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TECHNICAL REPORT
December 1, 1992 through February 28, 1993

Project Title: **REMOVAL OF CO_2 FROM FLUE GASES BY ALGAE**
DE-FC22-92PC92521

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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. The research program involves the determination of the biocatalytic characteristics of free and immobilized cultures of Botryococcus braunii in bench-scale studies, and the feasibility study and economic analysis of the Botryococcus braunii culture systems for the conversion of flue gas CO_2 to hydrocarbons.

The objective of the second quarter of this research program was to determine the growth and hydrocarbon formation characteristics of free cells of Botryococcus braunii in bench-scale photobioreactors and to initiate immobilized cell studies. Sealed bottle and raceway type photobioreactors were used. The free cell studies with air and CO_2 enriched air [10 and 15% (v/v) CO_2 in air] in media with initial pH values of 7.3, 8, 9 and 10, and in media enriched by Na_2CO_3 and $NaHCO_3$ were conducted. The highest extractable oil productivity was about 20 gram oil per 100 grams of cell dry weight. This production level was achieved with 10% (v/v) CO_2 enriched air and an initial pH of 10.

DISCLAIMER

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. The research program involves determination of the biocatalytic characteristics of free and immobilized cultures of Botryococcus braunii in bench-scale studies, and the feasibility study and economic analysis of the Botryococcus braunii culture systems for the conversion of flue gas CO_2 to hydrocarbons.

The objective of the second quarter of this research program was to determine the growth and hydrocarbon formation characteristics of free cells of Botryococcus braunii in bench-scale photobioreactors and to initiate immobilized cell studies. Sealed bottle and raceway type photobioreactors were used. The bioreactors were illuminated with two 42 inch 40 watt fluorescent lights. The free cell studies with air and CO_2 enriched air [(10 and 15% (v/v) CO_2 in air] in media with initial pH values of 7.3, 8, 9 and 10, and in media enriched by Na_2CO_3 and $NaHCO_3$ were conducted in sealed bottle bioreactors. The highest extractable oil productivity was about 20 gram oil per 100 grams of cell dry weight. This production level was achieved with 10% (v/v) CO_2 enriched air and an initial pH of 10. The highest productivity conditions were applied to the raceway type bioreactor under intermittent injection of 10% (v/v) CO_2 enriched air and a productivity of of 15g oil per 100g cell dry weight was achieved. For the immobilized cell studies, Botryococcus braunii cells were embedded in beads of calcium alginate gel.

The next quarter of this research program will involve continuation of the free cell and immobilized cell studies in the photobioreactors.

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 free and immobilized cultures of Botryococcus braunii will be 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 will be analyzed.

The research program involves 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.

The work in this quarter included only the third task.

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 will be 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 is 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. 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. Evaluation of feasibility and economic merits of using these culture systems for the removal of CO_2 from flue gases is planned for 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

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. The photobioreactor was continuously illuminated by two 40 watt fluorescent lights. 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.

Test Media: Bold's Bristol's modified medium was used as a the growth medium, buffered at various pH levels as given in Appendix 1.

Inoculum Preparation: Initial inoculum of Botryococcus braunii 572 was prepared from the stock cultures by using soil extract medium and Bold's Bristol's Medium as described in the Appendix 1. The inoculated liquid media indicated steady growth of Botryococcus braunii in all growth bottles and brought up to six 1000 ml volumes in six Fernbach flasks by using pH 10 CAPS buffered Bold's Bristol's medium with 10% CO_2

enriched headspace. Strict adherence to aseptic techniques had to be practiced in order to avoid competing algal contamination.

Immobilized Cells: The free cells of Botryococcus braunii 572 were immobilized in calcium alginate gels to form beads of approximately 2 mm diameter. The details of the procedure used for the immobilization of Botryococcus braunii are given in Appendix 2.

Determination of Biomass Dry Weight: At the end of the incubation period, the bioreactor medium 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.

Determination of Hydrocarbons and other Oily Products: The dried biomass was extracted with hexane. The solvent was evaporated under air stream. Oily material remaining after solvent evaporation is weighed after the evaporation of the solvent. The amount of oily material 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.

Other determinations: 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. No contamination occurred in the sealed bottle photobioreactors. Growth of bacteria and protozoa was observed in the raceway type of photobioreactor at increasing levels as the incubation period progressed. In the raceway type photobioreactor the gas flow rates, CO_2/air ratio, and the pH of the medium were monitored and, if needed, adjusted daily.

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 inches for the sealed bottle photo-bioreactors. The raceway photobioreactor was illuminated continuously. The sealed bottle photobioreactors were illuminated with 8/16 hours light/dark cycles.

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.

Adjustment of Medium pH: 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 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.

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. Each test was run in triplicate.

RESULTS AND DISCUSSION

Free Cell Studies: The visual daily observation of the sealed bottle bioreactors indicated growth in most of the samples 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 2). 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 2. 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 percent. The highest oil content was 22 percent and achieved in the medium that contained 0.2 g $NaHCO_3$ per 100 ml.

Studies in the Raceway Photobioreactor: 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.

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 the 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. This immobilized cell study is still in progress.

CONCLUSIONS AND RECOMMENDATIONS

The reported second quarter studies confirmed our preliminary observations that Botryococcus braunii can tolerate and grow well in flue gas CO_2 concentrations of 10 to 15%, and produce oil. The highest extracted oil was observed in 10% CO_2 enriched air. Initial pH of the medium at or near 10 pH is favorable to cell growth probably by stimulating the CO_2 solubilization in the medium. This is also indicated in Botryococcus braunii growth and oil formation in $NaHCO_3$ added medium. The lack of growth in pH 8 and 9 media is attributed to inhibition by TRIS buffer rather than to pH effect and this will be confirmed in our third quarter studies. The lack of growth in Na_2CO_3 containing media was probably due to high pH. This matter will also be reinvestigated in our third quarter studies. The $CaCO_3$ precipitation from the Ca^{++} gelled alginate beads indicate the need for alternative immobilization systems. But the attachment of the Botryococcus braunii cells to the bottom inner surfaces of the photobioreactors may eliminate the need for gel entrapment systems as the immobilization matrices. In the third quarter studies, surface adhesion immobilization systems will be included in immobilized cell investigations. Attachment of the Botryococcus braunii cells to the bottom inner surfaces of the photobioreactors, rather than remaining in the suspension, reduces the significance of self shadowing and related liquid height (thickness) effect. The capability of Botryococcus braunii to grow in $NaHCO_3$ solutions is very encouraging toward development of an alkaline scrubbing system for the flue gas followed by removal of the CO_2 from the alkaline solution. In such a system the pH 10 is the currently observed upper limit. We will reexamine the effect of pH further in the in the third quarter studies.

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.

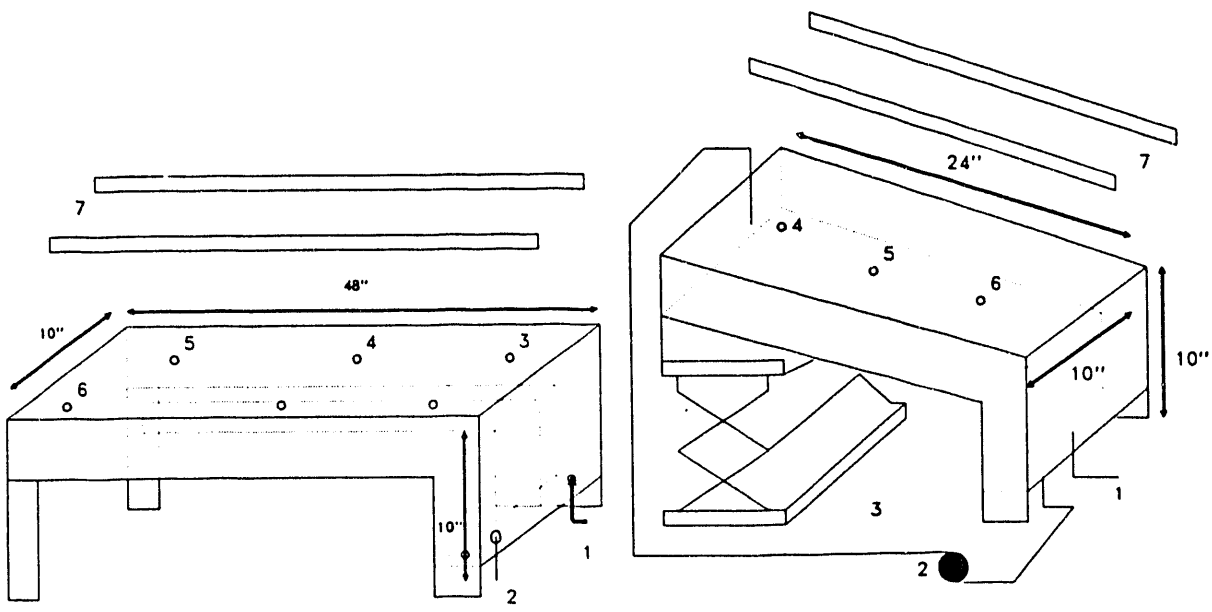
† Equivalent to 1% CO_2.

Table 2. 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	22.0
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.



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 1. SCHEMATIC ILLUSTRATION AND APPROXIMATE DIMENSIONS OF THE ALGAE PHOTOBIOREACTORS ASSEMBLED BY USING 1/2 INCH THICK PLEXIGLASS PLATE

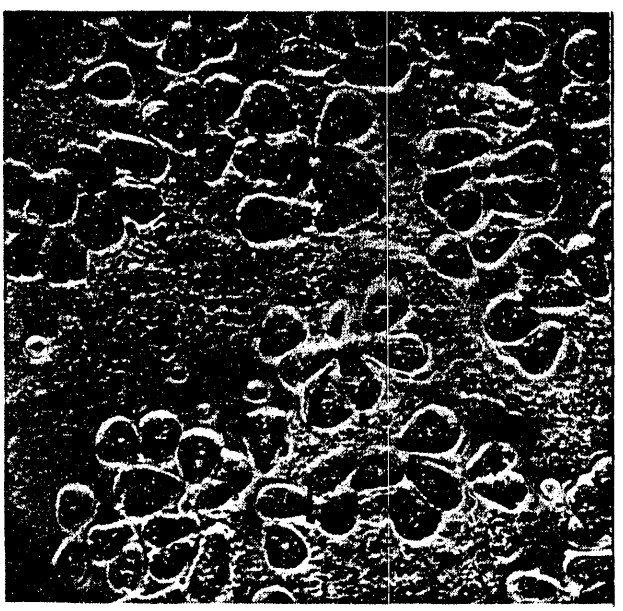


Figure 2. Botryococcus braunii CELLS WITH OIL DROPLETS AS SEEN UNDER MICROSCOPE

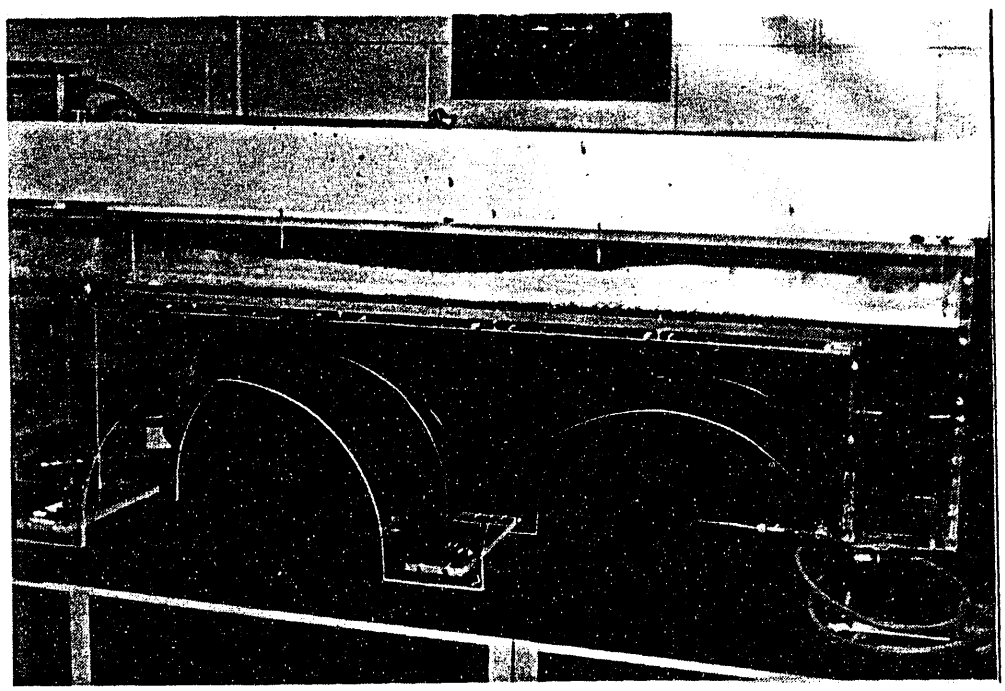


Figure 3. RACEWAY BIOREACTOR WITH Boryococcus braunii CULTURE

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 (milli-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.

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. Autoclave solution biotin.*

* 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.

APPENDIX 2

IMMOBILIZATION OF Botryococcus braunni

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 resupsended in Bold's Bristol's Medium (pH 10, CAPS buffer) and added to the bioreactor.

PROJECT MANAGEMENT REPORT
December 1, 1992 through February 28, 1993

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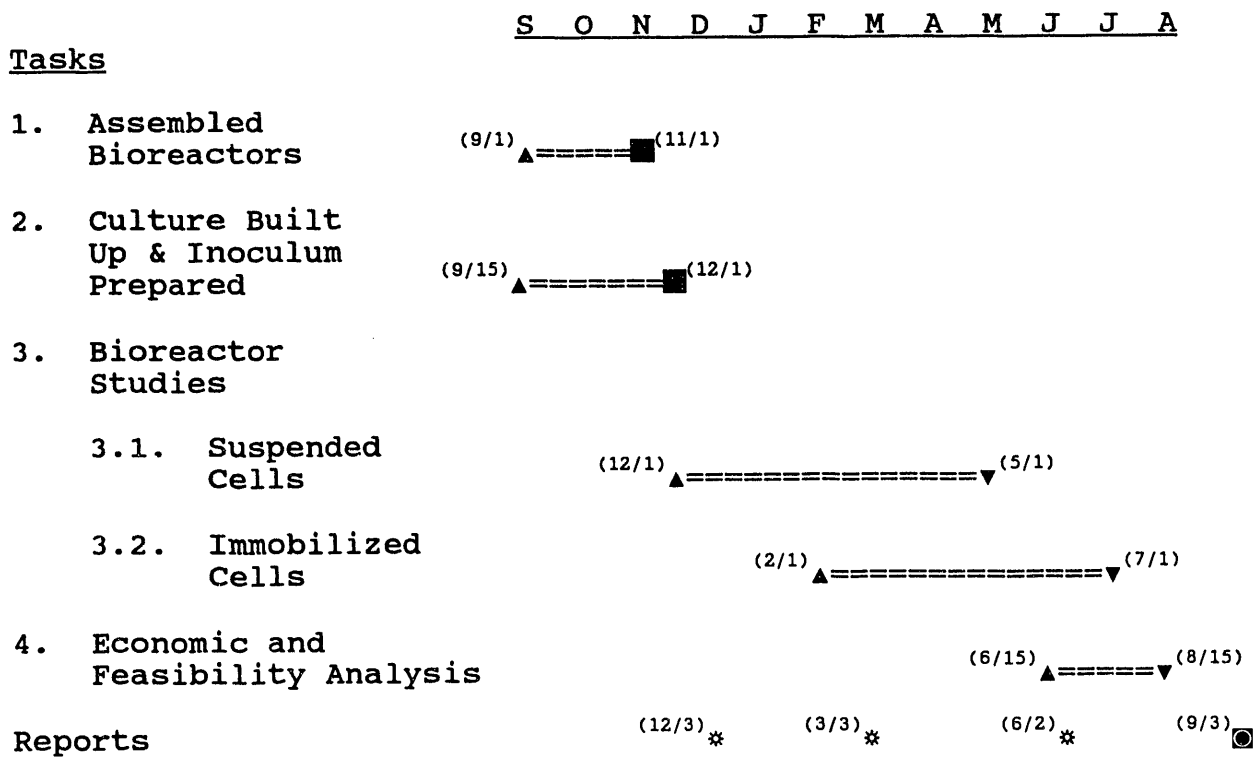
Principal Investigator: Cavit Akin
Institute of Gas Technology
Other Investigators: Andrea Maka and Salil Pradhan
Institute of Gas Technology
Project Manager: Dr. Dan Banerjee
Illinois Clean Coal Institute

COMMENTS

There were no significant deviations of estimated actual cost from the projected cost. No technical personnel changes took place. All equipment and cultures were in time. The research work progressed as planned in the proposal.

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
6/17/93