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DIVISION of FISHERIES and OCEANOGRAPHY

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FOR MEASURING THE PHOTOSYNTHESIS OF PHYTOPLANKTON

By H. R. Jitts and B. D. Scott

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Abstract

Two incubators used for the study of phytoplankton photosynthesis are described in this report. Both incubators are illuminated by high pressure xenon arc lamps, which are similar in level of irradiance and spectrum to that of sunlight. The temperature of the samples in both incubators can be controlled within the range normally found in nature, to ± 0.1°C.

INTRODUCTION

Most laboratory studies of the photosynthesis of marine phytoplankton have been accomplished in the past using incubators illuminated by incandescent or fluorescent lamps. The spectral characteristics of these sources of radiance are quite dissimilar to that of the "white" radiation from the sun, and the energy available from these sources is often much lower than that found in the natural environment. It is also often difficult to measure the energy received by the phytoplankton samples in these incubators due to the geometry of the source of radiance. Other studies have been made using the light fron the sun near noon, but this method can only be used when the sky is clear, and furthermore, samples can only be taken and incubated at high levels of irradiance close to noon.

High pressure xenon arc lamps have a spectrum in the photosynthetically usable portion of the spectrum (350-700 nm) which is similar to that of sunlight. Such lamps are capable of producing levels of irradiance higher than that of sunlight. The xenon lamps also have the advantage of being almost a point source of light, so that the measurement of irradiance at the sample position presents no problems of geometry. The high levels of irradiance of the xenon lamp permits the measurement of action spectra of phytoplankton photosynthesis by using narrow band width (20 nm) interference filters. These lamps can thus produce levels of irradiance with spectral characteristics easily related to those found in nature.

Two incubators are described in this report which incorporate xenon lamps, and also control the temperature at which the samples are incubated. The incubators have been used for long periods at sea, and have performed satisfactorily.

I. XENON LAMP, WATER BATH INCUBATOR

General Description

This incubator consists of four water baths which can be separately temperature controlled, but all four are illuminated by a single high pressure xenon arc lamp. The samples are held in 50 cm^3 pyrex flasks in light proof containers with a single filtered window. These containers are placed on racks under water. The levels of irradiance to which the samples are exposed, are controlled by placing the containers at different distances from the lamp, and by inserting neutral filters in the windows. The spectrum of the irradiance is controlled by using suitable spectral filters.

Construction

The incubator consists of three main sections, which are indicated in Fig. 1. The top section contains the xenon lamp and the water baths with their temperature controls. The middle section houses the controls and the circuitry for the xenon lamp, and also the controls and pumps for the water circulation system. The bottom section contains a refrigeration unit, which produces cold water for the temperature regulation of the baths. The overall dimensions of the incubator are 1.20 m high, 1.05 m long and 0.45 m wide, and the total weight empty is about 400 kg.

The baths in the top section are made from brass sheet, strengthened with brass angles, and insulated on the inside by rigid polystyrene foam, attached to the walls and bottom. The baths are wedge shaped, to take advantage of the radiating light, and also to provide space between these wedges along the longitudinal axis, for pipes and control devices. The top of the bath is made of wood, and can be unbolted and removed for maintenance. For routine introduction and removal of samples, a small hinged hatch is provided on the top of each of the four baths. The surfaces inside the baths are coated with non-reflective black paint. In the centre of the four baths there is a cylindrical pyrex glass well, within which the xenon lamp is mounted. The joints between the bath walls, the wooden top and the glass well are made water tight by means of rubber mouldings.

Inside each of the four water baths there is a rack, as indicated in Fig. 2. This is composed of a brass frame with six rails, which radiate from the light source. These rails have 3 mm holes drilled at 10 mm intervals along their length, to enable the sample flask holders to be accurately located in predetermined positions.

The sample flask holders are 50 mm diam. brass cylinders, which are slightly larger than the sample flasks. They are fitted with a blank cap at one end to allow entry for the sample flasks, and at the other end they are fitted with a square cap with a circular window, into which the clear, neutral or spectral filters can be placed. On one side of the holder there are two clips, one of which has a short pin, allowing the holder to be positioned securely on the racks in the bath. The sample flasks which fit into the holders are round bottomed pyrex glass flasks, 50 cm^3, with B19 ground glass stoppers. The stopper is held in place by a rubber band over the top of the stopper, secured by two hooks on the neck of the flask.

The water in each of the four baths is stirred and circulated by means of four separate centrifugal pumps located in the middle section of the incubator.

Water is removed from the bottom of each bath and pumped back to a distribution manifold, mounted just below the water level in each bath. This circulation system is also used for regulating the water temperature of the baths. After the water leaves the bath, some cold water from the cold water supply is added to it, and the colder mixture is then adjusted to the required temperature with a thermostatically controlled heater (1000 watt), before it is pumped back to the bath. This temperature regulation is carried out in a chamber located in the wedge shaped space, between the baths in the top section of the incubator. The excess water introduced to the baths from the cold water supply, is removed by means of a ball valve level control located in the thermostat chamber, and returned to the cold water supply circuit.

The cold water supply in the bottom section of the incubator consists of a refrigerator (2900 watt), which cools water in a heat exchanger. This water is pumped through a filter (PALL, Ultipore® 9) to remove particulate matter greater than 0.5 μm before being metered to each of the four baths. The refrigerator produces water at temperatures down to 5°C, depending on the flow rate and the temperature of the water returned to it. When ethanol-water mixtures are used to fill the bath, the refrigerator can be switched to provide a coolant supply as low as -10°C.

All the pipes in the incubator are insulated to prevent heat loss, and also to prevent condensation which would be harmful to the electrical components. Distilled water is used to fill the bath to avoid scattering of the light, and also to minimise growths of algae or fungi in the bath. If the water does become cloudy after some weeks of use, it is drained out through a valve located in the middle section of the incubator, and then replaced with fresh distilled water, after first changing the filter. The total capacity of the baths and cooling unit is 115 dm^3.

The water cooled xenon arc lamp (Wotan, XBF, 1000 watt) is mounted in the centre of the pyrex glass well, in the top section of the incubator. The cooling water for the lamp is pumped around a closed circuit, going from the pump to a heat exchanger, cation exchange resin column, and finally to a bubble elimination chamber before reaching the lamp. The heat exchanger is cooled by mains water, or by a seawater supply if used on a ship. The cation exchange column is necessary to prevent the deposition of metallic compounds originating from the heat exchanger or other pipes, on the hot inner surface of the lamp jacket. If after some time of operation the lamp becomes blackened due to this cause, the deposit may be removed by filling the lamp jacket with concentrated hydrochloric acid for a minute or two, then rinsing with water before refilling the cooling circuit with distilled water. The cooling water circuit is provided with automatic devices which will switch off the xenon lamp if the water becomes too hot, or if the flow is reduced. The electrical components of the circuits needed to ignite and maintain the xenon arc are housed in the middle section of the incubator. Since this incubator was constructed, "ozone free" air cooled lamps have become available, which could replace the water cooled lamp described above.

Voltage fluctuations of the mains power supply can result in shortened lamp life, and also make the measurement of irradiance in the incubator very difficult. This problem is overcome by connecting an electromechanical voltage stabilizer in series with the incubator.

The spectrum of the radiance from the xenon lamp has a high proportion of its energy in the infra red region. To reduce the amount of infra red reaching the water baths a cylindrical reflective coated heat filter (Schott, type T6/11) is mounted around the lamp, and this in turn is cooled by a small fan located below the lamp. This fan also assists in ventilating the middle section of the incubator and preventing condensation.

Performance

The temperatures of the baths can be maintained within the range of 35°C to 8°C, although temperatures can be maintained down to -5°C when water-ethanol mixtures are used as the coolant. The time taken for the baths to reach the desired temperatures from ambient is about 20 minutes. When the required temperatures have been reached, the average fluctuation about that temperature is 0.1°C.

By varying the position of the sample flask holders in the bath, irradiances from 25 to 250 $W.m^{-2}$, between 400 and 700 nm, can be obtained. Lower levels of irradiance can be obtained using neutral filters in the flask holders. The spectrum of the irradiance in the bath, as determined with an ISCO spectro-radiometer (model SR), is shown in Fig. 3.

II. XENON LAMP, HIGH IRRADIANCE INCUBATOR

General Description

This incubator consists of a single, air cooled, high pressure xenon arc lamp, which illuminates eighteen samples held in 50 cm^3 flasks. The flasks are contained in individual water jackets, the temperatures of which are controlled by circulating water through the jackets from an external controlled temperature water bath.

The eighteen samples are located in two groups; twelve positions can receive levels of irradiance equivalent to 15% of full sunlight, and the other six can receive levels of irradiance of more than three times full summer sunlight. The level and spectra of the irradiance to which samples are exposed is controlled by using neutral or spectral filters inserted in a rack between the xenon lamp and the samples.

Construction

The incubator has a square wooden base, which supports the body of the incubator, made from brass angle and sheet. The body of the incubator encloses the lamp assembly which is suspended inside the body from three columns which are fixed to, and extend, above the body. A stainless steel tray, slightly larger than the base is fitted below the base to catch condensation or accidental spills. The incubator is 1.2 m high and 0.8 m square and weighs about 25 kg, not including the water and power supplies, which are separated from the incubator. A general view of the incubator is shown in Fig. 4.

The lamp assembly consists of the xenon lamp (Wotan, XBO, 900 W) mounted on a sub-frame surrounded by a cylindrical reflective coated heat filter (Schott, type T6/11), and outside this filter by six glass lenses, as shown in Fig. 5. These lenses (57 mm diam., 64 mm focal length) are mounted edge to edge around the horizontal plane of the xenon arc. The purpose of these lenses is to collect light from close to the lamp and collimate it, so that samples can be irradiated at a greater distance from the lamp without attenuation of the light. The ignition device for the xenon arc lamp is mounted on top of the lamp assembly. The lamp is cooled by a fan mounted on the wooden base below the lamp. To avoid the necessity of exhausting the cooling air from the laboratory, the "ozone free" type of lamp is used in this incubator. A fibrous filter similar to that used in air conditioners is fitted below the fan to remove dust from the air, which may otherwise deposit on the lamp and become burnt into the silica surface, reducing its life and brightness. This dust may also deposit on the heat filter and lenses and obscure them.

Around the body of the incubator there are two rings of sample positions. The six upper positions are those that correspond with the high irradiance collimated light beams. The twelve lower positions utilise non-collimated light which has passed through the lower half of the heat filter and is of a lower irradiance level due to the attenuation caused by the greater distance of the samples from the xenon lamp.

At each sample position an enclosed filter rack is fixed to the body of the incubator. Neutral or spectral filters (50 mm x 50 mm) mounted on square brass plates with a circular window can be placed into slots in this filter rack in order to modify the irradiance which reaches the sample. These racks are also provided with metal blanks which can be fitted into a filter slot to cut off the light to the samples. On the outer end of each filter rack a circular recess and a knurled head screw is provided to attach the sample flask jacket.

The sample flask jacket consists of a brass cylinder which is fitted with two threaded brass caps, made water tight with O rings. One of these caps has a circular glass window. The sample flasks which fit into these jackets are round bottomed pyrex glass flasks, 50 cm^3 with B19 ground glass stoppers. The flask stopper is held in place by a rubber band over the top of the stopper, secured by two hooks on the neck of the flask. Two small pipes on the jacket are provided for connection of the temperature regulated water supply. The water supply is distributed to the water jackets in parallel from two manifolds fixed below the wooden base, with individual tops for each jacket. One manifold is used as the inlet and the other as the outlet. The total volume of water in the incubator is 2500 cm^3. Distilled water is used as a coolant to avoid light scattering and also to minimise growths of algae and fungi in the water circuit.

Water is supplied to the incubator from an external constant temperature bath. If more than one incubation temperature were required, more baths and manifolds could be used.

All the internal parts of the incubator which are not optical elements are coated with non-reflective black paint. The metal parts on the outside of the incubator are treated in a similar manner to aid in the dispersion of heat created by the lamp.

The transformer-rectifier unit which produces the direct current supply for the xenon lamp is located in the proximity of the incubator, together with the electromechanical voltage stabiliser. Voltage stabilisation is necessary since fluctuations can result in shortened lamp life and also make the measurement of irradiance less precise.

Performance

The level of irradiance in all the six upper sample positions is about 1700 $W.m^{-2}$, and in the twelve lower positions 75 $W.m^{-2}$, within the wavelength range of 400 to 700 nm.

The spectra of the irradiances in these two locations are shown in Fig. 3. There is a slight difference in the two spectra which may be due either to the extra thickness of the glass lens interposed between the lamp and the sample in the six upper positions, or to the different angle of incidence on the reflective coated heat filter in the twelve lower positions.

The temperature range possible with this incubator is only limited by the cooling/heating and pumping capacity of the external constant temperature water bath. Temperatures which are much lower than ambient may produce condensation on the outside of the window of the sample flask jacket and cause attenuation of the light due to scattering.

COMPARISON OF THE TWO INCUBATORS

Both incubators are illuminated by xenon arc lamps, and have means for controlling the temperature of the samples, but their characteristics suit them for different applications.

The xenon lamp, water bath incubator, is more suited to investigations where growth *vs* irradiance *vs* temperature relationships are required. Although this bath incubator is heavy and requires a large volume of clean water it is compact and has reliable temperature control. Larger samples can be incubated if the racks in the bath are changed or removed. The type of neutral or spectral filters used in this incubator must be of a type which are not affected by immersion in water. Although the maximum sample irradiance possible in this bath is only about half that of full sunlight, it is sufficient for most photosynthesis *vs* irradiance studies. It is however difficult to irradiate many samples with the same level of irradiance due to the geometry of the incubator baths.

The xenon lamp, high irradiance incubator, is more suited to operation at temperatures within 7^{o}C of ambient, since the water jackets are not insulated. Broader temperature ranges can be used, but the control would be less precise, and the use of low temperatures may cause condensation on the glass window of the sample flask container. In this incubator it is possible to irradiate six samples at high irradiance or twelve samples at the same lower irradiance, and is therefore suited to applications which require the same level of irradiance at different wavelengths. The high level of irradiance also makes it possible to use spectral filters of 30 to 50% transmission to measure growth rates in wavelength bands, at levels of irradiance which are comparable to those of full sunlight, within those wavelength bands. Since these filters are not immersed in water, a greater variety of filter types may be used in this incubator. The measurement of irradiance in this incubator is also less complex, since the sensing device need not be waterproof.

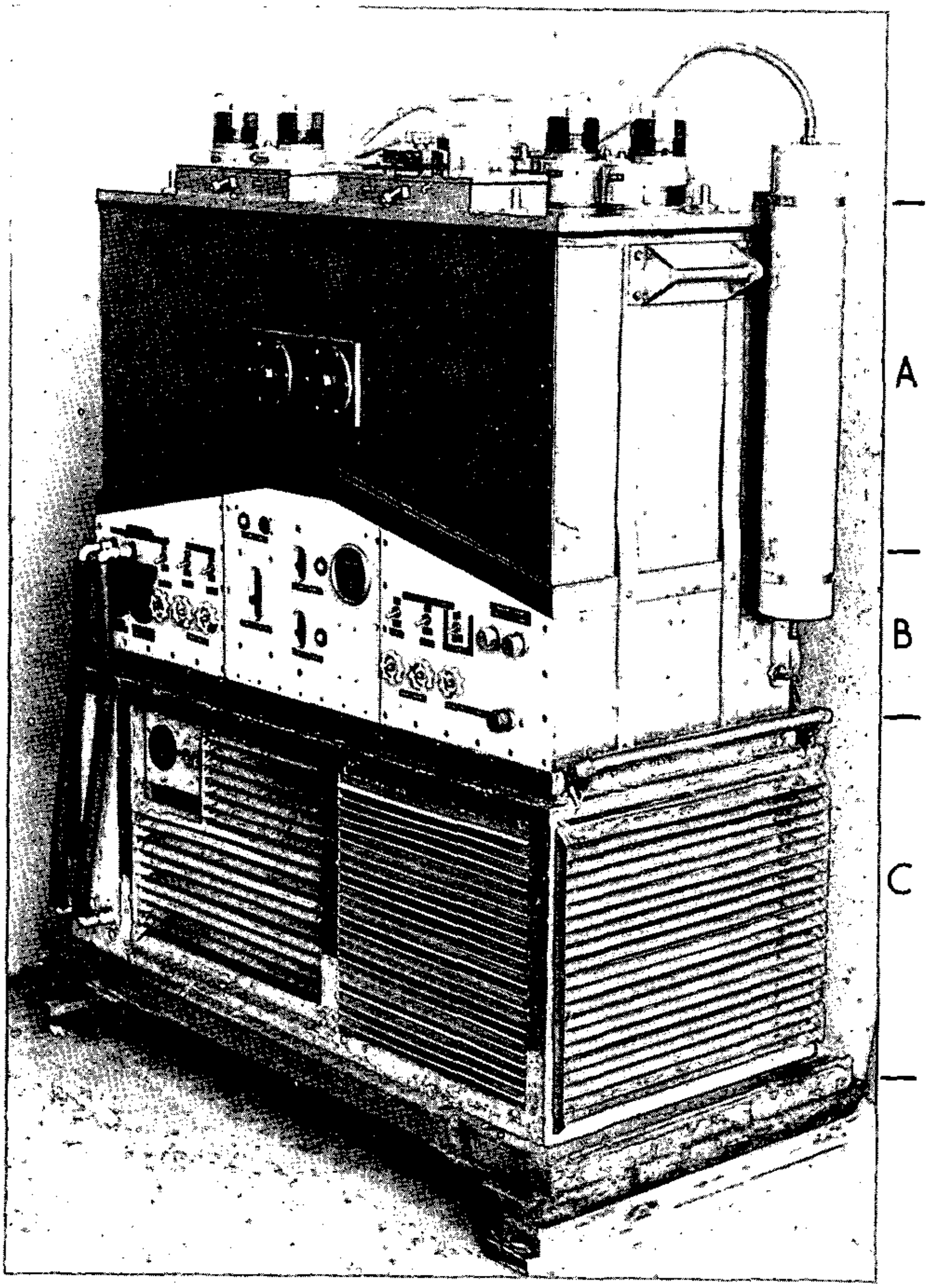

Figure 1. Xenon lamp, water bath incubator.

A. Top section, containing xenon lamp, water baths and temperature controls.
B. Middle section, containing controls and circuitry for lamp, and pumps for the water circulation.
C. Bottom section, containing the refrigeration unit.

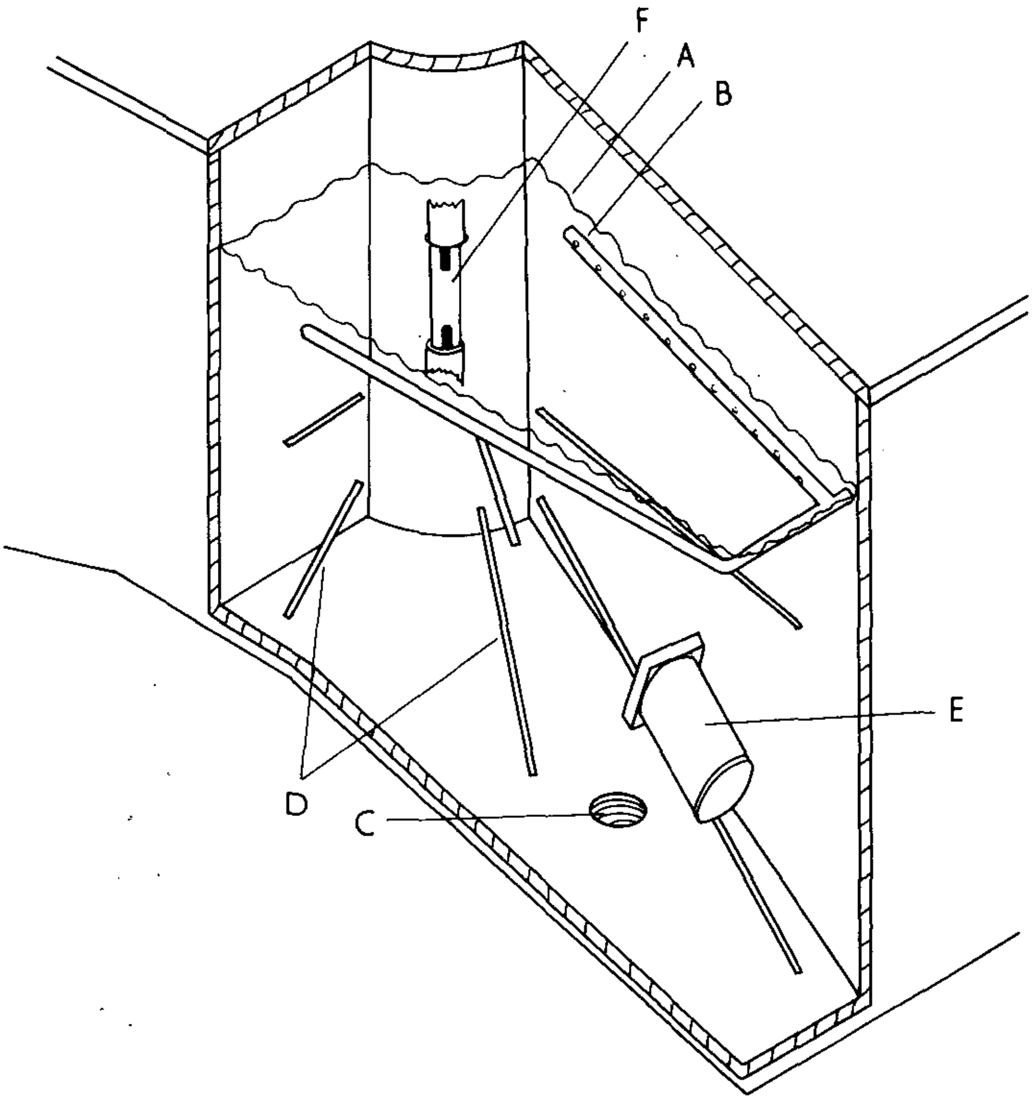

Figure 2. Xenon lamp, water bath incubator
A cut-away section of one of the four incubation compartments showing:

A. Water level, B. water inlet manifold, C. water outlet
D. sample flask holder racks, E. sample flask holder,
F. arc lamp, within the central glass well.

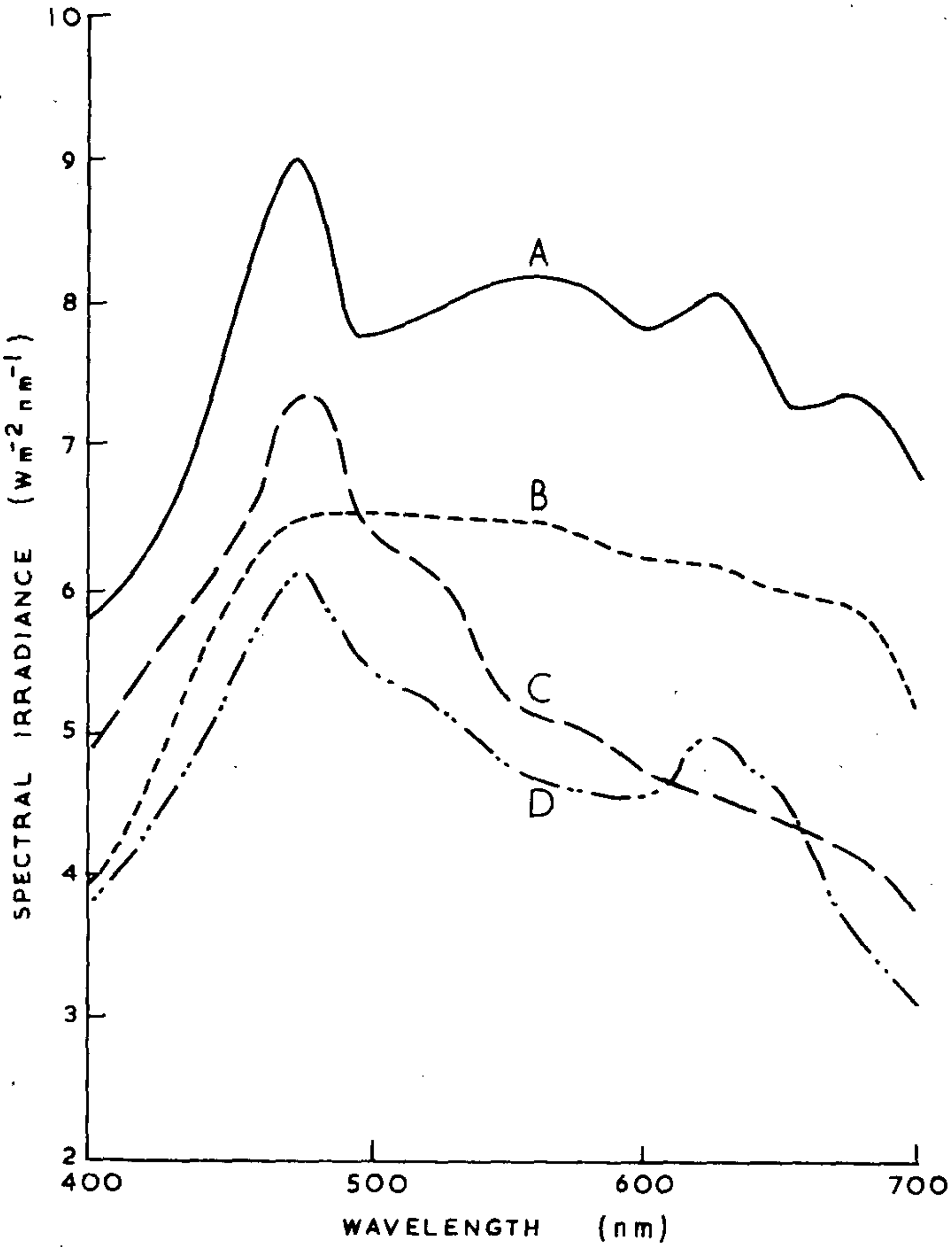

Figure 3. Spectral distribution of irradiance

A. Water bath incubator (x 10)
B. Typical solar irradiance (x 5)
C. High irradiance incubator, upper sample positions (x 1)
D. High irradiance incubator, lower sample positions (x 20)

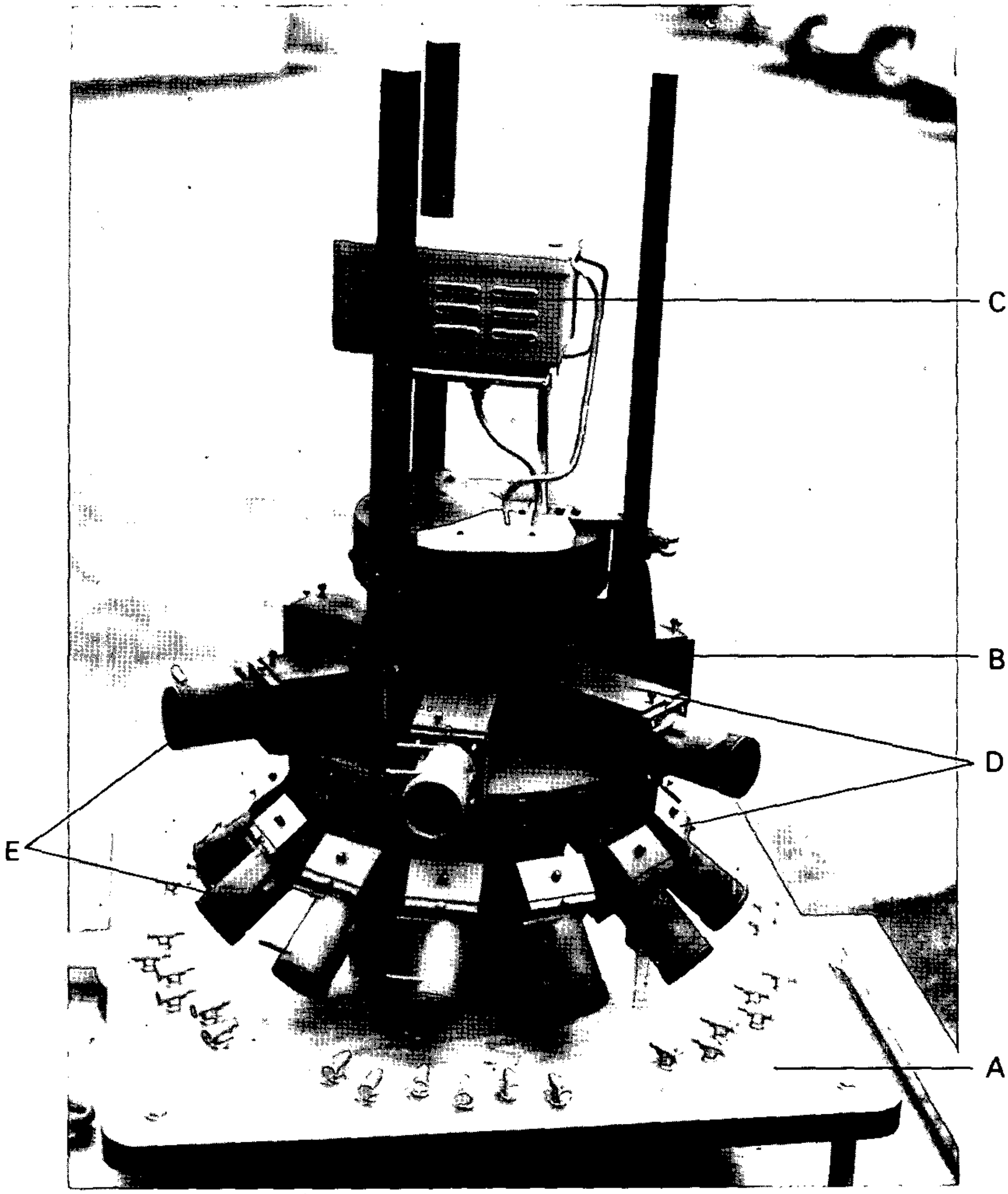

Figure 4. Xenon lamp, high irradiance incubator.

A. Wooden base
B. Body enclosing the lamp assembly
C. Drip tray
D. Ignition device
E. Filter rack

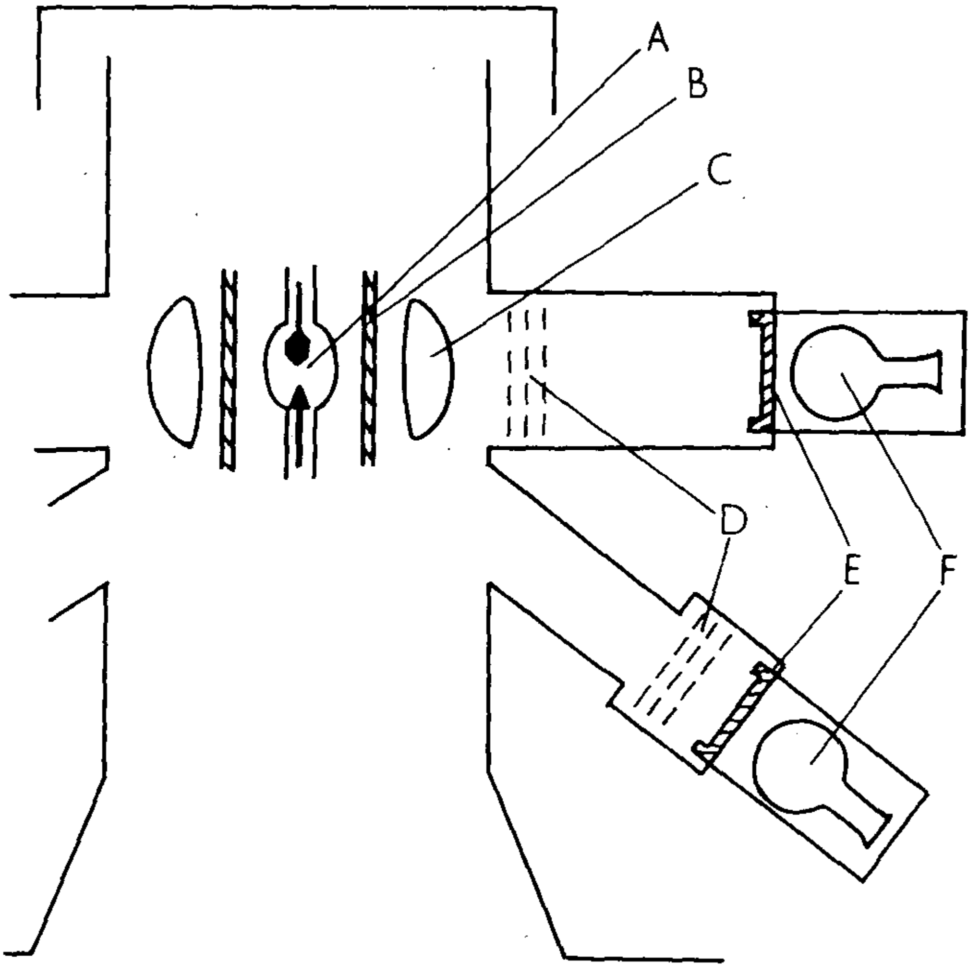

Figure 5. Xenon lamp, high irradiance incubator. Diagrammatic cross section showing optical components.

A. Xenon lamp, B. heat filter, C. condensing lens, D. filters, E. glass window, F. sample flask.