2. E. B. CHAIN, S. PALADINO, F. UGOLINI, D. S. CALLOW and J. Van der SLUIS (*). — **A laboratory fermenter for vortex and sparger aeration.**

Summary. — An all metal laboratory fermenter of 10 litres total capacity is described. It is suitable for aerobic fermentations with non pathogenic micro-organisms. Aeration may be effected either by the vortex method or by means of an air sparger in the presence or absence of baffles. The fermenter is easily handled and has been shown to be mechanically reliable and to give a low incidence of contamination when in routine use over a long period. Full constructional and operating details are given.

Riassunto. — Sono descritti ed illustrati fermentatori da laboratorio da 10 litri di capacità totale per fermentazioni con micro-organismi non patogeni. L'aerazione può essere effettuata sia con il sistema «vortice» che con distributore in presenza o meno di antivortici. Questi fermentatori hanno funzionato ed a lungo con un minimo numero d'inquinamento.

Résumé. — Les A. décrivent un fermentateur de laboratoire, entièrement métallique, d'une capacité de 10 litres. Il est destiné aux fermentations aérobiques de germes non-pathogènes. L'aération s'y effectue soit par le système vortex, soit par un disperseur d'air, avec ou sans panneaux de turbulence. Ce fermentateur est d'un maniement aisé et doté de qualités de confiance; l'incidence de contamination en travail continu est très faible. Les détails de construction et de manipulation sont minutiesement exposés.

Zusammenfassung. — Ein aus rostfreiem Stahl hergestelltes Laboratoriumsgärgefäss von 10 l Gesamtinhalt wird beschrieben. Es ist geeignet für aerobe Gärungen in Tiefkultur mit nicht pathogenen Mikroorganismen. Belüftung kann durch das Vortexsystem oder durch Luftverteilung mittels eines Luftverteilers in Gegenwart oder Abwesenheit von Prallblechen erfolgen. Das Gärgefäss ist einfach in der Handhabung und arbeitet mechanisch sehr zuverlässig. Verunreinigungen durch Fremdorganismen kommen sehr selten vor, wenn die nötigen Vorsichtsmassnahmen beachtet werden. Genaue Angaben der Konstruktionseinzelheiten der Gärgefässed und seiner Gebrauchsmethoden werden mitgeteilt.

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Introduction.

In a previous communication concerned with the study of different aeration devices and factors influencing aeration efficiency a « spargerless » method of aeration was described in which aeration was effected by sucking air into the liquid to be aerated from the atmosphere above by means of a vortex created by fast rotating propeller blades; the air is dispersed into fine bubbles by the blades at the apex of the vortex (Chain, Paladino, Callow, Ugolini and Van der Sluis, 1952) (*).

The present paper is concerned with the description of laboratory fermenters of 10 l capacity suitable for aerobic fermentations in which the aeration of the culture fluid can be effected either by the vortex method or by means of an air sparger in the presence or absence of baffles.

MATERIALS OF CONSTRUCTION

The following materials have been used in the construction of the fermenters and accessories described below.

1) Stainless steel 18/8.
2) Brass.
3) Iron standard pipe fittings.
4) Aluminium.
5) Glass, thick walled pyrex.
6) Rubber pressure tubing and stoppers of pure gum (para) rubber. This stands up well to repeated sterilisation with steam.
7) Rubber pressure hose, canvas reinforced for use up to 10 atm.

Components.

A cross section and photographs of the fermenter are given in fig. 1 and plates 1-4. It consists of a fermenter body and a head carrying the bearing and stuffing box for the propeller shaft and various accessories.

(*) It was recently pointed out to one of the present authors (personal communication from Dr. A. Finlay of Messers. Chas. Pfizer & Co., Brooklyn, New York, U.S.A.) that J. N. Currie and A. Finlay had applied for and obtained in 1932 an American Patent, No. 1908225, for an aeration method the principle of which is similar to that described in the paper referred to. This patent was unknown to us, and, in general, the method of vortex aeration does not seem to have been widely used. In order to make the vortex aeration method applicable to sterile aerobic fermentations numerous difficulties of mechanical nature have to be overcome. The methods by which this has been achieved, appear to the authors of sufficient novelty to warrant a detailed description.

Fermenter body.

The fermenter body (fig. 1) consists of a stainless steel cylinder to one end of which a dished bottom and to the other end a flange is welded. Six holes are drilled into the flange through which pass the stainless steel bolts connecting the fermenter body with the fermenter head. The heads of the bolts are welded to the underside of the flange. The flange is provided with a recess to accomodate a square section rubber gasket. The fermenter head is made tight on the body by means of six stainless steel hexagonal nuts or wing nuts and stainless steel washers. A number serving as reference mark is punched on all fermenter heads and bodies in order to avoid interchange and ensure that bolts and holes coincide.

Fermenter head plate. Spacing of holes.

The head consists of a stainless steel plate, diameter 250 mm, thickness 12 mm. It is drilled as indicated in fig. 2. The six unthreaded peripheral clearance holes, serve for passage of the bolts used for fastening the head to the body of the fermenter. The central hole, (1), is threaded to take the stuffing box (see below). The 6 holes (2-7 fig.2), are drilled equidistant 75 mm from the centre of the head. Hole 8 is drilled 50 mm from the centre of the head. Holes 2 and 3 serve for the air inlet and outlet tubes, holes 4 and 5 for addition vessels, hole 6 for the introduction of the inoculum or the occasional addition of solutions and hole 7 for the sampler tube. Hole 8, threaded, serves for the insertion of a sparking plug, acting as foam detecting electrode (see below p. 69). The spacing of the holes in the above manner is important; on it depends the ease of the manipulation of the fermenter. The position of holes 7 and 8 is critical. If the vortex system of aeration is used the sampler tube which passes through hole 7 must be as near as possible to the wall of the fermenter in order to reduce its baffling effect. On the other hand the position of hole 8 must be such that the foam detecting electrode passing through it is not so near the wall of the fermenter that it may be easily short-circuited.

Air inlet.

The arrangement for the air inlet is illustrated in fig. 3. For all connections, wherever possible, standard 1/4" pipe fittings have been used. Since these could not easily be obtained in stainless steel, or-

dinary steel fittings have been employed and have given no trouble. The filter tube, however, was made from stainless steel as experience with tubes made of iron showed that repeated sterilization gave rise to excessive rusting. The air enters from the distribution line through a short length of rubber pressure hose connected by means of a rapid coupling (1 fig. 3) screwed to an air filter by means of a bend (2 fig. 3). The filter consists of a length of stainless steel tubing (3 fig. 3) threaded externally at one end to take the 1″ pipe cap (4 fig. 3) for closure. The pipe cap is drilled and tapped for connection with the bend. The other end of the filter tube is closed by a welded plate drilled and tapped for connection through a nipple (5 fig. 3) to a tee (6 fig. 3) and bend (7 fig. 3). The latter is connected to a short length of stainless steel tubing (9 fig. 3) through a globe valve (8 fig. 3). Tube 9 fig. 3 is threaded internally so that it can be connected with an air sparger (see 1 fig. 5) if required. The tee (6 fig. 3) is also connected through a bend (10 fig. 3) and a short length of pressure tubing to the side arm of the sampler, as indicated in fig. 6. This by-pass serves to blow air through the sampling tube into the fermenter in order to free it from stagnant culture fluid prior to sample taking.

Air outlet.

The air leaves the fermenter through the stainless steel elbow (11 fig. 3) the stainless steel filter tube (12 fig. 3) constructed as the inlet filter tube (3 fig. 3) but shorter, the tee (13 fig. 3) to which a pressure gauge measuring 0 to 4 atm. is attached, the bend (14 fig. 3) and the globe valve (15 fig. 3) to which is screwed a short connection tube for a rubber tube leading to an air flowmeter.

Packing of air filters.

Tightly packed fine Corning Fiberglass is used for the filters [30 gms for the air entry filter (3 fig. 3), 20 gms for the air exit filter (12 fig. 3)]. It was found with the Bourdillon slit sampler (Bourdillon, Lidwell and Schuster, 1948) that the air leaving the compressor was practically sterile. This observation, part of a series of unpublished studies on air sterilisation in this laboratory, is in agreement with the findings of Stark and Pohler (1950). The air filters on the fermenters were essentially considered to function as a safety device; their efficiency of air sterilization was not determined quantitatively.

Contaminations arising from the air supply have never been encountered.

Bearing and stuffing box unit.

In the construction of the bearing and stuffing box unit due consideration had to be given to three main points:

1) With the vortex system of aeration the stirrer has to rotate at fairly high speed (700-1500 r.p.m.).

2) For many fermentations a period of at least one week is necessary during which time no maintenance other than greasing is possible.

3) For fermentations carried out under positive pressure leakage must be reduced to a minimum.

In order to meet these requirements the following construction has been developed (fig. 4).

The stuffing box is turned from a brass bar to the shape indicated in the figure (2 fig. 4, for details see 2 fig. 4-a). On the flange the threaded hole (a) serves for introducing grease for the lubrication of the lower ball bearing. This hole is normally closed by means of a screw and a graphite-asbestos washer of the Klingerit type. The flange is slotted in two places (bb) to allow the insertion of a pin spanner for tightening the complete stuffing box unit through the cap (5 fig. 4) to the fermenter head.

The stuffing box is externally threaded above and below the flange. The upper thread serves for holding the knurled brass cap (3 fig. 4, for details see 3 fig. 4-a) the purpose of which is to compress the packing material and to carry the upper ball bearing. To the inner race of the latter is fitted the chuck (6 fig. 4, for details see 6 fig. 4-b) which grips the shaft (1 fig. 4) by means of the knurled lock nut (8 fig. 4). This arrangement fixes the upper ball race on the shaft but allows it to follow the movement of the cap (3 fig. 4) during compression of the packing material to the desired degree, whilst at the same time the inner and outer races of the upper ball bearing are blocked, the former by means of the small lock ring (9 fig. 4, for details see 9 fig. 4-b), the latter by means of the threaded cover plate (4 fig. 4, for details see 4 fig. 4-a). Packing rings (with square section) of the type « triplex star » (made in Italy) were used, consisting of a mixture of hemp, asbestos and graphite soaked in a solution of silicone.

The lower ball bearing is inserted into the cylindrical recess below the flange of the stuffing box. The outer race is held in position and prevented from turning by a ring (10 fig. 4, for details see 10 fig. 4-b) which blocks the race between the inner shoulder of the externally and internally threaded cap (5 fig. 4) and the lower shoulder of the stuffing box. The inner race is blocked between the lower shoulder of the widest part of the propeller shaft and the inner tube of the grease cup (7 fig. 4, for details see 7 fig. 4-b) which is screwed to the shaft.

Cap 5 (fig. 4, for details see 5 fig. 4-a) is constructed of stainless steel as it is exposed to the atmosphere inside the fermenter. The internal thread of cap 5 fits the lower external thread of the stuffing box unit and is tightened to it by means of a pin spanner inserted into two recesses drilled for this purpose into the lower surface of the cap.

Propeller shaft.

The propeller shaft consists of a stainless steel rod (1 fig. 4) threaded at the lower end and at a point corresponding to the position immediately below the lower ball bearing. The latter thread connects cup 7 serving for collecting excess grease and preventing it from dropping into the culture medium. The lower thread of the shaft serves for connecting the propeller (15 fig. 4-c).

A keyway is cut into the upper part of the shaft. A pin inserted into it connects the shaft with the inner race of the upper ball bearing and prevents the shaft from rotating independently of the ball bearing. The unthreaded upper extremity of the shaft carries one half of a flexible coupling (11 fig. 4) the other half of which carries the floating shaft of the transmission (see page 73).

Propeller.

Stainless steel propellers of different diameters, of the disc turbine type as previously described (Chain et al., 1952), were used throughout. The approximate overall diameters were 65,75 and 90 mm. Constructional details of the propeller with the smallest diameter are given in 15 fig. 4 and 15 fig. 4-c. The larger sizes were made in geometrical proportion except for the height of the propeller blades which was kept constant at 15 mm. The propellers are held on the shaft by the hexagonal stainless steel nut (14-b fig. 4) and the stainless steel washer (14-a fig. 4).

Air sparger and baffle plates.

If required for aeration by the fully baffled system an air sparger can readily be fitted by screwing to the short threaded tube (plate 5) of the air inlet. The sparger (2 fig. 5) consists of a stainless steel tube shaped so that the air leaves the tube centrally below the propeller. The air outlet points upward and is externally threaded to take different nozzles. The type of nozzle which has been generally used is shown in 2 fig. 5; four equidistantly spaced holes of 1 mm ∅ are drilled into its upper surface.

The baffle plates consist of four equidistantly spaced strips of stainless steel (210 mm long. 15mm wide and 1 mm thick). They are held together by two strips of the same metal 550 mm long, 10 mm wide and 2 mm thick welded at the top and the bottom and bent to a circle (plate 4). The baffle unit is inserted into the fermenter body and the natural spring of the metal is sufficient to hold it firmly in position against the fermenter wall.

Arrangement for sterile sampling.

Sterile samples of the culture fluid are taken under positive pressure through the stainless steel sampling tube (1 fig. 5) which passes to the bottom of the fermenter through hole 7 of the fermenter head. The part of the sampling tube above the fermenter head is bent as shown so as to give room for a normal laboratory boss head and clamp on its vertical part to hold the sampler unit.

The sampling tube is connected to the sampler unit (fig. 6) by means of a length of rubber pressure tubing which during the fermentation is kept closed by means of the special clip (fig. 7) (see also plates 1 and 2). The construction of the sampler unit is visible from fig. 6. The receiver made of thick walled pyrex glass has a side arm which is connected by means of rubber pressure tubing to the by-pass tube (10 fig. 3) of the air inlet filter. It is closed by means of a rubber bung through which a short length of stainless steel tubing (9 fig. 6) passes, connecting the glass receiver with the upper end of the sampling tube (1 fig. 5). The rubber bung is prevented from moving upwards by the small metal ring (13 fig. 6) welded to stainless steel tube (9 fig. 6). A short length of rubber tubing is slipped on tube 9 above the small metal ring; it serves to give a better grip to the clamp connecting the receiver assembly to the vertical tract of the sampling tube.

The end of the receiver is connected through a short length of rubber pressure tubing to the externally threaded stainless steel tube 6 (fig. 6) on which is held the metal hood (2 fig. 6) by means of two 1/4″ nuts (8 fig. 6). Tube 6 is kept closed during the fermentation by means of a 1/4″ pipe cap (7 fig. 6).

All rubber tube connections are tightly wired on the respective tubes. The receiver and the hood connected to it is prevented from coming off the rubber bung by means of the clamp arrangement illustrated in fig. 6.

The three brass rods (10 fig. 6) connect two aluminium rings (5 & 11 fig. 6) one resting on hood (4 fig. 6), the other held by a small rubber ring (15 fig. 6) placed on the tube below the wider part of the receiver; this rubber ring protects the glass receiver against pressure exerted by the three wing nuts (12 fig. 6) by means of which the receiver assembly may be tightened against the rubber bung to the desired degree. The purpose of hood 4 is to reduce the risk of contamination should the receiver assembly momentarily, through overpressure from the fermenter, during the operation of freeing the sampler tube from stagnant culture fluid, be dislocated from the rubber bung. The risk of contamination through such an event is however, very small as a stream of sterile air under positive pressure would in this case always pass from the by-pass through the receiver.

For opening and closing the rubber tubing for the purpose of sampling a special spring clip is used the constructional details of which are given in fig. 7. The function of this clip is to enable the operator to open and close the tube rapidly and smoothly and thereby to regulate at will the volume of the sample. It is difficult to achieve this aim by any commercial valve or clip. The special clip, normally held closed by a steel spring, fits comfortably into the palm of the hand and can thus be easily regulated.

Simplified sampler.

For working at overpressures of 1 to 2 atmospheres the sampling arrangement has been modified (see plate 3). The glass receiver has been eliminated and substituted by a 1/4″ metal tee the upper end of which is connected to the sampling tube by means of rubber pressure tubing, the lower end to the hooded tube (6 fig. 6), while the side arm is connected by means of a 1/4″ stainless steel tube and a 1/4″ globe valve to the by-pass tube (10 fig. 3). This modification has proved

so successful that it is now used also in routine fermentations at the normal overpressure of 0.5 atm.

A still further simplification of the sampler has been introduced while this paper was in the press, and has proved very successful. The rubber tubing which on occasions was blown off or burst during the fermentation owing to excessive overpressure has been completely eliminated and replaced by a stainless steel tube. The special spring clips and the Hoffman clips have been replaced by Klinger cocks (AB10) of brass. (These were recommended to us by Dr. R. Elsworth of the Experimental Station, Ministry of Supply, Porton U. K.). The new arrangement is illustrated in plate 7.

Foam detecting electrode.

Aeration of culture media is nearly always accompanied by a more or less intense foam formation. With the vortex system of aeration usually little mechanical trouble is experienced through foaming though the aeration efficiency may be greatly reduced by microfoam formation even if the foam does not rise to the top of the fermenter (see CHAIN *et al.*, 1952 and CHAIN and GUALANDI, 1954). When aeration is effected by means of an air sparger foaming always presents a serious problem on the effective control of which the success of the fermentation often depends. It is therefore of considerable importance to detect foaming before the foam reaches the top of the fermenter. Foam detecting electrodes which close a circuit on contact with foam have been described by several authors (ANDERSON, WHITMORE, BROWN, PETERSON, CHURCHILL, ROEGNER, CAMPBELL, BACKUS and STAUFFER, 1953; BROWN and PETERSON, 1950a). The present authors have used as foam detecting electrode a spark plug (1 fig. 8) screwed into hole 8 of the fermenter head (fig. 2). A stainless steel wire of 2 mm thickness and 150 mm length is welded to the lower end of the central spark plug electrode, the protruding end of the other electrode having been removed. When the foam reaches this wire an electric current passes, the stainless steel body of the fermenter acting as the other electrode, and closes an electronic relay. In order to avoid short circuit through condensed water between the stainless steel wire and the metal body of the spark plug (which being in direct contact with the fermenter head acts as the second electrode) a short piece of rubber tubing is inserted to completely fill the space between them; furthermore a close fitting rubber sleeve is put on the stainless steel wire along 2/3 of its

length (see 1 fig. 8 and plate 5) and all the surface of the rubber tubing as well as the protruding metal part of the spark plug and a circular area on the underside of the fermenter head immediately adjacent to the spark plug is coated with silicone grease.

The characteristics of the relay must meet the following requirements: 1) It must be sensitive to currents of the order of approximately 50 μ A, as the current passing through the foam (which consists mainly of air) is very small. 2) It is desirable for safety reasons that the voltage of the passing current be low, not higher than 40 V.

For the above reasons an electronic relay is most suitable. The relay used in the present work closed when a current of 15 μ A passed, with an applied voltage of about 24 V.

One single electronic relay is used for a battery of fermenters. An automatic searcher periodically establishes contact with the foam detecting electrode of each fermenter in turn, and in those fermenters where the foam level has risen to the height of the foam detecting electrode the relay closes. This actuates a warning bell and at the same time the number of the fermenter concerned appears on an indicator board as used in domestic bell systems. When the warning bell indicates foam formation, antifoam agent is added manually from the vessels described below. The amount of antifoam added varies with the fermentation under investigation. The foam detecting system as described above has been tested by the authors in penicillin fermentations only and in their experience has been found to function satisfactorily if the antifoam agent added is really effective, so that the foam is never allowed to reach the top of the fermenter. Once the foam has touched the fermenter head it is difficult or impossible to detect and regulate subsequent foam formation owing to the fact that the mycelium deposited by the foam on the fermenter head and electrode rapidly forms a continuous surface growth which short-circuits the electrode (plate 6).

A certain amount of surface growth always forms on the stainless steel rod of the electrode once it has been touched by the foam, but owing to the sensitivity of the relay this does not interfere with the efficiency of the electrode.

Addition vessels.

Details for the construction of vessels for the sterile addition of solutions during the fermentation are given in fig. 9. Two sizes are used, one of about 250 ml capacity with the dimensions indicated in

fig. 9, the other of about 500 ml capacity, with the same height, but 70 mm internal diameter. The addition vessels consist of a stainless steel cylinder (1 fig. 9) closed at both ends by welded plates (2 fig. 9). A short length of stainless steel tubing (3 fig. 9) of sufficient diameter to accomodate an internal 1/4″ gas thread is welded centrally into both plates. The upper tube, normally closed by a stainless steel cap screw (4 fig. 9) serves for introducing solutions into the addition vessels; the lower tube through which the solution passes into the fermenter is connected through a nipple (5 fig. 9) with a 1/4″ seatless piston valve (6 fig. 9) the internal part of which (piston and lining) are made of stainless steel, the housing of bronze). To the latter a short length of stainless steel tubing (7 fig. 9) is screwed; its lower end is threaded and carries a 3/8″ half coupling (8 fig. 9; the other half of the coupling is screwed to a stainless steel tube (2 fig. 8) welded to the fermenter head in hole (4 fig. 2). To stainless steel tube (7 fig. 9) is welded, half way along its length, the narrow stainless steel tube (10 fig. 9) which enters the addition vessel near the top. This by-pass tube connects the atmosphere above the culture fluid inside the fermenter with the atmosphere above the solution in the addition vessel and equalises their pressures. By this arrangement solutions can be introduced into the fermenter by gravity during the course of a fermentation in a sterile manner, on opening valve (6 fig. 9) while the fermenter remains under working pressure.

A glass level gauge (11 fig. 9) is fitted to the addition vessel in the way indicated in fig. 9. The glass tubing (thick walled 8 mm pyrex) is held in two packing glands in which the asbestos string packing contained in the stainless steel cylinder (12 fig. 9) is compressed by the threaded brass nut (13 fig. 9).

Stainless steel cylinder (12 fig. 9) is welded to stainless steel tube (14 fig. 9) which in turn is welded to the top and bottom plates (2 fig. 9) of the addition vessel. Slight leakage around the point of entry of the glass tubing into the packing glands frequently occurs, but has never given trouble with regard to contamination. In the latest model the asbestos packing has been replaced by a small rubber O-ring and no leakage has been observed.

Injection boss for the addition of small amounts of solution.

When the volumes of the solution to be added are relatively small (up to 10 ml) or the additions infrequent it is preferable to effect the

addition by means of a syringe instead of by means of the standard addition vessel. An ordinary hypodermic syringe and needle are used. The injections are made through a rubber diaphragm fastened in a modified half coupling (fig. 10) which is screwed onto the inoculation port (3 fig. 8) of the fermenter head after the fermenter has been inoculated. The diaphragm (1 fig. 10) which is held by the knurled brass cap (2 fig. 10) consists of a rubber cap from a commercial penicillin vial and is easily replaced when necessary. Before making an injection the diaphragm should be swabbed with 70% ethanol or dilute formalin and it is well to reduce the overpressure in the fermenter to about 0.1 atm.

Dispensing device for inoculum.

When a series of fermenters is to be seeded with one microbial strain it is convenient to use one fermenter for the preparation of the inoculum which then provides a uniform seed for the whole series of fermenters. (For details of inoculating see page 79). In order to transfer the inoculum from the seed fermenter to the fermenters to be inoculated and to measure the volume of the inoculum a simple dispensing device is used (fig. 11). This consists of a stout walled pyrex bolt head flask (10 fig. 11) (of sufficient size to contain the desired inoculum for one fermenter) fitted with a three holed rubber stopper, through which pass three pieces of stainless steel tubing of 10 mm ∅. One of these tubes reaches right to the bottom of the flask when the stopper is firmly in place and has attached to it a long length of rubber pressure tubing (which must be sufficient to reach from the seed fermenter to all fermenters to be seeded). The rubber tubing terminates in a piece of stainless steel tubing 150 mm long, 10 mm ∅ fitted with a hood (7 fig. 11). The other two tubes passing through the stopper are short. To one of them is connected a piece of rubber pressure tubing about 50 cm long terminating in a short piece of stainless steel tubing fitted with a 1/4″ half coupling. The other half of this coupling is screwed to the lower part of the outlet tube of the sampling unit of the seed fermenter (6 fig. 11). The second short tube carries a few centimetres of rubber pressure tubing and a small air filter (11 fig. 11) consisting of a glass « calcium chloride » tube tightly packed with fine Corning Fiberglass.

Temperature control.

The temperature of the fermenters is controlled by immersing them to about 2/3 of their height in a water bath (fig. 12 and plate 8). They are supported and kept in position by rectangular frames resting on the rim of the bath. The water baths are rectangular containers made of sheet iron, reinforced by a frame of angle iron. Each bath has room for a group of fermenters, the size of the bath being conditioned by the available space. The standard unit houses eight fermenters. Its dimensions are as follows: length approximately 3 m, width 0.45 m, height 0.37 m, with a working volume of about 400 l.

Cooling of the water bath is achieved by the admission of mains water of +12 to +15° C through a solenoid valve (1 fig. 12) operated by a bimetallic thermoregulator or a contact thermometer (2 fig. 12) and a relay. If the temperature of the room is below the working temperature an electric immersion heater (3 fig. 12)is brought into circuit. The water is circulated by means of a pump (4 fig. 12) with a throughput of about 100 l per minute. The inlet of the circulating water (at 5 fig. 12) is constricted to 1/4″ so as to increase its velocity in order to make the circulation, and with it the temperature regulation, more efficient.

The bath can be drained through the cock (6 fig. 12).

Air line.

Air is furnished by a reciprocating piston compressor at a pressure of 3 to 4 atmospheres. It arrives through the trap (7 fig. 12) through which condensed water can be removed on opening the small needle valve (8 fig. 12) and passes through the reduction valve (9 fig. 12) to the distributor (10 fig. 12) then via 3/8″ globe valves to the air inlets of the individual fermenters through lengths of rubber pressure hose.

Transmission.

The water baths were placed against a wall wherever possible so that the driving mechanism for the propeller shaft of the fermenters could be anchored to the wall. The transmission is effected as follows (fig. 13). One 1/5 hp three phase motor (1 fig. 13) is used for two fermenters. It is held on a frame (2 fig. 13) between two arms (3 fig. 13) cemented into the wall. It is connected by means of a V-transmission

belt with two pulley units (4 fig. 13, for details see 4 fig. 13-a). The tension of the belt is adjusted by sliding the motor frame (2 fig. 13) along the arms (3 fig. 13) and locking in the desired position by means of wing nuts. The pulley unit (4 fig. 13) is coupled to the fermenter shaft by means of a floating steel shaft (length 405 mm, diam. 10 mm) with commercial pin and rubber bush flexible couplings (5 fig. 13-a). Any small misalignment of the transmission may be corrected by moving the pulley on the hinge (6 fig. 13 and 13-a).

Speed regulation is effected by means of suitable pulley combinations.

Technique of operation of the fermenters

Checking and assembly of fermenters.

Before assembly the fermenters are carefully inspected with regard to the following:

a) Cleanliness, with particular reference to all tubes passing through the lid and the screwthreads used for attaching the addition vessels (for cleaning see page 83).

b) Adequate lubrication of stuffing box bearings (for lubricating see page 83).

c) Straightness of stirrer shaft. (When handling fermenter heads care must be taken at all times to avoid bending of the shaft).

d) Insulation of foam detecting electrode and adjacent area on fermenter head with a thin film of silicone grease as described above.

e) Cleanliness and dryness of inlet and outlet filters.

f) Soundness of the rubber gasket and of all pressure tubing connections.

These conditions being satisfied the desired propeller is fitted to the propeller shaft . If sparger aeration is used, the air sparger is fitted to the air entry tube and, if required, the baffle unit is now inserted into the fermenter body. The fermenter is now closed care being taken that the reference numbers on lid and body coincide both as to number and position. The lid is screwed down with an even tightening of the nuts and the three entry ports are closed with appropriate metal caps (4 fig. 8). The latter should be examined to see that packing washers are present.

Check of pressure tightness.

Next the assembled fermenter is checked for pressure tightness. The sampling tube and by-pass tube are closed by Hoffman screw clips and a pressure gauge screwed in place. The fermenter is now connected to an air line at a pressure of about 1 atm. and the air inlet valve opened. With the fermenter inlet valve open and the outlet also open it is first checked that there is a free flow of air through the filters. Next the air outlet is closed and a rough test of tightness made, any gross leaks being detected by ear. Finally the air inlet is also closed and the rapidity with which the fermenter pressure gauge falls to zero is noted. Normally the time taken for the fermenter to reach atmospheric pressure from 1 atm. should be not less than five minutes. For special purposes selected fermenters can be further improved by extra tightening of the stuffing box and other sources of leakage. On completion of the pressure test the air line is disconnected.

Preparation of fermenters for sterilisation.

The fermenter is now prepared for sterilising in the autoclave. The medium is introduced through one of the filling ports and the following operations performed:

a) The three metal caps closing the entry ports on the lid and the small cap closing the lower end of the sampler are loosened so as to allow free passage of steam during sterilisation and avoid the danger of air pockets at these points.

b) The air inlet valve is tightly closed. This is particularly important when a sparger is fitted in order to prevent the sucking back of medium into the filter.

c) The pressure gauge is removed from the tee on the air outlet. The air outlet valve is best left open so that any condensed water falling into the open arm of the tee drains away without entering the outlet filter.

d) The two Hoffman clips closing the by-pass and the lower tube of the sampling unit are removed to allow complete sterilising of the sampler.

e) The sampling tube is clamped off tightly by means of the two Hoffman clips removed from the sampling unit. The special clip is not used for this purpose as its spring gets damaged by repeated sterili-

sations. It appears unnecessary to completely fill the sampling tube up to the clips with liquid to avoid the presence of an air pocket difficult to sterilise. In the early part of the work this precaution was routinely observed but has now been discontinued without any incidence of contamination.

The fermenter is now ready for sterilising.

Preparation of accessories for sterilisation.

1) *Addition vessels.*

The vessels for the addition of solutions to the fermenters during a fermentation may either be sterilised in position on the fermenters if an autoclave of sufficient height is available or may be sterilised independently. After filling through the hole at the top each vessel should be checked with regard to the following:

a) The outlet valve should be in good working order.

b) The liquid level gauge should be clean and smooth flowing when observed during filling.

c) The packing washers on the cap screws closing the filling hole and the hole at the top of the level gauge should be sound and the screws tight.

When the vessels are to be sterilised in position on the fermenters they are placed on the appropriate ports and the coupling nuts loosely fastened so that air may escape from this point during sterilising. Antifoam vessels are always sterilised separately from the fermenters as the oil requires a considerably longer time to sterilise than do aqueous solutions.

If the vessels are to be sterilised separately from the fermenters the half couplings below the valves must be wrapped in gauze and cotton protected by kraft paper or aluminum caps. Stout wire S hooks are useful for hanging the vessels on a frame for filling and sterilising.

2) *Device for dispensing inoculum.*

The device for dispensing inoculum is prepared for sterilisation by putting a small amount of suitable antifoam agent in the bolt head flask serving for measuring the inoculum, tying or wiring the rubber stopper firmly into the flask and wrapping the half coupling and the hooded tube first with gauze and then with cotton and kraft paper (in culture media which do not foam the addition of antifoam to the flask

may be omitted). The rubber tubing should be neatly coiled and must be free from kinks.

Sterilisation.

During the early work with small fermenters in this laboratory the only available autoclave was of such dimensions that only two fermenters without accessories could be sterilised at one time. Later a special horizontal autoclave able to take a battery of eight fermenters with addition vessels mounted in position was constructed and is now in routine use. The fermenters are supported in a frame made of angle iron which slides into the autoclave on runners fastened to its wall. (See plate 9).

In both cases the same conditions of sterilisation are used. The fermenters containing the culture media are sterilised for 15' at 100° C flowing steam followed by a period of 20-40' at 120° C, according to the nature of the culture media. The preliminary 15' with flowing steam serves two purposes: 1) It flushes all air from the autoclave. 2) It brings the fermenters and their contents up to 100° C. Tests with a maximum thermometer placed inside the fermenter containing 5 l of culture medium showed that the latter reached 100° C within 15'. Similar tests with a 10 l pyrex glass bottle containing 5 l liquid showed that twice the time was necessary in that case.

The addition vessels containing aqueous solutions are usually sterilised for the same time period as the fermenters, but the minimum period of 20' is sufficient to achieve their sterilisation. The addition vessels containing antifoam oil are sterilised for 15' at 100° C flowing steam followed by a period of 90' at 120° C.

When the required sterilising time has elapsed the autoclave is brought down to normal pressure as quickly as possible without causing the boiling out of liquids from the sterilised vessels. The autoclave is then opened, the frame with its battery of 8 fermenters removed to its trolley and all caps which have been left loose for the sterilisation are immediately tightened. The fermenters are now placed in the water bath and made ready for the fermentation.

Connection of the fermenters to the air line.

The following sequence of operations is performed:

a) The special clip is placed on the upper rubber tube of the sampling unit.

b) The two Hoffman clips are removed from the upper rubber tube of the sampling unit and placed one on the by-pass tube and the other on the lower rubber tube of the sampling unit. All these clips are closed.

c) A pressure gauge is screwed to the upper arm of the tee on the air outlet of the fermenter.

d) The distributor air line pressure is adjusted to 0.7-0.8 atm. by means of the reduction valve.

e) The rubber hose connection is made between air distributor and fermenter.

f) The globe valve of the air distributor is opened.

g) The fermenter air inlet valve is opened.

h) The fermenter air outlet valve is closed.

This sequence of operations is performed on each fermenter in turn until all are under pressure of sterile air. It should be noted that the air outlet valve is left open until after the inlet valve has been opened. This is important as during all the above operations the fermenter has been cooling rapidly in the waterbath. Leaving the outlet valve open allows sterile air to be sucked in through the air filter (which has negligible resistance) instead of non-sterile air through small leaks in the stuffing box or elsewhere.

Mounting of accessories.

a) Addition vessels.

When the addition vessels have been sterilised separately from the fermenters they are most conviently mounted before the steel shafts connecting pulley units with fermenters are placed in position. The operation of mounting the addition vessels on the fermenters is best done by two persons but, after a little practice, may be done by one. The sterilised vessels are hung on to the rim of the water bath in front of the fermenter to which they are to be fitted so that they are ready to hand. One operator then loosens the pipe cap closing the half coupling of the port on the fermenter head while a second operator loosens the wrapping which covers the half coupling on the addition vessel and brings the vessel near to the fermenter head. Now the first operator removes the pipe cap while simultaneously the second operator frees the half coupling from its wrapping and immediately places it

in position on the half coupling of the port on the fermenter head. The coupling is quickly tightened by hand and finally by spanner. During the whole of the above operation the air supply to the fermenter is not interrupted. Thus at the moment of removal of the pipe cap from the fermenter head a stream of sterile air emerges and the possibility of contamination is reduced to a minimum. Flaming of connections is not usually done but a lighted bunsen is always kept nearby in case some hitch should occur in the operation.

b) Pulleys and shafts.

The pulleys and V belts appropriate to the desired agitation speed are conveniently mounted during the interval when the fermenters are being sterilised. After mounting pulleys, belts and shafts the pulleys are turned by hand to check alignment of all components. If this is satisfactory the motors may be switched on. The speed of each fermenter should then be checked with a tachometer to see that it is at the desired value and no belt slip is occurring. The speed should also be checked occasionally during the fermentation.

Sterility test.

The fermenters are generally run for 48 hours under working conditions prior to inoculation. After 24 hours running a sample is taken from each fermenter for a test of sterility (for sampling see page 82). From each sample a loopful is streaked onto two agar slants and a 0.1 ml aliquot added to each of two tubes of nutrient broth. One agar and one broth tube are incubated at 24° C, the other pair at 37° C. These tubes are carefully examined after not less than 24 hours incubation. Contaminations are rarely encountered if sterilisation of fermenters and accessories has been efficient. In a recent series of twenty eight fermentations involving a total number of approximately 250 fermenters only two have been lost through contamination due to faulty manipulation. After the initial sterility test samples are taken immediately after inoculation and thereafter every twenty four hours throughout the fermentation for ascertaining the absence of contamination.

Inoculation.

If only one or two fermenters are to be inoculated the operation is done by introducing a sterile pipette through the inoculating port on the head of the fermenter. During this operation the air supply to the fermenter is not interrupted.

When a battery of fermenters is to be inoculated from a seed fermenter the dispensing device described above is used to transfer the inoculum into the fermenters and to measure its volume. The assembly, previously sterilised in the autoclave, is placed close to the seed fermenter with the measuring flask clamped to a laboratory retort stand.

The following sequence of operations is then carried out:

a) The cap at the lower end of the sampling unit of the seed fermenter is removed and the exposed half coupling well flamed.

b) The half coupling of the short length of rubber tubing of the measuring flask is unwrapped and connected with sterile precautions to the half coupling of the sampling unit of the seed fermenter and screwed dead tight.

c) Special clips (fig. 7) of the type used on the upper rubber tubing of the sampling units are connected to 1) the by-pass of the sampling unit of the seed fermenter, 2) the long rubber tube of the measuring flask at a point as near as possible to the flask, 3) the small piece of rubber tube between the measuring flask and the glass air filter.

d) The two Hoffman clips on the sampling unit are removed. The situation should now be as shown in the sketch (fig. 11). Two operators are now necessary.

Operator A: (A) manipulates the clips controlling the flow of inoculum from the seed fermenter to the fermenters to be inoculated.

Operator B: (B) connects the fermenters to be seeded one at a time with the hooded tube attached to the long rubber tube of the bolt-head flask. The details of the operation are as follows: Operator (A) opens the special clip (3 fig. 11) on the upper rubber tube of the sampling unit of the seed fermenter and at the same time opens the special clip (9 fig. 11) on the air filter of the bolt-head flask. The clips are not removed from the rubber tubes but held open by hand pressure, one clip in each hand. The bolt-head flask will now fill with the culture from the seed fermenter (1 fig. 11) since the latter is under overpressure. The flow of culture is controlled by the clips until the flask is full to the desired level when both clips are closed. In the meantine operator (B) unwraps the hooded tube (7 fig. 11) and inserts it into a sterile Erlenmeyer flask retaining it in place with his hand.

Now operator (A) opens the other pair of special clips (4 and 8 fig. 11) i.e. the one on the by-pass tube of the sampling unit of the seed fermenter and the one on the long rubber tube carrying the hooded

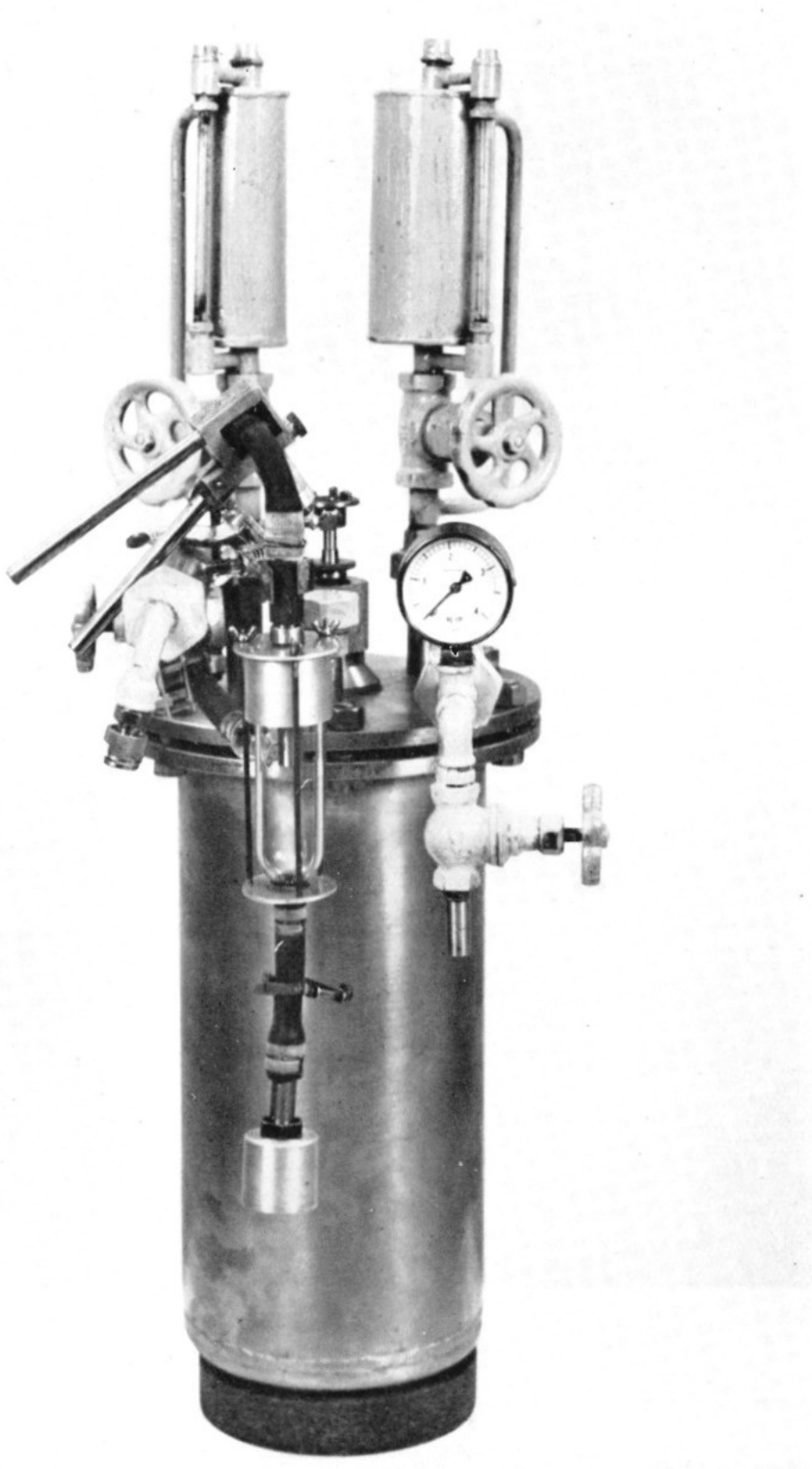

Plate 1. - Complete fermenter (with glass sampler) with addition vessels in place. Front view.

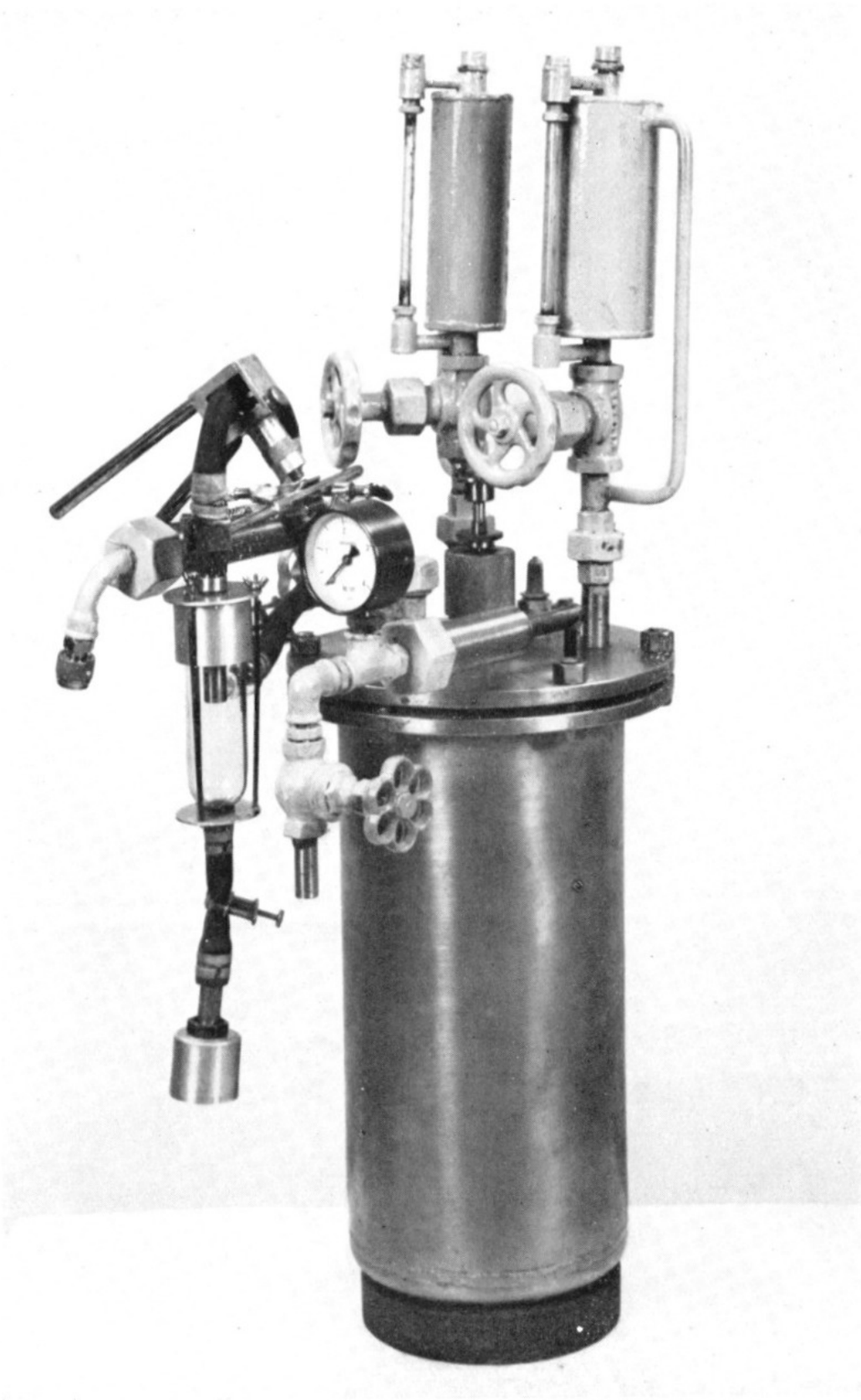

Plate 2. - Complete fermenter (with glass sampler) with addition vessels in place. Side view.

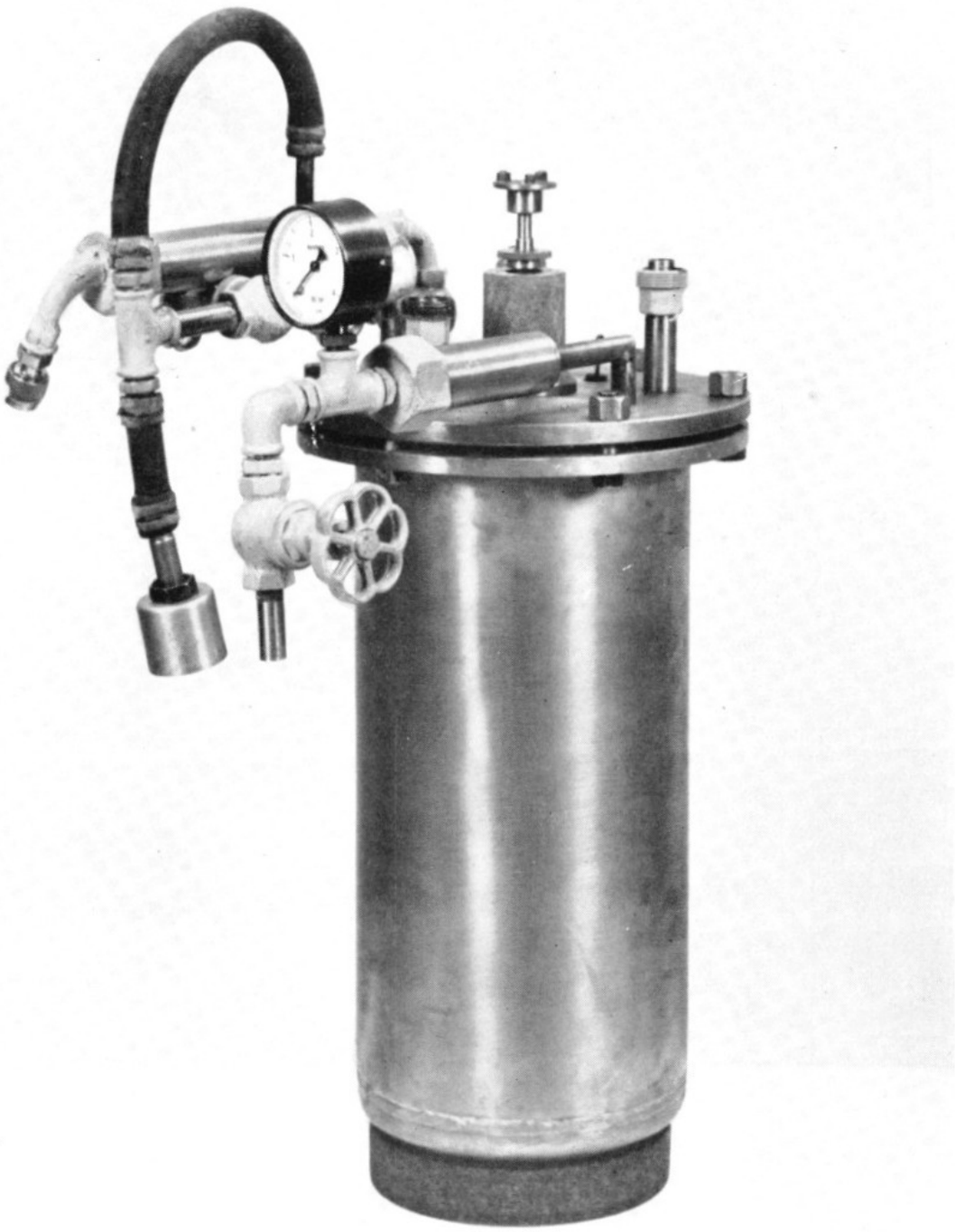

Plate 3. - Fermenter without addition vessels, modified for higher working pressures. Side view.

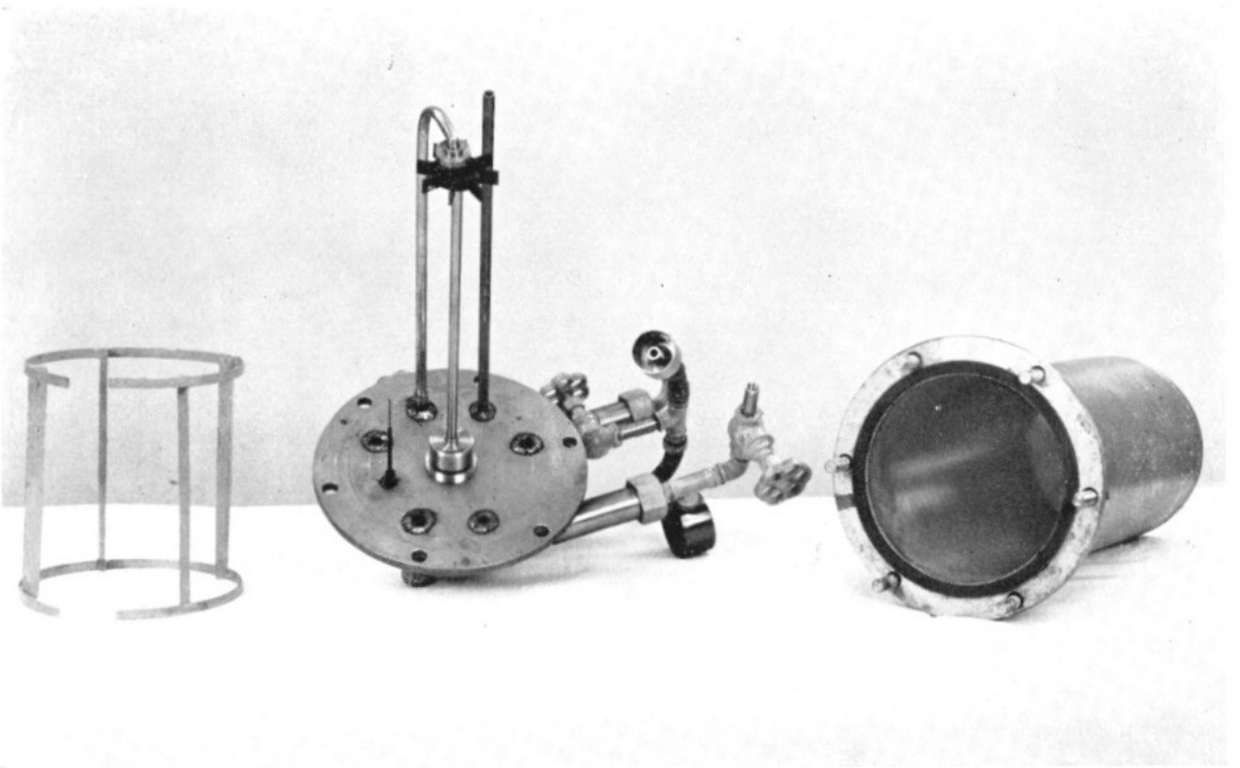

Plate 4. - Fermenter body, fermenter head with sparger in place and baffle plates.

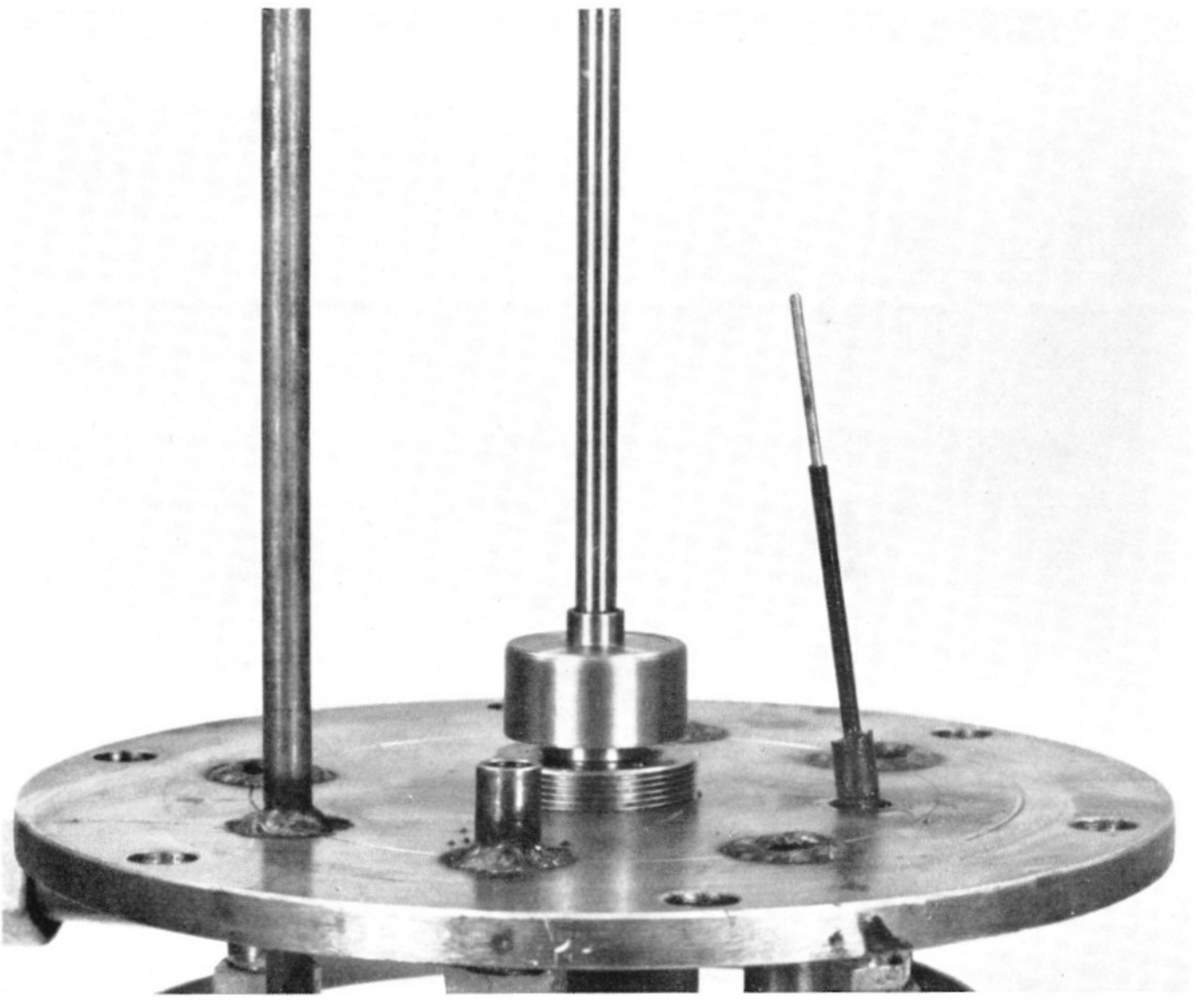

Plate 5. - Detail of fermenter head showing electrode, grease cup, sampling tube and point of attachment for sparger.

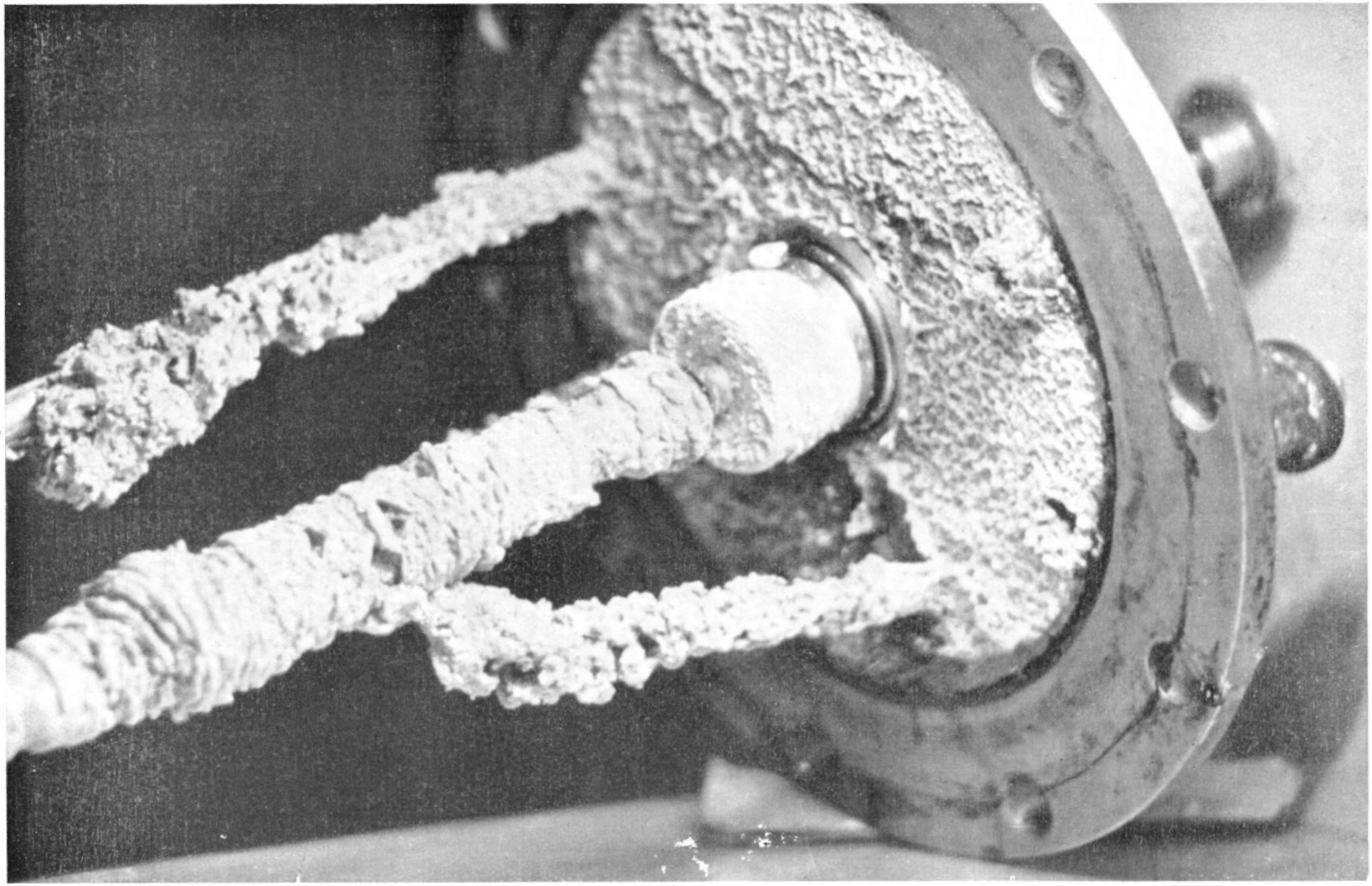

Plate 6. - Fermenter head after fermentation which has foamed excessively.

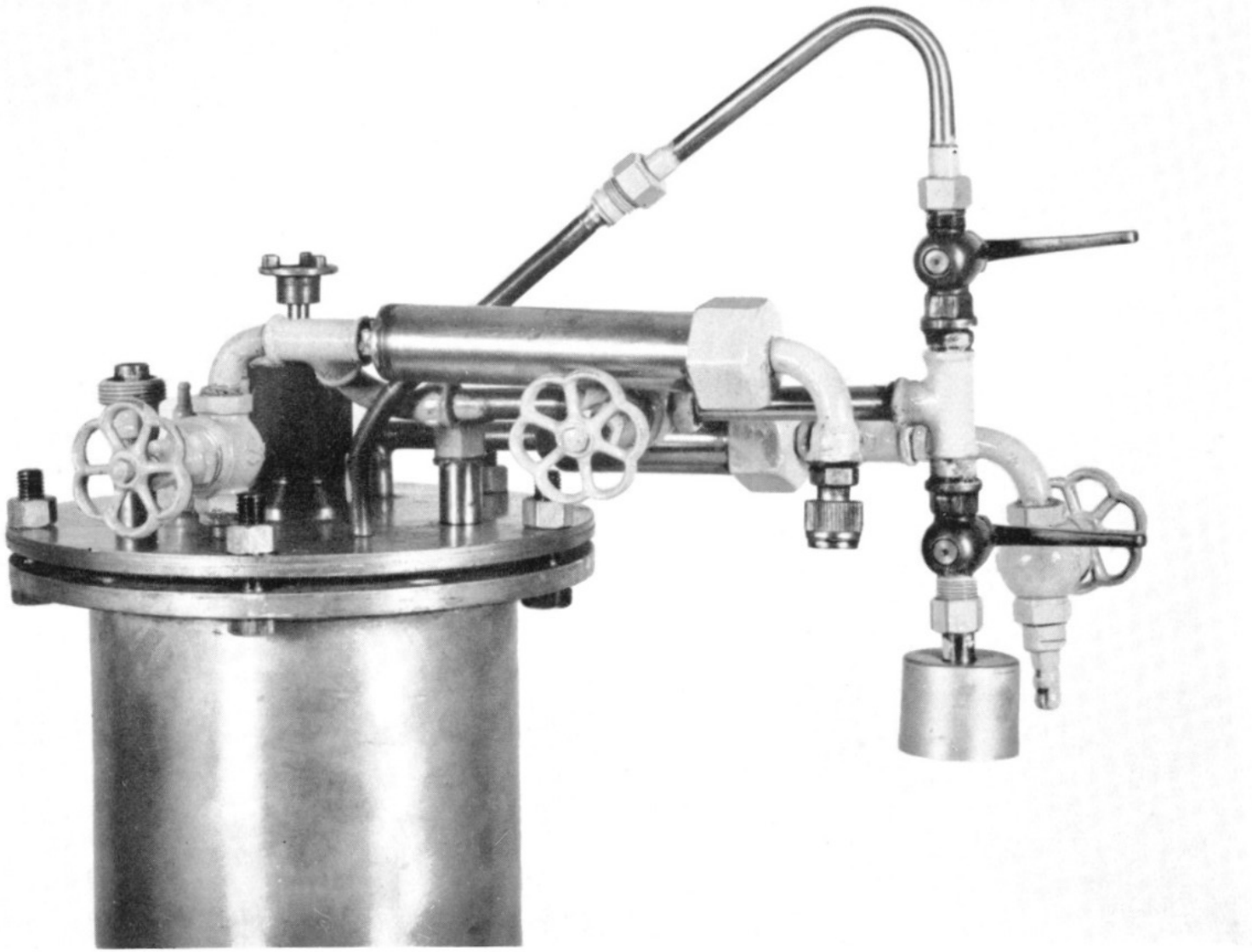

Plate 7. - Fermenter head with all metal sampler.

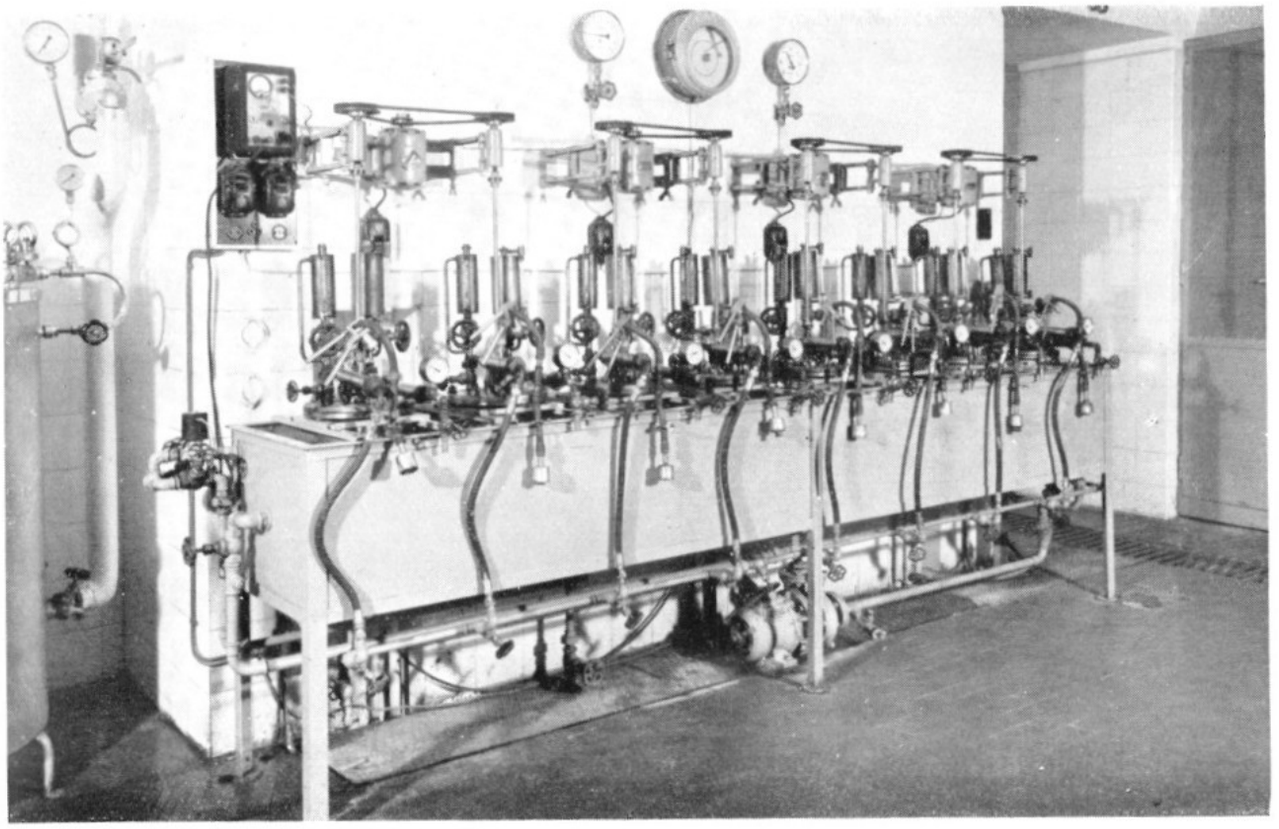

Plate 8. - Group of fermenters during fermentation.

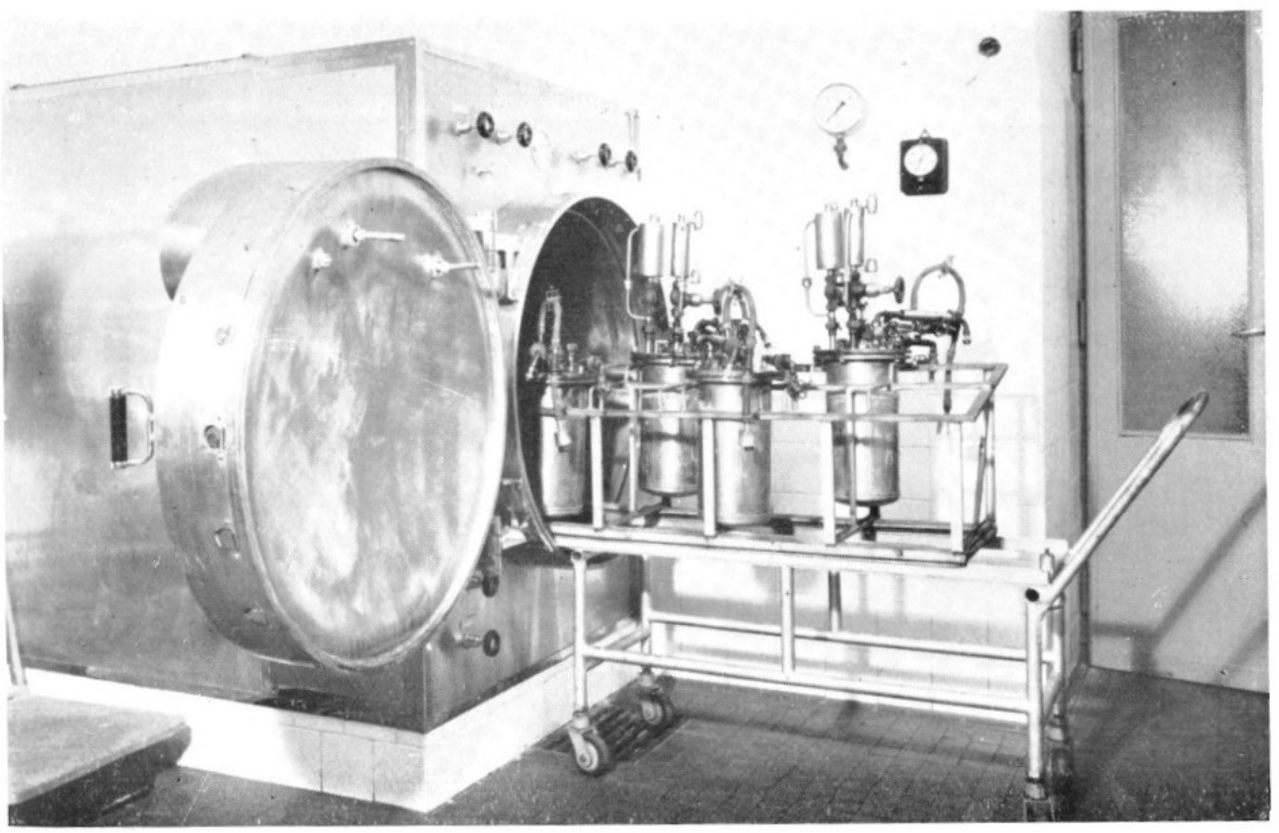

Plate 9. - Group of fermenters ready for sterilisation.

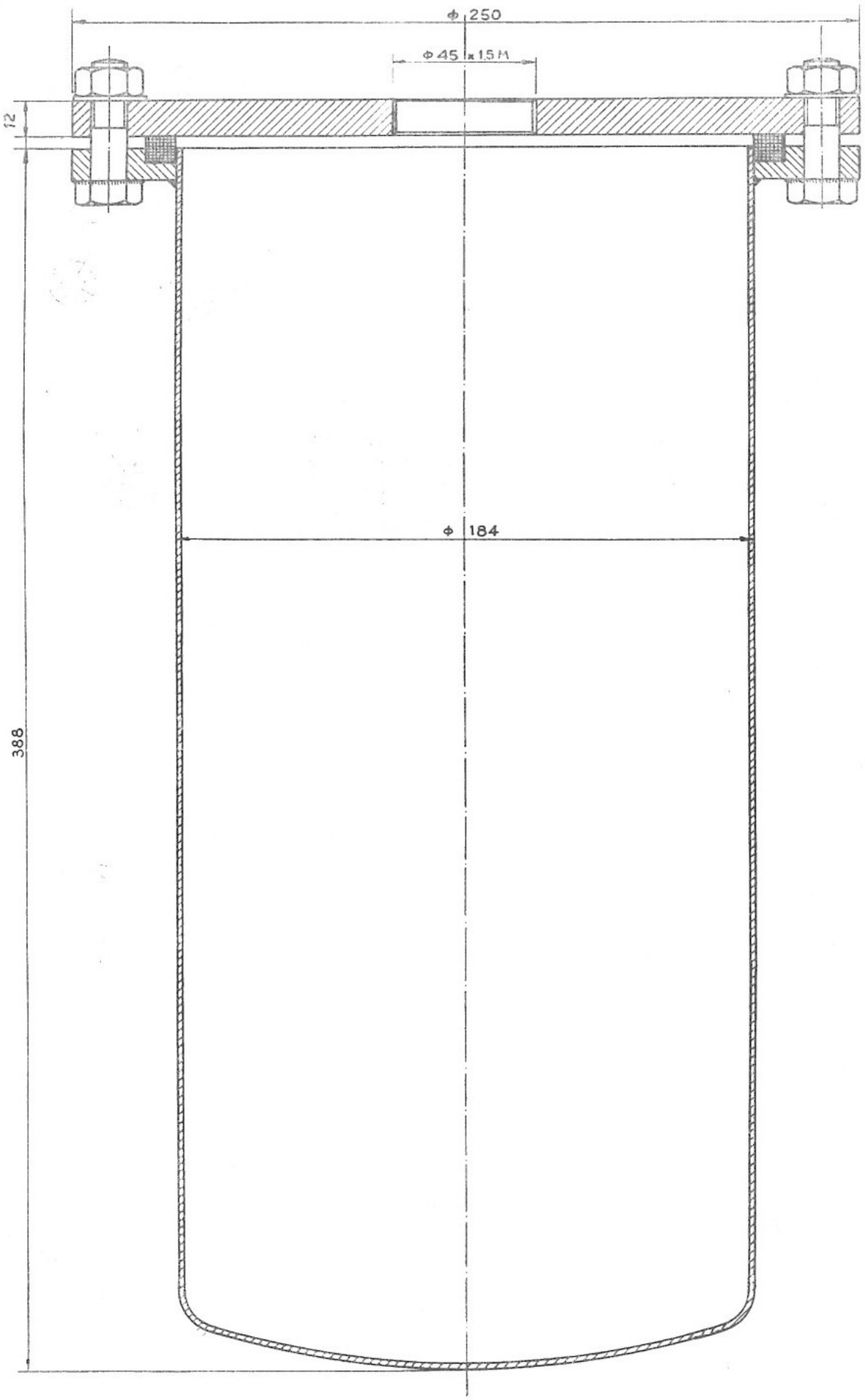

Fig. 1. - Section through fermenter head and body without accessories.

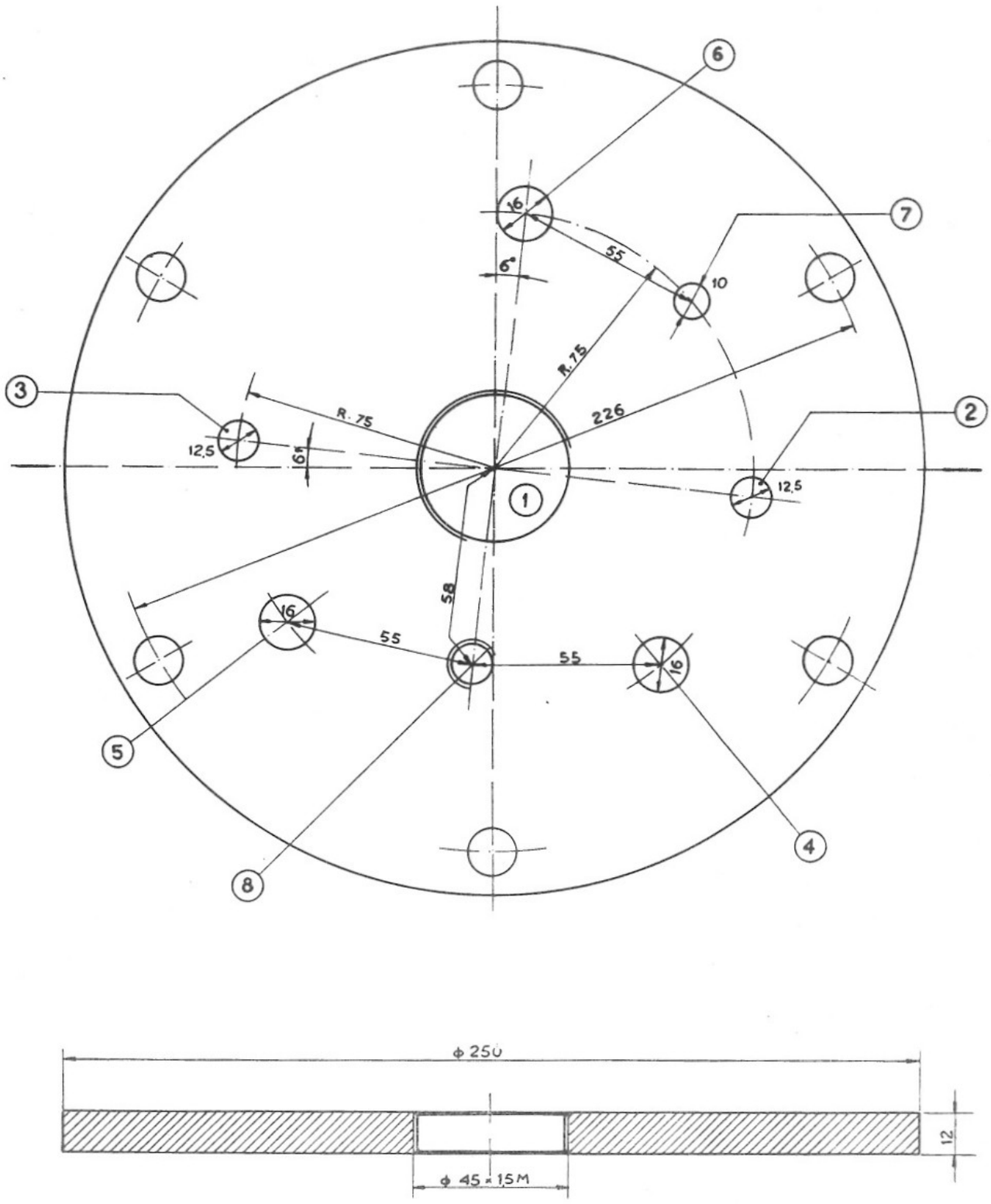

Fig. 2 - Fermenter head plate. Drilling template. Holes for:

1) Agitation unit.
2) Air inlet.
3) Air outlet.
4) Addition port.
5) Addition port.
6) Inoculation port.
7) Sampling tube.
8) Foam detecting electrode.

The unnumbered peripheral holes are for bolts.

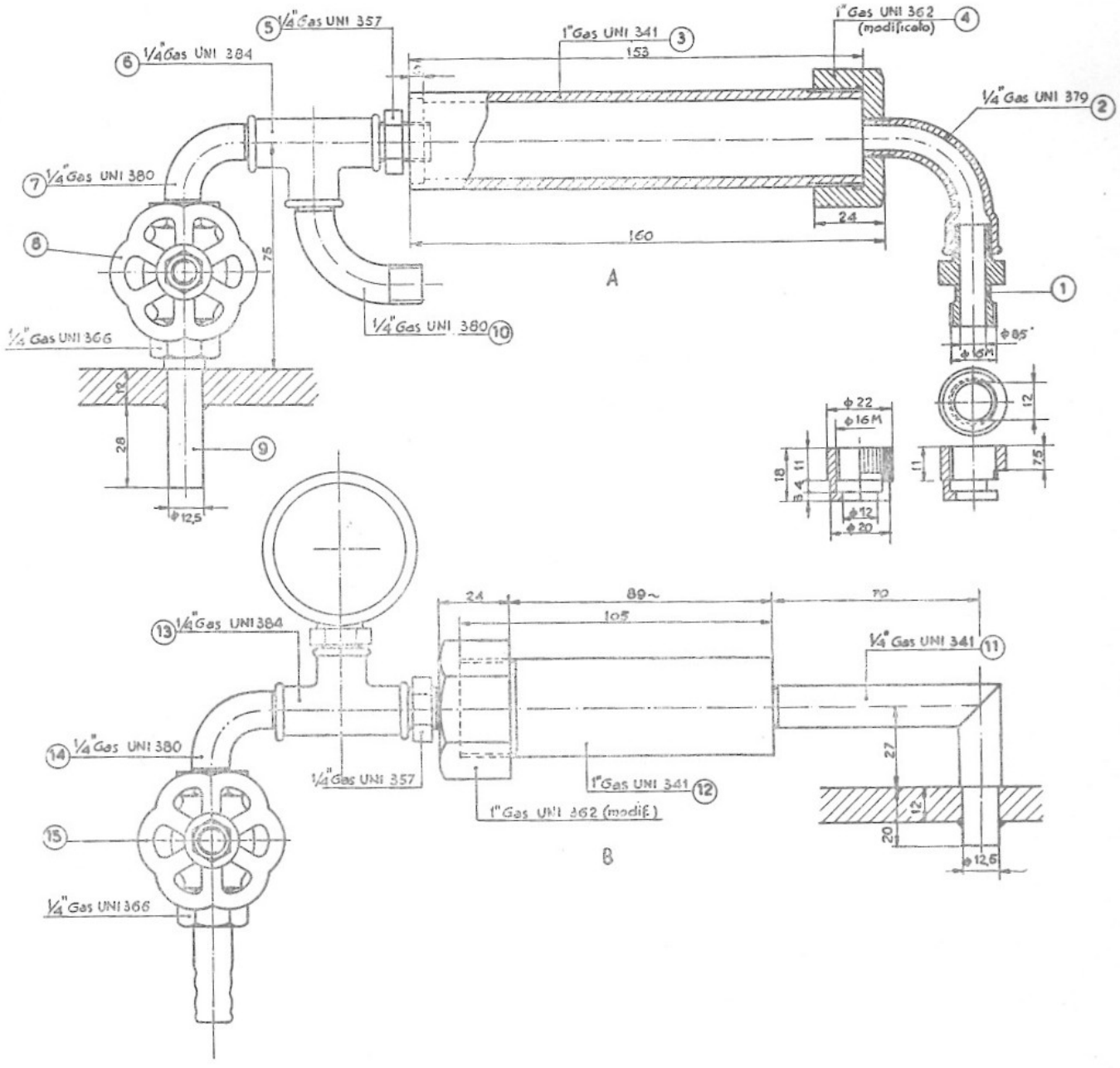

Fig. 3 - Constructional details of (A) air inlet and (B) outlet.

1) Rapid hose coupling.
2) Bend.
3) Filter tube.
4) Pipe cap.
5) Nipple.
6) Tee.
7) Bend.
8) Globe valve.
9) Threaded tube for attachment of sparger.
10) By-pass bend.
11) Elbow.
12) Filter tube.
13) Tee carrying pressure gauge
14) Bend.
15) Globe valve.

Fig. 4 - Agitation unit.

	Component	*Material*
1)	Shaft.	Stainless steel.
2)	Stuffing box.	Brass.
3)	Upper cap.	Brass.
4)	Lock ring.	Brass.
5)	Lower cap.	Stainless steel.
6)	Chuck.	Brass.
7)	Grease cup.	Stainless steel.
8)	Lock ring.	Brass.
9)	Lock nut.	Brass.
10)	Distance ring.	Stainless steel.
11)	Half coupling.	Brass.
12)	Half coupling.	Steel.
13)	Half coupling.	Rubber.
14-*a*)	Washer.	Stainless steel.
14-*b*)	Nut.	Stainless steel.
15)	Propeller.	Stainless steel.
16)	Shim.	

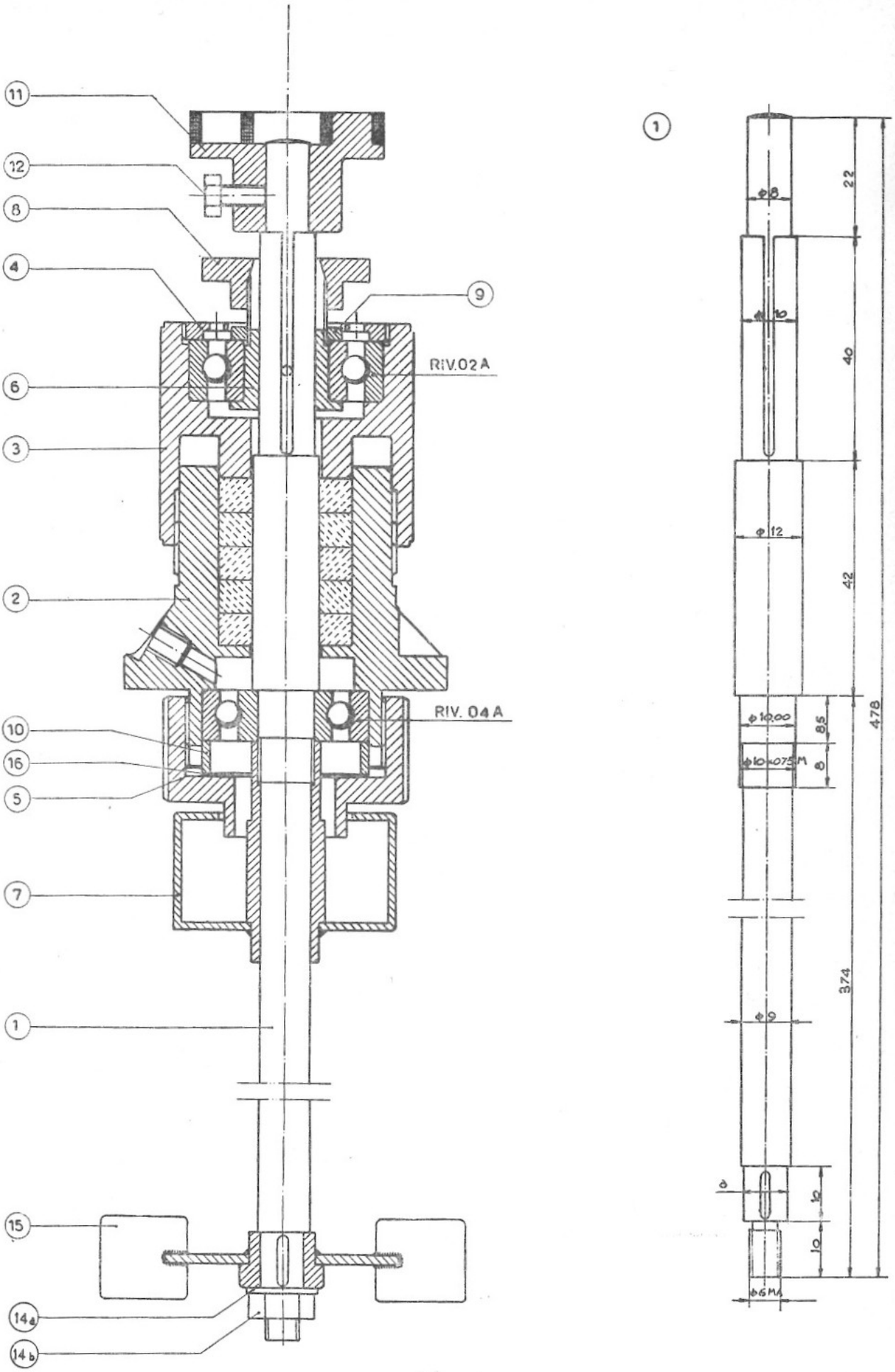
11
12
8
4
9
RIV.02 A
6
3
2
RIV. 04 A
10
16
5
7
1
15
14a
14b
1
22
40
42
478
374
10
M6

Fig. 4.

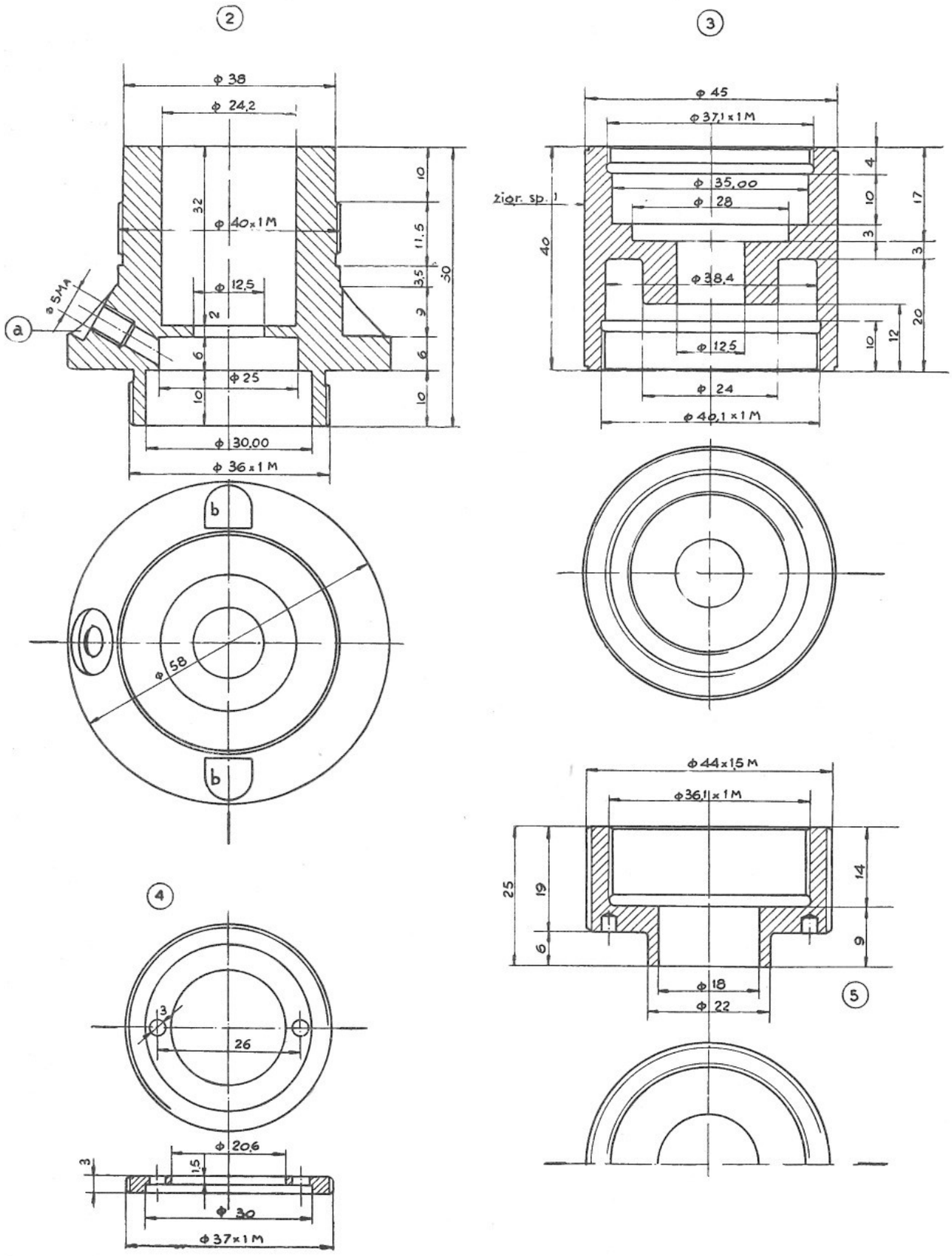

Fig. 4-a - Details of 2, 3, 4, and 5 fig. 4.

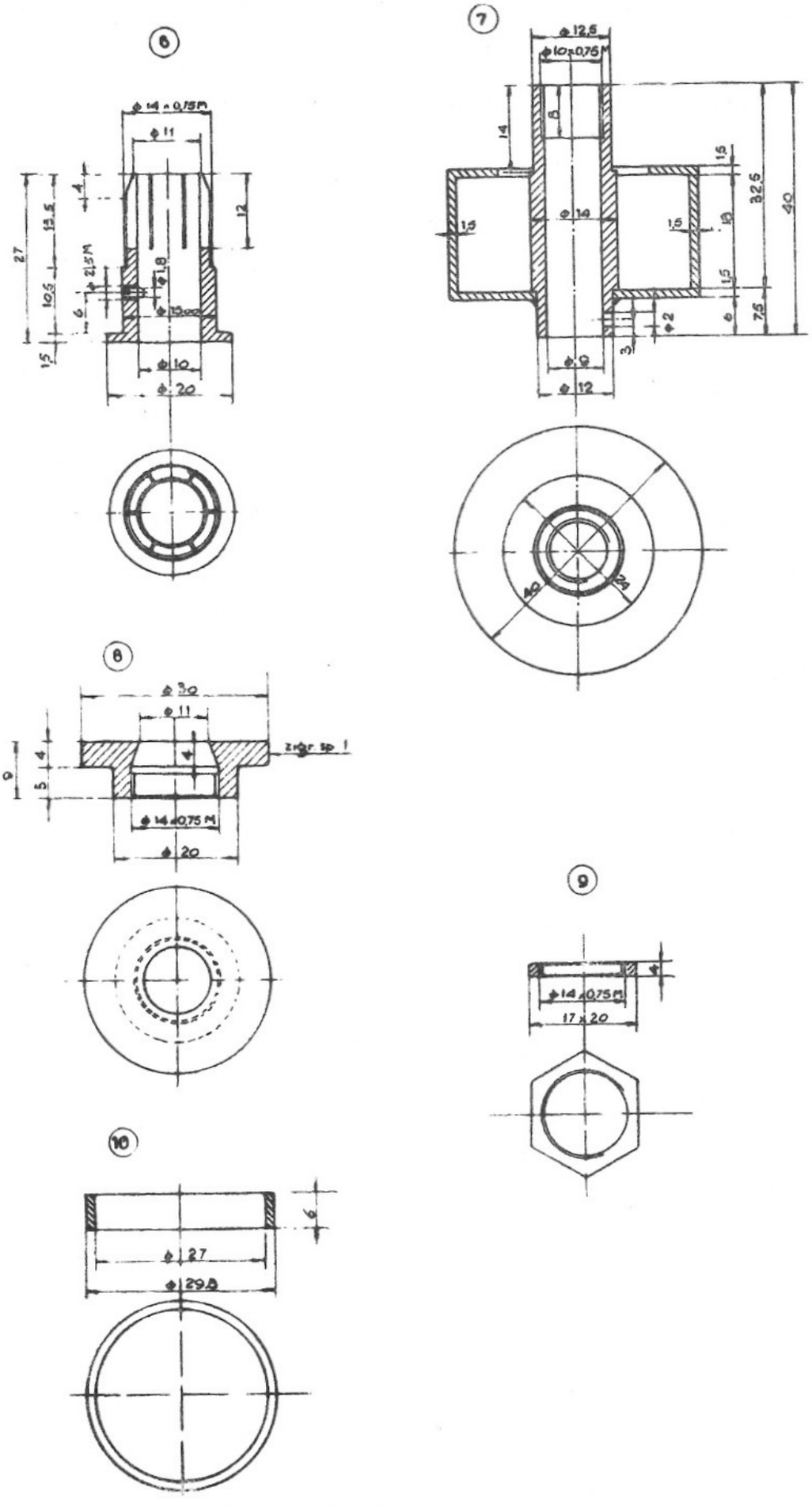

Fig. 4-b - Details of 6, 7, 8, 9, and 10 fig. 4.

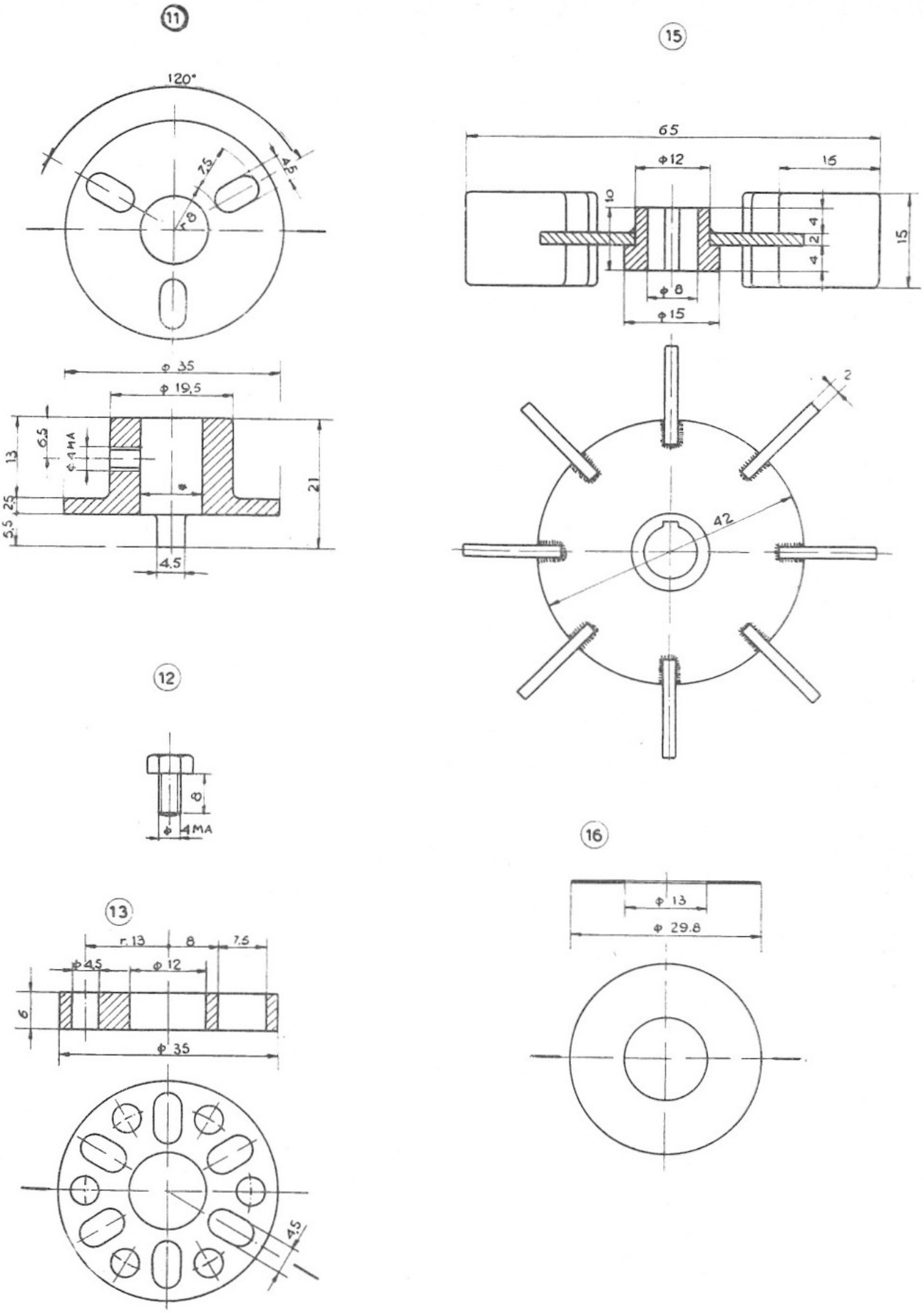

Fig. 4-c - Details of 11, 12, 13, 15 and 16 Fig. 4.

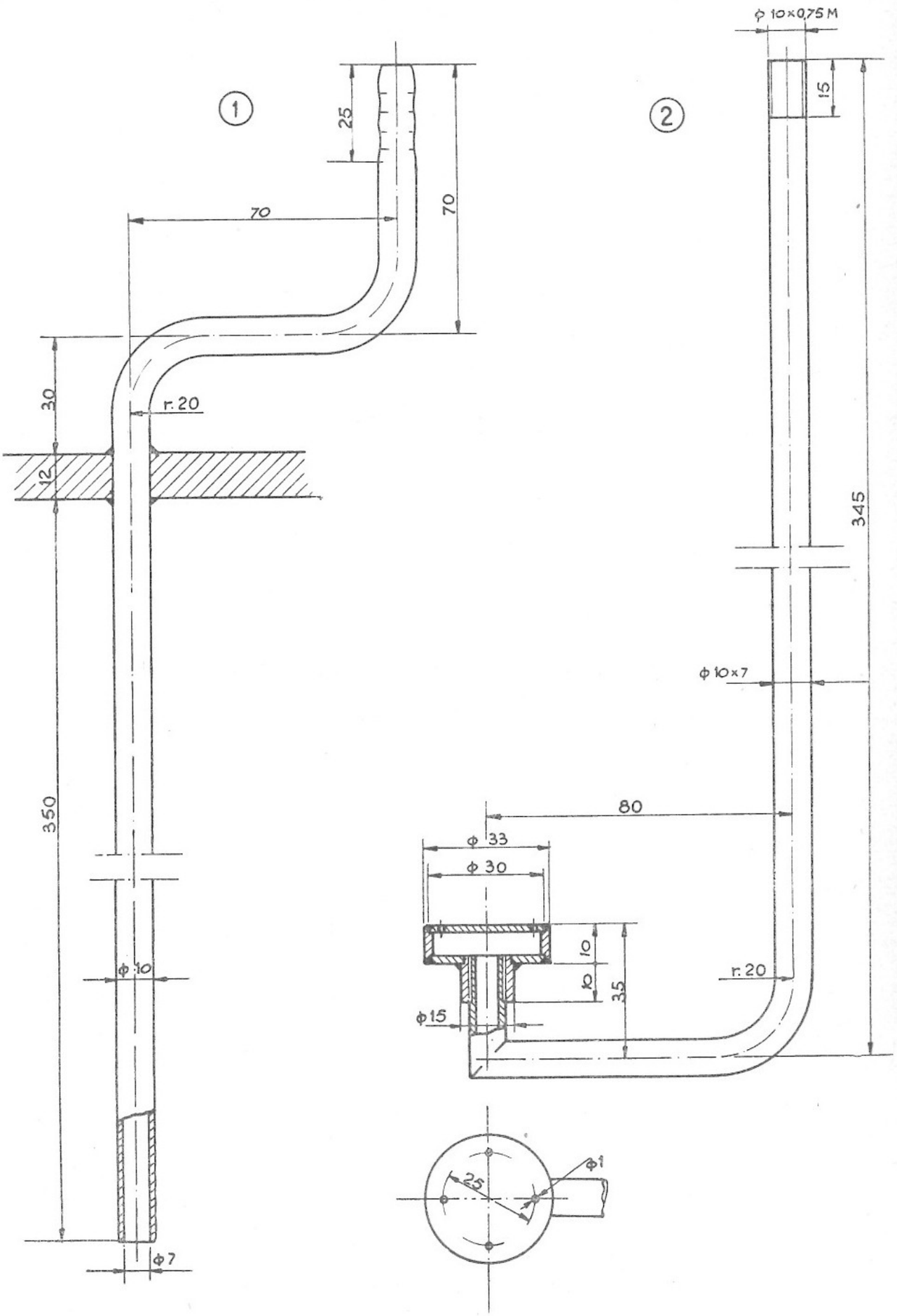

Fig. 5 - 1) Sampling tube and 2) Air sparger.

Fig. 6 - Sampler assembly.

Component	*Material*
1) Receiver.	Glass (pyrex).
2) Hood.	Aluminum.
3) Stopper	Rubber.
4) Hood.	Aluminum.
5) Disc.	Aluminum.
6) Outlet tube.	Stainless steel.
7) Pipe cap.	Iron.
8) Lock nuts (2).	Iron.
9) Tube.	Stainless steel.
10) Clamping rods (3).	Brass.
11) Disc.	Aluminum.
12) Wing nuts (3).	Iron.
13) Retaining ring.	Stainless steel.
14) Connecting tubes (3).	Thick walled rubber.
15) Cushion.	Rubber.
16) Sleeve.	Rubber.

A) To sampling tube (1 fig. 5).
B) To by-pass of air inlet filter (10 fig. 3).

A laboratory fermenter for vortex and sparger aeration.

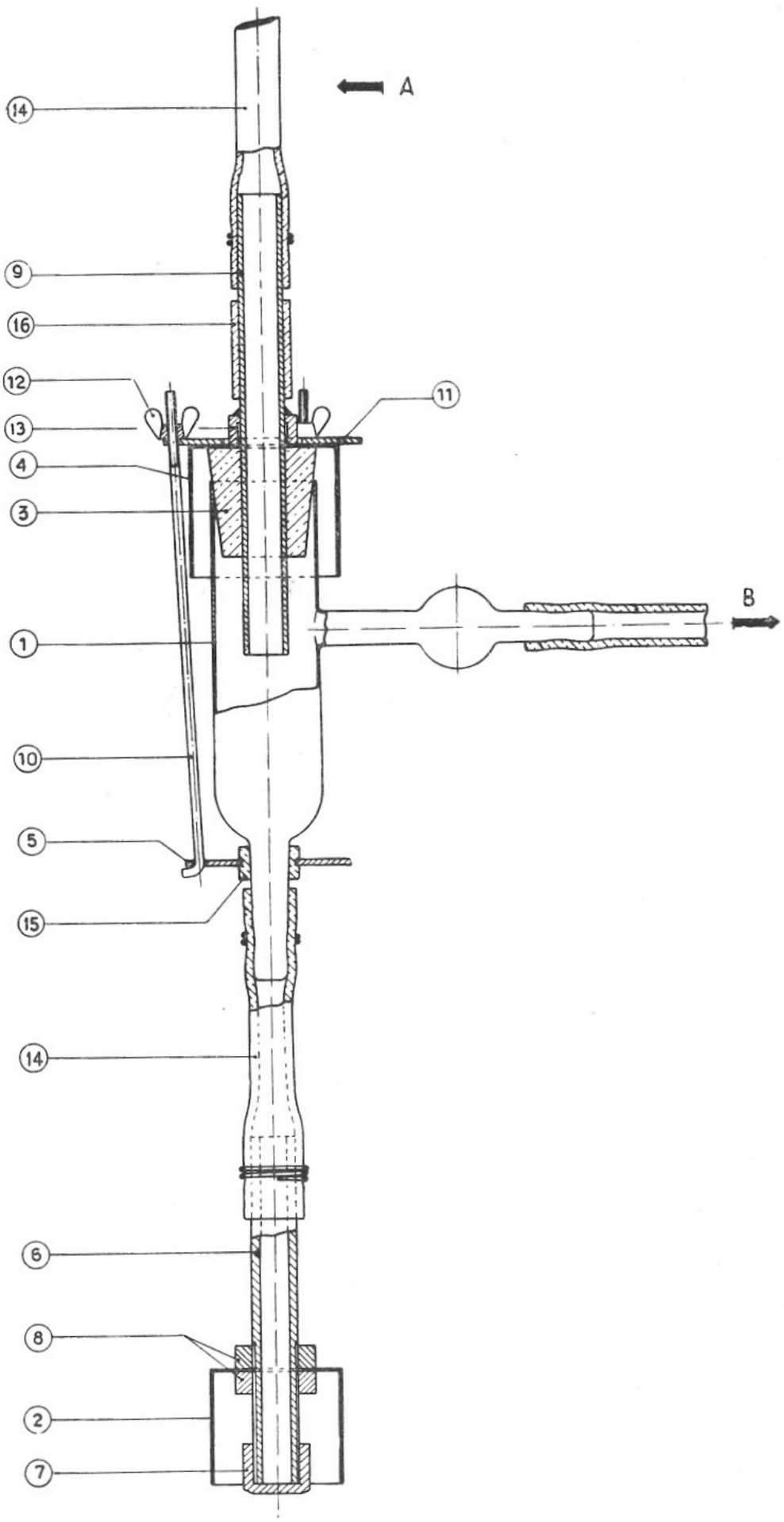

Fig. 6.

Fig. 7. - Special clip for sampling.

Component	*Material*
1) Body.	Aluminum.
2) Pressure plate.	Brass.
3) Hinge pin.	Steel.
4) Retaining plate.	Aluminum.
5) Handle (fixed).	Steel tube.
6) Handle (hinged).	Steel tube.
7) Pressure transmitter.	Steel.
8) Pressure regulating cap.	Aluminum.
9) Spring.	Steel.
10) Fastener for retaining plate.	Steel.

A laboratory fermenter for vortex and sparger aeration.

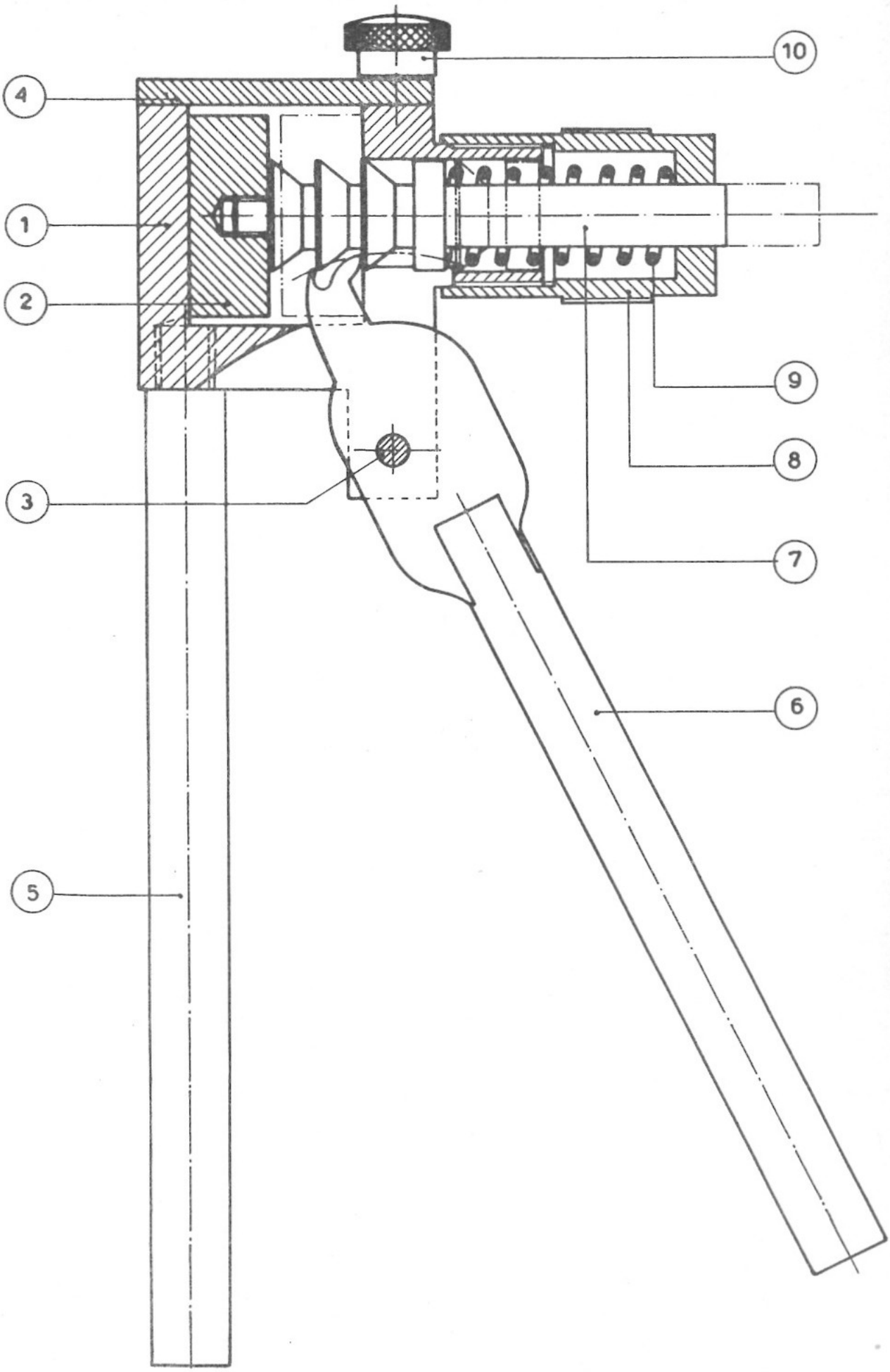

Fig. 7.

Fig. 8. - Details of:

1) Foam detecting electrode.
2) Ports for addition vessels (2).
3) Port for inoculum.
4) Cap for closing ports.

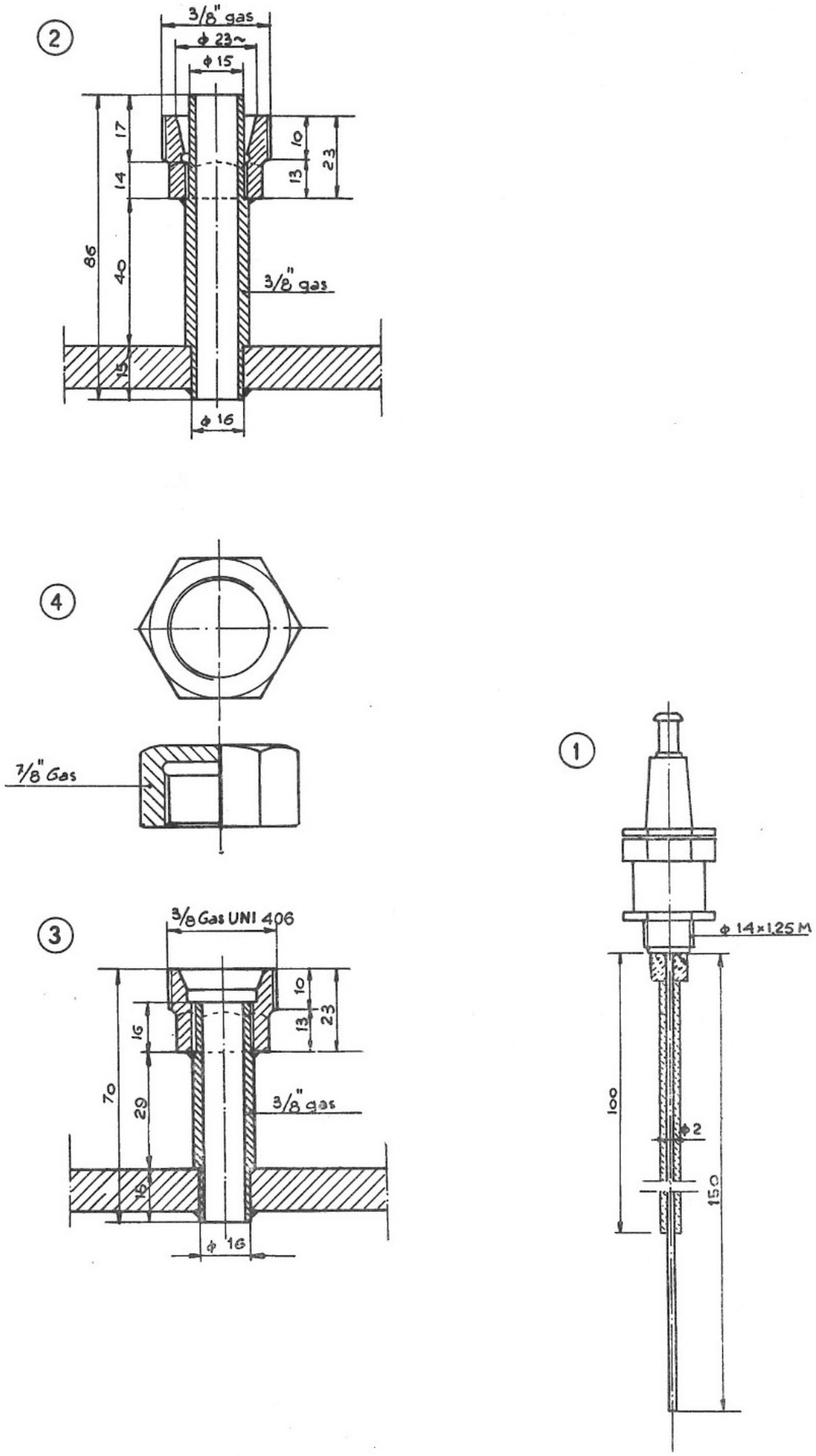
2
3/8" gas
ϕ 23~
ϕ 15
17
14
10
13
23
86
40
3/8" gas
15
ϕ 16
4
7/8" Gas
3
3/8 Gas UNI 406
10
13
23
16
70
29
3/8" gas
15
ϕ 16
1
ϕ 14×1,25 M
100
ϕ 2
150

Fig. 8.

Fig. 9. - Details of construction of addition vessels.

Component	*Material*
1) Cylindrical body.	Stainless steel.
2) End plates (2).	Stainless steel.
3) Filling port and outlet.	Stainless steel.
4) Cap screw.	Stainless steel.
5) Nipple.	Stainless steel.
6) Cylindrical plug valve.	Stainless steel plunger and liner with bronze housing.
7) Tee tube.	Stainless steel.
8) Half coupling.	Iron.
9) Entry tube on fermenter head	Stainless steel.
10) By-pass tube.	Stainless steel.
11) Level gauge.	Glass (pyrex thick wall).
12) Stuffing boxes (2).	Stainless steel.
13) Stuffing nuts (2).	Brass.
14) Connecting tubes (2).	Stainless steel.

A laboratory fermenter for vortex and sparger aeration.

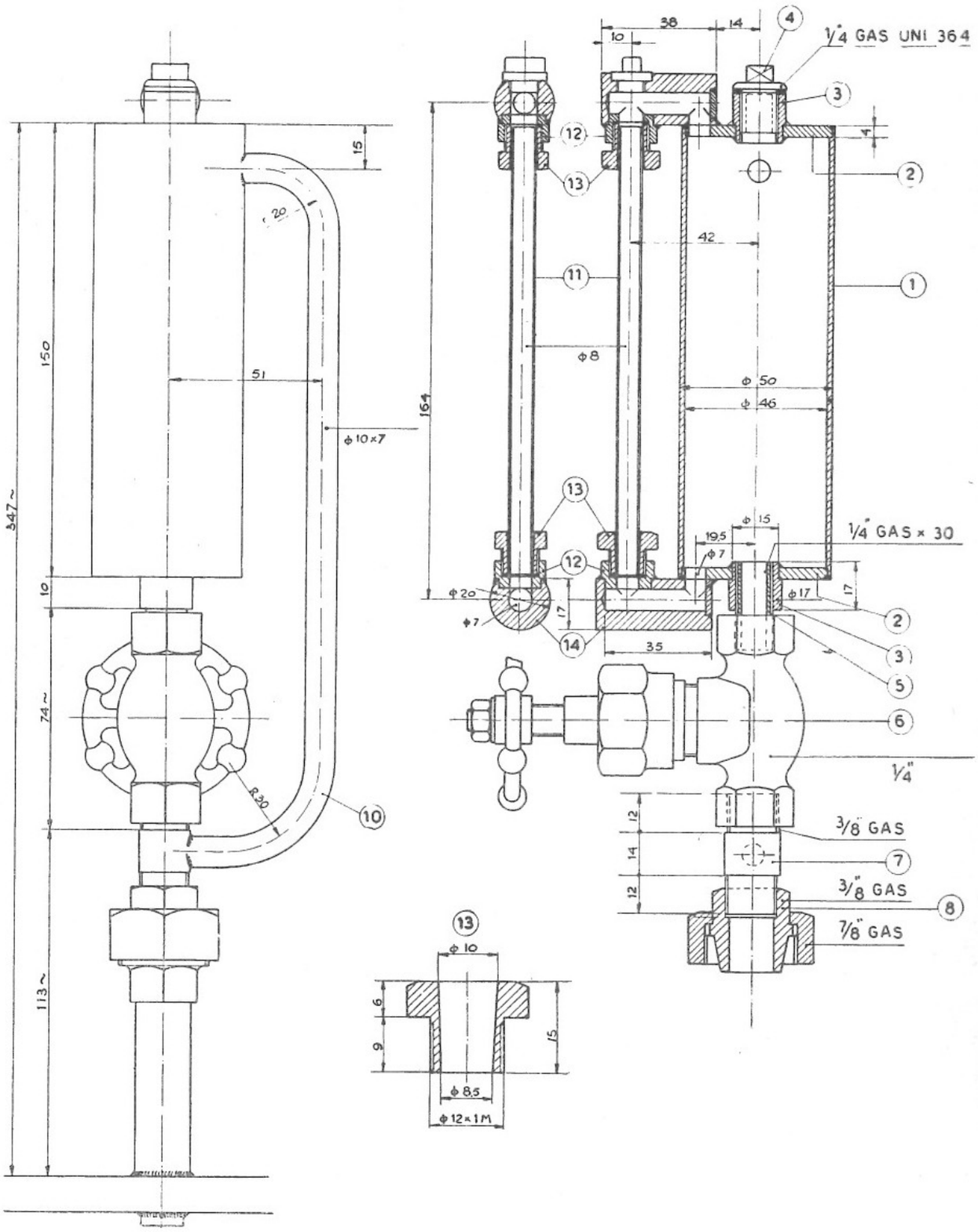

Fig. 9.

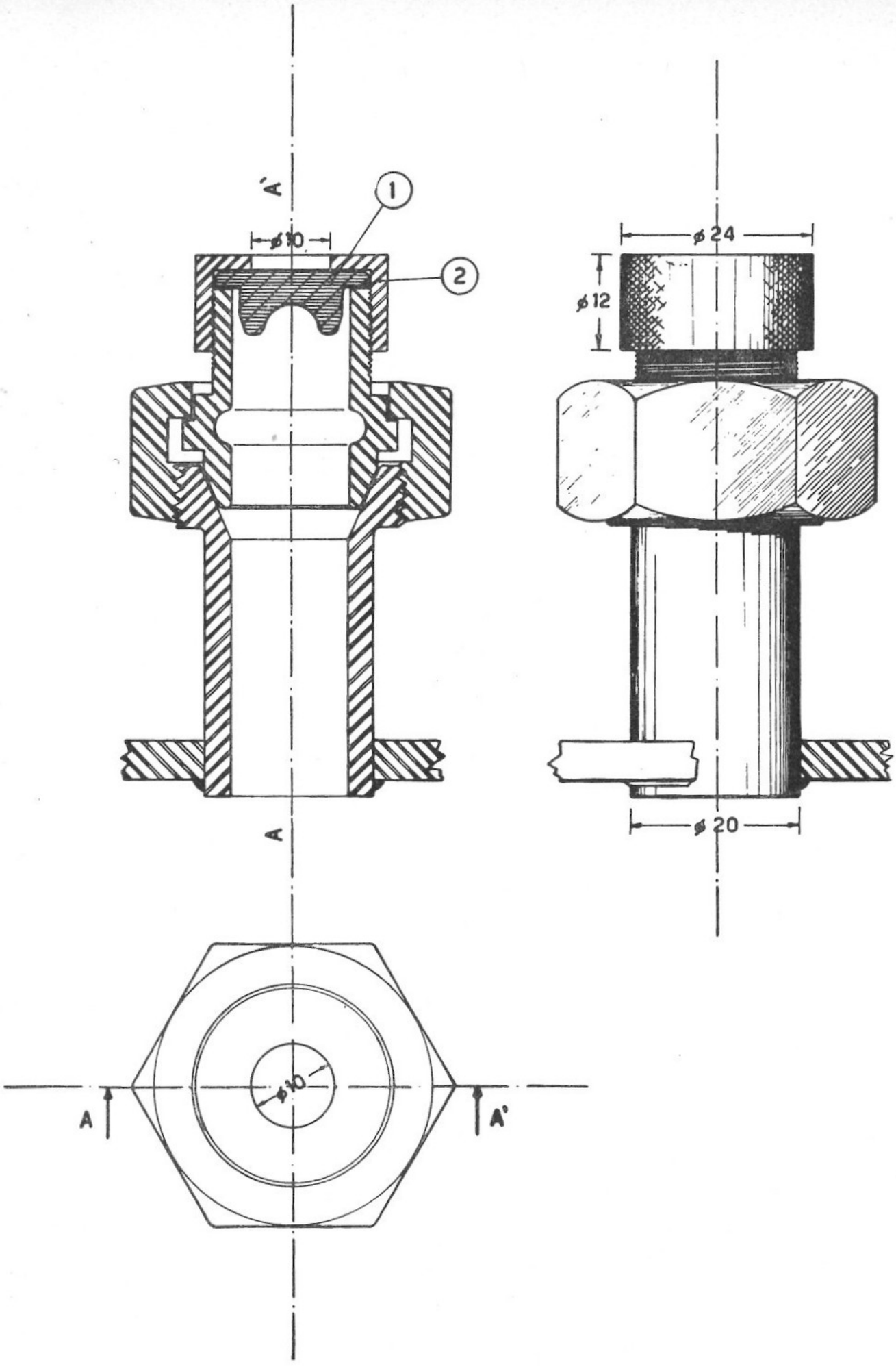

Fig. 10 - Injection boss for addition of solutions by hypodermic syringe.

1) Rubber diaphragm.
2) Brass cap.

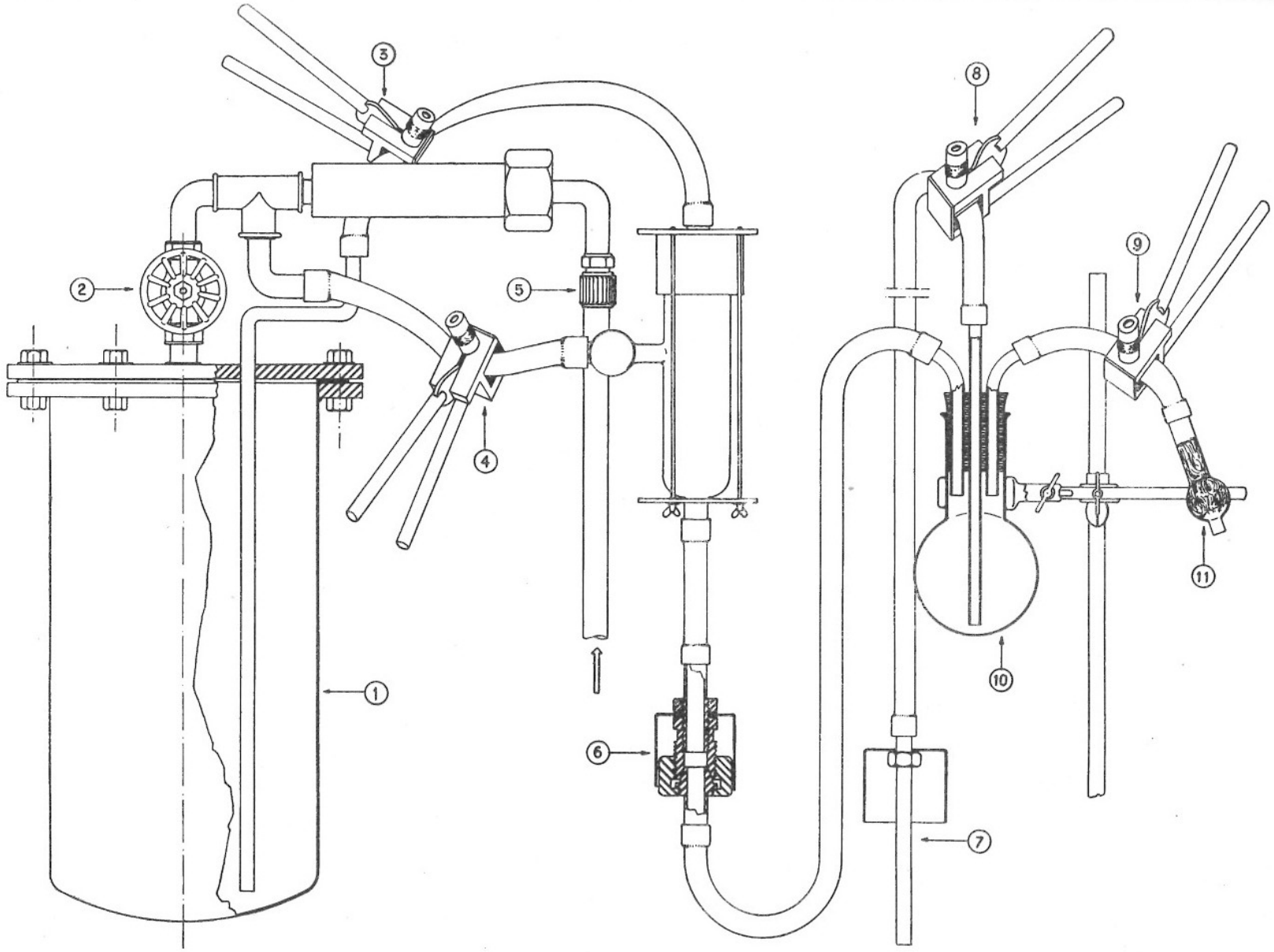

Fig. 11 - Arrangement for transfer of inoculum.

1) Seed fermenter.
2) Air inlet valve of seed fermenter.
3) Clip on sampling tube.
4) Clip on by-pass tube.
5) Air inlet fom distributor line.
6) Coupling.
7) Hooded inoculation tube.
8) Clip on inoculation tube.
9) Clip on air vent tube.
10) Measuring vessel (bolt head flask).
11) Air vent filter.

Fig. 12 - General arrangement of a group of fermenters in a waterbath.

1) Electromagnetic valve on water inlet.
2) Bimetallic helix or contact thermometer.
3) Immersion heater.
4) Electrically driven circulating pump.
5) Circulating water inlet.
6) Drain cock.
7) Condensate trap in air line.
8) Needle bleed valve.
9) Reduction valve.
10) Air distribution line.
11) Overflow tube.
12) Leads to electronic relay.
13) Cold water inlet from mains.

⇨ Air.

➡ Water.

A laboratory fermenter for vortex and sparger aeration.

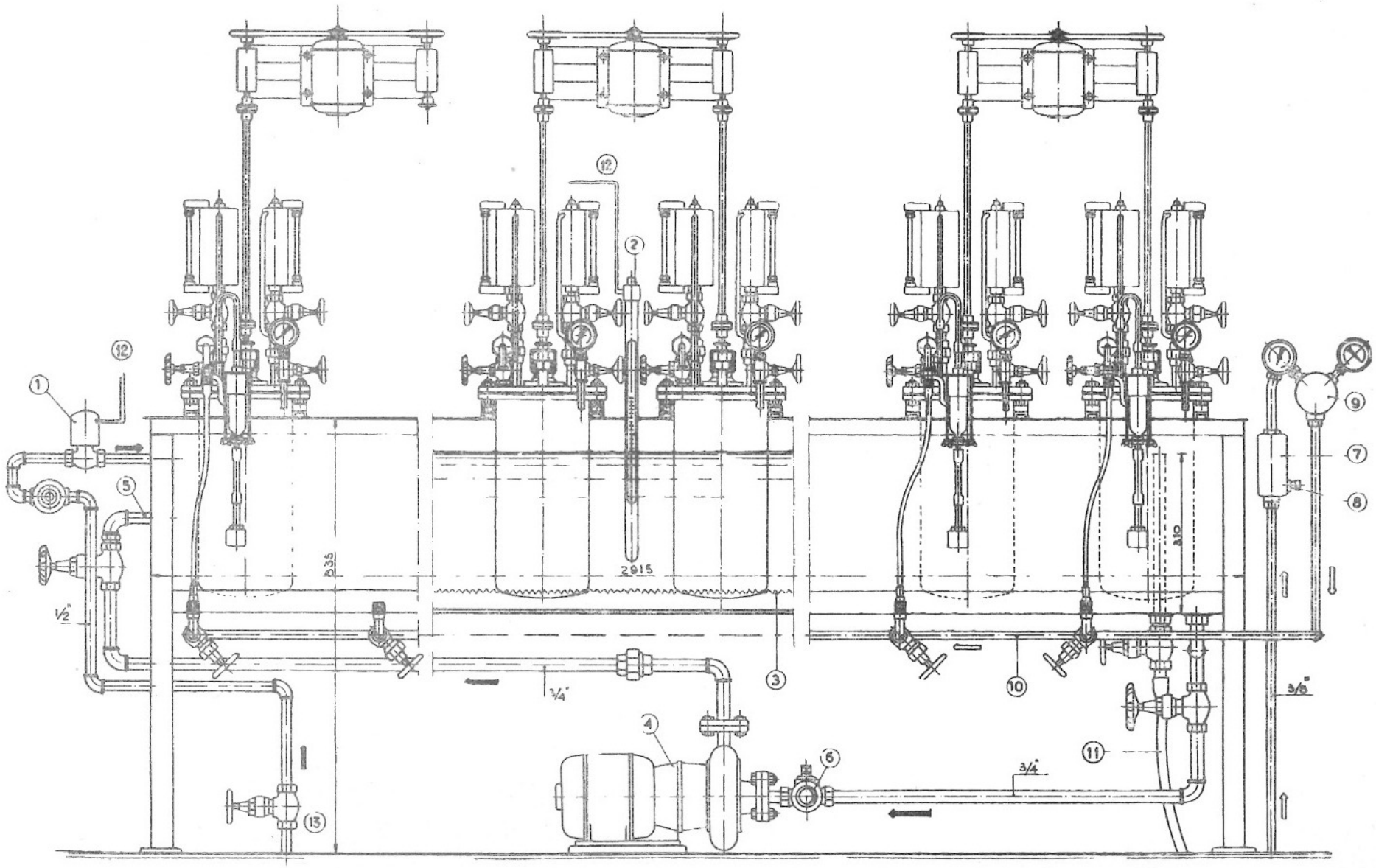

Fig. 12.

Fig. 13 - Transmission.

1) Electric motor.
2) Sliding frame.
3) Supporting arms.
4) Pulley unit.
5) Pin and rubber bush flexible coupling (see also 11, 12, 13 fig. 4-c).
6) Pivot bolt.

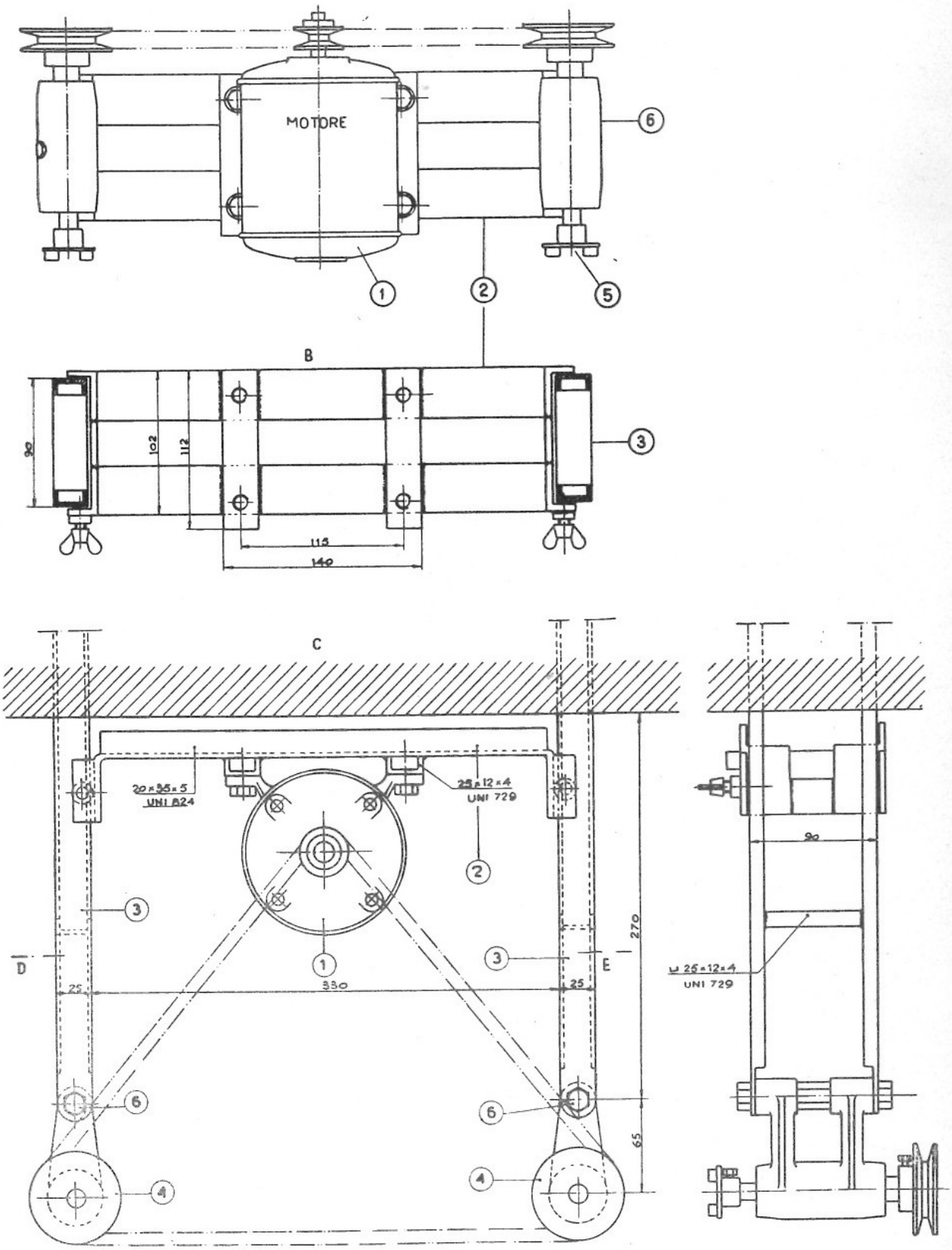
MOTORE
6
1
2
5
B
3
30
102
112
115
140
C
20×35×5
UNI 624
25×12×4
UNI 729
2
3
1
3
D
E
25
330
25
270
6
6
4
4
65
90
25×12×4
UNI 729

Fig. 13.

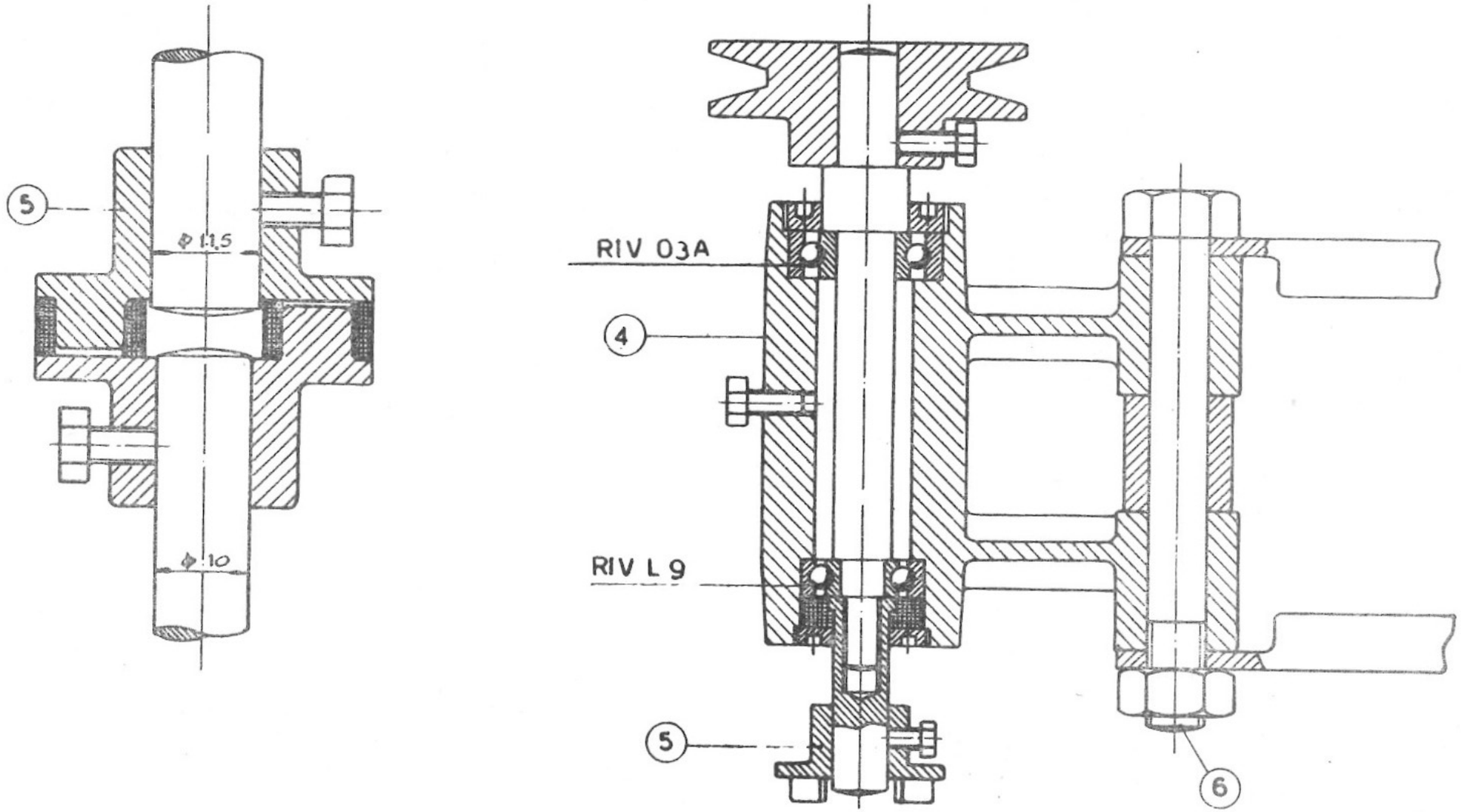

Fig. 13-a - Details of 4, 5, and 6 fig. 13.

metal tube. This causes the contents of the bolt-head flask to be expelled into the Erlenmeyer flask due to the air pressure from the by-pass tube of the sampling unit of the seed fermenter.

Next operator (A) releases his hold on the clips he is operating (4 and 8 fig. 11) and again takes up the first pair of clips (3 and 9 fig. 11) in order to refill the bolt-head flask with the culture from the seed fermenter. While he is doing this, operator (B) carries the hooded metal tube (still in the Erlenmeyer flask) to the first fermenter to be inoculated. He (B) then removes the cap from the inoculating port of the fermenter and slips the hooded tube from the Erlenmeyer into the port. [The culture in the Erlenmeyer flask is discarded. This preliminary sample serves to wet the internal walls of the rubber tubing (whose length may be up to 10 metres) and the bolt-head flask of the dispensing device. If the first sample is not discarded in this way the first fermenter inoculated will not receive the desired volume of inoculum].

When operator (A) has filled the bolt-head flask a second time and sees that operator (B) has inserted the hooded tube into the fermenter to be inoculated he opens the second pair of clips (4 and 8 fig. 11) and thus transfers the measured volume of inoculum from the flask to the fermenter. He (operator A) retains the clips in the open position until he sees a small wave travel along the rubber tube and reach the hooded metal tube or until he hears a bubbling sound. This indicates that all liquid has been expelled from the tube and only air is passing.

Now operator (A) again fills the bolt-head flask from the seed fermenter while operator (B) transfers the hooded metal tube to the next fermenter to be inoculated. This operator (B) does by first loosening the cap of the second fermenter with one hand and with the other taking hold of but not removing the hooded metal tube connected to the first fermenter. Then with a simultaneous crossover movement of both hands he transfers the hooded metal tube from the first fermenter to the second and the pipe cap from the second to the first.

The cycle of operations is now repeated by operators (A) and (B) until all fermenters have been inoculated. After the inoculum has entered the last fermenter the pipe cap coming originally from the first fermenter (which has been closed after inoculation by the pipe cap from the second fermenter) is used to close it. Since this cap has been exposed for some minutes to the atmosphere it is necessary to well flame it before closing the inoculating port. Apart from this and the moment at the beginning of the cycle of operations when the dispensing device is connected to the seed fermenter the operations are all performed without the use of a flame. It has been found that working

without a flame enables the operations of inoculation to be carried out more quickly and smoothly and does not lead to contaminations provided that the air supply to the fermenter is maintained at all times. The inoculation of eight fermenters is completed in 15 minutes. When all fermenters have been inoculated the dispensing device is disconnected from the seed fermenter and immediately flushed thoroughly with detergent solution and water. The seed fermenter is dismantled and cleaned as described under « Dismantling and cleaning » (page 83).

Sampling.

In order to take a sterile sample from a fermenter during fermentation the procedure is as follows:

a) The air inlet valve of the fermenter is closed.

b) The air outlet valve is opened and pressure reduced until it is well below the working pressure but not zero. The valve is then closed.

c) The by-pass clip of the sampling unit is opened.

d) The special clip of the upper rubber tube of the sampling unit is opened until the pressure in the fermenter rises as indicated by the pressure gauge. This clears the sampling tube of stagnant culture fluid.

e) The special clip and the by-pass clip are closed.

f) The air inlet valve of the fermenter is opened.

g) The 1/4″ pipe cap at the bottom of the sampling unit is removed and the exposed tube flamed.

h) The Hoffman clip on the lower tube of the sampling unit is opened. At this moment it is as well to have some vessel under the end of the sampling unit as a few ml of fluid may be expelled.

i) The sample is taken by placing the desired receiving vessel under the hood of the outlet of the sampling unit and opening the special clip until the required volume has been obtained.

j) The Hoffman clip on the lower tube of the sampling unit is closed.

k) The end of the terminal tube and hood of the sampling unit are well flamed.

l) The 1/4'' pipe cap is held with tongs or pliers, well flamed and replaced on the terminal tube of the sampling unit. This completes the sampling operation.

Essentially the same procedure as above is also applicable to the sampling units without glass parts described under « simplified sampler » (page 68).

Dismantling and cleaning.

On completion of the fermentation the fermenters and accessories are dismantled following roughly the reverse order from that used in their assembly. The fermenter contents are transferred to a storage vessel if required for subsequent investigation or otherwise discarded by flushing to waste. Baffles, if present, are removed and the fermenter bodies are then washed with hot water from a hose, scrubbed with a brush and detergent solution and thoroughly rinsed. They are then left upside down to drain.

Before cleaning the fermenter heads the spargers, if present, are removed and cleaned separately. The air inlet and outlet holes on the underside of the heads are plugged with corks to prevent the filters becoming wet and the heads are then given the same cleaning treatment as the bodies. A medium size bottle brush is useful for scouring the three ports and a wire brush for cleaning the threads of the couplings.

The vessels used for addition of aqueous solutions to the fermenters during fermentation are flushed with water and stored hung on a frame by S hooks with the valves open to allow complete drainage. Vessels used for antifoam oil are not usually cleaned between fermentations as they are used solely for this purpose. Should the glass level gauges become dirty they can most easily be cleaned by removing the cap screw from the top of the gauge and inserting a pipe cleaner into the glass tube.

Greasing and General Maintenance

1) *Bearings.* — The ballbearings of the bearing and stuffing box units on the fermenter heads are greased at the end of every fermentation immediately after all cleaning has been completed. A small grease gun filled with high melting grease and a short flexible connector threaded to fit the threaded hole on the flange of the bearing and stuffing box units are used for the purpose. The bearings are checked for play and adjusted if necessary.

If required the packing pressure of the stuffing box is adjusted by means of the knurled cap. The bearings of the pulley units need only occasional greasing.

2) *Air filters.* — It sometimes happens that the air filters become wetted with culture fluid during the fermentations. This may happen to the inlet filter if culture fluid is sucked back through the air spargers due to a fall in pressure of the main air line, while the outlet filter may become wetted through uncontrolled foaming. In such cases it is often possible to save the fermentation by flaming the metal tubes of the filters and thus drying the Fiberglass. In all cases when the filters are known to have been wetted during the fermentation they should be replaced before starting a new fermentation.

Discussion.

For many problems of chemical microbiology the availability of laboratory fermenters provided with mechanical agitation for obtaining high aeration rates is useful, if not essential. Substitution of fermenters by shake flask is very often impossible, particularly if high aeration rates are required.

In devising a laboratory fermenter a compromise must be struck between simplicity of design and efficiency of operation. The fermenter must be mechanically sound, it must be adaptable to different types of fermentations with varying oxygen demands, and above all, the risk of contamination during sample taking and the addition of solutions during the fermentation must be reduced to a minimum. As each fermentation has to be done at least in duplicate the smallest fermenter unit should contain not less than eight fermenters.

To improvise a laboratory fermenter unit fulfilling these conditions from ordinary laboratory glassware is not possible. It has been tried in various laboratories including those of the authors, but has never given really satisfactory results. Generally speaking the more improvised the fermenter equipment is, the smaller is its flexibility and the greater the hazard of contaminations.

The fermenter described in this communication was developed especially for the vortex system of aeration, but can equally well be used for sparger aeration. As far as versatility, simplicity of operation and reliability are concerned it compares favourably with other models described in the literature (Humfeld, 1947; Brown and Peterson, 1950b; Rivett, Johnson and Peterson, 1950; Calam, Driver and Bowers, 1951; Lumb and Fawcett, 1951; Kelly, Miller and Hale, 1952; Nelson, Traufler, Kelley and Lockwood, 1952). It is completely made of metal, stainless steel being used for all parts which may come in contact with

the culture fluid. In the literature most laboratory fermenter models which have been described have a metal head and a pyrex glass body, but the authors have preferred all-metal fermenters in order to abolish the risk of breakage, particularly during the sterilization.

The volume of the fermenter was chosen to be 10 litres, with a working volume of 5-7 l, because it was considered that with larger working volumes the fermenters would have become too heavy to handle with ease. The present fermenter, filled with 5 l of culture medium and with the addition vessels attached has a weight of 12 Kg. The construction of the bearing and stuffing box, which is novel in details, allows rotation of the propeller shaft at high speeds for prolonged periods. By suitable selection of propeller diameter and propeller speed any desired aeration rate, up to 350 ml 0_2 per 100 ml solution per hour, can readily be obtained at the normal working over-pressure of 0.5 atmosphere. The construction of the fermenter easily allows the application of an overpressure of 1.5 atmospheres so that aeration rates can be further increased by a factor of two.

Addition of solutions during the fermentation is effected in a particularly simple manner by opening the outlet valve of the addition vessels without the necessity of applying overpressure or releasing the working pressure of the fermenter.

With the aid of the special clip described above withdrawal of samples from the fermenter is also a simple and easily regulated operation. The reproducibility of the results in fermentations is satisfactory if foaming is controlled; examples for penicillin fermentations will be given in a following paper (Chain, Callow and Sekulic, 1954). The authors, at the time of writing, with their latest fermenter model as described above, have carried out 28 fermentations, each with 8-10 fermenters. In these experiments, involving a total of about 250 fermenters, the agitation system has never given the slightest trouble. No maintenance of the stuffing box was required apart from greasing. The percentage of fermenters which became contaminated during the fermentation run was low. Apart from one experiment, where 9 fermenters were lost because of insufficient sterilization of the culture medium, only two fermenters became contaminated, owing to faulty manipulation.

The fermenter has been successfuly used for fermentations with numerous filamentous fungi, actinomycetes, yeasts and bacteria. It is not suitable, however, for working with pathogens.

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