide a good starting point for further carbon capture and conversion technology R&D. The observation that the rate of CO2 capture and conversion slows somewhat as cultures spend time in stationary phase is not unexpected and indicates the need for process optimization. Several avenues are available to improve the CO2 uptake abilities of Oakbio's microbes, including:

- Synthetic biology: for instance, engineering their RubisCO enzymes to improve the efficiency and rate of CO2 uptake.
- Process engineering: for instance, optimizing gas delivery to the bioreactor to supply the most nutritious gas composition for each stage of growth.
- Bioreactor optimization: for instance, maximization of mass-transfer between the gas and liquid phases, so that H2 is efficiently dissolved into water and available to the microbes.

Efforts in these areas form the backbone of Oakbio's scale-up and commercialization strategy.

3.7 Limitations of Microbial Growth on Cement Flue Gas

Two experts in chemoautotrophic microbes, Jian Yu of the University of Hawaii and F. Robert Tabita of The Ohio State University, have demonstrated that optimal conditions for chemoautotrophic growth of its microbes include maintaining a 1:1 ratio of CO2:O2 in the gas feed stream, and maintaining culture temperature between 30° and 35°C.

Oakbio's experience in growing its cultures on CO2 and O2 in the flue gas of the Lehigh cement factory over the past three years has been invaluable in identifying opportunities for process improvement.

Lehigh Flue Gas is Cooled to ~30°C

- Oakbio's field lab (discussed above) was originally an environmental testing station that sampled flue gases directly from the kiln exhaust system. This stream of kiln exhaust was cooled to ~30°C in order to protect the instruments within the testing station.
- The gas supply was therefore maintained at a consistent temperature appropriate for Oakbio's microbes.
- Cooling of flue gases to ambient temperature will be necessary at commercial scale. This represents a source of operational expenditure for the technology described here.

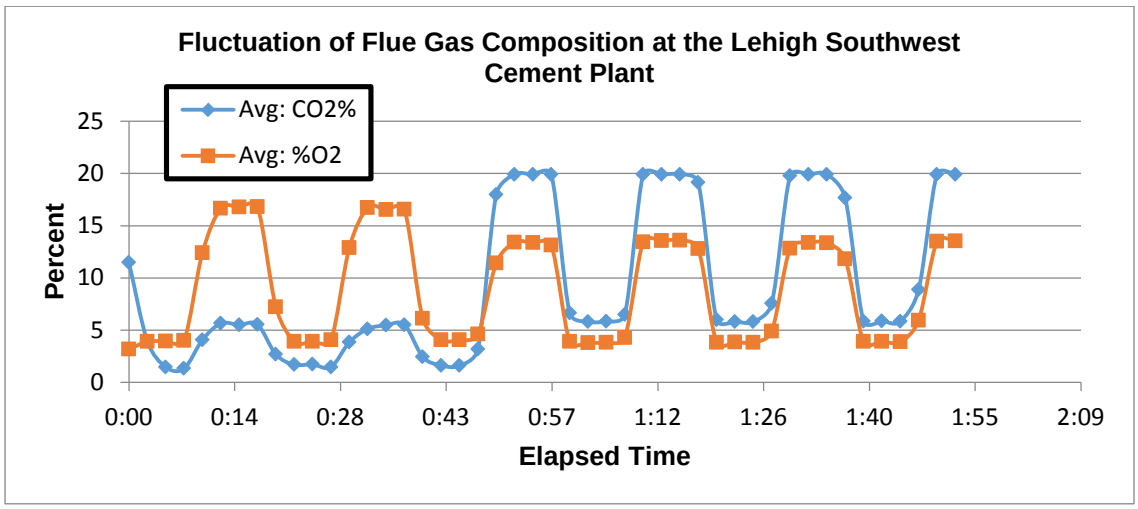
Flue Gas Composition Varies with Point Source in a Given Plant

Sampling directly from the kiln exhaust will provide flue gas of a different composition, including CO2:O2 ratio, than sampling at the top of the flue stack, or at other points in the cement manufacturing process.

Lehigh Flue Gas Does Not Provide CO2 and O2 in a Consistent Ratio

The ratio of CO2:O2 in flue gas will vary with the production cycle (startup vs. high productivity vs. shutdown), with the fuel used for combustion, and with other factors. An extreme case of this variance, observed on 4 December, 2013, is shown in Figure 3.8. These factors can cause significant changes in flue gas composition over a period of hours.

Figure 3.8: Fluctuation of Composition of Lehigh Flue Gas



Source: Oakbio internal data

- Over the course of two hours, the concentration of O2 in Lehigh flue gas cycled repeatedly from ~5% to ~15%; over the same period of time, the concentration of CO2 in Lehigh flue gas cycled from a low of ~2% to highs of 20%.
- Any culture exposed to such gas supplies will spend at least some of its growth period outside of the optimal conditions discussed above. This will reduce total biomass, CO2 uptake, and productivity.

Industrial Operations Experience Unplanned Downtime

- The Lehigh plant experienced ~20% downtime over the course of the Energy Commission grant, approximately one week out of five.
- Some downtime was scheduled, but much was unforeseeable.
    - Oakbio's microbes do not grow or generate product without a supply of CO2.
- This has also been the case with PG&E's Gateway power plant. The plant is spun up and spun down in response to increases and decreases in electricity demand, respectively. Activation or deactivation decisions are made within a period of 24 hours. This is not enough notice to operate a biologically-based industrial process.

Solutions

Oakbio has developed a tank system for storage and transport of flue gases in order to address these shortcomings (described in detail in Chapter 4). Oakbio can utilize this tank system to collect flue gases directly from an industrial point source, and compress and transport them to its Sunnyvale lab for use in growth and production experiments.

At commercial scale, there are a number of solutions to the limitations above. For example, one could:

- Design front end of process with storage tanks to hold gases in reserve, so that cultures can be fed during interruptions in gas flow.
- Provide additional sources of CO2 in order to smooth out variations in the CO2 content of flue gases.
- Mix atmosphere (containing 21% O2) as necessary to smooth out variations in the O2 content of flue gases.

Efficiently addressing these limitations will be an area of active research as Oakbio develops its CO2 Capture and Conversion technology for commercialization.

3.8 Testing the Oakbio Process on Cement Flue Gas - Conclusions

OB311 grows faster and to higher cell density than OB213 and its parent microbial strain on cement flue gas. This assessment was supported by culture dry weight measurements. Oakbio's adaptation of OB311 to cement flue gas conditions has created a microbe well-suited for industrial CCC. Faster growth means faster process cycle time. Higher cell density means higher productivity per unit volume. Shorter process cycle time and higher productivity per unit volume increase the efficiency and profitability of a fermentation process.

OB311 consumes ~40% of the CO2 present in cement flue gas after one pass through a fermenter, and an additional 30% after 20 minutes of headspace recycling. This result was confirmed by Curtis & Tompkins Environmental Testing Agency. The result shows that a large percentage of CO2 present in a cement flue gas stream can be captured in one pass through solution. With a large enough bioreactor, a significant percentage of total plant CO2 emissions could therefore be captured and converted to chemical products, especially when a head gas recycling system is installed. A gas recycling system will also smooth the observed variation in gas composition inherent to cement production process.

In OB311 cultures that were fed cement flue gas, PHA accounted for a minimum of 24% of total biomass after several days in stationary phase. Under more ideal conditions (lab gas, larger gas fermenter, longer fermentation time), PHA accumulated to 75% total dry biomass weight. A next step in process development should include full characterization of OB311's bioplastic production, including the maximum amount of time a culture may spend in stationary phase before process costs of continuing the batch culture outweigh the value of productivity gains.

OB311 was also shown to grow on CO2 from natural gas-fired power plant emissions at more than twice the rate it grows on cement flue gas. While naturally-occurring chemoautotrophic microbes grow well on optimized lab gas, the impurities and toxins (such as CO, SOx, NOx) present in Lehigh cement gas have a significant deleterious effect.

CHAPTER 4:
Testing the Oakbio Process with Natural Gas and Coal Flue Gas

4.1 Primary Objective

Determine how variation in flue gas composition affects Oakbio's OB311 and OB213 microbes, using gas from coal- and natural gas-fired power plants. Of necessity, these experiments were performed in the Sunnyvale laboratory. Table 4.1 illustrates the differences in composition of various flue gases.

Table 4.1: Comparison of Composition of Lab Gas and Multiple Industrial Flue Gases

	CO2 (%)	O2 (%)	H2O (%)	N (%)	CO (ppm)	Others (ppm)	H2 (%)
Oakbio Lab Gas	20	10		5			65
Cement Plant Gas	19	8	13	60		NOx SOx	
Natural Gas-Fired Power Plant Gas	9-11	2-3	15	73-74	200-300	NOx	
Coal-Fired Power Plant Gas	12	7	6	76-77	50	NOx H2S	

Source: Oakbio internal data and *testo Flue Gas Analysis in Industry* report (testo Corp., 2014)

Note that both coal- and natural gas-fired power plant flue gases have about half the CO2 content of cement flue gas as a percentage of total emissions. Additionally, power plant flue gases contain CO in measurable amounts (on the order of parts per million), and, in the case of coal, even contain hydrogen sulfide (H2S). Table 4.1 also shows that cement flue gas generally has a higher concentration of CO2 and of O2, when compared to natural gas-fired power plant gas. These contaminants create a significantly different environment for testing the CCC capabilities of Oakbio's microbes.

4.2 Experimental Strategy

Three separate categories of experiments were performed:

- Microbial Growth on Natural Gas-Fired Power Plant Flue Gas

Natural gas is commonly combusted to generate electricity, and the largest supplier of electricity in the San Francisco Bay Area is Pacific Gas & Electric (PG&E). Oakbio contacted PG&E in order to acquire natural gas-fired power plant flue gas, and PG&E graciously offered to support the company's work under the auspices of the Energy Commission grant. PG&E gave Oakbio access to their Gateway Generating Station in Antioch, CA, so that the company

could collect emissions directly from their flue stacks. Oakbio was able to sample flue gas from two separate locations at the Gateway plant over the course of three separate visits.

- Microbial Growth on Bituminous Coal Combustion Products

Nationwide, coal-fired power plants account for approximately 40% of all electricity generation, and emit millions of tons of CO2 annually. Oakbio was unable to identify a partner in Northern California, and so the company purchased bituminous coal, combusted it, and collected the resulting gases in order to test microbial growth.

- Variation of CO2:O2 Ratio

Oakbio varied the ratio of CO2:O2 in its lab gas to mimic CO2:O2 ratios in potentially important flue gas mixtures while eliminating the presence of impurities such as CO, SOx, and NOx. This experiment provides evidence to distinguish between the effects of impurities and the effects of variations in CO2:O2 ratio.

Oakbio used broadly the same experimental strategy here as was described in the previous chapter, with one primary difference. Instead of performing experiments at the Lehigh field lab on cement flue gases, flue gas from a natural gas-fired power plant was used.

The same three key experiments described in Chapter 3 were used:

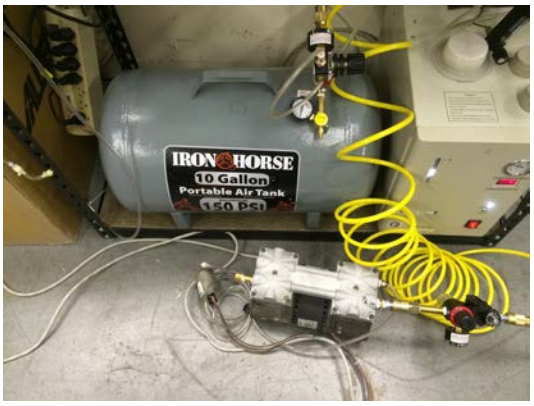
1. Monitor microbial growth.
2. Measure CO2 concentration in flue gases before and after exposure to microbial cultures.
3. Measure the chemical content of fully-grown microbial cultures.

In order to facilitate these experiments, Oakbio developed a tank system for collecting samples of flue gases from industrial sites that could then be safely and efficiently transported back to the Sunnyvale lab. This tank system was then connected to Oakbio's existing gas-handling equipment, and used as the CO2 source for microbial growth experiments. This portable system was used to collect samples of flue gases from combustion of natural gas and bituminous coal.

4.3 Construction of a System for Transporting Flue Gas Samples

Figure 4.1, right shows the simplest configuration of the tank system that Oakbio created for collection and transport of flue gas samples. Here, one 10-gallon portable tank is connected to a portable compressor that is rigged with a pressure cutoff switch set to 125 psi. The source gases that Oakbio accessed with this system were generally hotter than 100°F, and therefore needed to be cooled to ambient temperature before entering the storage tanks. A 3-foot steel environmental sampling rod was therefore attached to approximately ten feet of copper tubing, and this was used to feed flue gas to the compressor. The flue gas cooled to ambient temperature as it traversed the coiled copper. The compression system can be seen in operation on-site at PG&E's Gateway Station in Figure 4.1, right.

Figure 4.1: Compression System for Collection and Transportation of Flue Gas Samples



Compression system for collection and transportation of flue gas samples.

Source: Oakbio internal photograph.

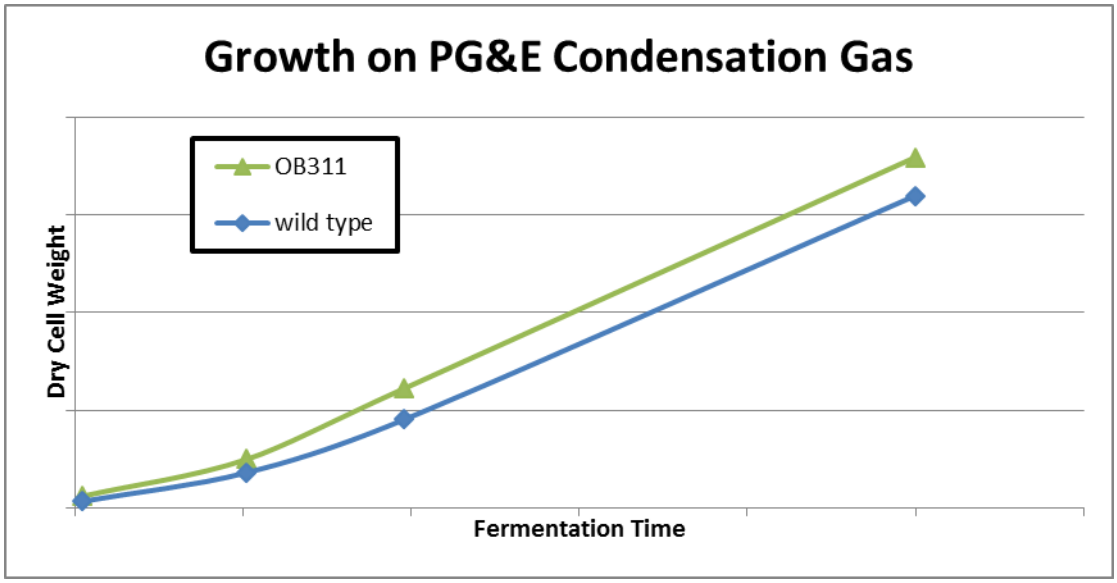
Compression system in operation on-site at PG&E's Gateway Power Station, Antioch, CA.

4.4 Results for Natural Gas Combustion

4.4.1 Results for OB311

Monitor Growth of Cultures

Figure 4.2: Growth of OB311 and Its Parent Strain on Natural Gas Combustion Flue Gas



Source: Oakbio internal data

Oakbio was able to collect several days of growth performance data at once for OB311 growing on natural gas combustion products due to the capacity of the tank system previously described. Figure 4.2 shows that, over the course of these growth experiments, OB311 and its parent strain performed similarly, with OB311 perhaps growing ~10% faster. OB311 growing on

cement flue gas required a much longer time to reach this same dry cell weight. There is little difference between OB311 and its parent strain when grown for several days on PG&E gas. This stands in marked contrast to the difference between OB311 and its parent strain grown on cement flue gas.

These results suggest that cement flue gas contains more potentially toxic impurities than does PG&E gas (higher levels of NOx and SOx, perhaps, or another contaminant not mentioned in Table 4.1. The parent strain of OB311 grew well on PG&E gas, which contains less CO2 than cement gas, strongly suggesting that there is a significant amount of detrimental chemicals present in the cement gas; otherwise, the parent strain would be expected to grow better on the higher-CO2 gas.

This also suggests that Oakbio's engineering of OB311 to grow on cement flue gas centered on resistance to those impurities. If Oakbio's adaptation of OB311 had instead already improved the microbe's inherent chemoautotrophic metabolism, it would have grown significantly faster than its parent strain in the cleaner PG&E gas, unless it was limited by CO2 supply. Once again, this is likely not to be the case, given OB311's equivalent performance on the higher concentration of CO2 in cement gas.

Measure CO2 Uptake by Microbial Cultures

The "One-Pass" experiment was performed as described in Chapter 3, except natural gas combustion flue gas was used instead of cement flue gas.

Table 4.2: Relative OB311 CO2 Uptake from Natural Gas Combustion Products

Culture (1L Volume)	%CO2 Decrease from Gas Analysis (Oakbio)	%CO2 Decrease from Gas Analysis (Third Party)
OB311	31%	36%

Source: Oakbio internal data and attached report from Curtis & Tompkins Environmental Testing Agency.

OB311 took up 36% of the CO2 present in PG&E gas in the context of the "one-pass" CO2 uptake experiment, as measured by the Curtis & Tompkins Environmental Testing Agency (Table 4.2). Oakbio's internal results report 31% uptake of CO2 present in PG&E gas. This result is notable because OB311 took up 43% of the CO2 from cement flue gas, as shown in Table 3.1.

As discussed previously, CO2 uptake results from the Curtis & Tompkins Environmental Testing Agency are expected to be more precise and accurate due to their specialized equipment. In particular, access to a mass spectrometer linked to a gas chromatograph gives Curtis & Tompkins the ability to positively identify and quantify each component present in a gas sample, as opposed to Oakbio's ability to monitor relative concentrations of CO2 and O2.

The finding that OB311 took up less CO2 from PG&E gas experiment than it took up from cement flue gas raises a number of questions. First, the cement flue gas experiment was performed on a culture that had reached stationary phase after an extended period of growth,

while the PG&E gas was, of necessity, performed on a culture still in exponential phase with only several days of growth. Therefore, the difference in CO2 uptake rate might be attributable to differences in the density of the microbial population.

On the other hand, natural gas combustion products generally contain ~50% of the CO2 contained in cement flue gas – perhaps the lower concentration of CO2 in PG&E gas reduced OB311's overall ability to consume CO2. This result points to the next step of identifying the optimal ratios of gas components for OB311's growth (particularly, the ratio of CO2:O2:H2). This trifactorial optimization should be carried out in the presence and the absence of the traditional impurities in combustion products: CO, SOx, and NOx. This data set will unambiguously characterize the optimum gas composition for OB311 at a given level of impurities, enabling us to predict OB311's growth behavior and production capabilities given the emission profiles of specific industries.

Note that CO2 may be obtained industrially from a number of sources, including MEA-CC, and used to supplement flue gases if necessary. O2 is essentially free, as it comprises 21% of the atmosphere. Blending these two sources of gas, along with H2 as the energy supply for OB311, into an industrial flue gas will create optimal conditions for microbial CCC.

Measure Bioplastic Content of Biomass

OB311 generated a small but significant percentage of its total biomass as PHB bioplastic after just a few days of growth on PG&E gas. This is a promising result when compared with OB311's bioplastic-generating performance from cement flue gas, as the natural gas cultures grew for a significantly shorter period of time. OB311's parent strain performed more than twice as well in terms of bioplastic generation on PG&E gas as it did on cement flue gas.

These results are consistent with the growth data that suggest that PG&E gas is less deleterious to growth for the WT parent than is cement flue gas. These results also suggest that several days of growth on PG&E gas enough time for OB311 to reach stationary phase and begin accumulating PHB bioplastic.

This result is significant because it emphasizes that, even though OB311 is adapted to grow on cement flue gas, impurities such as CO, SOx, and NOx have a significant detrimental effect on its growth, and therefore they lengthen production time. Consequently, further diminishing the growth-retarding effect of these impurities will be a critical component of Oakbio's technology development.

4.4.2 Results for OB213

Initial results suggest that PG&E gas has minimal, if any, deleterious effect on OB213 growth as compared to lab gas. OB213 cultures that were grown into exponential phase and then switched to natural gas combustion products (plus hydrogen) for ten hours showed no significant decrease in exponential growth rate. Confirmatory experiments are underway (data not shown).

Taken together with the observation of OB311's growth behavior on PG&E gas, this result suggests that natural gas combustion products do not contain as many impurities (such as CO, SOx, or NOx) as do coal or cement manufacturing gases. As a result, OB213 might conceivably

form the heart of a CCC process fed with natural gas combustion products; this is not the case for a CCC process fed with cement flue gas.

"One-Pass" Experiment with OB213 on Natural Gas Combustion Flue Gases

The results of the "One-Pass" experiment show that OB213 took up more CO2 from PG&E gas, as a percent of total, than did OB311. This is also the same relative uptake that was observed for OB213 from cement flue gas. As discussed above, Oakbio believes that the %CO2 uptake reported by Curtis & Tompkins is a more accurate and precise measurement.

In the one-pass experiment, OB213 was grown on lab gas until it reached stationary phase, then equilibrated with PG&E gas for two hours before measurements were taken. Therefore, OB213 had the opportunity to reach its maximum cell density attainable via chemoautotrophic growth. This result, consistent with that shown above, suggests that OB213 has the ability to consume CO2 at a high rate. Of course, OB213 does not grow well on flue gases containing levels of impurities higher than that seen in natural gas combustion. Together these results point to OB213's potential utility for CCC on lab-type gas compositions. That is, gases that are primarily mixtures of CO2, O2 and H2.

Based on the raw data, OB213 appears to consume CO2 faster than OB311. This is likely an artifact of data analysis for the one-pass experiment. OB213 most likely takes up *less* CO2 than OB311, not more.

Specifically, one-pass data are analyzed by assuming that N2 is neither consumed nor produced during the experiment – N2 serves as an internal standard against which other gas populations are normalized. A literature search reveals that the parent strain of OB213 is in fact able to consume N2 gas as part of its normal metabolism (Masepohl and Hallenbeck, 2010). If the amount of an internal standard assumed to be constant actually decreases, this will manifest as an increase in amount of all other chemical species, which is what is observed for the case of OB213.

As with OB311, the next step for further exploration of OB213's potential is conduct a trifactorial optimization, in the presence and the absence of inhibitors, in order to identify industrial emissions to which OB213 might best be suited.

4.5 Results for Coal Combustion

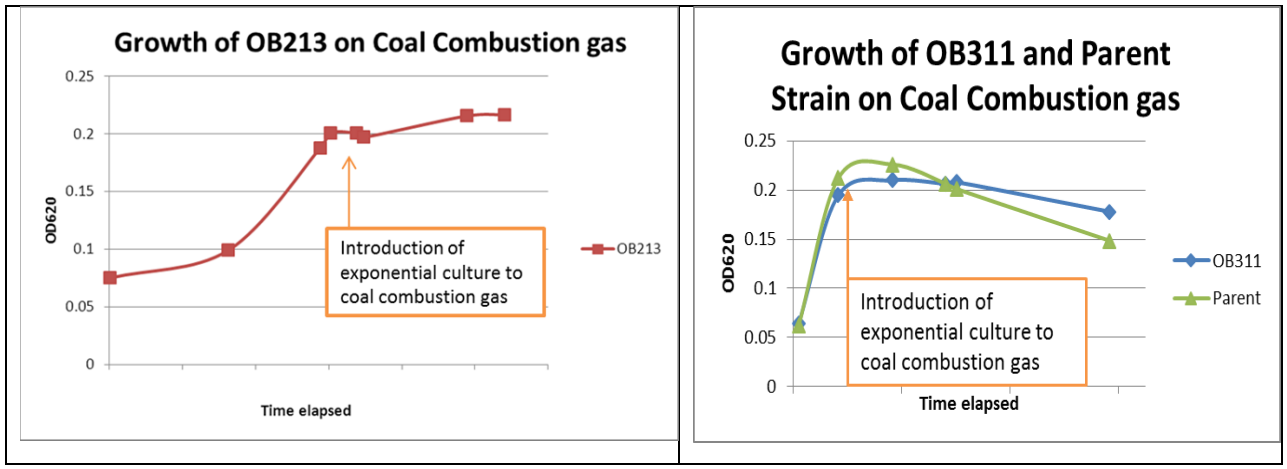
Monitor Growth of Cultures by Optical Density

Bituminous coal was combusted on-site at Oakbio's Sunnyvale lab, and the gaseous combustion products were collected using the tank system described earlier. At the same time, OB213 and the parent strain of OB311 were each grown for 48 hours on lab gas. Once in exponential phase, each culture was switched from growing on lab gas to coal combustion gas. Growth was then observed for a subsequent period of several days.

Figure 4.3 shows the growth curves of OB213, OB311 and the parent strain of OB311 grown initially on lab gas, and subsequent switched to coal combustion products. OB213, OB311 and

the parent strain were all observed to effectively stop growing after the switch from lab gas to coal combustion gas. This experiment has been repeated, with similar results.

Figure 4.3: Growth of Oakbio Microbes on Coal Combustion Products



Source: Oakbio internal data

It is clear from these graphs that one or more chemical species present in the bituminous coal combustion gas have a significant deleterious effect on all three microbes. However, this result does not rule out the possibility of developing a CCC process supplied by a coal-fired power plant. This is because Oakbio combusted bituminous coal, collected the gases without any treatment, and utilized those gases for the above experiments. In contrast, a coal-fired power plant does extensive post-combustion processing of its flue gases to reduce the concentrations of chemicals such as CO, SOx, NOx, and hydrogen sulfide.

While raw coal combustion gas appears to be quite deleterious to Oakbio's microbes, OB213 and/or OB311 may be able to utilize CO2 from this source after US Environmental Protection Agency-mandated processing of the gas stream and before final emission. Obtaining flue gas samples from an operating coal-fired power plant, perhaps in Southern California, is therefore a priority effort, so that this hypothesis can be tested.

4.6 Testing with Natural Gas and Coal Flue Gas - Conclusions

OB311 grows roughly twice as fast on PG&E flue gas as it grows on cement flue gas. When fed PG&E flue gas as a carbon source, OB311 and its parent strain grow similarly, unlike on cement flue gas. This result suggests that emissions from natural gas-fired power plants contain fewer toxins or contaminants than do emissions from cement manufacturing. The percentage of CO2 taken up by OB311 and OB213 from PG&E gas is broadly equivalent to the corresponding percentage taken up from cement flue gas. This result suggests that the inhibitors present in cement flue gas affect microbial growth, but not necessarily the microbial CO2 uptake pathway.

CHAPTER 5:
Technology Development and Commercialization Path

5.1 Primary Objective

Apply the results discussed in Chapters 3 and 4 to Oakbio's techno-economic model to describe the economic performance of the technology.

5.2 Process Development and Scale-up

Oakbio has created a technoeconomic model of its projected commercial-scale CCC process. The purpose of this model is to quantify the process' potential to profitably reduce GHG emissions using reasonable, benchmarked assumptions for the baseline scenario. This model, its assumptions, and the baseline scenario are analyzed in detail in Section 5.5.

Three of the key variables in this model represent features of Oakbio's technology that are under active, internal development. They are presented below, along with their values for the baseline scenario:

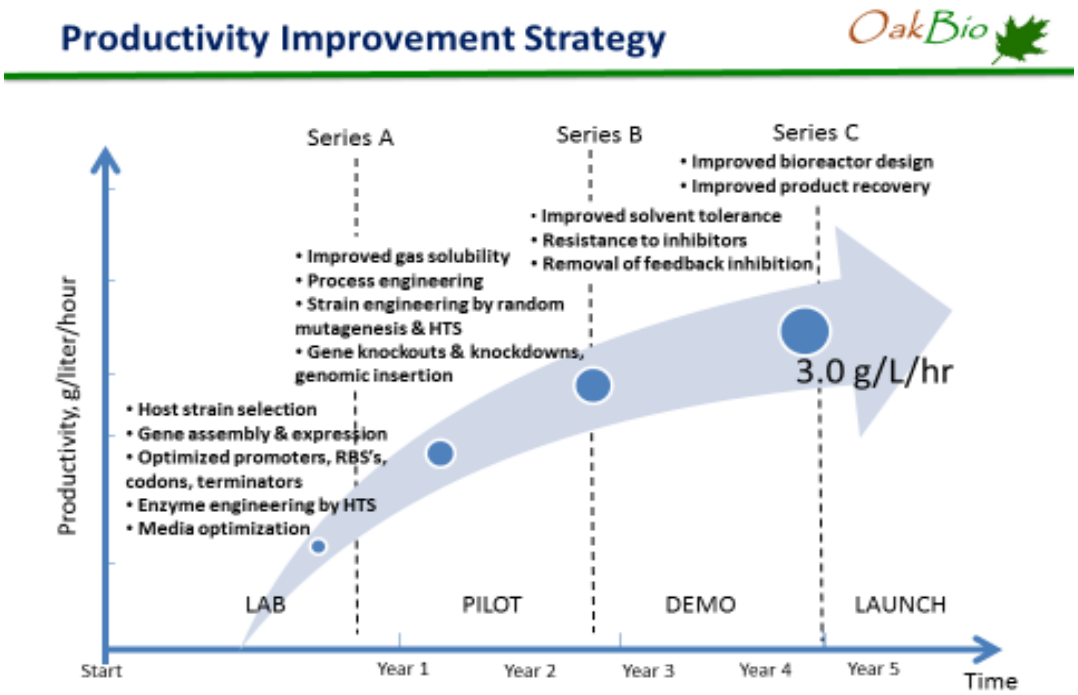
- Biomass generated per hour (3 g/L/hr)
- Batch duration (4 days)
- PHA as a percentage of biomass (70%)

These three variables each represent distinct axes of performance for Oakbio's microbe OB311. Note that a significant reduction in batch duration and biomass generation per batch will be required to develop this technology from its current proof-of-concept status to meet the baseline CCC scenario given above. The next section discusses how Oakbio will develop its technology to deliver this level of process improvement.

5.2.1 Oakbio Process Development Plan

Oakbio will simultaneously pursue three distinct technology development strategies to improve OB311's CCC process performance. Each of these strategies may improve OB311's performance in each of the three categories discussed above (Volova 2009), which will maximize Oakbio's chances of developing a profitable CCC process. It should be noted that the laboratory-based strain and fermentation improvements will complement the work performed at the pilot and demonstration plants, 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. A general scheme for productivity improvement over time is shown in Figure 5.1.

Figure 5.1: Microbial Productivity Improvement Strategy



Source: Oakbio internal document

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 how to enhance the inflow of carbon and energy to the bioplastic production apparatus and decrease consumption of the product:
- Increase activation of the PHA synthase by enzyme engineering and random mutagenesis (Pfeiffer and Jendrossek, 2014).
- Double the production of PHA by expressing the gene for NAD kinase in OB311 to increase the cellular supply of NADP, which is ultimately needed for CO2 fixation (see Li *et al*., 2009).
- Express additional enzymes to increase the production of acetyl-CoA (an essential precursor) and thereby increase PHA accumulation (shown to provide a 16-fold improvement in yeast; Kocharin *et al*., 2012).
- Reduce degradation of PHAs up to 4-fold by inactivating the gene for PHA depolymerase (*phaZ1*) (Povolo *et al*., 2010).Eliminate factors that limit the growth rate of OB311 on flue gas. In particular, it will be important to continue to enhance the

tolerance of strain OB311 to toxic flue gas components. These improvements will lower the production cost by accelerating the growth rate, thereby reducing the process cycle time.

- Examples of industrial strain improvement programs that have significantly increased product yields and reduced costs:
- 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, it is anticipated 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) In much the same way, Oakbio's bioplastics platform will help to guide the development of a related biofuels platform.

Media and Feedstock Development

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

Objective: optimize the composition of the OB311 growth medium, as well as the gas feedstock mixtures.

- Proper control of the composition of the growth medium is crucial to bioplastics fermentation because the metabolic pathways that generate PHA are activated by certain nutrient limitations (e.g., nitrogen and/or phosphorus; Volova, 2009).
- Volova (2009) has shown that the growth rate of cultures can be maximized by optimizing the ratio of the component gases in the feedstock mixture. Oakbio will therefore adjust the molar ratio of critical gases in the feedstock mixture (i.e., CO2, H2, O2) to provide the highest growth rate and PHA production rate for this specific

process. Oakbio can use the published information and procedures to begin to optimize the feeding strategy for the company's chosen product.

- Oakbio will seek to improve the availability of energy and carbon to the bacteria by increasing the solubility of CO2 and H2 in the aqueous growth medium (Volova, 2009). For example, it is well known that the solubility of gases in water decreases as the temperature increases. It will therefore be important to optimize the growth temperature of the fermentation (i.e., the best temperature for bacterial metabolism) with the temperature-solubility profile of the feedstock gases. Using cost analysis and design-of-experiments to substitute cheaper and more effective media components (*e.g.*, nitrogen sources; Wendell *et al*., 2014).

Process Design

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

- Improvements due to scale-up: Volova has shown that increasing the culture volume to the pilot scale or greater increases the growth rate, due to improved mass transfer of the feedstock gases (Volova, 2009). It was observed that this phenomenon in Oakbio's research when the culture volume was scaled-up from 1-L to 20-L. Oakbio expects to obtain higher yields as the scale of the PHB process increases.

- 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). These methods will be applied to the systematic improvement of OB311 performance.

- Fermenter design: There has been considerable investment over the past 30 years in improving the efficiency of microbial gas fermentations. For example, there are various vertical-loop fermenter configurations designed by NorFerm A/S and others that vastly improve the delivery of gas to the microbes and can operate at the 10,000 metric tonnes per annum level (for a review see, *e.g.*, Faust and Sittig, 2005, Al Tawfeel *et al*., 2012). Calysta, Inc. (a methane-to-biofuels company in Menlo Park, CA) has reported "8-fold improved performance over traditional fermentation technologies" using a high-mass transfer bioreactor specifically designed for gas fermentation and originally developed by BioProtein A/S of Stavanger, Norway (Calysta website, 2014). Coskata, Inc. has also developed an effective large-scale CO-based fermentation system that uses chains of hollow-fiber membrane bioreactors to improve gas delivery to the growth solution (Michaels *et al*., 1984; Hickey *et al*., 2008). By incorporating these process and bioreactor design improvements in the system, researchers expect to see increased biomass generation and decreased batch duration because optimized fermentation systems will deliver precisely the required nutrients, minerals, and gases in the optimum amount for each stage of microbial growth.

- 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. Oakbio will seek to incorporate these procedures into their process design.
- Improvements in downstream processing: Current PHA extraction methods comprise various steps, such as: concentrating the culture to cell paste using vacuum evaporation and centrifugation, extracting the polymer (using turbine-type stirrer, settling tanks, and a sedimenter), and drying the product (Volova, 2009). Improved methods for polymer extraction using high temperature and hydrogen peroxide to lyse the cells (De Rooy *et al*., 2007) will be investigated, as these may help to reduce the amount of organic solvent needed to separate the PHA from the cell debris.

A summary of some of these potential technical hurdles, as well as the technology and methods that might be employed to overcome them is shown in Table 5.1.

Table 5.1: Potential Technical Hurdles and Proposed Solutions

Technical Hurdle/Risk	Proposed Solution
Bacterial host strain is not optimal for producing PHA from flue gas.	Evaluate multiple strains for their suitability and select the best performers (already done).
Native enzymes in the host strain are not optimal for producing PHA at industrial scale.	Evaluate alternative (heterologous) enzyme genes based on their activity and express them in the desired host. Engineer enhanced specific activity in the enzymes.
Expression of enzyme genes in the PHA synthesis pathway is not sufficient for the desired level of production.	Engineer promoters, ribosome binding sites and terminators to improve expression; optimize codon usage for the desired host strain.
Solubility of feedstock gases in aqueous solution is insufficient to sustain high-productivity of fermentation.	Improve solubility by engineering mass transfer; employ emulsions to improve solubility.
Overall productivity of fermentation is low.	Improve productivity by standard process engineering techniques (medium optimization, fed-batch vs. continuous, improved gas feeding, better process control).
Productivity is low due to misdirection of carbon flow within the host bacterium.	Improve productivity by employing targeted gene knockouts and knockdowns, as well as global random mutagenesis.
Production is limited due to feedback inhibition of enzymes at high product or high substrate concentration.	Engineer enzymes to remove product and/or substrate inhibition.
Bacterial growth/productivity is reduced by toxic products generated during fermentation.	Devise methods to remove inhibitors from the fermentation broth or engineer key enzymes in the bacterium to resist inhibitors.
Bioreactor is inefficient for large-scale production.	Improve bioreactor design (tank geometry and gas/solution mixing).
Product recovery is inefficient/expensive.	Develop alternative methods for PHA extraction and purification (improved solvent extraction, separation from biomass, and product recovery).

Source: Oakbio internal document

Summary

Simultaneously pursuing each of these three technology development strategies can reasonably be expected to generate a CCC performance, based on peer-reviewed studies and the published materials of comparable companies. This compares favorably with the fold improvement required to meet the baseline modeling scenario.

5.3 Oakbio Research and Development Timeline

The general timeline of Oakbio's technology development plan, incorporating the three strategies discussed above, is shown in Figure 5.2. 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.

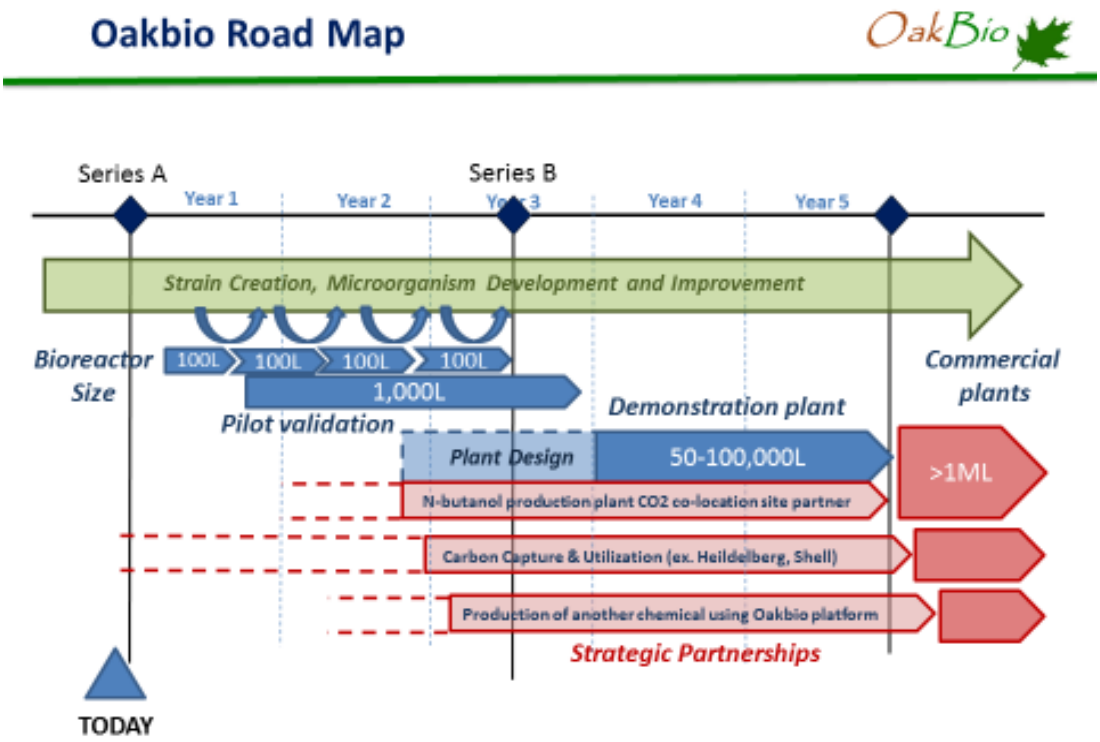
Oakbio envisions a 3-4 year technology development process that will scale-up its technology from the laboratory through a successful commercial demonstration. This four-year development period will encompass two major process scales defined by the fermentation capacity studied in each stage:

1. Pilot scale 1,000L-5,000L
2. Demonstration scale 50,000L-100,000L

The first two years of technology development will occur primarily at lab (100L) and pilot scales. 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.

Figure 5.2: Development of Oakbio's CCC System for Commercial Deployment



Source: Oakbio internal document

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 tonnes of CO2 and convert it into profitable petroleum replacements (see model discussion below).

5.4 Projected Performance at Commercial Scale

5.4.1 Techno-Economic Model

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 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 can be designed different ways depending on the goal(s) Oakbio and its industrial partners want to achieve or depending on the major constraints of the project. The selected starting point then drives the flow of calculations. For instance, the model could be designed based on achieving a certain level of carbon capture or design the model based on achieving the production of a certain quantity of product. Oakbio selected the quantity of CO2 released by the industrial partner as the starting point of calculations, as it represents the maximum amount of carbon to capture. Oakbio also proposed using the cost/profit of capturing CO2 (in $/metric tonne CO2 captured) as the economic measurement of the success of Oakbio CCC technology. This measure is used frequently in specialized field literature, albeit with different methods of calculations.

5.4.2 Representative Case study at Commercial Scale

The overall objective is to determine the value of Oakbio CCC technology at a representative CO2-emitting site at commercial scale. The Lehigh cement plant located in Cupertino, CA, was used as a representative industrial CO2 emitter who might be interested in setting up Oakbio's commercial unit to abate their carbon emissions. Lehigh released 914,000 t CO2 in 2012 (California Air Resources Board, 2013).

The model assumes that at the time of implementation, Oakbio technology has been fully developed to scale. R&D costs are considered sunk cost and are not included in the economic analysis. The model evaluates the system performance independently of who is operating it, whether it is Oakbio, Lehigh, a JV between the two companies, or a third party. The entity builds an Oakbio CCC commercial size plant on site at Lehigh cement, operates it and sells the resulting product(s).

Oakbio used a 10 year period for the techno-economic evaluation, with a terminal value added for the business at the end the period. Capital Cost expenditure for plant set-up is upfront with

straight line depreciation over the ensuing 10 years of use. Production is half of full capacity in first year of operation, which means that revenues and expenses are half of steady state situation. Inflation rate projected at 2.5%.

To populate the model, Oakbio selected a set of values representative of a realistic scenario. A sensitivity analysis was performed to identify the key variables most impacting techno-economic performances and studied their individual impact of overall system performance

5.4.3 Inputs into the model (Methods, Assumptions and Values)

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.

Key assumptions:

CO2 Cost: Oakbio assumed that the cost of access to CO2 is zero. CO2 utilized by Oakbio CCC is included in the industrial waste gas. It is noted that the Oakbio process does not require pretreatment of the cement flue gas before utilization. Unlike other carbon capture processes (e.g. algal), the Oakbio process also does not require separation and concentration of CO2 before utilization. The only alteration to the exhaust gas, at a relatively small cost, is the cooling down of the flue gas before bubbling it into Oakbio bioreactors (gas fermenters).

CO2 capture specifies the quantity of CO2 absorbed by Oakbio microbes after one pass through the fermenters. This value is based on Oakbio's data obtained from repeated experiments. In the model, researchers assume 50% CO2 capture, which includes 40% in first pass through and an additional 10% through recycling the headspace gas at the top of the fermenter. Internal data demonstrated additional CO2 capture potential of 30% when headspace gas is recycled in the same fermenter for 20 min (see Chapter 3); however Oakbio needed to conduct additional experiments to evaluate the technical and cost impact of head space gas recycling on various parameters, such as fermenter design, gas control system and overall fermenter volume capacity.

H2 cost: Hydrogen is the source of energy used by the Oakbio microbes to capture CO2. H2 cost represents the price paid by Oakbio per kg of H2. H2 price and GHG impact varies significantly depending on source and volume required. H2 can be obtained from many sources, each affecting not only the H2 cost/kg but also the life-cycle GHG emissions. For the model which represents a large scale operation, the company assumes a base cost of $2/kg.

Bioreactor volume determines the Oakbio plant's total fermentation capacity for a given fermentation batch. Since the goal is to maximize carbon capture, the case study assumes that all CO2 emissions from the cement plant are fed into the Oakbio system, which is built at adequate volume capacity. A capacity of 10,000,000 L allows the Oakbio process to maximize CO2 capture for a CO2 emissions level of 914,000 t at Lehigh cement plant. This capacity is comparable to the volume required for a small- to medium-sized commercial bioethanol plant producing 50-60 million gallons per year (NREL, 2011).

Biomass Production

The Oakbio model assumes a batch production system. Other fermentation techniques that could be potentially more productive will need to be investigated. The total amount of biomass produced during one batch run of Oakbio's process depends on the performance factors enumerated in Table 5.2.

Table 5.2: Modeled Microbial Performance Factors

Batch Growth Duration	100	Hr
Yield	297	dry wt. g/L
Productivity	3.0	g/L/hr
H2/CO2 Mole Balance	3.4	Calculated

Source: Various (discussed in detail in the text)

PHA Production

The performance factors affecting PHA production, and included in Oakbio's model, are given in Table 5.3.

Table 5.3: Modeled Bioplastic Productivity Factors

Yield	70.0%	% biomass
Productivity	2.1	g/L/hr
Market price	2.6	$/kg
Product Recovery	85%	% available
Residual value in biomass	0.40	$/kg

Source: Various (discussed in detail in the text)

PHA retail price has been reported between $1.76 and $11 per kg, depending upon the commercial application of the final product (Metabolix, 2012):

There are many different categories of plastics sold in the market today, but they are generally categorized into two broad groups: commodity polymers and engineering polymers. The most commonly known commodity polymers include polyethylene, polypropylene, polystyrene, PET and polyvinyl chloride. The commodity polymers are high volume resins which tend to be lower value-added materials produced in volumes of hundreds of billions of kilograms per year. Engineering specialty polymer pricing varies widely based on type of resin and the performance characteristics offered by the material. However, these resins are typically priced at a premium compared to commodity plastics and according to Plastic News (http://www.plasticsnews.com/resin-pricing/all-resins.html), engineering thermoplastics purchased at annual volumes of 140,000-230,000 kg were selling at values starting at $2.4/kg and reaching, in some cases, $9.3 /in Feb 2012. At smaller volumes, prices can be higher. By

contrast, the commodity resins purchased at annual volumes of 800,000-2,200,000 kg were priced between $1.6 and $3.7 per kg.

In the model, a price of $2.60 /kg is assumed, which is in the middle of the range for commodity polymer, and at the low end of the range for engineering specialty polymer. Oakbio believes, however, that it is possible to position its product as a premium priced specialty material catering to the customers who want to match the functionality of the petroleum-based plastic with the added dimension of environmental responsibility for their products and brands.

Residual value in Biomass: the lower possible value for residual biomass would be using it as combustible, i.e., burning it for its BTU value (ASPs $100/t). Cement manufacture commonly utilizes coal or petroleum coke as combustible to heat the kiln at the cement plant; the use of dried biomass would represent a $ saving, and could also lower the release of contaminants sometimes contained in fossil fuels, henceforth adding the total GHG reductions obtained from the Oakbio process. Several commercially valuable products, such as fatty acids, could be extracted at low cost from the residual biomass. These could generate significant additional income. For the base case, the company estimated a conservative value of $400/t for OB311 biomass, with is the low end of its use for agricultural purpose. Further studies needs to be undertaken to fully evaluate and test how much value can be extracted from the residual biomass after PHA extraction.

5.5 Overall Discussion on System Performance

5.5.1 Carbon Capture Performance

Carbon capture potential of 50+% CO2 emissions (largely based on Oakbio's data) and carbon conversion to PHA at a profit of $51 per metric tonne of CO2 captured. The latter value is based on overall NPV divided by the quantity of CO2 captured during the forecasting time period. This value compares favorably with figures provided by other emerging methods of CCC. It is, however, difficult to compare Oakbio's performance with that of other CCC technologies, as the methods of calculations are most often different and 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 will be linked to Capex, operating costs will likely also be significant, at roughly $5-$7/t in variable costs and $1-$5/t 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 $1 billion, which would represent a 40-60% increase in the capital cost of the plant and which would exclude downstream investments in infrastructure to transport and store the CO2.

When evaluating the GHG reduction performance or the combined cement manufacture + Oakbio CCC, the carbon cost of all components in the process value chain must be considered. In the Oakbio case, the source of H2 plays a major role in assessing net GHG reductions. H2 can be obtained from many sources, with different impacts on net GHG reductions. Several methods for obtaining H2 are discussed below in terms of their respective GHG footprints.

These sources are steam reformation of methane (SMR), electrolysis, by-product H2, and nuclear H2.

- SMR is a commercial H2 production technology currently seeing wide use. It creates demand for electricity and consumes natural gas (CH4) and water to generate 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 produced through wind, solar, geothermal, or hydroelectric generation, the process is nearly carbon neutral.
- Byproduct H2 is produced in a number of industrial processes, including Cl2 and styrene manufacturing. It is also produced during remediation of agricultural waste from the livestock industry. This may provide feedstock H2 to the Oakbio process in a net GHG-reducing manner.
- Nuclear generation of H2 provides several advantages over other strategies. It requires no fossil fuels, results in lower GHG emissions and other pollutants, and lends itself to large-scale production. The International Agency for Atomic Energy and The US Department of Energy are actively exploring alternative nuclear technologies explicitly for H2 generation, including thermochemical and hybrid processes (US DOE, 2004).

5.5.2 Economic Performance

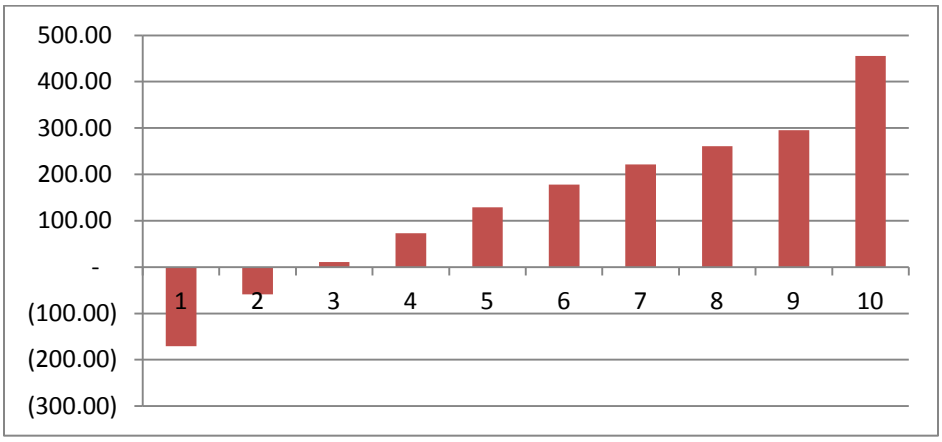
An upfront investment $244 million in an Oakbio CCC plant is required in order to maximize CCC at an industrial site emitting 914,330,000 kg/year. Break-even on initial investment will be achieved after three years of operation and the business shows a positive NPV of $455 million and IRR of 46% over its lifetime (see Table 5.5 and Figure 5.3).

Table 5.4: Summary of Oakbio Process Financial Performance

PHA market price	2.60	$/Kg Product
Production Cost	1.66	$/Kg Product
Product margin	0.94	36%
NPV (net of plant cost)	$455	million
Value of CO2 captured	51	$/tonne
IRR	46%	

Source: Oakbio internal modeling

Figure 5.3: Breakeven Analysis Based on Cumulative NPV



Source: Oakbio internal modeling

5.5.3 Sensitivity Analysis

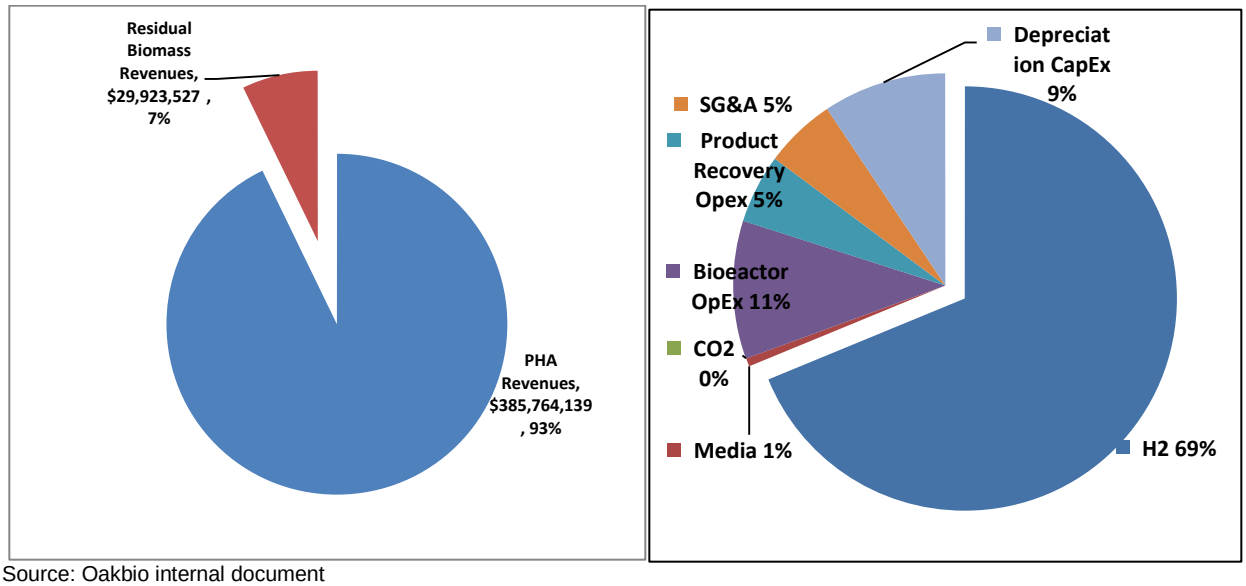
Economic performances are most affected by:

1) The cost of H2 needed to fuel the process, which is linked to the price of H2 supply and the potential losses during the process itself and

2) The market value of the PHA products and of the potential additional value in the residual biomass after PHA extraction.

At 69% of Opex, H2 is the major cost in the Oakbio process (see Figure 5.4, right).

Figure 5.4: Modeled Project Revenues (left) and Expenses (right) at Commercial Scale.



Source: Oakbio internal document

This level of cost for raw materials is not uncommon in the production of commodity chemicals, either produced from sugar (bioethanol) or from crude oil (petrochemicals). Base case H2 cost is $2/kg. An H2 price of $1/kg (pipeline access) would increase the profit/metric tonne CO2 to $107/metric tonne CO2 (NPV $953 million), whereas a high H2 price of $2.7/kg would bring the NPV to zero (a zero cost CCC process – still an attractive proposition compared to current alternatives of carbon capture which have a negative value – a cost – per ton CO2 captured.

Oakbio used a PHA market value of $2.6/kg for the model's base case. A PHA market value of $3/kg would increase the CCC value to $77/metric tonne CO2 (NPV $685million) and a $4/kg to $141/metric tonne CO2 (NPV $1,258 million). There is huge value upside linked to the market value of PHA. Finding the right commercial applications for the PHA, and/or the potential to add more value after extraction from biomass through on-site processing before sale, should be explored aggressively to maximize the overall value of the cement-to-bioplastics proposition.

The model is economically less sensitive to the carbon captured as % total CO2 available to the microbes. Variations from the base case of 50% capture, show decrease of CCC value to $47/metric tonne CO2 at 40% and $56/metric tonne CO2 at 60%. However, in the Oakbio model design, the amount of biomass produced is directly calculated from the overall amount of carbon captured. So there is a correlation between size of bioreactor and productivity levels to match that level of biomass production. At identical biomass yield at harvest time, faster batch duration would allow significant reduction in Capex (to capture same quantity of CO2 available). And reciprocally, a slower batch duration with same yield or same batch duration at lower yield of harvest would require additional bioreactor capacity (and higher Capex) – not modeled.

The percentage of PHA content in biomass is also moderate in its economic impact. The base case assumes 70% PHA content in biomass. An upside of 80% would increase the CCC value to $61/metric tonne CO2, whereas the %PHA would have to go down to 35% for neutralizing the NPV. Improving the product recovery post-fermentation from base case 85% to 90% would increase value to $59/metric tonne CO2.

5.5.4 Full Value of Oakbio technology to CO2 emitters

It is important to note that several value streams can be generated directly and indirectly from Oakbio's Carbon Capture and Conversion. In the example of cement production in California, these include (Figure 5.5):

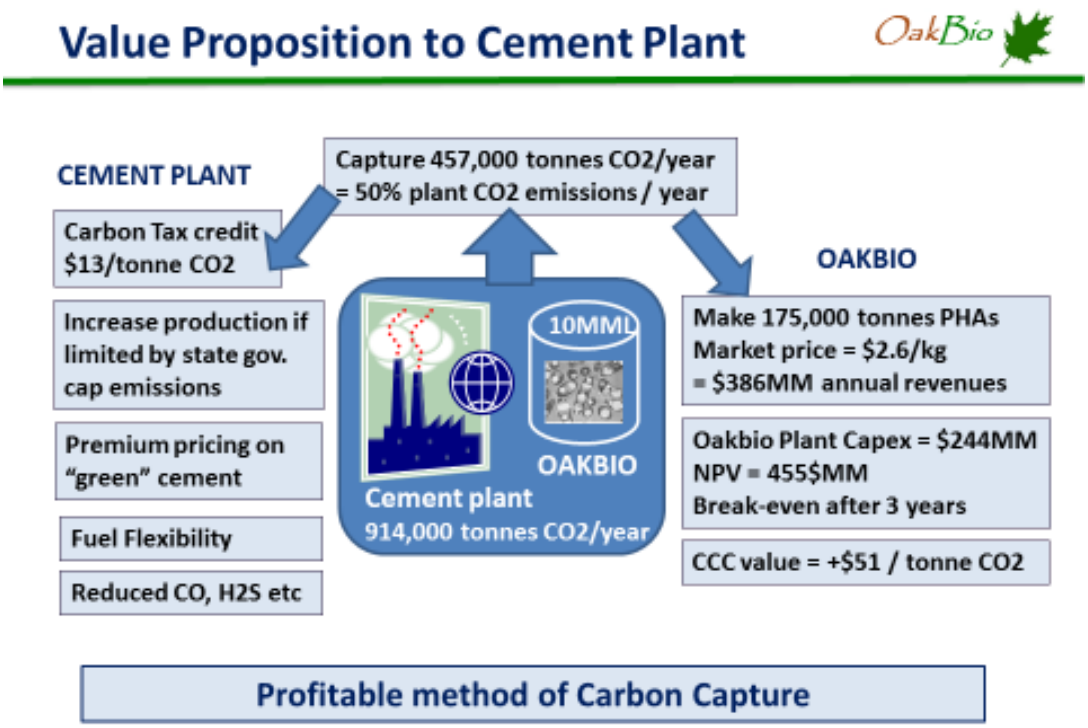
1. Sales of primary product (PHA in this case) from carbon conversion
2. Sales of secondary products (residual biomass and other products contained in it)
3. Value of tax credits / tax savings from resulting carbon capture
4. Revenues from additional sales of cement if plant output was capped by a certain level of carbon emissions
5. Extra-value given by some market segments for "green(er)" cement

6. Others (remediation of toxic components, less use of fossil fuel combustibles, etc.)

In this base case analysis, Oakbio only considered value point # 1 (and # 2).

Tax credit/savings represents the amount given/taken by the local government as incentives/tax for carbon emission/ reduction. It is expressed in $/metric tonne CO2 captured/emitted. In California current value for CO2 capture is $12-13 / t CO2 (California Carbon Dashboard, 2014). The European Commission is considering imposing by 2018 a cap of CO2 emission for cement producers, which then would have to buy carbon credit at 25-30 euros /metric tonne CO2 in order to continue producing at current levels.

Figure 5.5: Value Proposition of Oakbio CCC Process for Industrial Partners



Source: Oakbio internal modeling

From an economic standpoint, the opportunity to continue producing cement at high level (value point # 4 above) is fundamental to cement producers. Cement manufacture involves a tremendous amount of capital expenditure and a high level of fixed costs. Any incremental production above fixed cost amortization generates profit for the company. This point alone is enough of an incentive for producers to be willing to do something about carbon capture. They are motivated to be proactive about it and take advantage of government funding support for developing new technology addressing this issue.

Oakbio's industrial collaborator on this grant was also motivated by the possibility to decrease the presence of other toxic components in their flue gas. With Oakbio's process, this can happen in two ways. First, the residual microbial biomass after extraction of the PHAs could be

dried by the heat generated by the kiln and then used as a combustible, to replace current use of petroleum coke. This is saves the cost of buying combustibles and reduces GHG release from their use. Second, Oakbio organisms have the capacity to utilize carbon monoxide (CO) and hydrogen sulfide (H2S) as feedstock, removing it from the flue gas, and helping further reduce GHG emissions in the process.

All these additional sources of value are not included in the technoeconomic analysis, but add very significant upside to the overall value of the Oakbio proposition.

5.6 Techno-Economic Analysis - Conclusions

Based on the experimental data, Oakbio's CCC system at commercial scale has the potential to capture 50+% of the carbon emissions for a representative cement plant. The captured CO2 will then be converted into bioplastic (PHA), which will be sold to generate revenues and profit. The techno-economic modeling estimates a CCC value of $51 /metric tonne CO2. This value is calculated from net present value divided by the quantity of CO2 captured during the forecasting period. Several additional benefits, such as carbon tax credit, potential increase of cement production over emission limits, potential price premium on "green" cement product and bioremediation linked to Oakbio's CCC system were not included in these calculations.

The initial cost of installing a scaled-up Oakbio's system at a CO2 emitting site is estimated at $244 million based on a 10,000,000L fermenter capacity capable of maximizing carbon capture for emissions of 914,330 metric tonnes CO2/year. Over its lifetime, the CCC business shows a positive NPV of $455million. Overall economic performance is sensitive to the cost of the H2 supply, which represents the bulk of feedstock cost for the Oakbio system. Economics are also sensitive to the market value of PHA. The calculations based on the expected value range for these two factors increased Oakbio's confidence that their system will be a profitable method of CCC. The bioplastic market is expanding fast, both in the lower price commodity polymer market but also in the higher price / lower volume engineered specialty polymers market segments. H2 is readily available and can be obtained at stable to decreasing prices from many sources, from natural gas reformation to electrolysis of water. When H2 can be sourced from renewable resources, the net carbon balance of the Oakbio process is very positive.

A techno-economic analysis based on using natural gas combustion would produce similar results. Because the NG combustion flue gas contains less contaminants than flue gas from cement manufacturing, the starting base technical productivity is higher and requires less improvement to reach commercial scale. The natural gas combustion process also generates more stable gas composition, which is an advantage for process efficiency. The major difference in gas content is the higher concentration of CO2 in cement flue gas than in natural gas combustion, although the effective CO2 concentration in the latter system could be increased through the use of head space gas recycling.

5.7 Overall Project Conclusions and Recommendations

The goal of this agreement was for Oakbio to demonstrate that its technology can reduce the cost of CO2 compliance by generating revenues from CO2 capture. This will be achieved by converting captured CO2 into PHA bioplastics, a commodity chemical. The objectives were to determine the technical and economic feasibility of a novel microbe-based process for capture and conversion of flue gas CO2 to bioplastics, using an already operating test system at a Cupertino cement plant. Key quantitative indices of system performance were measured, including CO2 capture, microbial biomass production and bioplastics production. Oakbio's final report, including a techno-economic analysis model, informs the cement industry and the CO2-emitting energy industry on the economic feasibility of Oakbio's system at large scale.

Oakbio's technical success in this PIER proposal could lead to development scale of their process for CO2 capture in the cement and power generation industries. Furthermore, to explore even broader economic potential, Oakbio hopes to set-up in situ pilot test gas fermenter units at several CO2-emitting locations in California and elsewhere. A profitable process for production of valuable chemicals a lower cost will lead to further investment in California technology process development, local jobs creation, reduced CO2 release from critical local industries, and potentially, dramatic expansion of the bioplastics industry driven by large scale production at lower cost. Commercial success would lead to environmentally beneficial conversion of flue gas CO2 to multiple plastic products, not only sequestering CO2 in manufactured goods, but decreasing use of non-renewable petroleum oil for production of plastic products from petrochemicals.

GLOSSARY

Term	Definition
3HB	3-hydroxybutyrate, the monomeric form of poly(hydroxybutyrate)
Bioreactor	Synonym to gas fermenter (in which gas is bubbled into solution to become available as feedstock for microorganisms growing in solution
CCC	Carbon Capture and Conversion
CCS	Carbon Capture and Sequestration
CO	Carbon Monoxide
CO2	Carbon Dioxide
GC	Gas Chromatograph, an analytical instrument that identifies and quantifies gases
GRAS	Generally Recognized As Safe, US Food and Drug Administration designation
H2	Hydrogen (molecular form)
MEA-CC	Methyl ethanolamine-based carbon capture, the existing industry standard method for scrubbing gas streams of carbon dioxide
N2	Nitrogen (molecular form)
NOx	Nitrogen Oxides, catch-all term for multiple chemical species
O2	Oxygen (molecular form)
OB213	Oakbio-proprietary microbe adapted for chemoautotrophic growth from *R. capsulatus*
OB311	Oakbio-proprietary microbe adapted from its parent strain to grow effectively on cement flue gas
OD	Optical density
PG&E	Pacific Gas & Electric Company
PHA	Polyhydroxyalkanoate, a class of biodegradable plastic produced by chemoautotrophic hydrogen bacteria in order to store energy and carbon
PHB	Poly(hydroxybutyrate), one member of the polyhydroxyalkanoate class of biodegradable plastics
psig	Pounds per square inch above atmospheric pressure

R. capsulatus	The parent microbial strain of OB213
SOx	Sulfur Oxides, catch-all term for multiple chemical species
WT	wild-type
wt%	Percent of a material consisting of one component by weight

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