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Project Title: **REMOVAL OF CO_2 FROM FLUE GASES BY ALGAE**
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ABSTRACT

The objective of this research program is to determine the feasibility of the alga Botryococcus braunii as a biocatalyst for the photosynthetic conversion of flue gas CO_2 to hydrocarbons. Free and immobilized cells of Botryococcus braunii were grown in aqueous medium supplemented with nitrogen, phosphorus and mineral nutrients. Air and CO_2 enriched air [10% to 15% (v/v) CO_2] in the gas phase and 0.2% to 2% $NaHCO_3$ in the liquid medium served as the carbon source. Growth and hydrocarbon formation characteristics of free and immobilized cultures of Botryococcus braunii were determined in bench-scale photobioreactors. Technical and economic feasibility of the conversion of flue gas CO_2 to hydrocarbons by Botryococcus braunii culture systems was evaluated. In free cell systems, the hexane extractable oil productivity was about 15 to 37 grams of oil per 100 grams of cell dry weight. In immobilized cell systems, the oil production ranged between 5% and 47% at different immobilization systems and immobilized surface locations, with an average of 19% of cell biomass dry weight.

The feasibility and economic evaluation estimated the cost of oil produced from flue gas CO_2 by algae to range between $45 and $75 per barrel assuming that a hydrocarbon yield of about 50% of the biomass weight is achievable and a credit of $60 per ton of carbon removed is available.

A future research program leading to development of a multistage process, consisting of closed systems for heavy inoculum buildup followed by lower cost open systems for oil production is recommended.

EXECUTIVE SUMMARY

An increased attention is now being given on the potential global warming effect of CO_2 released to the atmosphere from fossil fuel burning. Various options that are suggested for the removal of CO_2 from flue gas include biological removal of CO_2, photosynthetic conversion of CO_2 to biomass, utilization of CO_2 for making chemicals, etc. The objective of this research program is to determine the feasibility of the alga Botryococcus braunii as a biocatalyst for the photosynthetic conversion of flue gas CO_2 to hydrocarbons.

Alga Botryococcus braunii converts CO_2 by photosynthesis to cell components and hydrocarbons. It produces linear alkenes, mostly C_{27}, C_{29}, and C_{31} dienes, branched alkenes (botryococcenes), C_{30}-C_{37} isoprenoids, with a general formula of C_nH_2n-10 form. The actual physiological conditions that trigger the production of hydrocarbons by Botryococcus braunii are not known.

In a conceptual process, CO_2 is scrubbed from the flue gases by using an alkaline solution and transferred to a bioreactor where, Botryococcus braunii serves as the biocatalysts (Figure 1). The solar energy is used as the energy source.

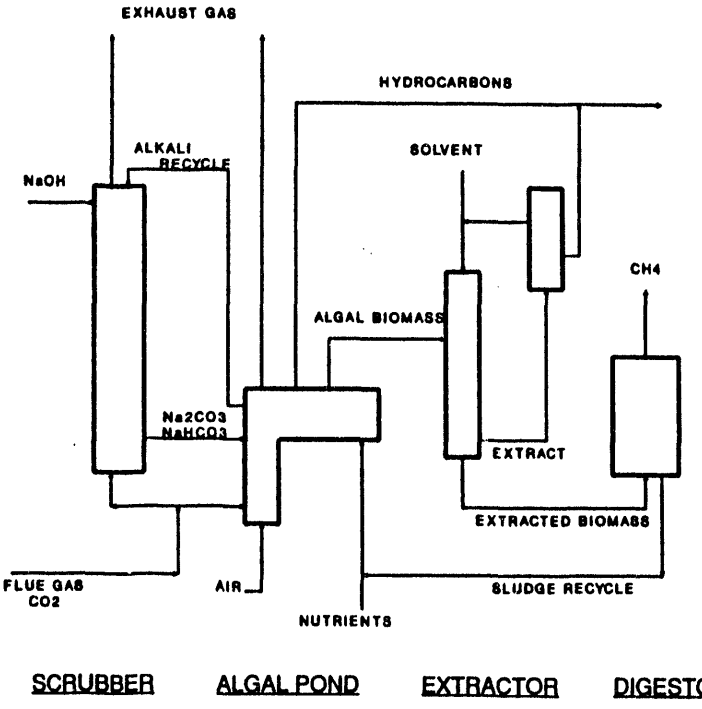
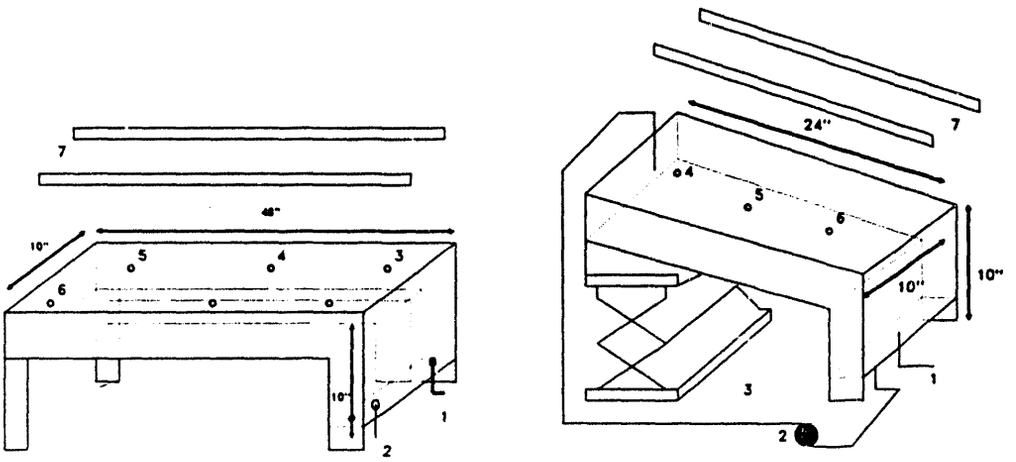


Figure 1. CONCEPTUAL PROCESS FOR THE CONVERSION OF FLUE-GAS CO_2 TO HYDROCARBONS BY ALGAE

Diesel-grade hydrocarbons, some biomass, and oxygen are produced in the bioreactor. As a first step toward achieving this long term process development goal, this research program is undertaken. Free and immobilized cells of Botryococcus braunii were grown in aqueous medium supplemented with nitrogen, phosphorus and mineral nutrients. Air and CO_2 enriched air [10% to 15% (v/v) CO_2] in the gas phase and 0.2% to 2% $NaHCO_3$ in the liquid medium served as the carbon source. Growth and hydrocarbon formation characteristics of free and immobilized cultures of Botryococcus braunii were determined in bench-scale photobioreactors. A raceway type bioreactor and a variable inclined-surface type bioreactor were used for the study of free cells and immobilized cells respectively (Figure 2). The type cultures of Botryococcus braunii were obtained from the Algae Culture Collection of the University of Texas, Austin. We observed that Botryococcus braunii could grow in air as well as CO_2 enriched air (CO_2 concentrations ranging between 1 to 15%(v/v) CO_2 in air); soil extract medium or a defined mineral salts medium could be used as the growth media. We also observed that the free cell cultures of Botryococcus braunii preferred to grow as lose clumps at the



RACEWAY TYPE ALGAE PHOTOBIOREACTOR
1 GAS INLET
2-5 SAMPLING PORTS
6 GAS OUTLET
7 LIGHT SOURCE
(NOT TO SCALE)

VARIABLE INCLINED-SURFACE ALGAE PHOTOBIOREACTOR
1 GAS INLET
2 LIQUID MEDIUM RECIRCULATION SYSTEM
3 HEIGHT ADJUSTMENT STAND
4 GAS OUTLET
5-6 SAMPLING PORTS
7 LIGHT SOURCE
(NOT TO SCALE)

Figure 2. SCHEMATIC ILLUSTRATION AND APPROXIMATE DIMENSIONS OF THE ALGAE PHOTO-BIOREACTORS ASSEMBLED BY USING 1/2 INCH THICK PLEXIGLASS PLATE

bottom of the growth flasks. Microscopic examination of the cultures indicated formation of oil by this algae (Figure 3). In free cell systems, the hexane extractable oil productivity was about 15 to 37 grams of oil per 100 grams of cell dry weight. In immobilized cell systems, the oil production ranged between 5% and 47% at different immobilization systems and immobilized surface locations, with an average of 19% of cell biomass dry weight. The actual environmental conditions that trigger the formation of oil in high concentrations are not clear. Culture buildup requires closed aseptic systems in order to eliminate contamination with other algae.

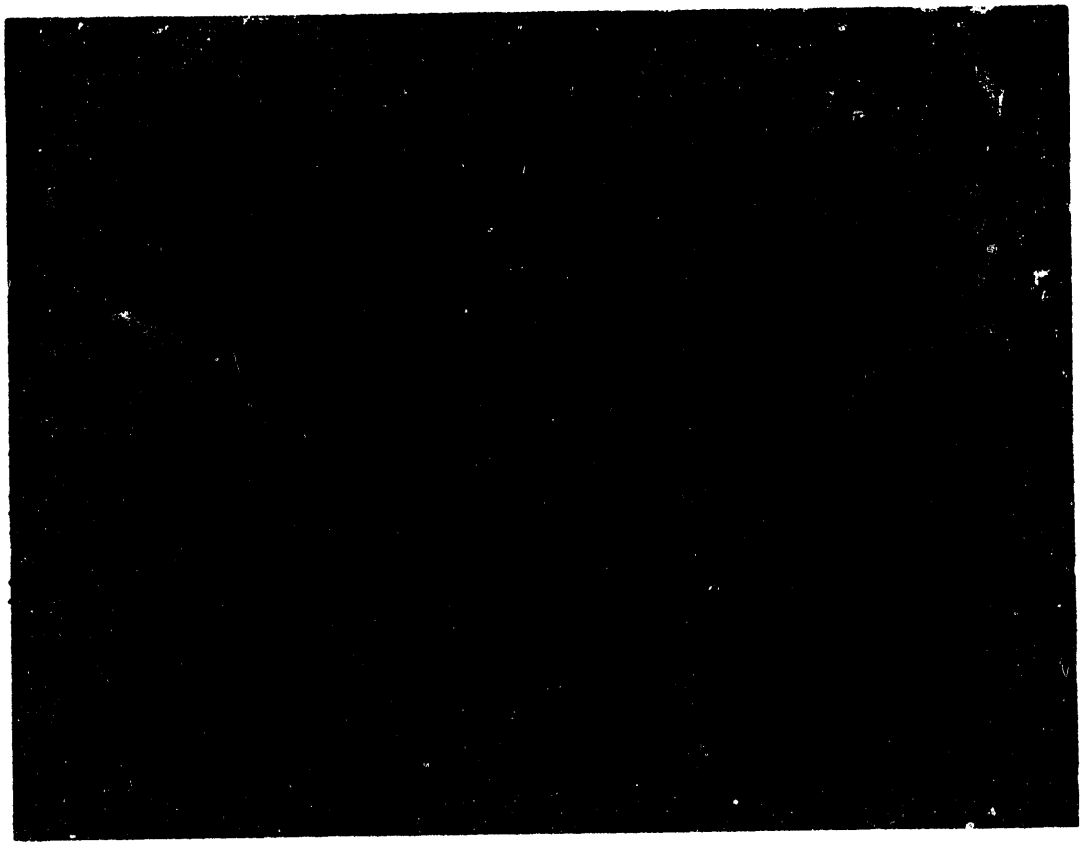


Figure 3. Botryococcus braunii CELLS WITH OIL DROPLETS AS SEEN UNDER MICROSCOPE

The feasibility and economic evaluation estimated the cost of oil produced from flue gas CO_2 by algae to range between $45 and $75 per barrel assuming that a hydrocarbon yield of about 50% of the biomass weight is achievable and a credit of $60 per ton of carbon removed is available.

A future research program leading to development of a multistage process, consisting of closed systems for heavy inoculum buildup followed by lower cost open systems for oil production is recommended.

OBJECTIVES

The overall objective of this research program is to determine the feasibility of the alga Botryococcus braunii as a biocatalyst for the photosynthetic conversion of flue gas CO_2 to hydrocarbons. In this research program, the biocatalytic characteristics of suspended and immobilized cultures of Botryococcus braunii were determined in bench-scale studies, and the feasibility and economic merits of Botryococcus braunii culture systems for the conversion of flue gas CO_2 to hydrocarbons were analyzed.

The research program involved four tasks covering the following specific objectives:

1. Assemble photobioreactors for the study of free and immobilized cultures of Botryococcus braunii.

2. Obtain type cultures of Botryococcus braunii from the algae culture collections, and build up the inocula for free and immobilized cell cultures.

3. Determine biocatalytic characteristics of Botryococcus braunii cultures for the conversion of flue gas CO_2 to hydrocarbons.

    3.1. Establish a baseline of the biocatalytic characteristics of Botryococcus braunii in free cell culture in thin aqueous layers.

    3.2. Determine the biocatalytic properties of Botryococcus braunii immobilized in calcium alginate beads for the conversion of CO_2 to hydrocarbons.

4. Conduct a feasibility and economic analyses of the conversion of flue gas CO_2 to hydrocarbons by free and immobilized cultures of Botryococcus braunii and make recommendations for future research needs toward development of the proposed conceptual process for the algae conversion of flue gas CO_2 to hydrocarbons.

INTRODUCTION AND BACKGROUND

This research program addresses the research priority No. 7.1B. The objective of the research program is to determine the feasibility of the alga Botryococcus braunii as a biocatalyst for the photosynthetic conversion of flue gas CO_2 to hydrocarbons. Bench-scale studies were performed to determine the feasibility of free and immobilized cell

cultures of Botryococcus braunii for the conversion of flue gas CO_2 to hydrocarbons. The duration of the research program was 12 months.

CO_2 released to the atmosphere from burning fossil fuels has been receiving increased attention recently because of a potential global warming effect. There is a tendency toward establishing international regulations restricting the CO_2 release from the power plants to 1990 emission levels. We present a conceptual process for the algal removal of CO_2 from the flue gases. The ultimate goal of this conceptual process is to convert CO_2 from the flue gases to liquid hydrocarbons for fuel applications. In this process, CO_2 is scrubbed from the flue gases by using an alkaline solution and transferred to a bioreactor where, Botryococcus braunii serves as the biocatalyst (Figure 1). The solar energy is used as the energy source. Diesel-grade hydrocarbons, some biomass, and oxygen are produced in the bioreactor. Botryococcus braunii which will be used as the biocatalyst for the photosynthetic conversion of CO_2 is reported to produce up to 86% of its dry weight as hydrocarbons. It is generally considered as the source of the hydrocarbons in many Torbanite shales. As a first step toward achieving this long term process development goal, this research program is undertaken. In this program, bench-scale studies are conducted to compare the biocatalytic characteristics of the free and immobilized cell cultures of Botryococcus braunii. Feasibility and economic merits of using these culture systems for the removal of CO_2 from flue gases were evaluated during the last quarter of the research program.

The research has the potential to have a positive influence for development of an environmentally friendly process for the removal of CO_2 from flue gases. Development of an economical process for flue gas CO_2 removal would eliminate potential restrictions for application of coal in electric power generation and would be an economic benefit to Illinois.

EXPERIMENTAL PROCEDURES

Stock Culture: Botryococcus braunii 572 type culture was purchased from University of Texas, Austin algae culture collection, was transferred to soil extract agar slants, and stored in 3°C refrigerator for eventual use in various tests.

Growth Media: Soil extract medium and Bold's Bristol's modified medium, with or without the addition of Na_2CO_3 or $NaHCO_3$, were used as the growth media (Appendix 1). The initial pH of the media was adjusted to either 7.3, 8, 9, or 10 by using various buffers (Table 1). Since CO_2 has a strong

Table 1. TEST MATRIX FOR GROWTH AND HYDROCARBON PRODUCTION STUDIES WITH THE FREE CELLS OF Botryococcus Braunii

Test Run	pH Initial	pH After CO_2 Add'n	Buffer	Carbon Source: CO_2 % (v/v) in Air	Carbon Source: Na_2CO_3 g/100 ml	Carbon Source: $NaHCO_3$ Medium
1	7.3	7.3	Phosphate	10	--	--
2	8.0	7.5	TRIS[a]	10	--	--
3	9.3	7.8	TRIS	10	--	--
4	10.0	8.2	CAPS[b]	10	--	--
5	10.8	10.8	--	air[c]	0.24*	--
6	10.8	10.9	--	air[c]	0.72**	--
7	11.0	11.1	--	air[c]	2.4†	--
8	9.1	10.0	--	air[c]	--	0.20*
9	8.8	9.7	--	air[c]	--	0.60**
10	8.9	9.0	--	air[c]	--	2.4†
11	7.3	7.3	Phosphate	15	--	--
12	8.0	7.5	Tris	15	--	--
13	9.3	7.7	Tris	15	--	--
14	10.0	7.5	CAPS	15	--	--

[a] Tris: Tris(hydroxymethyl)aminomethane.

[b] CAPS: 3-[Cyclohexyl amino] - 1 propanesulfonic acid.

[c] air: CO_2 source was air.

* Equivalent to 0.1% CO_2.

** Equivalent to 0.3% CO_2.

† Equivalent to 1% CO_2.

buffering effect, the pH of all media dropped to near 6.5 upon addition of CO_2. In the sealed bottle photobioreactors, the pH of the medium gradually increased to the initial pH level as the CO_2 is consumed. In the raceway type bioreactor, although the initial pH was 10, as 10% CO_2 enriched air sparged through the medium the pH dropped to 6.5. By intermittent sparging of CO_2, the pH in the raceway type photobioreactor could be maintained between 6.5 and 7.3. Although we selected buffers for their biocompatibilty we observed that media buffered with TRIS buffer at pH 8 and 9 inhibited the growth of Botryococcus braunii. Since adequate growth at a higher initial pH of 10 could be achieved with CAPS buffer, and since in all buffered systems the media pH was dominated by the buffering effect of CO_2, no further attempt was made to identify another biocompatible buffer for

the pH 8 and 9 media. Initial pH of the media is also affected by the addition of Na_2CO_3 and $NaHCO_3$ and ranged from 8.83 to 11.04. In the Bold's Bristol's modified medium with 2% $NaHCO_3$ the initial pH was about 10, and dropped to 8.3 upon sparging with 10% CO_2.

Inoculum Preparation: Soil extract medium and Bold's modified Bristol's Medium were used for culture build up and inoculum preparation. The procedures for the preparation of these media are given in Appendix 1. Initial transfer from the stock culture was made to 4 ml liquid media in 60 ml serum bottles. When culture indicated adequate growth as judged by the chlorophyll color intensity, 3 ml of the culture suspension was transferred to 17 ml liquid medium in 160 ml serum bottle, and finally to 1000 ml liquid medium in a 4000 ml Fernbach flask by using aseptic technique. The serum bottles were crimp sealed with sterilized rubber caps. The Fernbach flasks were plugged with cheese cloth/cotton plugs which allowed diffusion of air while keeping the flask contents free of contamination. The purity of the culture was checked by microscopic examination prior to transfers into larger volumes. Only those cultures that were judged to be Botryococcus braunii and uncontaminated by other algae were transferred. Occasional presence of bacteria was noticed but found not to be detrimental to growth of Botryococcus braunii. We also have taken advantage of large number of inoculum flasks to conduct a preliminary study of the effect of the media pH, and the CO_2 concentration on the growth of Botryococcus braunii. This study was conducted by adjusting the medium pH in various bottles to 7.3, 8, 9, and 10, and injecting CO_2 to the headspace of the bottles to achieve CO_2 enrichment levels of 1 to 15% in air. The serum bottles were illuminated by a fluorescent shop lamp located 24 inches above the bottles for 16 hours and kept in dark for 8 hours for each 24 hour period. The large volume Fernbach flask cultures were exposed to light for 24 hours a day. The head space in the Fernbach flask cultures was air. All of the bottles and flasks were incubated at room temperature under stationary conditions with only gentle manual swirling of the bottles once a day.

Photobioreactors: Two types of bench-scale photobioreactors were assembled by using 1/2 inch thick plexiglass plate as the construction material: A raceway type bioreactor for the study of free cell cultures and a variable inclined surface type bioreactor suitable for the study of immobilized cells. The schematic illustration and the approximate dimensions of these photobioreactors are given in Figure 1. In each set of the bioreactor study, the bioreactors and attached recirculation systems are sanitized by washing the bioreactors with milli-Q water, then keeping the bioreactor filled with a 1%

bleach solution for sixteen to twenty hours, removing the bleach solution and washing the reactor twice with milli-Q water. After this sanitation steps warm (about 55°C) growth medium is added to the bioreactor and allowed to cool before inoculation. The medium circulation system and attached connectors were steam sterilized. Inoculation and addition of liquid and gas, and sampling were done under aseptic conditions. Samples were taken at zero time for biomass determinations, and after two to three weeks of incubation for biomass and extractable oil determination. All determinations are made in triplicate samples. For the free cell study, 12 liters of steam sterilized Bristol medium (pH 10, buffered with CAPS buffer) at about 55°C was pumped into the bioreactor. When the medium cooled to room temperature (about 23°to 25°C), Botryococcus braunii inoculum (1100 ml) was added to the medium. A gas mixture containing 90% air and 10% CO_2 was sparged through the well section of the photobioreactor at a flow rate of 370 ml per minute. Daily samples were taken for pH measurement and for the microscopic examination of the culture. Since the pH of the medium was dominated by the acidity of CO_2 at about pH 6.5, CO_2 was injected intermittently with 8 hours on and 16 hours off cycles. Preliminary studies with the raceway type bioreactor indicated that inoculum concentration has to be high in order to reach a desirable biomass concentration without contamination with other alga. Screening studies at various initial pH, CO_2 concentration and media with Na_2CO_3 and $NaHCO_3$ were conducted in sealed bottle bioreactors under sterile conditions, while building up a large volume inoculum for the raceway type photobioreactor. The sealed bottle photobioreactors consisted of 250 ml serum bottles with 150 ml liquid medium and 100 ml headspace for the gas mixtures. The bottles and seals were dry sterilized, and the media was added to the bottles by using aseptic techniques and the bottles were then crimp sealed. Inoculum and head space gas CO_2 were introduced to the bottles and the samples were taken from the bottles by using sterile syringes.

Illumination: The bioreactors were illuminated by fluorescent shop lights with two 40 watt bulbs. The lights were located at 6 inches from the top of the liquid medium for the raceway type bioreactor, and 12 to 24 inches for the sealed bottle photobioreactors. The raceway and inclined surface photo-bioreactors were illuminated continuously. The sealed bottle photobioreactors were illuminated with 8/16 hours light/dark cycles. Illumination of the raceway and inclined surface photo-bioreactors by locating the light source beneath the bottom surface was also tested. Such bottom illumination was found to be undesirable because it resulted in excessive heating of the culture medium.

Immobilized Cells: Free cells of Botryococcus braunii 572 were immobilized in calcium alginate gels to form beads of approximately 2 mm diameter (Appendix 2). In preliminary studies the cells immobilized in calcium alginate beads indicated growth and oil formation but the exposure of these beads to gas streams with 10% CO_2 resulted in sequestering of Ca^{++} as $CaCO_3$, and thus deterioration of the bead structure. A thin layer of solidified agar was tested and found satisfactory as the immobilization surface. The agar media was prepared as described in the Appendix 1 and added to the photobioreactor to form a solidified gel layer of about 0.5 cm thickness. Botryococcus braunii suspension was spread over the agar surface and the cells were allowed to grow and become attached on the agar surface. Immobilization on cheese cloth fibers was also tested by spreading the Botryococcus braunii suspension over the cloth and allowing the cells to grow while the immobilization surface was flooded by the liquid medium.

Determination of Biomass Dry Weight: At the end of the incubation period, the bioreactor medium with free cells is centrifuged. The residue was washed twice with milli-Q water. Washed residue was dried in an oven at 80°C to a constant dry weight. The biomass determination in the calcium alginate immobilized cells was made after solubilizing the calcium alginate by suspending the beads in 2% sodium citrate solution in water and centrifuging, washing the cell residue and drying as in the free cell biomass determination. The residue was washed twice with milli-Q water. Surface immobilized cells were collected by scraping and washed twice. Washed residue was dried in an oven at 80°C to a constant dry weight.

Determination of Hydrocarbons and other Oily Products: The dried cell biomass was extracted with hexane. The solvent was evaporated under air stream. The quantity of oily material remaining after solvent evaporation is determined by weight and is expressed as percent of cell dry weight. In one test the cell free supernatant was also extracted with hexane. No oil was recovered from the supernatant by this hexane extraction, indicating that all of the oil produced by the algae remained within the centrifuged cell biomass. The C, H, O, N contents of the oil product were determined by using LECO CHN 600 carbon, hydrogen, nitrogen analyzer. Oxygen is estimated by difference assuming that there was no sulfur.

Other Determinations: The gas flow rates, CO_2/air ratio, and the pH of the medium were monitored and, if needed, adjusted daily in all bench-scale photo-bioreactors. Bioreactor samples were observed under the microscope daily to follow the growth stage of the Botryococcus braunii, absence of algal contamination, and visible oil formation. In the Bold's Bristol's modified medium, growth of bacteria and protozoa was

observed in the raceway type of photobioreactor at increasing levels as the incubation period progressed. No contamination occurred in the 2% $NaHCO_3$ containing media for an eight week run in the raceway type bioreactor.

Temperature: The bioreactors were located on the laboratory bench. Special temperature control was not applied. All test runs were conducted at room temperature which ranged between 23° to 25°C.

General Test Matrix: In order to select the test parameters to be applied in the raceway type photobioreactors, a series of tests were conducted in the sealed bottle bioreactors. The test matrix is given in Table 1. Tests were also conducted in Bold's Bristol's modified medium, with or without the addition of $NaHCO_3$, in free cell and immobilized cell systems. The test matrix is given in Table 2. Each test was run in duplicate.

Table 2. TEST MATRIX FOR GROWTH AND HYDROCARBON PRODUCTION STUDIES WITH THE FREE AND IMMOBILIZED CELLS OF Botryococcus Braunii

Test Run	pH Initial	pH After CO_2 Add'n	Buffer	Carbon Source: CO_2 % (v/v) in Air	Carbon Source: $NaHCO_3$ g/100 ml Medium
1	7.3	7.3	Phosphate	10	--
2	7.3	7.3	Phosphate	10	--
3[a]	10	8.2	CAPS[b]	10	--
4	10	8.3	--	10	2.0
5[c]	10	8.3	--	10	2.0

[a] Cells were immobilized in calcium alginate matrix.

[b] CAPS: 3-[Cyclohexyl amino] - 1 propanesulfonic acid.

[c] Cells were immobilized on solidified agar surface.

RESULTS AND DISCUSSION

Free Cell Studies: The preliminary studies in sealed bottle bioreactors indicated that Botryococcus braunii could grow at pH 7.3, 8, 9, and 10,and air and 1 to 15% CO_2 enriched air. This pH and CO_2 tolerance of Botryococcus braunii will be very useful in eventual industrial applications of Botryococcus braunii for the removal of CO_2 from flue gases. In subsequent series of experiments with sealed bottle bioreactors, visual daily observations indicated growth of Botryococcus braunii in

most of the bioreactors except in the test sets where Na_2CO_3 was used as the carbon source, and in test sets which were buffered with TRIS buffer. In those sets where cell growth took place, the cells accumulated at the inner bottom surface of the bioreactor. Most of these cells could be suspended in the liquid medium by gentle shaking of the bottle. If the bottles were left without daily stirring, the algal cells attached themselves firmly on the glass surface. The microscopic examination of the samples were limited to those bioreactors where cell growth took place. Microscopic examination indicated presence of typical single cells and clusters of cells of Botryococcus braunii. Starting with the second week of growth, the oil droplets were visible under the microscope and could be squeezed out of the cells by pressing the cover slip over the microscope slide (Figure 3). There were no contamination in these photobioreactors by bacteria, protozoa or other algae. After 19 days of incubation, the liquid with algal cells was centrifuged at 10,000 rpm 10 minutes, the residue was washed twice with milli-Q water and dried. Dry biomass weight was determined, and the dry cell biomass was extracted with hexane and the oil recovered and weighed. The results are summarized in Table 3. The highest growth of Botryococcus braunii was 30 mg per 100 ml and observed in CAPS buffered medium with an initial pH of 10 and a final pH of 10.1, and with 100 ml of 10% CO_2 enriched air in the head space. The pH of this medium dropped to 8.2 upon addition of the CO_2 to the head space as the CO_2 became dissolved in the medium. As the CO_2 become consumed by the growing algae, the pH returned to the buffered level of 10. The oil content of this culture was about 20%. The highest oil content was 22% and achieved in the medium that contained 0.2 g $NaHCO_3$ per 100 ml.

Studies in the Raceway Photobioreactor: In preliminary studies in the Raceway Photobioreactor (Figure 4), twelve liters of Bold's Bristol's medium was pumped into the sanitized raceway photobioreactor at about 55°C. When the medium temperature cooled down to the room temperature (about 22°C), 1100 ml of Botryococcus braunii inoculum was added. Air mixed with 10% CO_2 was sparged continuously through the well portion of the raceway photobioreactor at 370 ml/minute. The pH of the medium was measured and the culture was examined under the microscope daily. In the phosphate buffered medium, pH of the medium remained at 7.3. Botryococcus braunii cell growth was profuse and oil production was visible under the microscope. Contamination with a fast growing alga was observed after three weeks of incubation. At this point, the cells were harvested and the biomass weight and the oil content were determined. The wet pellet weight was 28 grams, dry biomass weight was 2.8 grams, and the cell oil concentration was 15 grams per 100 gram of cell dry weight. In the

Table 3. GROWTH AND HYDROCARBON PRODUCTION BY THE FREE CELLS OF Botryococcus braunii

Test Run	pH Initial	pH Final	Buffer	Carbon Source: CO_2 %(v/v) in air	Carbon Source: Na_2CO_3 g/100 ml	Carbon Source: $NaHCO_3$ Medium	Biomass Dry wt mg	Oil[e] g/100
1	7.3	7.3	Phosphate	10	--	--	13	16.3
2	8.0	7.5	TRIS[a]	10	--	--	NG[d]	
3	9.3	7.8	TRIS	10	--	--	NG[d]	
4	10.0	10.1	CAPS[b]	10	--	--	30	19.9
5	10.8	10.8	--	air[c]	0.24*	--	NG[d]	
6	10.8	10.9	--	air[c]	0.72**	--	NG[d]	
7	11.0	11.1	--	air[c]	2.4†	--	NG[d]	
8	9.1	10.0	--	air[c]	--	0.20*	17	22
9	8.8	9.7	--	air[c]	--	0.60**	21	15.1
10	8.9	9.0	--	air[c]	--	2.4†	22	18.3
11	7.3	8.1	Phosphate	15	--	--	19	14.9
12	8.0	--	TRIS	15	--	--	NG[d]	
13	9.3	--	TRIS	15	--	--	NG[d]	
14	10.0	9.4	CAPS	15	--	--	22	16.0

[a] TRIS: Tris(hydroxymethyl)aminomethane.

[b] CAPS: 3-[Cyclohexyl amino] — 1 propanesulfonic acid.

[c] air: CO_2 source was air.

[d] NG: No Growth.

[e] Oil is expressed as grams of hexane extractable matter per 100 g of biomass dry weight.

* Equivalent to 0.1% CO_2.

** Equivalent to 0.3% CO_2.

† Equivalent to 1% CO_2.

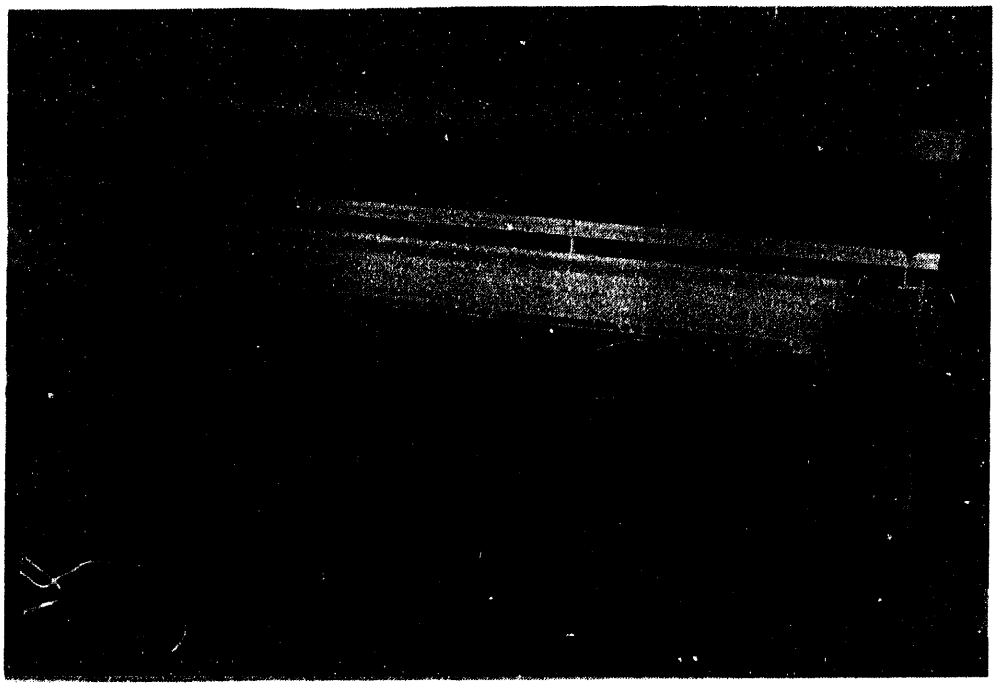


Figure 4. RACEWAY BIOREACTOR WITH Botryococcus braunii CULTURE

follow up studies, twelve liters of Bold's Bristol's medium buffered with CAPS buffer at pH 10 was pumped into the sanitized raceway photobioreactor at about 55°C. When the medium temperature cooled down to the room temperature (about 22°C), 1100 ml of Botryococcus braunii inoculum was added. Air mixed with 10% CO_2 was sparged through the well portion of the raceway photobioreactor at 370 ml/minute. The pH of the medium was measured and the culture was examined under the microscope daily. The pH of the medium dropped to 6.5 when the CO_2 injection was initiated and remained at that level when the 10% CO_2 sparging was continuous. When CO_2 enrichment was applied intermittently 8 hours on 16 hours off daily the pH of the medium varied between 6.8 and 7.3. Botryococcus braunii cell growth was profuse and oil production was visible under the microscope. Contamination with bacteria and protozoa was observed as the Botryococcus braunii cell population increased. All Botryococcus braunii cells remained attached to the inner bottom surface of the raceway and of the well section of the bioreactor. After 19 days of incubation, the cells were harvested and the biomass weight and the oil content were determined. The wet pellet weight was 27.7 grams, dry biomass weight was 2.8 grams, and the cell oil concentration was 15 grams per 100 gram of cell dry weight. The visual daily observation of the raceway type photobioreactors indicated that the cells became settled at the bottom

of the bioreactor right after inoculation and a layer of near uniformly distributed growth of Botryococcus braunii cells attached to the inner bottom surface of the bioreactor took place when the Bold's Bristol's modified medium was used. Microscopic examination indicated presence of typical single cells and clusters of cells of Botryococcus braunii. In subsequent runs we encountered significant contamination by other types of algae with spherical, spindle, and filamentous cell morphologies starting with the second week of incubation. Presence of the foreign algae diluted the Botryococcus braunii biomass. In these contaminated systems the test was terminated at the end of the second week at which time nearly 50% of the cell biomass was algae other than the Botryococcus braunii. The oil content of the biomass was about 6 g oil/100 g of dry biomass. When the medium contained 2% $NaHCO_3$, the cell growth was localized in large and thick clusters attached to the inner bottom surface of the bioreactor along the fast flow zones of the circulating medium. No suspended cells could be observed. The bacterial contamination and the protozoa activity was suppressed and no algal contamination was observed over eight weeks of incubation period. The biomass content of the samples taken from the thick algal mat sections had about 200 mg cell dry weight/100 ml medium. The oil content of these cells was similar to the cells grown in the $NaHCO_3$ free medium: 15 g oil/100 g of cell dry weight.

Immobilized Cell Studies: Botryococcus braunii cells were immobilized in calcium alginate beads as described in Appendix 2. The beads were put in a raceway photobioreactor to form a monolayer at the inner bottom surface of the raceway. Bold's Bristol's medium buffered at pH 10 with CAPS buffer was used. When air enriched with 10% CO_2 was sparged through the medium the Ca^{++} ions used in the gel formation of alginate diffused out of the gel and a white fluffy precipitate of $CaCO_3$ formed and settled at the bottom of the well section of the photobioreactor. In spite of the removal of some of the Ca^{++} ions the beads remained intact and growth of the algae became visually detectable by the formation, and increase in intensity, of the green color of chlorophyll in the beads. The beads shrank in size as the $CaCO_3$ formation increased and as the incubation period advanced. After three weeks the remaining beads were removed. Biomass dry weight and the oil content were determined (Table 4). The biomass was 178 mg dry weight/100 gram wet bead, and the oil content was 5 g oil/100 g of cell dry weight. Because of the calcium alginate bead structure deterioration in presence of 10% CO_2, immobilization with this system was abandoned, and immobilization of cells on solidified agar surface was tested. Since the Botryococcus braunii cells grew into agar and remained attached on the agar surface in clusters, this system was

Table 4. GROWTH AND HYDROCARBON PRODUCTION BY THE FREE AND IMMOBILIZED CELLS OF Botryococcus braunii

Test Run	pH After CO_2 Add'n	Carbon Source: CO_2 % (v/v) in Air	Carbon Source: $NaHCO_3$ g/100 ml	Biomass Dry wt mg/100 ml	Oil[a] g/100
1	7.3	10	--	23	15
2	7.3	10	--	23	6
3[b]	8.2	10	--	178[c]	5
4	8.3	10	2.0	200[d]	15
5[e]	8.3	10	2.0	--	15 to 47

[a] Oil is expressed as grams of hexane extractable matter per 100 g of biomass dry weight.

[b] Cells were immobilized in calcium alginate matrix.

[c] mg cell dry weight/100g wet beads.

[d] Concentration of cells in 30 ml sample taken from the concentrated cell mat location in the raceway bioreactor.

[e] Cells were immobilized on solidified agar surface.

satisfactory for the immobilized cell studies in the inclined surface photobioreactors. After four weeks of incubation with continuous flooding with Bold's Bristol medium with 2% $NaHCO_3$ sparged with 10% CO_2, cells were scraped from the agar surface and the oil content was determined. The oil production was as high as 47% of the cell biomass dry weight at different immobilized surface locations, with an average oil content of 19%. The actual environmental conditions that trigger the formation of oil in high concentrations are not clear. Since the Botryococcus braunii cells attach themselves to the available surfaces readily, immobilization on cheese cloth fibers was also tested. The inclined surface photobioreactors were set at 30° angle with horizontal plane. Two layers of cheese cloth were spread over the inner bottom surface of the bioreactor. The bioreactor was sanitized by adding 55°C soil extract medium. When the medium cooled to room temperature Botryococcus braunii cell suspension was spread over the cheese cloth and allowed to settle. After 24 hours, trickling with the CO_2 sparged medium was initiated by pumping out the medium from the well and spraying at higest level of the bioreactor and allowing it to flow over the cheese cloth and collecting again in the well for CO_2 sparging and recycle. This operation was anologous to the large scale conceptual process. Within three weeks of operation a layer of algae has

grown attached to the cheese cloth fibers. There was no evidence of algae sluff off from the cheese cloth fibers nor algae accumulation in the well.

Other Observations: We observed occasional presence of bacterial cells in Botryococcus braunii growth systems, particularly in the soil extract medium. The presence of bacteria did not adversely effect the growth of Botryococcus braunii. We were informed by the Austin Algae Collection scientists that the co-existence of Botryococcus braunii and the bacteria is expected, and that bacteria provides some unknown nutrient factors to Botryococcus braunii and thus beneficial. Microscopic examination of the inoculum revealed the presence of hydrocarbon around the cell clusters in all tested samples. The visible amount of oil varied at different periods of growth of this algae. Older cells appeared to have a higher quantity of oil. Increased amounts of visible oil could be squeezed out of the cell clusters by gentle pressing of the cover slip over the microscope slide. Elemental analysis of the composite oil sample indicated 78% carbon, 13% hydrogen, 0.1% nitrogen and, by difference, 9% oxygen. We also noticed that in free cell systems Botryococcus braunii cells prefer stationary liquid culture, and they settle to the bottom surface of the container in clusters. Because of this property of Botryococcus braunii liquid height in the bioreactors become insignificant for the self shadowing effects. Also high density thin layer biofilms of this algae can be developed by taking advantage of its cluster formation capability.

Feasibility Study and Economic Analysis: (This section is summarized from a report prepared by Dr. John Benemann, Consultant to IGT).

Any B. braunii hydrocarbon production process must, at this stage, be almost entirely conceptual, constrained by available data but assuming that current limitations could be overcome through directed basic and applied R&D. Of course, the major focus of such an effort would be to develop B. braunii strains that meet the requirements of very high photosynthetic efficiency, high content of hydrocarbons (at least 50%), adaptation to the pond environment and to local conditions (water quality and temperatures). In the development of this conceptual process it is assumed that such strains can be developed. Indeed, a major objective of the present exercise is to set the requirements for such a strain development program. The conceptual process developed herein would have the following components:

- An Inoculum Production Subsystem. It is assumed that B. braunii would be, like perhaps most algae cultivated in such mass culture systems and selected for high biomass

productivity (which does not equate with high competitiveness), subject to invasions and contamination. This has not been explicitly recognized in prior studies of, for example, lipid production by microalgae (e.g. Table 1). Thus, this is the major new component of such a system.

- Alga Growth Ponds. There is at present no apparent alternative to the shallow, raceway, mixed, earthworks ponds for low cost algal culture. Unit size of about 10 ha, with a single baffle and single mixing device (paddle wheel) are considered to be both feasible and provide optimum economics of size.

- Algal Harvesting Process. In prior analysis of fuel production by microalgae the harvesting process involved a bioflocculation-settling pond. The basic concept was that the culture would be discharged (once a day) into a large pond, where it would be allowed to flocculate and the algae settle. The volume of this pond was dictated by the maximum daily harvest detention time (typically 40% of the culture volume per day) and the depth of the pond would be limited by construction costs and settling velocities (average) of the algae flocs.

- Algae Processing. The algal biomass recovered from the settling ponds would be concentrated further by centrifugation and the biomass paste thus produced subjected to oil extraction with a solvent (e.g. hexane) similar to soybean oil extraction. The cost of the centrifuges were allocated to the harvesting subsystem and the cost of algal oil extraction was based on soybean oil extraction costs, multiplied by a factor of about 3 to account for the problem of the high water content of the algal biomass. However, this was highly speculative, as there is little or no data on which to develop cost data for an algal lipid extraction and processing to biodiesel. In the case of B. braunii a different process is likely to be used.

- Support Systems. Into this general category fall all of the other subsystem of this process, including methane fermentation of the residual biomass, water recycle, CO_2 supply, etc. These are similar to those of lipid production systems with some modifications discussed below.

Herein a very favorable conceptual Botryococcus production process (Figure 5) is proposed. It is believed that achievement of the assumed properties of such a process is possible with current biotechnology, but it would be a long-term R&D project, requiring some "breakthroughs"

Stage	Area
Starter Culture	--
↓	
Axenic Cultivation	10 m^2
↓	
Controlled Cultures	100 m^2
↓	
Inoculum Production	0.1 ha
↓	
Tubular Reactors	10 ha
↓	
Covered Ponds	100 ha
↓	
Open Ponds	1,000 ha
↓	
Harvesting Ponds	50 ha
↓	
Hydrocarbon Extraction	--
↓	
Hydrocarbon Processing	
↓	
FUEL	

Figure 5. SCHEMATIC OF THE PROPOSED Botryococcus PROCESS

which although logical and plausible, can not be clearly or confidently forecast.

Process Design and Cost Estimates — Inoculum Production:

The objective of the present feasibility analysis is not to arrive at precise economics of such a process, but to establish the relative costs of such a process compared to, for example, other concepts of microalgae fuels production. This would allow identification of areas where significant cost reductions may be feasible through applied R&D. Of course, such an exercise is also needed to determine if the present concept is at least feasible in principle.

The cost estimation of the open ponds, and even the harvesting, processing, and waste treatment subsystems can be directly based on prior studies. That analysis can also be adapted for the costs of water supply and disposal, utilities, CO_2 supply, and other general support systems. Thus, the emphasis in this feasibility analysis is on the inoculum production subsystem, which involves both tubular reactors and covered ponds, and which present novel challenges.

In the case of Botryococcus, inoculum would need to be about 5 to 10% of the total biomass produced. This would mean that approximately this much (or more) of the total growth area would be devoted to inoculum production.

Initial Scale-up of Axenic Cultures:

To achieve a 10% inoculation goal it would be necessary to build up this culture by a factor of 100,000 fold under relatively controlled conditions that avoid contamination by other algae. That is the central problem.

To avoid overwhelming the economics of production, average production costs for the inoculum must be no higher than $0.5/kg, which by itself would add 20% to the costs of the overall process, which is probably all that could be considered for such a fuel production process.

Scale-up Considerations:

Basically two major concepts for microalgae production in enclosed photobioreactors area available: tubular reactors and covered ponds. Tubular reactors appear to be most suitable for the first stage of scale-up.

The costs of tubular reactors can only be provided in approximate terms. A total cost of $10,000 per module would appear reasonable, giving total costs of about $40/$m^2$. To

this would need to be added engineering and contingencies, which would increase costs by about 25%.

The tubular reactors would be followed by plastic lined and covered ponds. The reason for a change in design is that covered ponds are considerably cheaper than tubular reactors. The open, unlined, ponds proposed for the large-scale production of microalgae are projected to cost about $\$2.75/m^2$, based on reasonably detailed analysis of the various cost factors involved.

The final major cost is the structure. Covering algal ponds is much cheaper than conventional greenhouses, as there would need to be essentially no "head" space, and the structures, to which the covers would be attached, would be essentially bend plastic PVC piping, with attachments for the plastic cover. Such structures would be quite cheap, with costs of about $\$2/m^2$ estimated. Finally the chlorination and blower system would probably add about $\$0.50/m^2$ for total costs for the covered ponds of about $\$12/m^2$, exclusive of engineering and contingencies.

Operations:

The small-scale cultures produced under axenic and then highly controlled conditions would be used to inoculate the tubular arrays. A total of 10 ha of such systems are proposed, which involves a 100 fold scale-up in culture. This is considered the better alternative than a 100 fold scale up in the covered ponds because of the greater control over the culture in the tubular reactors. However, this is, of course, a matter of experimental study and experience.

The cultures from the covered inoculum ponds would be transferred to a 10 ha growth pond, where it would grow as a continuous (once a day dilution) culture, with the dilution set to maximize productivity (depending on solar input and temperature), but with a maximal dilution of about one third per day (compared with almost 50% for other algal cultures).

About 11% of the culture area would be devoted to inoculum production, which is not counted in the overall systems productivity. (Perhaps closer to 15% of the total land area, would be used for inoculum production, due to relatively greater land wastage by the inoculum system). The assumption is that the inoculum production system would not be as efficient (productive) as the open pond cultures (e.g. 5% vs 10%, respectively). The total growth in the cultivation ponds would only involve a little over four generations (doubling of biomass), particularly as the inoculum is actually counted as growth pond productivity.

This is the central fact in the production of large amounts of inoculum under controlled conditions: it would allow with high assurance pure culture production, as any contaminant would need to make a major inroad in the system within a relatively short time. In this example, with the Botryococcus doubling in biomass every three days, and the culture carried for a period of 14 days, a contaminating alga able to doubled once a day, could increase its relative biomass by about a thousand fold. (These are rounded numbers). Thus, the contaminant would need to be present at the time of inoculation, either in the inoculum or in the ponds, at a viable concentration of 0.1%, which is both a rather high contamination level and easily detected. However, a ten fold decrease in inoculum production would provide a further thousand fold potential advantage to the contaminant, which probably sets a lower limit on inoculum production.

In conclusion, the inoculum system, at over 10% of the total growth area, and providing about 5% of the final biomass, and costing overall over half of the capital and operating costs of the growth pond themselves, represents a major input to the production system, and a significant departure from prior process designs. However, only with such a relatively large inoculation system is the production of Botryococcus at high hydrocarbon productivity relatively assured.

Growth Ponds and Harvesting System:

The economics of microalgae production in open ponds was estimated based on the previous design consisting of a shallow (30 cm), unlined (except for a thin clay cover) paddle wheel mixed pond. Mixing velocities would be about 20 cm/sec. Mixing power inputs are relatively low and do not represent a significant operating cost. The water source is not further specified, it can be either fresh or saline, or brackish, waters. The amount of alkalinity needed should be the subject of future analysis and optimization. It is assumed that the plant will be located near a power plant and that CO_2 availability or cost is not an issue.

The open ponds consists of a set of 10 hectare individual growth ponds (length to width ratio of the ponds about 10:1). Four sumps per growth pond provides for carbonation, paddle wheels span a narrowed section of the ponds, to minimize costs and improve hydraulics. Harvest and dilution lines allow for filling the settling ponds and re-filling with water. The ponds are arranged in two sets of 10 adjoining ponds with a central corridor containing the algal settling, hydrocarbon processing, inoculum ponds, etc.

The harvesting system involves a deep pond into which the culture to be harvested is placed on a daily basis. The algae would be allowed to float, based on their hydrocarbon content. The harvesting system consists of below grade, plastic lined, 3 m deep, 1 ha surface area ponds (one per 10 ha growth pond). If the average depth of the settling ponds is ten times that of the growth ponds, then the area of the harvesting pond would be about 4%, assuming about a 3 day retention time and some land area for berms, etc. The water from the harvesting ponds remaining after skimming off the algae would be directly recycled to the growth ponds.

Hydrocarbon Extraction and Processing:

The cost estimates for the hydrocarbon extraction and processing reflect the cost of a soybean oil extraction system, multiplied by approximately three fold to account for the difficulty of contacting the aqueous phase algal biomass with the hexane.

Anaerobic Digestion and Waste Treatment:

The amount of biomass residue remaining after hydrocarbon extraction is about half of the total dry weight (but only about 40% of total C and one third of the heat of combustion). The residue would need to be treated before discharge or re-use. This could be accomplished by anaerobic digestion.

Operating and Overall Process Costs:

The average productivity is projected at 15 to 30 g hydrocarbon/m^2/day. This refers to extractable hydrocarbons. This is based on an insolation of 500 langleys (5,000 Kcal/m^2/d) and an overall 5 to 10% conversion efficiency into recoverable hydrocarbons. This basic datum allows calculation of the rate of CO_2 supply required, which sets such basic inputs as pipe sizes, blower requirements, number of carbonation stations (depending on alkalinity, pH range, and allowable outgassing).

The incorporation of a major inoculum production subsystem increases capital costs about $20,000 per hectare, or 15 to 20% depending on productivity assumption. It also increases operating costs by $1,500 per hectare/year for the replacement covers (Table 5).

Additional operating costs would be incurred for the inoculum system due to, at least, higher labor costs. For example. The power inputs required for the tubular reactors has not been defined. Considerable uncertainty exists about these costs, and they require further study. Although they are not

Table 5. CAPITAL AND OPERATING COSTS FOR *BOTRYOCOCCUS* PRODUCTION

(Based on 1,000 hectares of algae growth ponds and 110 ha of inoculum production. Productivity is based on growth ponds only, but includes the inoculum).

Growth Pond Productivity Assumed: (ash-free dry weight)	Current Projected	Maximum Theoretical
Average Daily:	30 g/m^2-d	60 g/m^2/d
Annual:	110 mt/ha-yr	220 mt/ha/yr
Capital Costs ($/ha):		
Inoculum System: Tubular Reactors, 10 ha	4,000	4,000
Covered Ponds, 100 ha	12,000	12,000
Growth Ponds (earthworks, CO_2 sumps, mixing)	27,500	33,000
Harvesting (settling ponds, centrifuges)	12,500	17,000
System-wide Costs (water, CO_2 supply, etc.)	30,000	40,000
Processing (hydrocarbon extraction, digestion)	10,000	20,000
Engineering, Contingencies (25% of above)	24,000	31,500
Total Capital Costs ($/ha)	120,000	157,500
Capital Costs $/t-yr	1,090	716
Barrels of Oil/ha-yr (@ 3.5 bar./t)	385	770
Capital Costs $/Barrel-yr	310	205
Operating Costs ($/ha-yr):		
Power, nutrients, labor, overheads, etc.	11,500	17,000
Credit for methane produced	-3,000	-6,000
Net Operating Costs $/ha-yr	8,500	11,000
Net Operating Costs $/barrel oil	22	14
CO_2 Mitigation Credits ($60/tC)	-10	-10
Annualized Capital Costs (0.2 x Capital)	62	41
Total Costs $/Barrel	74	45

Assumptions: (All figures rounded)
Alae composition: 50% hydrocarbons, 25% carbohydrate, 25% protein. Algae heat of Combustion: 7.5 Kcal/g (60% C in biomass). Avg. Annual Solar Insolation: 500 Langleys, 45% visible. Multiply growth pond area by 1.4 for inoculum system, roads, piping, etc. to obtain total system area required.

expected to increase overall costs by a large factor, they may still be significant. Indeed, other system components require more design and analysis, such as the harvesting and processing subsytems. However, overall, the major issue in Botryococcus braunii production is the ability to produce sufficient inoculum to overcome the potential for contamination. Although this analysis suggests that this is possible in principle, in practice considerable more R&D is required, as discussed next.

CONCLUSIONS AND R&D NEEDS

The cost analysis of the conceptual process as outlined above is only an initial and preliminary assessment of the relative economics of such a system, and many details still remain to be explored and addressed.

In the development of a hydrocarbon production process a major, immediate, issue is the choice of algal strain, or strains, on which to base future development efforts. There are probably about a dozen strains that have been studied to at least some extent in the laboratory. Most of these have been kept in the laboratory for a relatively long period, which could call into question whether genetic selection and even drift under such conditions may have affected actual physiological capabilities and genetic diversity and adaptation potential. Although such strains are useful in terms of hydrocarbon biosynthesis studies, and for laboratory studies, they may be deficient in terms of characteristics useful in outdoor production, including adaptation to fluctuating light and temperature regimes.

Thus the first recommendation is to carry out a strain selection effort. Two approaches could be considered: the use of actual small-scale outdoor ponds or laboratory cultures that simulated to the extent realistically feasible, the outdoor pond environment in the laboratory. The two major conditions that could be simulated are light and temperature. Light is the harder one, as it involves a combination of high intensity, quality, and periodicities, none of which are easy to simulate in the laboratory. However, there are significant advantages to any laboratory selection process, as it is more controllable and easier to carry out. Thus a laboratory selection process is recommended for initial R&D efforts.

The major objective would be to select for cultures that maximize productivity (cell, or more correctly, pigment density). Note that the objective is not to select for the fastest growing alga, and thus dilution rate should be kept within bounds. Thus a 33% per day dilution rate, corresponding to a doubling of biomass every two days, would

appear to be the maximum dilution to be aimed at, with initial work starting out at lower dilutions (to avoid washing out the cultures). Too fast of a growth rate may result in rapid loss of hydrocarbon production potential. Indeed, loss of hydrocarbon production would be expected even at the lower dilutions. Thus, these type of experiments must be carefully monitored for both total biomass and specifically hydrocarbon productivity. Any evidence of reduction in hydrocarbon biosynthesis would require returning to an earlier point in the selection process, for which it would be important to archive cultures throughout the selection process.

Many other aspects of the proposed process require investigation. For example hydrocarbon extraction and processing must be investigated. It can be argued that alternative processes, such as those using algal cultures in biofilms or artificially immobilized, could be more advantageous. For another example, it has been suggested that the algae can be re-used after hydrocarbon extraction. Although these may have potential, they are far from current practice, data is too limited, and such proposals do not allow any realistic assessment of their feasibility. Indeed, the most immediate recommendation is to carry out a more detailed analysis of the presently proposed process, and possible alternatives. This analysis should integrate the biological/biochemical issues with the engineering design and economics.

APPENDIX 1

Soil Extract Medium:

The soil extract medium is prepared in a 160 ml serum bottle as follows:

garden soil placed 1 cm in bottom of bottle
deionized (mili-q) water 125 ml
a pinch of $CaCO_3$

Cover medium container and steam for 1-1/2 hours each of 3 consecutive days. After steaming add a pinch of sterile NH_4MgPO_4 to medium. Transfer aliquots to sterile vials or bottles.

Soil Extract Agar:

Add noble agar to above extract to obtain 0.5 to 0.75% agar concentration.

Bold's Bristol's Medium:

Six stock solutions, 400 ml in volume each, are employed. Each solution contains one of the following in milli-Q water:

$NaNO_3$	10.0 g
$CaCl_2 \cdot 2H_2O$	1.0 g
$Mg \cdot SO4 \cdot 7H_2O$	3.0 g
K_2HPO_4	3.0 g
KH_2PO_4	7.0 g
NaCl	1.0 g

Ten ml of each stock solution is added to 940 ml of milli-Q water. To this add a drop of 1.0% $FeCl_3$ solution and 6 ml PIV metal solution.

PIV metal solution. To 1000 ml of mail-q water add, 0.75g of Na_2 EDTA, and dissolve fully. Add the following salts:

$FeCl_3 \cdot 6H_2O$	97 mg
$ZnCl_2 \cdot 4H_2O$	41 mg
$ZnCl_2$	5 mg
$CoCl_2 \cdot 6H_2O$	2 mg
$Na_2MoO_4 \cdot 2H_2O$	4 mg

Vitamin Stock Solutions:

Thiamin	10 mg/l
B_{12}	15 X 10^{-4} g/l
Biotin	25 X 10^{-4} g/l

Filter-Sterilize each solution. Aseptically add 0.01 ml of B_{12} and Biotin and 0.1 ml of thiamin to sterile Bold's Bristol's medium.

Buffered Media:

For pH 7.3, Bold's Bristol's medium as described above is utilized (0.001M phosphate buffer). For pH 8 and 9, Bold's Bristol's medium without the phosphate buffer is prepared and TRIS buffer (0.001M) was added. For pH 10, Bold's Bristol's medium without the phosphate buffer is prepared and CAPS buffer (0.001M) was added.

Na_2CO_3 Added Media:

Bold's Bristol's medium is utilized and Na_2CO_3 is added to achieve concentrations of either 0.24%, 0.72%, or 2.4% which correspond to equivalent CO_2 concentrations of 0.1%, 0.3% and 1% respectively.

$NaHCO_3$ Added Media:

Bold's Bristol's medium is utilized and $NaHCO_3$ is added to achieve concentrations of either 0.2%, 0.6%, or 2% which correspond to equivalent CO_2 concentrations of 0.1%, 0.3% and 1% respectively.

Agar Media for Cell Immobilization:

One gram agar (Difco) was added to 100 ml Bold's Bristol's medium or the Bold's Bristol's medium with 2% $NaHCO_3$. The mixture was steamed to dissolve the agar and then steam sterilized.

APPENDIX 2

IMMOBILIZATION OF Botryococcus braunii IN CALCIUM ALGINATE MATRIX

A 8% sodium alginate solution (final volume 200 ml) and a 0.2M solution of $CaCl_2$ (final volume 1000 ml) were prepared. The solutions were autoclaved and cooled to room temperature. Botryococcus braunii inoculum (300 ml) was added to the 8% sodium alginate solution. With the aid of a peristaltic pump, the sodium alginate and algae solution was added dropwise into the $CaCl_2$ solution to form the immobilized cells embedded in beads of calcium alginate gel. The beads were kept in the $CaCl_2$ solution overnight at about 4°C. Afterwards the liquid was decanted and the immobilized cell beads were washed three times in a sterile saline solution. The beads were resuspended in Bold's Bristol's Medium (pH 10, CAPS buffer) and added to the bioreactor.

IMMOBILIZATION OF Botryococcus braunii ON SOLIDIFIED AGAR SURFACE

Agar media was prepared as described in the Appendix 1 and added to the photobioreactor to form a layer of about 0.5 cm thickness and allowed to solidify. Botryococcus braunii suspension was spread over the agar surface and the cells were allowed to grow and attach themselves on the agar surface.

PROJECT MANAGEMENT REPORT
June 1, 1993 through August 31, 1993

Project Title: **REMOVAL OF CO_2 FROM FLUE GASES BY ALGAE**

Principal Investigator:	Cavit Akin, Institute of Gas Technology
Other Investigators:	A. Maka, S. Patel, and J. Conrad, Institute of Gas Technology J. Benemann, Consultant to Institute of Gas Technology
Project Manager:	Daniel Banerjee, Illinois Clean Coal Institute

COMMENTS

There were no significant deviations of estimated actual cost from the projected cost. Co-investigator Dr. Andrea Maka left IGT at mid March. Dr. Akin took over the laboratory work which was conducted by Dr. Maka. John Conrad handled the bioreactor assembly and maintenance which was originally planned for S. Pradhan to handle. All equipment and cultures were in time. The research work progressed as planned in the proposal.

EXPENDITURES - EXHIBIT B

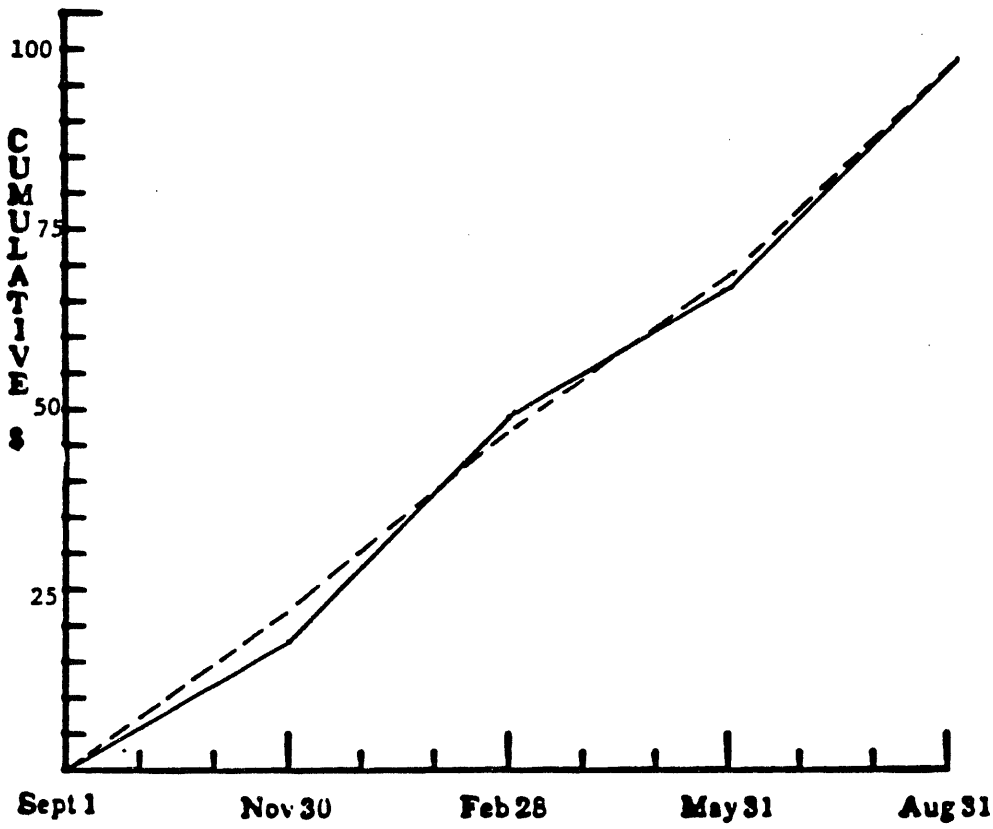
Cummulative Projected and Estimated Actual Expenditures by Quarter

Quarter *	Types of Cost	Direct Labor	Materials and Supplies	Travel	Major Equipment	Other Direct Costs	Indirect Costs	Total
Sept. 1, 1992 to Nov. 30, 1992	Projected	7,500	570	-	-	-	13,674	21,744
	Estimated Actual	6,000	600	-	-	-	10,675	17,275
Sept. 1, 1992 to Feb. 28, 1993	Projected	15,000	1,895	-	-	-	27,506	44,401
	Estimated Actual	17,000	700	-	-	-	30,026	47,726
Sept. 1, 1992 to May 31, 1993	Projected	23,000	3,220	268	-	-	42,298	68,786
	Estimated Actual	23,500	1,500	-	-	-	41,624	66,624
Sept. 1, 1992 to Aug. 31, 1993	Projected	30,351	3,710	268	-	8,000	57,365	99,694
	Estimated Actual	32,985	1,170	-	-	6,000	59,539	99,694

* Cumulative by quarter

IGT Project No. 40327

CUMMULATIVE COSTS BY QUARTER - EXHIBIT C



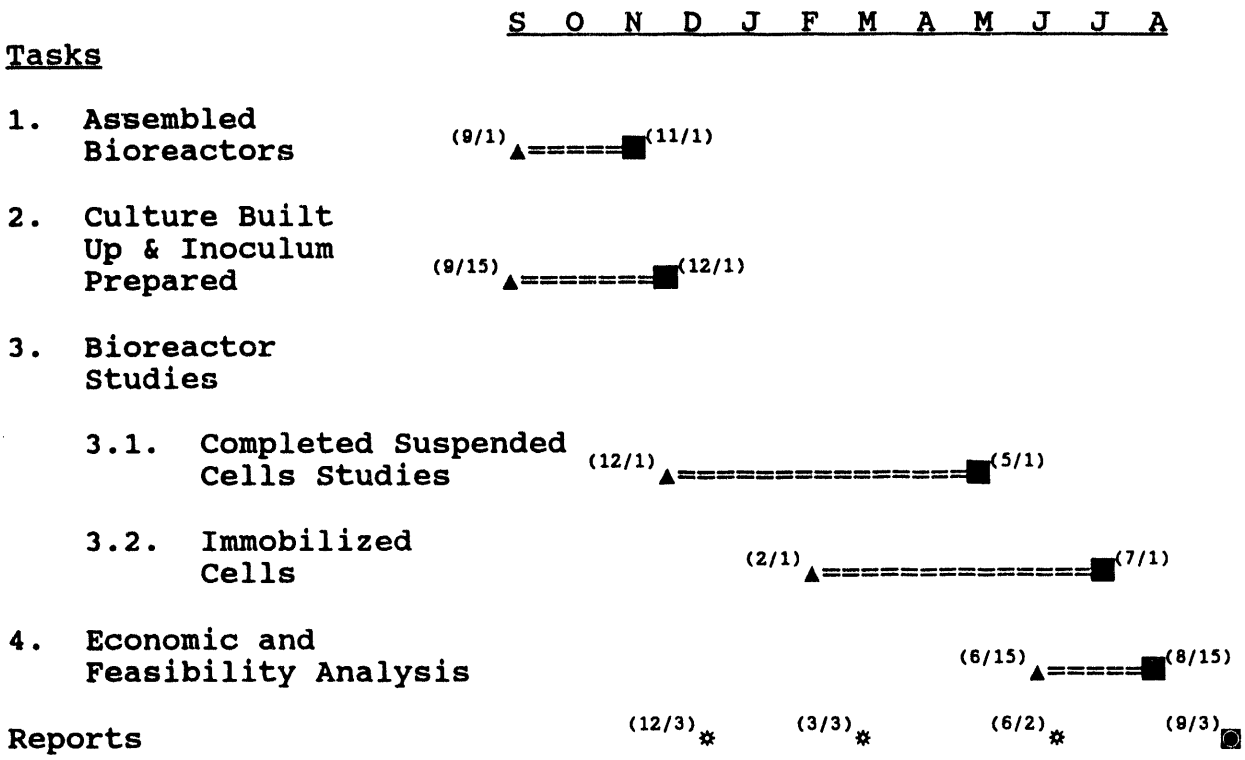
Months and Quarters

0 = Projected Expenditures ——————

Δ = Actual Expenditures ——————

Total CRSC Award $ 99,694

SCHEDULE OF PROJECT MILESTONES



Legend

▲ Beginning of Task
▼ Completion of Task
✲ Quarterly Technical Progress and Project Management Reports
■ Annual (final) Technical Report
■ Completed

(/) numbers in parenthesis indicate the beginning or the completion dates month/day) for the tasks and the delivery dates for the reports.

END

DATE FILMED

4/11/94