DOE/PC/92521--T41

TECHNICAL REPORT
March 1, 1993 through May 31, 1993

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

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

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 third quarter of this research program was to determine the growth and hydrocarbon formation characteristics of free and immobilized cells of Botryococcus braunii in bench-scale photobioreactors. Raceway and inclined surface type bioreactors were used for free cell and immobilized cell studies respectively. The free cell studies with air and CO_2 enriched air [10% (v/v) CO_2 in air] in media with and without $NaHCO_3$ were conducted. In the free cell system the extractable oil productivity was about 15 grams of oil per 100 gram of cell dry weight. This production level was achieved in 2% $NaHCO_3$ medium with 10% (v/v) CO_2 enriched air. In the 2% $NaHCO_3$ containing medium protozoa activity has disappeared, and bacterial and algal contaminations were suppressed and the system could be run contamination free for over 8 weeks. In the calcium alginate immobilized system, the support matrix lost its textural integrity in gas streams with 10% CO_2. A thin layer of solidified agar on the bottom surface of the photobioreactor was tested and found satisfactory as an immobilization surface for the algae.

U.S. DOE Patent Clearance is NOT required prior to the publication of this document.

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. 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 third quarter of this research program was to determine the growth and hydrocarbon formation characteristics of free and immobilized cells of Botryococcus braunii in bench-scale photobioreactors. Raceway and inclined surface type bioreactors were used for free cell and immobilized cell studies respectively.

The free cell studies with air and CO_2 enriched air [10% (v/v) CO_2 in air] in media with and without $NaHCO_3$ were conducted. In the free cell system the extractable oil productivity was about 15 grams of oil per 100 gram of cell dry weight. This production level was achieved in 2% $NaHCO_3$ medium with 10% (v/v) CO_2 enriched air. In the 2% $NaHCO_3$ containing medium protozoa activity has disappeared, and bacterial and algal contaminations were suppressed and the system could be run contamination free for over 8 weeks. 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 on the bottom surface of the photobioreactor could serve as the immobilization surface for the cells in place of the alginate beads.

This 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.

The next quarter of this research program will involve continuation of the immobilized cell studies and the preliminary feasibility and economic analyses of the conversion of flue gas CO_2 to hydrocarbons by free and immobilized cultures of Botryococcus braunii, and identify areas of research emphasis for development of an economically viable process.

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: 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 were used. The schematic illustrations and the operation procedures of these photobioreactors were given in the second quarterly report.

Test Media: Bold's Bristol's modified medium, with or without the addition of $NaHCO_3$, was used as a the growth medium (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.

Studies in the Raceway Photobioreactor: 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. After three weeks of incubation, 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.

Immobilized Cells: Initially 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.

Determination of Biomass Dry Weight: In free cell systems algae cells were harvested from the bioreactor medium by centrifugation. 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.

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. 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 percent $NaHCO_3$ containing media. 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 photobioreactors.

Media pH: The initial pH of the Bold's Bristol's modified medium was 7.3 and maintained this level in the phosphate buffered system. Since CO_2 has a strong buffering effect, the pH of the CAPS buffered medium dropped form 10 to 8.2 upon sparging 10 percent CO_2. In the Bold's Bristol's modified medium with 2 percent $NaHCO_3$ the initial pH was about 10, and dropped to 8.3 upon sparging with 10 percent CO_2.

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.

Illumination: The bioreactors were illuminated continuously 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 and inclined surface bioreactors. Illumination of the 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.

General Test Matrix: Tests were 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 1. Each test was run in duplicate.

RESULTS AND DISCUSSION

Free Cell Studies: 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. 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. 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. Contamination by other algal cells was observed toward the end of the third week at which time the contents of the bioreactor was collected, 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 biomass concentration was 23 mg/100 ml, and the oil content was 15 g/100 g dry biomass. 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. (This reactor is still operating without any contamination). 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. 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 satisfactory for the immobilized cell studies. Agar surface immobilized cell studies are in progress.

CONCLUSIONS AND RECOMMENDATIONS

The reported third quarter studies confirmed our second quarter observations that Botryococcus braunii can tolerate and grow well in 10% CO_2 and produce oil. The capability of Botryococcus braunii to grow in 2% $NaHCO_3$ solution and suppression of the bacterial and algal contamination and the protozoa activity 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 by Botryococcus braunii. Calcium alginate immobilization is not practical in presence of 10% CO_2 and it would be similarly inconvenient in the presence of $NaHCO_3$. Other surface immobilization techniques need be developed. Oil productivity we observed so far is low and conditions that improve the oil productivity need be identified. In the forth quarter studies, surface adhesion immobilization systems will be continued and the economic and feasibility of the conceptual process for the removal of CO_2 from flue gases by algae will be analyzed and research needs for development of an economical process will be suggested.

Table 1. 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.

Table 2. 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[b]	7.3	10	--	23	6
3	8.2	10	--	178[c]	5
4	8.3	10	2.0	200[d]	15
5[e]	8.3	10	2.0	In Progress	

[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.

APPENDIX 1

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.

$NaHCO_3$ Added Media:

Bold's Bristol's medium is utilized and $NaHCO_3$ is added to achieve 2% $NaHCO_3$ concentration.

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
March 1, 1993 through May 31, 1993

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

Principal Investigator: Cavit Akin
Institute of Gas Technology
Other Investigators: 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. Co-investigator Dr. Andrea Maka left IGT at mid March. Dr. Akin took over the laboratory work which was conducted by Dr. Maka. All equipment and cultures were in time. The research work progressed as planned in the proposal.

EXPENDITURES - EXHIBIT B

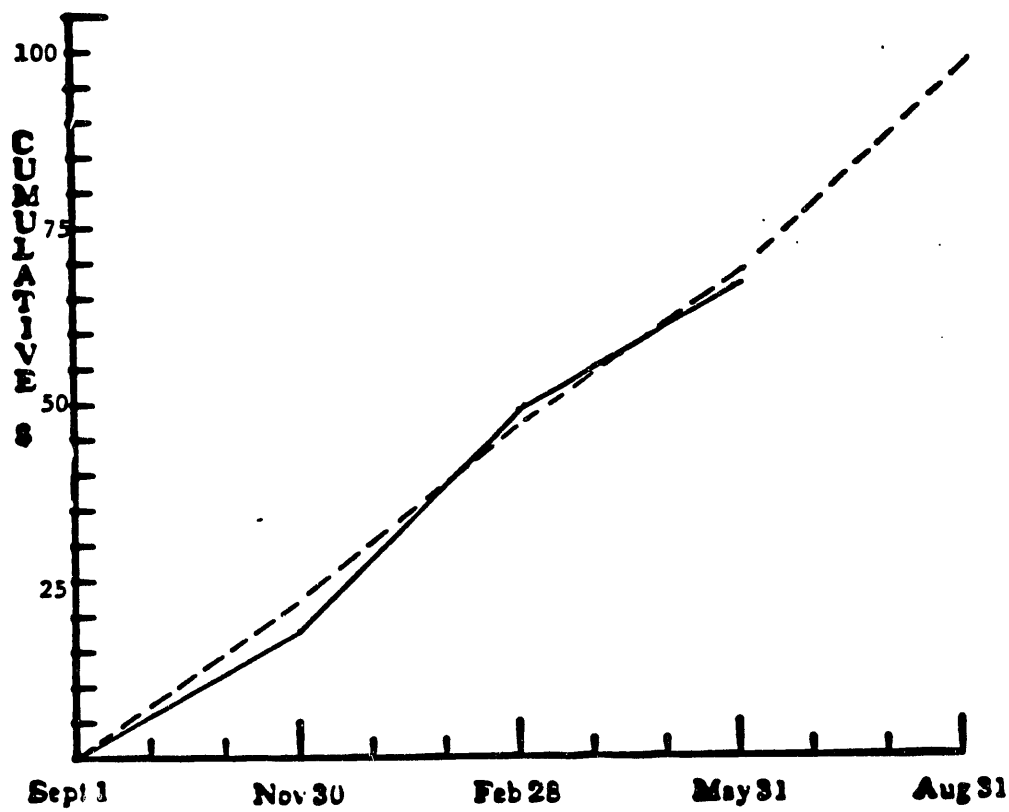
Cumulative Projected and Estimated Actual Expenditures by Quarter

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							

* Cumulative by quarter

IGT Project No. 40327

CUMMULATIVE COSTS BY QUARTER - EXHIBIT C



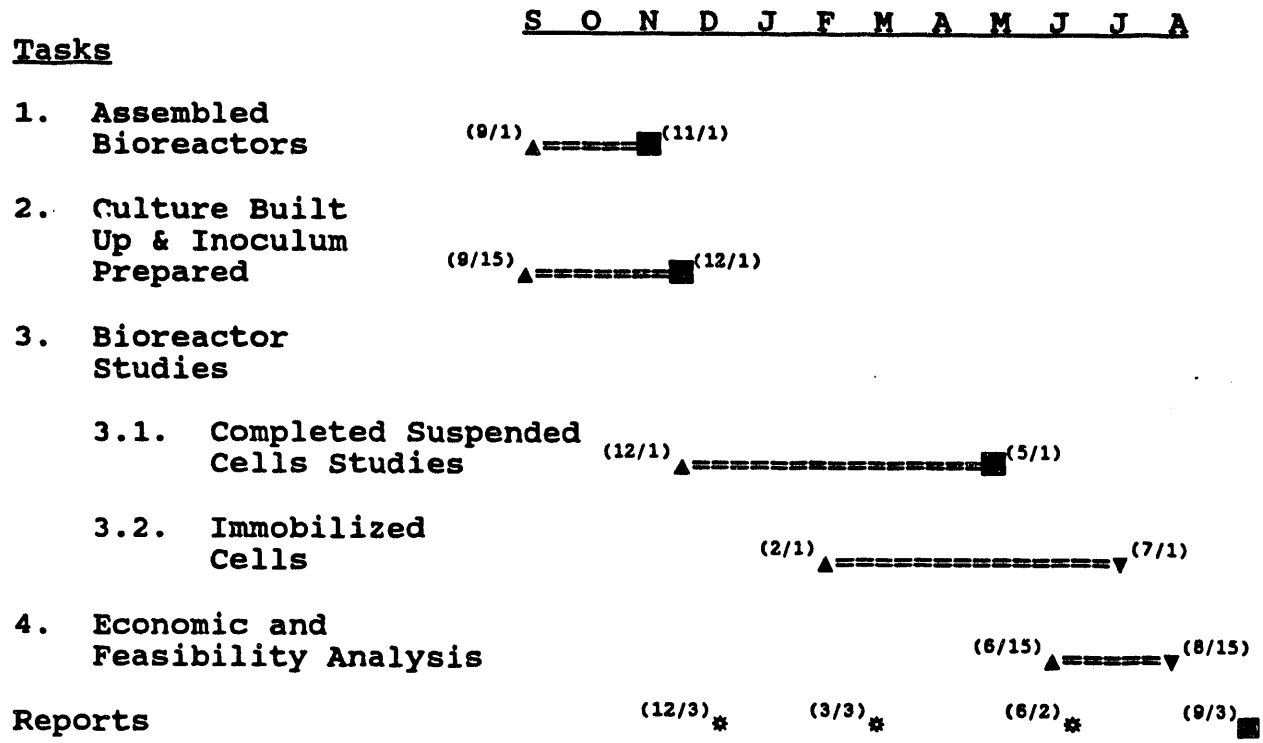
Months and Quarters

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

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