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Presented here is an investigation and comparison of two entrapment methods of algae. Conventional calcium alginate bead entrapment is compared to a recently described modified bead entrapment method, utilizing poly(ethylene glycol) and calcium alginate, which results in void or capillary formation within the beads. It is shown that algal growth rates within these modified beads is higher than in conventional calcium alginate beads. The conventional system resulted in a maximum growth rate of 1.0×10^7 (cells/ml beads)/day, while the modified beads resulted in a maximum growth rate of 1.8×10^7 (cells/ml beads)/day. Limiting the amount of cell escape from entrapped beads is an important aspect of this investigation. It could not be properly determined whether cell escape was reduced through use of modified beads, due to experimental complications, however, the use of a larger growth vessel reduced cell leakage due to a lessening of frictional forces acting on the beads.

Entrapment of Micro-Algae in Modified Alginate Beads for Oxygen and Nutrient Source

by

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

Page

LIST OF FIGURES

LIST OF APPENDICES

LIST OF APPENDIX TABLES

Entrapment of Micro-Algae in Modified Alginate Beads for Oxygen and Nutrient Source

INTRODUCTION

Problem Definition

Algae are among the smallest living organisms, and yet play one of the most important roles in sustaining life on earth. The photosynthetic function of algae is beneficial as a sink for carbon dioxide and a producer of oxygen, which could be utilized for space (Wharton, Smernoff, & Averner, 1988) and submarine life-support (Benoit, 1964). However, algae are also valuable for a number of other reasons. Algae can play a significant role in wastewater treatment and nitrogen fixation, as well as the production of protein, carotenoids, fatty acids, sugars, vitamins, and antibiotics for human use (Borowitzka, 2001). Algae can also be utilized as a source of fuel, such as methane, ethanol, and hydrogen (Calvin & Taylor, 1989). With the ever-increasing use of genetic engineering, algal cells can be manipulated into manufacturing a wide variety of organic compounds (van der Meer, 1988), which forces investigators to look for methods of growing algae in new and unique ways to take advantage of these new developments.

The use of bioreactors (growth vessels) has been extensively developed, but bioreactors still have limitations. Unlike many bioreactor systems, light plays a major role in the function of algal growth vessels. Therefore, reactor biofouling (the immobilization of cells on the inside wall of the reactor vessel) is of major concern in algal cell growth, as well as sedimentation of algal cells. To alleviate these limitations, cells may be

immobilized purposefully through the use of entrapment or encapsulation techniques (Joo, 2001), whereby the cells are enclosed within polymerized beads to prevent accumulation of cells in the liquid medium. As cell concentration increases in the liquid medium, light transmission to cells in the beads, in particular cells in the center of the growth vessels becomes greatly limited. Cell entrapment allows small molecules that are released extracellularly to diffuse out of the bead matrix, whereas molecules that are required by the algae may diffuse through the gel matrix from the liquid medium to nourish the cells, effectively acting as a filtration system. For molecules that are contained within the algal cells, harvesting of the cells is made easy as the cells are contained within the beads, and filtration or centrifugation of the medium is not needed.

Gel entrapment was explored in this investigation as a cost-effective and relatively simple method for production of gel beads. One commonly used gel polymer is sodium alginate (Figure 1), a polysaccharide consisting of glycosidically linked α-L-guluronic acid (G) and its C-5 epimer β-D-mannuronic acid (M), obtained from brown seaweeds (McHugh, 1987). The properties inherent in sodium alginate allow for a gel to form when sodium

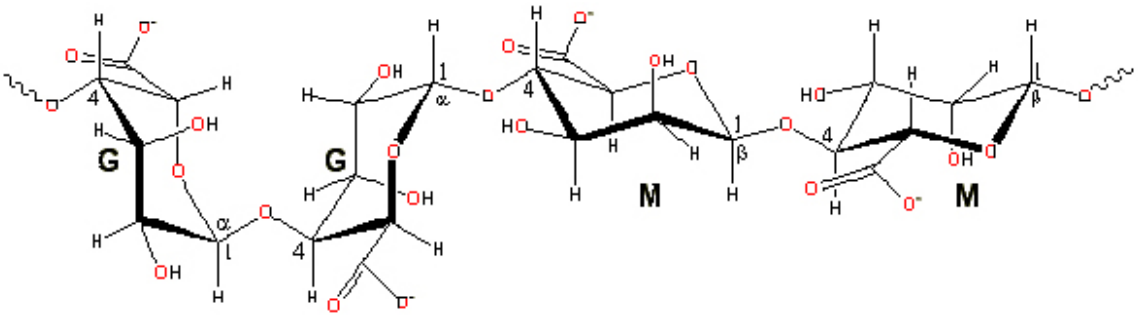

Figure 1. The Structure of Alginate (Chaplin, 2003).

alginate is introduced to a multivalent cation solution, such as calcium chloride. A gel is formed via cross-linking in a method commonly called the “egg-box model” (Figure 2), in which G chains cross-link, displacing sodium ions (Grant, 1973). In particular, sodium alginate added drop-wise to calcium chloride will produce a spherical calcium alginate gel bead closely correlated to the size of the sodium alginate drop.

One of the drawbacks of this simple procedure is that algal cells can escape the bead through frictional wear on the surface of the bead, as well as from the pressure of additional cell growth within the bead. A modified bead may be developed that would eliminate algal cell escape from the bead.

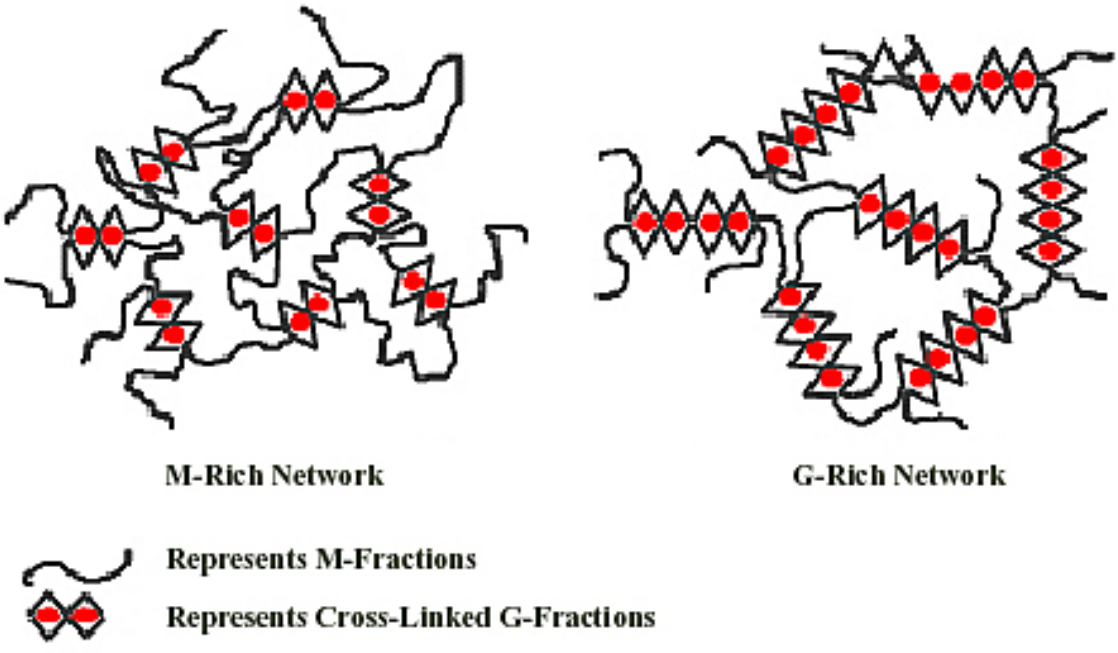

Figure 2. The egg-box model of alginate cross-linking (Novamatrix, 2003).

Statement of Purpose

Through entrapment with modified calcium alginate beads, which could be labeled a one-step encapsulation method, algae can be more securely contained within the gel bead and prevented from surface fouling and sedimentation. One method to combat algal escape from gel beads is encapsulation, but this proposed new method is in effect encapsulation. However, traditional methods of encapsulation require three steps to form a capsule, but with the method explored in this investigation, only one step is required to achieve a capsule-like environment. This method, to effectively reduce bead leakage was

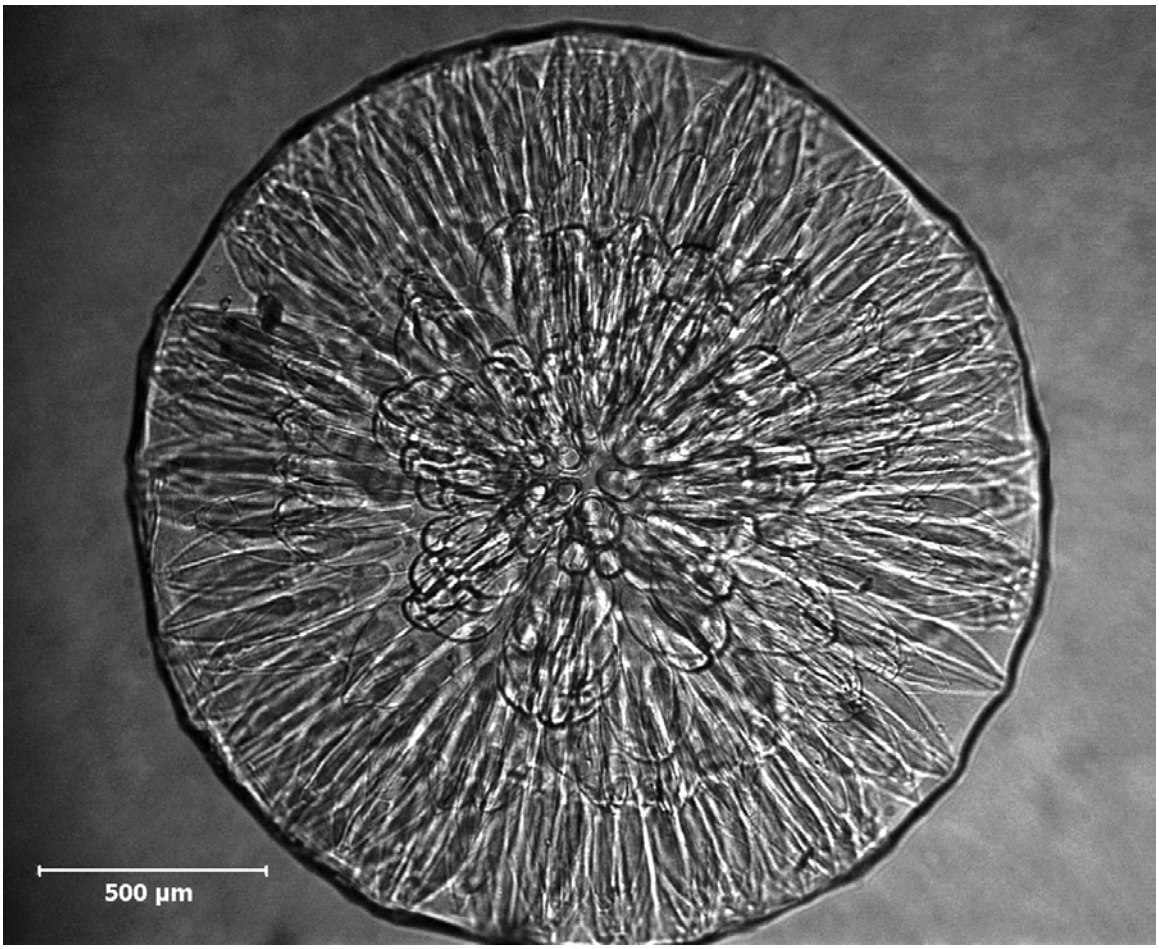

Figure 3. Voids in modified bead, formed with 10% PEG.

introduced by Seifert and Phillips (1997), in which poly(ethylene glycol) (PEG) is utilized to form void spaces, sometimes referred to as capillaries, within the interior of the bead (Figure 3). These voids are oriented radially from the center towards the surface of the sphere (Figure 4), and allow cells to propagate within the spaces to achieve a

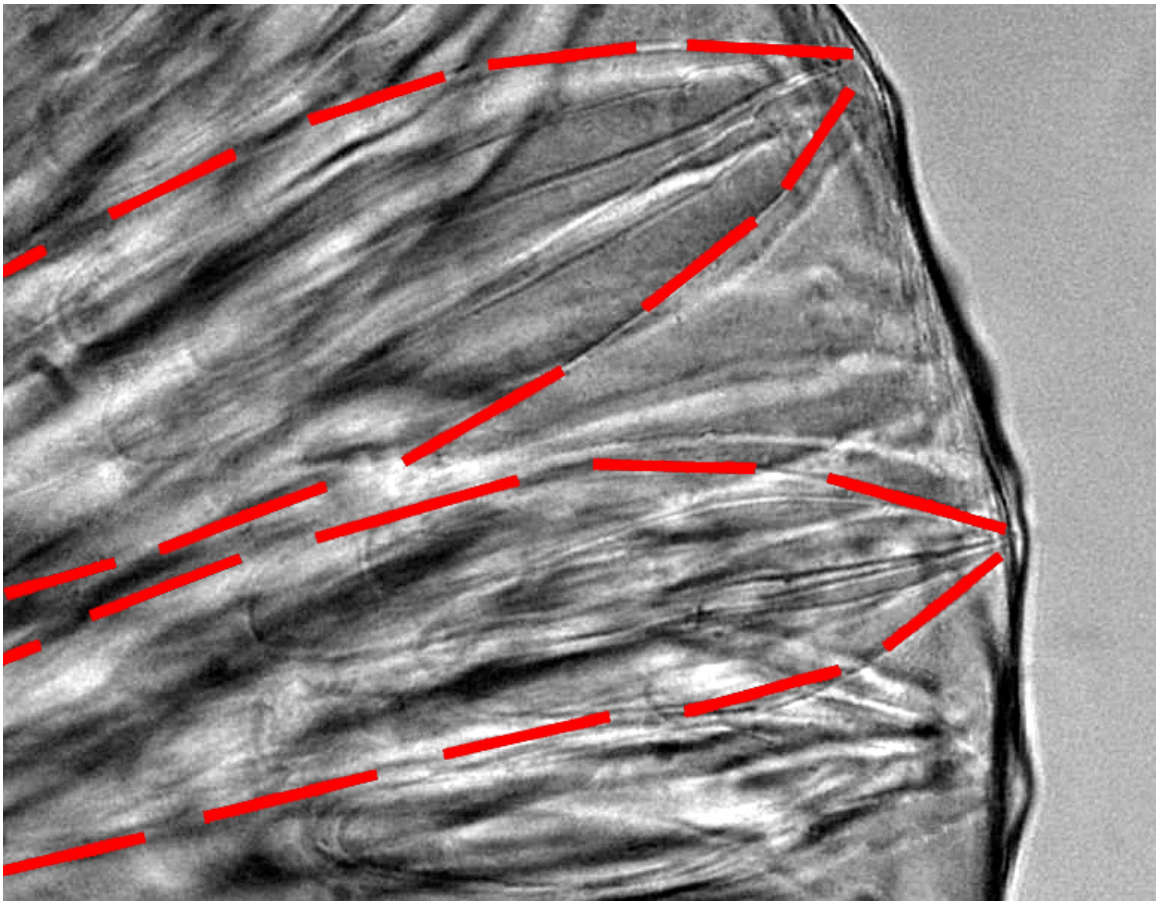

Figure 4. Voids in PEG-modified bead. Two voids are outlined in red. Width of image is 400 micrometers.

higher cell concentration than with ordinary calcium alginate beads. This method is especially useful for anchorage-dependent cells, effectively increasing the surface area of the bead many-fold. The calcium alginate also forms a denser gel, which is stronger and

can better withstand frictional effects and contain algal cells within the interior of the bead.

Seifert and Phillips also found that the PEG-modified beads had more rapid cell growth than with conventional calcium alginate beads, with growth similar to that observed in conventionally encapsulated cells. The various types of entrapment and encapsulation vary, however, in their diffusional limitations, whereby diffusion into PEG-modified beads is not limited by cells attached to the interior surface of the bead as occurs in capsules. The authors also calculated a theoretical maximum concentration of hybridoma cells based on cell volume (Figure 5), whereby the volume of PEG-induced voids was

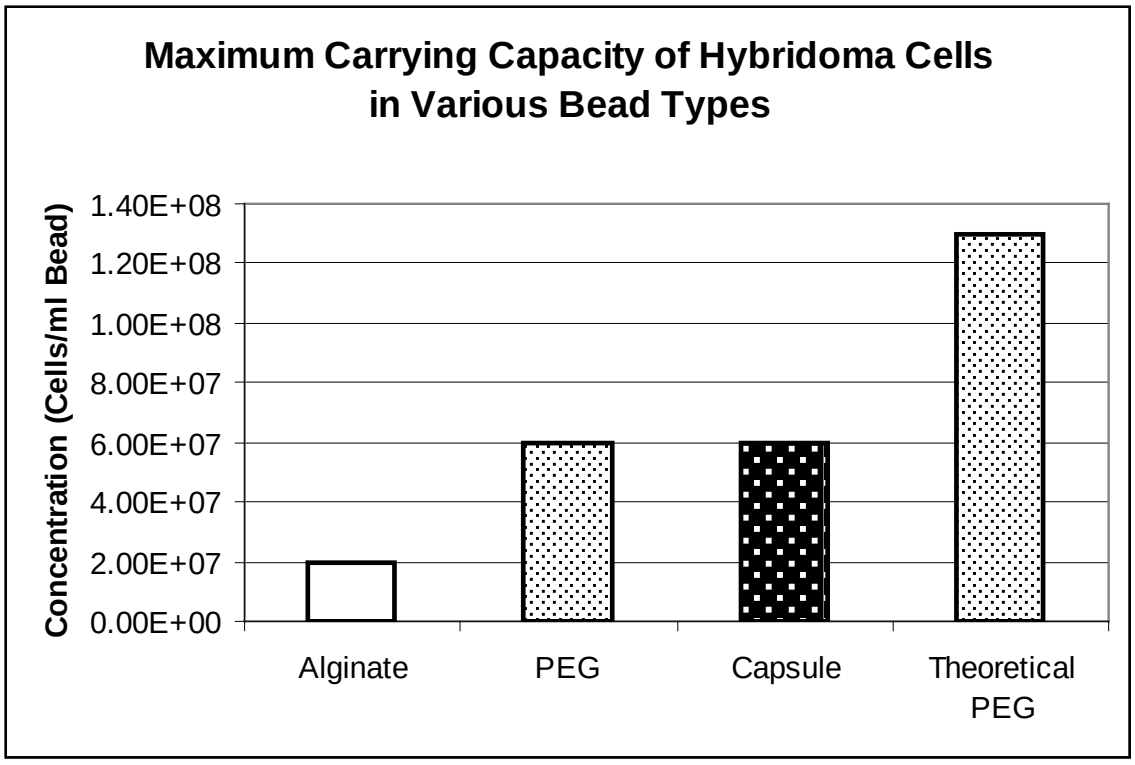

Figure 5. Carrying capacity of hybridoma cells for various bead types. Alginate = conventional calcium alginate entrapment, PEG = PEG-modified entrapment, Capsule = encapsulation, Theoretical PEG = calculated theoretical maximum concentration of hybridoma in PEG-modified beads.

approximated and this volume was divided by the volume of a single hybridoma cell. Through comparison of algal cell volume with hybridoma cell volume, a theoretical maximum concentration of algal cells in PEG-modified beads can be obtained (presented later), neglecting any diffusional limitations that may occur at so dense a concentration.

In this work, PEG-modified calcium alginate entrapment will be compared with the traditional calcium alginate method, in order to maximize the growth rate and carrying capacity of algal cells in the beads. The effectiveness of containment will also be analyzed, because it is important to limit biofouling of the reactor walls.

MATERIALS AND METHODS

Materials

The sodium alginate used was Keltone HV (average molecular weight 150,000 – 180,000) from Kelco (San Diego, CA). Poly(ethylene glycol) 400 was from Union Carbide (Danbury, CT). Calcium chloride dihydrate, powder (#4160) was from Mallinckrodt (Phillipsburg, NJ). Sodium citrate dihydrate, crystal (#0754) was from Mallinckrodt (Phillipsburg, NJ). Pluronic® F108 detergent was obtained from BASF Co. (Parsippany, NJ). The alga was *Oocystis* and the growth medium was ASM-1 (modified), both obtained from Eric Henry, PhD (Department of Botany and Plant Pathology, Oregon State University), specifications for the growth medium are listed in Appendix A. The spectrophotometer used was an EMDS Unipak 500 utilizing 1.50 ml cuvettes at a wavelength of 546 nanometers. This wavelength is not the optimal absorbance for *Oocystis*, but was the most convenient as the spectrophotometer only recorded absorbance at this one wavelength.

Calcium Alginate Beads (Control 1 & 2)

Oocystis stock was obtained for two separate control trials at optical densities of 0.461 and 0.413, respectively, at a wavelength of 546 nm. Sodium alginate and R. O. water were combined to form a final 1.0 wt % solution following the addition of algae stock to water at a ratio of 1:9.

Figure 6. 40 ml Syringe with 22 gauge, modified needle dropping sodium alginate into calcium chloride.

Figure 7. Syringe pump facilitating alginate extrusion into slow-mixing $CaCl_2$.

This mixture was loaded into a 40 ml syringe fitted with a 22-gauge needle shortened to a length of 1.5 millimeters to decrease the pressure required to pass the solution through the needle (Figure 6). The mixture was then added drop-wise to 500 mL, 0.10 M calcium chloride (under slow mixing to prevent beads undergoing gelation from cross-linking with each other, but still slow enough to not disrupt bead formation). An automatic syringe pump was used in this extrusion process (Figure 7). Average bead diameters of 2.33 mm, +/- 0.14 mm for Control Trial 1, and 2.29 mm, +/- 0.10 mm for Control Trial 2

(following equilibration with growth medium) were achieved. Beads were allowed to cure for two hours, and were then washed with approximately ten times the bead volume with ASM-1 medium for 1 hour, followed by a second, identical wash for 2 hours before introduction into growth vessels. Control Trial 1 was batched in 125 ml reactors, while Control Trial 2 was batched in 500 ml reactors, to be explained later.

PEG-Modified Calcium Alginate Beads

Oocystis stock was obtained at an optical density of 0.277 at a wavelength of 546 nm. A resulting 1.0 wt % solution of sodium alginate with 10.0 wt % PEG 400 was prepared. Sodium alginate, R. O. water, PEG and algae stock were combined to result in a 1.0 wt % solution of sodium alginate with 10.0 wt % PEG. The ratio of algae stock to water was 1:8.2. This difference in ratio from the control is an attempt at balancing the solution volume, because PEG is a liquid at molecular weight 400, adding more volume to the overall solution. The mixture was loaded into a 40 ml syringe fitted with a 22-gauge needle. The mixture was then added drop-wise to 0.10 M calcium chloride resulting in an average bead diameter of 2.54 mm, +/- 0.12 mm (following equilibration with growth medium). Beads were allowed to cure for two hours, and washed for three hours in ASM-1 medium prior to introduction to the growth vessels.

Bioreactor Operation

Two different types of growth vessels were utilized in this investigation.

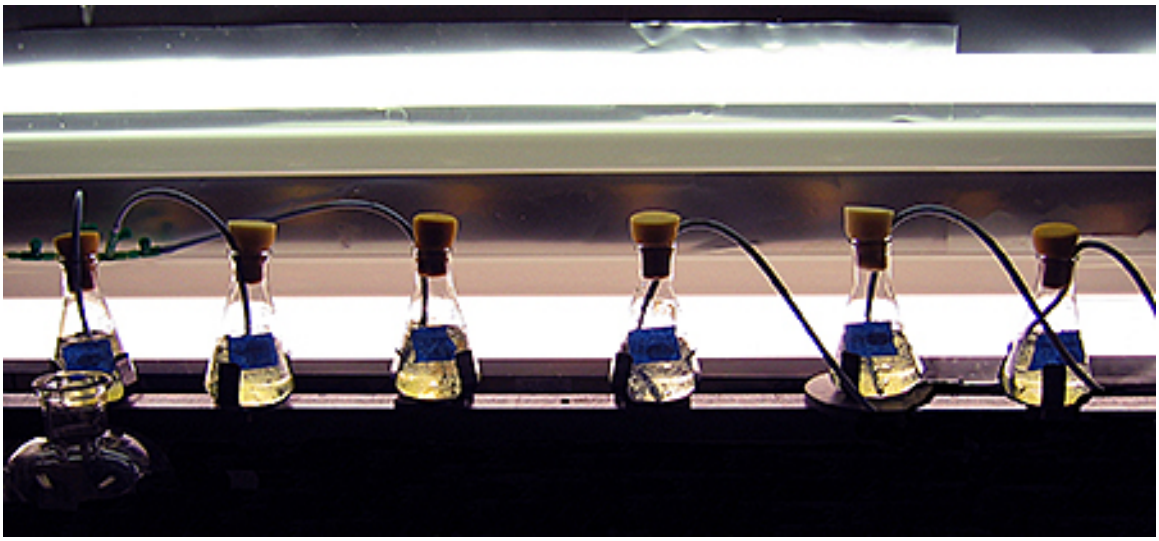

Figure 8. Erlenmeyer flask growth vessels (bioreactors) of 125 ml.

Erlenmeyer flasks of 125 ml were used for the growth of Control 1 and PEG-modified calcium alginate beads (Figures 8 & 9).

Tissue cell culture flasks with a volume of 500 ml were used for growth of Control 2 (Figures 10 & 11). The larger growth vessels were used to attempt to maximize light transmittance into the reactor. A trial with PEG-modified beads was also run in these larger vessels but failed to grow, probably due to PEG toxicity, which will be explained later.

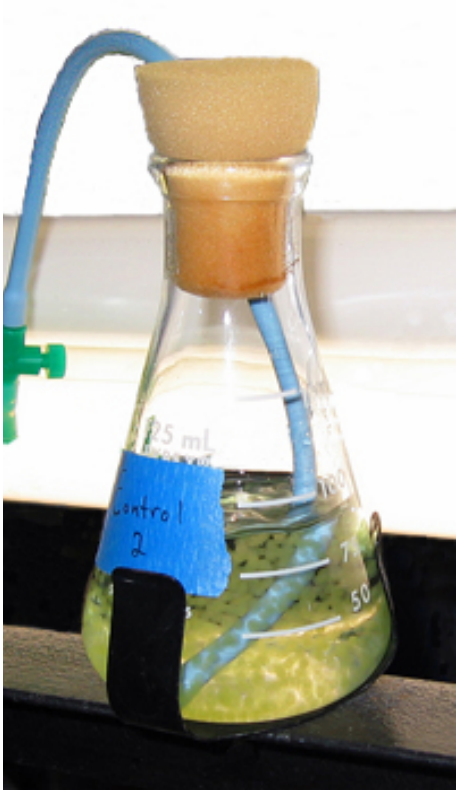

Figure 9. 125 ml growth vessel.

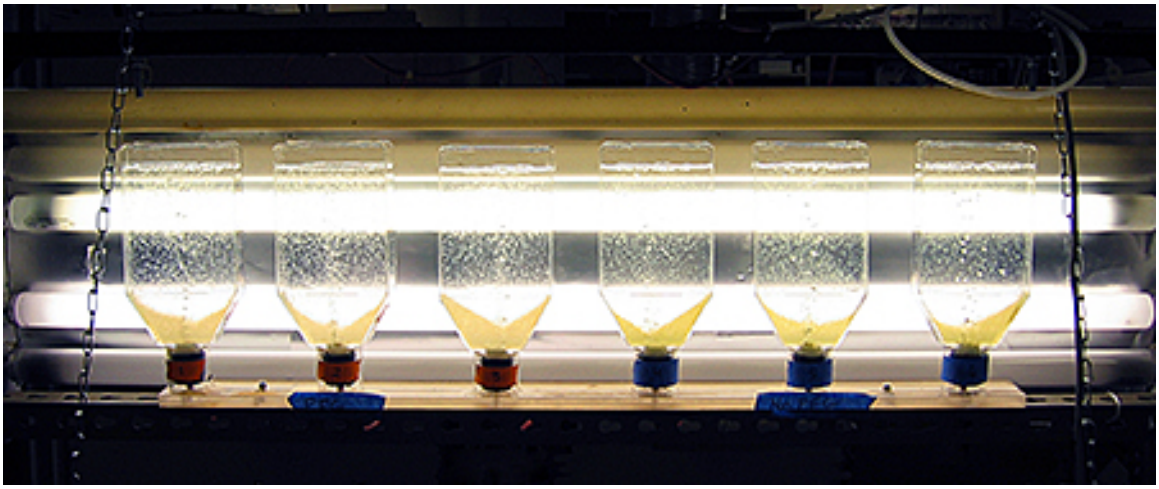

Figure 10. Tissue cell culture flask growth vessels (bioreactors) of 500 ml.

The growth vessels were aligned in front of two 40-inch, 40 watt, cool white, fluorescent tubes with metallic reflector. The tubes were in constant operation. The 125 ml flasks were stoppered with foam, and beads were agitated by a bubble stream from a small tube that passed through the foam and sparged air at the bottom of the vessels. The airflow rate was maintained at the lowest rate that would maintain constant movement of beads, to limit sedimentation of the beads. The 500 ml flasks were positioned inverted with the wide flat side of the flask facing the light source. A small hole was bored into the (now top) end opposite the cap. A hole was also bored through the cap

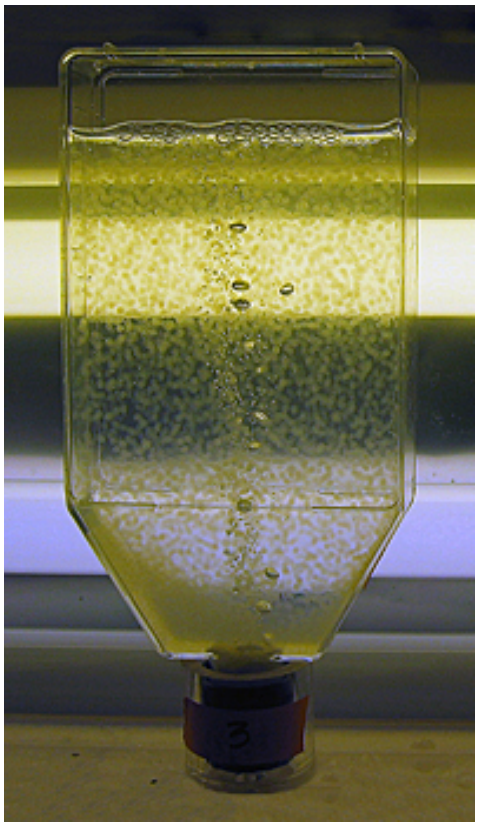

Figure 11. 500 ml growth vessel.

itself and a tube with a sparger was inserted inside the flask so that the protruding end of the sparger was level with the opening of the main body of the flask. Air was supplied at the lowest rate that would maintain constant movement of the beads. Due to the larger size of the tissue cell culture flasks, more beads were produced for this system. For both systems, the ratio of bead volume to culture medium was approximately 1:5. Medium was added as needed to compensate for evaporation caused by the airflow.

Dissolution of Gel Beads

Beads were first washed with water to attempt to remove algal cells that may have been attached to the exterior of the bead. Beads were dissolved by addition of a small number of beads to a known volume of 0.10 M sodium citrate, which chelates Ca++ and disrupts the gel structure. In a 5 ml beaker, aided by agitation with a magnetic “flea”, beads usually dissolved within 5 minutes.

Determination of Algal Cell Concentrations

Algal concentrations were determined through use of a spectrophotometer and a standard calibration curve (Figure 12). The spectrophotometer was calibrated with manual counts of algal cells (with a hemacytometer) of suspensions of known optical densities.

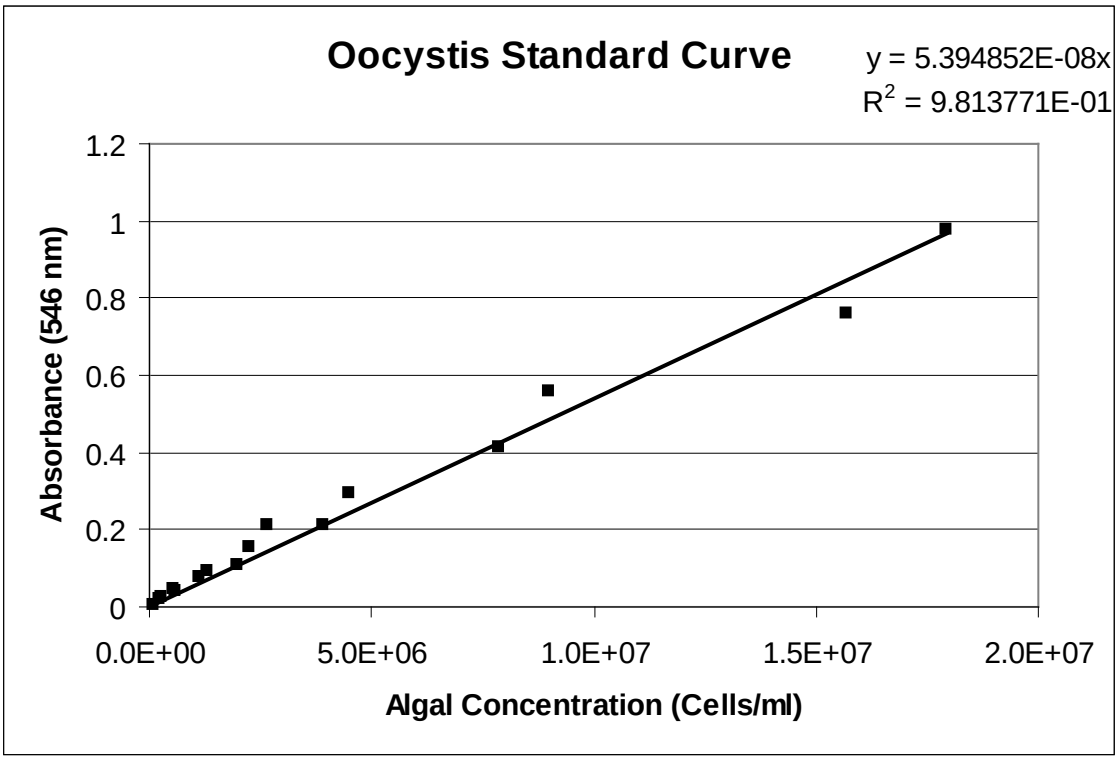

Figure 12. Standard spectrophotometric calibration curve for *Oocystis* at 546 nm.

Algal concentrations were determined by selecting 15 beads at random from a growth vessel. The dissolved solution was then placed in a 1.5 ml cuvette. The absorbance of the dissolved solution was measured. The optical density of calcium alginate dissolved in sodium citrate is 0.10, so this value was subtracted from the optical densities recorded for the dissolved beads. This is not the most accurate assumption, but the resulting density values are more conservative, rather than liberal. The optical density was interpolated into a concentration value through use of the standard curve.

The concentration was then determined in units of algal cells per ml of beads. This was done by determining the volume of the 15 beads, assuming the beads to be perfect spheres, by measuring the diameter of a sample bead population by microscope. The

dilution with sodium citrate was taken into account, and the resulting concentration of cells per ml of bead volume was obtained (Appendix B).

Cell escape or leakage from the beads into the medium was determined by spectrophotometer at 546 nm, utilizing a water blank. This is not an accurate measure of cell escape due to cell divisions occurring after cells escape into the medium, but it allows for a first approximation of the amount of escape occurring.

RESULTS

Two trials were run, one in 125 ml vessels and the other in 500 ml vessels for a period of 15 to 20 days. Conventional calcium alginate beads (Control) were compared with PEG-modified beads. The trials for these systems were run in triplicate. The three bead systems were not run at the same time due to death of algal cells entrapped in PEG-modified beads. This algal death has been attributed to toxic effects of PEG when in contact with algae, and the time algal cells spend in PEG should be as limited as possible. Complications with control 3 were also experienced as a result of the reactor medium evaporating due to an airflow rate greater than anticipated.

Using the standard curve and methods previously mentioned, growth rates were obtained for algae within the two bead systems (Figure 13). Cell escape or leakage from the beads was also determined as growth rate in the medium (Figure 14). Three sets of data are represented on this plot to show the differences in leakage between the two growth vessel types. As cellular concentration within the medium increased, medium was changed to maximize light transmittance. These changes in medium are reflected on the figure representing cell leakage. Cell leakage was found to be minimal in the control and PEG-modified systems of the 125 ml reactors until about 5 days into the experiment. Cell leakage was minimal for the entirety of the trial in the 500 ml vessels.

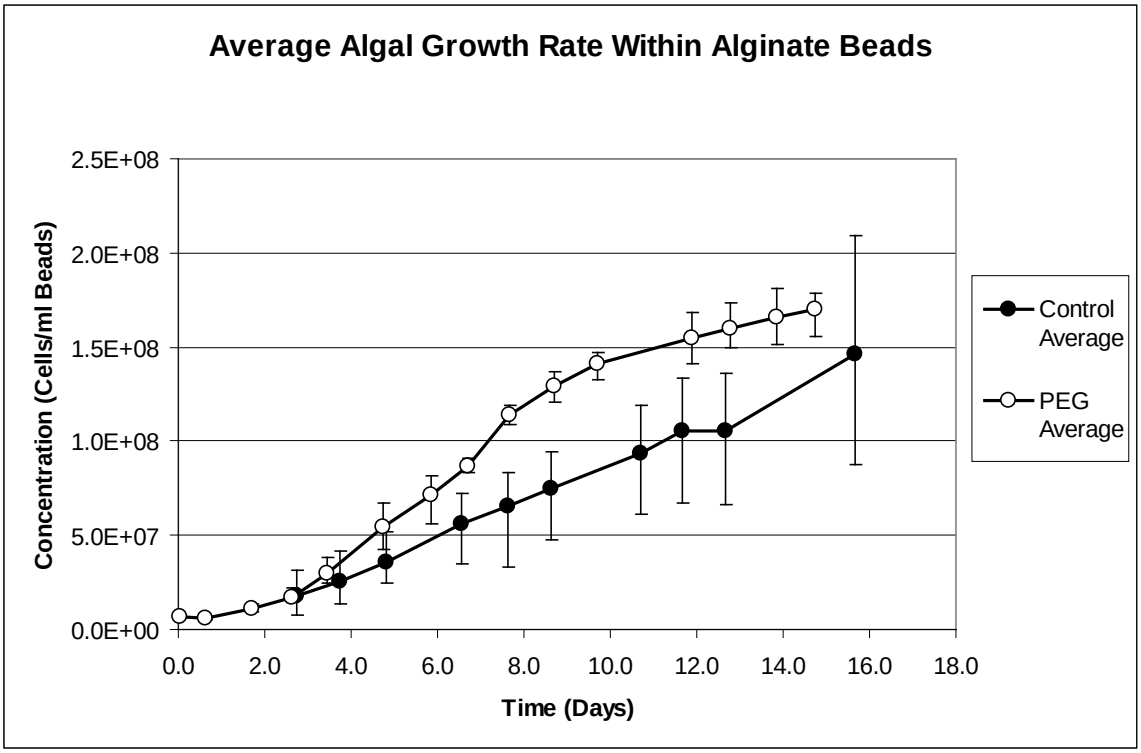

Figure 13. Average algal growth rates within alginate beads.

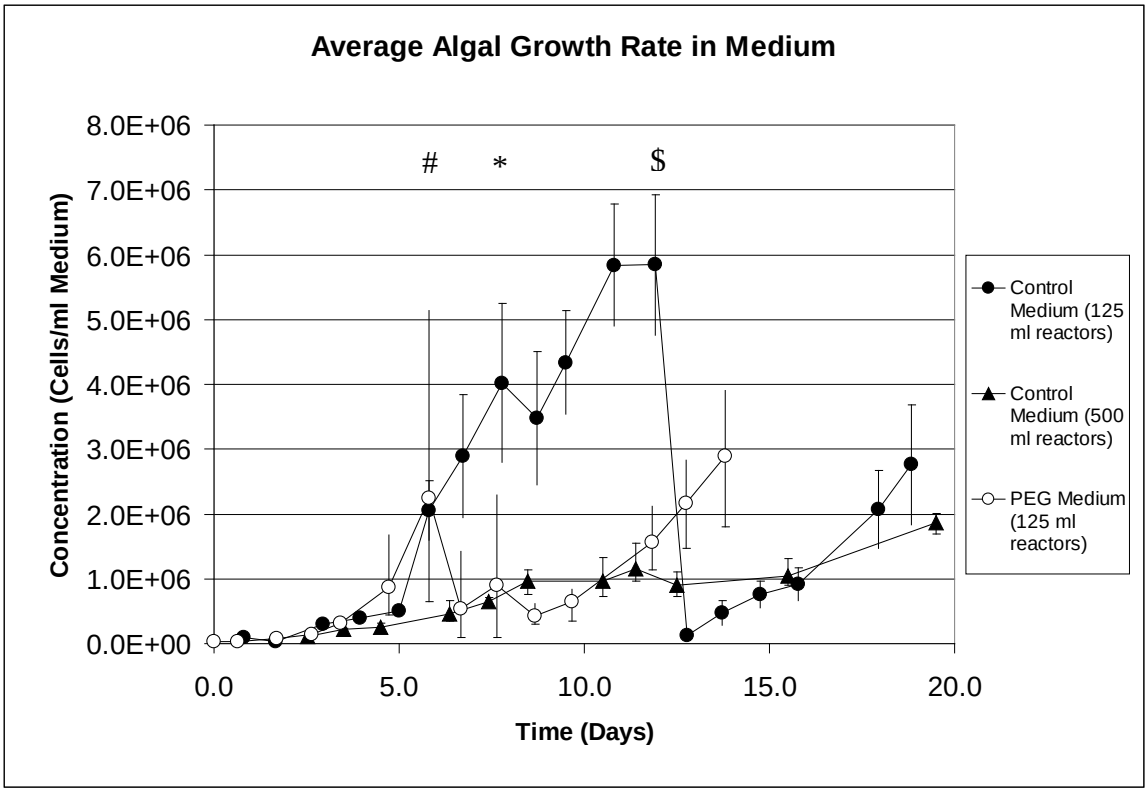

Figure 14. Average algal growth rates in the medium (algal escape rate). At times denoted by these symbols, #, PEG Medium changed; *, PEG Medium and Control Medium (125 ml reactors) changed; $, Control Medium (125 ml reactor) changed.

DISCUSSION

Comparison of Growth Rates

The two entrapment systems were found to permit the growth of algal cells. It was also found that algae within the PEG-modified beads had a higher growth rate. To quantify the data, growth rates were derived from the linear slope of the data obtained in Figure 13 (Figure 15). These data were obtained using six data points within this linear region,

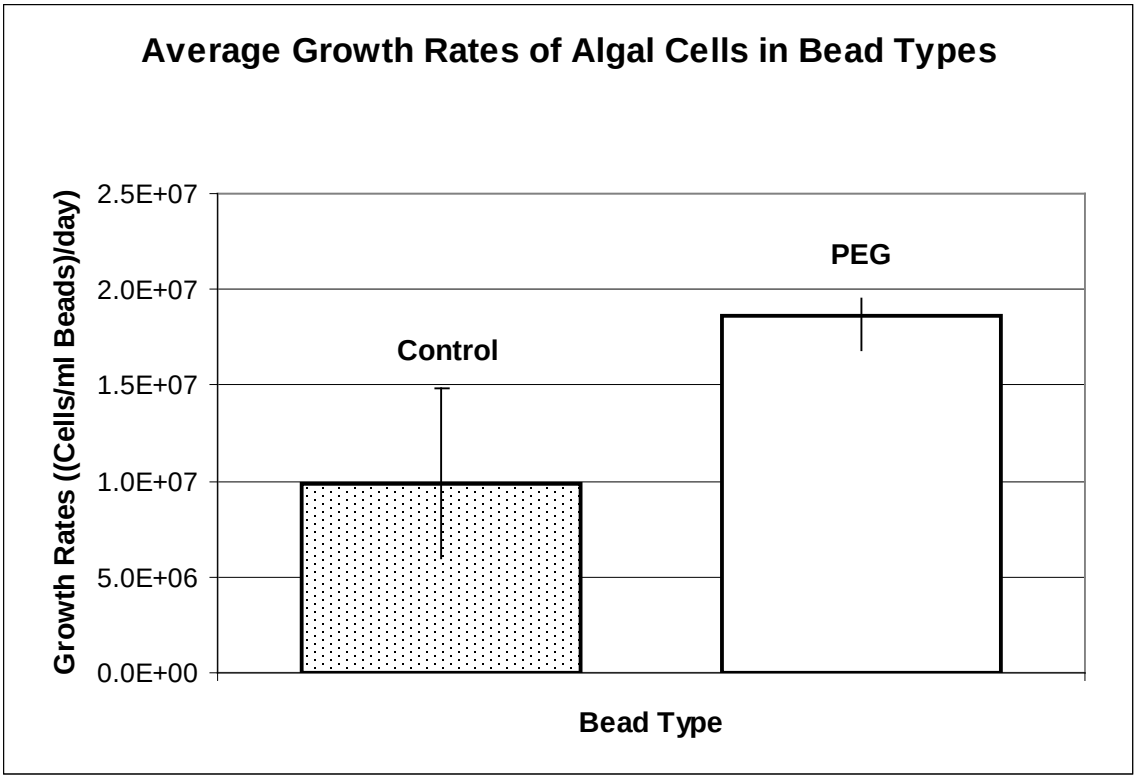

Figure 15. Comparison of average algal growth rates.

immediately following the initial lag phase. As evidenced, there is a substantial difference between growth rates between the two bead systems. When averaging the maximum growth rates the control was found to be about 1.0×10^7 cells/ml beads/day,

whereas the maximum growth rate within the PEG-modified beads was over 1.8×10^7 cells/ml beads/day. Statistical analysis, a Student's t-test, utilizing Microsoft Excel was done on growth rates (Appendix C). From this analysis it was found that the difference between the mean maximum growth rate of the algal cells entrapped within PEG-modified beads was significantly different from the mean maximum growth rate of the algal cells entrapped in the control beads, with $p<0.01$.

Distribution of Cells within Beads

As seen in Day 11 of Figure 16, the growth of algal cells in conventional alginate beads (control) resulted in bundles of cells distributed throughout the bead. Also seen in the

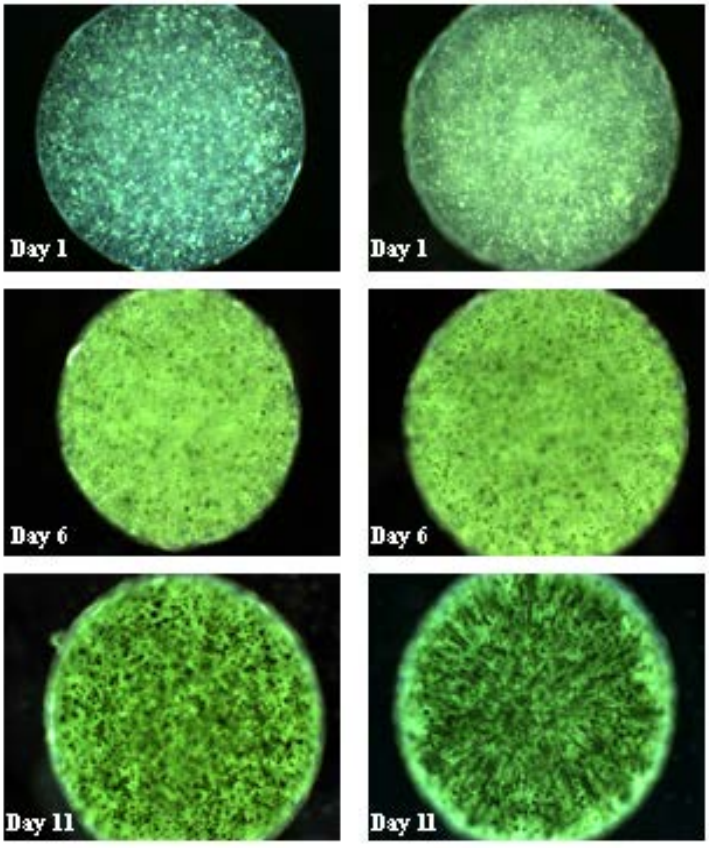

Figure 16. Growth of *Oocystis* in control (left) and PEG-modified (right) (magnification 40x).

figure is the distribution of cells into the voids of the PEG-modified beads. When comparing the two entrapment methods, the PEG-modified beads are less restrictive and allow cells more room to grow, which probably results in the higher growth rates obtained. Clumped cells also appear in the PEG-modified beads, where the cells have been trapped in the alginate gel and not in the void spaces. Figure 17, shows a comparison between cell distributions in voids and in the gel. The image is taken from a PEG-modified bead to illustrate both clumped and dispersed cells in the beads.

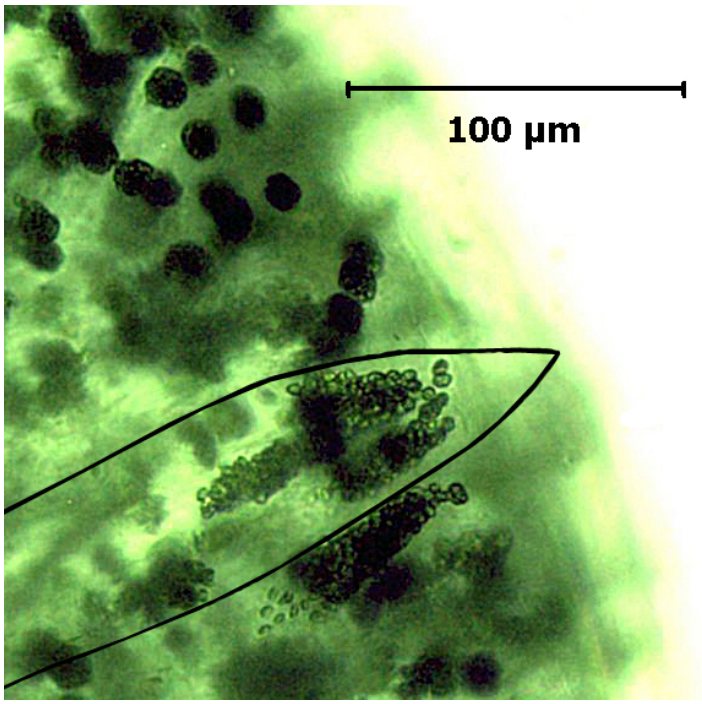

Figure 17. Comparison of cell distribution within voids (lower half), and within gel (upper half). Void outlined in black shows dispersed cells.

Analysis of Cell Leakage

The analysis of cell leakage was inconclusive, but it was determined that the effects of cell leakage, i.e. appearance of a substantial number of cells in the medium, did not occur until about 5 days into the experiment, for the control and PEG-modified beads in 125 ml reactors. As seen in Figure 14, the rate of cell leakage could not be determined because of a large amount of variance in the data. One possible way to decrease the amount of cell leakage in the PEG-modified beads would be to increase the concentration of alginate, thereby creating a stronger matrix to attempt to stabilize the void spaces near the surface of the bead. Another method to strengthen the alginate matrix would be through the use of a different multivalent cation, such as aluminum (Rochefort, 1986). It also appears that the void spaces have small holes or weaknesses at the surface of the bead through which algae could escape, but better microscopical techniques need to be used to determine whether this is true. Conventional encapsulation with poly-L-lysine could also be considered to eliminate cell leakage (Strand, 2002), but with encapsulation, a more time-consuming procedure is required and diffusion limitations of nutrients increase. A major difference the PEG-modified and control bead types had with their leakage rates was the type of growth vessel used. When the larger 500 ml tissue cell culture flasks were used, cell leakage was found to be very low, thus larger flasks may result in less frictional wear on the beads.

Carrying Capacity

The carrying capacity of the beads was determined and compared to earlier work by Seifert and Phillips (1997). The estimated carrying capacity for hybridoma cells as

theorized by the researchers was 1.3 x 10^8 cells/ml beads. They were able to achieve 6.0 x 10^7 cells/ml beads, 46% of the theoretical maximum. As carrying capacity was not a focus of this investigation, the bead systems were not run until cell death, but were stopped once satisfactory results had been obtained for cell growth rates. However, at the time the experiments were completed, the control system had achieved over 2.0 x 10^8 cells/ml beads. The PEG-modified bead system was not run as long as the controls, but a concentration of nearly 1.7 x 10^8 cells per ml beads was obtained. A higher concentration was obtained with algal cells (diameter ~10 μm), probably due to their smaller size compared to hybridoma cells (diameter ~15 μm). Accounting for the differences in cell size, the theoretical carrying capacity for algal cells would be around 4.4 x 10^8 cells per ml beads, about 3.5 times that of hybridoma cells (Figure 4). The amount obtained in this experiment resulted in 45% of this theoretical maximum.

Void Formation in Conventional Beads

It was found through examination of the control beads of conventional calcium alginate that even in these, a small number of voids were formed. Few studies have been carried out to attempt to explain such void formation, but it is possible that water present in the alginate sol is dehydrated or forced from the forming gel to aggregate in the sol, as the gelling process occurs towards the center of the bead. This water continues to dehydrate from the gel, forming voids or capillaries (Thiele & Hallich, 1958; Baardseth, 1965; Thumbs & Kohler, 1995; Treml, Woelki, & Kohler, 2003). This same process is expected to be at work in the PEG-modified beads, but the PEG increases this dehydration phenomenon dramatically.

An Attempt at Partitioning Cells into Voids

Surface modification of the algal cells was carried out with the detergent Pluronic® F108 to attempt an increased rate of cell partitioning into the void spaces, by increasing the hydrophilicity of the algal cell surface (Neff, 1998). This action did not result in the desired effect and was not pursued further. Pluronic® F108 has a hydrophobic backbone of poly(propylene oxide), and two hydrophilic tails of PEG. It was thought that the hydrophobic backbone would favor the algal cell surface and therefore cause the algal cell surface to become highly hydrophilic, which should be advantageous in partitioning the cells into the hydrophilic PEG void spaces. This would only work however, if the algae were mixed with the PEG following treatment with F108 before addition of alginate, since both PEG and alginate are hydrophilic aqueous solutions.

CONCLUSION

The PEG-modification of calcium alginate beads is a simple way of increasing the growth rates for algal cell lines, as well as other cell types as shown by Seifert and Phillips (1997). One drawback to the process used in this investigation is the size of the beads used. To decrease diffusional limitations, a smaller bead diameter should be utilized to increase the surface to volume ratio. Future work should focus on partitioning algal cells into the voids only, whereas the current method allows distribution of cells into both the alginate matrix as well as the void spaces. Also, characterization of the volume, diameter, length, and shape of the void spaces resulting from varying concentrations of the different reagents should also be explored. Methods to increase the strength of the alginate beads, while not causing toxicity, through the use of different multivalent cations for cross-linking may be utilized, which may result in less cell escape. If suitable methods for strengthening the alginate matrix cannot be achieved, hardening agents such as poly-L-lysine (Strand, 2002) or chitosan may be utilized to encapsulate the alginate bead (Yoo, 1996). If cell leakage can be controlled more efficiently with modified calcium alginate beads, conventional encapsulation of beads would not be necessary and would permit cheaper bead production with equivalent and perhaps higher cell growth.

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APPENDIX A

Variant of ASM-1 medium

1. Mix A:	**major metals**	**2000X stock; use 0.5 ml/l**	**(conc. In medium)**
magnesium sulfate	$MgSO_4 \cdot 7H_2O$	98.6 g/l	**(200 µM)**
magnesium chloride	$MgCl_2 \cdot 6H_2O$	81.4 g/l	**(200 µM)**
boric acid	H_3BO_3	4.8 g/l	**(40 µM)**

2. Mix B: **chelate + trace metals** **2000X stock; use 0.5 ml/l**

-----Add ingredients in this order, **with stirring**, allowing each to dissolve first--

potassium hydroxide	KOH	5.0 g/l	**(45 µM)**
disodium EDTA	$Na_2EDTA \cdot 2H_2O$	15.0 g/l	**(20 µM)**
ferric chloride	$FeCl_3 \cdot 6H_2O$	2.0 g/l	**(4 µM)**
manganese chloride	$MnCl_2 \cdot 4H_2O$	2.8 g/l	**(7 µM)**
zinc chloride	$ZnCl_2$	0.8 g/l	**(3.2 µM)**
cobalt chloride	$CoCl_2 \cdot 6H_2O$	0.04 g/l	**(0.08 µM)**
sodium molybdate	$NaMoO_4 \cdot 2H_2O$	0.048 g/l	**(0.1 µM)**
copper sulfate	$CuSO_4 \cdot 5H_2O$	0.0004 g/l	**(0.0008 µM)**

3. calcium chloride	$CaCl_2 \cdot 2H_20$	**2000X stock = 60.0 g/l**	**use 0.5 ml/l**	**(200 µM)**
4. sodium nitrate	$NaNO_3$	**2000X stock = 340.0 g/l**	**use 0.5 ml/l**	**(2mM)**
5. potassium phosphate	K_2HPO_4	**1M stock = 174.2 g/l**	**use 0.2 ml/l**	**(200 µM)**
6. sodium bicarbonate	$NaHCO_3$	**1M stock = 84 g/l**	**use 2.5 ml/l**	**(2.5 mM)**

APPENDIX B

Data Tables

Table 1. Collected Data and Analysis of Algal Concentrations within Beads.

Control = No Peg, No Voids
PEG = PEG Initiates Void Formation
Absorbance taken at 546 nm

Control Trial Begun on April 29, 2004

Control 1

	Hour	Day	Absorbance	Diameter	Concentration
	20	0.83	0.024	2.29	7.619E+06
	40.5	1.69	0.053	2.28	1.683E+07
	70.5	2.94	0.1	2.18	3.175E+07
	94.75	3.95	0.132	2.16	4.191E+07
	122.25	5.09	0.163	2.07	5.175E+07
	140.75	5.86	0.183	2.1	5.810E+07
	163	6.79	0.227	2.18	7.207E+07
	188.5	7.85	0.263	2.22	8.349E+07
**	211	8.79	0.29	2.13	9.207E+07
	230.25	9.59	0.322	2.32	1.022E+08
	261.25	10.89	0.364	2.26	1.156E+08
	287.75	11.99	0.42	2.24	1.333E+08
**	308.5	12.85	0.428	2.29	1.359E+08
*	331.25	13.80	0.535	2.29	1.698E+08
	356	14.83	0.614	2.34	1.949E+08
	380.5	15.85	0.658	2.29	2.089E+08
	432.5	18.02	0.712	2.34	2.260E+08
	454.25	18.93	0.75		2.381E+08

*light increased
**media change

Control 2

	Hour	Day	Absorbance	Diameter	Concentration
	20	0.83	0.03	2.28	7.477E+06
	40.5	1.69	0.058	2.29	1.446E+07
	70.5	2.94	0.114	2.36	2.841E+07
	94.75	3.95	0.163	2.29	4.063E+07
	122.25	5.09	0.199	2.32	4.960E+07
	140.75	5.86	0.23	2.28	5.733E+07
	163	6.79	0.272	2.31	6.779E+07
	188.5	7.85	0.317	2.59	7.901E+07
**	211	8.79	0.329	2.51	8.200E+07
	230.25	9.59	0.366	2.59	9.122E+07
	261.25	10.89	0.399	2.64	9.945E+07

	287.75	11.99	0.428	2.57	1.067E+08
**	308.5	12.85	0.469	2.6	1.169E+08
*	331.25	13.80	0.534	2.43	1.331E+08
	356	14.83	0.591	2.3	1.473E+08
	380.5	15.85	0.649	2.36	1.618E+08
	432.5	18.02	0.67	2.45	1.670E+08
	454.25	18.93	0.659		1.643E+08

*light increased
**media change

Control
3

	Hour	Day	Absorbance	Diameter	Concentration
	20	0.83	0.033	2.2	9.162E+06
	40.5	1.69	0.059	2.26	1.638E+07
	70.5	2.94	0.208	2.15	5.775E+07
	94.75	3.95	0.342	2.22	9.495E+07
	122.25	5.09	0.413	2.31	1.147E+08
	140.75	5.86	0.46		1.277E+08
	163	6.79	0.518	2.34	1.438E+08
	188.5	7.85	0.556	2.32	1.544E+08
**	211	8.79	0.576	2.19	1.599E+08
	230.25	9.59	0.636	2.37	1.766E+08
	261.25	10.89	0.658	2.38	1.827E+08
	287.75	11.99	0.684	2.39	1.899E+08
**	308.5	12.85	0.725	2.41	2.013E+08
*	331.25	13.80	0.823	2.36	2.285E+08
	356	14.83	0.859	2.4	2.385E+08
	380.5	15.85	0.916	2.5	2.543E+08
	432.5	18.02	0.971	2.58	2.696E+08
	454.25	18.93	0.987		2.740E+08

*light increased
**media change

2nd Control Trial begun on October 25, 2004

Control
4

Hour	Day	Absorbance	Diameter	Concentration
60.5	2.52	0.022	2.257	7.310E+06
84	3.50	0.048	2.221	1.595E+07
108	4.50	0.08	2.212	2.658E+07
152.5	6.35	0.106	2.191	3.522E+07
* 178	7.42	0.101	2.212	3.356E+07
203.25	8.47	0.144	2.304	4.785E+07
252	10.50	0.183	2.131	6.081E+07
273	11.38	0.236	2.232	7.842E+07
300	12.50	0.236	2.217	7.842E+07
372	15.50	0.321	2.126	1.067E+08
468	19.50	0.456	2.194	1.515E+08
*Light reduced			2.108	

Control
5

Hour	Day	Absorbance	Diameter	Concentration
60.5	2.52	0.033	2.242	9.092E+06
84	3.50	0.048	2.349	1.322E+07

	108	4.50	0.09	2.287	2.480E+07
	152.5	6.35	0.156	2.32	4.298E+07
*	178	7.42	0.181	2.467	4.987E+07
	203.25	8.47	0.192	2.409	5.290E+07
	252	10.50	0.244	2.24	6.722E+07
	273	11.38	0.245	2.29	6.750E+07
	300	12.50	0.24	2.245	6.612E+07
	372	15.50	0.317	2.381	8.733E+07
	468	19.50	0.485	2.437	1.336E+08
	*Light reduced			2.44	

Control
6

Hour	Day	Absorbance	Diameter	Concentration
60.5	2.52	0.038	2.272	1.046E+07
84	3.50	0.06	2.295	1.651E+07
108	4.50	0.089	2.384	2.450E+07
152.5	6.35	0.213	2.328	5.863E+07
* 178	7.42	0.288	2.326	7.927E+07
203.25	8.47	0.344	2.284	9.468E+07
252	10.50	0.431	2.28	1.186E+08
273	11.38	0.458	2.267	1.261E+08
300	12.50	0.436	2.404	1.200E+08
372	15.50	0.518	2.483	1.426E+08
468	19.50	0.563	2.45	1.550E+08

*Light reduced

PEG Trial begun on May 10, 2004

PEG 1

Hour	Day	Absorbance	Diameter	Concentration
0.5	0.02	0.032	2.21	7.856E+06
15.25	0.64	0.023	2.58	5.647E+06
40.75	1.70	0.037	2.45	9.084E+06
63.25	2.64	0.059	2.37	1.448E+07
82.25	3.43	0.101	2.38	2.480E+07
113.5	4.73	0.172	2.36	4.223E+07
140.5	5.85	0.228	2.37	5.598E+07
** 161	6.71	0.339	2.32	8.323E+07
* 183.75	7.66	0.486	2.46	1.193E+08
208.5	8.69	0.559	2.51	1.372E+08
233	9.71	0.598	2.48	1.468E+08
285	11.88	0.686	2.55	1.684E+08
306.75	12.78	0.705	2.53	1.731E+08
332.25	13.84	0.737	2.46	1.809E+08
353.5	14.73	0.727	2.48	1.785E+08

*light increased
**media change

PEG 2

Hour	Day	Absorbance	Diameter	Concentration
0.5	0.02	0.026	2.34	5.608E+06
15.25	0.64	0.025	2.6	5.392E+06
40.75	1.70	0.044	2.51	9.491E+06
63.25	2.64	0.072	2.42	1.553E+07
82.25	3.43	0.121	2.44	2.610E+07

	113.5	4.73	0.248	2.44	5.349E+07
	140.25	5.84	0.36	2.51	7.765E+07
**	161	6.71	0.405	2.3	8.736E+07
*	183.75	7.66	0.526	2.52	1.135E+08
	208.5	8.69	0.599	2.58	1.292E+08
	233	9.71	0.661	2.6	1.426E+08
	285	11.88	0.717	2.66	1.547E+08
	306.75	12.78	0.731	2.8	1.577E+08
	332.25	13.84	0.768	2.73	1.657E+08
	353.5	14.73	0.814	2.67	1.756E+08

*light increased
**media change

PEG 3

	Hour	Day	Absorbance	Diameter	Concentration
	0.5	0.02	0.028	2.34	6.931E+06
	15.25	0.64	0.025	2.62	6.188E+06
	40.75	1.70	0.054	2.4	1.337E+07
	63.25	2.64	0.09	2.36	2.228E+07
	82.25	3.43	0.154	2.58	3.812E+07
	113.5	4.73	0.272	2.42	6.733E+07
	140	5.83	0.329	2.55	8.144E+07
**	161	6.71	0.367	2.29	9.084E+07
*	183.75	7.66	0.439	2.39	1.087E+08
**	208.5	8.69	0.487	2.36	1.205E+08
	233	9.71	0.537	2.42	1.329E+08
	285	11.88	0.57	2.41	1.411E+08
	306.75	12.78	0.603	2.46	1.493E+08
	332.25	13.84	0.613	2.44	1.517E+08
	353.5	14.73	0.627	2.37	1.552E+08

*light increased
**media change

Table 2. Collected Data and Analysis of Algal Concentrations in Medium.

Control = No Peg, No Voids
PEG = PEG Initiates Void Formation
Absorbance taken at 486 nm

Control Trial Begun on April 29, 2004

Control Medium 1

	Hour	Day	Absorbance	Concentration (Cells/mL)
	19.00	0.79	0.005	9.268E+04
	40.00	1.67	0.002	3.707E+04
	70.50	2.94	0.013	2.410E+05
	94.50	3.94	0.017	3.151E+05
	120.25	5.01	0.024	4.449E+05
	139.25	5.80	0.086	1.594E+06
	161.00	6.71	0.105	1.946E+06
	186.50	7.77	0.151	2.799E+06
**	209.00	8.71	0.132	2.447E+06
	228.25	9.51	0.191	3.540E+06
	259.25	10.80	0.264	4.894E+06
	285.75	11.91	0.257	4.764E+06
**	306.50	12.77	0.008	1.483E+05
*	329.25	13.72	0.036	6.673E+05
	354.00	14.75	0.052	9.639E+05
	378.50	15.77	0.063	1.168E+06
	430.50	17.94	0.144	2.669E+06
	452.25	18.84	0.199	3.689E+06

*light increased
**media change

Control Medium 2

	Hour	Day	Absorbance	Concentration (Cells/mL)
	19.00	0.79	0.006	1.112E+05
	40.00	1.67	0.002	3.707E+04
	70.50	2.94	0.020	3.707E+05
	94.50	3.94	0.025	4.634E+05
	120.25	5.01	0.030	5.561E+05
	139.25	5.80	0.136	2.521E+06
	161.00	6.71	0.207	3.837E+06
	186.50	7.77	0.283	5.246E+06
**	209.00	8.71	0.243	4.504E+06
	228.25	9.51	0.277	5.135E+06
	259.25	10.80	0.366	6.784E+06
	285.75	11.91	0.374	6.933E+06
**	306.50	12.77	0.006	1.112E+05
*	329.25	13.72	0.015	2.780E+05
	354.00	14.75	0.030	5.561E+05

	378.50	15.77	0.036	6.673E+05
	430.50	17.94	0.079	1.464E+06
	452.25	18.84	0.099	1.835E+06

*light increased

**media change

Control Medium 3

	Hour	Day	Absorbance	Concentration (Cells/mL)
	19.00	0.79	0.006	1.112E+05
	40.00	1.67	0.000	0.000E+00
	70.50	2.94	0.044	8.156E+05
	94.50	3.94	0.098	1.817E+06
	120.25	5.01	0.210	3.893E+06
	139.25	5.80	0.229	4.245E+06
	161.00	6.71	0.319	5.913E+06
	186.50	7.77	0.502	9.305E+06
**	209.00	8.71	0.391	7.248E+06
	228.25	9.51	0.494	9.157E+06
	259.25	10.80	0.532	9.861E+06
	285.75	11.91	0.450	8.341E+06
**	306.50	12.77	0.009	1.668E+05
*	329.25	13.72	0.013	2.410E+05
	354.00	14.75	0.036	6.673E+05
	378.50	15.77	0.027	5.005E+05
	430.50	17.94	0.060	1.112E+06
	452.25	18.84	0.073	1.353E+06

*light increased

**media change

2nd Control Trial begun on October 25, 2004

Control Medium 4

	Hour	Day	Absorbance	Concentration (Cells/mL)
	60.50	2.52	0.006	1.112E+05
	84.00	3.50	0.011	2.039E+05
	108.00	4.50	0.011	2.039E+05
	152.50	6.35	0.02	3.707E+05
*	178.00	7.42	0.031	5.746E+05
	203.25	8.47	0.041	7.600E+05
	252.00	10.50	0.039	7.229E+05
	273.00	11.38	0.052	9.639E+05
	300.00	12.50	0.048	8.897E+05
	372.00	15.50	0.05	9.268E+05
	468.00	19.50	0.108	2.002E+06

*Light reduced

Control Medium 5

Hour	Day	Absorbance	Concentration (Cells/mL)
60.50	2.52	0.003	5.561E+04

	84.00	3.50	0.012	2.224E+05
	108.00	4.50	0.013	2.410E+05
	152.50	6.35	0.019	3.522E+05
*	178.00	7.42	0.036	6.673E+05
	203.25	8.47	0.054	1.001E+06
	252.00	10.50	0.044	8.156E+05
	273.00	11.38	0.052	9.639E+05
	300.00	12.50	0.039	7.229E+05
	372.00	15.50	0.049	9.083E+05
	468.00	19.50	0.104	1.928E+06

*Light reduced

Control Medium 6

	Hour	Day	Absorbance	Concentration (Cells/mL)
	60.50	2.52	0.008	1.483E+05
	84.00	3.50	0.013	2.410E+05
	108.00	4.50	0.017	3.151E+05
	152.50	6.35	0.036	6.673E+05
*	178.00	7.42	0.038	7.044E+05
	203.25	8.47	0.061	1.131E+06
	252.00	10.50	0.072	1.335E+06
	273.00	11.38	0.084	1.557E+06
	300.00	12.50	0.06	1.112E+06
	372.00	15.50	0.071	1.316E+06
	468.00	19.50	0.091	1.687E+06

PEG Trial begun on May 10, 2004

PEG Medium 1

	Hour	Day	Absorbance	Concentration (Cells/mL)
	0	0.00	0	0.000E+00
	15	0.63	0.002	3.707E+04
	40.5	1.69	0.006	1.112E+05
	63	2.63	0.01	1.854E+05
	82	3.42	0.016	2.966E+05
	113.25	4.72	0.024	4.449E+05
	139.75	5.82	0.035	6.488E+05
**	160.25	6.68	0.006	1.112E+05
*	183	7.63	0.016	2.966E+05
	207.75	8.66	0.033	6.117E+05
	232.25	9.68	0.041	7.600E+05
	284.25	11.84	0.078	1.446E+06
	306	12.75	0.118	2.187E+06
	331.5	13.81	0.159	2.947E+06

*light increased

**media change

PEG Medium 2

	Hour	Day	Absorbance	Concentration (Cells/mL)
	0	0.00	0.003	5.561E+04
	15	0.63	0.002	3.707E+04

	40.5	1.69	0.002	3.707E+04
	63	2.63	0.005	9.268E+04
	82	3.42	0.016	2.966E+05
	113.25	4.72	0.026	4.819E+05
	139.5	5.81	0.051	9.453E+05
**	160.25	6.68	0.005	9.268E+04
	183	7.63	0.005	9.268E+04
*	207.75	8.66	0.016	2.966E+05
	232.25	9.68	0.019	3.522E+05
	284.25	11.84	0.061	1.131E+06
	306	12.75	0.079	1.464E+06
	331.5	13.81	0.097	1.798E+06

*light increased

**media change

PEG Medium 3

	Hour	Day	Absorbance	Concentration (Cells/mL)
	0	0.00	0.002	3.707E+04
	15	0.63	0	0.000E+00
	40.5	1.69	0.004	7.414E+04
	63	2.63	0.007	1.298E+05
	82	3.42	0.019	3.522E+05
	113.25	4.72	0.09	1.668E+06
	139.5	5.81	0.277	5.135E+06
**	160.25	6.68	0.077	1.427E+06
*	183	7.63	0.124	2.298E+06
**	207.75	8.66	0.019	3.522E+05
	232.25	9.68	0.045	8.341E+05
	284.25	11.84	0.114	2.113E+06
	306	12.75	0.153	2.836E+06
	331.5	13.81	0.211	3.911E+06

*light increased

**media change

APPENDIX C

Statistical Analysis Utilizing Student's t-test

Table 3. Statistical Analysis.

	Growth Rate		Growth Rate
Growth Rate of Control 1	1.048E+07	Growth Rate of PEG 1	1.955E+07
Growth Rate of Control 2	1.009E+07	Growth Rate of PEG 2	1.938E+07
Growth Rate of Control 4	6.013E+06	Growth Rate of PEG 3	1.681E+07
Growth Rate of Control 5	8.075E+06		
Growth Rate of Control 6	1.489E+07		

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	*Variable 2*
Mean	1.86E+07	9.91E+06
Variance	2.36E+12	1.09E+13
Observations	3	5
Pooled Variance	8.07E+12	
Hypothesized Mean Difference	0	
Degrees of Freedom	6	
t Stat	4.18	
P(T<=t) one-tail	2.91E-03	
t Critical one-tail	1.94	
P(T<=t) two-tail	5.82E-03	
t Critical two-tail	2.45	

According to the t-test statistical analysis, the difference between the mean growth rates of the control and PEG-modified beads is statistically significant, with $p < 0.01$. This can be determined from the above table, since the "t Stat" is larger than the "t Critical two-tail".