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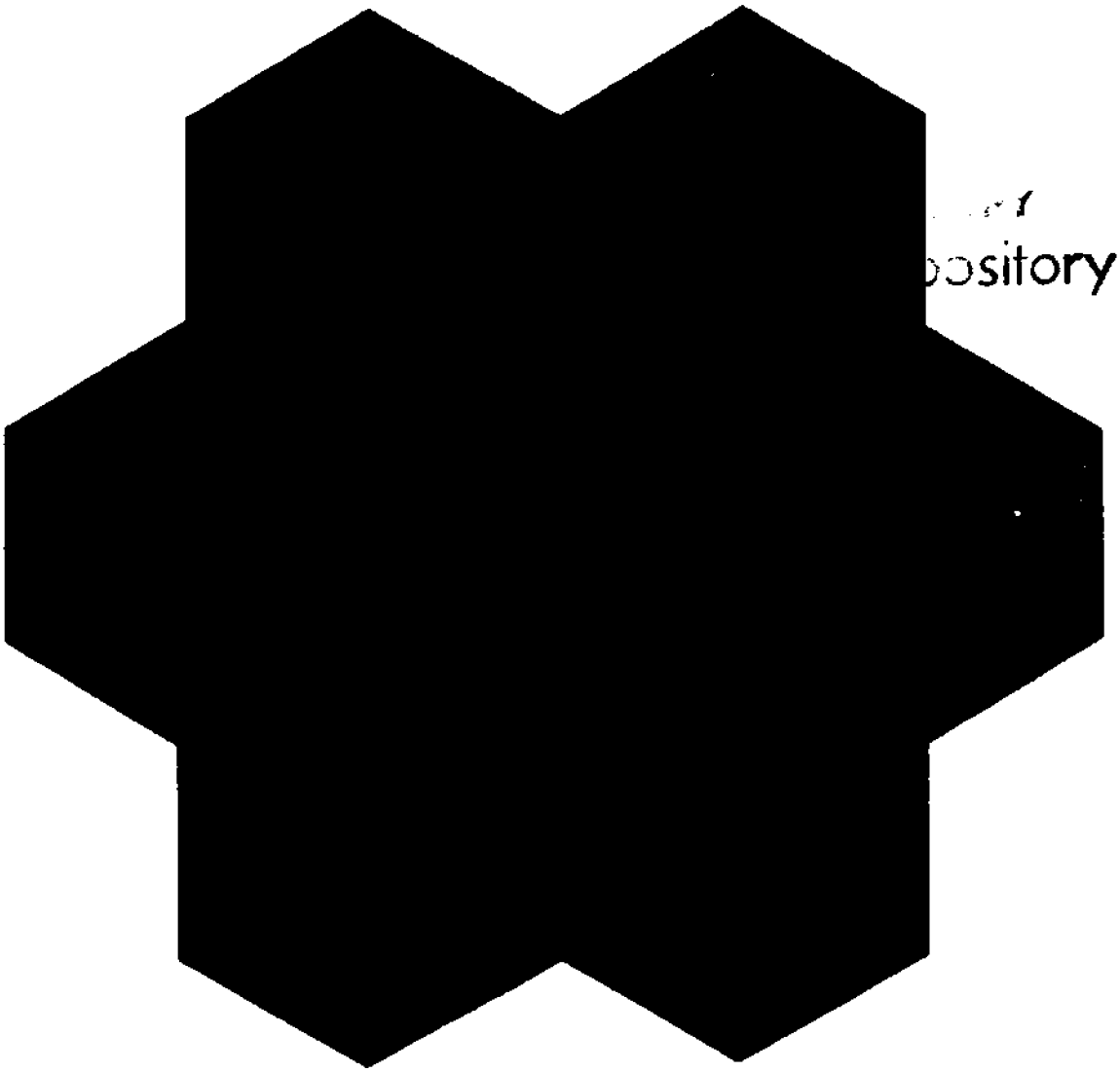

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Homer C. Dudley

Techniques and Instrumentation to Control Some Environmental Factors in Shellfish Nutritional Studies

College of Marine Studies University of Delaware Newark, Delaware 19711

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TECHNIQUES AND INSTRUMENTATION TO CONTROL SOME ENVIRONMENTAL FACTORS FOR SHELLFISH NUTRITIONAL STUDIES

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Ramesh C. Dwivedy

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ABSTRACT

Techniques and instrumentation were developed that allow positive control over environmental factors such as feed density, flow rate, flow volume, light etc. for oyster feeding studies in a recirculating system. They are also suitable for feeding studies using labelling materials such as radioactive tracers to determine acceptance, rejection and utilization level of a given feed by the oyster.

ACKNOWLEDGMENTS

The author wishes to thank Mr. Robert Hohn, Electronic Technician for constructing the feed density controller used in this study. Thanks are also due to Professors R. L. Salsbury, C. W. Woodmansee and C. Epifanio for critically reviewing the manuscript.

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TECHNIQUES AND INSTRUMENTATION TO CONTROL SOME ENVIRONMENTAL FACTORS FOR SHELLFISH NUTRITIONAL STUDIES

by

R. C. Dwivedy

INTRODUCTION

The literature regarding the food and feeding of an oyster as well as the influence of environmental factors upon feeding of this mollusk is very limited (1,2). Knowledge in these areas is necessary before satisfactory rearing of oysters in closed systems can be accomplished.

The objective of this research was to develop techniques and instrumentation that would allow oyster feeding under controlled environmental conditions. By labelling a given feed, one may determine acceptance, rejection and the utilization level of the feed by the oyster through the techniques and instrumentation developed. Similarly, the influence of several environmental factors such as light, feed density, etc. upon oyster feeding can be studied.

RELATED WORK

The feeding mechanism in an adult oyster depends on ciliary action of gills which drives a current of water through the ostia which are open spaces between two adjoining filaments of gills (Fig. 1). During passage, particulate material is filtered off, wrapped in mucous and is transported to the labial palps, where it is sorted out, based on its physical size and chemical property, before it is ingested (2, 3).

Review of the literature reveals that most of the work on oyster feeding studies has been concerned with the gut content of the oyster in natural environment (1, 2). It must be realized that oysters sometime may ingest material like sand particles, clay, carmine powder and similar other undigestible non-food material (2); therefore, any conclusion on food of the oyster by examining its gut content is very weak.

Some researchers in the past have based the measure of utilization of a given food by the oysters on their growth (4). A group of oysters fed a given food, natural or synthetic, showing more growth than the unfed control group is positive proof that the oysters are utilizing the material. However, there are certain difficulties associated with this kind of experiment, the long period of time necessary to complete the experiment being the most serious one. The use of labeled feed in such experiments offers many advantages. For example, if a given feed is labeled with a radioisotope to act as a tracer and is fed to oysters, any radioactivity found upon analysis of the tissues is definite proof that the oyster has utilized the labeled feed. Also analysis of feces and pseudofeces will indicate the amount of feed ingested and rejected by the oyster.

Very little work has been done to study the influence of environmental factors upon feeding behavior of oysters. Probably the most well understood parameter is temperature (2). The effect of photoenergy apparently has not been studied at all in this context.

Loosanoff and Engle (5) have studied the effect of feed density upon feeding of oysters but there is no mention whether other environmental factors such as light, flow volumes, flow rate and water

chemical factors were controlled during the experiment. Their finding was that the oyster will stop feeding or start producing large amounts of pseudofeces if the number of algae cells in the feeding medium is heavy.

MATERIAL AND METHODS

Feeding Apparatus:

An apparatus to hold individual oysters under equal flow rate in a closed system was designed and constructed. The design of this apparatus is a modification of the apparatus described by Haven et. al. (6). The operation of this apparatus is based on maintenance of a constant head pressure in a central tube by continuous recycling of water. Water flows to individual compartments around the tube through holes of equal diameter.

Acrylic plastic sheets, 1/8" thick, and a cylinder 4" long and 1 1/2" I.D., were cemented together to construct the apparatus (Figs. 2, 3, 4). Six equally spaced feeder holes, 3/16" diameter, were drilled around the periphery of the central cylinder. These holes, 3 1/4" above the base, open into an equal number of holding compartments. Each compartment has two vertical baffles and three vertical dividers. The outermost section of each compartment is not divided (Fig. 3). Vertical separators in the bottom of each compartment allow separate accumulation of oyster feces and pseudofeces. The base is a 14-inch square, 1/8" thick, plate.

Airlifts are used to recycle the water in this apparatus, working on the principle that the density of air-water mixture is less than that of the water and therefore the mixture rises. Three airlifts were used in this study, pumping the water into the top of the central

tube. Feeder holes in the central tube allow the water to flow out of the tube into individual compartments. The purpose of the baffles and vertical dividers is to control and regulate water flow pattern. The number of airlifts and size of feeder holes could be varied to change the rate of flow through each compartment.

It is important to space the baffles such that an even flow over the oysters is obtained. This prevents flushing of the feces and pseudofeces of the oyster.

Six oysters were placed in individual compartments as shown in Fig. 3. Since pseudofeces and feces are ejected separately on opposite sides of the shell, they also accumulate separately on opposite sides of the bottom separator plate. At the end of the experiment, biodeposits were collected by suction with a bulb aspirator.

Feed Density Controller:

An electronic unit using two photoconductive cells was constructed and used to control the feed density in the feeding apparatus. The design of the controller is based on the circuit described by Maddux and Jones (7).

The monitoring light source was a red flashlight lamp (Fig. 4). The parabolic flashlight reflector focused a light beam onto the two photoconductive cells, P 1 and P 2. The two photocells were arranged such that light beam fell on the photocell, P 2, directly, whereas the photocell P 1, got light after the beam has travelled through the feeding medium. Obviously, photocell P 1 acted as a reference whereas the other photocell acted as a measuring photocell. The red light source (peak emission around 700 mμ) was used to prevent any photosynthetic effect by the monitoring light and to eliminate errors due to pigment changes in algal feed.

The two photocells were connected in an a.c. bridge the output of which passed to an amplifier and phase detector, which activated a relay to operate a normally closed solenoid valve whenever the feed density in the feeding apparatus dropped below the level determined by the presetting of the bridge potentiometer. The opening of the solenoid valve activated the airlift in the feed jar which, in turn, pumped feed into the feeding apparatus until density increased to the preset level.

Density Controller Circuit:

Schematic diagram of the feed density controller circuit is given in Fig. 5. An a.c. signal, produced by unbalancing of the bridge, was amplified by the electronic tube and then compared with a reference a.c. voltage across the 270k resistor which was in parallel with a neon lamp (Ne 1). The reference voltage was not enough to light the lamp. If the intensity of light falling on photocell, P 1, increased due to reduced density of feed, the error signal was in phase with the reference signal and the neon bulb lit. The neon bulb was cemented to the face of the photocell, P 3, connected in the grid circuit of the second electronic tube which had the relay connected to its cathode. The photocell, P 3, and neon lamp (Ne 2) were wrapped in a light-tight covering of aluminum foil. The dark resistance of the cell, P 3 was about 12 megohms, but when illuminated by the neon bulb, its resistance dropped to about 300 k ohms. Variable resistor, R_1, had a resistance of 1/10 of the photocell light resistance. When the neon lamp (Ne2) was "on," the grid of the relay tube increased to about 25 volts, which activated the relay. The neon lamp (Ne 1) was always "on" but was so arranged that it did not illuminate the photocell, P 3, but served to prevent the "dark effect" in the control lamp (Ne 2). This arrangement assured a closely reproducible firing voltage.

Experimental Chamber:

Schematic diagram of the experimental chamber is shown in Fig. 6. An overall view of the setup in the experimental chamber is shown in Fig. 7. The chamber was a steel cabinet, 5' x 3' x 2', with the front end open. The chamber was located in an air conditioned room where the temperature could be controlled at 70°F ± 2°F. A scanning tele-thermometer and a laboratory recorder, both manufactured by Yellow Springs Instrument Company, were used to record the temperature of the feeding medium in several compartments of the feeding apparatus. A circular fluorescent lamp, 60 watt output provided illumination over the feeding apparatus. A 24-hour electrical timer operated the fluorescent lamp. This arrangement allowed the selection of several ratios of light to dark periods. As is obvious, the intensity of illumination could be easily varied by changing the size of the lamp. During the experiment, the front end of the chamber was covered with a black polyethylene curtain to avoid any disturbance to the oysters due to stray lights or by the turning "on" and "off" of the room light. If it is desired to study influence of sound energy on the feeding of the oysters, the experimental chamber should be placed in a soundproof room.

Continuous Algal Culture System:

A schematic diagram of the set up is shown in Fig. 8. An electronic density controller, similar to the design described previously, was used to control the density of algal culture in the growth chamber. Even a slight rise of the density in the culture over the preset value triggered the controller which, in turn, caused the normally closed solenoid valve to open, thus allowing the medium supply to flow in the culture until the density fell back to the preset value. The system allowed daily harvest of algal culture of equal density (equal number of cells/unit volume of

culture). The amount of harvest depends mainly upon the volume of growth chamber and the species of the algae. Using a 12-liter glass carboy as a growth chamber it is not difficult to harvest one liter of Nitzschia closterium (brown marine algae) every 24 hours with a density of 1.5 million cells per milliliter.

The system is suitable for the purpose of labelling algae with tracers such as radioactive material. The procedure for labelling is to inoculate the radioactive medium in the growth chamber with a small amount of unlabelled algae, and then to discard culture until the required amount of labelling has been reached.

The system was placed in an air-conditioned room. If an air-conditioned room is not available, a water bath could be used to control the temperature of the culture. Synthetic sea-salt should be used to make medium for the culture. Rila sea-salt, manufactured by Rila Products, Inc. was used in this work.

Oysters:

American oysters, Crassostrea Virginica, were used in this study. Approximate size of each oyster was 1.5" in height and 1.00" in length.

RESULTS

Testing accuracy of the density controller:

The density controller was tested for its accuracy to control feed density in the feeding apparatus. Typical examples of its operation are given as follows:

Example # 1.

Type of feed: Nitzschia closterium

Initial density of algal cells in the feeding apparatus: 0.47×10^6 cells/ml

Time	Number of cells in the feeding apparatus (cells/ml)	Position of solenoid valve	Remarks
09:30	0.47×10^6	close	
10:30	0.44×10^6	close	
12:10	0.43×10^6	open	Valve was open for 3 minutes, during this time algae was pumped into the feeding apparatus
12:13	0.48×10^6	close	
15:30	0.44×10^6	open	-Do-
15:33	0.48×10^6	close	
17:00	0.43×10^6	open	-Do-
17:03	0.48×10^6	close	Experiment terminated

Range of density controlled = $\frac{(0.48-0.43)}{0.47} \times 100$

= 10.6% of the set value

= $\pm$ 5% of the set value

Example # 2.

Type of feed: Corn starch

Initial density of feed in the feeding apparatus: 110 mg/l

Time	Density of corn starch in the feeding apparatus (mg/ l)	Position of solenoid valve	Remarks
10:45	110	close	
11:45	105	open	Valve was open for about 2 minutes during which corn starch was pumped into the feeding apparatus.
11:48	111	close	

13:00	105	open	-Do-
13:03	110	close	
14:00	106	open	-Do-
14:03	110	close	Experiment terminated

Range of density controlled = (111-104) x 100

= 5.4% of the set value

= $\pm$ 2.7% of the set value

Calculation of feed intake by Oysters:

Length of each experiment was twelve hours. As mentioned previously, the feeding apparatus held six oysters in individual compartments. Oysters were starved for about 24 hours before each experiment.

Disappearance of food from the feeding medium in the apparatus was accounted for by two factors; feed intake by oysters and settling of feed. A "control" experiment without any oysters in the apparatus was run to account for settling, prior to each actual experiment. It was assumed that the rates of settling in both "experimental" and "control" apparatuses were equal. Two examples to illustrate the technique for feed intake calculations are given as follows:

Example #1

Type of feed: Nitzschia closterium

Feed density maintained in the apparatus: 0.50×10^6 cells/ml

Capacity of feeding apparatus: 8 liters

Description	Experimental (with oysters)	Control (without oysters)
1. Total input of feed in twelve hours	8.0×10^6 cells	8.0×10^6 cells
2. Amount of feed remaining in the feeding medium at the end of 12 hours	Negligible	4.56×10^6 cells
3. Amount of feed intake by oysters + amount of feed settled	8.0×10^6 cells	
4. Amount settled in bottom		3.44×10^6 cells

5. Minimum amount of feed intake by six oysters (3) - (4) = (5)	4.56×10^6 cells
6. Minimum amount of feed intake by each oyster	0.76×10^6 cells

Example #2

Type of feed: corn starch

Feed density maintained in the apparatus: 0.5 gm/liter

Capacity of feeding apparatus: 8 liters

Description	Experimental (with oysters)	Control (without oysters)
1. Total input of feed in twelve hours	4.00 gms	4.00 gms
2. Amount of feed remaining in the feeding medium at the end of 12 hours	0.125 gm	1.85 gms
3. Amount of feed intake by oysters + amount of feed settled	3.875 gms	
4. Amount settled in bottom		2.15 gms
5. Minimum amount of feed intake by six oysters (3) - (4) = (5)	1.725 gms	
6. Minimum amount of feed intake by each oyster	0.288 gm	

Feces and pseudofeces of the oyster:

At the end of the twelve hour period, feces and pseudofeces were collected by suction using a bulb aspirator. Typical values of air-dried weights of feces and pseudofeces are given as follows:

Type of feed	Amount of feces per oyster in twelve hours	Amount of pseudofeces per oyster in twelve hours
Nitzschia closterium	0.04 gm	0.13 gm
Corn starch	0.06 gm	0.20 gm

Water chemical analysis of the feeding medium in feeding apparatus:

Water chemical analysis of the medium was run before the oysters were placed in the apparatus and at the end of each experiment. Analysis showed little variation in the water chemicals after a twelve hour period. A typical example of these values is given as follows:

Chemical tested	Initial value	Final value	Fluctuating range
Ammonia	0.10 ppm	0.12 ppm	+ 0.02 ppm
Nitrate	5.0 ppm	5.0 ppm	0.00
Nitrite	0.09 ppm	0.09 ppm	0.00
Ph	7.6	7.4	- 0.20
Salinity	26 ppt	26 ppt	0.00
Temperature	19°C	19°C	0.00
Dissolved oxygen	8 ppm	7 ppm	- 1.00 ppm

DISCUSSION

The results show that the density controller maintains feed density in the apparatus within a small range (± 5% of the set density for algae, ± 2.7% of the set density for corn starch). Although the range seems to be biased down to a value below the set density, the controller provided an adequate control. As indicated by comparison of the results of Examples 1 and 2, the density controller provided a better control of corn starch density than that of algae. The reason for this difference lies in the fact that corn starch particles are opaque whereas algal cells are probably translucent which allow undesirable refracted light energy to fall on the measuring photocell.

Since algal cells are buoyant as compared to corn starch particles, the amount of starch settled in the bottom of the apparatus during an experiment was appreciably more than that of algae. Air bubbling in the oyster compartments was tested so as to reduce settling rate but since the indications were that bubbling inhibited oyster feeding due to physical disturbance, this method was not used in actual experiments. Although there is a way to account for the amount of feed lost due to settling, it would be helpful to devise some means of preventing or at least reducing the feed loss.

It is recognized that the instrumentation of this study does not provide any control over water chemicals such as pH, ammonia, etc. In spite of this, the results indicate that the values of these chemicals did not change appreciably. This stability of the water chemicals was due to the two facts. First, duration of each experiment was short (12 hours) and second, fresh feed was periodically added to the feeding

apparatus as commanded by the settling of feed along with oyster feeding rate. Nutritional experiments of this type are usually of short duration, where objective of the experimenter is to investigate ingestion, rejection, and utilization of feed by oysters. Therefore, the instrumentation and techniques of this study can be used without any problem. However, if it is desired to run experiments for longer periods of time, some device to control water chemicals will have to be devised and used along with the present instrumentation.

CONCLUSIONS

1. The instrumentation developed in this study allows a positive control over the environmental factors such as light intensity, feed density, light to dark ratio, flow rate, flow volume and others for oyster nutritional studies.
2. The techniques and instrumentation are suitable for feeding studies using labelling material such as radioactive tracers.
3. With slight modification, the techniques and instrumentation can be used to study food and feeding of any shellfish.

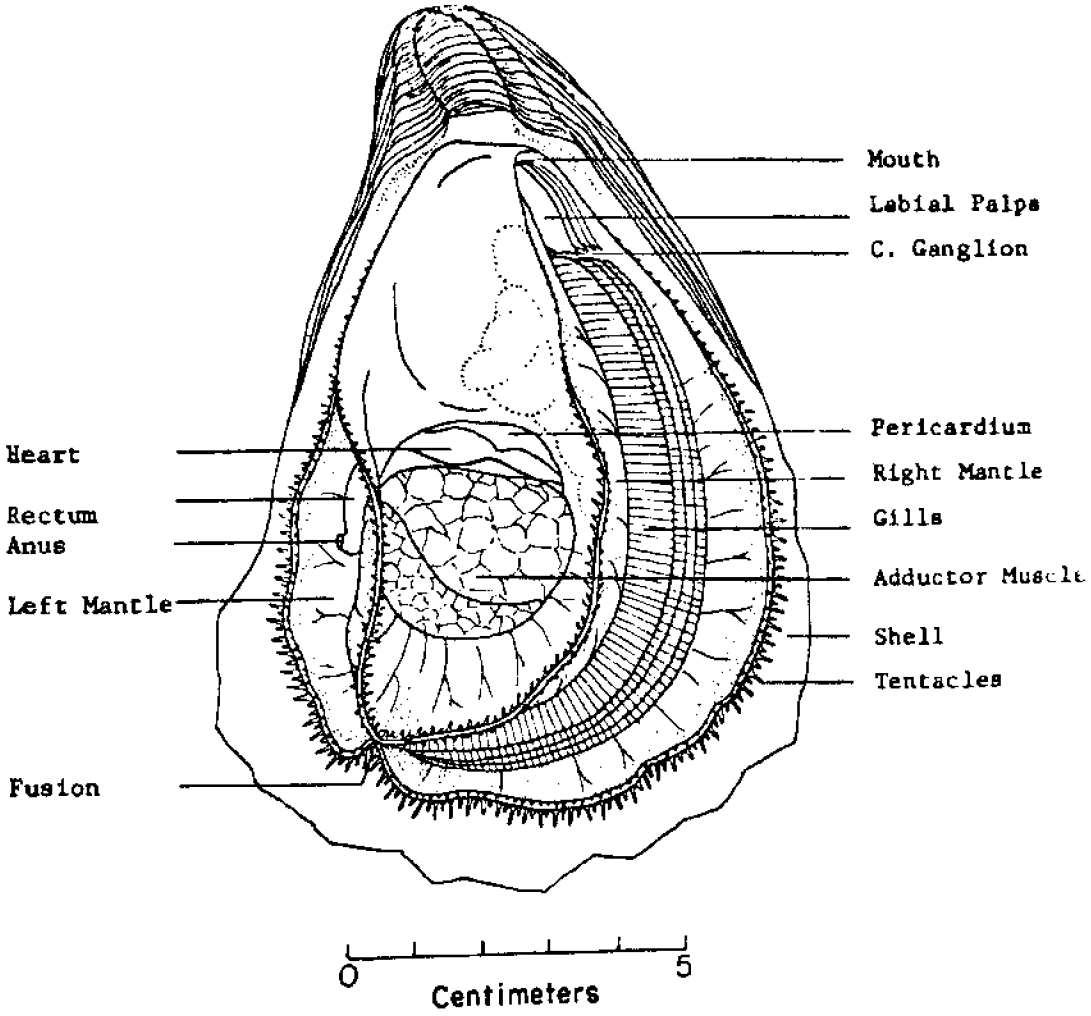


Fig. 1. Internal Organs of the American Oyster (Redrawn from Ref. #2)

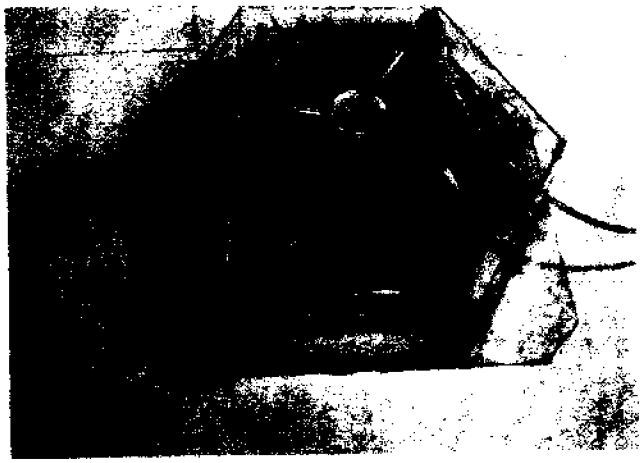
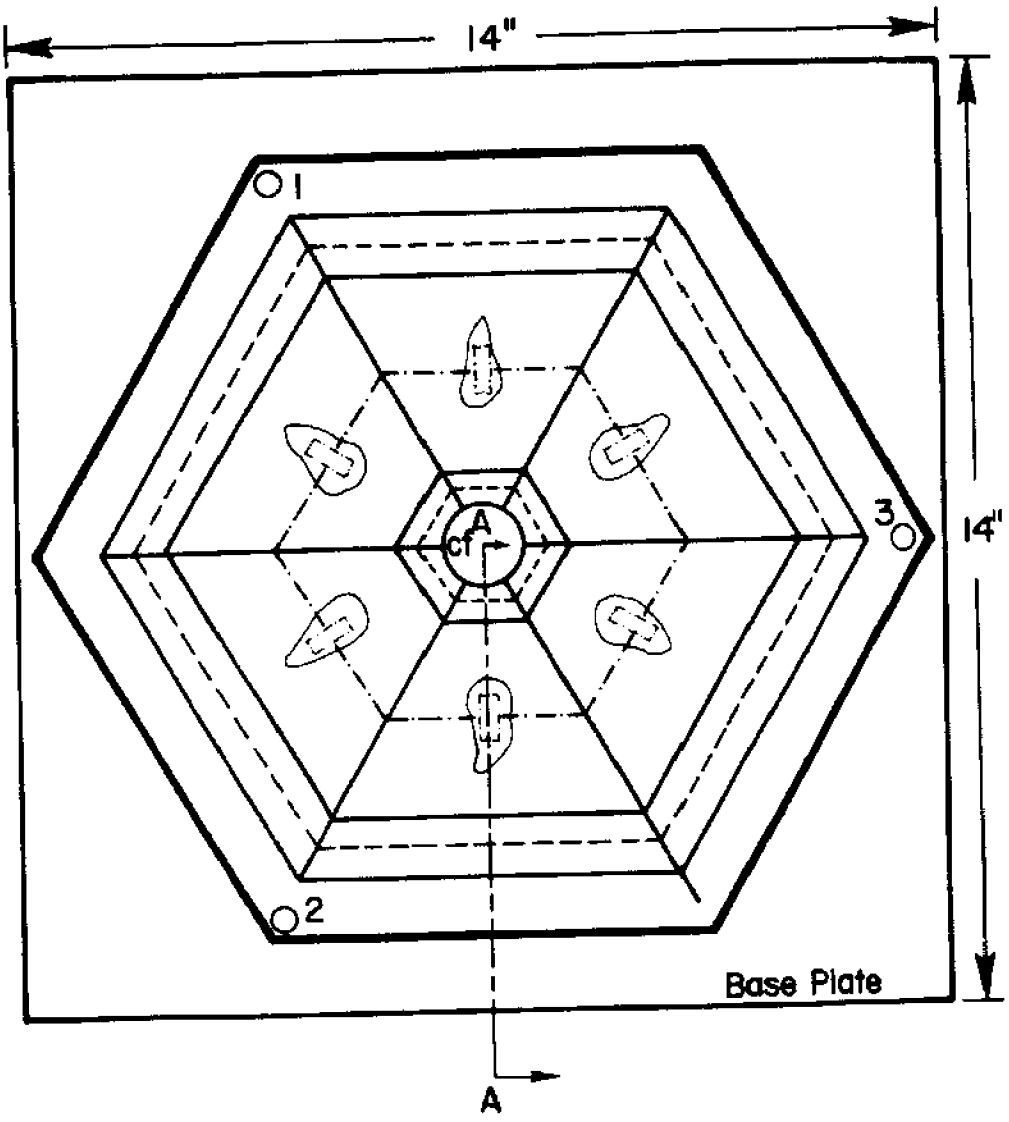


Fig. 2. Feeding Apparatus. For details, see Figs. 3 and 4.



1,2,3 Air lifts for Circulation of Feed

—— Compartment Dividers

--- Baffles

—·— Feces–Pseudofeces Separators

cf Central Feeder Tube (1 1/2" diameter)

Fig. 3 Top view of Hexagonal Feeding Apparatus. Oysters are shown in place.

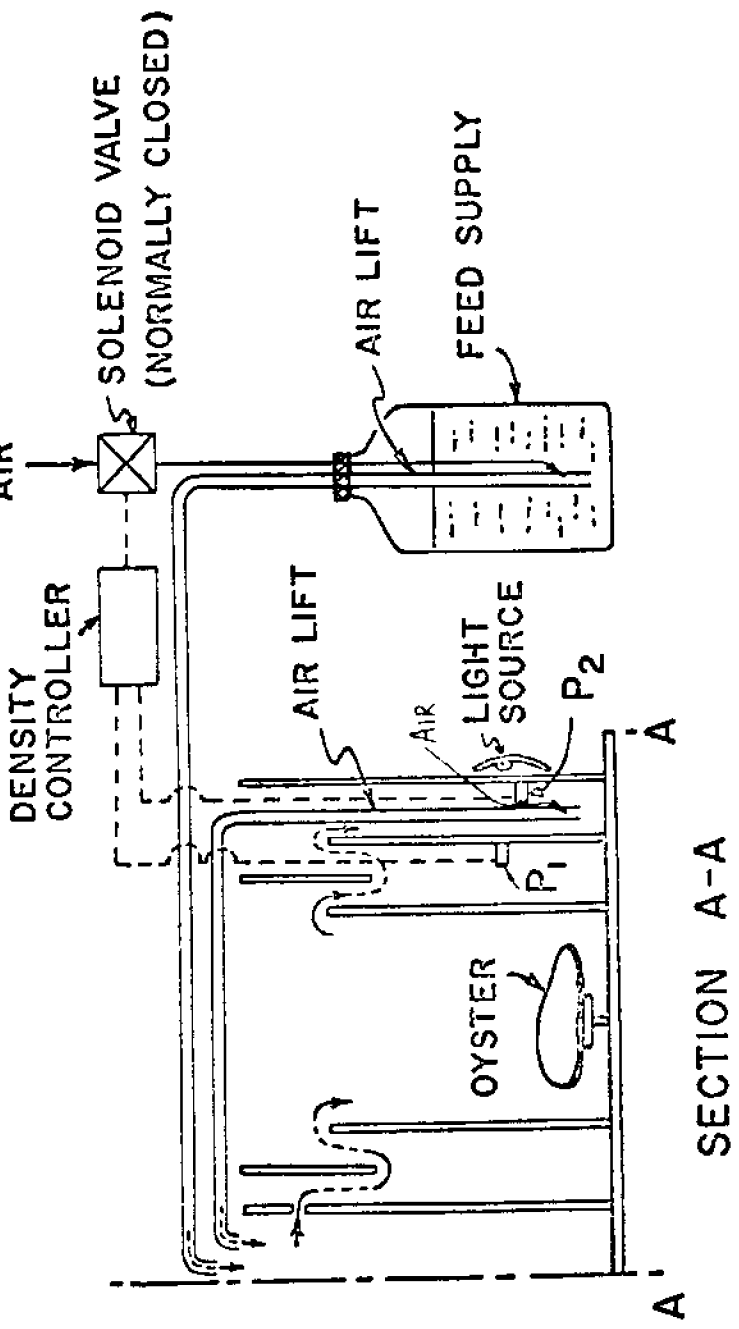


Fig: 4. A schematic cut-view of feeding apparatus (section through A-A). P_1, P_2 are photocells, ⟶ indicates feed flow.

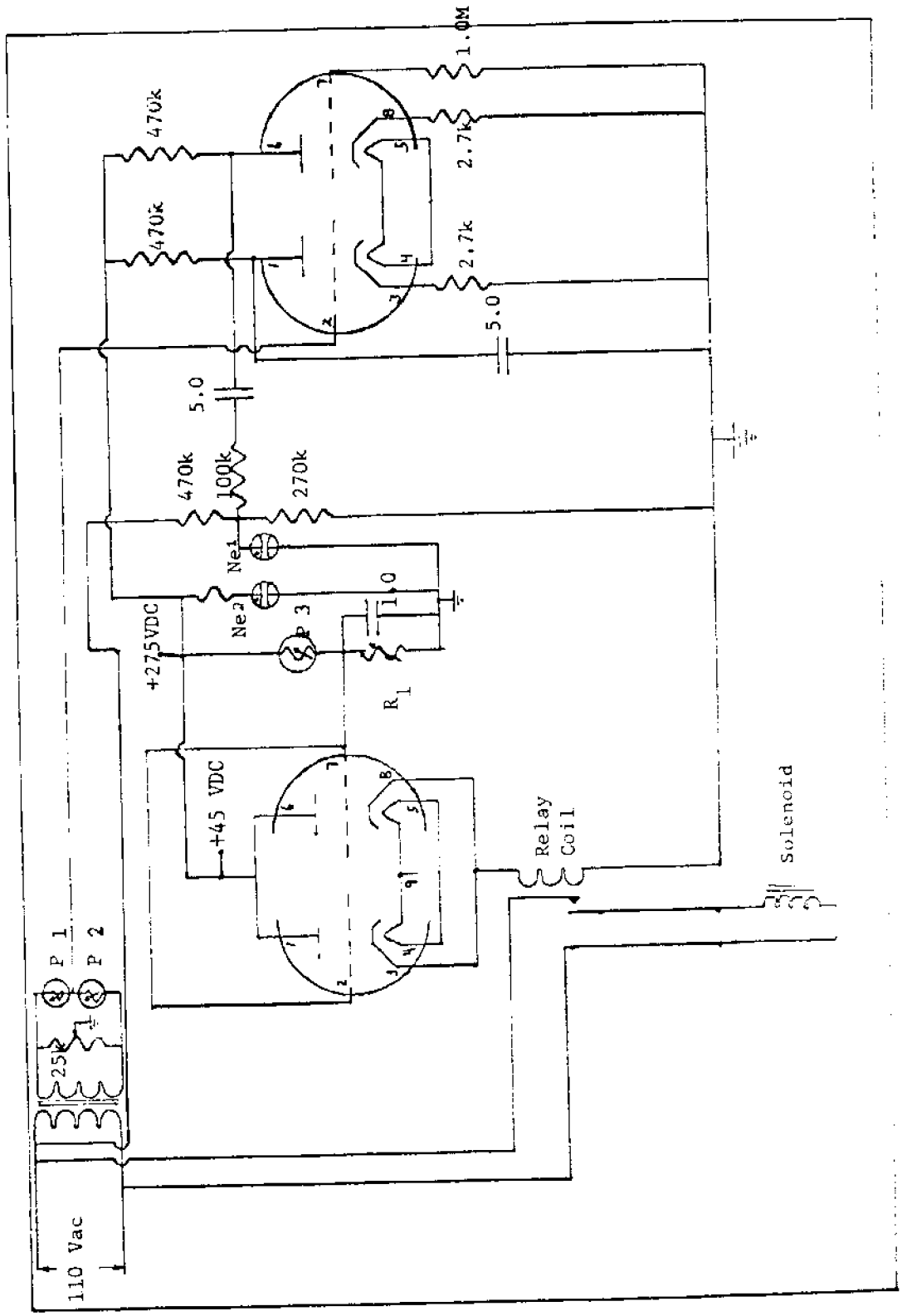


Fig. 5. Circuit Diagram of Feed Density Controller. For explanation, see text.

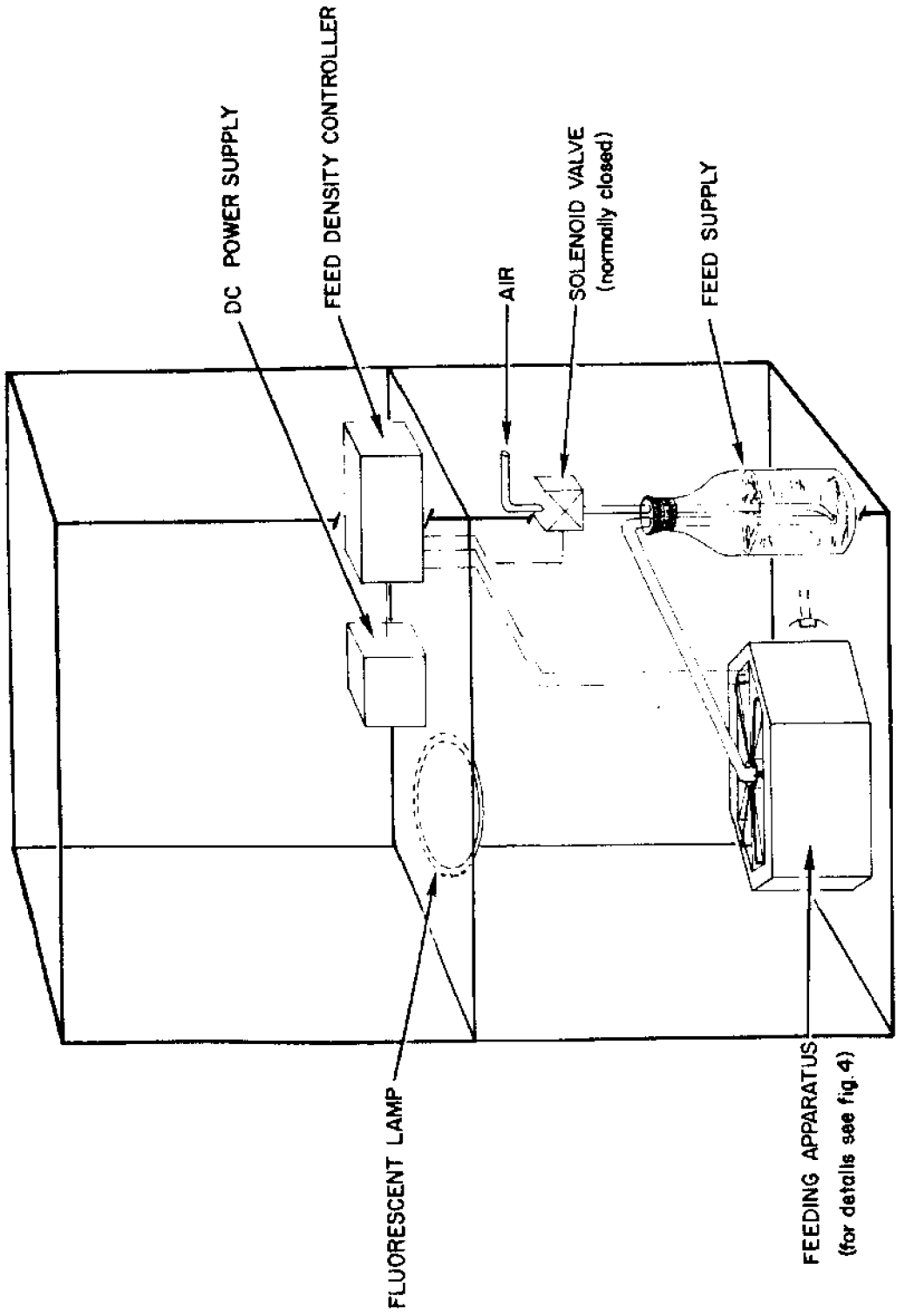


Fig. 6. Environmentally Controlled Experimental Chamber

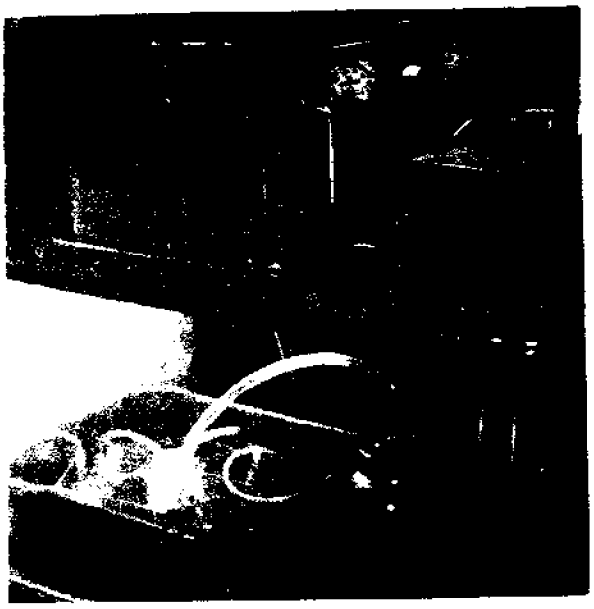


Fig. 7. An overall view of the experimental set-up.

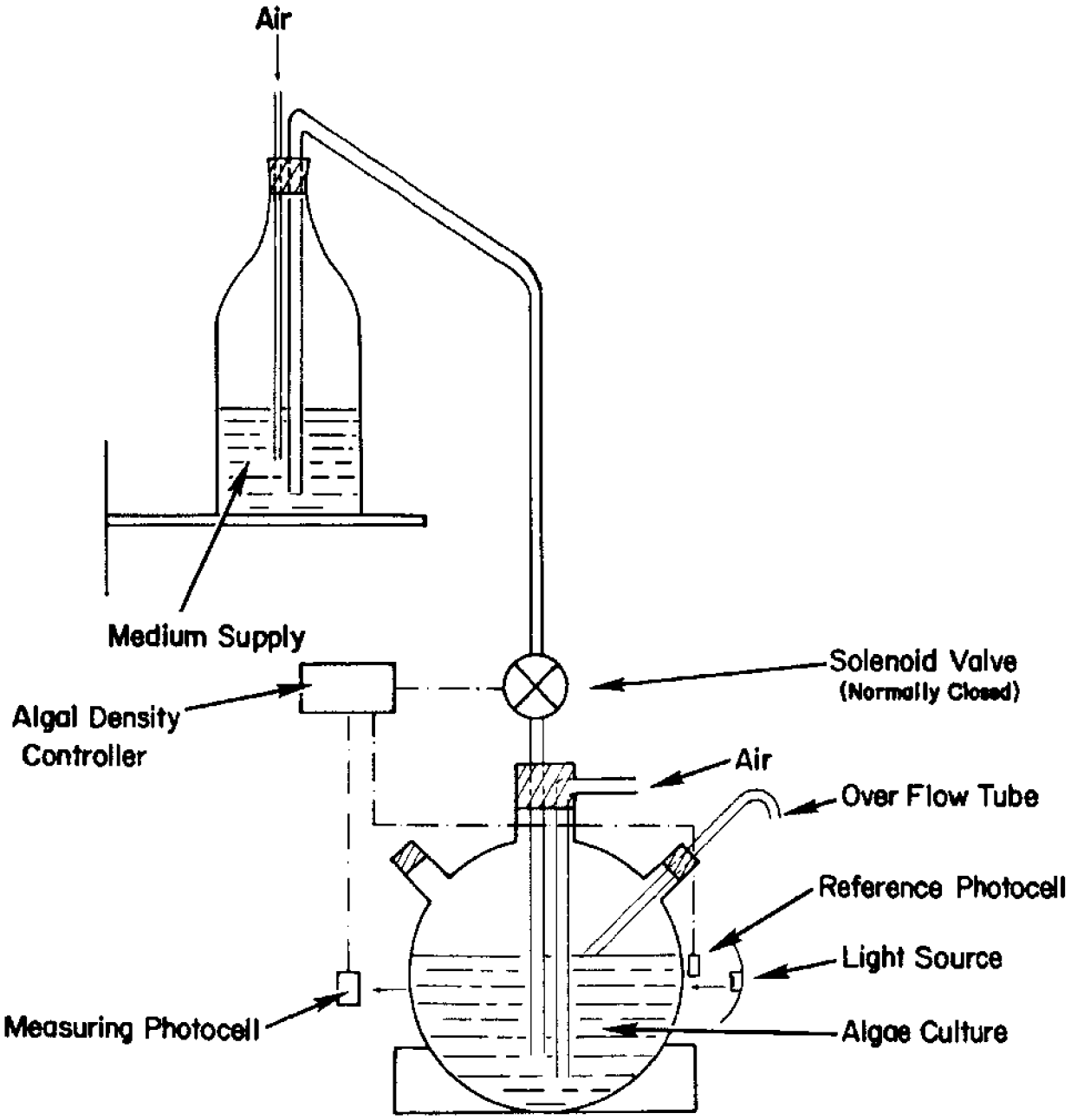


Fig. 8. Controlled Density Algal Culture System

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